Hydrolytic Pretreatment of Macauba Shells for Anaerobic Digestion

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Abstract

This experiment examined the effect of sub-critical water hydrolysis as a pretreatment for anaerobic digestion of macauba nut shells for production of renewable methane. Renewable natural gas was made from macauba nut shells with and without pretreatment using subcritical water hydrolysis as the pretreatment. The control reactor had an average solid content of 16.9%, an accumulated volume of biogas of 17.6 liters and an average methane composition of 38%. The pretreatment reactor had an average solids content of 4.4%, an accumulated volume of 22.5 liters and an average methane composition of 67%. The accumulated energy of the pretreated reactor was 97.9 MJ/kg and the control reactor was 4.69 MJ/kg. This shows that the pretreated reactor produced almost 21 times more energy per kilogram of macauba shells used to feed and start up the digestor, meaning that this pretreatment performed far better than the control.

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1.0 Introduction

Due to the negative environmental impact of fossil fuels, many countries are finding new renewable energy sources to shift their dependence on the negative energy sources [11]. The greenhouse gas effect of methane and carbon dioxide is requiring that countries find alternative energy sources that reduce the harmful effect of these gases. Brazil is one of the world's leading countries in renewable energy sources [7]. The country passes many policies in favor of companies and citizens switching to renewable energy sources. Brazil has a large agriculture industry which makes producing bioenergy from this renewable source an appealing option. Biodiesel and biogas are both renewable energy sources of particular interest in Brazil and around the world [13]. This project looked specifically at the production of biogas using anaerobic digestion.

Biogas is a combination of methane and carbon dioxide and can be used for mechanical and thermal energy. It can be produced from either anerobic digestion or composting and there are benefits to both. Anaerobic digestion is the breakdown of organic material by microorganisms in the absence of oxygen [8]. It occurs in four main steps where feedstock enters the digestor as carbohydrates and proteins and slowly breaks down into methane and carbon dioxide. The largest limiting factor of anaerobic digestion is the slow rate of breakdown of material [7]. Because anaerobic digestion relies heavily on the breakdown of material, steps can be taken to speed up the process [10]. Many feedstocks are pretreated to either break them down or make it easier for the organisms to break it down [18]. There are many different types of pretreatment and one of the difficulties of anaerobic digestion is matching the correct pretreatment to the feedstock selected [11]. Anaerobic digestion has a high start up cost and with the wrong pretreatment, it prevents the process from being scaled up and economically feasible [23]. This experiment was designed to test the feasibility of a certain pretreatment for a given material.

Macauba nut shells are good candidates to be used as a raw material as a feedstock for anaerobic digestion. Macauba nuts are found in many countries in South America and are very common in Brazil. The nuts are used in the cosmetic, chemical, energy and biodiesel industry. Only the cake part of the macauba nut is used to produce biodiesel meaning the shells are discarded as waste. Using the residue from biodiesel reduces the overall amount of waste and increases the energy production from a single macauba nut [7]. Currently in Brazil, soybeans are the highest producer of renewable energy sources, because they are used to produced biodiesel. Soybeans have more edible applications than macauba nuts, so they are more readily available [14]. There is a lack of research into the ability of macauba nuts to produce renewable energy.

This project examined the feasibility of a particular pretreatment for anaerobic digestion of the macauba nut. Sub-critical water hydrolysis is a pretreatment that focuses on breaking down the raw material into a liquid state for the digestor [12]. A control reactor with ground macauba shells was compared to a pretreated reactor with hydrolysis used as the raw material. Daily characteristics were taken from each of the digestors, including volume and composition of biogas produced.

Every 2-3 days a full characterization of the digestors was performed which included total solids content, pH, alkalinity, ammoniacal nitrogen content and chemical oxygen demand.

2.0 Background

2.1 Biogas

Pollution prevention and human health issues require increasingly sustainable energy solutions [11]. Due to this increase in awareness for environmental safety, renewable energy is an ever-growing industry. One industry in particular that has experienced this growth is the biofuel industry. Biofuel is defined as a fuel source that is derived directly from living matter. There are many manners to produce biofuel, but two main ways are the production of biogas and the production of biodiesel.

Biogas it a mixture of carbon dioxide and methane [3]. It is produced from a process called methanogenesis, where organic material degrades into methane and carbon dioxide. Raw material for this process can be found from many different sources such as animal or food byproducts [11]. These substrates naturally produce methane and left unused, this methane is released into the atmosphere. The greenhouse effect of methane is roughly 27 times higher than carbon dioxide so there is a strong environmental advantage to using the biogas since this process occurs naturally already [3]. Two of the most common ways to produce biogas are from anerobic digestion and composting, where anaerobic digestion occurs in the absence of oxygen like the name suggests.

Biogas production stabilizes waste and has a net energy production making it a viable option for a renewable energy source. It is also carbon neutral which means it does not increase the overall level of carbon dioxide. As a fuel source, biogas is used for different power plants and can be used for heating in industrial settings.

2.2 Macauba Nut

One agricultural source for raw material for the production of biogas is Macauba oil. Macauba is a palm native to Brazil that is used for human consumption, cosmetics, and a source for alternative fuel [13]. Macauba has a high oil content with an average between 23 and 34% which makes it a promising source for biodiesel production. There are four common parts to the macauba nut, the epicarp, mesocarp, endocarp and endosperm. Figure 2.1 shows the four layers of the macauba nut. The epicarp is the shell of the nut and can be used in anerobic digestion to produce biogas, used to produce thermic energy or used in animal feed. The mesocarp and the endosperm are both cake like layers that have a high oil content. They can be used to produce biodiesel, in cosmetics or in the chemical industry. The endocarp layer can be used to produce energy in the form of coal. All parts of the macauba nut are useful in various industries except the food industry. Due to the lack of edible applications of the nut, it is not as widely produced in countries like Brazil.

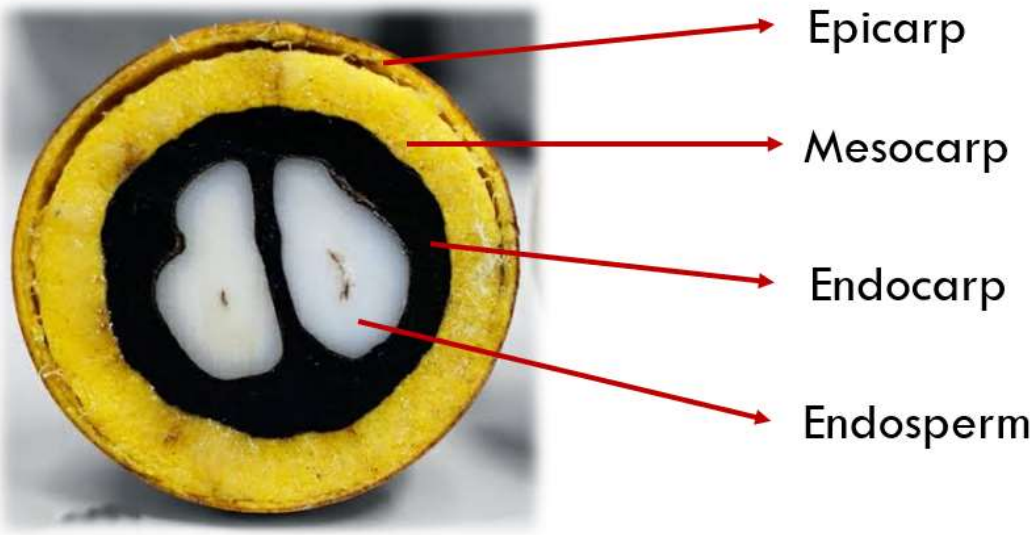

Figure 2.1: The four layers of the Macauba Shell

2.2.1 Macauba Oil for Biodiesel Production

Currently in Brazil macauba is not the leading producer of biodiesel. Soybeans are the largest producer of biodiesel in Brazil because of the abundance of the crop [19]. Macauba nuts have less food applications and are not as widely produced, they are however, easier to grow than soybeans. The nut has a high resistance to pests and has the ability to grow in areas of low rainfall [17]. Because of this, it is cheaper to produce macauba then to produce soybeans.

Biodiesel is different from biogas but is another promising renewable energy source due to the similarities with regular diesel [13]. It is a biodegradable fuel that can be used in preexisting engines without modifications [17]. Currently about 80% of the biodiesel production in Brazil is from soybean oil, however macauba oil is much cheaper and is growing as a source of raw material for biodiesel [16]. To make biodiesel, a transesterification reaction occurs to produce fatty acid methyl esters (FAMEs) and glycerol [16]. The FAMEs are the desired product of the reaction with glycerol being an undesired side product. The residue left over after the production contains a mixture of sugars, acids and glycerol [7]. This residue can be used to produce ethanol or can be used to biogas. With macauba nuts, only the endosperm and mesocarp layers can be used to produce biodiesel, leaving the epicarp and endocarp layers as byproducts of the production that can be used in other forms of energy production.

A lot of research is being done into the applications of Macauba as a renewable energy source. When Macauba is used for biodiesel production only the nuts are used and the shells are waste. These shells can be used as raw material in other methods of producing bioenergy.

2.3 Anaerobic Digestion

A popular method for converting raw material to biogas is through anaerobic digestion. This process, also commonly known as biomethanation, allows energy to be produced in the form of biogas [3]. This process works by degrading raw material using microorganisms and bacteria. In order to produce methane the reactor contains an active inoculum of microorganisms to digest the material. This is a common process that occurs many places in nature. One place of particular interest is in landfills because this process produces methane and carbon dioxide which are both harmful greenhouse gases. Biogas production can not only reduce the release of these harmful chemicals into the environment but also harness these gases into a renewable energy source [18].

Anaerobic digestion occurs in four steps [8]. The first step is hydrolysis where the raw material, mostly made up of proteins, fats and carbohydrates, is broken down into sugars, fatty acids and amino acids. In hydrolysis, water is typically used to break down the organic matter. This leads into the second step which is acidogenesis [4]. This is where the molecules are broken down further after hydrolysis. Bacteria breaks down the sugars and fatty acids into more acidic byproducts like ammonia, carbonic acids and alcohols. The mixture is still too large for the ultimate end goal of producing methane and needs to be digested further.

In the third step, acetogens take the products produced in the second step and create acetate. This is why this step is named acetogenesis. The products created in this step are carbon dioxide, hydrogen and acetate. This step is the most important to occur in an anaerobic environment due to the thermodynamics of the reactions taking place. The last step is methanogenesis where methanogens finally produce methane from the products of the third step. The main way to produce methane is from acetic acid produced in the third step, however, some of the products produced in step two can also be used by the methanogens to produce methane. The two chemical reactions to produce methane can be seen in equations 1 and 2 below.

$$CO_2 + 4H_2 \xrightarrow{yields} CH_4 + 2H_2O \quad (1)$$

$$CH_3COOH \xrightarrow{yields} CH_4 + CO_2 \quad (2)$$

There are many different factors that affect the efficiency of biogas via anaerobic digestion production including mixing rate, reactor design, pretreatment steps and pH of the solution. The reaction can occur in one or multiple reactors and can occur as a batch or continuous process. Different types of reactors help in different aspects of the reaction [10]. A continuous stirred-tank reactor (CSTR) can be used to ensure constant mixing within the process. A plug flow reactor is another viable option for equal mixing because the feed flows through a bed packed with particles to mix it [8].

The pH of the system is an important factor to consider with reactor conditions. The methanogens perform most efficiently in a pH between 6.8 and 7.2 [8]. However, other steps do not perform

optimally at this pH so some variation from this pH range is needed. Temperature is an important factor as well. Most reactors operate in either mesophilic or thermophilic temperatures with maximums at 35 degrees Celsius or 55 degrees Celsius [22]. Although thermophilic temperatures are more favorable for endergonic reactions, they are also affected more by changes in the surroundings.

2.3.1 Pretreatment Options

Depending on the type of feedstock being harvested, different pretreatment steps can be taken to produce a higher yield of biogas. Feedstock of raw material is harvested and then commonly physically pretreated or shredded before it is fed into the reactor [22]. Pretreatment is common in many biomass feedstocks because it can break down proteins like cellulose and lignin. Common pretreatments include, ultrasonic, alkali and thermal hydrolysis.

There are many different options for feedstock in anaerobic digestion. Historically, manure, sewage sludge and food waste have been optimal raw material for anaerobic digestion [24]. Due to the differences in all of these materials, selecting the correct method for pretreatment is critical for the success of methane production. The pretreatment option is based on the composition of the feedstock and what steps are necessary to prepare the sample for methanogenesis. There are four different types of pretreatments, physical, chemical, biological and physical-chemical.

Raw materials that are large in size and cannot be put directly in the reactor have to be physically pretreated by grinding or other methods. These materials include waste activated sludge and some food byproducts like the macauba residue used in this experiment.

2.3.2 Sub-critical Water Hydrolysis

Another pretreatment option is subcritical water hydrolysis. This is an alternative to using corrosive acids and organic solvents that are more harmful to the environment [25]. The primary goal of pretreating the biomass is to alter the structure of the raw material and make it more easily digestible by the microorganisms [15].

This technique uses sub-critical water which is water that is between 100 degrees Celsius and 374 degrees Celsius but kept under a high pressure in order to maintain the liquid state of the water [15]. The high temperature supports the conversion of feedstock into simple sugars needed for the production of biogas [12]. The use of water as the medium for hydrolysis presents many advantages over using more traditional mediums such as acid, alkali or enzymes. Water produces less waste and undesirable byproducts and is also less toxic than the other solvents [21]. The main use of sub-critical water hydrolysis is to convert organic waste into useful products, however, it can also be used as a pretreatment for lignocellulose biomass to produce biogas through anaerobic digestion.

