ANAEROBIC DIGESTION OF BREWERS' SPENT GRAIN IN A NOVEL PLUG FLOW REACTOR SYSTEM

by

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(Under the Direction of Keshav Das)

ABSTRACT

Beer brewing generates large amounts of spent grain which is currently used mostly as an animal feed. Anaerobic digestion offers an opportunity to generate methane from this low-value by-product, potentially increasing the value of this waste and diversifying its usage streams. A novel modified plug-flow UASB dual reactor was proposed which allows for different hydraulic and solids retention times, which decreases by-product inhibition in the anaerobic digestion process. The effect of by-product inhibition as an important factor in anaerobic systems was tested. Also, an analysis of other potential options for downstream uses of the digestate liquid and digested spent grain are discussed.

INDEX WORDS: Anaerobic digestion, Agricultural residue, Nutrition, Barley, Spent grain, Methane, Biogas, Volatile fatty acids, Acetate, Propionate, Butyrate, By-product inhibition

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DEDICATION

This work is dedicated to my new-born son, Noah. For nine months, you were with Kelly and me as we journeyed through life. I would come home, tired from work, but the anticipation of your arrival would rejuvinate me. Though we did not know your name, we both loved you from the very beginning of your life. While I have done great work in the past two years, creating and loving you and your mother have been the most important and the most enjoyable. As the final push to complete my thesis came, so did you. I am excited to enter this new time of transition in life: a new child, the completion of my thesis, and the beginning of a new degree. By the time I finish school, you may be starting school yourself. May you always know that you are our beloved son. We love you, Noah.

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CHAPTER 1

INTRODUCTION

"Wastes are… resources out of place" (Taiganides, 1979). This memorable quote rings especially true in the brewing industry. Many other industries have realized that their waste streams can be converted into profitable sources of revenue. For instance, the poultry industry currently sells poultry litter, the animal excreta mixed with a bedding material, to farmers as a nutrient-rich fertilizer. Similarly, dairy farmers use cattle excreta as a fertilizer or as a feedstock for anaerobic digestion. Converting a waste into a resource requires creativity as well as a comprehensive understanding of the by-product streams that the industry is generating.

Beer brewing creates an array of different wastes. Between 4 and 20 liters of water are required to make 1 liter of beer, which means water wastes are high (Mussatto et al, 2006). Brewers' spent grain (BSG), another by-product of the beer brewing process, is produced at a rate of 200 g/L beer (Mussatto and Roberto, 2006). Of the original malted barley that goes into the mashing tun, about 30% remains as BSG. Most of what is extracted are proteins and newly hydrolyzed simple sugars (Robertson et al 2010). As a reference, a small local brewery in Athens produces 14000 barrels, or approximately 1,600,000 L per year. Based on that conversion factor, they produce over 320 tons of spent grain per year. Contrast that to the average BSG wastes coming from a MillerCoors, LLC brewery: anywhere from 16-30 truck-loads per day with 50,000 lb per truck-load (Teague, 2010). For both the smaller local brewery as well as the nationally-recognized brands, this by-product has to be moved. For both of the

breweries mentioned above and for approximately 95% of all spent grain, the final destination is a farm for use as an animal feed (Mussatto et al, 2006). This one-dimensional usage of spent grain links the value of this potentially valuable by-product to that of other commodities used in animal feeding operations. Spent grain has characteristics that make it a prime candidate for many other important and valuable uses. As a human food additive or a nutriceutical product, spent grain would command a premium over its current usage. As an energy source for a fermentation process, it could produce not only a valuable fuel, but also nutrient-rich effluents and solid organic matter for composting. Generally, diversification of the end-usage of this product could lead to more efficient use of resources.

CHAPTER 2

LITERATURE REVIEW

To determine if anaerobic digestion of brewers' spent grain will yield an economic advantage over the currently preferred usage, a deeper understanding of the characteristics of spent grain must be established. Next, a thorough evaluation of the current state-of-the-art of anaerobic digestion reactors and other factors that may affect digestion of BSG will be explored. Lastly, the potentially valuable products that come from anaerobic digestion like methane as well as the many volatile fatty acids (VFA) will be described.

Brewers' spent grain

A study by Santos et al (2003) looked at intra-brewery variation of BSG. The study concluded that the percent composition of BSG from eight different samples had similar levels of fat and protein with practically negligible difference in ash and total phenolics. Important to note from this study, though, is that the level of barley and corn in the eight different samples was constant at 80:20. This is not necessarily the case for other breweries. The grain bill for mashing can be anywhere from 50-100% barley, with up to 50% adjuncts as diverse in composition as corn, wheat, rice, etc. Either a two row or six row cultivar of barley can be used, and the difference in protein and carbohydrate spectra are different in these different substrates (Robertson et al, 2010). For a thorough treatment of the composition of brewers' spent grain as well as the microbial ecology at the site of production, see Robertson et al (2010).

Generally, BSG is composed of (dry weight composition) protein (31%), pentosans (19%), lignin (16%), starch and beta-glucans (12%), cellulose (9%), lipids (9%) and ash (4%) (Santos et al 2003). Another, more recent source estimates the following percentages: hemicellulose (28.4%), lignin (27.8%), cellulose (16.8%), and protein (15.3%) (Mussatto and Roberto, 2006). Another study cites cellulose (25%), protein (24%) arabinoxylose (22%), lignin (12%), lipid (11%), and ash (2%). Seen here, the composition of brewers' spent grain can vary widely depending on origin and processing (malting and mashing) of the barley as well as the addition of adjuncts to the grain bill of the brewery (Santos et al, 2003). Many different sources have been compiled and an average has been determined and will be used for reference in this work (see Table 1).

Table 1: Composition of brewer's spent grain

Composition (% dry weight)	**Kanauchi et al (2001)**	**Mussatto and Roberto (2006)**	**Santos et al (2003)**	**Average**
Protein	24	15.2	31	**23.4**
Beta-glucans	25.4	16.8	21	**21.1**
Arabinoxylose	21.8	17	19	**19.3**
Lignin	11.9	27.8	16	**18.5**
Lipid	10.6	nd	9	**10.5**
Ash	2.4	4.6	4	**3.7**

Anaerobic digestion

Most basically, anaerobic digestion is the decomposition of a substrate in the absence of oxygen. In anaerobic digestion, microbes use a molecule other than oxygen as the terminal

electron acceptor (TEA). This process rarely occurs when oxygen in the environment is abundant because using oxygen as a TEA is almost always the means by which the most chemical energy can be harvested from a substrate. Anaerobic digestion usually occurs in a vessel closed off from the atmosphere so that oxygen cannot enter the system.

Many different digester types may be employed. Reactor choice is dependent upon the characteristics of the substrate being digested and the goals for anaerobic digestion. For instance, an up-flow anaerobic sludge blanket is generally regarded as the most efficient reactor design, but would be inappropriate for digesting a high suspended solids waste like raw brewers' spent grain. Another type of digester, the anaerobic lagoon, is long pit or trough in which the substrate sits or moves slowly, and the natural anaerobic bacteria degrade it. This is a common method for the stabilization of animal wastes. A plug flow reactor is usually a long tubular reactor in which the addition of substrate at one end forces the previously digested material out of the other end. In practice, the two aforementioned reactors have many similarities. One last type of reactor is the continuous stirred tank reactor (CSTR), which can vary greatly in size and relies on some sort of mechanical mixing to maintain a relatively homogeneous digester. In theory, the mixing prevents the local accumulation of by-products and helps physically move the anaerobic bacterial throughout the digester and brings them in contact with new substrate.

Factors affecting anaerobic digestion

Many parameters of an anaerobic system may affect the performance of the reactor. These factors may be physical, chemical, or biological, or some combination of the three. Below, important factors are discussed in detail.

Effect of Temperature

Anaerobic digestion can take place in mesophilic conditions (temperature range of 20˚C to 41˚C [68 ˚F to 105 ˚F]) or in thermophilic conditions (43˚C to 71˚C [110˚F to 160˚F]) (Burke, 2001). Under thermophilic conditions, a temperature fluctuation may adversely affect methane production (Kelleher et al., 2002). Under mesophilic conditions both digestion and co-digestion of cattle and broiler manure were better at 35˚C than ambient temperature (Demirci and Demirer, 2004). As a general rule, the higher the temperature, the quicker the digestion takes place (Dubrovskis et al, 2008). At ambient temperature (especially in colder climates), the digestion efficiency decreases because the anaerobes are sensitive to low temperature.

