# Batch Reactor Pretreatment of Brewer's Spent Grain Enables Bioenergy Production from Continuous Anaerobic Digestion

A Major Qualifying Project Report

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# Introduction

The world needs green energy sources because the current petroleum dependence is not a sustainable practice. Current popular energy strategies include the combustion of natural gas and use of petroleum-based products. These methods contribute largely to the buildup of greenhouse gases in the atmosphere, contributing to the global climate crisis. Research is currently being done to identify potential renewable energy sources that can replace natural gas and petroleum.

Brazil is one of the world's leaders in producing and utilizing renewable energy sources, such as biogas[1]. Appealing options for sources of biogas are the residues leftover from the large agricultural industry in Brazil. Using residues from industrial agriculture in renewable energy production, can reduce carbon emissions and waste production. Biogas can be used to create chemical, thermal, and mechanical energy; it is a combination of hydrogen, methane, and carbon dioxide[1, 2]. Biogas is naturally produced through anaerobic digestion, when microorganisms break down organic matter without the presence of oxygen[3].

Legal decisions and updates have incentivized the commercialization of biogas in Brazil. The National Oil Agency established the first set of quality control standards for biogas derived energy and encouraged the use of energy for heating homes and powering vehicles. The National Oil Agency is joined by many other federal agencies in promoting the usage of biogas[1, 4, 5]. 2017 was the first year that biogas appeared as a major presence on Brazil's Ten-Year Energy Expansion Plan. In 2016, 165 operational biogas plants produced about 5,300 GWh of energy, where 1 in three plants used anaerobic digestion as a method of producing biogas. The recent development and promotion for the use of biogas derived energy demonstrates the potential for anaerobic digestion as a mechanism for ubiquitous energy production in Brazil[3].

The inherent barriers to the commercialization of biogas as a renewable energy source are the slow production rate and the low yields of biogas per amount of agricultural waste substrate. Researchers have suggested pretreating substrates as a method of mitigating the barriers to commercialization. Pretreatment has the potential to degrade the structure of the cell walls so that they are more available for the microorganisms to digest[6-8]. Research on pretreatment methods focuses on optimizing methods to produce biogas to determine if there is an economic way to convert agricultural waste into biogas via anaerobic digestion.

This experiment focused on using a batch reactor approach to the pretreatment of barley bagasse to produce biogas. Barley bagasse is a byproduct of the beer brewing process, also known as brewer's spent grain. The raw material for this project was donated by the largest brewer in South Americas Ambev, a branch of AB InBev. Ambev currently uses their 130-250 tons/day of spent grain as animal feed[9]. Brazil is among the world's highest beer producers. By using their spent grain as feedstock to produce biogas, Brazilian brewers can establish more sustainable and lower cost processes.

# Background

## Anaerobic Digestion

Anaerobic digestion is the process microorganisms undertake when they digest organic material without the presence of oxygen[3, 7, 10]. In this work, an anaerobic digestion reactor was used to convert barley bagasse into biogas. Biogas can be used as a source of renewable energy; it is a combination of methane, carbon dioxide, and hydrogen[2]. Anaerobic digestion occurs in four stages, but the general process can be seen below[3].

The first stage of anaerobic digestion is hydrolysis: where lipids, proteins, and polysaccharides are converted into fatty acids, amino acids, and monosaccharides[3, 11]. Hydrolysis refers to the splitting of a large organic molecule into a simpler molecule in the presence of water. This first step is typically the rate limiting step during anaerobic digestion[12]. The rate of the hydrolysis step is dependent on both the organic material. The speed of hydrolysis can be impeded if the cellulose in the organic material is highly crystallized or intertwined with lignin[13].

The products of hydrolysis continue through acidogenesis[2, 11]. Acidogenic bacteria convert the monomer units of hydrolysis to alcohols and ketones. The products of acidogenesis include carbon dioxide, hydrogen, and acetic, butyric, and propionic acid. The products of acidogenesis are often not found in the final products of anaerobic digestion because they are converted to different compounds in later stages[13]. Acidogenesis is considered the fast step of anerobic digestion. This step is only limited by the rate of the previous step, which is the slowest stage of anerobic digestion[12]. The products of acidogenesis proceed to acetogenesis.

Acetogenesis, the third step of anaerobic digestion, refers to the process which converts the alcohols and ketones of acidogenesis into hydrogen, carbon dioxide, and acetate ions[3].

Acetogenesis is only thermodynamically favorable during anaerobic digestion, not at standard temperature and pressure[14]. Researchers have found that the acetate ions produced in acetogenesis are the main source of methane in the final stage of anaerobic digestion[15]. Decreasing the presence of hydrogen in this step can increase the final production of methane[3].

The final step of anaerobic digestion in methanogenesis. During methanogenesis, acetate ions are converted to methane and carbon dioxide by acetoclastic methanogenic archaea. This process is responsible for 70% of the methane produced by anaerobic digestion. Methane may also be produced from hydrogen going through hydrogenotrophic methanogenic archaea[3].

## Biogas

Anaerobic digestion is a favorable method of procuring biogas. Anaerobic digestion can produce biogas in a single process, with no external drying or processing necessary[3]. The four stages can occur at both mesophilic (30°C) and thermophilic (50°C) conditions, which are accepted as the optimal conditions for microbial reactions. Hydrolysis presents the largest difficulty in using anaerobic digestion to obtain biogas[2]. Hydrolysis requires external energy to be applied to the system so that the cell walls of the organic material can be broken down: because of this requirement, hydrolysis is considered to be the rate limiting step in the kinetic modeling of anaerobic digestion[6].

Biogas is a mixture of other gases that is valuable for its properties as a renewable energy source. Biogas is composed of 50-70% methane and carbon dioxide: its other components depend on the original organic material and can be hydrogen sulfide, nitrogen, and hydrogen[2, 14]. Biogas is viable for its uses in thermal, chemical, and electrical energy. Currently, biogas is currently used in some power plants that provide both heat and power[2, 16]. Biogas can be used as a direct energy source when combusted. Technologies that utilize biogas are currently in early design stages[11].

Biogas production from anaerobic digestion can be affected by the reaction temperature, retention time, and properties of the organic feedstock. Cellulose, fats, and proteins can undergo the anaerobic digestion process to produce biogas[16]. The characteristics of the organic feedstock can affect the final composition of the biogas. Fat produces the highest biogas yield, methane yield, and methane composition: cellulose preforms the worst by these metrics. Lignin cannot be used as an organic feedstock for anaerobic digestion. In this work, biogas is a renewable energy source because it is derived from barley bagasse, a byproduct of the brewing industry. The digestate remaining from the process can be used as fertilizer. For the agricultural industry, anaerobic digestion to produce biogas can be a viable waste management strategy and sustainability achievement method.

Energy reports for biogas typically come in units of energy per amount of substrate or volatile solid feed. An experimental biogas yield can be calculated using the ideal gas law with heating data for the combustible components, usually methane and hydrogen. Experimental biogas yields are usually poor because of non-optimized conditions leading to incomplete digestion.

## Barley Bagasse

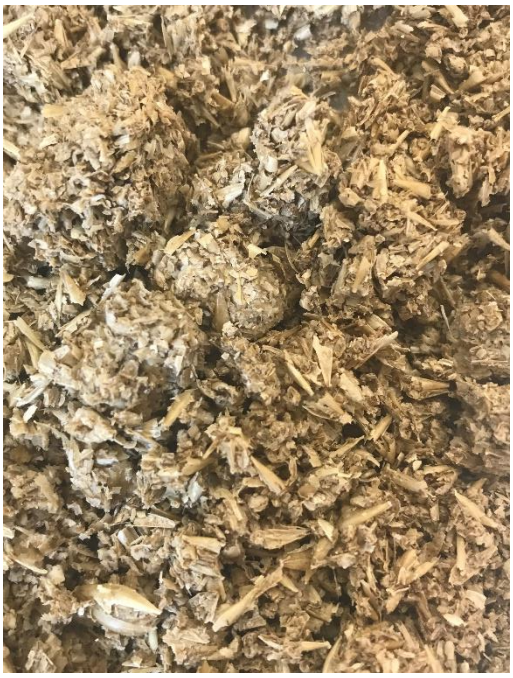

*Figure 1: Barley Bagasse*

Barley bagasse, also known as brewers, spent grain, is a byproduct of the brewing process[17](Figure 1). Barley bagasse is primarily composed of the malt and grain remaining from the mashing and lautering stages of the brewing process[9, 12]. Barley bagasse can represent as much as 85% of the byproducts of brewing. Barley bagasse's substate is approximately 70% fiber and 20% protein, classifying it as a type of lignocellulosic biomass[9, 18, 19]. The fibrous portion of barley bagasse is comprised of cellulose, hemicellulose, and lignin. Bagasse with high proportions of lignin are not optimal candidates for anaerobic digestion: they require high amounts of energy to break down cell walls during hydrolysis[18, 20]. Bagasse with a greater proportion of cellulose and hemicellulose is preferred for anerobic digestion. Currently, barley bagasse is sold as animal feed, a low value application. Barley bagasse is not a viable candidate for combustion because of the presence of Nitrogen and Sulfur in its chemical composition. If combusted, barley bagasse will product NOx and Sox, which pose serious environmental threats[9]. Barley bagasse is a good organic material for anaerobic digestion because of its low cost and high availability[12].

