WPI

Determining the Effect of Ultrasonic Pretreatment on Anaerobic Digestion of Barley Bagasse

A Major Qualifying Project
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Submitted to:
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Abstract

This experiment analyzed the effect of ultrasonic pretreatment on the anaerobic digestion of barley bagasse. Anaerobic digestion is the breakdown of biodegradable substrates by microorganisms in an absence of oxygen, resulting in the production of biogas. Biogas, a combination of hydrogen, methane, and carbon dioxide, can be used to produce electrical, thermal, or mechanical energy. This process can be enhanced with pretreatment methods such as ultrasonic irradiation of the digestible substrate. The experiment determined differences in the anaerobic digestion of untreated and ultrasound-pretreated barley bagasse over 50-days. Differences between the experiments were defined by daily characterizations of the biogas product and the digestate. Characterization of the digestate in the pretreated reactor had variable results due to a change in the biogas collection method. The reactor yielded an average of 164 mL of biogas per day with an average 55% methane composition. Initial energy calculations suggest that sonication parameters must be optimized for the system to be an energy-efficient pretreatment method. The reactor with untreated barley bagasse recently began operation, with full data for a comparison expected in June.

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1.0 Introduction

Brazil is one of the world leaders in energy production from renewable energy sources. Within renewable energy, biogas production and utilization is a growing sector.[1] The large agricultural industry makes bioenergy production from these residues an appealing option as it reduces carbon emissions from other energy sources and is a productive waste management strategy. Biogas, a combination of hydrogen, methane, and carbon dioxide, can be used to produce electrical, thermal, or mechanical energy.[1, 2] Because biogas is produced from wastes of agricultural materials, it is a renewable energy source. Natural production of biogas results from the microorganistic-assisted breakdown of organic materials in the absence of oxygen in a process called anaerobic digestion. A general overall reaction for the conversion of carbohydrates in anaerobic digestion can be seen below.[3]

$$C_6H_{12}O_6 \rightarrow 3CO_2 + 3CH_4 \qquad (1)$$

The largest limiting factors to anaerobic digestion are the slow rate at which it occurs and the quantity of biogas produced per amount of substrate used. Recent research suggests that pretreatment of the substrates used in anaerobic digestion can mitigate these challenges by weakening the cell walls of the substrate, making them easier for the microorganisms to break down.[4-6] Although pretreatment can incur high utility expenses, it has the potential to lower overall process costs and increase efficiency of anaerobic digestion.[3] Research on different pretreatment techniques and their optimization is necessary to understand the economic feasibility of widespread anaerobic digestion.

This experiment focused on the effect of ultrasonic pretreatment on the anaerobic digestion process of barley bagasse. Barley bagasse, also known as brewers' spent grain, is a byproduct of the brewing process. The feedstock for this study was obtained from AmBev, the Latin American branch of AB InBev and the largest brewer in South America. AmBev production yields 130-250 tons/day of barley bagasse which is currently used as animal feed. Brazil is one of the top five countries in the world in beer production, citing a production of 1.7 million tons of spent grain in 2002.[7] Recovering resources from this barley bagasse waste is a desirable production goal for process sustainability and to lower process operational costs.

Policy updates and new incentives in Brazil have increased domestic focus on production and use of biogas as a fuel source. Biomethane definitions and regulations established by the National Oil Agency encouraged the use of biogas for heating and electrical in residences and for use as a vehicle and commercial shipping fuel as it established the first set of official quality control standards.[1, 8, 9] New initiatives from the Ministry of Cities, Ministry of Agrarian development, Brazilian Biogas Association, National Policy on Biofuels, and Electric Energy National Agency help with the promotion of biogas as a fuel source.[10] For the first time in 2017, Brazil's Ten Year Energy Expansion Plan recognized biogas as a significant part of the electirc matrix.[1, 11] As of 2016, there were 165 operational biogas plants in Brazil with an annual energy output of 5,219 GWh. Within these plants 33% used industrial byproducts as a feedstock substrate for anaerobic digestion in biogas production. The Energy Research Office within the Ministry of Mines and Energy estimates that Brazil has a biomethane production potential of 18.5 million Nm^3/day[1] while other sources put this estimate at 80 million Nm^3/day.[9] The recent recognition and emergence of biogas as an energy source shows the growth and potential for this energy from anaerobic digestion in Brazil in the near future.

Despite new initiatives and support for biogas production, high initial start-up costs and insufficient research and exploratory funding are obstacles to widespread implementation.[3] A complete infrastructure for biogas production and use is in the beginning phases, thus information and other support for farmers or companies looking to implement small biogas plants is not widespread.[3] Another deterrent is the difficulty for small biogas plants to sell their extra carbon credits on the market. Possible financial incentives could

possibly include taking acetate, propionate, and butyrate as value-added by-products from the anaerobic digestion reaction.[3] An efficiently-optimized pretreatment process could make anaerobic digestion an energy-efficient and feasible process, encouraging widespread implementation. This experiment sought to test the viability of ultrasonic irradiation as one such pretreatment alternative.

The experiment compared the anaerobic digestion of untreated and ultrasound-pretreated barley bagasse over a 50-day period. Differences between the experiments were defined by a series of characterizations of the biogas product and the reactant mixture samples during each experimental condition. Daily characterization included quantity and composition of the biogas produced and monitoring the pH of the reactant mixture. Every 3-4 days, a full characterization of the reactant mixture was completed with evaluations of total and volatile solids, ammoniacal nitrogen content, alkalinity, and chemical oxygen demand (COD). These parameters were compared between the control and pretreated-substrate experiment.

2.0 Background:

2.1 Anaerobic Digestion

Anaerobic digestion is a process for the breakdown of biodegradable substrates by microorganisms in an environment with an absence of oxygen.[3, 5] This process was employed in an anaerobic digestion reactor for the conversion of barley bagasse feedstock into biogas. Biogas is a combustible product composed of methane, hydrogen, and carbon dioxide, which can be used as a renewable energy source.[2] The general overall reaction for anaerobic digestion can be seen below.[3]

$$C_6H_{12}O_6 \rightarrow 3CO_2 + 3CH_4 \quad (1)$$

The process of anaerobic digestion occurs in four steps. The first step is hydrolysis, where carbohydrates, lipids and proteins forming the biogas are broken down into smaller organic molecules; primarily sugars, fatty acids, and amino acids.[3, 12] Hydrolysis is generally the mechanism of water breaking organic molecules into smaller monomers and is often the rate-limiting step within the anaerobic digestion process.[7] The rate of hydrolysis is dependent on crystallinity of the cellulose and degree of association with lignin and cellulase activity of microbial process applied.[13]

$$biomass + H_2O \rightarrow monomers + H_2 \quad (2)$$

The second step of anaerobic digestion is acidogenesis, where sugars, fatty acids, and amino acids are converted into alcohols and ketones by acidogenic bacteria.[2, 12] The products of this step include acetic, propionic, and butyric acid; carbon dioxide; and hydrogen. These compounds are typically identified in larger quantities towards the beginning of the anaerobic digestion process. Acidogenic reactions are thermodynamically advantageous, occurring the most readily. Slow acidogenic reactions are thus attributed to limitation by the previous hydrolysis step.[3, 7] Examples of sugars reacting with water and hydrogen to form the acids and carbon dioxide are shown below as a subset of the acidogenic reactions.

Acidogenic Reactions (3)

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COO^- + 2CO_2 + 2H^+ + 4H_2 \quad \text{(a)}$$
$$C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COO^- + 2H_2O + 2H^+ \quad \text{(b)}$$
$$C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COO^- + 2CO_2 + H^+ + 3H_2 \quad \text{(c)}$$

The products of acidogenesis then undergo acetogenesis, where acetogenic bacteria converts the molecules into acetate ions, hydrogen, and carbon dioxide. These reactions compete for hydrogen. It was found that 70% of methane production comes from reaction of acetate ions, thus efficient reactors incorporate hydrogen removal to facilitate higher production of acetate.[3, 14, 15] Methane production was found to be higher at lower partial pressures of hydrogen within the reactor. Acetogenesis reactions are only thermodynamically favorable in conditions found in anaerobic digesters, but not under standard conditions.[3]

Acetogenic Reactions (4)

$$CH_3CH_2COO^- + 3H_2O \rightarrow \boldsymbol{CH_3COO^-} + HCO_3^- + H^+ + 3H_2 \quad \text{(a)}$$
$$CH_3CH_2COO^- + 2HCO_3^- \rightarrow \boldsymbol{CH_3COO^-} + H^+ + 3HCOO^- \quad \text{(b)}$$
$$CH_3CH_2CH_2COO^- + 2H_2O \rightarrow \boldsymbol{2CH_3COO^-} + H^+ + 2H_2 \quad \text{(c)}$$

The final step is methanogenesis in which the acetate ions are converted to methane and carbon dioxide by acetoclastic methanogenic archaea (70% methane produced through acetate ions). Methane can also be produced from hydrogen by hydrogenotrophic methanogenic archaea, but this is a lesser reaction.[3]

Methanogenic Reactions (5)

$$\boldsymbol{CH_3COO^-} + H_2O \rightarrow \boldsymbol{CH_4} + HCO_3^- + 2H_2 \quad \text{(a)}$$

$$4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O \quad \text{(b)}$$

$$4HCOO^- + H_2O + H^+ \rightarrow CH_4 + 3HCO_3^- \quad \text{(c)}$$

In the presence of sulfides in the inoculum or substrate, a side reaction can occur instead of methanogenesis in which hydrogen sulfide and bisulfide can be produced by sulphate-reducing bacteria.

Anaerobic digestion is a desirable process for conversion of biomass to biogas as it is a single process which eliminates the need for an energy-intensive drying. The process is most limited by the energy required to break down the complex cell wall structure during hydrolysis, designating this as the rate limiting step in kinetic modeling.[4] Anaerobic digestion occurs best under either mesophilic or thermophilic conditions, which define optimal temperatures for microbial reactions. Thermophilic conditions are defined as reactor environments at 50°C and mesophilic conditions at 30°C.[2, 3]

2.2 Biogas

Biogas is a mixture of 50-70% methane and carbon dioxide.[2, 14] Depending on the chemical composition of the initial substrate material, biogas can additionally contain small amounts of hydrogen sulfide, nitrogen, and often some amount of hydrogen. Biogas can be converted to a source of thermal or electrical energy. It is often used in combined heat and power (CHP) plants.[2, 16] As a fuel, biogas can be burned with the resulting energy used as a direct fuel source. Boilers utilizing a biogas fuel source are in the design stages.[12]

The potential biogas production from a substrate is dependent on the reactor temperature and the retention time of the digestate.[2] Cellulose, fats and proteins are the macromolecules which can undergo anaerobic digestion to produce methane and other biogas constituents. Both the type and quantity of specific macromolecules and their interactions determine the biogas production potential. Lignin cannot be broken anaerobically to produce biogas. The following reactions show the general chemical equations for this process and the associated potential gas yield at STP.[12]

Table 1: Potential biogas yield and composition from breakdown of macromolecules[12]

Macromolecule	Chemical Reaction	Potential biogas yield (mL gas/g)*	Potential methane yield (mL CH_4/g)*	Potential CH_4 Composition (%)*
Cellulose	$(C_6H_{10}O_5)_n + nH_2O \rightarrow 3nCH_4 + 3nCO_2$	830	415	50
Protein	$2C_5H_7NO_2 + 8H_2O \rightarrow 5CH_4 + 3CO_2 + 2(NH_4)(HCO_3)$	793	504	63.6
Fat	$C_{57}H_{104}O_6 + 28H_2O \rightarrow 40CH_4 + 17CO_2$	1,444	1,014	70.2

**At Standard Temperature and Pressure conditions (0°C, 1atm)*

Biogas produced in this experiment is a renewable energy source as it is produced from barley bagasse, a by-product of the brewing process which uses agricultural feedstock. Biogas is a versatile energy source as the physical location of an anaerobic digester can be independent of geography and allows for easy storage

of biogas. The process is very sustainable as a waste management strategy, as the end digestate product can be used as an agricultural fertilizer.[16]

The energy available from the biogas produced is often reported as energy produced per amount of substrate used or amount of volatile solid fed. The experimental biogas yield can be calculated using the ideal gas law and the heating value for methane and hydrogen as those are the combustible components. The experimental yield of biogas is typically lower than the absolute production potential due to poor maintenance of optimal environmental factors which result in incomplete digestion.

2.3 Barley Bagasse

Barley bagasse, also known as brewers' spent grain, is a byproduct of the brewing process. The material is the malt and grain residue that is left in the kettle after the mashing and lautering processes.[17] Barley bagasse can be up to 85% of the waste byproduct remaining from brewing.[7, 18] The substrate is a combination of approximately 70% fiber and 20% protein, qualifying it as a lignocellulosic product.[7, 19, 20] The fibrous portion of barley bagasse is cellulose, hemicellulose, and lignin with hemicellulose as the predominant component, typically comprising 40%-50% of the bagasse.[19, 21] The lignin composition of the barley bagasse hinders digestion as there is a high energy requirement to break the cell wall, however the cellulose and hemicellulose components enhance anaerobic digestion as they are easier to break down and provide high levels of digestible organic material.

Barley bagasse is currently used as animal feed and is a cheap material to purchase.[7] Barley bagasse cannot directly be combusted due to the potential for nitrogen and sulfur compounds within the substrate. When combusted, these compounds could form NOx and SOx, posing environmental concerns.[7]

Due to its low cost and high availability from industry, barley bagasse is a viable substrate for use in anaerobic digestion for energy production.

2.4 Anaerobic Digestion of Barley Bagasse

A few lab-scale and pilot plants have researched the anaerobic digestion behavior and potential of barley bagasse. A lab study investigated the kinetics of barley bagasse anaerobic digestion over a 15-day period. The digestion produced a total of 3.48 L of biogas with a 66% methane composition. This study found there was a 60% degradation of the initial cellulose and 40% degradation of the initial lignin during the digestion process. Kinetic models applied to this digestion determined the digestion reaction to be first order, dependent on the acetate concentration for the methanogenic step.[13]

A pilot plant with anaerobic digestion of barley bagasse in Austria found a methane production of 75 Nm^3 per ton of substrate[22], although theoretical calculations predict up to a 98 Nm^3 methane/ton barley bagasse production potential.[21] Biogas produced in the anaerobic digestion of barley bagasse could potentially recover 50-60% of the electrical energy demand and 60% of the thermal energy demand in a typical brewing, storage, and bottling process.[22]

These two studies yielded results supporting the feasibility of using barley bagasse as a substrate to produce biogas.