Angelidaki and Ahring (1994) showed that at higher ammonia loads, process stability is more greatly affected by higher temperatures. That is, substrates with high potential for ammonia production should use mesophilic temperatures if greater process stability is desired. Psychrophilic anaerobic digestion (<20 °C) has not been widely studied. Safley and Westerman (1990) found that achieving steady state conditions at temperatures below 20˚C was difficult even though the digester inoculum was collected from a lagoon during the winter months. To optimize both stability and performance, mesophilic temperatures were investigated in the current research.

Effect of pH and Alkalinity

Anaerobes prefer an environment with a pH range between 6.8 and 8.5 (Burke, 2001) and any variation from this value results in decreased bacterial activity. The acidity of the digester system depends on several factors: the presence of digestion products like VFAs, the buffering capacity of the feedstock, and others.

Effect of Retention Time: Hydraulic (HRT) and Solid (SRT)

The number of days the liquid phase stays in the digester is called the hydraulic retention time, where as solids retention time is the ratio of quantity of solids maintained in the digester to the quantity of solids exiting the digester each day (Burke, 2001). In most reactors, these two values are the same. In one very efficient type of reactor, the up-flow anaerobic sludge blanket (UASB), the solids retention time is much higher than the hydraulic retention time, preventing wash-out of the methanogenic and syntrophic bacteria.

Generally, more complex substrates (especially high particulate organic loadings) will require a longer SRT. However, the long retention times used in, for instance, a continuously stirred tank reactor (CSTR), can lead to accumulation of inhibitory by-products.

Other Factors Controlling Anaerobic Process Performance

Researchers have employed many method modifications and amendment additions to increase the anaerobic digestion and methanogenesis for a variety of substrates. In a study by Rao and Seenayya (1994), the addition of iron to fed-batch reactors containing poultry litter led to increased process characteristics. Additions of 5 to 50 mM ferrous sulfate were used in the study. The highest level of ferrous sulfate yielded the maximum biogas production rate. At the 20 mM ferrous sulfate level, the poultry litter waste digester showed an increase in the concentration of methanogenic cells by three orders of magnitude. Further, upon overloading the reactors, the system loaded with iron sulfate returned to normal functioning, whereas the control group reactors went into system failure.

Co-digestion with other substrates often helps the rates and yields in anaerobic digestion of agricultural and food processing residues. The brewing industry has many waste products

besides spent grain that are good candidates for co-digestion and may increase yields. These include residual yeast, spent hops, and equipment rinsing water. However, co-digestion of BSG has not been addressed in this work.

The carbon to nitrogen (C: N) ratio is important in the anaerobic digestion process. A ratio that is too low (too much nitrogen) or too high can have a negative effects on system performance. The C:N ratio for spent grain is around 15 (Huotari et al, 2008), which would generally be considered a good ratio. The optimal ratio for anaerobic digestion is approximately 20-25.

Previous ideas in anaerobic digestion: insights and shortcomings

One of the research questions is whether or not this newly proposed method for anaerobic digestion will be an improvement on either: 1) the technology that is already available for anaerobic digestion or 2) the usage of brewers' spent grain. Concerning the former, the standard method for treating a waste stream with high particulate solids is a CSTR. In this type of reactor, all the processes from hydrolysis to methanogenesis occur in the same location. This is not ideal because each clade of microorganisms have different optimum environmental conditions and can only function within certain ranges. For instance, in one study concerning anaerobic digestion in landfills, clear stratification occurs in which the various bacterial clades segregate and work in their optimum environment (Martin et al, 2003). With regard to the latter, standard usage for the disposal of this waste stream (brewers' spent grain) is almost exclusively as a cattle feed which is a low-value commodity whose price will be linked to that of other animal feeds, namely corn which costs about 70-110 $US per ton.

The anaerobic digestion process can be artificially segregated into four component phases: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. A substrate such as brewers' spent grain is high in lignocellulosic material, whereas most of the smaller, soluble molecules are dissolved and left in the brewing process. Hydrolysis of the large organic molecules creates readily degradable compounds like simple sugars. The simple sugars, such as glucose, arabinose, and xylose, produced in the hydrolysis step are then broken down into acidic molecules such as volatile fatty acids (VFAs) and alcohols. Later, a different group of bacteria converts the alcohols and volatile fatty acids into acetic acid, the simplest VFA. Lastly, methanogens use the acetate to make methane.

The rate of hydrolysis is often the rate-limiting step in the anaerobic digestion of organic matter in which complex molecules and large polymers compose most of the biomass, as is the case with brewers' spent grain (GonCalves et al, 1994; Xiros and Christakopoulos, 2009).

GonCalves et al (1994) proposed a new reactor for the processing of domestic wastewater. The purpose of this system is not the generation of methane-rich biogas, rather the desired products are the volatile fatty acids for use in denitrification and phosphorous removal. With this in mind, it is important to remember that although the final goal of this research is to produce methane as an alternative fuel source, it will also generate intermediates that can be used for other applications.

There has been much work done on anaerobic digestion of waste streams from various industries for the purpose of waste reduction, waste stabilization, as well as value added product generation (like methane-rich biogas). The insights from this previous research have been drawn upon to generate this experimental work, but the shortfalls and incompatibilities are also noted.

1. Different bacteria have different optimum environments. Researchers have proposed the separation of phases of digestion. Demirel and Yenigun (2002) completed an extensive review on two-phase anaerobic digestion which highlights the benefits and drawbacks of such a system. Two ways in which a two-phase system can be implemented are as follows:

    a) Separate reaction vessels (Cohen et al, 1979; Ghosh et al, 1975). Liu (1998) claims that the this is not optimal because the physical separation leads to the disturbance of interspecies transfer of hydrogen gas.

    b) Taking advantage of natural stratification. Liu (1998) created a reactor that performed in this way. An unfavorable drop in the pH occurred near the beginning of the experiment, but the system did recover. While the system did not go into complete failure, the time that it was operating at a pH well below five could have decreased system performance. There would be a benefit with respect to reactor stability if a novel design could prevent such a drop in pH.

2. Rapid drops in pH lead to complete reactor failure. Changes, specifically drops, in pH are one of the most common culprits in reactor failure (Chen et al, 2008). Potential solutions that have been developed include:

    a) Recirculation of effluent may help to maintain balance. This is undesirable, however, because recirculation means that the treatment time of the waste stream is longer. Consequently, a larger reactor would be needed to treat the same amount of waste.

b) Addition of buffering agents. This solution is very costly and would probably make anaerobic digestion of brewers' spent grain economically unattractive.

3. Slow degradation time of more complex substrates

a) Various pre-treatments can increase breakdown efficiency and effectiveness. Acid hydrolysis, whereby the substrate is heated in the presence of an acid, has been shown to break the substrate into simpler molecules. Supercritical carbon dioxide or steam explosion are also options. However, many pretreatments are so expensive as to be cost-prohibitive.

b) Use of larger vessels. This is also not a great option because it increases the footprint of the treatment facility and also adds to the cost of the plant.

4. Reactors with high flow rates (low HRT) lead to wash-out of methanogens. Methanogens, the essential, methane-producing organisms, generally have a slow growth rate. Consequently, if a low strength wastewater is treated and flow rates are maintained at a high level to prevent the need for such large reactor volumes, the methanogens can wash out of the system. This occurs because the dilution rate (the medium flow rate over the volume of the reactor) is higher than the specific growth rate of the microbe.

a) An anaerobic filter is not appropriate for streams with high suspended solids.

b) UASB system. The UASB reactor addresses this washout problem by creating a layer of sludge granules within which the methanogens live and metabolize. The sludge granules have good settle-ability and thus the flow rate can be maintained

at a high level without washout of the integral bacteria. Many studies show the effectiveness of using reactors with a HRT that is less than that of the SRT.