## Anaerobic Digestion of Barley Bagasse

Research labs and pilot plants have investigated the possibility of using anaerobic digestion to convert barley bagasse to biogas[21, 22]. One lab studied the production of biogas from barley bagasse over a 2-week period, looking to study the biogas composition, biomass degradation, and

kinetics. The experimented resulted in 3.5L of biogas that was over 66% methane[21]. From the initial substrate, 60% of the cellulose and 40% of the lignin was degraded. A pilot plant in Austria attended to product biogas from barley bagasse on the industrial scale. The theoretical methane yield of the plant was 98$Nm^3$ per ton of substrate, but the actual yield was 75 $Nm^3$. The energy from the biogas created from barley bagasse recovers 60% of the electrical and thermal energy required to brew, bottle, and store beer[21]. Experiments such as these support the continued research into the anerobic digestion of barley bagasse[13].

## Characterization

### *Gas Chromatograph*

Gas Chromatography (GC) is one of the techniques used to analyze the composition of biogas produced by anaerobic digestion[23]. GC identifies the type and relative quantity of compounds in gas based on their polarities. The technique uses an inert gas carrier, called the mobile phase, and a polymer with a high boiling point, known as the stationary phase, to line the capillary sampling tubes.

Compounds are identified based on their interaction with the stationary phase. Compounds with polarities similar to the stationary phase have stronger interactions, resulting in slower retention times through the column. Compounds with polarities that differ significantly from the stationary phase experience weaker interactions and faster retention times. In a GC, components of a gas can separate based on different factors: vapor pressure, column temperature, flow rate, amount of sample, and length of column[23].

The quality of results will be affected by adjusting the influencing factors in a GC. Columns with higher vapor pressure, temperatures, and carrier gas flow rates will see shorter retention times for samples. Shorter retention times can result in peaks that are difficult to differentiate. Inversely, a long column can increase separation and retention time of components. Exceedingly long retention times can lead to broad peaks, making results difficult to analyze. GC results should appear to be symmetric, clearly defined peaks. Injecting too much sample can lead to the tailing and deforming of peaks[2, 21].

A biogas profile from a GC reflects the composition and quantities of different chemical species present. Methane is the most prevalent component of biogas, usually found in quantities of 50-80%[2, 14]. Carbon dioxide is the other major component of biogas, representing a significant peak on most GC results. Hydrogen gas may also be present in GC results, if any remains from acidogenesis. Oxygen should not be present in biogas GC analysis because anerobic digestion requires the absence of oxygen, if it is noted on results it is most likely due to flawed sampling methods.

***pH***

During anaerobic digestion, microbes are very sensitive to changes in pH due to the formation of fatty acids during acidogenesis and the inoculum[13]. The pH of the system naturally increases as the fatty acids are produced and then consumed in later stages of anaerobic digestion. The early stages of anaerobic digestion have a low pH: but when carbon dioxide reacts with hydroxide ions to form carbonate ions the pH becomes more neutral as the system enters an auto-buffering cycle[13]. When methane production is less than the rate of fatty acid formation, the pH of the system and biogas production rate decrease while carbon dioxide formation increases[13]. The

optimal pH for the anaerobic digestion process is between 6.8 and 7.4, but the four stages are sensitive to pH change[24]. The optimal pH for acidogenesis occurs between 5.5 and 6.5. Methanogenic is optimal at a pH of 7.0 but can withstand a range of 6.5-8.2[25].

***Alkalinity***

In the analysis of anerobic digesting, alkalinity is used to measure the ability of the digestate to neutralize acids in a method more sensitive than pH testing[26]. Alkalinity is measured in units of mg$CaCO_3$/L of digestate. Alkalinity is measured on a linear scale, where pH is measured on a logarithmic scale, making it easier to detect small environmental changes. Alkalinity results refer to the presence of specific ions in solution: $CO_3^{2-}$,$HCO_3^-$, $OH^-$[13, 26]. These ions are significant because they are involved in the auto-buffering cycle, which increases methane production[26, 27]. The optimal alkalinity for anaerobic digestion is 2,000-4,000 mg $CaCO_3$/L[3, 13].

***Solids***

The solid content of the anaerobic digestion system can expose insights into the optimum organic content for biogas production. Solid analysis can directly measure the amount of total and fixed solids in the system, from this we can indirectly measure the amount of volatile solids. Total solids include suspended solids and those dissolved in the digestate[26].

Fixed solids are the mass that remains after ignition of volatile solids. Volatile solids indicate the organic matter in the digestate: the more organic matter in the system increases the production of biogas because there is more material available for the bacteria to process. The ignition involved in the analysis of volatile solids decomposes inorganic salts present in the digestate. In digestates with high volumes of inorganic salts, volatile solid and chemical oxygen demand analysis should be combined to determine the amount of organic matter available for

microbes. Barley bagasse does not have many inorganic salts; therefore a volatile solid analysis is sufficient as an indicator of organics in the digestate[26].

***Chemical Oxygen Demand***

Chemical Oxygen Demand (COD) is a measure of the organic content in the digestate. When microbes degrade organic matter, the organic presence in the reactor decreases which results as a decrease in COD[7]. The initial COD of the reactor depends on the feed, but the COD should decrease overtime. The COD should stabilize after time with slightly increases associated with the addition of daily organic feeds to the reactor[3, 11, 22]. Theoretically, anaerobic digestion should produce 0.35 $m^3$ of biogas per kg COD; this quantity is usually not reached because biogas production is also dependent on temperature, pressure, and other environmental conditions. COD analysis measures the amount of oxidant that reacts with the organic material. In this experiment, the oxidant used to measure COD was $Cr_2O_7^{2-}$, which was reduced by organics to $Cr_3^{+11, 26}$.

***Ammoniacal Nitrogen***

During anaerobic digestion, the main source of ammonia comes from proteins in the cell walls of the organic material[7]. The initial amount of ammonia is dependent on the organic matter. Anaerobic digestion can see the presence of some nitrites and nitrates, but nitrogen is most commonly in ammoniacal form[11, 28]. The presence of nitrogen in anaerobic digestion is essential for the bacteria in the inoculum to grow. However, an overabundance of nitrogen is toxic to the bacteria. When the bacteria is flooded with nitrogen, methanogenesis cannot occur which causes insufficient utilization of carbon sources[25]. Ammonical nitrogen has fewer toxic effects at mesophilic conditions, where it is easier for the bacteria to adjust to environmental changes. Ammonical nitrogen is measured as the carbon to nitrogen ratio (C/N) in the reactor. A C/N of

under 30/1 is recommended to prevent harmful conditions for bacteria. The most common C/N ratio is 25, with most studies ranging between 20 and 30[25]. Tolerable levels of ammonia vary by feed, temperature, pressure, and environment. Some anaerobic digesters can tolerate up to 6,000 mg/L $N\text{-}NH_3$, however it is usually maintained under 3,000 mg/L[26].

# Materials & Methods:

The following section dictates the specific materials and methods used in the analysis of this project. The continuous reactor was operated for a 35-day period at the BIOTAR laboratory at the University of Campinas. At the end of the 35-day period, worked stopped because of Brazilian Carnival, which coincided with the departure of the MQP students from the laboratory: any samples from the final days of the 35-day period were intended to be analyzed at a later date, and the results communicated for this MQP report. Unfortunately, due to the coronavirus outbreak and preventative measures, all laboratories at the University of Campinas were closed for the remainder of the semester. This report shares the results that were analyzed and the methods that were consistently used in the operation of this project.

## Raw Materials

Barley bagasse and mesophilic inoculum for this project were provided by the AMBEV Brewing Company, from Jaguariúna, São Paulo, Brazil. Before this project, the barley bagasse was oven dried at 105℃ for 8 hours. It was then frozen at -18℃ in a plastic bag before use.

## Batch Reactor Conditions

The batch reactor was a 3.5 L glass vessel. The reactor was temperature controlled by a thermostatic water bath at 25℃ ± 2℃. The batch reactor was mixed by a two finned agitator, connected a shaft in the center of the reactor. The agitator was used for two minutes daily and set at 150 rpm: however, the digestate was often too thick to allow the agitator to move freely and manual mixing was necessary to aid the mechanical agitator. All liquid samples were taken from

a sampling port at the top of the reactor. The gas phase samples were taken from a rubber septum attached to the biogas collection bag. 40% (1L) of the reactor was left empty, as headspace for the biogas produced by the reactor: 60% (2.5L) of the reactor was filled with the digestate. The digestate of the batch reactor was comprised of 750 mL mesophilic inoculum (758 g), 500 mL water (497g), and 840 mL barley bagasse (183g). The batch reactor was filled three times to sustain feeding the continuous reactor. The first two were done in the previously mentioned proportion. The final fill was done in one and a half the proportion of the previously mentioned mixture, to sustain the reactor system for the last two weeks of the project.