2.4 Analytical Techniques

2.4.1 Gas Chromatography

Gas chromatography is a technology used to help separate and characterize volatile compounds [9]. Chromatography is the separation of compounds using two phases, stationary and mobile. This technique is used to determine the specific compounds present in a sample and in what quantity. The chromatograph calculates the time a sample spends in the column and uses this as the retention time. The gas chromatograph produces a plot of retention time versus intensity and based on the integrated area of the peaks, the percentage of compounds present can be calculated. The retention time is how to determine which compounds are present, since each compound has a specific retention time. In this experiment, the levels of hydrogen, oxygen, methane and carbon dioxide were measured on each of the samples. Table X shows the approximate retention times of each of these species. In addition to the retention time the chromatograms also show the area of each peak. The area of each peaked is used to determine the composition of each component of the sample.

SPECIES	RETENTION TIME (MINUTES)
HYDROGEN	3
OXYGEN	7
METHANE	15
CARBON DIOXIDE	23

The gas chromatograms in this report were analyzed for different components of biogas; methane, carbon dioxide and hydrogen as well as monitoring for oxygen [9]. Because this digestion was done anaerobically, there should be no oxygen present in the reactor, however some chromatograms showed oxygen was present due to exposure during feeding and sampling of the reactor.

2.4.2 Total Solids

Total solids analysis is used to determine the moisture content of a certain sample. There are many techniques to do total solids analysis, but an oven method was used for this report. The sample is entered into an oven for a specific period of time and the loss of weight is determined as the moisture content. The formula for the calculation of the total solids content can be seen below in Equation 3. The total solids (TS) are defined as the solids that are left over after drying in an oven.

$$TS = \frac{wt\ wet\ sample - wt\ of\ dry\ sample}{wt\ of\ wet\ sample} * 100 \quad (3)$$

In addition to total solids analysis, volatile solids analysis can also help to determine the optimal moisture content of the mixture. Fixed solids are defined as the solids that remain after the sample is both dried in the over and then incinerated [2]. Volatile solids analysis is the difference between the fixed solids and the total solids. The equations for fixed solids (FS) and volatile solids (VS) are shown below in equations 4 and 5.

$$FS = \frac{wt\ of\ wet\ sample - wt\ of\ incinerated\ sample}{wt\ of\ wet\ sample} * 100 \quad (4)$$

$$VS = TS - FS \quad (5)$$

2.4.3 pH

The microorganisms used for anaerobic digestion are sensitive to the pH of the environment of the reactor. Because of the different steps in anaerobic digestion, the preferred pH varies depending on what step in the process is being targeted [22]. Generally a pH of 6.8-7.2 is preferred for anaerobic digestion but some reactors produce more methane under a pH range of 7-8. For this reason, it is necessary to test the pH of the reactor regularly and correct it using a base. Over the process the pH increases naturally as the acid are digested.

2.4.4 Alkalinity

Alkalinity is the buffer capacity of the mixture in the reactor [22]. Alkalinity measures the amount of carbonate, bicarbonate and hydroxide ions that are present because all of these ions are key to creating a self-buffering mixture. This is important because the microorganisms are so sensitive to changes in pH that the more buffer capacity the mixture has, the better production of methane that will occur because the pH can be more easily maintained at a favorable level. Alkalinity is usually measured in milligrams of calcium carbonate per liter of sample. The alkalinity is similar to the pH, however, it shows a more in depth picture of what is happening in the reactor and picks up on more minute changes in the system. It is typically measured in milligrams of calcium chloride per liter.

2.4.5 Chemical Oxygen Demand

The chemical oxygen demand of a system is the measured amount of organic matter in a system [1]. To determine the chemical oxygen demand samples are prepared with oxidants that digest the organic matter in the system. A macro or micro method of digestion can be used. After the sample is digested the chemical oxygen demand can be determined either by titration or by absorbency. Chemical oxygen demand is typically measured in milligrams of oxygen per liter of sample. In this experiment the oxidant used was a chromate ion ($Cr_2O_{-2}^{7}$).

In anaerobic digestion the bacteria in the reactor digests the organic material causing a decrease in chemical oxygen demand over time [1]. Depending on the feed rate there can be a stabilization of the chemical oxygen demand over time as the organic matter is reintroduced in the feed.

2.4.6 Ammoniacal Nitrogen

Nitrogen is an important part of anerobic digestion because it helps the bacteria grow and develops the process further. Nitrogen can appear in many forms in the digestive material but it occurs most commonly as ammoniacal nitrogen [12]. This is why it is important to get a measurement of how much ammoniacal nitrogen is in the reactor to see if the bacteria have the optimal environment to continue the process of anaerobic digestion [22]. Ammonia can be harmful to anerobic digestion

in high quantities but is still necessary for the process [12]. The optimal amount of nitrogen and ammonia depend on the feedstock and other conditions in the reactor. There are also different types of tests for ammoniacal nitrogen content. If high concentrations are anticipated, a method including titration and distillation should be used.

3.0 Materials and Methods

3.1 Raw Materials

Solid residues (Macauba Nut Shells) from biofuel production were provided by a biodiesel production plant located in Campinas, Brazil. Inoculum was provided by AmBev Beer Company in Sao Paulo, Brazil. The Macauba shells were grinded as a physical pretreatment to the raw material. The before and after pictures of the physical pretreatment can be seen in Figures 3.1 and 3.2 below.

Figure 3.2: Macauba Shells from biodiesel production plant

Figure 3.3: Physically Pretreated Macauba Shells

3.2 Pretreatment

Sub-critical water hydrolysis was used to pretreat the Macauba shells. 10 grams of Macauba shells were hydrolyzed in the hydrolysis machine to produce 500 ml of hydrolysate. The reactor was run at 200ºC and 15 MPa with a flow rate of 10 ml/min. A picture of the hydrolysis reactor can be seen in Figure 3.3 below. The hydrolysate was collected in 50 ml Falcon tubes and the residue from the reactor was collected in a beaker. When preparing the feedings for the reactor all the hydrolysate

was mixed together with the residue. The feed and initial digestor material were taken from the mixture.

Figure 3.4: Sub-critical Water Hydrolysis Machine

3.3 Reactor Set Up

Two reactors were used to test the pretreatment of the Macauba shells. The first reactor was the pretreated reactor and the second reactor was the control reactor. Both reactors were set up in the same fashion and Figure 3.4 shows the setup of both reactors in the lab. Both reactors were 4.3 liters in size and contained 60% liquid and 40% head space left for gas. The digestor experiments were run for 30 days. Samples of 110 ml were taken every Monday, Wednesday and Friday during the experiment. Every time the digestors were sampled, a feed of 110 ml was also introduced to the reactor to replace the sample. Both reactors were attached to 10 liter Supelco Analytical biogas collection bags.

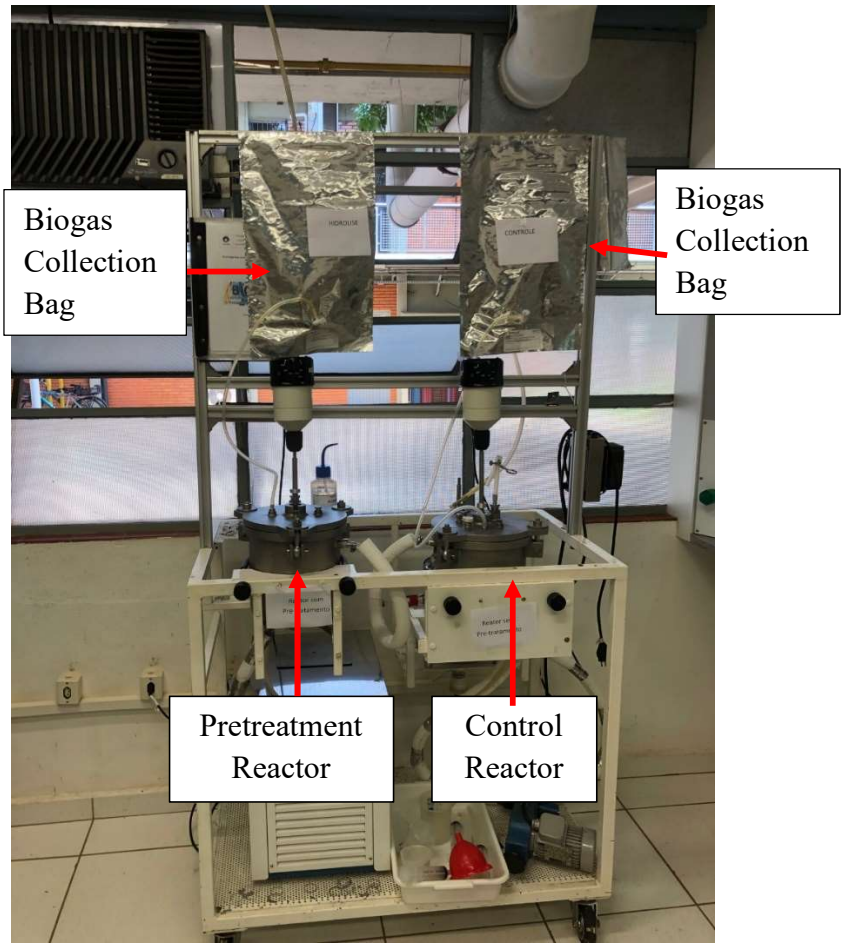

Figure 3.5: Set up of anaerobic digestors

3.3.1 Pretreated Reactor

Figure 3.5 shows the setup of the pretreated reactor. The pretreated reactor was loaded with 2.58 liters of a liquid mixture to match the 60% of the reactor. Of the liquid portion 0.774 liters were inoculum and 1.806 liters were hydrolysate from the hydrolysis machine. The reactor has a gas sample port, biogas collection tube, reactor sample port and agitation motor.

The feed was composed only of 110 ml of hydrolysate from the pretreatment reactor.

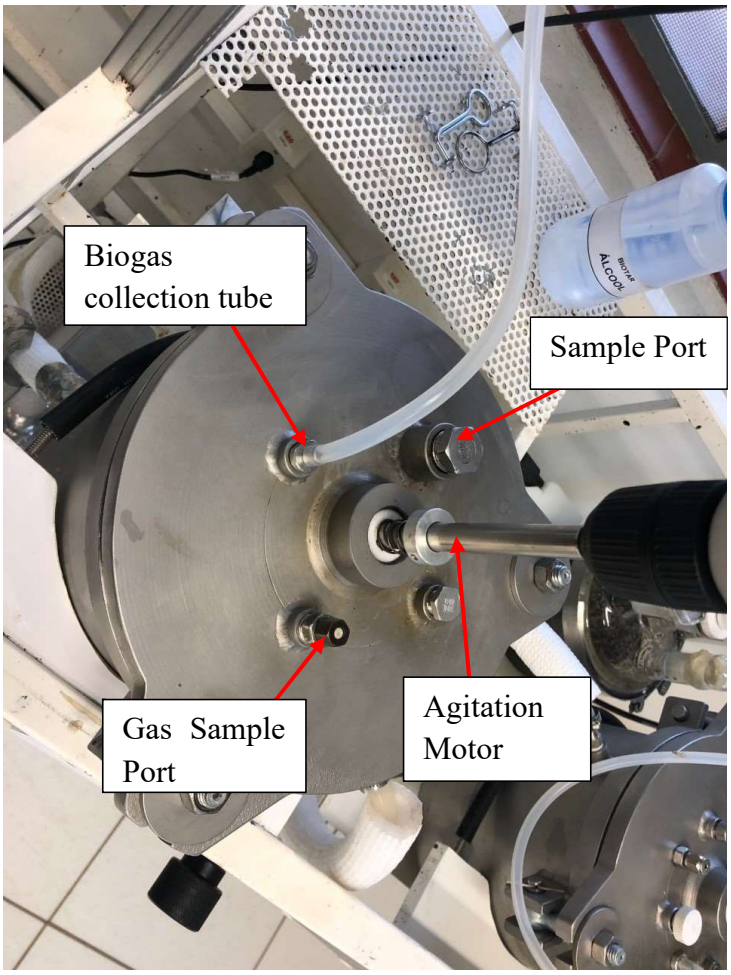

Figure 3.6 Setup of Pretreatment Reactor

3.3.2 Control Reactor

The control reactor was setup in an identical digestor to the pretreatment reactor. The Macauba used in this reactor was only physically pretreated but not placed through a hydrolysis machine. The control reactor was initially loaded with 2.58 liters of material which was 35% Macauba and 65% liquid. The liquid was composed of 60% inoculum and 40% water. The reactor has a gas sample port, biogas collection tube, reactor sample port and agitation motor. Figure 3.6 below shows the set up of the control reactor.

The feed was a mixture of 65% water and 35% ground macauba shells.