- A currently-used technology is a system that contains dual UASB reactors: one for the acidogenic phase, and one for the methanogenic stage. Both of the reactors rely on the gravity settling of the particulate material, sludge. The concept employed in the UASB type reactor is that the SRT will be greater than the HRT. Consequently, the solids (which often are the matrix upon which the bacteria affix themselves) stay in the system for a longer period of time. While this approach has been shown to be optimal for the methanogenic stage when treating relatively low-strength waste waters with low suspended particulate matter, it does not logically seem to be the best choice for a substrate such as brewers' spent grain. GonCalves et al (1994) were working with wastewater treatment which has a low total suspended solids contrasted to the high solids that will be used in this study. Even working with such low suspended solids, the flushing of excess sludge was necessary on a regular basis. For this reason, a UASB reactor would not be appropriate for this kind of substrate. Furthermore, another disadvantage to using a UASB is that the solids retention time is a dependent variable that can only be indirectly manipulated.

Important products of anaerobic digestion: volatile fatty acids

The short-chain fatty acids, acetic, propionic, n- and iso-butyric, and n- and iso-valeric are important intermediate compounds in the process of converting complex polymers and other large molecules into methane. At neutral pH, these acids exist most abundantly in their ionic forms of acetate, propionate, butyrate, and valerate, respectively. Throughout the course of this work, these names will be used interchangeably with the understanding that the actual form that the molecule takes in the aqueous phase of the reactor is dependent upon the pH of the environment in which they occur.

Acetic acid is a major intermediate in the methanogenesis of various feedstocks (Batstone et al, 2003). Both propionic and butyric acids yield acetic acid during a successful anaerobic digestion. The effect that increasing levels of acetate have on methanogenesis from acetate and other VFAs as well as the composition and volume of the biogas varies from study to study. Acetic acid levels of 15-30 mM had a moderately inhibitory effect on propionate utilization (Mawson et al, 1991). In one study, increasing levels of acetic acid levels may increase both the methane content in and the volume of biogas (Pind et al 2003). However, increasing levels of acetic acid lower the pH below a critical threshold and stop methanogenesis altogether.

Propionic acid is one of the most common VFAs found in anaerobic digestion systems. Its accumulation in the aqueous phase, however, can both lead to and be a result of reactor instability. If propionate levels rise to extreme levels, further process inhibition can take place and lead to further reactor failure. Propionate conversion to methane, which is relatively thermodynamically unfavorable, is catalyzed by obligate syntrophic bacteria, which require the

concordant functioning of other bacterial species to efficiently remove the pH-lowering fatty acid (Ozturk 1993).

Butyric acid, another important intermediate in the production of methane from food-processing wastes, is commonly produced by the breakdown of various amino acids like histidine, lysine, threonine, valine, and glutamic acid. It is expected that since a large fraction of BSG is protein, butyrate will be in a higher concentration than in digestions with more carbohydrate-rich feedstocks (Batstone et al, 2003).

Previously, Lata et al (2002) anaerobically digested two different substrates, tea waste and vegetable market waste, and found that the levels of VFAs differed. For the vegetable waste, the level of production of VFAs were acetic > propionic > butyric > (valeric, iso-butyric). For the tea waste the order was acetic > iso-butyric > iso-valeric > propionic. The protein content of the spent grain should yield some iso-valeric acid, which is commonly produced from leucine during anaerobic digestion (Batstone et al, 2003).

As with ammonia and sulfide, the toxicity of VFAs depends, in part, on the pH of the surrounding environment (Chen et al, 2008; Mawson et al, 1991). The inhibitory effects of VFAs are generally attributed to the undissociated acids. Consequently, a system where the pH is kept close to neutral will have less inhibition due to increases in the levels of VFAs. Phase II of this work demonstrates the power of dilution in preventing the accumulation of acids in one region, and thus preventing inhibition.

Potential uses for brewers' spent grain

The market price of BSG is intricately linked to its most common current usage: animal feed. The cost of BSG from larger breweries like MillerCoors, LLC can vary from 0-40 $US/ton wet grain at the brewery dock, and 20-80$US/ton wet grain delivered. This vast price difference depends on the laws of supply and demand and can determine profitability or economic loss for a company using the spent grain as an integral part of operation (Teague, 2010). This study hopes to elucidate alternative possibilities for BSG usage and to determine the most economically lucrative means of using it.

Human nutrition

With rising costs of food in the American supermarket, having a lower-cost raw material for processed food would serve as a great resource to the food production industry and may have the effect of reducing the costs born by the consumer in the US and abroad. Brewers' spent grain, because of its high nutritive value, has been evaluated for its usage in the manufacture of breakfast cereals, bread and other baked goods, and snacks. Unfortunately, brewer's spent grain usually must be milled into a flour before use in food products because the grains are too coarse otherwise (Mussatto et al 2006). This requires the drying of the grain which is energy intensive.

Most bacterial contamination residually present in the grain immediately after mashing would not be pathogenic to humans. A bacterial species that could withstand temperatures of 65-70oC for many hours in the kettle boil would have to be thermo-tolerant if not thermophilic, and currently, no thermophilic bacterium has been shown to be pathogenic to humans (Robertson et al, 2010). Contamination after removal from the mashing (and lauter) tun and subsequent

environmental exposure would be possible. Furthermore, the bacteria that could survive in the lauter tun would not have an optimal activity at room temperature. The concern then is the environmental contamination after removal from the lauter tun.

An inoculation of an appropriate bacterial consortium could lead to a controlled fermentation which would preserve the grain for either a foodstuff (human or animal) or as a substrate for value-added products or energy production. This would circumvent the need for drying to preserve the grain effectively using natural biological processes to save money, time and resources. Currently, many farmers generate silage using a similar method. In human nutrition, food fermentation has been a method of preservation for many years.

One drawback for human consumption is that BSG's color and flavor reduce desirability of the material for foodstuffs because of social norms and cultural preferences, especially in the US. BSG's brown color prevent its use in white products, which decreases its versatility in human food consumption. Mussatto et al (2006) believe that because of its nutritive value, BSG could be a potentially important food ingredient in developing countries where food resources are more scarce. BSG could also provide great benefit to the developed nations where the number of cases of inflammatory bowel disease is on the rise.

Prentice et al (1978) demonstrated that up to 15% addition of BSG was acceptable for addition into cookies and did not significantly alter the organoleptic properties. The organoleptic properties are those perceived by the senses. In another study, Prentice and D'Appolonia (1977) tested BSG's acceptability by substituting it for a fraction of flour in breads. Up to 10% addition of BSG produced an acceptable product. When more than 10% spent grain was incorporated into the baked goods, it formed a less-desirable product. Important to note is that this study

reports on BSG and not on the derived product, germinated barley foodstuff (GBF). GBF is produced by removing the husk fraction by milling and sieving. GBF may provide a more palatable product with more desirable organoleptic properties. Even if BSG addition to processed foodstuffs is undesirable for the general public, usage in prebiotic or nutriceutical foods may be acceptable when considering the benefits that BSG and its derivatives provide to people suffering from inflammatory bowel disease and other gastrointestinal distress as well as its benefits for cardiovascular health.

Many studies have found a diverse array of health benefits by adding BSG to the diet. BSG and derived products increases fecal weight, excretion of cholesterol and fat, and decreases occurrence of gallstones. BSG has been shown to alleviate constipation and diarrhea, the former by supplying insoluble fiber and the latter by increasing stool water-holding capacity (Mussatto et al., 2006). Zhang et al (1991) showed that the addition of brewer's spent grain to subjects' diets increased their excretion of cholesterol, nitrogen, fat and energy. Because the subjects were excreting more of these molecules that can be harmful in excess, less were reabsorbed by the body. This article suggests that BSG can lower cholesterol and would serve as a supplemental therapy for individuals with high cholesterol and increased risk of cardiovascular disease.

People who suffer from heart disease can benefit from increases of BSG in the diet, but the health benefits are not limited to this application. GBF or BSG could find a niche market as a nutriceutical for GI disease treatment. Current treatment for inflammatory bowel disease (IBD) focuses almost exclusively on symptom management, whereas various functional foods that actually improve the gastrointestinal (GI) health of affected individuals would be a great benefit. Germinated barley foodstuffs which are derived from brewer's spent grain have many beneficial

effects in ameliorating the symptoms of inflammatory bowel disease in model rodents. The readily-available hemicellulose in the spent grain products are converted by gut bacteria into butyrate and other volatile fatty acids. Butyrate, in particular, has been shown to have a protective effect in patients with GI problems perhaps because it serves as an important energy source for the colon and increases the growth of colonic epithelial cells which are essential for a healthy GI tract. In the rodent model, the feeding of GBF leads to a significant decrease in the levels of fecal occult blood, which implies an improving health with this supplement (Kanauchi et al, 1999). If marketed as a nutriceutical product, GBF could have a value much greater than other grain counterparts and also would be buffered from the fluctuations present in other commodity grain markets like corn which do not have the health benefits of BSG.