## Continuous Reactor

The continuous reactor was a 6.8 L vessel. The reactor was temperature controlled by a thermostatic bath at 35℃±2℃. The thermostatic bath was fed to a heating jacket that heated the lowest 2L of the reactor. To aid in heat transfer in the digestate, an insulating cloth was kept around the reactor except during sampling and feeding times. The contents of the reactor were stirred by a two finned agitator connected to a shaft in the center of the reactor. All liquid samples were taken from an opening at the top of the reactor; all gas phase samples were connected from a septum located between the reactor and a biogas collection bag. A mylar biogas collection bag was connected to the reactor by a system of rubber tubing. Like the batch reactor, 40% (2.72L) of the reactor volume was left empty to leave space for the biogas and 60% (4.08L) was left for the digestate. The digestate of the continuous reactor consisted of two mixtures of barley bagasse, mesophilic inoculum, and water and a daily feeding of 180 mL of the bath reactor digestate. The first mixture was comprised of 1.4 L of mesophilic inoculum (1542 g), 0.97 L of water (1497g), and 1.6 L of barley bagasse (560g). The second mixture was comprised of 0.59 L of mesophilic

inoculum (592 g), 0.354 L of water (341 g), and 0.236 L of barley bagasse (50.85 g). Each day 180 mL of the reactor digestate was removed before 180 mL of the batch reactor digestate was added.

## Characterization Methods

The biogas and reactor contents were characterized and measured daily. These characterizations and measurements included those which quantified the amount of moisture, biogas compositions, and presence of chemical factors in the reactors. Figure 2 below shows the sampling scheme used to analyze the stability metrics of the continuous reactor.

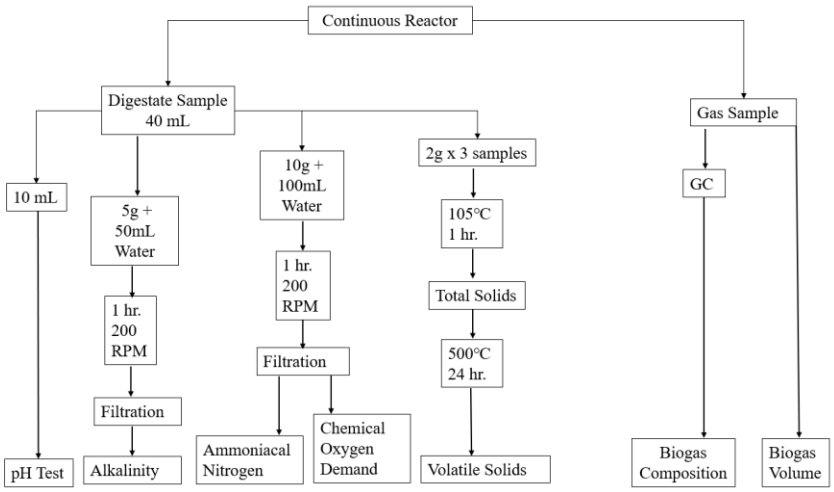


*Figure 2: Sampling and Analytics Used on the Continuous Reactor*

### *Gas Chromatography*

The chemical components of the biogas were analyzed via Gas Chromatography, in a GC 2014 Shimadzu Corporation. The chromatograph used a packed column and thermal activity detected at 200°C, ShinCarbon ST 50/80 mesh. The column temperature increased in intervals of 5°C/min, from 50°C to 180°C. The mobile phase was nitrogen gas at 5bar, being pumped at 35 mL/min. The

complete analysis took 35 minutes. A 5mL sample of biogas from the reactor was analyzed in the gas chromatography each day for the presence of methane, carbon dioxide, hydrogen, and oxygen.

***Biogas Volume***

The volume of biogas produced by the reactor was measured both in a mylar collection bag and the reactor. Ten 60mL syringes of biogas were removed from the reactor each day to avoid a pressure build up inside of the reactor. Syringes were used to remove biogas from the collection bag. The number of syringes was used to record the daily volume of biogas produced by the reactor.

***Energy Production***

The potential energy production of the continuous reactor was calculated using the data from the biogas volume and gas chromatography efforts. The ideal gas law and lower heating value for methane were used in these calculations. Equations 1a-1c describe the process used to calculate the energy produced by the continuous reactor daily.

| | | |
|---|---|---|
| Volume of Methane Produced | $V_{CH_4} = V_{biogas} * x_{CH_4}$ | 1a |
| Moles of Methane Produced | $n_{CH_4} = \frac{PV_{CH_4}}{RT}$ | 1b |
| Energy Produced | $E_p = n_{CH_4} * \frac{HV_L}{MW}$ | 1c |

***pH Monitoring***

The microorganisms involved in anaerobic digestion are very sensitive to changes in pH. The optimal pH for anaerobic digestion is between 6.5-8.5. The pH was monitored daily and adjusted

with NaOH to ensure the reactors were operating within the optimal range. A 10mL sample of the digestate was taken and measured for pH. Drops of 6N NaOH were added to the sample until a pH of 8.0 was achieved. The volume of NaOH added was then scaled up to adjust the entire reactor. The scale up calculation was tabulated using Equation 2.

$$\frac{20\ mL}{Reactor\ Volume\ (mL)} * \frac{\#\ of\ Drops\ NaOH}{25\ drops\frac{NaOH}{mL}} = mL\ NaOH \quad (2)$$

***Total Solids***

The digestate of both reactors was tested three times a week for total, volatile, and fixed solid contents. The methods of testing solid content are consistent those documented in: NREL Determination of Total Solids and Ash in Algal Biomass: 2.00g of each sample was taken and tested in triplicate, the average results were used to represent the results of the digestate overall. Equation 3 and 4 below describe the process of calculating the total and volatile solid content.

$$Total\ Solids = \frac{mass\ of\ dried\ digestate\ sample}{mass\ of\ wet\ digestate\ sample} * 100 \quad (3)$$

$$Volatile\ Solids = \left(1 - \frac{mass\ of\ muffle\ dried\ sample}{nass\ of\ dried\ digestate\ sample}\right) * 100 \quad (4)$$

## Characterization Methods

Samples of the digestate were taken three times a week for characterization. Forty milliliter samples were stored in a -18°C freezer. All characterization methods shared a preparation technique. Five grams of digestate was diluted in 50 mL of deionized water and shaken for 1 hour

at 200 rpm. The samples were then gravity filtered through cotton to remove any suspended large solids. Then, the samples were vacuum filtered through a Buchner funnel to remove any particulates for the solution.

### *Alkalinity*

The reactor was tested for alkalinity via 10 mL portions of the prepared samples. The samples were titrated while being agitated with a magnetic stir bar. The pH of the sample was monitored with a pH meter. The sample was titrated with 0.02 M H2SO4 until the pH fell within the range of 4.3-4.7. The alkalinity was then calculated with Equation 5 below.

$$Alkalinity = \frac{M_{H_2SO_4} * V_{H_2SO_4} * 5000}{10\ mL} \quad (5)$$

### *Chemical Oxygen Demand*

The chemical oxygen demand of the samples was measured using digestive and catalytic solutions. A solution was created by adding 2.5 mL of the prepared sample, 1.5 mL of a digestive solution, and 3.5 mL of a catalytic solution. The solutions were then heated and mixed. The absorbance of each sample was measured at 610 nm in a Hatch spectrometer. The chemical oxygen demand was calculated using Equation 6 below.

$$COD = absorbance\ wavelength * standard\ slope \quad (6$$

### *Ammoniacal Nitrogen*

The ammoniacal nitrogen concentration in the reactor was measured every three days using a distillation and titration-based procedure. Multiple solutions were used in the process of measuring

the ammoniacal nitrogen concentration: among these was a borate buffer solution made from sodium hydroxide and hydrated sodium tetraborate. A boric acid absorbent solution received the distillated samples: the solution was by dissolving 20g of H3BO3 in a liter of water and then mixing an indicator solution (methyl red and methylene blue in 95% isopropanol). The samples were distillated with 0.5M NaOH to maintain a pH of 9.5, preventing hydrolysis of nitrogen compounds in the sample. The distillate and borate buffer solution were titrated with 0.02 M H2SO4 until the solution became faintly pink.

# Results & Discussion

## Batch Reactor Behavior

This project required both a batch and continuous reactor for the anaerobic digestion of barley bagasse. The batch reactor was used to feed the continuous reactor. Results for the characterization of the batch reactor can be found in Appendix 1A-1G.

## Continuous Reactor Behavior

The continuous reactor was operated as expected according to the methods included in Section 3. There were no unexpected changes in operation of the reactor. The following pages detail the characterization of the biogas and digestate of the continuous reactor. Data for the characterization of the continuous reactor can be found in Appendix 2A-2G.

### *Biogas Production*

Figure 3 displays the daily biogas production measured from the continuous reactor. Over a 35-day period, the reactor produced over 20L of biogas, averaging 745 mL of biogas produced per day. The composition of the biogas production is explored in the following section. The 20 L of biogas in 35 days was achieved by 13.1 L of digestate, this includes the original mix of the continuous reactor and the 180 mL feedings necessary to sustain the reactor. The stabilization of volumetric production after day 15 suggests that the reactor began a quasi-steady state period.