2.5 Ultrasonic Irradiation

Ultrasonic irradiation, also known as sonication, is a pretreatment technique which uses high-frequency waves to clean or damage substrates. The goal of the sonication pretreatment of the bagasse was to damage or break-down the cell walls to decrease limitations of anaerobic digestion during the hydrolysis step.[23, 24] Sonication is a cavitation mechanism in which high-energy bubbles are formed in a liquid medium and explode, releasing high amounts of concentrated energy and damaging nearby solid structures. Ultrasonic waves cause expansion and contraction of the liquid medium as they pass through. This movement causes dissolved gases in the liquid to form bubbles. When the bubbles gain enough energy to reach an unstable size, they explode and cause a concentrated, localized energy release (Figure 1). This energy release is a cavitation mechanism, causing cell wall degradation of the organic substrate.[23] Lower frequencies create smaller bubbles with the same amount of energy, and thus have a greater cavitation effect.[25]

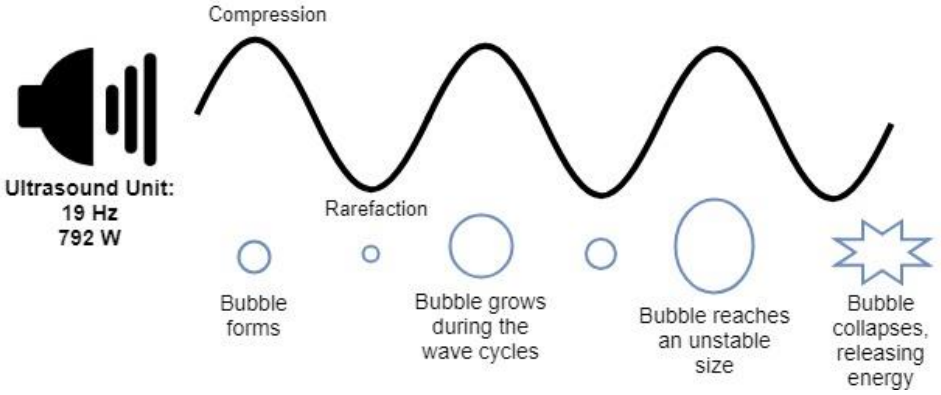

Figure 1: Schematic representation of ultrasonic cavitation mechanism

2.6 Ultrasonic Irradiation as a Pretreatment

Ultrasonic irradiation can be used as a mechanical pretreatment method to break down the cell wall of the substrate, thereby assisting the hydrolysis step in anaerobic digestion.[23, 24] Degradation of the cell wall and membrane would also yield a higher soluble chemical oxygen demand (COD) by increasing the accessible organic material to react and form biogas. This would also increase the possible methane yield of the substrate. Ultrasonic pretreatment can disintegrate microbiological biomass and increase access to cellulose in organic matter but does not disintegrate lignocellulosic material.[23] The relative simplicity and lower cost of ultrasonic technology makes it a desirable pretreatment method.[23]

In a comparison of ultrasonic and thermal pretreatments on anaerobic digestion of algal biomass, Gonzalez-Fernandez et. al. suggest that the degradation of the cell wall of the biomass during ultrasonic pretreatment is likely the result of both the sonication and the temperature increase experienced by the substrate during the sonication process.[6] This study found that compared to a control substrate without pretreatment, the pretreated biomass yielded a higher overall methane production, particularly during the first 2-4 days of digestion. While ultrasonic pretreatment sped up the rate of anaerobic digestion of the biomass, it only yielded 3% more methane than the untreated control substrate.[6] Many experiments show yield a higher biogas or methane yield and initial substrate degradation rate with a reduction of the particle size of the substrate.[24] Other studies suggest that ultrasonic pretreatment may not be enough to disrupt the cell wall.[5] There are no publications of ultrasonic pretreatment of barley bagasse in anaerobic digestion, thus specific findings of the effect of ultrasound irradiation pretreatment should be determined for this substrate.

2.7 Characterization Techniques

2.7.1 Gas Chromatography:

Gas chromatography was completed daily to assess the composition of biogas produced throughout the reaction. This analytical technique is used to determine the type and relative quantity of specific compounds present in a sample based on polarity of the components. Gas chromatographs have a mobile phase of an inert carrier gas and a stationary phase of a high-boiling temperature polymer lining the walls of the capillary sample tube. Compounds in the gas chromatograph are detected and identified based on the strength of their interaction with the stationary phase. Constituents with similar polarities to the stationary phase have a stronger interaction with this phase and thus pass at a slower rate through the column. These compounds have a longer retention time and can be identified based on this measurement and the stationary-phase chemistry. Constituents with a different in polarity from the stationary phase have a shorter retention time in the column.[26]

Separation of constituents in gas chromatography can be based on the compound vapor pressure, column temperature, flow rate of the carrier gas through the column, the length of the column, and the amount of sample gas injected. High vapor pressure constituents spend more time in the gas phase and have shorter retention times in the column. A high column temperature expedites the gas chromatography process by decreasing retention times but yields poor separation as constituents have limited interaction with the stationary phase as they stay mostly in the gas phase. The low retention times additionally make it difficult to discern between compound peaks. A similar effect is experienced with high carrier gas flow rates causing low retention times. Longer columns increase separation and increase retention times. This can result in broadening of the peaks on the chromatograph. The amount of sample to be injected should yield a chromatogram with a symmetric shape, indicating a proper amount injected. Injection of too much sample will yield tailing, resulting in a worse separation.[26]

The gas chromatograms produced were analyzed for the constituents of biogas; hydrogen, carbon dioxide, and methane in addition to monitoring for oxygen and hydrogen sulfide. An example chromatogram can be seen with labelled constituent peaks in Figure 2.

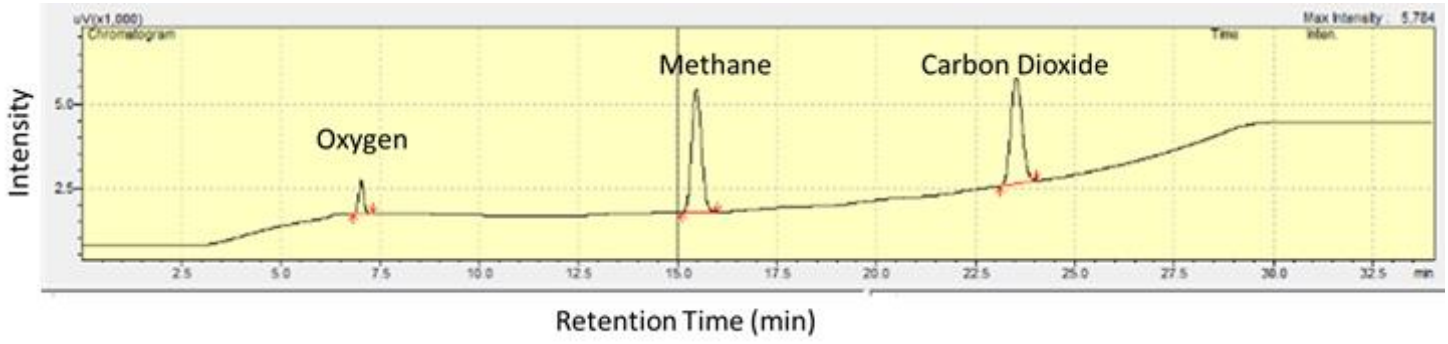

Figure 2: Example chromatogram (Day 22) with labeled constituent peaks. The biogas this day was equal parts methane and carbon dioxide (47%) with the remaining as oxygen (6%) as determined by relative integrated areas of the peaks.

Optimal biogas production has a methane concentration within the range of 50-80%.[2, 14] As the digester is anaerobic, it should not exhibit any oxygen, although small amounts may be present as introduced by inconsistent gas sampling practices. Carbon dioxide should comprise most of the rest of the gas sample, and hydrogen may also be present as the product of acidogenesis (Equations 3a-3c). Analyzed species and associated retention times are listed in Table 2.

Table 2: Gaseous chemical species and associated retention time on gas chromatograph

Species	Retention Time (min)
Hydrogen (H_2)	3
Oxygen (O_2)	7
Methane (CH_4)	15
Carbon Dioxide (CO_2)	23

2.7.2 pH:

Anaerobic digesters are very sensitive to changes in pH due to reactions in the acidogenic step to form fatty acids and the sensitivity of the bacteria in the inoculum. The pH of the digestate naturally increases through the digestion process as the acids produced and consumed in their conversion into methane. The mechanism of anaerobic digestion predicts a low digestate pH towards the beginning of the reaction as intermediary organic acids are produced in the acidogenic step (Equation 3a-3c). The reaction of carbon dioxide with hydroxide ions forms carbonate ions, returning the reactor to a more neutral pH. The production of carbonate ions additionally stabilizes the process as it makes it an auto-buffering cycle between carbonic acid and carbonate/bicarbonate ion production. When the rate of methane production is lower than that of acid formation, the pH decreases, causing biogas production to decrease and an increase in carbon dioxide formation.[27] The bacteria in anaerobic digestion is inhibited by excessive acidity and yields optimal methane production with a pH of 7-8 in the reactor.[5, 27] Methanogenic bacteria specifically thrives in an environment with a pH in a range of 8.0-8.5.[28]

2.7.3 Alkalinity:

Alkalinity in anaerobic digestors was calculated in addition to the pH as a more sensitive method to measure the ability of the digestate to neutralize acids.[29] The measurement of alkalinity is typically expressed in units of mg $CaCO_3$/L of digestate. The results indicate the presence of carbonate (CO_3^{2-}), bicarbonate (HCO_3^-), and hydroxide (OH^-) ions present in the solution.[29] As described in 2.7.2 pH, these ions can help the reactor self-buffer to the desired pH, increasing methane production. Optimal alkalinity ranges for anaerobic digestion are 2,000-4,000 mg $CaCO_3$/L.[27, 29, 30] Monitoring of pH is an simple lab procedure, but provides measurements on a log scale, making it difficult to discern small changes in digestate conditions. Alkalinity is thus important to monitor in addition to pH as is a linear scale and will show smaller environmental changes.[3]

2.7.4 Solids:

The solids content of the digestate was measured to maintain optimal conditions for biogas production and organic content for biogas production potential. Total solids and fixed solids were measured, and a calculation was completed to determine the volatile solids. Total solids of the digestate include both the total suspended solids and total dissolved solids measured as the residue after the evaporation of moisture in a digestate sample through drying in an oven at 105°C. The fixed solids are determined as the remaining digestate after ignition of the volatile solids in an oven at 550°C. Volatile solids, the difference between total and fixed solids, are an indication of the organic matter in the digestate. The bacteria in the inoculum interacts with the organic matter to produce biogas, thus a higher volatile solid content corresponds with a higher potential to produce biogas. Because the ignition of digestate at such high temperatures also causes decomposition of inorganic minerals and salts, volatile solid results should be used in conjunction with a measure of the chemical oxygen demand for a complete analysis of the quantity or organic matter

available.[29] Barley bagasse has low levels of inorganic mineral salts, thus use of volatile solids is a strong indication of the amount of organics in the digestate.[29]

2.7.5 COD

Chemical oxygen demand (COD) is a measure of the organic content of the digestate. As the bacteria interacts with the organic matter in the anaerobic digester, the amount of organics decreases, detected as a decrease in the COD. The amount of COD is dependent on the feed rate to the reactor, but should significantly decrease over time.[5] The initial COD level in the reactor should grow in the beginning days as the bacteria only begins to react with the large initial amount of organics and there is a daily feed of new organic content. As time progresses, the bacteria have reacted with most of the initial feedstock, thus the COD is predominantly based on the daily feed rate, yielding in a stabilization or decrease of COD in the reactor. For every kilogram of COD, there is a biogas production possibility of 0.35 m^3.[12, 24] This is only a production possibility as COD is not entirely digested in the process and the amount of biogas produced is also dependent on temperature, pressure, and other enviornmental conditions.[12]

Analytical testing for COD measures the amount of an oxidant that reacts with the organics in a sample. In this case, the oxidant to react with the organics was a chromate ion, $Cr_2O_7^{2-}$, which was reduced to Cr^{3+} as it reacted with the organics. In the process, both organic and inorganic components can oxidize but the amount of oxidation of organics is much higher than that of any inorganic compounds which might be present. Error in the measurments is inherent as oxidation can occur on as low as 95% of the available species. Additional error may present as nitrogen from nitrite can cause additional oxidation of organic species. This value is typically considered to have negligible effect on the total COD measurement.[29]

2.7.6 Ammoniacal Nitrogen

Ammonia in the digestate typically comes from proteins in the cell wall of the feed material and thus is highly dependent on this substrate material.[5] Nitrogen appears in different compounds in the digestate including nitrites and nitrates, but typically appears in the highest concentrations as ammoniacal nitrogen. Nitrogen is required for growth of the bacteria from the inoculum, but too much nitrogen, especially that in ammonia form, can be toxic to the bacteria. The possible negative effects of ammoniacal nitrogen are lower at mesophilic temperatures as there is more time for the digestate to adjust to changing conditions. Nitrogen is often expressed in terms of a carbon to nitrogen ration (C/N), which should be maintained to under 30/1 to prevent nitrogen from becoming the limiting factor for bacteria growth or inhibiting the process.[12] Inhibition of anaerobic digestion by ammoniacal nitrogen varies based on organic feed rate, temperature, and other factors, and has been shown to be tolerable up to concentrations of 6000 mg/L $N\text{-}NH_3$ although typical inhibition of methanogenic bacteria occurs below the 3000 mg/L region.[31]

The type of test for ammoniacal nitrogen should be chosen based off expected concentrations, and where high concentrations are anticipated, a distillation/titration method should be completed. Tests for ammoniacal nitrogen can have possible interference from especially urea and cyanates as they hydrolyze in distillation. Typically these compounds hydrolyze to less than 10% in distillation thus this error is considered negligible.[29]

3.0 Methods and Materials

3.1 Raw Materials

Solid residues (barley bagasse) and mesophilic inoculum were provided by the AMBEV CSC Brewery (Jaguariúna, São Paulo, Brazil). The barley bagasse was oven-dried (Fenem, Model 315 SE) at 105 °C for 8 hours, packed in a plastic bag and stored in a freezer at -18 °C for later use in the experiment.