An exact economic analysis for the value of BSG used in human nutrition supplementation is difficult to determine. Certainly, as the proportion of income that is spent on pharmaceuticals increases, alternative methods for maintaining both intestinal and cardiovascular health will command a premium.

Animal feed supplement

Spent grain, with its good protein and energy levels, makes a good supplement to an animal's food rations. The most common application for BSG is as an animal feed. Most often used as a cattle feed, BSG has been shown to increase milk yields and total solids in the milk in dairy production operations (Mussatto et al 2006). For a cattle or dairy producer, both the economic as well as the product quality concerns will determine usage of spent grain as a feed.

In one study, a 33% substitution of rye-grass silage for BSD led to an increase in milk yield in lactating Holstein/Fresian dairy cattle. The cattle also ate more of the BSG mix than of the control, perhaps because of the increased palatability of the BSG and the high moisture content (Phipps et al, 1995). Another study with pigs found that although the amount of time to slaughter weight increased linearly with increasing levels of BSG (which replaced maize), the quality of the meat remained the same. Further, the percentage of ham from the pigs increased quadratically with increasing levels of BSG. As an additional benefit, the pigs required a decreasing quantity of feed as the the percentage BSG in the feed increased (Yaakugh et al, 1994).

Corn is the main feed that BSG replaces, and the current price for corn is $130/ton at approximately 15% moisture (Teague, 2010). The hypothetical cost for one ton of dry corn would be $153. The price for delivered spent grain is between $20-80/ton at 80% moisture which translates to $100-400 per ton dry BSG. From this analysis, the price of commodities determine the viability of BSG as an animal feed.

While animal feed is the process in which 95% of all spent grain is used, it is not without challenges. The spent grain is received wet which presents many problems. The cost of freight (on a per calorie basis) for moving the spent grain at 80% moisture is considerable more than transporting corn and soybean feeds at 15% moisture. The wet grain must be used within 10 days for dairy operations or 3 weeks for beef production. Even when the economics of transporting the spent grain are comparable, the amount of manure produced when cattle are fed spent grain increases and must be dealt with accordingly. Year-long contracts with large breweries for their waste resources do offer a way to hedge against high prices of animal feed,

but in years where corn and soy prices are low, a locked-in contract for the spent grain can be costly for the farmer (Teague, 2010). The often unpredictable fluctuations in the value of commodities are a disadvantage for this end-use of BSG. The previous option, human nutriceutical, could prevent or at least moderate these price fluctuations.

Energy source

Farmers, especially those with cattle operations, use spent grain because it is a good source of both protein and energy. Feeding spent grain to cattle leads to the transformation of calories of BSG into calories of meat or dairy, which have a perceived value that is higher than the starting product. In the three unique ways discussed below, the energy stored in the spend grain can be transformed into an array of different energy forms that are useful for humans.

Direct combustion

The direct combustion of brewers' spent grain requires only a few pretreatment steps to decrease the moisture content before the combustion phase. Wartsila, a Finnish engine manufacturer, has built a power plant that uses BSG as a feedstock. They have also investigated the co-combustion of BSG with other biomass sources such as wood chips. The plant produces both heat and electricity from spent grain. The grain must be dried to around 55% moisture (from initial levels of around 80% moisture) before it is a suitable substrate for combustion (Huotari et al, 2008). The table below shows the composition of spent grain in comparison with wood chip, another common combustible substrate (see table 2). A mix of these two substrates yields a combustible product.

One of the drawbacks to direct combustion of BSG is the production of SOx and NOx. Some communities in which biomass combustion plants have been receiving permits have spoken out about concerns over the wide array of different emissions that these plants would release into the atmosphere (Henihan et al, 2003). This public concern represents a non-economic disincentive and may deter other companies or municipalities from investing in this technology (Turnell et al, 2007).

Table 2. Composition of spent grain, wood chips, and a mix (from Huotari et al, 2008)

Component	**Unit**	**Spent Grain**	**Wood Chips**	**Mix**
Moisture	%	58	45	52
Heat value, dry	Mj/kg	20	19	19
Heat value, wet	MJ/kg	7	9	8
Bulk density	kg/m3	257	236	247
Volume ratio, wet	%	55	45	100
Elements, dry				
C	%	51.20	50.90	51.10
H	%	7.00	6.30	6.70
N	%	3.63	0.10	1.90
S	%	0.27	0.02	0.15
Cl	%	0.02	0.01	0.01
O	%	34.49	41.17	37.80
Ash	%	3.40	1.50	2.45
Minerals in dry fuel				
Na	%	0.0083	0.0064	0.0074
K	%	0.0293	0.0500	0.0396
Ca	%	0.1930	0.0676	0.1303
Si	%	0.1452	0.0058	0.0755
Al	%	0.0007	0.0016	0.0011
P	%	0.4290	0.0045	0.2168
Mg	%	0.1220	0.0111	0.0666

Ethanol

Another promising alternative fuel technology, the production of ethanol from BSG, a lignocellulosic feedstock has potential, but many hurdles must be overcome to make this a viable technology.

One benefit of using BSG over corn is that BSG in not currently directly used as a human foodstuff like corn. However, like corn, BSG is used as a cattle feed and so diverting BSG from animal feed operations to energy production will have an impact on the cost of human food, thus presenting not only economic but also humanitarian barriers to implementation and widespread adoption as a viable alternative. This is the so-called food versus fuel controversy.

One difference between most lignocellulosic feedstocks and BSG is that BSG would not require the addition of urea as a nitrogen source because of the high levels of protein present in the spent grain (Banerjee et al, 2009).

Most yeasts used in commercial ethanol production cannot digest complex substrates, and so to enable yeasts to ferment the BSG to ethanol, the complex carbohydrates in BSG must be hydrolyzed to more simple sugars like glucose, xylose, and arabinose by some other means (White et al, 2008). Hydrolysis is often achieved industrially by acid pretreatments combined with some level of heating. One experiment cites the use of 0.16 N HNO_3 at 121ºC for 15 minutes coupled with enzymatic breakdown of BSG (White et al, 2008). The combination of acid and enzymatic treatment is beneficial because acid hydrolysis does not hydrolyze cellulose as well as it does hemicellulose. This research demonstrates the potential of various strains of yeast for the production of ethanol from BSG. The ethanol yield was found to be 4.2g ethanol per 100g of spent grain.

Currently, the large amount of enzymes required for the manufacture of fuel ethanol greatly increase the cost of this alternative technology. Co-digestion with high carbohydrate feedstocks like corn cobs have been investigated. Xiros and Christakopoulos (2009) showed that co-digesting BSG with another carbon source (corn cobs) significantly increased the levels of xylanase and endogluconase as well as feruloyl esterase, cellobiohydrase, and Beta-D-xylosidase. All of these enzymes are essential in the hydrolysis of larger polysaccharides into smaller, fermentable sugars.

One drawback to this way of producing a fuel is that ethanol is in solution with water and must subsequently be made anhydrous before use in internal combustion engines. Yet another major drawback to ethanol from a lignocellulosic biomass like BSG is that there are currently no commercial plants that have successfully implemented a lignocellulose to ethanol process for BSG on a commercial scale.

The bottlenecks and road-blocks of the lignocellulose to ethanol field, in general, are the same barriers to implementation of the specific case of BSG to ethanol. Many steps are required: pre-treatment, hydrolysis to simple substrates, inhibition during fermentation, finding suitable strains to ferment all the simple sugar types, as well as the challenge of ethanol recovery are all currently in the process of optimization. While synthetic biology has led to the creation and usage of many different genetically-modified microbes, these ideas are still at the bench-scale and have yet to be proved in larger-scale facilities.

Methane

Methanogenesis from waste streams has been a common treatment option for many years. Unlike ethanol production, methane need not be manually separated from its aqueous medium because it is a gas and naturally separates.