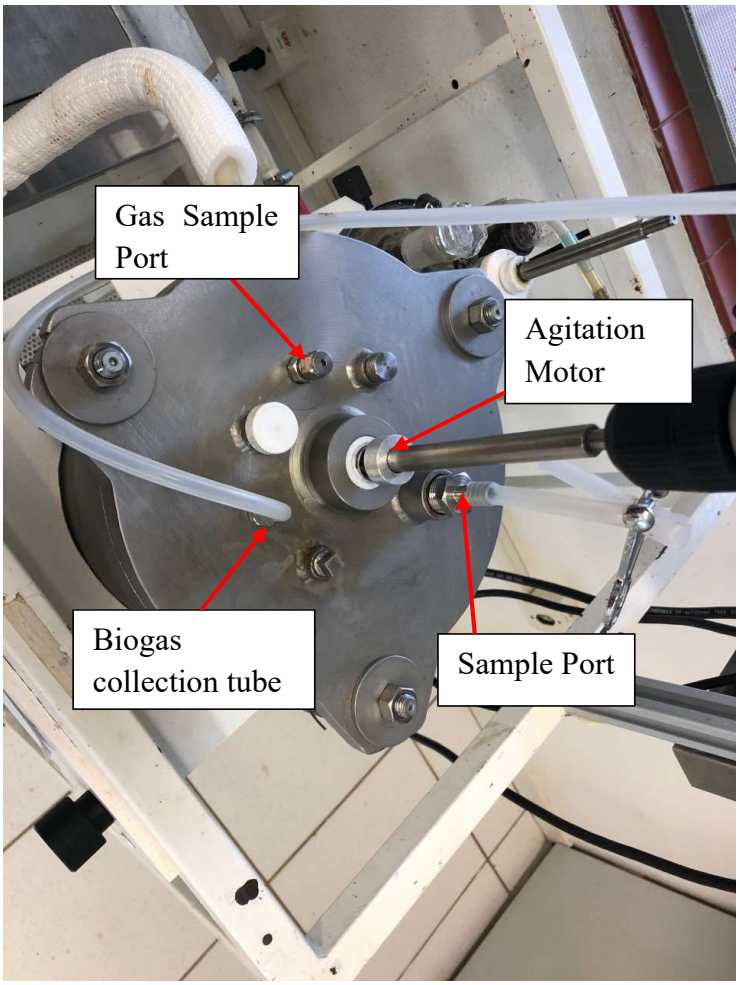

Figure 3.7: Setup of Control Reactor

3.4 Analytical Techniques

3.4.1 Total Solids Content

The total and volatile solids were analyzed for each sample from both reactors. Samples were taken in triplicate and placed in crucibles. The crucibles were first dried in an oven at 105°C and then the mass of each was recorded. Approximately 2 grams of sample was placed in a crucible and the exact mass was recorded. The sample was then placed in the oven at 105ºC for 24 hours and the mass after drying was recorded. The total solids content was calculated using equation 6 below.

$$TS = \frac{mass\ dry\ sample - mass\ of\ dry\ crucible}{mass\ of\ wet\ sample} * 100 \quad (6)$$

The volatile solids content was found after the total solids analysis. Each crucible was then placed in the mufla to be incinerated. The temperature of the mufla was gradually increased to 550ºC where the sample stayed for 2 hours. After the sample was moved the mass was recorded. This

allowed the fixed solids content to be found using equation 7 and equation 8 shows how volatile solids content was found.

$$FS = \frac{mass\ of\ incinerated\ sample - mas\ \ of\ dry\ crucible}{mass\ of\ wet\ sample} * 100 \quad (7)$$

$$VS = TS - FS \quad (8)$$

3.4.2 Volume of Biogas Produced

Every day the amount of biogas produced was recorded. A 60 ml syringe was used to empty the biogas from the reactor and the gas collection bag every day. 600 ml of biogas was taken from each reactor every day of the trial to alleviate the pressure of the head space and the gas collection bag was emptied in full and both amounts of biogas were recorded.

3.4.3 Composition of Biogas

Every day the composition of biogas was also analyzed. A 0.5 ml sample of biogas was taken from the headspace in the reactor and placed into a gas chromatograph (GC-2014) and analyzed daily. The temperature of the gas chromatograph was set at 250ºC and 35 ml/min. The retention time, area and ratio of area to height were recorded for hydrogen, oxygen, methane and carbon dioxide.

3.4.4 pH

The optimal pH range for anaerobic digestion is between 6.5 and 8.0 [22]. Every Monday, Wednesday and Friday when the reactors were sampled, the pH was also monitored and corrected. The pH was monitored with a Sinergia W3B pH meter with calibrations at 4 and 7. A 20 ml sample was taken and drops of 2 M sodium hydroxide were added until the pH was between 7.0 and 8.0. This was then converted to an amount that would correct the overall pH in the reactor based on the ratio between the number of drops that corresponds to 1 milliliter. This number was then scaled up to the amount of liquid in the reactor. The sodium hydroxide was then mixed with the feed and introduced into the reactor.

3.4.5 Alkalinity

The alkalinity was measured every Monday, Wednesday and Friday that samples were taken. 5 grams of sample was taken and diluted with 50 ml of deionized water in a 250 ml Erlenmeyer flask. The flask was placed in a Tecnal TE-421 shaker at 200 rpm, 25°C for 1 hour. 10 ml of the diluted sample was placed into a 40 ml beaker with a magnetic stir bar. The pH of the sample was recorded, and the magnetic stirrer was turned on. 0.2 M sulfuric acid was added to the sample until the pH was between 4.3 and 4.7. The volume of sulfuric acid added was recorded. The alkalinity was calculated using equation 10 [22].

$$Alkalinity = \frac{M_{H_2SO_4} * V_{H_2SO_4} * 50000}{10\ ml} \quad (10)$$

3.4.6 Chemical Oxygen Demand (COD)

The chemical oxygen demand was calculated for all of the samples taken from the reactors. 5 grams of sample were placed into a 250 ml Erlenmeyer flask and diluted with 50 ml of deionized water. The flask was placed in a Tecnal TE-421 shaker at 200 rpm, 25°C for 1 hour. The samples were then filtered by cotton and by vacuum. The HACH tubes were rined with 2 M sulfuric acid solution. 2.5 ml of the filtered solution was placed into the HACH tubes three times. 1.5 ml of digestive solution and 3.5 ml of catalytic solution were added to the HACH tube and then it was vortexed. The tubes were placed into a block digestor at 150ºC for 2 hours. The samples were removed from the digestor and placed in the dark to cool for 1 hour. The absorbance of the samples was then read in a spectrophotometer at 610 nm. A standard curve was also made the first time preparing the samples by analyzing samples of 25 mg COD/ liter, 100 mg COD/liter, 500 mg COD/ liter, 700 mg COD/ liter, 900 mg COD/ liter, and 1100 mg COD/liter. The absorbance of these samples were plotted on a graph and a line of best fit was found. The slope and y-intercept of this standard line was used to calculate the COD of the rest of the samples using equation 9 below [1].

$$COD = \frac{absorbance - y - intercept}{slope} \quad (9)$$

3.4.7 Ammoniacal Nitrogen

The ammoniacal nitrogen content was found for each sample taken from both reactors. 5 grams of the sample was placed into a 250 ml Erlenmeyer flask and diluted with 50 ml of deionized water. The flask was placed in a Tecnal TE-421 shaker at 200 rpm, 25°C for 1 hour. The sample was then filtered by cotton into a new 250 ml Erlenmeyer flask and then filtered by vacuum. 5 ml of the sample was then placed into a 30 ml beaker along with 5 ml of a borate buffer. The pH was adjusted to 9.5 by adding drops of 6 M sodium hydroxide and the number of drops added were recorded. The solutions were then transferred to a Kjeldahl digestion buffer tube. 10 ml of absorbance solution was placed into 250 ml Erlenmeyer flask.

A Marconi nitrogen distiller was used to connect the samples. First a tube of distilled water and a sample of distilled water was connected to heat up the machine. Then a blank sample of 5 ml of distilled water and 5 ml of borate buffer were connected and run through the nitrogen distiller until the flask filled to 100 ml. The tube and flask were disconnected from the distiller and left to cool. The flask was then titrated with 0.2 M sulfuric acid until the solution turned pink. This process was then repeated with the triplicate samples. After removing them from the distiller we titrated until the color matched that of the blank, recording how much sulfuric acid was added to each sample. Equation 10 was used to find the ammonia concentration in the sample [10].

$$Ammoniacal\ Nitrogen = \frac{(A-B)*14*M_{H2SO4}}{V_{Sampl}} \quad (10)$$

Where A is the volume of sulfuric acid added to each of the samples and B is the volume of sulfuric acid added to the blank.

4.0 Results and Discussion

4.1 Total and Volatile Solids

The total and volatile solids were collected every Monday, Wednesday and Friday from the samples. The total solids were found after leaving the samples in the oven and the fixed solids were found after incinerating the samples. The volatile solids were then calculated based on those two values. The total solid and volatile solid content of the reactor allows for a better understanding of the composition of the reactor and how much it changes over time.

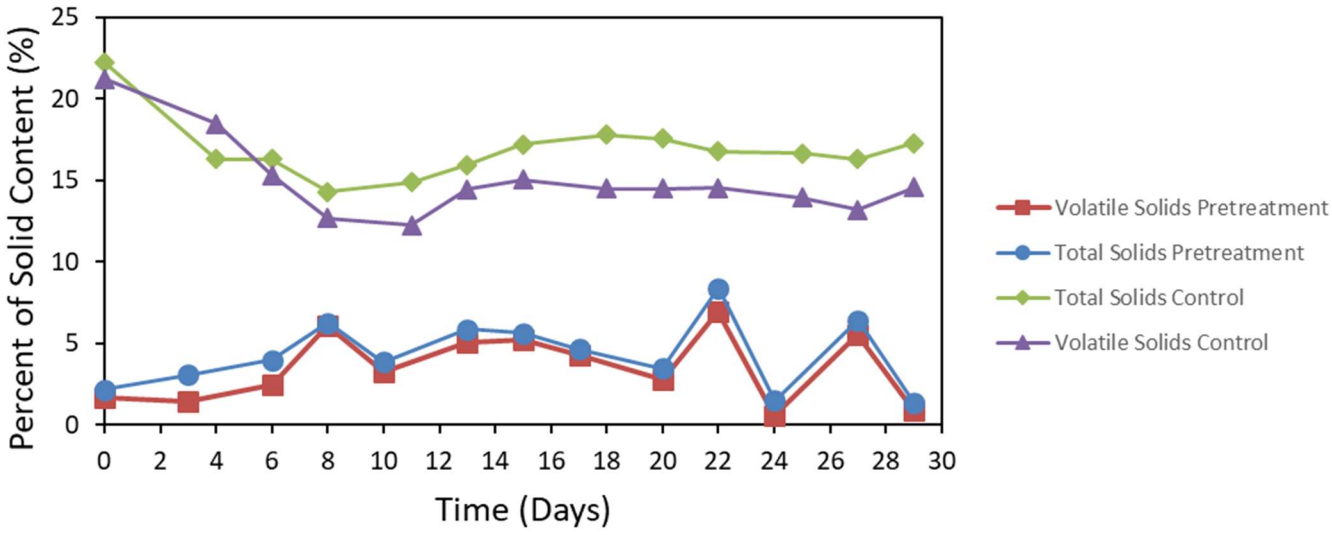

Figure 4.8:Total and Volatile Solids Measurements for both Control and Pretreatment Reactors

Figure 4.1 shows the total and volatile solids contents of both reactors over 30 days. The Control reactor had a much greater solids content than the pretreatment reactor. This is due to the initial conditions and feed conditions for both reactors. The control reactor was fed with and initially contained solid macauba shells. The pretreatment reactor was fed with and initially contained macauba shell hydrolysis. In general, the solid content of both reactors did not fluctuate much because they were being fed with a similar content than they initially contained. The control reactor was more stable in the solid content because each sample taken was a more consistent consistency whereas, the pretreatment reactor was more separated, and it was hard to get a sample with the same amount of solid content each time.

4.2 Chemical Oxygen Demand

The chemical oxygen demand was collected every Monday, Wednesday and Friday. The COD measurement is taken to assess the amount of organic content within the reactor to see how much potential of the reactor has to produce biogas. The chemical oxygen demand measures the intermediates between the feedstock and the biogas.

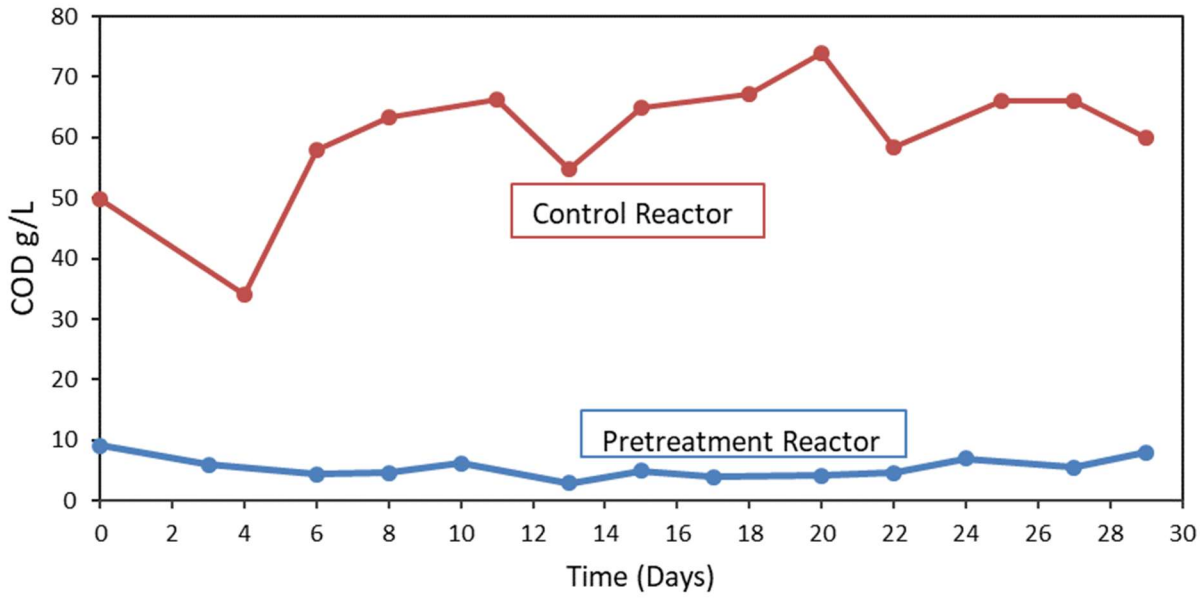

Figure 4.9: Chemical Oxygen Demand of Pretreated and Control Reactor

Figure 4.2 shows the chemical oxygen demand of both reactors over 30 days. The control reactor had a much greater chemical oxygen demand over the trial period. Both reactors had a very stable chemical oxygen demand over the 30 days. The pretreatment reactor had much less chemical oxygen demand than the control reactor. Usually a greater chemical oxygen demand corelates to a greater yield in biogas. However, having a greater amount of chemical oxygen demand over the entire trial period means that not all of the organic content in the reactor is used because it stays as the intermediates between feedstock and biogas. The pretreatment reactor does have lower chemical oxygen demand but that is indicative of the reactor not containing the intermediates that are between the raw material and the final product of the biogas. This could be because the pretreatment reactor produced biogas at a faster rate and therefore contained less intermediates than the control reactor.