3.2 Sonication Pretreatment

Sonication requires a liquid medium for the wave to pass through to the substrate. In this experiment, a mixture of 1.43 L of water and 1.23 L of bagasse was sonicated. An ultrasonic probe from Unique Industrial and Commercial Products, Brazil was used for sonication (Figure 3). The probe operated at 99% of 800 W of power (792 W) at a 19 Hz frequency. The initial feed mixture was separated into 9 beakers with approximately 400 mL of solution in each beaker to increase the proximity of all parts of the bagasse mixture to the ultrasound probe. Each beaker was sonicated for 15 minutes and then cooled in ambient air for 15 minutes. This cycle was done four times for each beaker for a total sonification time of 1 hour.

Figure 3: Ultrasonic Probe Controller Unit

A platform was set up to control the probe depth in the mixture during sonication. The experimental setup can be seen in Figure 4. The reactants before and after pretreatment are seen in Figure 5.

Figure 4: Set-up with ultrasonic probe for pretreatment of water and barley bagasse mixture.

Figure 5: Barley bagasse-water mixture before (left) and after (middle) ultrasonic pretreatment for one hour. The mesophilic inoculum is also seen (right).

3.3 Bioreactor

The same bioreactor was used in the anaerobic digestion for both the control and pretreated experiments. The reactor temperature was controlled with a heating jacket supplied by a thermostatic bath set to 25°C. The heating jacket only insulated the bottom two liters of the reactor, depending on mixing and heat transfer to bring the rest of the digestate to mesophilic temperatures. An insulating cloth was kept around the reactor except during sampling to better facilitate heat transfer and maintain the digestate temperature. The reactor temperature was set to mesophilic conditions at 35°C +/- 2°C. Two finned agitator attachments connected to a central shaft mixed the reaction with agitation set to 40-50 rpm. All gas and liquid samples were taken from small ports in the top of the reactor, which was otherwise sealed off to prevent exposure to the air (Figure 6b). A gas collection bag was attached to an outlet port of the reactor (Figure 6a).

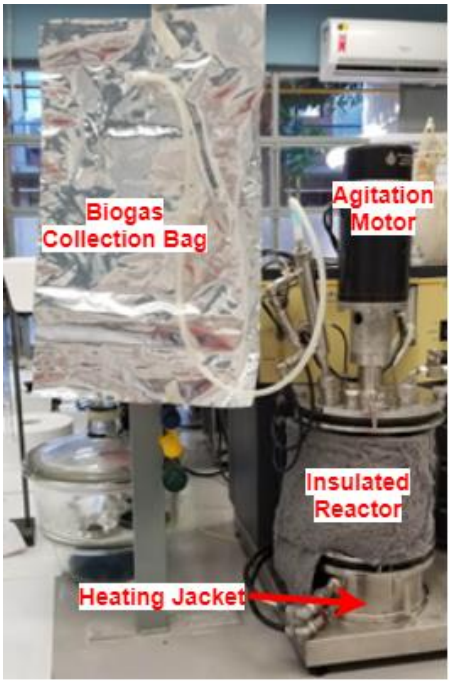

Figure 6a: Anaerobic digestion reactor and gas collection set-up (Day 1-19 pretreated trial).

Figure 6b: Sample collection on anaerobic digestor (Day 1-19 pretreated trial).

On Day 19 of the pretreated trial, the biogas collection method was changed from a collection bag to a water displacement method. There were concerns with the entry valve on the gas collection bag; when it was opened slightly too far, it allowed for entry of air in the biogas product during volume measurements. When the valve was closed to prevent this, it prevented biogas collection and caused a pressure buildup in the reactor. Water displacement was deemed a suitable alternative to measure the volume of biogas produced, prompting a slight change in set-up of the reactor and sampling ports.

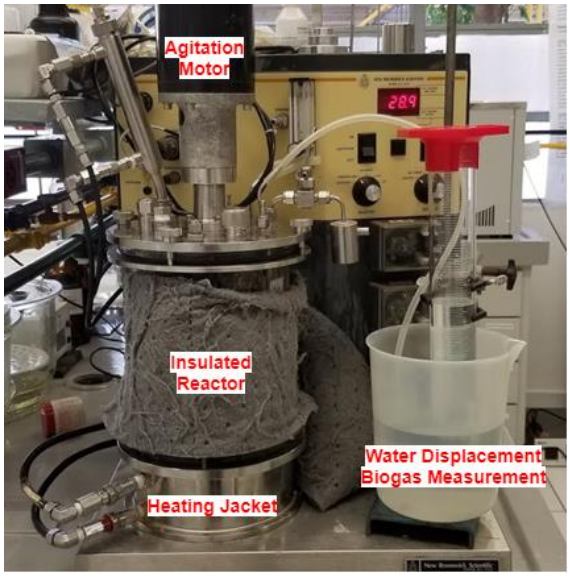

Figure 7a: Revised gas collection procedure of water displacement method (Day 19-40 pretreated trial and Day 1-40 control trial).

Figure 7b: Revised sample collection (Day 19-40 pretreated trial and Day 1-40 control trial).

Initial feed conditions for the reactor were designed for 65% feed and 35% headspace by volume for optimal biogas production.[5] Total reactor volume was 6.8 L, yielding the proportions outlined in Figure 8. Of the 65% initial feed load; 28% was barley bagasse substrate, 39% inoculum, and 33% water.

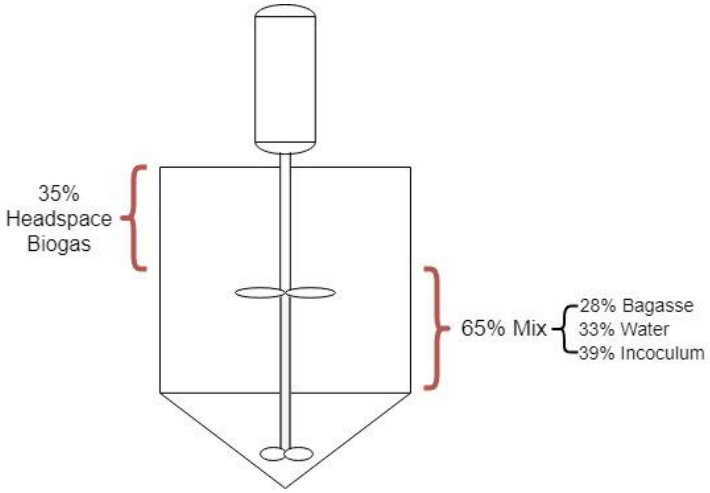

Figure 8: Theoretical volume proportions of reactor and mixed reactants

Because of the low density and high absorptivity of the bagasse, the digestate volume was much lower than designed for. As the bagasse was sonicated, it absorbed more water and compacted, yielding a lower actual volume. An actual volume of 3.5 L was noted in the reactor, yielding an actual 50% feedstock and headspace during operation. The volume proportions added to the initial feedstock were calculated based on constituent density, thus those percentages were added as designed.

3.2.1 Feed Conditions

The reactor can be classified as semi-continuous with a sonicated bagasse-water feedstock added every day. The amount of feedstock added per day was calculated from a heuristic for average residence time of 10-

30 days.[24] The feedstock "flow rate" defined as amount per day was calculated using a residence time of 29 days and Equation 6.

$$\tau = \frac{V}{u} \quad (6)$$

$\tau = residence\ time\ (days)$

$V = reaction\ volume\ (mL)$

$u = flow\ rate\ (\frac{mL}{day})$

Feed Calculation:

$$29\ days = \frac{3500\ mL}{u}$$

$$u = 120\frac{mL}{day}$$

A feed rate of 120 mL of the sonicated bagasse water mixture was calculated. The daily feed maintained proportions of 30% bagasse and 70% water, yielding 36 mL of bagasse and 84mL of water. With the low density of the bagasse and its absorbance of water, the actual total volumetric feed rate and digestate removal rate was 87.5 mL/day. A constant feed rate helped to assimilate the bacteria in the system.[12]

Upon initial set-up there were challenges with mixing and heat transfer. This was remediated by adding a second set of agitation fins to increase mixing throughout the reaction instead of just at the bottom of the reactor. When new feedstock was added, agitation was increased six-fold for a few minutes to mix the new bagasse into the reaction.

3.3 Characterization Procedures:

Characterization and reaction monitoring procedures were completed daily. These included a measurement of the volume of biogas produced, characterization of the biogas composition with gas chromatography, and pH monitoring.

3.3.1 Amount of Biogas Produced

For days 1-19 of the pretreated trial, the biogas collection bag was emptied daily with a 60 mL syringe to measure the volume of biogas produced by the reactor. The number of 60 mL syringes of gas removed was totaled and added to 600 mL of biogas which was removed daily from the headspace of the reactor to avoid pressure buildup. Concerns with the biogas production data prompted a methodology switch to a water displacement measurement set-up for volume measurement. An 500 mL graduated cylinder full of water was inverted in a large beaker, and the gas outlet tube from the top of the reactor was fed into the graduated cylinder. Gas produced in the anaerobic reactor travelled through the tube into the graduated cylinder, forcing water out and into the beaker. The amount of gas was then measured as the volume of water displaced from the graduated cylinder.

3.3.2 Gas Chromatography

Gas composition was measured on a gas chromatograph (GC 2014 Shimadzu Corporation) to analyze for the chemical components of biogas. The chromatogram contained a thermal conductivity detector and a packed column (ShinCarbon ST 50/80 mesh). The injector and detector temperatures were set to 200°C. The initial column temperature was 50°C and increased to 180°C in increments of 5°C/min. An inert nitrogen carrier gas at 5 bar, 35 mL/min was used as the mobile phase. The total analysis time was 35 minutes.[5] Results were analyzed for hydrogen, carbon dioxide, methane, hydrogen sulfide, and oxygen gas as possible products of anaerobic digestion or species in the reactor. A 0.5 mL sample of biogas was taken daily from the headspace in the reactor and injected for analysis.

The biogas energy potential was calculated using the volume and percentage of methane produced, ideal gas law and lower heating value of methane to yield a conservative estimate. The following equations outline the process for production calculations.

Volume of Methane Produced (V_m)	$V_m = V_{biogas} * x_m$	(7)	x_m the volume fraction of methane (from chromatography)
Moles of Methane Produced (n_m)	$n_m = \frac{PV_m}{RT}$	(8)	$P = reactor\ pressure$ $R = gas\ constant$ $T = reactor\ temperature$
Energy Produced (E_p)	$E_p = \frac{n_m * HV_L}{MW}$	(9)	$HV_L = low\ heating\ value$[32] $CH_4 = 50 \frac{MJ}{kg}$ $MW = methane\ molecular\ weight$
Energy Used in Sonication (E_u)	$E_u = P * t * N$	(10)	$P = ultrasound\ power$ $t = time\ of\ sonication\ per\ beaker$ $N = number\ of\ beakers\ sonicated$

3.3.3 pH Monitoring

Optimal conditions for methane production are when the reaction is in the range of 6.5-8 for production of the intermediary acids.[28] The pH was thus monitored every day and regulated with the addition of NaOH to maintain the reactor between 7.0-8.0. The pH of a 20 mL sample of the digestate was measured. The number of drops of NaOH required to increase the pH to 8.0 was determined by adding one drop of NaOH at a time to the 20 mL sample and mixing with constant pH monitoring. The volume of NaOH to add to the reactor was calculated as Equation 11 and then added to the reactor.

$$\frac{20mL}{3500mL} * \frac{\#\ drops\ NaOH}{25\ drops \frac{NaOH}{mL}} = x\ mL\ NaOH\ added \qquad (11)$$

A full characterization of the reaction mixture was completed twice a week to monitor changes in the digestate. Full characterization included tests and calculations for total and volatile solids, chemical oxygen demand (COD), alkalinity, and ammoniacal nitrogen.

3.3.4 Solids:

Solids characterization tests were performed and calculated using the analytical lab procedures outlined in NREL Determination of Total Solids and Ash in Algal Biomass.[33] Adjustments to the procedure were made to use 2.000 g for each sample. Each test was performed in triplicate and averaged in calculations. Total solids (TS) and volatile solids (VS) were calculated as per Equations 12 and 13.

$$TS = \frac{mass\ of\ dry\ substrate}{(mass\ of\ wet\ substrate\ mix)} * 100 \quad [=] \frac{g\ solids}{kg\ reactor\ sludge} \qquad (12)$$

$$VS = (1 - \frac{mass\ of\ incinerated\ substrate}{mass\ of\ dry\ substrate}) * TS \quad [=] \frac{g\ volatile\ solids}{g\ total\ solids} \qquad (13)$$

Standard procedures for COD, alkalinity, and ammoniacal nitrogen characterization tests were taken from the 20th edition of Standard Methods for the Examination of Water and Wastewater.[29] These tests all underwent the same sample preparation. Five gram samples of the digestate were filtered first through cotton (all) to remove the larger of the suspended solids and then through a Büchner Funnel/vacuum pump system (alkalinity and nitrogen) to yield liquid samples for the tests. The samples were well-mixed in a shaker for an hour. Filtered samples were then frozen for 1-2 weeks in accordance with the Standard Methods for the Examination of Water and Wastewater, thawed and tested later.