The major advantage of thermal gasification is its ability to achieve total conversion of organic matter at rapid rates, but its limitation is the energy requirements related to evaporation of moisture of the feedstock. On the other hand biological gasification or anaerobic digestion takes place at relatively low temperatures in wet or dry feedstock but usually results in only 50% conversion of organic solids at significantly slower rates than those of thermal gasification (Chynoweth and Isaacson, 1987).

While anaerobic digestion may not utilize as much of the substrate for energy product generation, the by products of the process could be used for other high value applications. For instance, the residual lignin-rich biomass left after AD would be a prime candidate for mushroom cultivation because the digested spent grain would have much lower levels of bacterially-fermentable substrate, and thus the fungi would have much fewer competitors. This secondary usage of BSG by-product would increase the value of the BSG.

One large barrier to the adoption of anaerobic digestion of BSG as a treatment option is the cost. Initial and maintenance costs for anaerobic digesters can be very high. As a reference, a digester that processes 8 million gallons of a hog and heifer manure mixture and produces 28 million cubic feet of biogas annually cost one million dollars. This price includes the digestion tank, the liquid storage tank, and construction and other costs.

Summary

The simplicity of the process is essential for commercial adoption. Direct combustion of biomass or use as an animal feed are the simplest. For combustion, just allow the biomass to dry to sufficient levels and then burn it. As an animal feed, the infrastructure and the general practices are already in place. Anaerobic digestion to form methane and use as a human foodstuff are next. Anaerobic digestion requires a large up-front investment and the technology requires operator knowledge. As a human foodstuff, the regulatory hurdles may be numerous, but breweries are already producing a human consumable. The most difficult of the options is fuel ethanol. First, the grain (a substrate high in lignocellulosic material) must have its cellulose and hemicellulose hydrolyzed. This is really a two-step process where the lignocellulose is physically, chemically or biologically transformed first into cellulose and hemicellulose (with other by-products not important to the generation of ethanol), and subsequently, cellulose and hemicellulose are then converted into simple sugars that can then be fermented into ethanol. Furthermore, the most common industrialized strains of microorganisms that ferment sugars into ethanol will not use pentose sugars as substrates. At a laboratory scale, many organisms have been engineered and shown to be capable of fermenting pentoses to ethanol, but currently, no commercial-scale microbe has been used for this purpose.

One compromise would be to anaerobically digest the grain first, and then belt press the digested spent grain. This digested spent grain would be high in lignin and would have a higher heating value than the raw material and the cellulosic, hemicellulosic, proteinaceous, and fatty components would most likely be degraded in the digester. Furthermore, the effluent from the reactor could be used to grow algae. Chlorophyll has 0.122% magnesium and 0.43%

phosphorus, and the addition of BSG digestate liquid to algal growth media could have a beneficial effect on yield. Many of the nutrients like nitrogen and numerous mineral nutrients in the spent grain are water soluble. After anaerobic digestion of BSG, the liquid effluent could then be used as a fertilizer. One possible usage would be as a nutrient supply for algae cultivation, another very promising biofuel field. Currently, algal research is being conducted in the same facility as this anaerobic digestion study.

Summary of challenge in methanogenesis

Some of the challenges faced in anaerobic digestion are as follows: different environmental optima for different bacteria; rapid pH changes leading to inhibition or process failure; slow degradation time for more complex substrates; and high hydraulic flow rates leading to washout of slow growing bacteria. Proposed here is a modified plug-flow reactor in series with a UASB for efficient conversion of substrates to methane. This system will overcome the previously mentioned difficulties faced in anaerobic digestion in the following ways: the dual reactor design allows for control of the different reactors to optimize the environment for each bacterial process; the high flow-rate of water will prevent the accumulation of acid; the solids of the BSG will be allowed to digest for longer periods of time and methanogenesis will be occurring concurrently downstream; and lastly, the implementation of the UASB for methanogenesis will prevent methanogen wash-out.

Objectives

To test the efficacy of this novel reactor design un to elucidate potential processes that may optimize the digestion, the following objectives will be evaluated:

-To evaluate the progress of the anaerobic digestion of spent grain over time

-To evaluate the effect of grinding as a pretreatment and inoculum choice on digestion

-To evaluate and quantify the effect of by-product inhibition on AD of BSG

-To test various methodologies of high water flow rates and levels on AD of BSG

CHAPTER 3

MATERIALS AND METHODS

Phase I: Basics of anaerobic digestion of brewers' spent grain

In the first set of experiments, ten gram aliquots of spent grain were added to each reactor. After analyzing some of the results, it was noted that by-product inhibition may be confounding the data, and so a lower level of grain, two percent, was used for the second set of experiments (table 3).

Anaerobic digestion of brewers' spent grain at different time points

Very little research has been published on the anaerobic digestion of brewer's spent grain. Consequently, little is known about how fast the initially-insoluble grain is hydrolyzed and transformed into smaller, more readily-digestible molecules. The goal of this experiment is to determine what has happened in the process of digestion at specific time points. To elucidate the rates of breakdown of grain, the following experiment was performed. In the first of two experiments, twelve reactor vessels (which represent three replicates for each of 4 distinct digestion times) were filled with 10 grams of grain and 100mL of oxygen-free water, making the total solids level of this experiment 10%. After 10 days, the first set of flasks was frozen to stop the digestion process. Subsequently, sets of flasks were also frozen after 16 and 30 days. The final set of flasks was allowed to run until April 14, which allowed for 44 days of digestion.

Table 3. Schematic summary of experimental phases in thesis research. Response variable for all treatments were solids digested and volatile fatty acids produced.

Phase	Solids level	Parameter evaluated	Levels
Phase I	10% solids (Set 1)	Time series	10,16,30,44 days
		Inoculum	Rumen fluid,Cow manure, UASB sludge, and a Mixture of the three
		Grinding	No grind (control), 2mm, 0.75mm
		Water volume	2,5,10,20% solids
	2% solids (Set 2)	Time series	5,12,20,26 days
		Inoculum	Rumen fluid,Cow manure, UASB sludge, and a Mixture of the three
		Grinding	No grind (control), 2mm, 1mm, 0.5mm
		Water volume	1,1.4,2,5,10% solids
	Water delivery method	**Water flow rate**	
Phase II	Submerge and purge	1L/day	
		3L/day	
	Drip	1L/day	
		3L/day	

In a second experiment, sets of flasks were frozen after 5, 12, 20 and 26 days of digestion. Furthermore, 2 grams of grain were used instead of ten, making the total solids for this experiment only 2%. At the end of the experiment, sets of frozen flasks were allowed to thaw at room temperature. A sample of the aqueous phase was taken for analysis of VFA content and concentration. The remaining grain was filtered and dried. Three replicated were performed for all of the phase I experiments.

The effect of grinding on AD of BSG

For this experiment and the subsequent two, two different durations were used: short (approximately 20 days) and long (approximately 44 days) duration. The short duration samples contained 2 grams of dried spent grain at the start date (except the percent solids experiment which contained 5 grams). The long duration samples all contained 10 grams of dry spent grain at the beginning of the experiments. All of the following experiments were conducted in 250mL Erlenmeyer flasks with red rubber stoppers unless otherwise noted. With the exception of the inoculum experiments, all samples were inoculated with 1mL of a solution of rumen fluid that had been diluted down ten times. Three replicates were performed for each treatment.

A grinding pretreatment could have an important effect on many factors in anaerobic digestion of spent grain. If a higher proportion of the grain can be degraded after grinding, there is less waste from the process. Furthermore, the increase in surface area of the spent grain could lead to a much quicker digestion if hydrolysis of the larger macromolecules is rate-limiting. By increasing the surface area to volume ratio, the cellulase enzymes would have less steric hindrance which makes the cellulose more available for degradation. Also, the modified rate of substrate usage could shift the production ratios of volatile fatty acids. The grain was prepared as above. The grain was then ground to the described sizes using a Retsch SM200 knife mill, and screens for the appropriate particle size. In the first experiment (long duration), the grind sizes used were 2.0mm and 0.75mm with a "no grind" control group. In the second experiment (short duration), the grind sizes used were 2.0mm, 1.0mm, and 0.5mm with a "no grind" control group.

The effect of inoculum type on AD of BSG

Different inocula will have different levels of the various types of bacteria. In both experiments, the types of inocula tested were rumen fluid, cow manure, granular sludge from a UASB reactor, and a equal-parts mixture of all three inocula. In this experiment, each of the flasks was inoculated with 1mL of a solution containing 10% of the respective inocula. In the mixed culture, 0.33mL of each of the inocula were used.