4.3 Ammoniacal Nitrogen

Ammoniacal nitrogen content was measured every Monday, Wednesday and Friday. This is similar to chemical oxygen demand as it also measures the intermediates in the steps of anaerobic digestion. Typically, within anaerobic digestion, a greater ammoniacal nitrogen content correlates to a greater yield of biogas because there are more intermediates to turn into carbon dioxide and methane [20].

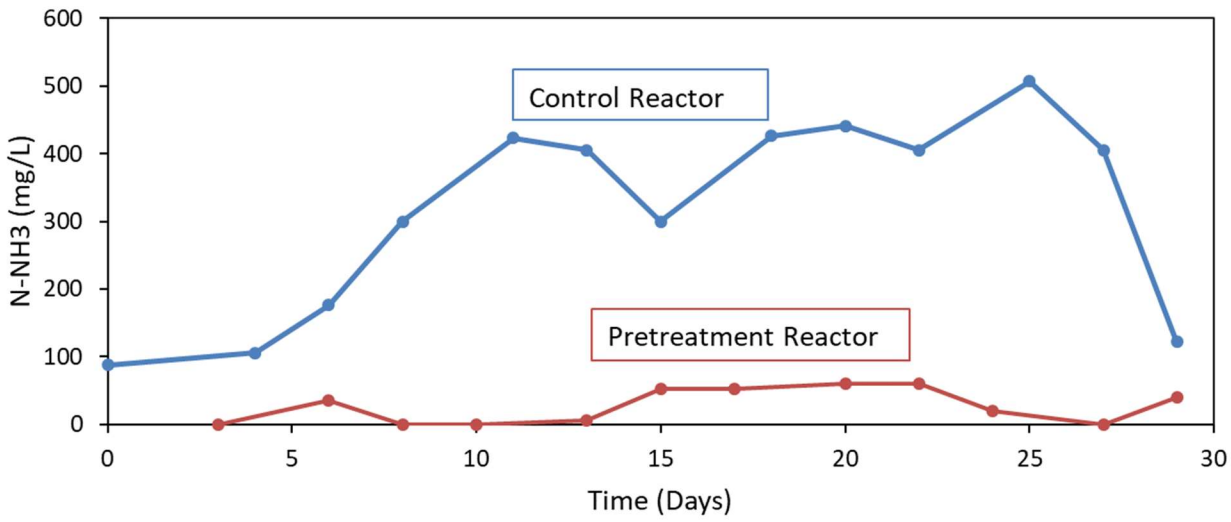

Figure 4.10: Ammoniacal Nitrogen Content of Pretreated and Control Reactors

Figure 4.3 shows the ammoniacal nitrogen content in both reactors. This graph shows the parallel between the chemical oxygen demand and ammoniacal nitrogen content because both of these are measures of the intermediate steps in anaerobic digestion. The pretreatment reactor had the same trend in ammoniacal nitrogen that it did with chemical oxygen demand. It was less throughout the entire trial period and that is indicative that the reactor did not spend any time in the intermediate stages. Although ammoniacal nitrogen content is a measure of the intermediate steps of anaerobic digestion, having too much nitrogen in the reactor can hinder anaerobic digestion more than it can help [10].

4.4 Alkalinity

The alkalinity is the measure of the buffer capacity in any reactor [22]. It gives a more complete picture of what is occurring in the reactor.

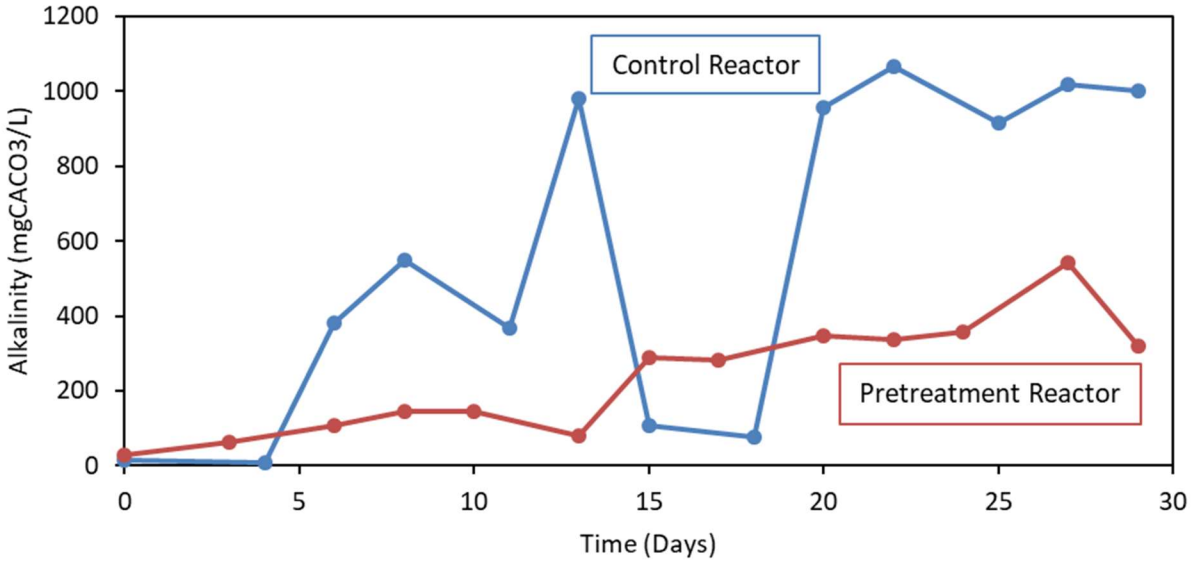

Figure 4.11: Alkalinity of the Control and Pretreatment Reactors over 30 days

Figure 4.4 shows the alkalinity in both reactors over the 30-day trial period. The alkalinity of the pretreated reactor was consistently less than the control reactor and steadily grew over time. The two low points of the alkalinity of the control reactor occurred on days where a different pH meter had to be used. The alkalinity came out less, but these could be outlier data points. The alkalinity is a measure of the buffer capacity and the control reactor had a greater alkalinity for the average of the time.

4.5 pH

The pH was measured in both reactors every Monday, Wednesday and Friday. The optimal pH of an anaerobic digestor is between 6.5 and 8 [22]. The pH was taken to make sure the reactor had the optimal conditions throughout the trial period and if the conditions inside needed to be corrected.

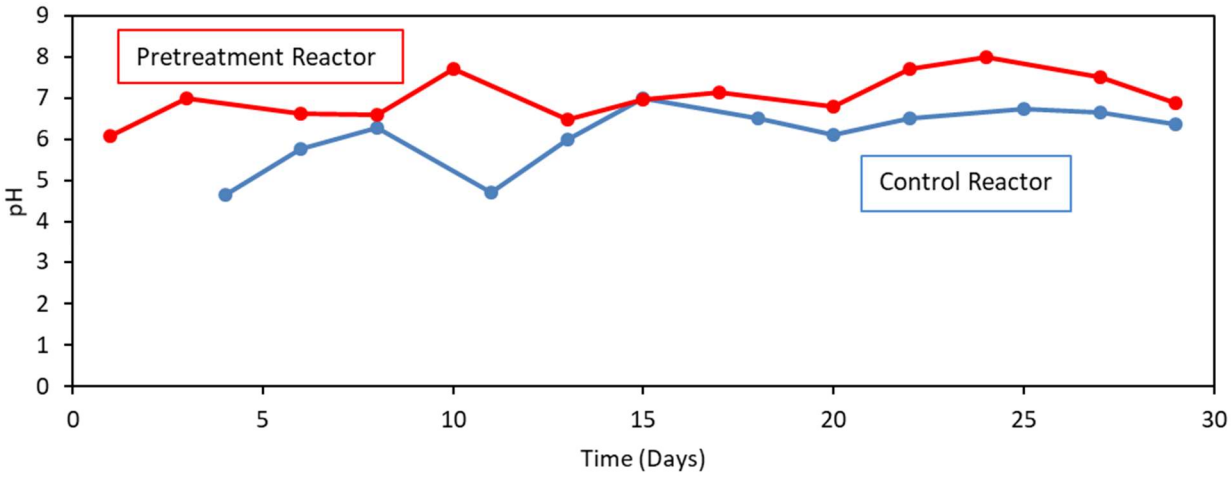

Figure 4.12:pH of the Control and Pretreated Reactors over 30 Days

Figure 4.5 shows the pH in both reactors over the 30-day trial period. The pretreatment reactor had a higher pH over the trial period than the control reactor. This lends itself towards a more conducive environment for biogas production and should have a higher yield of methane. Both reactors held a relatively stable pH throughout the trial period as well. The pretreated reactor did not need to be corrected as many times as the control reactor. The control reactor had a greater alkalinity than the pretreated reactor which means it had a better ability to control the pH. This can be shown in Figure 4.5 where the control reactor was consistently below the range of 7-8, the ideal pH for anaerobic digestion, despite being corrected with sodium hydroxide each time.

4.6 Volume of Biogas

The volume of biogas was measured every weekday from each reactor. 600 ml of biogas was taken from each reactor ever time. The entire contents of the collection bag were emptied each day as well.

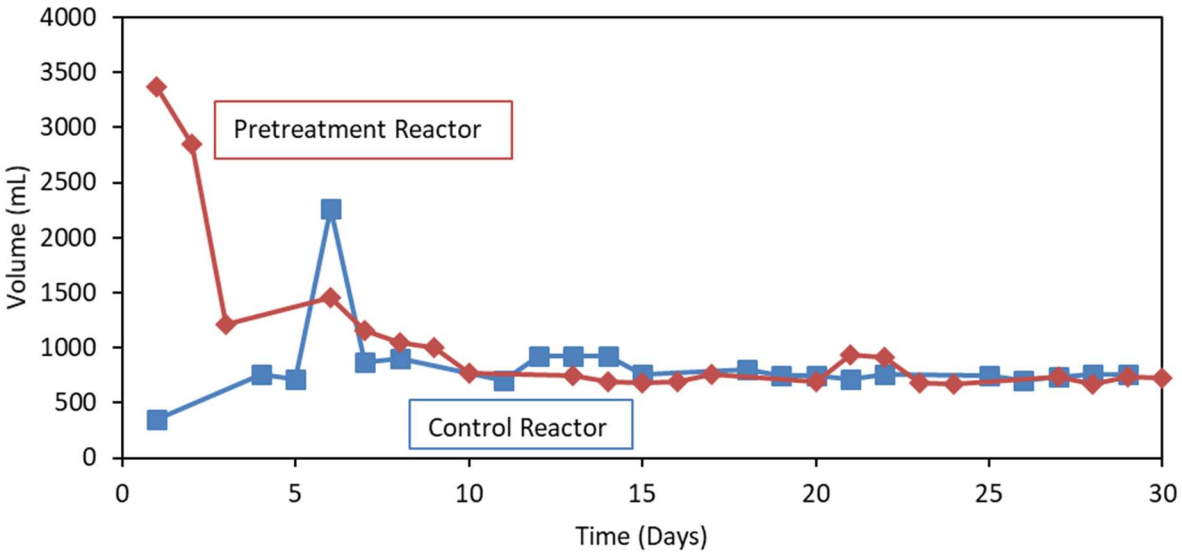

Figure 4.13: Biogas Production of Control and Pretreated Reactor over 30 Days

Figure 4.6 shows the biogas production each day of the trial period. The pretreated reactor produced much more biogas at the beginning of the 30 days but both reactors stabled out after about 10 days an produced the same amount of biogas.

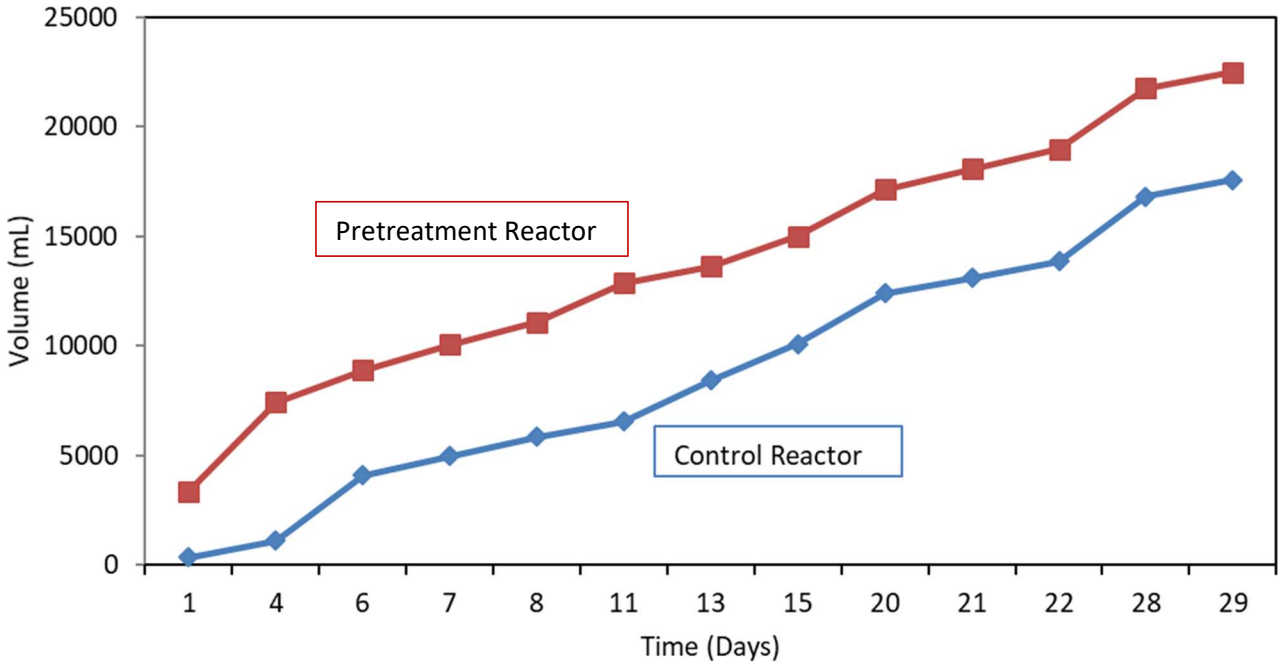

Figure 4.14: Accumulated Volume of Biogas over 30-day Trial Period for the Control and Pretreated Reactor

Figure 4.7 shows the accumulated biogas volume for both reactors over the entire trial. This graph shows that the pretreated reactor started up quicker than the control reactor. The two reactors, after startup, grew at essentially the same slope but the pretreated reactor had more overall biogas produced.