3.3.5 Chemical Oxygen Demand:

An analysis of the chemical oxygen demand (COD) required a digestive solution and a catalytic solution. A standard solution made from potassium biftalato was used to make a standard curve for COD testing (See Appendix I for standard curve). The filtered reaction samples were mixed and heated with a potassium dichromate digestive solution and a silver (II) sulfate/sulfuric acid catalytic solution to allow for reaction and read in a Hach spectrophotometer at 610 nm.

$$COD\left(\frac{mg\ O_2}{L}\right) = absorbance * standard\ slope \quad (14)$$

3.3.6 Ammoniacal Nitrogen:

A distillation and titration procedure were used to determine ammoniacal nitrogen concentration every three days in the reaction mixture. The combination of the distillation and titration procedure set was selected to increase the precision of the nitrogen readings and to allow for detection of ammoniacal nitrogen concentrations greater than 5 mg/L. Several solutions were made and used in the procedures, including a *borate buffer solution* of sodium hydroxide and hydrated sodium tetraborate. A *boric acid absorbent solution* was made by dissolving 20g of H_3BO_3 in a liter of water. A mixed indicator of methyl red and methylene blue in 95% isopropyl alcohol was added to the boric acid absorbent solution. The filtered reactant sample to be distilled was buffered with 0.5 M NaOH to a pH of 9.5 to prevent hydrolysis of other nitrogenous organic compounds during distillation. The filtered reactant sample was added in equal part to the borate buffer and distilled into the boric acid absorbent solution. The distillate in borate buffer was titrated with 0.02 M H_2SO_4 until the solution became pink.[29]

The distillation apparatus was cleaned by running a sample of deionized water through the column. A blank was completed to facilitate corrections on the trials. Additionally, the 0.02 M H_2SO_4 was standardized against a solution made with 2.0 g Na_2CO_3. A new indicator solution and borate buffer solution was made every month to minimize the effects of color changes with solution aging.

3.3.7 Alkalinity:

Alkalinity was measured in 10 mL of the prepared sample. An agitated titration setup with a pH meter was used. The initial pH of the sample was measured to ensure it was less than 8.3. In all cases, the pH met this criterion. The reaction sample was titrated with the standardized 0.02 M H_2SO_4 until the pH was 4.3-4.7. The alkalinity was calculated using Equation 15.

$$alkalinity = \frac{M_{H_2SO_4} * V_{H_2SO_4} * 50000}{10\ mL}[=]\frac{mg\ CaCO_3}{L} \quad (15)$$

3.4 Initial Bagasse Substrate Characterization

Moisture, total solids, ash, total extractives, carbohydrates, lignin and protein analyses were performed on the barley bagasse using methodologies prescribed by the National Renewable Energy Laboratory (NREL).[33-36] Measurement of these components in the initial substrate material was important to determine the biogas production potential of the barley bagasse.

The protein content within the sample is difficult to measure directly and is thus estimated based on the total (Kjeldahl) nitrogen. With the total nitrogen determined, a nitrogen factor can be calculated to relate the nitrogen content to protein level. A nitrogen factor of 6.25 is used for biomass except wheat grains. A nitrogen-to-protein conversion factor is then used to estimate the protein content of the biomass.[36]

Only evaluation of structural carbohydrates is possible in addition with determination of lignin content. The structural carbohydrates are bound within the biomass whereas the non-structural are removed in washing and extraction. Lignin is a complex, phenolic polymer. A hydrolysis is completed in two steps to break the biomass into acid-soluble and acid-insoluble components. The acid-soluble components and lignin are identified using UV-visible spectroscopy. The carbohydrates are hydrolyzed into soluble-polymers which allows for measurement with HPLC. This technique can also be used to measure the acetate content in the sample.[35]

4.0 Results

4.1 Control Reactor Behavior:

The experimental design called for a 50-day digestion period for ultrasound-pretreated barley bagasse and a 50-day digestion period of unpretreated barley bagasse. Although initially intended as a single-term project, this experimental design extended the experiment beyond the time constraints of a seven-week term. At the time of the completion of this paper the control reactor trial was just started, thus no data from the control was available for analysis in this paper. Full data and analysis of both data sets will be available later in a UNICAMP publication.

4.2 Pretreated Bagasse Reactor Behavior:

The pretreated reactor was run with consistent operating conditions as described in Chapter 3 for a 50-day period. The only major, unanticipated change to reactor conditions was the change of gas collection method at day 19 as outlined in Section 3.3.1. The reactor appeared to reach a steady-state operation after this sample procedure change. The effects of this change were first noticed on day 22, with an almost 10-fold decrease in alkalinity and ammoniacal nitrogen in the reactor digestate. The conditions resulted in biogas with a methane content consistently higher than 50% within the biogas, and higher than that of carbon dioxide. Day 22 was the first time a full characterization of the digestate was completed after the biogas collection procedure changed. The following section details the results for each characterization parameters measured and their effects on the biogas production of the reactor with the sonicated barley bagasse.

4.2.1 Biogas Production

The biogas production during the pretreatment trial had variable results, largely due to inconsistencies in the gas collection method (Figure 9). Gas entered the collection bag through a sensitive valve. Small adjustments in rotation of the valve either completely prevented entry of the gas into the bag or allowed for the leaching of gas into the atmosphere. It was measured that 4.8 L of biogas was collected from the bag on day 1 and day 2. The bag was known to have a full capacity of 5 L but did not have any volume expansion that would occur with 4.8 L of biogas. It was thus assumed that gas removed from the bag with the sample collection syringe was air entering the bag through the widely-opened valve. The valve was closed half a turn after day 2, yielding reasonable biogas collection results.

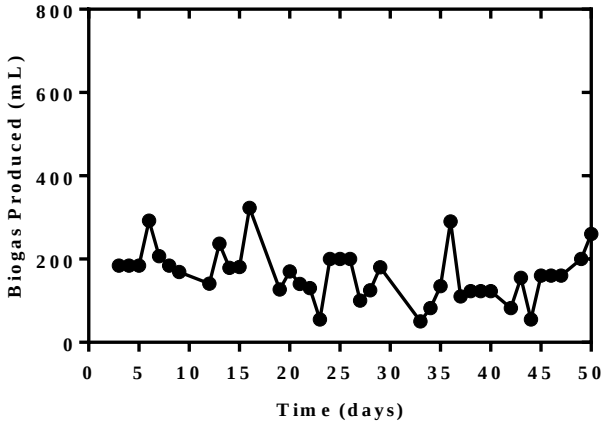

Figure 9: Volume of biogas produced over the 50-day trial with sonicated barley bagasse corrected for change in collection method. The quantity of biogas produced was not reliably measured during the first 2 days of operation.

To increase the accuracy of biogas volume measurements, the gas collection method was switched to water displacement in an inverted cylinder. Results from the gas collection bag were adjusted for the methodology change. When the gas collection bag was used, 600 mL of gas from the reactor headspace was added to the amount of biogas from the bag to yield a total volume of gas produced. Since we were unable to verify that the headspace of the reactor was full to the 600 mL capacity every day, we corrected the biogas volume data for the first 19 days of the trial by subtracting this 600 mL from the collected volume total. While likely some percentage of the reactor headspace was filled with biogas, it could not be verified that the entire 600 mL was biogas volume produced. When the biogas volume data is collected for the control reactor, a comparison should be done with 1) the corrected data, 2) the uncorrected data, and 3) the volume data for after day 19 to determine the difference between the control and pretreated reaction production potential and to verify the applied correction for the trial reactor. The uncorrected data assuming the 600 mL of reactor headspace was full of biogas, can be seen in Figure 10.

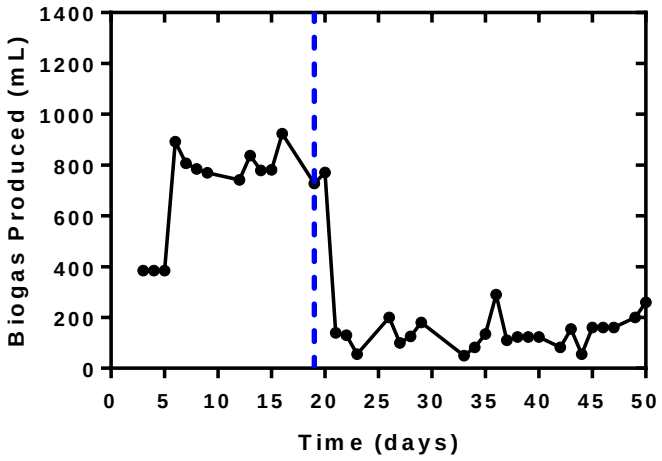

Figure 10: Volume of biogas produced over the 50-day trial with sonicated barley bagasse. Data is not corrected for the change in collection method at Day 19 marked with the blue line. The quantity of biogas produced was not reliably measured during the first 2 days of operation.

While the results were still variable, the standard deviation of the data decreased five-fold, supporting more consistent results of the biogas volume with the new method. Biogas production on the weekend was calculated as the volume of gas collected on Monday divided over the three days. The average daily volume of biogas produced was 163 mL. The average daily volume of biogas produced is difficult to compare with others found in the literature as it is dependent on the feedstock characterization, reactor size, and reactor operating conditions. The implications of this value will be later discussed in the comparative context of amount of energy produced per mass of substrate used. When data for the control reactor is collected, this will be an important comparison parameter between the two reactors. Literature suggests that the average daily amount of biogas produced will be higher for the pretreated reactor as the broken cell walls of the sonicated bagasse will yield better access for the methanogenic process (Chapter 2).[4-6]

4.2.2 Biogas Composition

The biogas composition was analyzed daily to track the methane content. As predicted by the acidogenic and methanogenic mechanisms, the produced biogas was predominantly a mixture of methane and carbon dioxide (Figure 11).

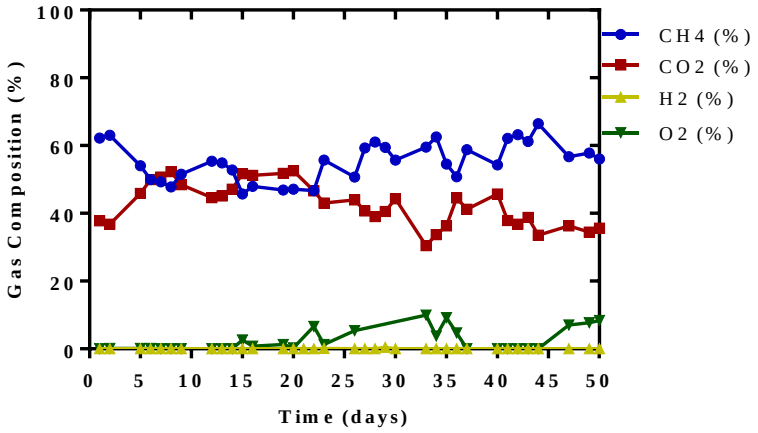

Figure 11: Biogas composition over the 50-day trial with sonicated barley bagasse. Hydrogen remained at unmeasurable levels, oxygen was typically at 0% with occasional increases to no more than 10%. Methane and carbon dioxide maintained around 50% each of the biogas composition.

After Day 22, the level of methane consistently remained above that of carbon dioxide. This is desirable as methane is the combustible constituent which provides the energy content of the biogas. Since anaerobic digesters are typically designed to run for long time periods, the final operation of the reactor producing a biogas product with a methane content above that of carbon dioxide shows the efficacy of this system at these experimental operating conditions.

Oxygen was occasionally present (day 20-36 and 46-50) in levels less than 10% of the biogas. As this was an anaerobic digester, the presence of oxygen was not predicted, as it could inhibit the ability of the bacteria in the inoculum to break down the bagasse. Since the quantities of oxygen in the biogas were less than 10%, it is predicted that oxygen was introduced into the reactor during imperfect operation procedures. The sample port at the top of the reactor (Figure 6b, 7b) was opened daily to feed the reactor and to take samples of the digestate for characterization. Feeding and sample collection procedures were completed as quickly as possible to limit the time the reactor was open to the air, but daily exposure to oxygen could account for some of the low oxygen levels in the biogas. Exposure to the air was typically less than 2 minutes during feeding and sampling, but there were instances where this was extended due to difficulties pulling the digestate into a syringe.

On at least one of the days during sampling, the tube connected to the syringe was pulled off into the reactor and took about five minutes to remove from the reactor. During this time, the reactor was open to the air. Small amounts of oxygen may appear in the chromatogram during the introduction of the gas sample into the column. If the gas syringe was at an angle as the sample was introduced to the column, a minute amount of air could be introduced into the column, resulting in a small amount of oxygen in the chromatograph. Since the levels of oxygen remained below 10% and it was not determined if this was representative of the quantity of oxygen in the biogas or if it was introduced during the gas sampling procedure, it was predicted to have little effect on the experiment. The presence of oxygen in biogas produced in the control reactor should be closely monitored and compared with this to determine if the oxygen may appear in the biogas during reactor sampling and feeding procedures.

The biogas product of anaerobic digesters typically has a small amount of hydrogen. Hydrogen was not measured in any quantity in the biogas produced from the pretreated reactor. The lack of hydrogen product

could suggest that reactions with hydrogen reactants (equation 3b-acidogenic, 5b-methanogenic) consumed the hydrogen produced in other processes (primarily equation 5a). This can be described as a symbiotic reaction with the bacteria in the reactor; when hydrogen is produced by one reaction, it is used in another process and thus does not appear in the biogas.

4.2.3 Ammoniacal Nitrogen

Results of the ammoniacal nitrogen levels in the reactor were the first indication of a large environmental change in the reactor. The initial ammoniacal nitrogen levels increased as expected. Nitrogen comes from the cells of organic matter in the reactor and should thus increases as the cell walls of both the barley bagasse are digested and as bacterium in the inoculum begin to die and accumulate in the reactor. There was a ten-fold drop of ammoniacal nitrogen levels at day 22 (Figure 12). Nitrogen is not used in any part of the anaerobic digestion mechanism; thus this drop was due to an environmental change.