The effect of by-product inhibition on anaerobic digestion of brewers' spent grain

Reversible chemical reactions tend toward an equilibrium of substrates and products. If the product(s) is continuously removed, then theoretically, a much higher level of substrate utilization can be achieved. In many biological systems, product inhibition leads to less than optimal rates of substrate utilization. Inhibition can occur for many reasons, as noted above in the section on inhibition of anaerobic digestion. Consequently, a more complete digestion of the spent grain may be possible if the by-products are diluted down to a less or non-inhibitory level.

In the first experiment, the solids levels tested were 20%, 10%, 5% and 2%. As in all the other long duration experiments, there were 10 grams of prepared grain added to each flask. Then 50, 100, 200 or 500mL of oxygen-free water was added.

In the second experiment, 10%, 5%, 2.5%, 1.4%, and 1% were tested. In this experiment, five grams of prepared grain were added to each flask followed by 50, 100, 200, 350, or 500mL of oxygen-free water was added, respectively.

Phase II: High water volume throughput reactors

In this set of experiments, two different techniques for a high water throughput were tested: drip irrigation, and submerge and purge. Both of these methods were tested at two different flow rates with four replicates each. All reactors contained 100g of spent grain at the beginning of the experiment. The inoculum used in all of these experiments was rumen fluid. Due to the quantity of water used in these experiments, the water was not purged of oxygen.

Drip irrigation

An apparatus was designed so that 2.5L reactors with an opening at both the top and bottom could be fitted with an inlet for water at the top and an exit port out of which the water would drain. Grain was added to each of the eight reactors. To begin this experiment, the outlet was sealed, the reactors were filled with a liter of water, and 1mL inoculum was added. The reactors were allowed to sit for 4 hours to allow adequate attachment time for the bacteria. Next, the outlet was unsealed and the water was allowed to drain. Dripping commenced for the two sets of reactors at a rate of approximately 2600mL/day and 890mL/day for the high and low flow rates, respectively. Daily samples of the liquid fraction were taken and stored in a freezer for later VFA analysis. At the end of the experiment, the grain was separated from the liquid fraction and then dried in an oven at 105ºC for 2 days. Final masses were recorded, and mass loss was then calculated.

Submerge and purge

In this experiment, the reactor vessels (one gallon glass jugs) were filled with spent grain, and one liter of deionized water was added along with 1mL of inoculum. The tops of the jugs were fitted with an air-lock device which prevents air from flowing into the reactor while at the same time allowing any over-pressure in the reactor head-space to be released. This device is commonly used in the brewing process which also requires anaerobic conditions. The reactors were then allowed to sit for four hours. At the end of four hours, 2L of water was added to the high throughput reactor for a total of 3 liters. No water was added to the low throughput reactor; its volume was maintained at 1L. Each weekday, 1L and 3L were removed and 1L and 3L of fresh, deionized water were added to the low and high throughput reactors, respectively. Samples of the aqueous fractions were taken and stored in a freezer for later VFA analysis.

Analytical methods

Preparation and analysis of the grain

All grain was collected from a local brewery in Athens, GA. The grain was then rinsed multiple times to remove any residual soluble organic matter. The grain was dried for 2 days at 105ºC. Its moisture content, volatile compounds, fixed carbon and ash were measured using a Leco TGA701 proximate analyzer using the method of ASTM D5142.

Alkalinity of brewer's spent grain

To test alkalinity, the ability of a solution to neutralize acids, the following procedure was used. Ten grams of BSG was added to 100mL deionized water. A pH probe was then used to

measure the initial pH of the solution. Then, 0.02M HCl was titrated into the spent grain solution. Drip-wise additions continue until a pH of 4.5 is reached. The volume titrated is then recorded, and alkalinity was calculated using the following formula:

mL HCl titrant * normality HCl * 50,000 / volume (mL) of the sample = alkalinity

Volume of biogas production

The volume of biogas produced was measured by water displacement. A needle connected to a tube leading to the apparatus was inserted through the rubber stoppers of each of the tubes. An over-pressure in the head-space of the reactor vessels, the Erlenmeyer flasks, led to a positive flow of biogas from the reactor into the head-space of the reactor until equilibrium was reached.

Biogas composition

The composition of the biogas was measured using an HP 5890 Series II gas chromatograph with the following procedure. A thirty microliter sample was taken from the head-space of the reactors and directly injected into a gas chromatograph with an Alltech Porapak Q 100/120 packed column of dimensions 6' X 1/8" X 0.85" stainless steel with oven, inlet and detector temperatures of 90ºC, 100ºC, and 140ºC, respectively. The detector used was a thermal conductivity detector, and runs were set for three minutes. The areas under the curve at the respective peak locations corresponded to the level of each of the gases.

Volatile fatty acid analysis of the samples

All volatile fatty acid concentrations were examined using an HP 5890 Series II gas chromatograph with an FFAP column with dimensions 30m X 0.25mm X 0.25um (film thickness) and a flame ionization detector. The oven, inlet and detector temperatures were 100ºC, 190ºC, and 200ºC, respectively. The flow rate was set to 2mL per minute, and the runs lasted ten minutes. A standard curve for each of the following fatty acids: acetic, propionic, n- and iso-butyric, n- and iso-valeric acids. The area under the curve at the location of each peak is proportional to the concentration of the acid.

Removing oxygen from the water

For the initial experiments, the water was heated to its boiling point and then nitrogen gas was bubbled through it for a period of at least 15 minutes. This was done to remove all oxygen from the containers to prevent harm to oxygen-sensitive microbes.

Measurement of the mass change of BSG

At the end of the experiments, the grain was filtered and then dried in an oven for 2 days at 105 degrees Celsius and then massed. The change in masses were used to assess microbial degradation.

Statistical analysis

All statistical analysis was performed using SAS 9.2 software distributed by the SAS Institute Inc. Primarily, multivariate analysis of variance (MANOVA) tests were performed.

CHAPTER 4

RESULTS AND DISCUSSION

Phase I

Two different sets of experiments for each of the four experimental groups were performed: two different levels of BSG were used, 10 grams in the set 1 experiments, and either 2 or 5 grams in the set 2 experiments. In order that the comparisons made herein are using an appropriate and equivalent measure of the differences occurring in the reactors, across the two experiments, the concentration of VFAs will be given in mM per gram BSG added. In all the following figures, data points are the average of three replicates, and the error bars represent one standard deviation above and below the average.

Time series

Biomass reduction over time

In the first set of experiments, there was no statistical difference in biomass degradation over time. Although, the last time point, 44 days, appears to have much more degradation than the other three (data not shown). The second study on the time series degradation of BSG yielded compelling data (figure 1). The data collected in this group has the greatest statistical power of any study that was performed ($p<0.0001$). The amount of mass removed through anaerobic digestion increases with time, however, most of the degradable biomass was digested

by day five. Even up to the last data point, however, the digestion of the BSG continued. For a more accurate picture of the progression of the digestion, a longer time series would need to be performed.

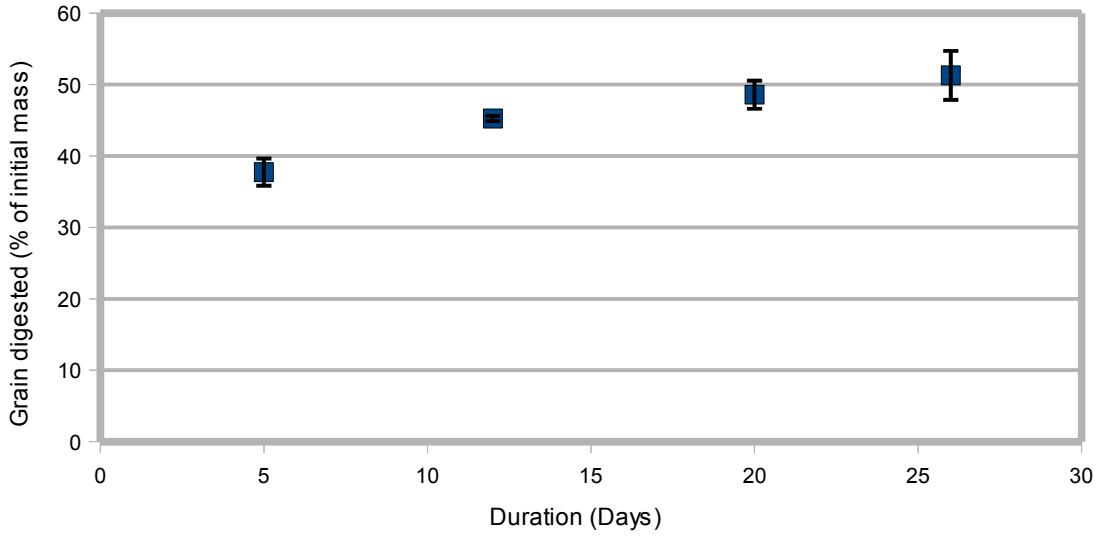

Figure 1. Level of digestion of spent grain at various time points

Volatile fatty acid production at various time points

Ten percent solids

By day 10 of the first time series experiment, the concentrations of both acetic and butyric acid had reached approximately 7.6 mM and 6.0 mM, respectively, which was close to the maximum concentration level for each of the two acids (figure 2). The level of these two acids increased marginally at day 16, but did not go up much after that point. Statistical analysis shows that there is no change in the levels of VFAs after day 10.