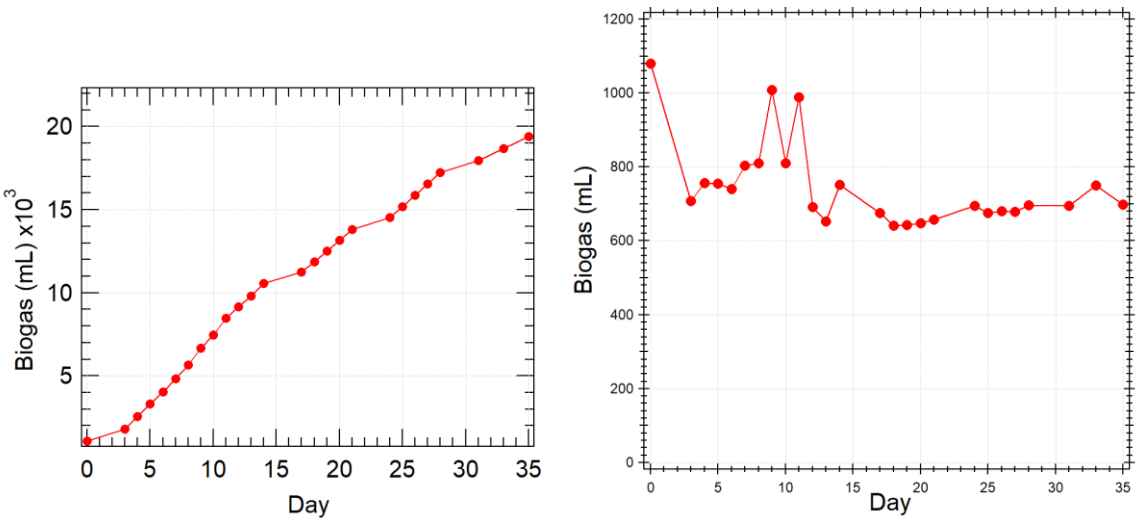


*Figure 3: Biogas Production Daily (Left) and Accumulative (Right)*

The amount of biogas produced is difficult to compare to literature because of dependence on substrate material, environmental conditions, reactor size, and collection methods. A comparison could be made to a former Major Qualifying Project completed in the BIOTAR Laboratory, focused on the anaerobic digestion of barley bagasse, which had been put through an ultrasound pretreatment process. The former project reported an average daily production of biogas of 163 mL, a much smaller value than the reactor discussed in this report. The former project used a water displacement method of collection, rather than a bag, for the last half of the project. The differences in biogas collection methods could be responsible for the major difference in average daily volume of biogas produced by the continuous reactor.

Measuring the volume of biogas produced by the reactor was a delicate task. The biogas collection bag was connected to the reactor via a system of rubber tubes. To measure the volume of biogas in the bag, the bag had to be disconnected from the rubber tubes which risked gas escaping to the atmosphere. Over the course of this project, four individuals measured the amount of biogas from the reactor, because there was no standard operating procedure for biogas production measurement, we can expect some deviations due to methods.

### *Biogas Composition*

Figure 4 displays the composition of biogas produced by the continuous reactor daily, as reported by a gas chromatograph. The composition saw an initial peak of methane composition as high as 75% in the first few days, however that peak started to slowly decrease. The amounts of methane and carbon dioxide were almost equivalent on day 23 but the proportion of methane began to increase after that. The biogas averaged a composition of 58% methane. The trend observed in this figure is consistent with those for similar materials. According to Professor Forester-Carneiro, materials that are highly biodegradable tend to product high amounts of methane in the beginning stages of anaerobic digestion, but these high amounts are not sustained through the lifetime of the reactor.

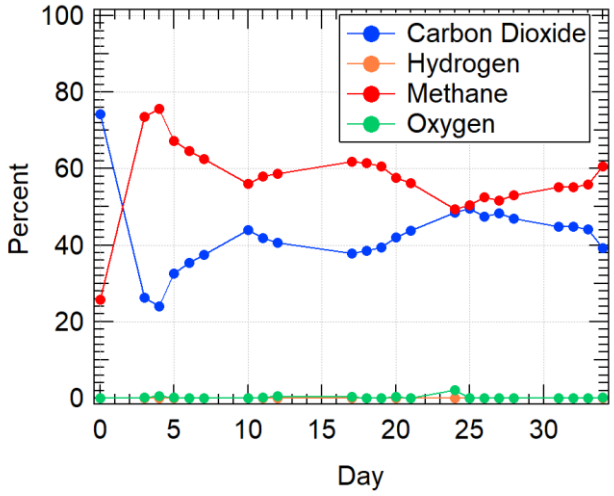


*Figure 4:Biogas Composition for the Continuous Reactor for 35 days*

The proportions of methane seen in this project are analogous to those with similar materials. In the former MQP on the anaerobic digestion of barley bagasse, the average percentage of methane was 61%[29]. The average methane composition from the anaerobic digestion of untreated corn stover was 59%[30]. The methane composition from the anaerobic digestion of untreated food waste yielded an average methane composition of 58%, the same as what is represented in this report[31].

On day 23, there was a minor presence of oxygen in the biogas composition. On that day, oxygen comprised less than 2% of the biogas sample. The presence of oxygen is not recorded in any other data point on Figure 4. The oxygen on day 23 is present due to error in operation of the reactor.

### *Ammoniacal Nitrogen*

Figure 5 represents the quantity of ammoniacal nitrogen present in the continuous reactor throughout the project. Over the course of a typical anaerobic digestion experiment, the amount of ammoniacal nitrogen is expected to increase as cell walls break down and as the expired bacteria began to accumulate in the reactor. In this reactor, the ammoniacal nitrogen content increases steadily and then decreases immediately following a refill of the batch reactor. This trend is to be expected in this project because a refill of the batch reactor implies the presence of cells walls that have not yet been degraded and bacteria that have not yet expired. The only outlier in this trend is the sudden decrease in ammoniacal nitrogen content at day 15. The magnitude of these quantities is consistent with those for similar anaerobic digestion experiments[31].

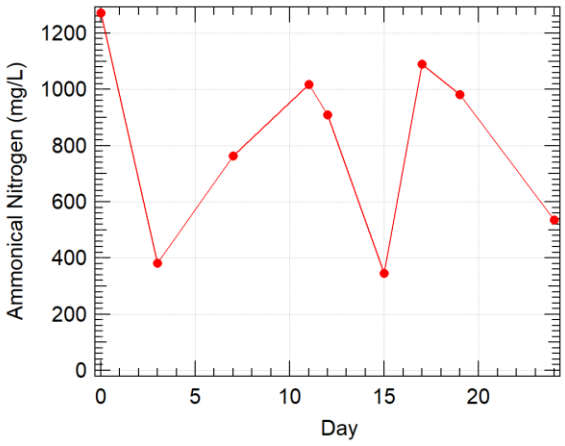


*Figure 5: Ammoniacal Nitrogen test results for the continuous reactor for 24 days*

### *Reactor pH and Alkalinity*

Figure 6 delineates the changes in pH over the 35-day period of the project. After day 11, the pH stabilized between 7.3 and 7.5. This was the first sign that the reactor had entered quasi-steady state. The stabilization of pH indicates that the bacteria in the digestate had begun the auto buffering cycle of anaerobic digestion, people to withstand and counterbalance changes in the pH of the system. This trend is also noticeable in the alkalinity results for the continuous reactor.

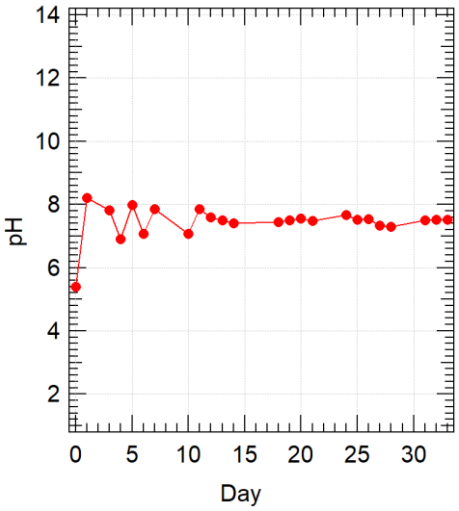


*Figure 6: pH results for the Continuous Reactor for 35 Days*

Figure 7 represents changes in the alkalinity of the digestate over the first 24 days of the 35-day period. Alkalinity is the ability of the digestate to withstand changes in the pH and ion balances in the reactor. After day 11, the alkalinity value appears to start to stabilize. This trend cannot be properly confirmed without the final 11 days of data, but the alkalinity values should follow the same patterns as the pH data. The magnitude of the alkalinity values is similar to other literature on anaerobic digestion.