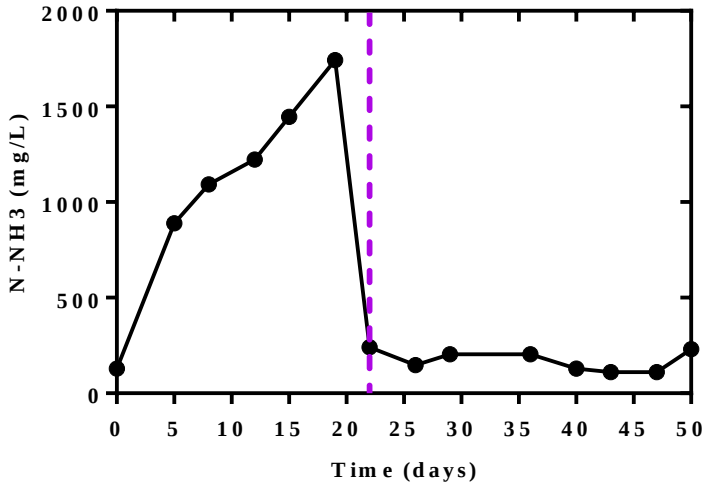

Figure 12: Ammoniacal nitrogen concentration in the pretreated bagasse reactor digestate. Day 22 is marked with the vertical purple line, corresponding with a significant drop in nitrogen levels.

It is predicted that the drop in ammoniacal nitrogen was an unanticipated result of the change in biogas collection method. Although this procedural change occurred on day 19, a full characterization of the digestate was not completed until day 22. The change in biogas collection method from the biogas collection bag to water displacement in an inverted cylinder relieved pressure in the reactor as observed by the rapid movement of gas into the inverted cylinder when the change was completed. A rapid decrease of pressure in the reactor would allow the ammonia forced into the liquid digestate to move into the gas phase by relieving the pressure in the confined reactor space. This explains a large and rapid decrease in ammonia content within the digestate.

Other possible environmental or operational factors which might account for a drastic change in ammoniacal nitrogen concentration include a temperature increase or a case in which the methanogenic bacteria grew at a rate rapid enough to consume the nitrogen from the other decomposing organic cells. The temperature of the reactor was controlled with a thermostatic water bath and monitored daily, thus this is not a plausible explanation for the change. A rapid and isolated increase in the growth of methanogenic bacterium is also unlikely and would likely result in a higher production of biogas volume, thus is not a plausible explanation for this change.

The large decrease in ammoniacal nitrogen resulted in a methane content consistently higher than that of carbon dioxide within the biogas (Figure 11 blue and red lines respectively), which is a more desirable composition. This suggests that the bacterial function of the inoculum was inhibited by the buildup of ammonia within the digestate.

4.2.4 Reactor pH and Alkalinity

The digestate alkalinity and pH was measured to follow the progression of the anaerobic digestion mechanism and ensure continuance of optimal environmental conditions (Figure 13). Based on the mechanisms of anerobic digestion, it is predicted that the alkalinity of the digestate should be lower during acidogenesis and acetogenesis (Equations 3, 4) as weak propionic, butyric, and acetic acids are produced. As the methanogenic mechanism occurs, it is predicted that the alkalinity in the reactor would increase as the hydrogen ions react with bicarbonate and formate to produce methane (Equations 5b, 5c). Anaerobic digestion is a kinetic process, thus the total alkalinity in the digestate is a function of the relative rate of the three mechanisms after hydrolysis. The slower rise in alkalinity could suggest that the relative rate of acidogenesis and acetogenesis to methanogenesis was greater for the first 5 days, and then the mechanism of methanogenesis became more dominant, increasing the consumption of hydrogen ions in the production of methane.

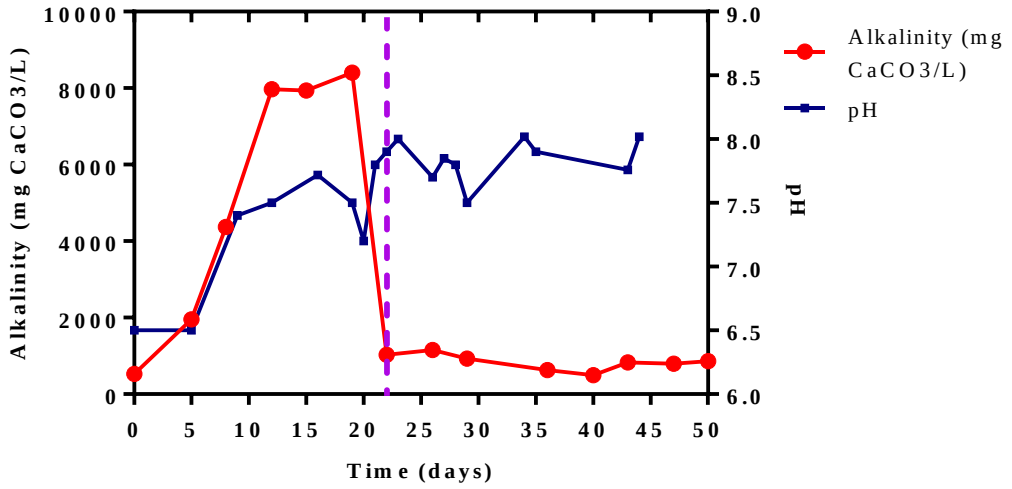

Figure 13: Alkalinity and pH levels in the pretreated bagasse reactor digestate. Day 22 is marked with the vertical purple line, corresponding with a significant drop in alkalinity levels

.

While alkalinity was monitored every 3-4 days, pH was taken daily. Since pH is measured on a logarithmic scale, small changes in pH correspond with more significant changes in alkalinity, thus the pH was corrected to 8.0, the high end of the optimal pH range found in literature for the methanogenic step in anaerobic digestion. After day 12, the reactor began to self-regulate pH, with the level never dropping below 7.5. The pH was still corrected up to 8.0 when necessary to ensure that alkalinity was not an inhibiting factor to biogas production.

The alkalinity level experienced a factor of 8 decrease on day 22. The corresponding drop in ammoniacal nitrogen could explain this high drop in alkalinity. A decrease of ammoniacal nitrogen yielded a decrease of ammonia in the digestate. As ammonia is a basic compound, its removal from the liquid phase and into the gas phase caused the alkalinity of the liquidous digestate to decrease. The drop in alkalinity was

somewhat reflected with greater decreases in daily pH levels between day 22 and day 30, although there is greater variability in the pH trends as it was artificially corrected daily.

4.2.5 Solids and Chemical Oxygen Demand

Total solids followed expected trends, increasing for the first five days of digestion and then having a general overall decreasing trend (Figure 14). The solids increased in the first few days as the reactor had to digest the initial feed load in addition to the daily feed. Results suggest that after five days, the rate of solids addition in the daily feed rate was lower than the rate of solids digestion in the operating reactor.

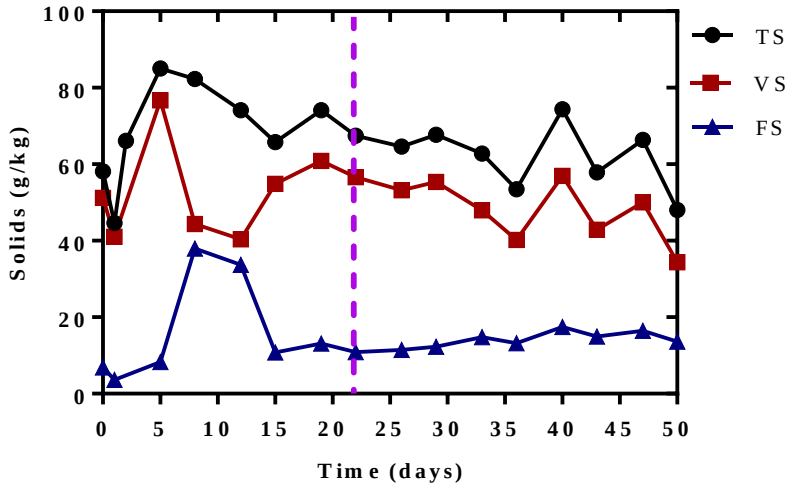

Figure 14: Total solids (TS), volatile solids (VS) and fixed solids (FS) in the pretreated bagasse reactor digestate. Day 22 is marked with the vertical purple line.

Total solids is a measurement of both the inorganic materials from the barley bagasse in addition to the organic materials measured as volatile solids. The daily feed was calculated to be 75% volatile solids, or 75% digestible organic material. After day 8, the volatile solids in the reactor comprised of 75% of the total solids, thus suggesting that the volatile solids from the inoculum and the initial feed were digested. The percentage of VS decreased after day 8 as the daily digestion of organics was greater than the organic load in the daily feed. The inorganic material remains as fixed solids after incineration and includes minerals and metal traces from the barley bagasse. After day 12, this value remained consistent at 13.5% ±3% of the total solids. A full characterization of the barley bagasse substrate will likely show an inorganic content around this value. This also suggests that within the 50-day trial, there was not a buildup of inorganic minerals and metals from a contaminated feed product, removing the possibility of these factors as possible contaminants within the reactor.

The total solids trends do not appear to be affected by the change in biogas collection method as day 22 occurs within an already-decreasing trend. This suggests that the suspected nitrogen buildup in the reactor inhibited the production capability of the organic material, not the amount of material digested as both the biogas volume and composition increased after this change but there was no observable effect on the trend of the solids in the reactor.

Similar to the volatile solids, chemical oxygen demand (COD) is an indicator of the amount of digestible organic material. The COD increased in the reactor as it digested both the organic load from the initial feed in addition to the organic load from the daily feed. As expected, the COD levels in the reactor rose for the

first few days of operation and then significantly decreased as new digestible organic matter only came from the daily feed load (Figure 15).

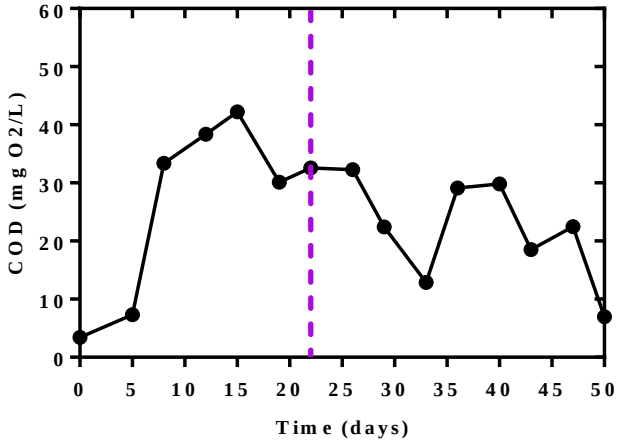

Figure 15: Chemical oxygen demand in the pretreated bagasse reactor digestate. Day 22 is marked with the vertical purple line.

The COD trends do not appear to be affected by the change in biogas collection method as day 22 occurs within an already-decreasing trend. This suggests that the suspected nitrogen buildup in the reactor inhibited the production capability of the organic material, not the amount of material digested as both the biogas volume and composition increased after this change but there was no observable effect on the trend of the solids in the reactor.

Volatile solids and chemical oxygen demand are both indirect measurements of the available amount of organic matter in the reactor which can be digested to produce biogas. As seen in Figure 16, these two parameters followed very similar trends after day 22 when the biogas collection method was changed.

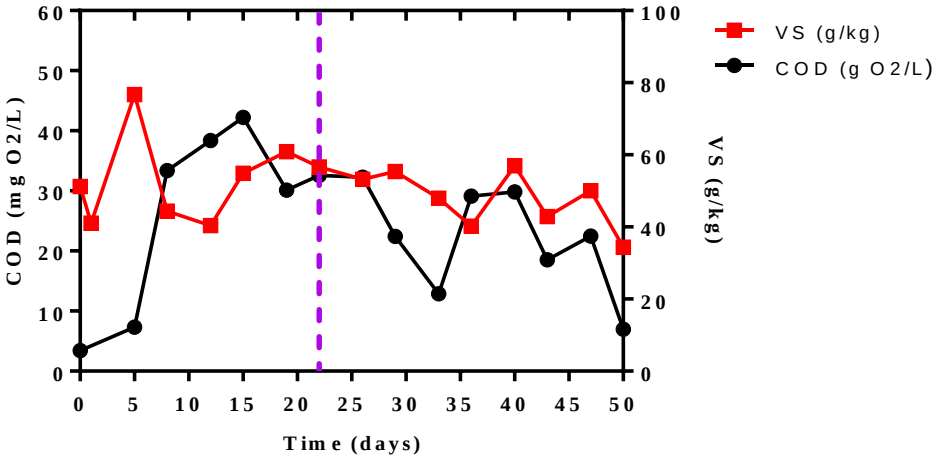

Figure 16: A comparison of COD and volatile solids as indicators of the amount of digestible organic material in the pretreated bagasse reactor digestate. Day 22 is marked with the vertical purple line.

Although the magnitude of the changes in the amount of COD and VS were not the same, the two parameters followed the same trends. The COD and VS both increased during the first week of anaerobic digestion as the cell walls of the initial feed load in addition to those of the daily feed of the bagasse underwent hydrolysis to release the organic material contained within the cell for digestion. There was an overall decrease in both parameters as the organic matter in the initial feed load was mostly digested and new organic matter was only available from the daily feed. The decreasing trends occur where the rate of hydrolysis is higher than the daily feed rate.

Days 19-36 show large differences between the COD and VS in the reactor. This could be due to the presence of small amounts of oxygen in the reactor during this time period (Figure 11) A higher amount of oxygen would result in a COD indicating a lower level of organic material than would be actually required by the organics as the COD of these species would be partially satisfied by the oxygen present in the reactor. While the VS tests would not be affected by the presence of oxygen, the COD would likely be lower than that indicating the actual amount of organics. Other slight increases in VS and COD (ex. Day 40, 46) may be the result of poor mixing in the reactor, resulting in pockets in the reactor of digestate with varied characteristics.

4.2.6 Energy Production Possibilities

A total of 0.03 kWh of energy was produced as biogas from the anaerobic digester (Figure 17). This amount was calculated using the lower heating value of methane and assuming the full methane content of the biogas would be combusted.