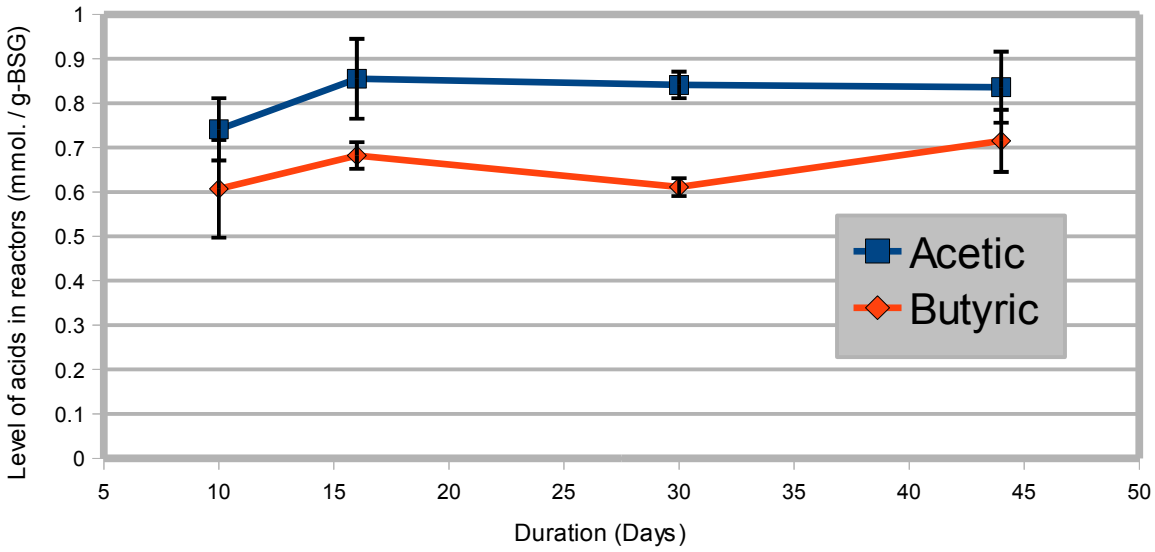

Figure 2. Concentration of acetic and butyric acids over the course of anaerobic digestion

Propionic acid, the VFA most commonly used to determine the state of reactor performance and stability, reached almost 1.5 mM by day 10 and decreased until day 30 (figure 3). The next sample, taken on day 44 at the end of the experiment, had a propionic acid concentration of over 0.3mM, double its previous maximum value. This could indicate that the osmotic stress of the acidic reactor was starting to decrease the activity of the relatively more sensitive propionate-degraders. Another explanation is that some of the complex substrates that lead to the formation of propionate were beginning to be broken down. This hypothesis is more plausible because propionate-degraders are usually less common in reactors, and propionate-utilization usually does not occur until acetate and butyrate have been utilized first.

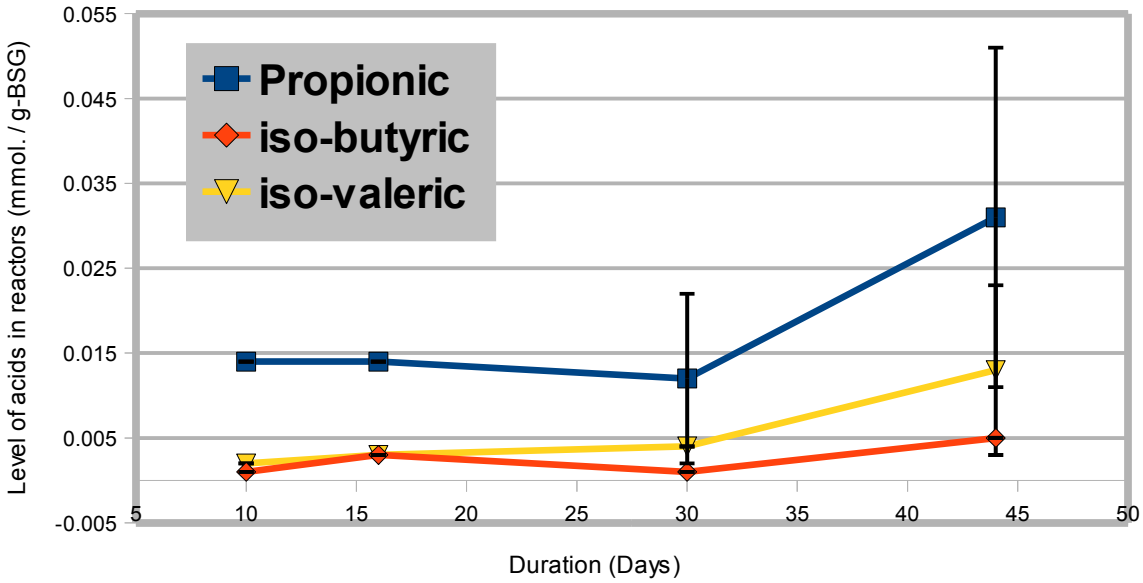

Figure 3. Concentration of less-predominant acids over time in digestion of spent grain

Two percent solids

In the second experiment investigating the breakdown of BGS over time, the initial level of BSG was 2%. This change had an important effect on the level of acids in solution. In the first experiment, the total level of the two most prevalent acids, acetic and butyric, reach a maximum after only 16 days. In this second experiment, the levels of both acids climbed for 20 days, and the level of acetic acid continued to increase until day 26 (figure 4). In the two experiments, the quantity of butyric acid produced per gram BSG added is about equal. However, the level of acetic acid in the set with 2% BSG is more than double that of the 10% BSG set after approximately four weeks.