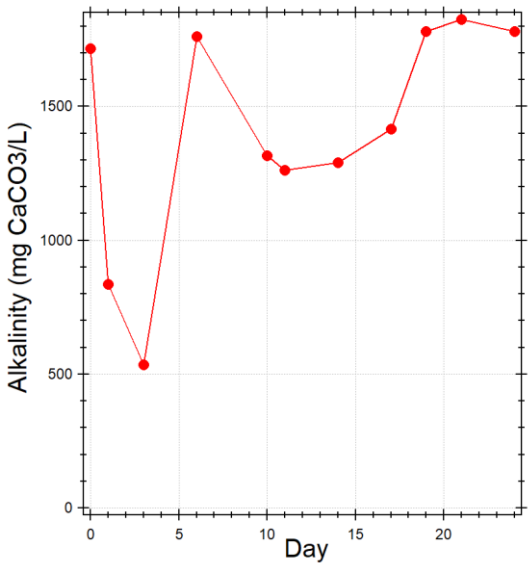


*Figure 7: Alkalinity Levels in the Continuous Reactor for 24 days*

### *Solids and Chemical Oxygen Demand*

Figure 8 represents the total solids testing of the reactor over the first 27 days of the 35-day period. The total solids represent the organic matter yet to be digested by the bacteria of the digestate. The fixed solids represent inorganic salts that are indigestible by the bacteria. The difference in those two amounts in the volatile solids present in the digestate: the amount of organic matter that could be digested but has not yet. In a typical anaerobic digestion experiment, there would be a steady downward trend in the total solids in the reactor. The peaks in this graph are directly correlated with days when the batch reactor was refilled. It is logical that the total solids would increase when a fresh batch was added because it means nearly fresh barley is being added to the continuous reactor. The quantities in this figure are considered consistent for *wet* anaerobic digestion (where the total solids should be between 8 % and 20% when the reactor is started).

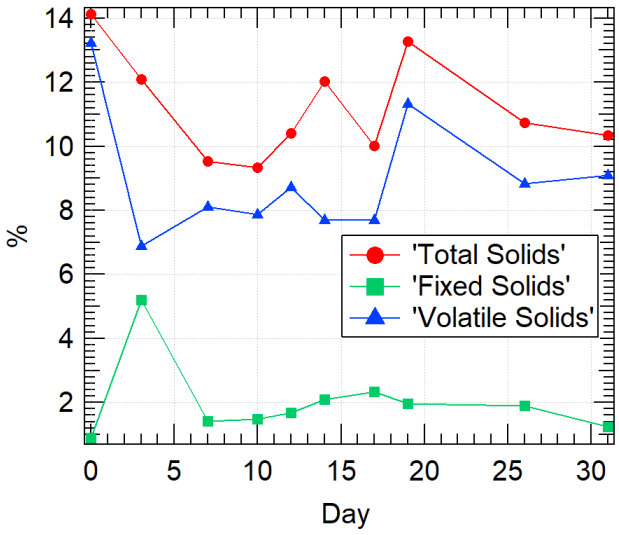


*Figure 8: Total, Volatile, and Fixed Solids Results for the Continuous Reactor for 32 Days*

Figure 9 shows the chemical oxygen demand data for the first 27 days of the 35-day period. Similar to the total solids data, there are decreases following refills of the batch reactor: this indicates that there was an increase in the organic matter present in the reactor that decreased as the bacteria began to thrive in the digestate. Although chemical oxygen demand tests were done every few days, as opposed to total solids which was tested daily, we can see that the two related quantities follow the same general trends. The magnitude of these values is consistent with previous MQPs and literature[29, 32].

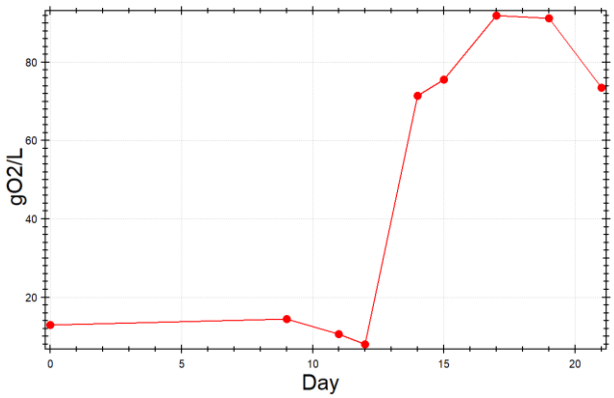


*Figure 9: Chemical Oxygen Demand results for 27 days*

### *Energy Production Potential*

A total of 0.00000035 MJ of energy was produced by the continuous reactor (Figure 10). Calculations for this value are included in Appendix 3. This value was obtained by assuming the biogas behaved as an ideal gas, all the methane was combustible, and using the lower heating value of methane. This value is most likely less than the total energy the reactor was able to produce because the 50-day experiment was cut down to only 30-days. There are some discrepancies in the collection and measurement of the biogas volume that are discussed in a future section. This value does not consider the energy expended in heating and stirring the reactor. With enough information on the operation of the reactor, the energy expenditure can be calculated and subtracted from the energy production to obtain a net energy gained by the system. The amount of energy produced by anaerobic digestion is often reported in terms of mass of the raw material: in this case it would be 0.0063 MJ/kg barley bagasse.

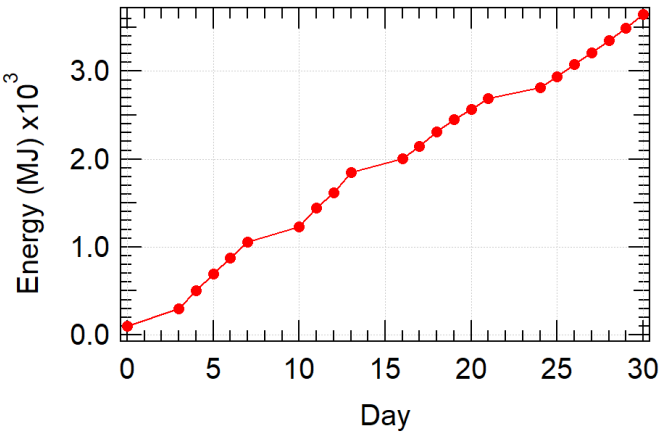


*Figure 10: Accumulative Energy Production*

The average brewery expends 200 MJ of energy producing 100 Liters of beer. The standard size for a bottle of beer is 355 mL[33]. These statistics reveal the continuous reactor produced enough energy to create 53% of one bottle of beer. A six-pack of beer would require 4.2 kg of barley bagasse to power via this pretreatment method for anaerobic digestion. This value can be brought into context by considering that the average brewing process creates 20 kg of barley bagasse for every 100 L of beer[33]. Through these values, it can be concluded that this pretreatment method for anaerobic digestion can use 60% of the barley

bagasse produced from one standard bottle of beer to supply the energy necessary to produce a six-pack of beer. Alternatively stated, the waste from one beer can be used to supply enough energy to produce 10 beers.

### *Mass Balance*

A mass balance was conducted on the continuous reactor to evaluate how much carbon from the brewers spent grain resulted in methane in the biogas. The carbon from both the initial loading of the continuous reactor and the daily feeds from the batch reactor. The mass balance found that 0.8% of the carbon in the continuous reactor was digested into methane. Presumably, the other 99.2% of the carbon became organic content in the liquid phase of the reactor. Calculations for the mass balance can be found in Appendix 4.

# Conclusions & Recommendations

The primary conclusion for this study lies in the energetic potential of this pretreatment method. This small-scale trial showed promising results as a renewable energy source for the brewing industry. Using barley bagasse as a renewable energy source would represent a large move toward a more sustainable process for the brewing industry. Future work should hold the digestate in the batch reactor for fixed periods of time, instead of refilling when the reactor is empty, to control for digestion in the pretreatment step. After the operation of the batch reactor has been standardized, I recommend work be done to optimize the conditions in each reactor.

Future work should focus on varying the parameters of this project to observe changes to the production of methane. The temperatures and pressures of the batch and continuous reactor should be systematically varied to select optimal environmental conditions. Changing the proportions of water in the original loading of both reactors change the mass balance results, further work should explore the effect this variable has on the methane production rate.

I recommend a technoeconomic analysis be done on the system to examine the costs associated with the industrial scale up of this system. This analysis should consider any energetic costs associated with the operation of both reactors. This analysis should also account for any financial gain possible by selling the digestate as fertilizer. A technoeconomic analysis is essential in the scale-up and industrialization of this process.