4.7 Composition of Biogas

The composition of biogas was also taken every weekday from both of the reactors. There were four components that were tested for in the biogas; methane, carbon dioxide, oxygen and hydrogen. Methane is the most important content of the biogas and a greater percent of methane is more useful within biogas production.

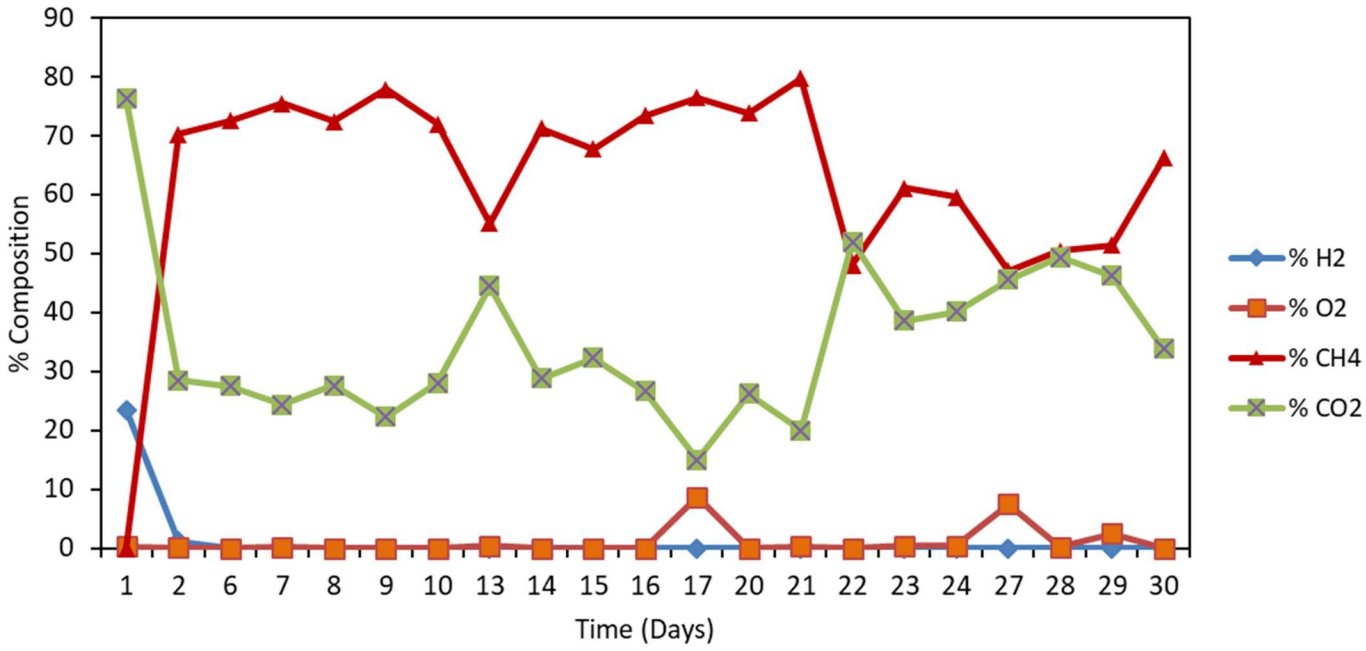

Figure 4.15: Composition of Biogas in Pretreated Reactor over 30 Days

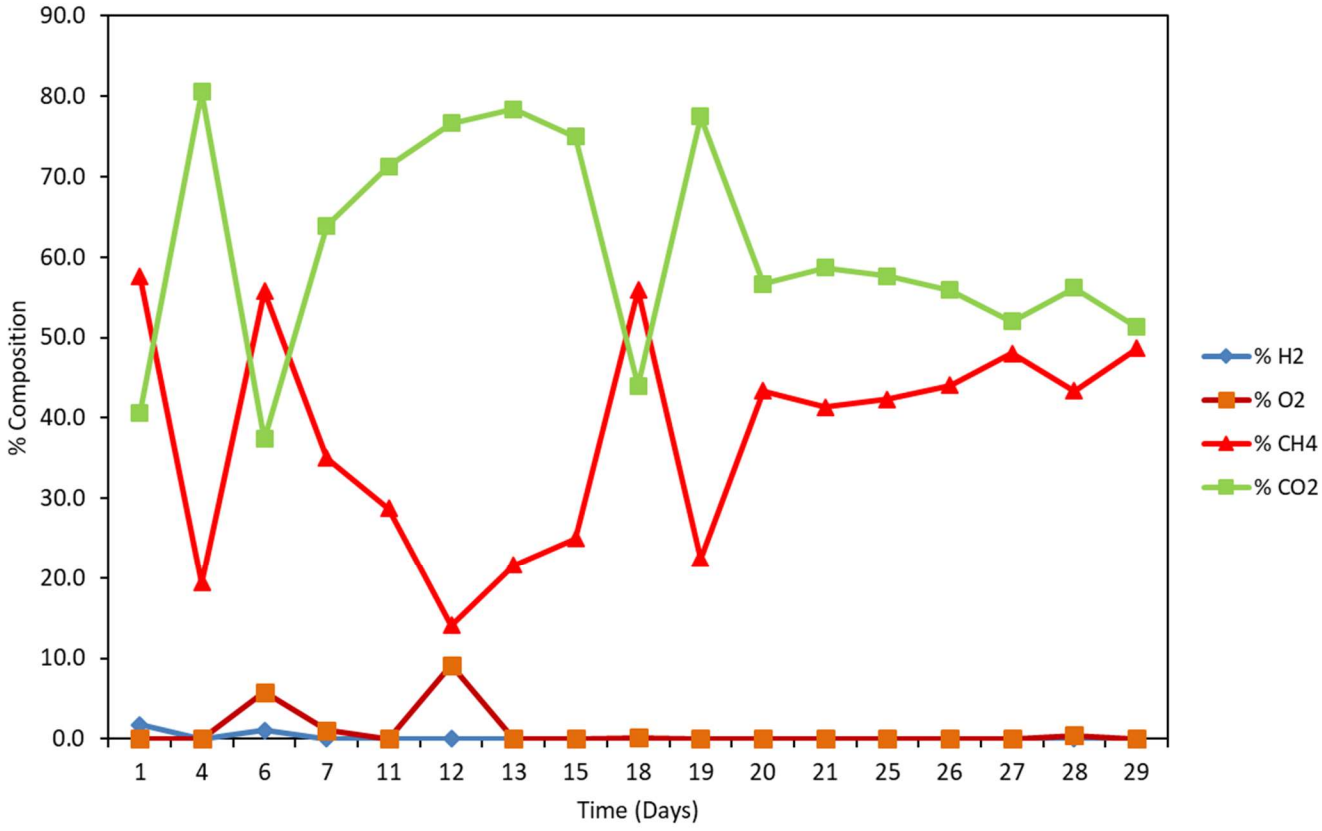

Figure 4.16:Composition of Biogas for the Control Reactor over 30 Days

Figure 4.8 and Figure 4.9 show the biogas composition for both reactors over 30 days. The pretreated reactor has a consistently greater methane composition than carbon dioxide within the 30 days. The control reactor starts with a significantly greater carbon dioxide composition than methane but then stabilizes out around day 19. In day 1 of the pretreated reactor a spike in hydrogen composition is seen on the figure. This is consistent with the process of anaerobic digestion where the second stage is acidogenesis and requires a large composition of hydrogen. Day 2 shows the start of the production of methane because it is produced in the last stage of anaerobic digestion called methanogenesis. Both reactors have two spikes in oxygen during the 30 days. This is inconsistent with anaerobic digestion because it is supposed to occur in the absence of oxygen. The oxygen present on those days can be attributed to oxygen leaks within the reactors from feeding and sampling the reactors the days prior.

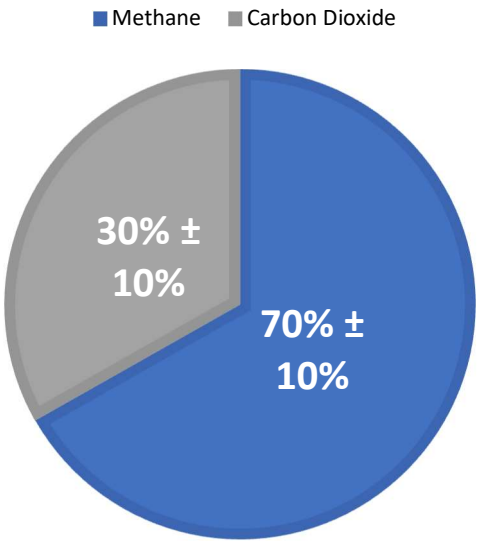

Figure 4.17: Average Methane and Carbon Dioxide Composition from Day 2 to Day 30 for the Pretreated Reactor

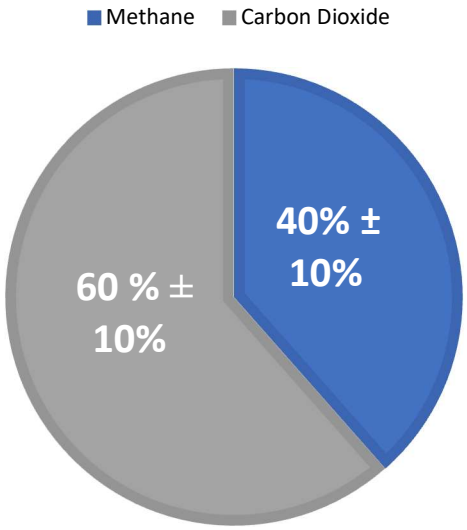

Figure 4.18: Average Composition of Biogas from Day 1 to Day 30 for the Control Reactor

Figure 4.10 and Figure 4.11 show the average compositions of carbon dioxide and methane in both reactors from day 2 to day 30. Day 1 is omitted because it included the acidogenesis stage for the pretreated reactor which does not have a large methane peak. These graphs are only through the stable periods of both reactors. The control reactor does not experience the same acidogenesis as the control reactor, so the average was taken for the entire trial period because day 1 methane was produced. These figures show that the pretreated reactor has a significantly greater average composition of methane throughout the 30 days and suggests the pretreatment was a better option than the untreated macauba for a feedstock.

4.8 Minimum Work Separation

Control Reactor	1.79 J/Kg
Pretreated Reactor	31.7 J/Kg

Table 1: Minimum work separation of the control and pretreated reactors

Table 1 shows the minimum work separation of both reactors based on the average compositions of the biogas produced shown in Figures 4.10 and 4.11. The minimum work separation was calculated assuming the biogas produced was and ideal gas. It was also normalized based on the mass of macauba shells used to initially feed each reactor. This value shows the amount of work it takes to separate the mixture of carbon dioxide and methane in each reactor based on the amount of solid macauba shells used. These numbers when compared to the total heat generated for each reactor are negligible and can be ignored.

4.9 Energy Produced

The accumulated energy produced by each reactor was calculated [5]. The volume of the gas produced each day and methane content were used to find the moles of methane using the ideal gas law. The gas was assumed to be ideal and the temperature and pressure were assumed to be standard. The moles of methane were multiplied with the heating value [6]. The total mass of raw material put into each reactor was calculated and used to normalize both of the energy values by kilogram.