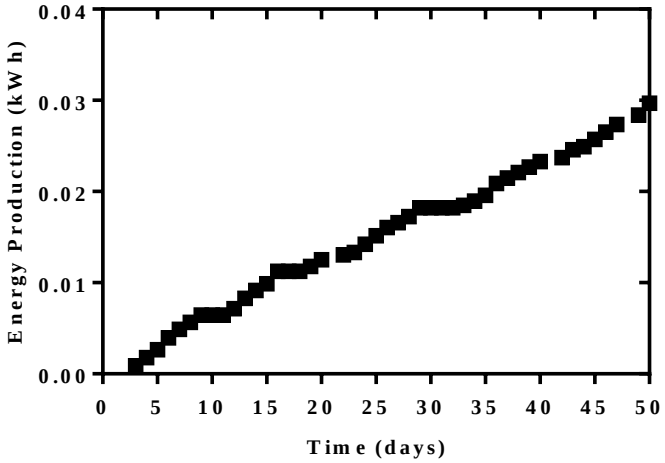

Figure 17: Cumulative energy production (kWh) calculated from volume and composition of biogas produced in anaerobic digester over 50-day period.

Since a reliable quantity of biogas produced was not collected during the first 2 days of operation, the cumulative energy value of the trial is likely lower than what was actually produced. The gaps in energy production prior to day 19 in addition to the lapse of data for the first 2 days was due to uncertainties resulting from the unreliable biogas collection bag. The cumulative energy results were based on the corrected biogas volume data, which also suggests that energy amounts are less than actual as there was likely some volume of gas produced in the 600 mL headspace of the reactor unaccounted for in these calculations as the amount could not be verified (Section 4.2.1).

A total of 7.13 kWh of energy was used to sonicate the barley bagasse used in the reactor, meaning that the amount of energy produced by the reactor was less than 0.5% of that used for the pretreatment process. Of the energy input into the bagasse mixture during sonication, some dissipated into heat, evidenced by an increase in the temperature of the mixtures. (Temperature rises can be found in Appendix H). The temperature rises in the bagasse mixture during sonication likely acted as a different form of pretreatment in addition to the cavitation mechanism from the ultrasound probe, which further weakened cell walls.

Some of the energy discrepancy can be attributed to the 50-day limit on the digestion reaction. An accurate energy comparison would be between the total amount of energy produced until the full biogas production potential of digestate reached and the digester stopped yielding biogas. The current biogas comparison is based on energy produced during an arbitrary time limit for biogas production.

A common metric to compare the efficiency of different digestible feedstocks is the energy produced per mass of feedstock. The pretreated barley bagasse was calculated to have an energy production of 0.17 kJ/g of bagasse. This value is likely lower than the actual potential of the feedstock as it is also based on the energy produced by incomplete digestion of the feed load within the reactor and only on the biogas produced during the 50-day trial. The energy production of the sonicated bagasse was significantly lower than that found in other studies, but those systems used milling as an additional pretreatment or had a much larger reactor capacity which may result in the higher energy production in those studies.[13, 22]

4.3 Expected Comparison Results:

Although there are no results for the control reactor, results can be predicted from trends exhibited in the literature and predictions based on the sonication mechanism. The sonication pretreatment should weaken the cell walls of the barley bagasse, lessening the barrier to the hydrolysis step in anaerobic digestion. Without the sonication to assist hydrolysis, the following trends are expected for the control reactor (in comparison to the pretreated trial reactor:

- Lower cumulative biogas volume production over 50-day period
- Longer time to reach a biogas composition greater than 50% methane
- Slower decrease in the total and volatile solids and COD
- Slower accumulation of $N\text{-}NH_3$
- Lower overall energy production within 50-days

4.4 Experimental and Methodology Errors:

There were a number of experimental and methodological errors which could affect the collected data. The largest experimental error was from the change in biogas collection method. The first two days of biogas volume data were entirely discounted because of the inaccuracy of the results. Day 3-19 of the data was adjusted to account for uncertainty of biogas in the reactor headspace, which would likely result in the reported volume and thus cumulative energy calculations to be lower than actually yielded by the reactor as discussed in 4.2.1 and 4.2.6. The change in collection procedure will make the comparison between the pretreated trial reactor data and the control data more difficult to compare. Results between the two reactors should first be compared from day 20-50 and to assess any differences and then compared for the first 20 days to see if the differences are maintained during this period where the behavior of the pretreated reactor was inhibited.

Some procedural errors occurred in isolated incidents while taking samples of the digestate. On day 6, a 2 mL plastic pipette tip fell off a sampling syringe into the digestate and was unrecoverable. Since this was a small item in comparison to the volume of the digestate, it likely had little to no effect on the anaerobic digestion process. Anaerobic digestion systems are also stable, so they are generally only affected by large system disruptions, not small incidents such as the introduction of a small pipette tip. A few weeks later, the hose used to pull the digestate into the syringe during sampling fell into the reactor. It took less than five minutes to remove the hose from the digestate, but during that time the reactor was open and exposed to the air. It is expected that this introduced oxygen from the air into the anaerobic system. As discussed in section 4.2.2, this level never reached above 10% of the biogas composition, so oxygen levels were low. The system was about three weeks into operation at this point, and the small amount of oxygen did not have a large effect on the system as seen by the characterization parameters. Following these incidents, a more secure apparatus was developed to take the digestate sample and avoid parts falling into the digestate. These incidents and their possible effects should be noted while performing a comparison with data from the control reactor.

Smaller errors involved in daily feeding procedures were that the barley bagasse, especially when sonicated, sticks to glassware. This resulted in less than the entire 87.5 mL feed added to the reactor every day as some of the mixture remained on the walls of the graduated cylinder. This error likely had no effect on overall measured trends as it was consistent daily. The only calculation this may affect is the energy production capacity per weight of substrate which was calculated from this experiment is likely slightly lower than actual. This error will remain with operation of the control reactor and will thus not affect the comparison of the pretreatment. To mitigate this challenge in future, it is recommended to not add the total required water before sonication of the bagasse but save some to wash the graduated cylinder used for the feed and ensure that all of the bagasse makes it into the reactor without adding too much water to change the intended proportions of the reactants.

Even after adjustments were made to the mixing apparatus within the reactor, much of the digestate did not experience proper mixing during the process. The stir rod was fitted with two fin assemblies, but the fins were too short to reach the outsides of the reactor. With a low rpm of 40-50, the digestate towards the outside of the reactor was not constantly stirred. To amend this, when the feed and NaOH was added to the reactor, the speed was increased for a few minutes to around 200 rpm to mix the new constituents. The effect of this mixing procedure on the bacterium in the reactor is unknown although since the speed increase was short in duration, there was likely no significant effect. The procedure will be continued with the introduction of feed and NaOH into the control reactor so the poor mixing remains constant between the treated and untreated trials.

An experimental error which may contribute to error in the magnitude of the results is the handling of the barley bagasse feedstock before use. The feedstock was dried and stored in a freezer then thawed before pretreatment. This was completed for all the feedstock; pretreated and unpretreated so would not affect the comparison between the reactors. The feedstock for the trial reactor was pretreated in two batches, not on a daily basis. The pretreated bagasse was stored in a freezer and then thawed before it was added to the reactor. This freezing-thawing process may have acted as an additional form of pretreatment, further stressing the cell walls of the barley bagasse.[24] This may have further increased the biogas output. If the unpretreated bagasse is also stored frozen and then thawed before feeding, this procedure should not affect the comparison between the results from the two reactors.

A final general source of error was introduced through a general lack of cleanliness in the laboratory. Gloves were not always worn by some working with the reactor or during characterization trials. If gloves were worn, they were almost always reused. Glassware was washed with generic dish soap and was not always given a final rinse with distilled water to limit contamination. There was general contamination in the laboratory, as the product of another experiment in the same laboratory was contaminated with bacteria from an anaerobic digester in near proximity. While this likely had little effect on the anaerobic digestion process itself, bacterial contamination easily could have affected some of the individual COD, nitrogen, and alkalinity tests.

While there were several sources of error, they will exist for both the pretreated trial reactor and the control reactor, affecting the results in the same capacity. The absolute magnitude of the biogas produced, the COD, alkalinity, or ammoniacal nitrogen content within the digestate, the comparison between the two reactors will be unaffected by the possible errors.

5.0 Conclusions

The largest conclusions on the effect of ultrasonic pretreatment of barley bagasse are forthcoming with a statistical, comparative analysis between the results from the control and pretreatment trial reactor. From the results of the trial reactor, it was concluded that the biogas collection method can significantly affect the anaerobic digestion process of barley bagasse, likely through ammoniacal inhibition of the process. While switching the collection procedure yielded an increased uncertainty in some results, it led to overall more consistent biogas composition, suggesting it was ultimately a better setup for the system. Based on the energy analysis of the pretreatment trial reactor, research is still required to determine under what parameters ultrasonic irradiation could be an energy-efficient pretreatment option to produce biogas from the anaerobic digestion of barley bagasse.

6.0 Recommendations

There are two sets of recommendations for this experiment; general recommendations for the operation of an anaerobic digester and conditional recommendations dependent on the results of the control reactor.

6.1 General Operational Recommendations:

The main operational recommendation is to run the reactors for a longer time period. Longer operation times allow the reactor to stabilize in interactions between bacteria in the inoculum and the feedstock. This additionally solidifies trends in characterization parameters over longer time periods and decreases variation in specific results. Another recommendation is to control the pH with the addition of sodium bicarbonate ($NaHCO_3$) instead of sodium hydroxide to allow it to auto-buffer. Sodium bicarbonate would better allow the reactor to self-regulate alkalinity as carbonate ions are naturally present in the acetogenic and methanogenic reactions whereas hydroxide ions are foreign to the system. Finally, the anaerobic digestion system can be optimized with regard to the daily feed rate of organics to the system.

6.2 Conditional Recommendations:

If the control reactor does not yield statistically different biogas volume or composition results from the pretreated reactor, the ultrasonic pretreatment was ineffective on barley bagasse, and other types of pretreatment should be explored. If there is a statistically-significant difference between the results of the pretreated and control reactor, the economic feasibility of the process should be determined through an optimization of sonication parameters. The goal of the optimization would be to minimize the energy used in sonication by changing the probe depth within the feedstock and the amount of feedstock to be sonicated at a time. The possibility of stirring the feedstock throughout the sonication process should be explored to determine if this affects the amount of disturbance to the cell walls or if there is equal distribution of the effects of sonication.[5] Previous studies suggest that ultrasonic waves may not be equally distributed throughout the substrate. The frequency and power of the ultrasound probe should be optimized to a level with minimum energy requirements and for a minimum time duration to achieve cell wall disturbance. A higher frequency of sonication would greatly increase the disturbance to the cell walls and likely allow for a lower power to be used to achieve similar or better results as the tested conditions.

Following optimization to make ultrasonic pretreatment a net energy positive process, the scale-up capability of the system should be determined. AMBEV has a daily production of 130-250 tons of barley bagasse waste product per day. This amounts to 7-14 million times the amount of feedstock used per day in the lab-scale reactor. A scale-up of this magnitude may require different feed rates and have different outcomes from the small reactor used in experiments. To determine the efficacy of this system on the industrial scale.

7.0 Future Work

The control reactor began operation in mid-April 2019. The reactor will operate under identical conditions to the trial reactor except the barley bagasse feedstock will not be pretreated with ultrasonic irradiation. The only difference between the operation of the two reactors is that the biogas production in the control reactor will be measured by the water displacement method for the entire 50-day trial. The same characterization procedures which were completed on the trial reactor will be completed for the control reactor.

In addition to operation and data collection of the control reactor, an initial characterization of the barley bagasse will be completed to determine the lipid, protein, and cellulose content. This information will help in comparison with anaerobic digestion of barley bagasse with other feedstocks and determine if the operating conditions of our reactor yielded better, worse or equivalent results to other studies of anaerobic digestion of barley bagasse.

Project partners at the University of Campinas will complete the project, including operation of the control reactor, characterization of the barley bagasse substrate, an analysis of volatile fatty acids and an analysis of the untreated and pretreated barley bagasse substrate using a scanning electron microscope (SEM analysis) or other imaging technique. An analysis of the volatile fatty acids will indicate when the rate of methanogenesis overtook that of acidogenesis. A SEM analysis would show the effect of the sonication pretreatment on the cell walls of the barley bagasse. If cell wall disruption of the pretreated bagasse is visible under the SEM or other imaging technique, an analysis could be used to predict the amount of sonication to increase biogas production. This may help to optimize the sonication parameters and minimize the energy needed in sonication of the barley bagasse.

Long-term future work is dependent on the results of the control reactor. Once data is collected for the characterization parameters from the control reactor, a statistical analysis should be completed to determine if the pretreated bagasse yielded different results from the control reactor. If the pretreated barley bagasse had a larger or faster production of biogas, there should be future work done to optimize the sonication pretreatment parameters. The optimal frequency, power, and time of sonication should be determined to have the greatest disruptive effect on the cell walls of the bagasse using the least energy to use the sonication probe. Optimized pretreatment parameters should then be employed in a full anaerobic digester trial to determine if the reactor can yield a net positive energy outcome with the pretreatment procedure. Finally, the scale-up applicability of the results should be explored.