# Acknowledgements

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# Appendix 1-A Batch Reactor Biogas Volume Data

| Data | Dia | Amonstra | Reactor | Sacola | Total | Accumulated |
|---|---|---|---|---|---|---|
| 11-Jan | 0 | A | 600 | 190.2 | 790.2 | 790.2 |
| 13-Jan | 5 | A | 600 | 410 | 1010 | 1800.2 |
| 14-Jan | 6 | A | 600 | 105 | 705 | 2505.2 |
| 15-Jan | 7 | A | 600 | 88 | 688 | 3193.2 |
| 16-Jan | 8 | A | 600 | 103 | 703 | 3896.2 |
| 17-Jan | 9 | A | 600 | 158 | 758 | 4654.2 |
| 20-Jan | 12 | A | 600 | 90 | 690 | 5344.2 |
| 21-Jan | 13 | A | 600 | 88 | 688 | 6032.2 |
| 22-Jan | 14 | A | 600 | 85 | 685 | 6717.2 |
| 23-Jan | 15 | A | 600 | 358 | 958 | 7675.2 |
| 24-Jan | 16 | A | 600 | 108 | 708 | 8383.2 |
| 27-Jan | 18 | A | 600 | 98 | 698 | 9081.2 |
| 28-Jan | 19 | A | 600 | 112 | 712 | 9793.2 |
| 29-Jan | 19 | A | 600 | 45 | 645 | 10438.2 |
| 30-Jan | 20 | A | 600 | 92 | 692 | 11130.2 |
| 31-Jan | 21 | A | 600 | 71 | 671 | 11801.2 |
| 3-Feb | 26 | A | 600 | 111 | 711 | 12512.2 |
| 4-Feb | 27 | A | 600 | 53 | 653 | 13165.2 |
| 5-Feb | 28 | A | 600 | 47.5 | 647.5 | 13812.7 |
| 6-Feb | 29 | A | 600 | 215 | 815 | 14627.7 |
| 7-Feb | 30 | A | 600 | 90 | 690 | 15317.7 |
| 10-Feb | 33 | A | 600 | 47.5 | 647.5 | 15965.2 |
| 11-Feb | 34 | A | 600 | 55 | 655 | 16620.2 |
| 12-Feb | 35 | A | 600 | 85 | 685 | 17305.2 |
| 13-Feb | 36 | A | 600 | 50 | 650 | 17955.2 |
| 14-Feb | 37 | A | 600 | 55 | 655 | 18610.2 |
| 17-Feb | 40 | A | 600 | 72 | 672 | 19282.2 |
| 19-Feb | 42 | A | 600 | 95 | 695 | 19977.2 |
| 21-Feb | 44 | A | 600 | 64 | 664 | 20641.2 |

# Appendix 1-B Batch Reactor Biogas Composition Data

| | Dia | Area H2 | Area O2 | Area CH4 | Area CO2 | Total Area | %H2 | %O2 | %CH4 | %CO2 |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 0 | 1573.4 | 488082.4 | 192315.9 | 681971.7 | 0 | 0.230713 | 71.5693 | 28.19998 |
| | 5 | 0 | 2389.6 | 329405.5 | 193225.5 | 525020.6 | 0 | 0.455144 | 62.74144 | 36.80341 |
| | 6 | 0 | 9999.9 | 119534.9 | 78124.5 | 207659.3 | 0 | 4.815532 | 57.56299 | 37.62148 |
| | 7 | 0 | 7895.1 | 113107.7 | 106626.7 | 227629.5 | 0 | 3.468399 | 49.68939 | 46.84222 |
| | 8 | 0 | 18664.7 | 140026.9 | 73436.2 | 232127.8 | 0 | 8.0407 | 60.32319 | 31.63611 |
| | 9 | 0 | 0 | 17713.8 | 124281.5 | 141995.3 | 0 | 0 | 12.47492 | 87.52508 |
| | 12 | 0 | 1430.7 | 24960.5 | 125581 | 151972.2 | 0 | 0.941422 | 16.42439 | 82.63419 |
| | 13 | 0 | 26907.9 | 51293.3 | 9312 | 87513.2 | 0 | 0 | 58.61207 | 10.64068 |
| | 14 | 0 | 6902.7 | 18018.8 | 53504.2 | 78425.7 | 0 | 0 | 22.97563 | 68.22279 |
| | 15 | 0 | 5196 | 31454.5 | 42040.3 | 78690.8 | 0 | 6.603059 | 39.97227 | 53.42467 |
| *New Feed | 16 | 1794.9 | 1631.7 | 497256.7 | 299782.8 | 800466.1 | 0.224232 | 0.203844 | 62.12089 | 37.45103 |
| | 19 | 0 | 22882 | 329597.6 | 265948.8 | 618428.4 | 0 | 3.700024 | 53.296 | 43.00398 |
| | 20 | 0 | 5233 | 108446.3 | 147396.9 | 261076.2 | 0 | 2.004396 | 41.53818 | 56.45743 |
| | 21 | 0 | 37365.6 | 27925 | 109069 | 174359.6 | 0 | 0 | 16.01575 | 62.55405 |
| | 22 | 0 | 0 | 161553.4 | 181767.2 | 343320.6 | 0 | 0 | 47.05613 | 52.94387 |
| | 23 | 0 | 9187.2 | 66307 | 106754.8 | 182249 | 0 | 5.041015 | 36.38264 | 58.57634 |
| | 26 | 0 | 8577.9 | 9809.9 | 88526.2 | 106914 | 0 | 8.023178 | 9.175506 | 82.80132 |
| | 27 | 0 | 18077.8 | 18800.2 | 130473.6 | 167351.6 | 0 | 10.80229 | 11.23395 | 77.96376 |
| | 28 | 0 | 31278.8 | 81129.6 | 153056.8 | 265465.2 | 0 | 11.78264 | 30.56129 | 57.65607 |
| *fresh feed | 29 | 0 | 2446.2 | 27832.7 | 144101.4 | 174380.3 | 0 | 1.402796 | 15.96092 | 82.63628 |
| | 33 | 17183.5 | 0 | 441419.3 | 295003.7 | 753606.5 | 2.280169 | 0 | 58.57424 | 39.14559 |
| | 34 | 0 | 1402.6 | 39400.1 | 298417 | 339219.7 | 0 | 0.413478 | 11.61492 | 87.9716 |
| | 35 | 0 | 35741.5 | 138716.9 | 138716.9 | 313175.3 | 0 | 11.41262 | 44.29369 | 44.29369 |
| | 36 | 0 | 6110 | 102851.1 | 176217.3 | 285178.4 | 0 | 2.142519 | 36.06553 | 61.79195 |
| | 37 | 0 | 11095 | 21498.5 | 86383.4 | 118976.9 | 0 | 9.32534 | 18.06947 | 72.60519 |
| | 40 | 0 | 43113 | 61319 | 140018.1 | 244450.1 | 0 | 17.63673 | 25.08447 | 57.27881 |
| | 42 | 0 | 1234 | 144964.8 | 213027 | 359225.8 | 0 | 0.343517 | 40.35479 | 59.3017 |
| | 43 | 0 | 0 | 19219.9 | 42720.4 | 61940.3 | 0 | 0 | 31.02972 | 68.97028 |
| | 44 | 0 | 0 | 176217.3 | 102851.1 | 279068.4 | 0 | 0 | 63.14484 | 36.85516 |

# Appendix 1-C Batch Reactor Total & Volatile Solids Data

| | Dia | ST Media | SV Media | SFT Media |
|---|---|---|---|---|
| | 0 | 19.01345 | 17.66368 | 1.349776 |
| 9-Jan | 1 | 12.46748 | 11.48272 | 0.984764 |
| 13-Jan | 5 | 12.20175 | 11.0925 | 1.10925 |
| 17-Jan | 8 | 9.469697 | 8.047896 | 1.421801 |
| 20-Jan | 12 | 12.12121 | 9.264069 | 2.857143 |
| 24-Jan | 15 | 10.01439 | 6.982801 | 3.03159 |
| 23-Jan | 15 | 10.01439 | 6.982801 | 3.03159 |
| 27-Jan | 18 | 11.21977 | 9.901772 | 1.318001 |
| 29-Jan | 21 | 11.94255 | 10.46598 | 1.476569 |
| 31-Jan | 23 | 11.56894 | 9.143647 | 2.425297 |
| 3-Feb | 29 | 11.11171 | 8.525307 | 2.586399 |
| 5-Feb | 33 | 12.73951 | 10.72123 | 2.018282 |
| 12-Feb | 35 | 10.79847 | 9.439447 | 1.359027 |
| 17-Feb | 40 | 11.09085 | 9.443942 | 1.646909 |

## Appendix 1-D Batch Reactor Alkalinity Data

| Dia | mg CaCO3/L |
|---|---|
| 0 | 136.215 |
| 5 | 590.265 |
| 12 | 1107.882 |
| 15 | 2088.63 |
| 18 | 835.452 |
| 21 | 1407.555 |
| 26 | 2470.032 |
| 28 | 1907.01 |
| 30 | 227.025 |
| 33 | 426.807 |

# Appendix 1-E Batch Reactor pH Data

| Dia | Amostra | pH | pH Correction |
|---|---|---|---|
| 5 | 1 | 6.96 | N/A |
| 7 | 1 | 6.7 | 7.39 |
| 8 | 1 | 7.02 | N/A |
| 11 | 1 | 7.65 | |
| 12 | 1 | 7.9 | 8.45 |
| 13 | 1 | 8.3 | 8.54 |
| 14 | 1 | 9.3 | N/A |
| 15 | 1 | 9.3 | N/A |
| 18 | 1 | 6.86 | 7.7 |
| 19 | 1 | 6.9 | 7.84 |
| 20 | 1 | 7.51 | 7.92 |
| 21 | 1 | 7.8 | |
| 22 | 1 | 7.7 | 8 |
| 25 | 1 | 7.4 | |
| 26 | 1 | 7.78 | |
| 27 | 1 | 8.02 | |
| 28 | 1 | 8.01 | |
| 29 | 1 | 7.66 | |
| 30 | 1 | 7.51 | |
| 33 | 1 | 6.83 | 7.43 |
| 34 | 1 | 6.78 | 7.5 |
| 35 | 1 | 6.7 | 7.5 |
| 36 | 1 | 6.97 | 7.42 |
| 37 | 1 | 7.43 | |
| 40 | 1 | 7 | |
| 41 | 1 | 7.2 | |
| 42 | 1 | 7.33 | 7.8 |