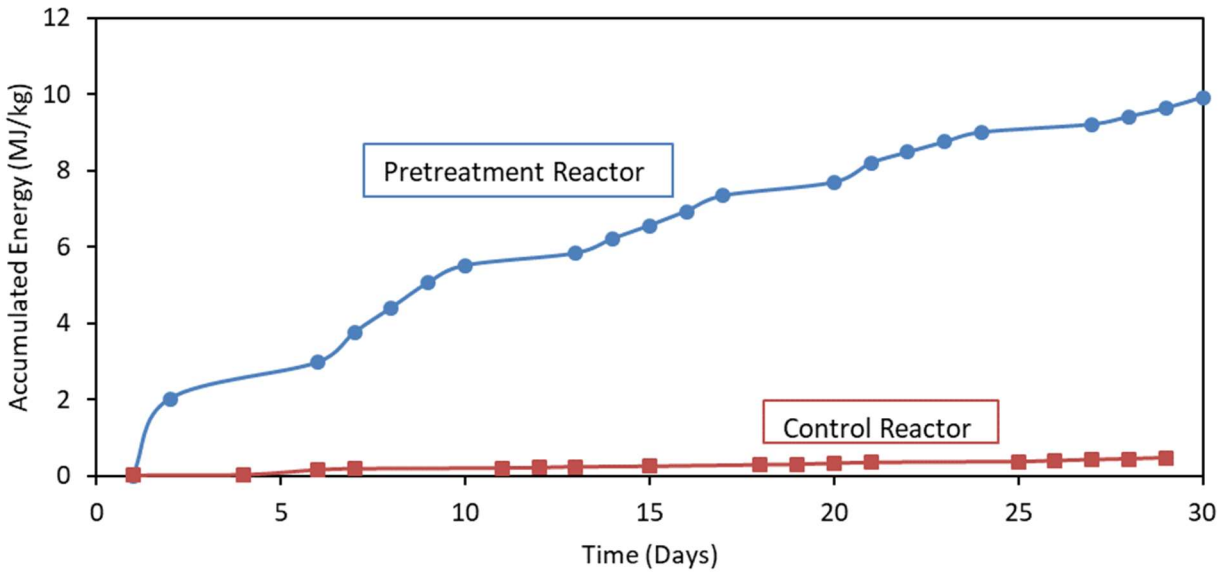

Figure 4.19: Accumulated Energy Output from Pretreated and Control Reactor over 30 Days

Figure 4.12 shows the accumulated energy output from both reactors over the trial period. The control reactor produced significantly less energy per kilogram of macauba fed into the reactor and this can be attributed to two things. First, the methane content of the control reactor was significantly lower than the pretreatment reactor which accounted for a low number of moles. Methane is the more useful component in biogas, and it is ideal to have a higher methane content which the control reactor did not. Second, the control reactor used a significantly greater amount of macauba shells in the initially feeding of the reactor and all subsequent feedings. This greatly reduced the energy output which was already lower than the pretreated reactor. This figure shows that this pretreatment is a better fit for anaerobic digestion than no pretreatment of the shells and should be used in the future to compare with other pretreatments.

5.0 Conclusions and Recommendations

The conclusion on the effect of sub-critical water hydrolysis as a pretreatment for anaerobic digestion of macauba shells is that it significantly enhances the digestion and the amount of energy to be produced. It can be concluded that the pretreatment increased the volume of biogas produced, the methane content of the biogas and therefore also the amount of energy produced per mass of raw material used. The hydrolysis broke down the feedstock in a way that sped up the anaerobic digestion and should be used in future anaerobic digestion trials with macauba shells.

General recommendations from the impact of this project would be to continue the study into the feasibility of macauba as a viable source for renewable energy. This trial with macauba shells and sub-critical water hydrolysis as the pretreatment could be duplicated and carried out for a longer trial period. For some of the data collected, it was observed that a stabilization started to appear later into the trial period and a longer period would allow for more stabilization. The biotar lab at the University of Campinas, carried out this experiment longer than the 30-day trial period used in this paper. Those results should be compared with this paper to determine if the stabilization trends still support these conclusions. This pretreatment did significantly increase energy output compared to the control reactor, however, other pretreatments such as sonification have been shown to also yield higher results and should be tested in order to determine the optimal pretreatment for macauba shells and anaerobic digestion.

The next step for the sub-critical water hydrolysis pretreatment would be to determine the economic feasibility of this process specifically for macauba shells. An optimization process could be undergone in order to minimize energy intake from the pretreatment but maximize energy output from the digestor. Varying parameters such as pH, continuous vs. batch process, and different amounts of raw material could be used in the start up of the process. The feasibility of using macauba as a feedstock should also be examined. Because macauba has little edible applications, it is not grown as widely as soybeans are in Brazil. A biorefinery process should be mapped for the lifecycle of the macauba plant. A cost benefit analysis can be performed to determine the economic gain potential held by using macauba for renewable energy such as biodiesel and biogas. Due to the fact that each part of the macauba nut can be used for some purpose, it is beneficial to determine the impact this plant could have on the renewable energy sector in Brazil and other South American Countries.

This process was carried out in a laboratory setting and following the optimization and economic feasibility process, a scale-up procedure should be determined. Different feed rates or operating procedures may need to be implemented to operate this process as a large-scale operation. The conclusions drawn from this experiment were promising for the use of sub-critical water hydrolysis as a pretreatment for anaerobic digestion. It should be examined for other feedstocks and determined how to perform this process on a large scale. Renewable energy is an important topic to continue to research and anaerobic digestion is an important technology within the field. Making plausible feedstock and pretreatment pairings is a step in the right direction to mitigate the

environmental impact that fossil fuels have. These harmful energy sources are not sustainable and require more research into sectors such as biogas and biodiesel. Macauba has promising potential in both applications and should be considered further as a viable candidate for large scale production.

6.0 Future Work

The control and pretreatment reactors will continue to operate as normal under a 50-day trial period where the same data will be collected. The preliminary results of this work are expected mid-April 2020 and will be further developed. In addition, the initial characterization of the macauba shells and the inoculum used in this project will be performed. All of this work will be performed by the project partners in the Biotar lab at the University of Campinas in the department of food engineering.

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- My project partner in Brazil- Larissa Ampese for assisting me in every aspect of this project and for continuing the project after I left
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Appendix A: Raw Data for Total Solids

Pretreated Reactor

Day	Sample	Po	P1	P2	ST	ST MEDIA	Umidade	SFT	SFT MEDIA	P3	SV	SV MEDIA	Umidade	ST
	1	26.62	2.02	26.68	2.9703		97.0297	0.49505		26.63	2.475247525		0.9703	2.9703
0	2	25.94	1.96	25.97	1.53061	2.16697	98.4694	0		25.94	1.530612245		0.98469	1.53061
	3	23.29	2	23.33	2		98	1	0.4983498	23.31	1	1.668619923	0.98	2
	1	27.77	2.08	27.83	2.88462		97.1154	0.96154		27.79	1.923076923		0.97115	2.88462
3	2	34.52	2.05	34.6	3.90244	3.08743	96.0976	1.95122		34.56	1.951219512		0.96098	3.90244
	3	23.75	2.02	23.8	2.47525		97.5248	1.9802	1.6309853	23.79	0.495049505	1.456448647	0.97525	2.47525
	1	23.28	2.03	23.34	2.95567		97.0443	1.97044		23.32	0.985221675		0.97044	2.95567
6	2	37.03	1.99	37.11	4.0201	4.0003	95.9799	1.50754		37.06	2.512562814		0.9598	4.0201
	3	25.94	1.99	26.04	5.02513		94.9749	1.00503	1.4943354	25.96	4.020100503	2.505961664	0.94975	5.02513
	1	30.83	2.04	30.93	4.90196		95.098	0		30.83	4.901960784		0.95098	4.90196
	2	40.18	2.04	40.33	7.35294	6.26252	92.6471	0.4902		40.19	6.862745098		0.92647	7.35294
8	3	60	1.99	60.13	6.53266		93.4673	0	0.1633987	60	6.532663317	6.099123066	0.93467	6.53266
	1	38.0468	2.0508	38.1394	4.51531		95.4847	0.64365		38.06	3.87165984		0.95485	4.51531
	2	23.2784	2.0513	23.3345	2.73485	3.85201	97.2651	0.49237		23.2885	2.242480378		0.97265	2.73485
10	3	26.6131	2.0623	26.7019	4.30587		95.6941	0.60127	0.5790975	26.6255	3.704601658	3.272913959	0.95694	4.30587
	1	37.257	2.0685	37.3983	6.83104		93.169	0.91854		37.276	5.912496978		0.93169	6.83104
	2	36.7299	2.006	36.8617	6.57029	5.87507	93.4297	0.77767		36.7455	5.792622134		0.9343	6.57029
13	3	59.9719	2.01	60.0568	4.22388		95.7761	0.69652	0.7975748	59.9859	3.527363184	5.077494099	0.95776	4.22388
	1	51.81	2.05	51.86	2.43902		97.561	-0.2878		51.8041	2.726829268		0.97561	2.43902
	2	40.19	2.02	40.34	7.42574	5.63331	92.5743	0.61386		40.2024	6.811881188		0.92574	7.42574
15	3	34.14	1.99	34.28	7.03518		92.9648	0.94975	0.4252684	34.1589	6.085427136	5.208045864	0.92965	7.03518
	1	27.7647	2.3129	27.837	3.12595		96.8741	0.39345		27.7738	2.732500324		0.96874	3.12595
	2	41.1098	1.9969	41.2306	6.04938	4.64344	93.9506	0.31048		41.116	5.738895288		0.93951	6.04938
17	3	32.2803	2.2061	32.3852	4.755		95.245	0.3037	0.3358767	32.287	4.451294139	4.30756325	0.95245	4.755
	1	30.2531	2.2707	30.3498	4.2586		95.7414	0.73105		30.2697	3.527546572		0.95741	4.2586
	2	32.3827	2.0938	32.4498	3.2047	3.46348	96.7953	0.64476		32.3962	2.559938867		0.96795	3.2047
20	3	33.6096	2.1147	33.6715	2.92713		97.0729	0.56273	0.6461801	33.6215	2.36440157	2.81729567	0.97073	2.92713
	1	36.73	2.0921	36.8932	7.80077		92.1992	1.28579		36.7569	6.514984943		0.92199	7.80077
	2	39.6244	1.9995	39.789	8.23206	8.37518	91.7679	1.48037		39.654	6.751687922		0.91768	8.23206
22	3	37.2575	2.017	37.4409	9.09271		90.9073	1.3882	1.3847866	37.2855	7.704511651	6.990394839	0.90907	9.09271
	1	40.1823	2.0614	40.2194	1.79975		98.2003	0.56758		40.194	1.23217231		0.982	1.79975
	2	41.6318	2.0074	41.6581	1.31015	1.49865	98.6898	0.52306		41.6423	0.787087775		0.9869	1.31015
24	3	32.3819	1.9985	32.4096	1.38604		98.614	0.72554	0.6053948	32.3964	0.660495372	0.893251819	0.98614	1.38604
	1	30.2528	2.0434	30.3396	4.24782		95.7522	0.70471		30.2672	3.543114417		0.95752	4.24782
	2	32.2718	2.0129	32.4415	8.43062	6.41867	91.5694	1.03333		32.2926	7.397287496		0.91569	8.43062
27	3	33.444	1.9901	33.5749	6.57756		93.4224	0.86428	0.8674403	33.4612	5.71328074	5.551227551	0.93422	6.57756
	1	35.0352	2.0142	35.0626	1.36034		98.6397	0.42697		35.0438	0.933373051		0.9864	1.36034
	2	56.1831	2.1054	56.2105	1.30142	1.33321	98.6986	0.39897		56.1915	0.902441341		0.98699	1.30142
29	3	40.0063	2.0032	40.0331	1.33786		98.6621	0.5641	0.4633467	40.0176	0.773761981	0.869858791	0.98662	1.33786

Control Reactor

Day	Sample	Po	P1	P2	ST	ST MEDIA	Umidade	SFT	SFT MEDIA	P3	SV	SV MEDIA	Umidade	ST
0	1	40.58	1.97	41.03	22.8426		77.1574	1.52284		40.61	21.31979695		0.77157	22.8426
	2	36.73	2.01	37.18	22.3881	22.2198	77.6119	0.99502		36.75	21.39303483		0.77612	22.3881
	3	51.82	1.96	52.24	21.4286		78.5714	0.5102	1.0093572	51.83	20.91836735	21.21039971	0.78571	21.4286
4	1	37.37	1.96	37.76	19.898		80.102	0.5102		37.38	19.3877551		0.80102	19.898
	2	40.08	2.02	40.47	19.3069	19.4656	80.6931	0.9901		40.1	18.31683168		0.80693	19.3069
	3	40.01	1.98	40.39	19.1919		80.8081	1.51515	1.0051515	40.04	17.67676768	18.46045149	0.80808	19.1919
6	1	23.76	1.98	24.0862	16.4747		83.5253	1.0101		23.78	15.46464646		0.83525	16.4747
	2	34.54	2.04	34.8631	15.8382	16.307	84.1618	0.4902		34.55	15.34803922		0.84162	15.8382
	3	27.77	1.99	28.1005	16.608		83.392	1.50754	1.0026116	27.8	15.10050251	15.30439606	0.83392	16.608
8	1	22.61	2.08	22.92	14.9038		85.0962	1.92308		22.65	12.98076923		0.85096	14.9038
	2	25.16	2.05	25.45	14.1463	14.3039	85.8537	1.46341		25.19	12.68292683		0.85854	14.1463
	3	26.61	2.02	26.89	13.8614		86.1386	1.48515	1.62388	26.64	12.37623762	12.67997789	0.86139	13.8614
11	1	38.05	1.99	38.346	14.8744		85.1256	2.01005		38.09	12.86432161		0.85126	14.8744
	2	40.85	2.05	41.1509	14.678	14.8822	85.322	3.41463		40.92	11.26341463		0.85322	14.678
	3	38.04	2.02	38.3449	15.0941		84.9059	2.47525	2.6333106	38.09	12.61881188	12.24884937	0.84906	15.0941
13	1	31.96	1.99	32.27	15.5779		84.4221	1.00503		31.98	14.57286432		0.84422	15.5779
	2	35.03	2	35.36	16.5	15.9471	83.5	1.5		35.06	15		0.835	16.5
	3	30.27	2.03	30.59	15.7635		84.2365	1.97044	1.4918228	30.31	13.79310345	14.45532259	0.84236	15.7635
15	1	56.1806	2.0413	56.5366	17.4399		82.5601	2.0967		56.2234	15.34316367		0.8256	17.4399
	2	54.5	2.4206	54.9256	17.5824	17.2003	82.4176	2.08213		54.5504	15.50028918		0.82418	17.5824
	3	22.5739	2.0098	22.9071	16.5788		83.4212	2.33854	2.1724575	22.6209	14.24022291	15.02789192	0.83421	16.5788
18	1	45.4042	2.0196	45.7654	17.8847		82.1153	4.89701		45.5031	12.98772034		0.82115	17.8847
	2	40.8476	2.0366	41.2092	17.7551	17.8016	82.2449	2.34705		40.8954	15.408033		0.82245	17.7551
	3	39.626	2.1126	40.0013	17.7648		82.2352	2.30048	3.1815137	39.6746	15.46435672	14.62003668	0.82235	17.7648
20	1	30.26	1.99	30.61	17.5879		82.4121	4.23116		30.3442	13.35678392		0.82412	17.5879
	2	40.07	1.97	40.42	17.7665	17.5378	82.2335	2.41117		40.1175	15.35532995		0.82234	17.7665
	3	38.71	1.97	39.05	17.2589		82.7411	2.49746	3.0465951	38.7592	14.76142132	14.4911784	0.82741	17.2589
22	1	34.5211	2.0359	34.8665	16.9655		83.0345	2.284		34.5676	14.68146766		0.83035	16.9655
	2	25.1541	2.0232	25.5031	17.2499	16.7617	82.7501	2.20443		25.1987	15.04547252		0.8275	17.2499
	3	25.9182	2.0187	26.2426	16.0697		83.9303	2.2143	2.2342424	25.9629	13.85545153	14.5274639	0.8393	16.0697
25	1	40.18	1.9777	40.5142	16.8984		83.1016	2.54336		40.2303	14.35505891		0.83102	16.8984
	2	40.0641	2.1411	40.4187	16.5616	16.6432	83.4384	3.20863		40.1328	13.35294942		0.83438	16.5616
	3	34.5037	2.0049	34.8339	16.4696		83.5304	2.39912	2.7170372	34.5518	14.07052721	13.92617851	0.8353	16.4696