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Appendix A: Raw Data for Gas Chromatography

	Day	H2 area	H2S area	O2 area	CH4 area	CO2 area	Total Area	H2 concentration (%)	H2S concentration (%)	O2 concentration (%)	CH4 concentration (%)	CO2 concentration (%)	Avg H2	Avg O2	Avg CH4	Avg CO2
10-Jan	1			6.993	457573	278130.5	735710	0	0	0	0.001	62.19	37.80	0	0.001	62.19	37.80
11-Jan	2	1023.8			503214.4	288086.3	792325	0.13	0.00	0.00	0.00	63.51	36.36				
11-Jan	2B			1466.4	469121.9	279085.4	749674	0.00	0.00	0.20	0.20	62.58	37.23	0.06	0.10	63.04	36.79
14-Jan	5				366853	307940.5	674794	0.00	0.00	0.00	0.00	54.37	45.63				
14-Jan	5B			1639.5	354047.7	304470.8	660158	0.00	0.00	0.25	0.25	53.63	46.12	0.00	0.12	54.00	45.88
15-Jan	6				289111.3	291679.8	580791	0.00	0.00	0.00	0.00	49.78	50.22				
15-Jan	6B			1338.9	293419	291096.2	585854	0.00	0.00	0.23	0.23	50.08	49.69	0.00	0.11	49.93	49.95
16-Jan	7				276080.5	284362.8	560443	0.00	0.00	0.00	0.00	49.26	50.74	0.00	0.00	49.26	50.74
17-Jan	8				206244.8	225690.8	431936	0.00	0.00	0.00	0.00	47.75	52.25	0.00	0.00	47.75	52.25
18-Jan	9				231083.9	217513.9	448598	0.00	0.00	0.00	0.00	51.51	48.49	0.00	0.00	51.51	48.49
21-Jan	12				272933.5	214349.4	487283	0.00	0.00	0.00	0.00	56.01	43.99	0.00	0.00		
	12B				251750	208233.3	459983	0.00	0.00	0.00	0.00	54.73	45.27	0.00	0.00	55.37	44.63
22-Jan	13				223662.2	183813.7	407476	0.00	0.00	0.00	0.00	54.89	45.11	0.00	0.00	54.89	45.11
23-Jan	14				210165.2	187928.6	398094	0.00	0.00	0.00	0.00	52.79	47.21	0.00	0.00	52.79	47.21
24-Jan	15			6889.6	119964.5	135816.6	262671	0.00	0.00	0.00	2.62	45.67	51.71	0.00	2.62	45.67	51.71
25-Jan	16			2498.7	146647.3	156559.9	305706	0.00	0.00	0.00	0.82	47.97	51.21	0.00	0.82	47.97	51.21
28-Jan	19			4093.5	145071.7	160298.6	309464	0.00	0.00	0.00	1.32	46.88	51.80		1.32	46.88	51.80
29-Jan	20			8933.6	88403.6	116360.6	213698	0.00	0.00	0.00	4.18	41.37	54.45	0.00	2.24		54.45
	20 b			1103.5	172321.9	191946.9	365372	0.00	0.00	0.00	0.30	47.16	52.53				
31-Jan	22			8934.8	62934.9	62788.8	134659	0.00	0.00	0.00	6.64	46.74	46.63		6.64	46.74	46.63
1-Feb	23	1159.7		3895	171587.7	132471.2	307954	0.15	0.00	0.00	1.26	55.72	43.02		1.26	55.72	43.02
4-Feb	26			8396.2	79742.1	69044.2	157183	0.00	0.00	0.00	5.34	50.73	43.93		5.34	50.73	43.93
5-Feb	27				272809.5	187007.5	459817	0.00	0.00	0.00	0.00	59.33	40.67		0.00	59.33	40.67
6-Feb	28				298393	190742.5	489136	0.00	#REF!		0.00	61.00	39.00		0.00	61.00	39.00
7-Feb	29	3319.4			263310.6	179561.7	442872	0.42	0.00	0.00	0.00	59.46	40.54		0.00	59.46	40.54
8-Feb	30				9043.4	7197.3	16241	0.00	0.00	0.00	0.00	55.68	44.32		0.00	55.68	44.32
11-Feb	33			28954.5	172910.6	88435.6	290301	0.00	0.00	0.00	9.97	59.56	30.46		9.97	59.56	30.46
12-Feb	34			2444.9	40824.7	21992.5	65262	0.00	0.00	0.00	3.75	62.55	33.70		3.75	62.55	33.70
13-Feb	35			2971	17606.2	11719.4	32297	0.00	0.00	0.00	9.20	54.51	36.29		9.20	54.51	36.29
14-Feb	36			1231.7	13338	11683.9	26254	0.00	0.00	0.00	4.69	50.80	44.50		4.69	50.80	44.50
15-Feb	37				293125.5	205448.3	498574	0.00	0.00	0.00	0.00	58.79	41.21		0.00	58.79	41.21
18-Feb	40				235637.3	198452.5	434090	0.00	0.00	0.00	0.00	54.28	45.72		0.00	54.28	45.72
19-Feb	41				307378	187624.9	495003	0.00	0.00	0.00	0.00	62.10	37.90		0.00	62.10	37.90
20-Feb	42				271509.5	158016.4	429526	0.00	0.00	0.00	0.00	63.21	36.79		0.00	63.21	36.79
21-Feb	43				272369.1	172667.4	445037	0.00	0.00	0.00	0.00	61.20	38.80		0.00	61.20	38.80
22-Feb	44				278873.4	140785.2	419659	0.00	0.00	0.00	0.00	66.45	33.55		0.00	66.45	33.55
25-Feb	47			29886	242119.4	154850.6	426856	0.00	0.00	0.00	7.00	56.72	36.28		7.00	56.72	36.28
27-Feb	49		26441.9	39739.2	297427.3	177113.1	514280	0.00	5.34	5.34	7.73	57.83	34.44		7.73	57.83	34.44
28-Feb	50		16528.8	43315.3	291072.2	185251.4	519639	0.00	3.85	3.85	8.34	56.01	35.65		8.34	56.01	35.65

Appendix B: Raw Data for Biogas Volume

Date	Day	Amount of Biogas in Collection Bag (mL)	Total Biogas Produced (mL)	Amount of Biogas Removed from Reactor (mL)	Total Biogas Produced (L)	Corrected Biogas Production (mL)
9-Jan	0					
10-Jan	1	1848	4268	600		1848
11-Jan	2			600		
12-Jan	**3**	184	386			
13-Jan	**4**	184	386			
14-Jan	5	184	386	600	0.386	184
15-Jan	6	292	892	600	1.278	292
16-Jan	7	207	807	600	2.085	207
17-Jan	8	184	784	600	2.869	184
18-Jan	9	169	769	600	3.638	169
21-Jan	12	141	741	600	4.379	141
22-Jan	13	237	837	600	5.216	237
23-Jan	14	179	779	600	5.995	179
24-Jan	15	181	781	600	6.776	181
25-Jan	16	323	923	600	7.699	323
28-Jan	19	127	727		8.426	127
29-Jan	20	n/a	770		9.196	Start water displacement collection method
30-Jan	21		140		9.336	140
31-Jan	22		130		9.466	130
1-Feb	23		55		9.521	55
5-Feb	27		100		9.621	100
6-Feb	28		125		9.746	125
	29		180		9.926	180
	33		50		9.976	50
	34		82		10.058	82
	35		135		10.193	135
	36		290		10.483	290
	37		110		10.593	110
	40		123		10.71633	123
	42		82		10.79833	82
	43		155		10.95333	155
	44		55		11.00833	55
	45		160		11.16833	160
	46		160		11.32833	160
	47		160		11.48833	160
	49		200		11.68833	200
	50		260		11.94833	260

Appendix C: Raw Data for Solids

Species	Sample #	P0: Crucible Weight (g)	Initial Sample Weight (g)	P1: Final Sample Weight (g)	Total Solids (g/kg)	Avg TS (g/kg)	Volatile Solids: Final Weight (g)	Fixed Total Solids (g/gTS)	Average Fixed Solids (g/gTS)	FS (g/kg TS)	Volatile Solids Calc. (g/gTS)	VS (g/kg TS)
Initial Inoculum	1	38.6995	2.0128	38.869	84.211		38.8170	0.6932				
	2	38.111	1.9761	38.3008	96.0478	90.3065	38.1405	0.1554				
	3	38.048	2.0119	38.2304	90.6606		38.0689	0.1146	0.1350	12.1918	0.8650	78.1146
Initial Bagasse	4	34.5023	2.0288	36.4875	978.509		35.0043	0.2529				
	5	29.7635	1.9913	31.7173	981.168	980.678	29.9935	0.1177				
	6	45.3946	2.0179	47.3769	982.358		46.1450	0.3786	0.2497	244.8887	0.7503	735.7897
Day 0 mix	1	30.8585	1.3584	30.9378	58.3775		30.8565	-0.0252				
	2	34.4791	1.4161	34.5572	55.1515	58.076	34.4858	0.0858				
	3	35.0461	1.9259	35.163	60.6989		35.0637	0.1506	0.1182	6.8629	0.8818	51.2130
Day 1 mix	1	54.2135	1.9214	54.3005	45.2795		54.2128	-0.0080				
	2	60.7581	1.9329	60.8448	44.8549	44.6186	60.7624	0.0496				
	3	23.3297	2.3421	23.4321	43.7214		23.3412	0.1123	0.0810	3.6119	0.9190	41.0067
Day 2 mix	1	34.5015	1.9357	34.6306	66.6942							
	2	29.763	2.0116	29.8869	61.5928	66.0859						
	3	33.4424	2.048	33.5857	69.9707					0.0000		
Day 5 mix	1	40.0621	2.0371	40.2438	89.1954		40.0728	0.0589				
	2	30.5608	1.9445	30.7292	86.6032	85.0235	30.5756	0.0879				
	3	38.0887	2.2139	38.2642	79.2719		38.1144	0.1464	0.0977	8.3100	0.9023	76.7135
Day 8	1	39.2536	2.1123	39.425	81.1438		39.2787	0.1464				
	2	39.6192	2.0253	39.7887	83.6913	82.3234	39.6997	0.4749				
	3	38.3131	2.0515	38.4816	82.135		38.3883	0.4463	0.4606	37.9188	0.5394	44.40452
Day 12	1	40.4494	2.0522	40.5986	72.7025		40.5187	0.464477				
	2	34.5667	2.4539	34.7531	75.9607	74.1432	34.6497	0.4453				
	3	35.5594	1.9982	35.7068	73.7664		35.5837	0.1649	0.45488	33.7261	0.545122	40.41708
Day 15	1	51.8156	2.0369	51.9389	60.5332		51.8495	0.274939				
	2	30.839	2.0747	30.9769	66.4674		30.8639	0.1806				
	3	37.0502	2.0348	37.1932	70.2772	65.7593	37.0714	0.1483	0.1644	10.8114	0.835591	54.94787
Day 19	1	45.3968	2.1439	45.5602	76.2162		45.4244	0.168911				
	2	40.8619	2.0622	41.009	71.3316		40.905	0.2930				
	3	40.4537	2.0303	40.6053	74.6688	74.0722	40.4819	0.1860	0.1775	13.1451	0.822537	60.92711
Day 22	1	38.2885	2.0351	38.4257	67.4168		38.3137	0.183673				
	2	37.2537	2.3781	37.3992	61.1833		37.2816	0.1918				
	3	38.0536	2.2376	38.2188	73.8291	67.4764	38.0767	0.1398	0.1618	10.9144	0.838248	56.56197
Day 26	1	30.8368	2.0065	30.9725	67.6302		30.8599	0.170228				
	2	45.3973	2.0131	45.5222	62.0436		45.4225	0.2018				
	3	40.4556	2.135	40.5927	64.2155	64.6298	40.4807	0.1831	0.1767	11.4171	0.823347	53.2127
Day 29	1	38.0933	2.0204	38.2366	70.9265		38.1177	0.170272				
	2	38.7638	2.0000	38.8984	67.3		38.7907	0.1999				
	3	40.1745	1.9731	40.3026	64.9232	67.7166	40.1993	0.1936	0.1819	12.3200	0.818065	55.39654
Day 33	1	40.3811	2.0029	40.5026	60.662		40.4099	0.237037				
	2	40.1336	2.0250	40.2634	64.0988		40.154	0.1572				
	3	35.0475	2.0722	35.1793	63.6039	62.7882	35.0785	0.2352	0.2361	14.8256	0.763879	47.96262
Day 36	1	40.862	2.0277	40.9711	53.8048		40.8885	0.242896				
	2	34.4791	1.9755	34.5799	51.0251		34.5034	0.2411				
	3	40.4571	2.0423	40.5703	55.4277	53.4192	40.4857	0.2527	0.2478	13.2358	0.752227	40.18334
Day 40	1	37.0276	2.052	37.1794	73.9766		37.0631	0.23386				
	2	30.8307	2.3174	31.0111	77.8459		30.8699	0.2173				
	3	30.5605	2.2739	30.7226	71.2872	74.3699	30.5986	0.2350	0.2345	17.4360	0.76555	56.93386
Day 43	1	38.3119	2.5935	38.4467	51.9761		38.3491	0.275964				
	2	35.0431	1.9016	35.1554	59.0555		35.0708	0.2467				
	3	37.2557	1.9945	37.3803	62.4718	57.8345	37.2859	0.2424	0.2592	14.9890	0.74083	42.84551
Day 47	1	40.3799	2.1724	40.5079	58.921		40.4136	0.263281				
	2	41.1034	2.7065	41.3017	73.2681		41.1505	0.2375				
	3	42.835	2.0813	42.9745	67.0254	66.4048	42.8673	0.2315	0.2474	16.4293	0.752589	49.97553
Day 50	1	38.0912	2.0919	38.1916	47.9946		38.1204	0.290837				
	2	36.7295	2.1544	36.8237	43.7245		36.7537	0.2569				
	3	41.632	2.2085	41.7474	52.2527	47.9906	41.6638	0.2756	0.2832	13.5909	0.7168	34.39966