# Appendix 1-F Batch Reactor Chemical Oxygen Demand Data

| Batch Reactor | |
|---|---|
| Day | g/L |
| 0 | 8.62334 |
| 5 | 0.36 |
| 12 | 7.98478 |
| 14 | 76.7922 |
| 15 | 122.381 |
| 18 | 69.7033 |
| 20 | 71.0478 |

# Appendix 1-G Batch Reactor Ammoniacal Nitrogen Data

| Day | mg/L |
|---|---|
| 0 | 1272.06 |
| 3 | 381.6 |
| 7 | 763.23 |
| 11 | 1017.65 |
| 12 | 908.6 |
| 15 | 345.2 |
| 17 | 1090 |
| 19 | 981.3 |
| 24 | 536 |

# Appendix 2-A Continuous Reactor Biogas Volume Data

| Data | Dia | Amonstra | Reactor | Sacola | Total | Accumulated | |
|---|---|---|---|---|---|---|---|
| 17-Jan | 0 | A | 900 | 180 | 1080 | 1080 | |
| 20-Jan | 3 | A | 600 | 108 | 708 | 1788 | |
| 21-Jan | 4 | A | 600 | 156 | 756 | 2544 | |
| 22-Jan | 5 | A | 600 | 155 | 755 | 3299 | |
| 23-Jan | 6 | A | 600 | 140 | 740 | 4039 | |
| 24-Jan | 7 | A | 600 | 203 | 803 | 4842 | |
| 25-Jan | 8 | A | 600 | 211 | 811 | 5653 | |
| 26-Jan | 9 | A | 600 | 409 | 1009 | 6662 | *fresh feed |
| 27-Jan | 10 | A | 600 | 211 | 811 | 7473 | |
| 28-Jan | 11 | A | 600 | 389 | 989 | 8462 | |
| 29-Jan | 12 | A | 600 | 92 | 692 | 9154 | |
| 30-Jan | 13 | A | 600 | 52.5 | 652.5 | 9806.5 | |
| 31-Jan | 14 | A | 600 | 152 | 752 | 10558.5 | |
| 3-Feb | 17 | A | 600 | 75 | 675 | 11233.5 | |
| 4-Feb | 18 | A | 600 | 42 | 642 | 11875.5 | |
| 5-Feb | 19 | A | 600 | 42.5 | 642.5 | 12518 | |
| 6-Feb | 20 | A | 600 | 47.5 | 647.5 | 13165.5 | *fresh feed (1.5) |
| 7-Feb | 21 | A | 600 | 57.5 | 657.5 | 13823 | |
| 10-Feb | 24 | A | 600 | 95 | 695 | 14518 | |
| 11-Feb | 25 | A | 600 | 75 | 675 | 15193 | |
| 12-Feb | 26 | A | 600 | 81 | 681 | 15874 | |
| 13-Feb | 27 | A | 600 | 78 | 678 | 16552 | |
| 14-Feb | 28 | A | 600 | 96 | 696 | 17248 | |
| 17-Feb | 31 | A | 600 | 95 | 695 | 17943 | |
| 19-Feb | 33 | A | 600 | 150 | 750 | 18693 | |
| 21-Feb | 35 | A | 600 | 99 | 699 | 19392 | |

# Appendix 2-B Continuous Reactor Biogas Composition Data

| Data | Area H2 | Area CH4 | Area CO2 | Area O2 | Total Area | %H2 | %CH4 | %CO2 | %O2 |
|---|---|---|---|---|---|---|---|---|---|
| 17-Jan | 0 | 890573.3 | 2575514 | 0 | 3466087 | 0 | 25.69391 | 74.30609 | 0 |
| 20-Jan | 0 | 555847.5 | 198883.5 | 1256.7 | 755987.7 | 0 | 73.526 | 26.30777 | 0.166233 |
| 21-Jan | 0 | 830329.8 | 262865.9 | 5189.6 | 1098385 | 0 | 75.59549 | 23.93203 | 0.472475 |
| 22-Jan | 0 | 530800.8 | 256360.3 | 1128.5 | 788289.6 | 0 | 67.33576 | 32.52108 | 0.143158 |
| 23-Jan | 0 | 471734.2 | 257730.4 | 0 | 729464.6 | 0 | 64.66855 | 35.33145 | 0 |
| 24-Jan | 0 | 476484.4 | 284709.9 | 0 | 761194.3 | 0 | 62.59695 | 37.40305 | 0 |
| 28-Jan | 0 | 345565.6 | 271412 | 0 | 616977.6 | 0 | 56.00942 | 43.99058 | 0 |
| 29-Jan | 0 | 336618.3 | 242637.1 | 1386.7 | 580642.1 | 0 | 57.97346 | 41.78772 | 0.238822 |
| 30-Jan | 0 | 310227.9 | 215265.2 | 3202.5 | 528695.6 | 0 | 58.67798 | 40.71628 | 0.605736 |
| 3-Feb | 0 | 459183.9 | 281034 | 2436.9 | 742654.8 | 0 | 61.83006 | 37.84181 | 0.328134 |
| 4-Feb | 0 | 357877.9 | 223550.2 | 0 | 581428.1 | 0 | 61.55153 | 38.44847 | 0 |
| 5-Feb | 0 | 362101.9 | 236203.7 | 0 | 598305.6 | 0 | 60.52123 | 39.47877 | 0 |
| 6-Feb | 0 | 325461 | 237693 | 2082 | 565236 | 0 | 57.57967 | 42.05199 | 0.368342 |
| 7-Feb | 0 | 299521.2 | 233866.9 | 0 | 533388.1 | 0 | 56.15446 | 43.84554 | 0 |
| 10-Feb | 0 | 194469.3 | 190727 | 8480.2 | 393676.5 | 0 | 49.39825 | 48.44765 | 2.154104 |
| 11-Feb | 0 | 274357.1 | 268663.6 | 0 | 543020.7 | 0 | 50.52424 | 49.47576 | 0 |
| 12-Feb | 0 | 286159.6 | 258993 | 0 | 545152.6 | 0 | 52.49165 | 47.50835 | 0 |
| 13-Feb | 0 | 299073.7 | 278944 | 0 | 578017.7 | 0 | 51.74127 | 48.25873 | 0 |
| 14-Feb | 0 | 344822.4 | 304924 | 0 | 649746.4 | 0 | 53.07031 | 46.92969 | 0 |
| 17-Feb | 0 | 375733.4 | 306328.6 | 0 | 682062 | 0 | 55.08787 | 44.91213 | 0 |
| 19-Feb | 0 | 418025.3 | 338951.1 | 0 | 756976.4 | 0 | 55.22303 | 44.77697 | 0 |
| 20-Feb | 0 | 342275 | 270974 | 0 | 613249 | 0 | 55.81338 | 44.18662 | 0 |
| 21-Feb | 0 | 542746.4 | 351197.9 | 1488 | 895432.3 | 0 | 60.61278 | 39.22104 | 0.166177 |

## Appendix 2-C Continuous Reactor Alkalinity Data

| Dia | mg CaCO3/L |
|---|---|
| 11 | 1262.259 |
| 14 | 1289.502 |
| 17 | 1416.636 |
| 19 | 1779.876 |
| 24 | 1779.876 |

# Appendix 2-D Continuous Reactor pH Data

| Dia | Amonstra | pH | pH Correction |
|---|---|---|---|
| 0 | 2 | 5.39 | 7.63 |
| 1 | 2 | 8.21 | N/A |
| 3 | 2 | 7.82 | N/A |
| 4 | 2 | 6.9 | 8.28 |
| 5 | 2 | 7.99 | N/A |
| 6 | 2 | 7.07 | 7.5 |
| 7 | 2 | 7.85 | N/A |
| 10 | 2 | 7.07 | 7.5 |
| 11 | 2 | 7.85 | |
| 12 | 2 | 7.6 | |
| 13 | 2 | 7.5 | 7.65 |
| 14 | 2 | 7.4 | 8 |
| 18 | 2 | 7.44 | |
| 19 | 2 | 7.5 | |
| 20 | 2 | 7.56 | |
| 21 | 2 | 7.49 | |
| 24 | 2 | 7.67 | |
| 25 | 2 | 7.52 | |
| 26 | 2 | 7.53 | |
| 27 | 2 | 7.33 | |
| 28 | 2 | 7.3 | |
| 31 | 2 | 7.5 | |
| 32 | 2 | 7.52 | |
| 33 | 2 | 7.51 | |

# Appendix 2-E Continuous Reactor Chemical Oxygen Demand Data

| Day | g/L |
|---|---|
| 0 | 12.9592 |
| 9 | 14.4992 |
| 11 | 10.5637 |
| 12 | 7.98478 |
| 14 | 71.4144 |
| 15 | 75.57 |
| 17 | 91.9478 |
| 19 | 91.2144 |
| 21 | 73.4922 |