	27	1	40.8486	2.1268	41.1654	14.8956		85.1044	2.62836		40.9045	12.26725597			0.85104	14.8956
		2	45.4043	2.0165	45.7576	17.5205	16.316	82.4795	4.35904		45.4922	13.1614183			0.8248	17.5205
		3	40.0085	1.9278	40.3272	16.5318		83.4682	2.43801	3.141804	40.0555	14.09378566	13.17415331		0.83468	16.5318
	29	1	33.6082	2.0198	33.9403	16.4422		83.5578	2.5151		33.659	13.9271215			0.83558	16.4422
		2	60.7524	2.0425	61.1032	17.175	17.2836	82.825	2.47246		60.8029	14.70257038			0.82825	17.175
		3	40.0631	1.9985	40.4275	18.2337		81.7663	3.14736	2.7116404	40.126	15.08631474	14.5720022		0.81766	18.2337

Appendix B: Raw Data for COD

Sample	Number	Absorbance	COD	Dilution	Average	STD DEV	Corrected COD(mgO2/L)	COD g/L
R2 F	1	0.288	953.667	5	971.444	39.76784482	53429.44444	53.4294
	2	0.285	943.667	5				
	3	0.307	1017	5				
R1 D0	1	0.249	823.667	1	825.889	3.849001795	9084.777778	9.08478
	2	0.249	823.667	1				
	3	0.251	830.333	1				
R2 D0	1	0.28	927	5	905.889	42.47003301	49823.88889	49.8239
	2	0.282	933.667	5				
	3	0.259	857	5				
R2 D4	1	0.178	587	5	620.333	31.79797338	34118.33333	34.1183
	2	0.197	650.333	5				
	3	0.189	623.667	5				
R1 D3	1	0.036	113.667	5	109.222	3.849001795	6007.222222	6.00722
	2	0.034	107	5				
	3	0.034	107	5				
R1 D6	1	0.028	87	5	80.3333	11.54700538	4418.333333	4.41833
	2	0.022	67	5				
	3	0.028	87	5				
R1 D8	1	0.028	87	5	84.7778	1.924500897	4662.777778	4.66278
	2	0.027	83.6667	5				
	3	0.027	83.6667	5				
R1 D10	1	0.049	157	5	112.556	39.76784482	6190.555556	6.19056
	2	0.032	100.333	5				
	3	0.026	80.3333	5				
R1 D13	1	0.027	83.6667	5	52.5556	31.68128318	2890.555556	2.89056
	2	0.008	20.3333	5				
	3	0.018	53.6667	5				
R2 D6	1	0.317	1050.33	5	1052.56	1.924500897	57890.55556	57.8906
	2	0.318	1053.67	5				
	3	0.318	1053.67	5				

R2 D8	1	0.338	1120.33	5	1152.56	29.12298316	63390.55556	63.3906
	2	0.35	1160.33	5				
	3	0.355	1177	5				
R2 D11	1	0.363	1203.67	5	1203.67	10	66201.66667	66.2017
	2	0.36	1193.67	5				
	3	0.366	1213.67	5				
R2 D 13	1	0.289	957	5	994.778	54.2968521	54712.77778	54.7128
	2	0.319	1057	5				
	3	0.293	970.333	5				
R2 D15	1	0.372	1233.67	5	1181.44	45.50132273	64979.44444	64.9794
	2	0.347	1150.33	5				
	3	0.35	1160.33	5				
R2 D18	1	0.377	1250.33	5	1221.44	25.23959265	67179.44444	67.1794
	2	0.365	1210.33	5				
	3	0.363	1203.67	5				
R1 D15	1	0.03	93.6667	5	90.3333	12.01850425	4968.333333	4.96833
	2	0.032	100.333	5				
	3	0.025	77	5				
R1 D 17	1	0.036	113.667	5	71.4444	36.56551705	3929.444444	3.92944
	2	0.017	50.3333	5				
	3	0.017	50.3333	5				
R1 D20	1	0.017	50.3333	5	75.8889	33.38884267	4173.888889	4.17389
	2	0.036	113.667	5				
	3	0.021	63.6667	5				
R1 D22	1	0.02	60.3333	5	84.7778	23.4125639	4662.777778	4.66278
	2	0.028	87	5				
	3	0.034	107	5				
R2 D20	1	0.402	1333.67	5	1344.78	10.18350154	73962.77778	73.9628
	2	0.406	1347	5				
	3	0.408	1353.67	5				

R2 D22	1	0.319	1057	5	1061.44	30.24590575	58379.44444	58.3794
	2	0.312	1033.67	5				
	3	0.33	1093.67	5				
R2 D25	1	0.342	1133.67	5	1102.56	28.73892701	60640.55556	60.6406
	2	0.331	1097	5				
	3	0.325	1077	5				
R2 D 27	1	0.359	1190.33	5	1202.56	21.16950987	66140.55556	66.1406
	2	0.37	1227	5				
	3	0.359	1190.33	5				
R1 D24	1	0.039	123.667	5	125.889	3.849001795	6923.888889	6.92389
	2	0.041	130.333	5				
	3	0.039	123.667	5				
R2 D29	1	0.333	1103.67	5	1090.33	23.09401077	59968.33333	59.9683
	2	0.321	1063.67	5				
	3	0.333	1103.67	5				
R1 D27	1	0.034	107	5	100.333	14.52966315	5518.333333	5.51833
	2	0.035	110.333	5				
	3	0.027	83.6667	5				
R1 D29	1	0.044	140.333	5	145.889	5.091750772	8023.888889	8.02389
	2	0.047	150.333	5				
	3	0.046	147	5				

Appendix C: Raw Data for Ammoniacal Nitrogen

Inóculo	1	5	0.3	0	14.432964		
	2	5	0.3	0	14.432964		
	3	5	0.3	0	14.432964	14.432964	158.762604
Macauba	1	5	0.1	0	4.810988		
	2	5	0.1	0	4.810988		
	3	5	0.1	0	4.810988	4.810988	52.920868
Feed R2	1	5	0.1	0	4.810988		
	2	5	0.05	0	2.405494		
	3	5	0	0	0	2.405494	26.460434
R2 D0	1	5	0.3	0	14.432964		
	2	5	0.1	0	4.810988		
	3	5	0.1	0	4.810988	8.018313333	88.20144667
R2 D4	1	5	0.2	0	9.621976		
	2	5	0.2	0	9.621976		
	3	5	0.2	0	9.621976	9.621976	105.841736
R1 D0	1	5	0.3	0.2	4.810988		
	2	5	0.3	0.2	4.810988		
	3	5	0.3	0.2	4.810988	4.810988	52.920868
R2 D6	1	5	0.6	0.2	19.243952		
	2	5	0.5	0.2	14.432964		
	3	5	0.5	0.2	14.432964	16.03662667	176.4028933
R1 D3	1	5	0.2	0.2	0		
	2	5	0.1	0.2	-4.810988		
	3	5	0.2	0.2	0	-1.603662667	-17.64028933
R2 D8	1	5	0.7	0.2	24.05494		
	2	5	0.8	0.2	28.865928		
	3	5	0.8	0.2	28.865928	27.26226533	299.8849187
R1 D6	1	5	0.3	0.2	4.810988		
	2	5	0.2	0.2	0		
	3	5	0.3	0.2	4.810988	3.207325333	35.28057867
R2 D11	1	5	1	0.2	38.487904		
	2	5	1	0.2	38.487904		
	3	5	1	0.2	38.487904	38.487904	423.366944

R1 D8	1	5	0.2	0.2	0		
	2	5	0.2	0.2	0		
	3	5	0.2	0.2	0	0	0
R2 D13	1	5	0.8	0.2	28.865928		
	2	5	0.9	0.2	33.676916		
	3	5	1.2	0.2	48.10988	36.88424133	405.7266547
R1 D10	1	5	0.2	0.2	0		
	2	5	0.2	0.2	0		
	3	5	0.2	0.2	0	0	0
R2 D15	1	5	0.9	0.2	33.676916		
	2	5	0.7	0.2	24.05494		
	3	5	0.7	0.2	24.05494	27.26226533	299.8849187
R1 D15	1	5	0.3	0.2	4.810988		
	2	5	0.3	0.2	4.810988		
	3	5	0.3	0.2	4.810988	4.810988	52.920868
R1 D17	1	5	0.3	0.2	4.810988		
	2	5	0.3	0.2	4.810988		
	3	5	0.3	0.2	4.810988	4.810988	52.920868
R2 D20	1	5	1	0.2	38.487904		
	2	5	1.1	0.2	43.298892		
	3	5	1	0.2	38.487904	40.09156667	441.0072333
R1 D20	1	5	0.3	0.2	5.533598		
	2	5	0.3	0.2	5.533598		
	3	5	0.3	0.2	5.533598	5.533598	60.869578
R1 D 22	1	5	0.4	0.2	11.067196		
	2	5	0.3	0.2	5.533598		
	3	5	0.2	0.2	0	5.533598	60.869578
R1 D 24	1	5	0.2	0.2	0		
	2	5	0.2	0.2	0		
	3	5	0.3	0.2	5.533598	1.844532667	20.28985933
R2 D18	1	5	0.9	0.2	38.735186		
	2	5	0.9	0.2	38.735186		
	3	5	0.9	0.2	38.735186	38.735186	426.087046
R2 D22	1	5	0.8	0.2	33.201588		
	2	5	0.9	0.2	38.735186		
	3	5	0.9	0.2	38.735186	36.89065333	405.7971867

	1	5	1	0.2	44.268784		
	2	5	1	0.2	44.268784		
R2 D25	3	5	1.1	0.2	49.802382	46.11331667	507.2464833
	1	5	0.4	0.3	5.533598		
	2	5	0.4	0.3	5.533598		
R1 D13	3	5	0.4	0.3	5.533598	5.533598	60.869578
	1	5	0.3	0.3	0		
	2	5	0.3	0.3	0		
R1 D27	3	5	0.3	0.3	0	0	0
	1	5	0.4	0.3	5.533598		
	2	5	0.4	0.3	5.533598		
R1 D29	3	5	0.3	0.3	0	3.689065333	40.57971867
	1	5	1	0.3	38.735186		
	2	5	1	0.3	38.735186		
R2 D27	3	5	0.9	0.3	33.201588	36.89065333	405.7971867
	1	5	0.8	0.3	27.66799		
	2	5	0.3	0.3	0		
R2 D29	3	5	0.4	0.3	5.533598	11.067196	121.739156

Appendix D: Raw Data for Alkalinity

Sample	Number	V(mL)	VH2SO4(mL)	Total Alkalinity (mgCaCO3/L)	Average	Corrected Alkalinity
Macauba	1	10	0.3	25.77315		
	2	10	0.3	25.77315		
	3	10	0.6	51.5463	34.3642	378.0062
Inoculo	1	10	0.8	68.7284		
	2	10	2.4	206.1852		
	3	10	1.2	103.0926	126.0021	1386.022733
Feed R2	1	10	0.1	8.59105		
	2	10	0.1	8.59105		
	3	10	0.1	8.59105	8.59105	94.50155
R2 Day 0	1	10	0.3	25.77315		
	2	10	0.1	8.59105		
	3	10	0.1	8.59105	14.31842	157.5025833
R1 Day 0	1	10	0.6	51.5463		
	2	10	0.2	17.1821		
	3	10	0.2	17.1821	28.63683	315.0051667
R2 Day 4	1	10	0.2	17.1821		
	2	10	0.05	4.295525		
	3	10	0.05	4.295525	8.59105	94.50155
R2 Day 6	1	10	2.4	206.1852		
	2	10	4.9	420.96145		
	3	10	6	515.463	380.8699	4189.568717
R1 Day 6	1	10	1.5	128.86575		
	2	10	1.5	128.86575		
	3	10	0.8	68.7284	108.82	1197.019633
R1 Day 8	1	10	1.9	163.22995		
	2	10	1.6	137.4568		
	3	10	1.6	137.4568	146.0479	1606.52635
R1 Day 3	1	10	0.7	60.13735		
	2	10	0.7	60.13735		
	3	10	0.8	68.7284	63.00103	693.0113667
R2 Day 13	1	10	11.4	979.3797		
	2	10	11.7	1005.15285		
	3	10	11.1	953.60655	979.3797	10773.1767
R2 Day 8	1	10	7.4	635.7377		
	2	10	6.3	541.23615		
	3	10	5.5	472.50775	549.8272	6048.0992
R2 Day 11	1	10	4.9	420.96145		
	2	10	4.3	369.41515		
	3	10	3.6	309.2778	366.5515	4032.066133
R1 Day 10	1	10	2.3	197.59415		
	2	10	0.7	60.13735		
	3	10	2.1	180.41205	146.0479	1606.52635
R2 Day 15	1	10	1.5	128.86575		
	2	10	0.6	51.5463		
	3	10	1.6	137.4568	105.9563	1165.519117
R1 Day 13	1	10	1.6	137.4568		
	2	10	0.9	77.31945		
	3	10	0.3	25.77315	80.18313	882.0144667
R2 Day 18	1	10	0.9	77.31945		
	2	10	0.8	68.7284		
	3	10	1	85.9105	77.31945	850.51395
R1 Day 15	1	10	3.3	283.50465		
	2	10	4	343.642		
	3	10	2.8	240.5494	289.232	3181.552183