Appendix D: Raw Data for Alkalinity and pH

Day	Test	V sample (mL)	V H2SO4 (mL)	pH Final	Alk (mg CaCO3/L)	Corrected Alk (mg CaCO3/L)	Average Alkalinity (mg CaCO3/L)	pH
Inoculum	1	10	0.9	4.7	89.3	893.4		
	2	10	1.2	4.45	119.1	1191.2	1042.3	
	3	10			0.0	0.0		
Bagasse	1	10			0.0	0.0		
	2	10			0.0	0.0		
	3	10			0.0	0.0		
	1	10	0.5	4.63	49.6	496.3		
	2	10	0.5	4.45	49.6	496.3		
0	3	10	0.6	4.7	59.6	595.6	529.4	6.5
	1	10	1.9	4.69	188.6	1886.1		
	2	10	2.1	4.52	208.5	2084.6		
5	3	10	1.9	4.7	188.6	1886.1	1952.2	6.5
	1	10	4.5	4.79	446.7	4467.0		
	2	10	4	4.77	397.1	3970.7		
8	3	10	4.7	4.78	466.6	4665.5	4367.7	
	1	10	7.8	4.61	774.3	7742.8		
	2	10	8.1	4.6	804.1	8040.6		
12	3	10	8.2	4.59	814.0	8139.9	7974.4	7.5
	1	10	8.1	4.55	804.1	8040.6		
	2	10	7.8	4.58	774.3	7742.8		
15	3	10	8.1	4.61	804.1	8040.6	7941.3	7.72
	1	10	8.5	4.55	843.8	8437.7		
	2	10	8.4	4.6	833.8	8338.4		
19	3	10	8.5	4.57	843.8	8437.7	8404.6	7.5
	1	10	1.2	4.49	119.1	1191.2		
	2	10	0.9	4.7	89.3	893.4		
22	3	10	1	4.55	99.3	992.7	1025.8	7.9
	1	10	1.2	4.4	119.1	1191.2		
	2	10	1.2	4.42	119.1	1191.2		
26	3	10	1.1	4.31	109.2	1091.9	1158.1	7.7
	1	10	1	4.52	99.3	992.7		
	2	10	0.8	4.62	79.4	794.1		
29	3	10	1	4.7	99.3	992.7	926.5	7.5
	1	10	0.6	4.61	59.6	595.6		
	2	10	0.7	4.59	69.5	694.9		
36	3	10	0.6	4.58	59.6	595.6	628.7	7.9
	1	10	0.5	4.5	49.6	496.3		
	2	10	0.5	4.42	49.6	496.3		
40	3	10	0.5	4.47	49.6	496.3	496.3	
	1	10	0.8	4.63	79.4	794.1		
	2	10	0.9	4.51	89.3	893.4		
43	3	10	0.8	4.55	79.4	794.1	827.2	7.76
	1	10	0.8	4.47	79.4	794.1		
	2	10	0.8	4.59	79.4	794.1		
47	3	10	0.8	4.62	79.4	794.1	794.1	
	1	10	0.9	4.45	89.3	893.4		
	2	10	0.9	4.41	89.3	893.4		
50	3	10	0.8	4.63	79.4	794.1	860.3	

Appendix E: Raw Data for Ammoniacal Nitrogen

Day	Sample#	V sample (mL)	A (mL)	B (mL)	N-NH3 (mg/L)	Corrected N-NH3 (mg/L)	Average N-NH3 (mg/L)
Inoculum Initial	1	5	0.2	0	11.1	111.2	111
	2	5	0.2	0	11.1	111.2	
	3	5	0.2	0	11.1	111.2	
Bagasse Initial	1	5	0.3	0	16.7	166.8	185.30
	2	5	0.4	0	22.2	222.4	
	3	5	0.3	0	16.7	166.8	
Day 0	1	5	0.2	0	11.1	111.2	129.71
	2	5	0.3	0	16.7	166.8	
	3	5	0.2	0	11.1	111.2	
Day 5	1	5	1.5	0	83.4	833.8	889.43
	2	5	1.7	0	94.5	945.0	
	3	5	1.6	0	88.9	889.4	
Day 8	1	5	1.8	0	100.1	1000.6	1093.26
	2	5	2	0	111.2	1111.8	
	3	5	2.1	0	116.7	1167.4	
Day 12	1	5	2.3	0	127.9	1278.6	1222.96
	2	5	2.2	0	122.3	1223.0	
	3	5	2.1	0	116.7	1167.4	
Day 15	1	5	2.7	0	150.1	1500.9	1445.32
	2	5	2.5	0	139.0	1389.7	
	3	5	2.6	0	144.5	1445.3	
Day 19	1	5	3.1	0	172.3	1723.3	1741.796
	2	5	3	0	166.8	1667.7	
	3	5	3.3	0	183.4	1834.4	
Day 22	1	5	0.5	0	27.8	277.9	240.8867
	2	5	0.4	0	22.2	222.4	
	3	5	0.4	0	22.2	222.4	
Day 26	1	5	0.3	0	16.7	166.8	148.238
	2	5	0.3	0	16.7	166.8	
	3	5	0.2	0	11.1	111.2	
Day 29	1	5	0.4	0	22.2	222.4	203.8272
	2	5	0.3	0	16.7	166.8	
	3	5	0.4	0	22.2	222.4	
Day 36	1	5	0.4	0	22.2	222.4	203.8272
	2	5	0.3	0	16.7	166.8	
	3	5	0.4	0	22.2	222.4	
Day 40	1	5	0.3	0	16.7	166.8	129.7082
	2	5	0.2	0	11.1	111.2	
	3	5	0.2	0	11.1	111.2	
Day 43	1	5	0.2	0	11.1	111.2	111.1785
	2	5	0.2	0	11.1	111.2	
	3	5	0.2	0	11.1	111.2	
Day 47	1	5	0.2	0	11.1	111.2	111.1785
	2	5	0.2	0	11.1	111.2	
	3	5	0.2	0	11.1	111.2	
Day 50	1	5	0.4	0	22.2	222.4	231.6218
	2	5	0.4	0	22.2	222.4	
	3	5	0.45	0	25.0	250.2	

Appendix F: Raw Data for COD

Day	Sample	Absorbance	COD (mg O2/L)	Dilution	Corrected COD (mg O2/kg)	Average (mg O2/L)	Average (g O2/L)
	A	0.017	39.6		396.0		
	B	0.024	55.9		559.1		
Inoculum	C	0.026	60.6		605.7	520.3	0.5202997
	A						
	B						
Bagasse	C						
	A	0.148	346.8	1	3467.6		
	B	0.145	339.7	1	3397.4		
0	C	0.146	342.1	1	3420.8	3428.6	3.42859
	A	0.306	717.0	1	7169.6		
	B	0.289	677.1	1	6771.3		
5	C	0.341	799.0	1	7989.6	7310.2	7.31016
	A	0.284	665.4	5	33270.6		
	B	0.281	658.4	5	32919.2		
8	C	0.289	677.1	5	33856.4	33348.7	33.3487
	A	0.176	412.4	10	41236.8		
	B	0.169	396.0	10	39596.7		
12	C	0.146	342.1	10	34207.8	38347.1	38.3471
	A	0.376	881.0	5	44048.4		
	B	0.366	857.5	5	42876.9		
15	C	0.339	794.3	5	39713.9	42213.1	42.21305
	A	0.132	309.3	10	30927.6		
	B	0.127	297.6	10	29756.1		
19	C	0.127	297.6	10	29756.1	30146.6	30.1466
	A	0.139	325.7	10	32567.7		
	B	0.141	330.4	10	33036.3		
22	C	0.137	321.0	10	32099.1	32567.7	32.5677
	A	0.147	344.4	10	34442.1		
	B	0.136	318.6	10	31864.8		
26	C	0.13	304.6	10	30459.0	32255.3	32.2553
	A	0.092	215.6	10	21555.6		
	B	0.095	222.6	10	22258.5		
29	C	0.1	234.3	10	23430.0	22414.7	22.4147
	A	0.055	128.9	10	12886.5		
	B	0.058	135.9	10	13589.4		
33	C	0.052	121.8	10	12183.6	12886.5	12.8865
	A	0.124	290.5	10	29053.2		
	B	0.126	295.2	10	29521.8		
36	C	0.123	288.2	10	28818.9	29131.3	29.1313
	A	0.122	285.8	10	28584.6		
	B	0.118	276.5	10	27647.4		
40	C	0.142	332.7	10	33270.6	29834.2	29.8342
	A	0.079	185.1	10	18509.7		
	B	0.072	168.7	10	16869.6		
43	C	0.086	201.5	10	20149.8	18509.7	18.5097
	A	0.101	236.6	10	23664.3		
	B	0.092	215.6	10	21555.6		
47	C	0.095	222.6	10	22258.5	22492.8	22.4928
	A	0.033	77.3	10	7731.9		
	B	0.027	63.3	10	6326.1		
50	C	0.029	67.9	10	6794.7	6950.9	6.9509

Appendix G: Raw Data for Energy Production Calculations

Day	Volume Biogas (mL)	Volume Biogas (m^3)	CH4 composition (%)	CH4 composition (1.0)	Volume of CH4 (mL)	Volume of CH4 (m^3)	Energy (MJ): Low HV	Energy (kJ)	Cummulative Energy (kJ)	Energy (kWh)	Cummulative Energy (kWh)
3	184	0.000184	54	0.54	99.36	0.000099	0.00319	3.19	3.2	0.00089	0.0009
4	184	0.000184	54	0.54	99.36	0.000099	0.00319	3.19	6.4	0.00089	0.0018
5	184	0.000184	54	0.54	99.36	0.000099	0.00319	3.19	9.6	0.00089	0.0027
6	292	0.000292	49.93	0.4993	145.7956	0.000146	0.00469	4.69	14.3	0.00130	0.0040
7	207	0.000207	49.26	0.4926	101.9682	0.000102	0.00328	3.28	17.5	0.00091	0.0049
8	184	0.000184	47.75	0.4775	87.86	0.000088	0.00282	2.82	20.4	0.00078	0.0057
9	169	0.000169	51.51	0.5151	87.0519	0.000087	0.00280	2.80	23.2	0.00078	0.0064
12	141	0.000141	55.37	0.5537	78.0717	0.000078	0.00251	2.51	25.7	0.00070	0.0071
13	237	0.000237	54.89	0.5489	130.0893	0.000130	0.00418	4.18	29.9	0.00116	0.0083
14	179	0.000179	52.79	0.5279	94.4941	0.000094	0.00304	3.04	32.9	0.00084	0.0091
15	181	0.000181	45.67	0.4567	82.6627	0.000083	0.00266	2.66	35.6	0.00074	0.0099
16	323	0.000323	47.97	0.4797	154.9431	0.000155	0.00498	4.98	40.5	0.00138	0.0113
19	127	0.000127	46.88	0.4688	59.5376	0.000060	0.00191	1.91	42.5	0.00053	0.0118
20	170	0.000170	47.16	0.4716	80.172	0.000080	0.00258	2.58	45.0	0.00072	0.0125
22	130	0.000130	46.74	0.4674	60.762	0.000061	0.00195	1.95	47.0	0.00054	0.0131
23	55	0.000055	55.72	0.5572	30.646	0.000031	0.00099	0.99	48.0	0.00027	0.0133
24	200	0.000200	50.73	0.5073	101.46	0.000101	0.00326	3.26	51.2	0.00091	0.0142
25	200	0.000200	50.73	0.5073	101.46	0.000101	0.00326	3.26	54.5	0.00091	0.0151
26	200	0.000200	50.73	0.5073	101.46	0.000101	0.00326	3.26	57.8	0.00091	0.0160
27	100	0.000100	59.33	0.5933	59.33	0.000059	0.00191	1.91	59.7	0.00053	0.0166
28	125	0.000125	61	0.61	76.25	0.000076	0.00245	2.45	62.1	0.00068	0.0173
29	180	0.000180	59.46	0.5946	107.028	0.000107	0.00344	3.44	65.6	0.00096	0.0182
33	50	0.000050	59.56	0.5956	29.78	0.000030	0.00096	0.96	66.5	0.00027	0.0185
34	82	0.000082	62.55	0.6255	51.291	0.000051	0.00165	1.65	68.2	0.00046	0.0189
35	135	0.000135	54.51	0.5451	73.5885	0.000074	0.00237	2.37	70.5	0.00066	0.0196
36	290	0.000290	50.48	0.5048	146.392	0.000146	0.00471	4.71	75.2	0.00131	0.0209
37	110	0.000110	58.79	0.5879	64.669	0.000065	0.00208	2.08	77.3	0.00058	0.0215
38	123	0.000123	54.28	0.5428	66.94533333	0.000067	0.00215	2.15	79.5	0.00060	0.0221
39	123	0.000123	54.28	0.5428	66.94533333	0.000067	0.00215	2.15	81.6	0.00060	0.0227
40	123	0.000123	54.28	0.5428	66.94533333	0.000067	0.00215	2.15	83.8	0.00060	0.0233
42	82	0.000082	63.21	0.6321	51.8322	0.000052	0.00167	1.67	85.4	0.00046	0.0237
43	155	0.000155	61.2	0.612	94.86	0.000095	0.00305	3.05	88.5	0.00085	0.0246
44	55	0.000055	66.45	0.6645	36.5475	0.000037	0.00117	1.17	89.7	0.00033	0.0249
45	160	0.000160	56.72	0.5672	90.752	0.000091	0.00292	2.92	92.6	0.00081	0.0257
46	160	0.000160	56.72	0.5672	90.752	0.000091	0.00292	2.92	95.5	0.00081	0.0265
47	160	0.000160	56.72	0.5672	90.752	0.000091	0.00292	2.92	98.4	0.00081	0.0273
49	200	0.000200	57.83	0.5783	115.66	0.000116	0.00372	3.72	102.1	0.00103	0.0284
50	260	0.000260	56.01	0.5601	145.626	0.000146	0.00468	4.68	106.8	0.00130	0.0297

Appendix H: Temperature Data during Sonication

			1 x			2x			3x			4x	
Room temp °C:		24.3	temp °C			temp °C			temp °C			temp °C	
Beaker	Time Started	temp °C	7.5 min	15 min	after waiting	7.5 min	15 min	after waiting	7.5 min	15 min	after waiting	7.5 min	15 min
1	10:56		43.3	55	43	63	70	31	53	59	40	48	68
2	11:11		34	55	38	52	63	31	56	61		54	53
3	15:05	24	41	53	41	48	52	46	46	51	48	49	54
4	15:22	25	39	42	38	47	51	43	46	56	46	51	52

Because of temperature rises during sonication, beakers of 400 mL of the bagasse-water mix were sonicated for 15 minutes at a time and then allowed to cool for 15 minutes. The temperature was monitored every 7.5 minutes during sonication to ensure that it did not get too high throughout the process. Temperature data was only available from the sonication of the initial feed loads. Similar temperature rises were noted during the sonication of the daily feed loads.

Appendix I: COD Standard Curve

The following data was collected from prepared standard samples with known COD concentrations. The absrobances of the known concentrations were plotted and the slope of this line was used to calculate the COD of the experimental samples.

Calibration Curve		
COD (mg/L)	*Absorbance*	*Average Absorbance*
25	0.033	
25	0.027	
25	0.036	0.032
100	0.05	
100	0.05	
100	0.046	0.048666667
500	0.25	
500	0.243	
500	0.249	0.247333333
700	0.324	
700	0.319	
700	0.321	0.321333333
900	0.35	
900	0.333	
900	0.336	0.3345