# Appendix 2-F Continuous Reactor Ammoniacal Nitrogen Data

| Continuous Reactor | |
|---|---|
| Day | mg/L |
| 0 | 1272.06 |
| 3 | 381.6 |
| 7 | 763.23 |
| 11 | 1017.65 |
| 12 | 908.6 |
| 15 | 345.2 |
| 17 | 1090 |
| 19 | 981.3 |
| 24 | 536 |

# Appendix 2-G Continuous Reactor Total & Volatile Solids Data

| Dia | ST MEDIA | SFT MEDIA | SV MEDIA |
|---|---|---|---|
| 0 | 14.1256 | 0.888713 | 13.23689 |
| 3 | 12.08791 | 5.203029 | 6.884883 |
| 7 | 9.528846 | 1.418367 | 8.110479 |
| 10 | 9.345 | 1.481982 | 7.863018 |
| 12 | 10.39631 | 1.67907 | 8.717244 |
| 14 | 12.02411 | 2.082077 | 7.688372 |
| 17 | 10.01395 | 2.325581 | 7.688372 |
| 19 | 13.28194 | 1.958763 | 11.32318 |
| 26 | 10.73232 | 1.892683 | 8.83964 |
| 31 | 10.34389 | 1.244344 | 9.099548 |

# Appendix 3 Energy Production Calculations

| Reacto | Bag | Total mL | CH4 Area | Total Area | CH4 Pr | CH4 Fract | Volume CH4 Produc | P/RT | Moles CH4 Pro | Lower Heating Value of Metha | Mass CH4 Produc | Energy Produced | Accumulated Energy | Produ | Day | Accumulated KilaWatt Hour |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 900 | 180 | 1080 | 890573.3 | 3466087.4 | 25.69 | 0.2569 | 277.494204 | 0.00012 | 0.032938879 | | 50 | 2.05355E-06 | 0.000102677 | | 0 | 0.000450734 |
| 600 | 108 | 708 | 555847.5 | 755987.7 | 73.53 | 0.73526 | 520.5640647 | 0.00012 | 0.06179155 | | 50 | 3.85234E-06 | 0.000192617 | | 3 | 0.001296288 |
| 600 | 156 | 756 | 830329.8 | 1098385.3 | 75.6 | 0.75595 | 571.5019391 | 0.00012 | 0.067837934 | | 50 | 4.2293E-06 | 0.000211465 | | 4 | 0.00222458 |
| 600 | 155 | 755 | 530800.8 | 788289.6 | 67.34 | 0.67336 | 508.3849946 | 0.00012 | 0.06034588 | | 50 | 3.76221E-06 | 0.000188111 | | 5 | 0.00305035 |
| 600 | 140 | 740 | 471734.2 | 729464.6 | 64.67 | 0.64669 | 478.5472907 | 0.00012 | 0.056804111 | | 50 | 3.5414E-06 | 0.00017707 | | 6 | 0.003827656 |
| 600 | 203 | 803 | 476484.4 | 761194.3 | 62.6 | 0.62597 | 502.6534923 | 0.00012 | 0.059665544 | | 50 | 3.7198E-06 | 0.00018599 | | 7 | 0.004644117 |
| 600 | 211 | 811 | 345565.6 | 616977.6 | 56.01 | 0.56009 | 454.2364287 | 0.00012 | 0.053918384 | | 50 | 3.3615E-06 | 0.000168075 | | 10 | 0.005381934 |
| 600 | 409 | 1009 | 336618.3 | 580642.1 | 57.97 | 0.57973 | 584.9521843 | 0.00012 | 0.069434493 | | 50 | 4.32883E-06 | 0.000216442 | | 11 | 0.006332073 |
| 600 | 211 | 811 | 310227.9 | 528695.6 | 58.68 | 0.58678 | 475.8784202 | 0.00012 | 0.056487313 | | 50 | 3.52165E-06 | 0.000176083 | | 12 | 0.007105044 |
| 600 | 389 | 989 | 459183.9 | 742654.8 | 61.83 | 0.6183 | 611.4992822 | 0.00012 | 0.072585664 | | 50 | 4.52529E-06 | 0.000226265 | | 13 | 0.008098303 |
| 600 | 92 | 692 | 357877.9 | 581428.1 | 61.55 | 0.61552 | 425.9365978 | 0.00012 | 0.050559161 | | 50 | 3.15207E-06 | 0.000157603 | | 16 | 0.008790153 |
| 600 | 52.5 | 652.5 | 362101.9 | 598305.6 | 60.52 | 0.60521 | 394.9010167 | 0.00012 | 0.046875202 | | 50 | 2.92239E-06 | 0.00014612 | | 17 | 0.009431592 |
| 600 | 152 | 752 | 325461 | 565236 | 57.58 | 0.5758 | 432.9990871 | 0.00012 | 0.051397487 | | 50 | 3.20433E-06 | 0.000160217 | | 18 | 0.010134913 |
| 600 | 75 | 675 | 299521.2 | 533388.1 | 56.15 | 0.56154 | 379.0425958 | 0.00012 | 0.04499279 | | 50 | 2.80504E-06 | 0.000140252 | | 19 | 0.010750593 |
| 600 | 42 | 642 | 194469.3 | 393676.5 | 49.4 | 0.49398 | 317.1367623 | 0.00012 | 0.037644496 | | 50 | 2.34691E-06 | 0.000117346 | | 20 | 0.011265719 |
| 600 | 42.5 | 642.5 | 274357.1 | 543020.7 | 50.52 | 0.50524 | 324.6182636 | 0.00012 | 0.038532559 | | 50 | 2.40228E-06 | 0.000120114 | | 21 | 0.011792997 |
| 600 | 47.5 | 647.5 | 286159.6 | 545152.6 | 52.49 | 0.52492 | 339.88344 | 0.00012 | 0.040344553 | | 50 | 2.51525E-06 | 0.000125762 | | 24 | 0.01234507 |
| 600 | 57.5 | 657.5 | 299073.7 | 578017.7 | 51.74 | 0.51741 | 340.1988516 | 0.00012 | 0.040381993 | | 50 | 2.51758E-06 | 0.000125879 | | 25 | 0.012897656 |
| 600 | 95 | 695 | 344822.4 | 649746.4 | 53.07 | 0.5307 | 368.8386238 | 0.00012 | 0.043781566 | | 50 | 2.72952E-06 | 0.000136476 | | 26 | 0.013496761 |
| 600 | 75 | 675 | 375733.4 | 682062 | 55.09 | 0.55088 | 371.8430949 | 0.00012 | 0.044138201 | | 50 | 2.75176E-06 | 0.000137588 | | 27 | 0.014100747 |
| 600 | 81 | 681 | 418025.3 | 756976.4 | 55.22 | 0.55223 | 376.0688303 | 0.00012 | 0.0446398 | | 50 | 2.78303E-06 | 0.000139151 | | 28 | 0.014711596 |
| 600 | 78 | 678 | 342275 | 613249 | 55.81 | 0.55813 | 378.4147222 | 0.00012 | 0.04491826 | | 50 | 2.80039E-06 | 0.00014002 | | 29 | 0.015326256 |
| 600 | 96 | 696 | 542746.4 | 895432.3 | 60.61 | 0.60613 | 421.864941 | 0.00012 | 0.050075851 | | 50 | 3.12194E-06 | 0.000156097 | | 30 | 0.016011492 |
| | | | | | | | | | 1.170091671 | | | | | | | |

| Digestate Volume (including Fe | Average Digestate | Digestate Mass (l | Total Energy per kg VS | | Energy per Kg Bagasse | |
|---|---|---|---|---|---|---|
| 8.515 | 0.55 | 4.68325 | 0.005990938 | MJ/ kg | 0.006245579 | MJ/kg |

Source: 200 MJ per 100 liters of beer= 2 MJ/L Beer

kg VS in digeste (^ 0.6088225 Standard Beer= 355 mL

# Appendix 4 Mass Balance Calculations

Mass Balance on Carbon

$$in - out + \cancel{generation} = accumulation$$

I am cancelling out generation term because I am balancing on Carbon, not methane.

$$in = original\ load + daily\ feed = (559g + 52\ g) + \left(28\ feeds\ * 40\%\ Barley\ * 180\ mL * 0.57\frac{g}{mL}\right) = 1770.6\ g\ C$$

Above: Finding the amount of carbon put into the continuous reactor by adding the original load and the carbon from the daily feedings

$$in = accumulation + out = C\ from\ biogas + total\ organics$$

$$acc + out = total\ organics + \left(1.17\ mol\ CH_4 * \left(\frac{1\ mol\ C}{1\ mol\ CH4}\right) * 12\frac{g}{mol}\right) = 14.04\ \text{g Carbon} + \text{Total Organics}$$

Moles of Methane Taken from Large spreadsheet with Calculations

$$1770.6\ g\ C = 14.04\ g\ C + Total\ Organics$$

$$Total\ Organics = 1756.6\ g\ C$$