R2 Day 20	1	10	10.8	927.8334		
	2	10	10.9	936.42445		
	3	10	11.7	1005.15285	956.4702	10521.17257
R1 Day 17	1	10	3.3	283.50465		
	2	10	3.3	283.50465		
	3	10	3.3	283.50465	283.5047	3118.55115
R1 Day 20	1	10	3.8	326.4599		
	2	10	4.1	352.23305		
	3	10	4.2	360.8241	346.5057	3811.562517
R2 Day 22	1	10	12.3	1056.69915		
	2	10	12.7	1091.06335		
	3	10	12.3	1056.69915	1068.154	11749.69272
R1 Day 24	1	10	3.6	355.7313		
	2	10	3.7	365.612725		
	3	10	3.6	355.7313	359.0251	3949.276192
R2 Day 25	1	10	9.8	968.37965		
	2	10	9.6	948.6168		
	3	10	8.4	830.0397	915.6787	10072.46588
R1 Day 27	1	10	3.1	306.324175		
	2	10	3.1	306.324175		
	3	10	10.3	1017.786775	543.4784	5978.262125
R2 Day 27	1	10	10.3	1017.786775		
	2	10	10.1	998.023925		
	3	10	10.5	1037.549625	1017.787	11195.65453
R1 Day22	1	10	3.4	335.96845		
	2	10	3.5	345.849875		
	3	10	3.3	326.087025	335.9685	3695.65295

R2 Day 29	1	10	10.3	1017.786775		
	2	10	10	988.1425		
	3	10	10.1	998.023925	1001.318	11014.49507
R1 Day 29	1	10	3.4	335.96845		
	2	10	3.2	316.2056		
	3	10	3.1	306.324175	319.4994	3514.493492

Appendix E: Raw Data for pH

Day	Control	Day	Pretreatment
4	4.66	1	6.08
6	5.77	3	7
8	6.27	6	6.62
11	4.72	8	6.59
13	5.99	10	7.7
15	6.99	13	6.47
18	6.51	15	6.96
20	6.1	17	7.15
22	6.5	20	6.79
25	6.75	22	7.7
27	6.65	24	8
29	6.38	27	7.52
		29	6.87

Appendix F: Raw Data for Volume of Biogas

Pretreatment Reactor

Data	Day	Volume in Reactor (mL)	Volume in Bag (mL)
15/01/2020	1	600	2766
	2	600	2250
17/01/2020	3	600	614
20/01/2020	6	600	860
21/01/2020	7	600	558
22/01/2020	8	600	444
23/01/2020	9	600	398
24/01/2020	10	600	176
27/01/2020	13	600	152
28/01/2020	14	600	92
29/01/2020	15	600	86
30/01/2020	16	600	92
31/01/2020	17	600	160
3/2/2020	20	600	98
4/2/2020	21	600	331
5/2/2020	22	600	312
6/2/2020	23	600	84
7/2/20202	24	600	74
10/2/2020	27	600	136
11/2/2020	28	600	75
12/2/2020	29	600	138

Control Reactor

Data	Day	Volume in reator (ml)	Volume in bag (ml)
11/1/2020	1	354	0
13/01/2020	4	600	164
14/01/2020	5	600	112
15/01/2020	6	600	1666
16/01/2020	7	600	266
17/01/2020	8	600	300
20/01/2020	11	600	102
21/01/2020	12	600	330
22/01/2020	13	600	324
23/01/2020	14	600	320
24/01/2020	15	600	158
27/01/2020	18	600	202
28/01/2020	19	600	150
29/01/2020	20	600	144
30/01/2020	21	600	112
31/01/2020	22	600	154
3/2/2020	25	600	147
4/2/2020	26	600	108
5/2/2020	27	600	140
6/2/2020	28	600	160
7/2/2020	29	600	164

Appendix G: Raw Data for Biogas Composition

Pretreatment Reactor

Day	area H2	area O2	area CH4	area CO2	area total	% H2	% O2	% CH4	% CO2
1	280515	3214.7	0	909678.8	1193408.8	23.5054	0.26937	0	76.2252465
2	9859.6	1091.7	597738.8	242088.8	850778.9	1.15889	0.12832	70.2578308	28.4549605
6	0	0	515291.7	195403.7	710695.4	0	0	72.5052814	27.4947186
7	0	1460.2	516885.3	166999.9	685345.4	0	0.21306	75.4196789	24.3672607
8	0	0	502427.1	191415.6	693842.7	0	0	72.4122485	27.5877515
9	0	0	460152.3	131410.9	591563.2	0	0	77.7858224	22.2141776
10	0	0	387480.8	150844.2	538325	0	0	71.9789718	28.0210282
13	0	2288.2	329597.6	265948.8	597834.6	0	0.38275	55.1319044	44.4853476
14	0	0	369931	149763.4	519694.4	0	0	71.1824103	28.8175897
15	0	0	352979.9	168422.3	521402.2	0	0	67.6981992	32.3018008
16	0	0	345113.7	125399.5	470513.2	0	0	73.3483566	26.6516434
17	0	20424.8	179135.6	35033.9	234594.3	0	8.70643	76.3597411	14.9338241
20	0	0	365876.2	129728.2	495604.4	0	0	73.8242437	26.1757563
21	0	1232.8	296496.8	74400.3	372129.9	0	0.33128	79.6756186	19.9930992
22	0	0	242597.7	262769.6	505367.3	0	0	48.0042338	51.9957662
23	0	1482.3	243182.6	153670.8	398335.7	0	0.37212	61.0496624	38.5782143
24	0	1459.3	209088.2	140820.7	351368.2	0	0.41532	59.5068649	40.0778158
27	0	12966.3	81935.5	79236.6	174138.4	0	7.44597	47.0519426	45.5020834
28	0	1014.4	215411	210422.9	426848.3	0	0.23765	50.4654698	49.2968814
29	0	10056.4	213189.4	191559.4	414805.2	0	2.42437	51.3950645	46.1805686
30	0	0	336223	171761.1	507984.1	0	0	66.1877015	33.8122985

Control Reactor

Day	area H2	area O2	area CH4	area CO2	Area Total	% H2	% O2	% CH4	% CO2
1	6643.7	0.0	213330.9	150663.3	370637.9	1.8	0.0	57.6	40.6
4	0.0	0.0	46776.4	194167.6	240944.0	0.0	0.0	19.4	80.6
6	2437.0	13445.8	129726.3	86979.1	232588.2	1.0	5.8	55.8	37.4
7	0.0	4980.0	158070.7	288958.6	452009.3	0.0	1.1	35.0	63.9
11	0.0	0.0	95804.9	237610.9	333415.8	0.0	0.0	28.7	71.3
12	0.0	12923.7	19890.1	107934.4	140748.2	0.0	9.2	14.1	76.7
13	0.0	0.0	63191.0	229111.0	292302.0	0.0	0.0	21.6	78.4
15	0.0	0.0	78154.0	234408.2	312562.2	0.0	0.0	25.0	75.0
18	0	1022.9	410305.9	322935.9	734264.7	0.0	0.1	55.9	44.0
19	0	0	62311.6	214968	277279.6	0.0	0.0	22.5	77.5
20	0	0	169960.9	221963	391923.9	0	0.0	43.4	56.6342
21	0	0	162813.3	231109.8	393923.1	0	0.0	41.3	58.6688
25	0	0	156151.8	212764.3	368916.1	0	0.0	42.3	57.6728
26	0	0	176664.4	224100.7	400765.1	0	0.0	44.1	55.9182
27	0	0	242597.7	262769.6	505367.3	0	0.0	48.0	51.9958
28	0	1994.7	178135	230932.2	411061.9	0	0.5	43.3	56.1794
29	0	0	240288.9	253324.1	493613.0	0	0.0	48.7	51.3204

Appendix H: Raw Data for Energy Calculation

Pretreatment Reactor

Day	Accumulated Mass (Kg)	Volume of Gas	Methane Content	Moles of Gas	Moles Methane	Total Heat	Accumulated heat	P	1	atm
1	0.03612	3.366	0	1.35858817	0	0	0	R	0.008314	kJ/mol*K
2	0.03612	2.85	0.702578308	1.15031975	0.808189703	19.9187884	19.9187884	T	298	K
6	0.04052	1.46	0.725052814	0.58928661	0.427263914	9.386941787	29.30573019	Heating Value	55.5	MJ/Kg
7	0.04052	1.158	0.754196789	0.46739308	0.352506358	7.7445264	37.05025659	Atomic Weight	16.04	g/mol
8	0.04272	1.044	0.724122485	0.42138029	0.30513094	6.358465958	43.40872254			
9	0.04272	0.998	0.777858224	0.40281372	0.313331967	6.529362907	49.93808545			
10	0.04492	0.776	0.719789718	0.31320987	0.225445243	4.467850937	54.40593639			
13	0.04712	0.752	0.551319044	0.30352297	0.167337991	3.161452171	57.56738856			
14	0.04712	0.692	0.711824103	0.27930571	0.198816535	3.756164163	61.32355272			
15	0.04932	0.686	0.676981992	0.27688398	0.187445469	3.383367919	64.70692064			
16	0.04932	0.692	0.733483566	0.27930571	0.204866146	3.697809019	68.40472966			
17	0.05152	0.76	0.763597411	0.30675193	0.234234982	4.047373163	72.45210282			
20	0.05372	0.698	0.738242437	0.28172743	0.207983147	3.446588923	75.89869175			
21	0.05372	0.931	0.796756186	0.37577112	0.299397963	4.96146788	80.86015963			
22	0.05592	0.912	0.480042338	0.36810232	0.176704698	2.813055368	83.67321499			
23	0.05592	0.684	0.610496624	0.27607674	0.168543917	2.683139596	86.35635459			
24	0.05812	0.674	0.595068649	0.27204053	0.161882791	2.479547451	88.83590204			
27	0.06032	0.736	0.470519426	0.29706503	0.139774867	2.062837904	90.89873995			
28	0.06032	0.675	0.504654698	0.27244415	0.137490221	2.02912043	92.92786038			
29	0.06252	0.738	0.513950645	0.29787227	0.153091646	2.179866366	95.10772674			
30	0.06252	0.728	0.661877015	0.29383606	0.194483336	2.769241126	97.87696787			

Control Reactor

Day	Accumulated Mass (Kg)	Volume of Gas	Methane Content	Moles of Gas	Moles Methane	Total Heat	Accumulated heat	P	1	atm
1	0.334	0.354	0.576	0.14288182	0.082239586	0.219195582	0.219195582	R	0.008314	kJ/mol*K
4	0.3503	0.764	0.194	0.30836642	0.059865657	0.15213704	0.371332622	T	298	K
6	0.3666	2.266	0.558	0.91460511	0.510121908	1.238736292	1.610068914	Heating Value	55.5	MJ/Kg
7	0.3666	0.866	0.350	0.34953576	0.12223501	0.296825016	1.906893931	Atomic Weight	16.04	g/mol
11	0.3992	0.702	0.287	0.28334192	0.08141649	0.181559588	2.088453518			
12	0.3992	0.93	0.141	0.3753675	0.053045773	0.118292605	2.206746123			
13	0.4155	0.924	0.216	0.37294577	0.080624889	0.172741007	2.37948713			
15	0.4318	0.758	0.250	0.30594469	0.076499338	0.157714777	2.537201907			
18	0.4481	0.802	0.559	0.32370401	0.180885267	0.359356578	2.896558486			
19	0.4481	0.75	0.225	0.30271572	0.068027727	0.135147609	3.031706094			
20	0.4644	0.744	0.434	0.300294	0.130224868	0.249631313	3.281337408			
21	0.4644	0.712	0.413	0.28737813	0.118776942	0.227686497	3.509023905			
25	0.497	0.747	0.423	0.30150486	0.12761852	0.22858865	3.737612554			
26	0.497	0.708	0.441	0.28576364	0.125969708	0.225635318	3.963247872			
27	0.5137	0.74	0.480	0.29867951	0.143378812	0.248469313	4.211717185			
28	0.5137	0.76	0.433	0.30675193	0.132931939	0.230365332	4.442082517			
29	0.53	0.764	0.487	0.30836642	0.150111579	0.252136472	4.694218989			

Appendix I: COD Standard Curve

Concentration	Absorbance	Average
25	0.004	-0.0077
	-0.014	
	-0.013	
100	0.04	0.04467
	0.045	
	0.049	
500	0.171	0.17233
	0.177	
	0.169	
700	0.234	0.23667
	0.23	
	0.246	
900	0.272	0.272
	0.266	
	0.278	
1100	0.351	0.35333
	0.353	
	0.356