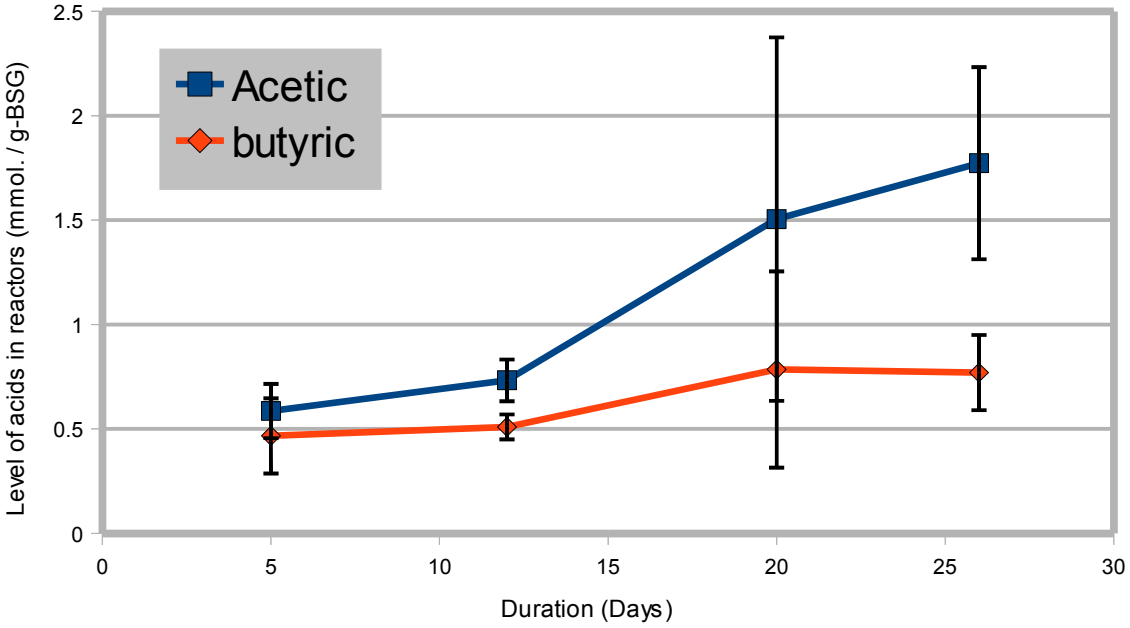

Figure 4. Concentration of acetic and butyric acids as a function of time

Inoculum

Biogas production as a function of inoculum type

Biogas volume in the various experiments has been variable and inconsistent with other data. Before beginning, it is important to reiterate that the most important measurements in these experiments were the total amount of grain solids that was digested and the level of VFAs in the aqueous phase in the reactors at the end of the experiments. Volume and content of biogas may be important supplementary information indicative of underlying processes (figure 5).

The methane/carbon dioxide analysis using the gas chromatograph revealed that the majority of the biogas appears to be carbon dioxide. No methane was detected in these samples. This finding shows that methanogenesis has yet to occur at an appreciable level. This is not a problem because, as mentioned before, the purpose of this work is to hydrolyze the substrate as

efficiently as possible to generate VFAs. The production of methane from VFAs will occur downstream from the work conducted in this research.

Biogas volumes in the shorter duration experiments were only measured a few times (data not shown). Furthermore, since it was determined in the first set of experiments that none of the biogas being produced contained methane and that the majority of the gases in the biogas were either nitrogen or carbon dioxide, no further measurements of biogas composition were made.

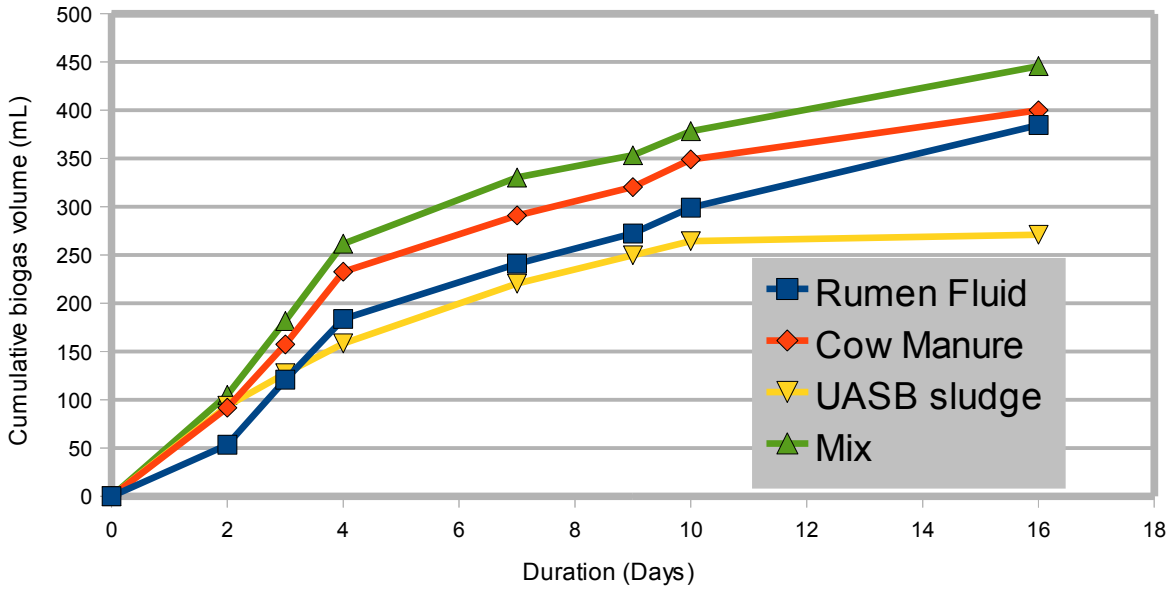

Figure 5. Cumulative biogas production levels of various inocula

In all trials, the biogas volume increased rapidly in the first seven days. After that point, the rates of gas production leveled off for some of the samples (figure 5). All samples increase slightly between day 10 and day 16 except the UASB sludge. One outlier exists in the data, and when it was removed, the cumulative gas production in the various inoculum types was significantly different at the 0.05 level. Furthermore, the removal of this outlier is perhaps

justified because the rubber stopper cap from that reactor had a crack in it. It could have been replaced, but it was noticed after a few days into the experiment, and replacement would have disturbed the reactor. Perhaps the over-pressure gases escaped, thus causing the erroneous measurement.

Biomass reduction as a function of inoculum type during anaerobic digestion

Ten percent solids

The effect of inoculum was not statistically significant in this set. However, as anticipated, the rumen fluid and cow manure performed similarly, while the UASB sludge led to less BSG mass loss.

Two percent solids

The second study of the effect of inoculum produced results similar to the first experiment (figure 6). The amount of biomass reduced by the UASB sludge was lower on average than the other three experiments. However, all mass losses were approximately 50%, and the means were not significantly different.

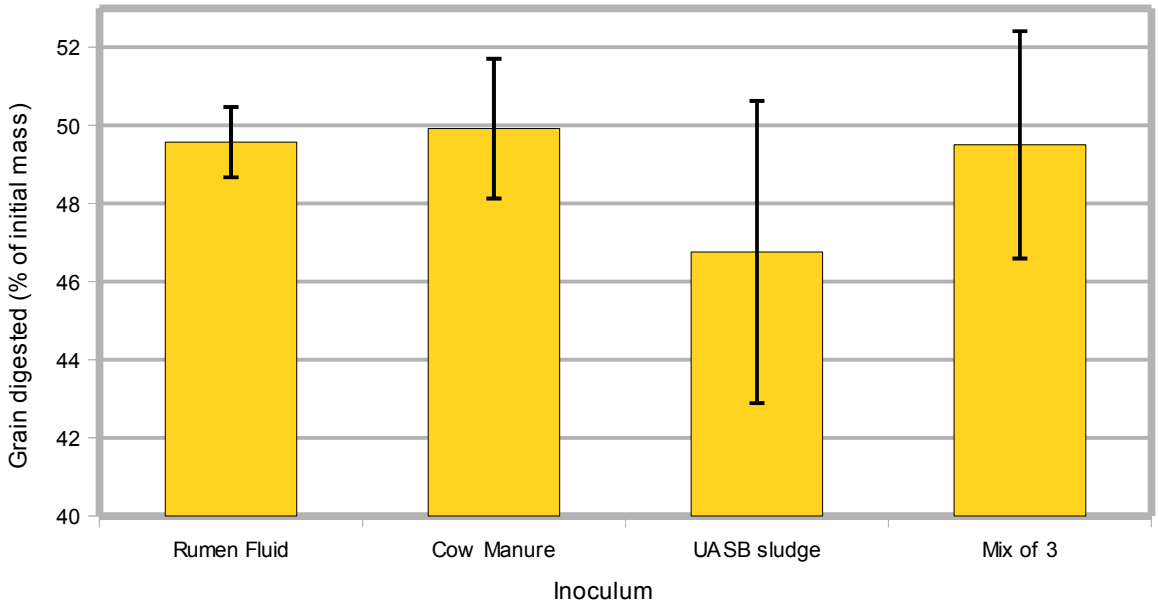

Figure 6. The effect of inoculum on the amount of grain digested

Volatile fatty acid production differences between various inoculum types

Ten percent solids

On day 4 of the first inoculum experiment, the acetic, propionic, and butyric acid levels were comparable throughout the various types of inoculum at levels of 2-3mM, 0.5mM, and 3-4mM, respectively. No statistical difference was apparent ($p=0.1417$). At the start of the second week, however, three other acids, iso-butyric, iso-valeric and n-valeric were present. Even then, there was no discernible difference in the levels of VFAs ($p=0.5103$). There was a discernible difference in the concentration of propionic acid when taken alone ($p=0.0108$, figure 7).

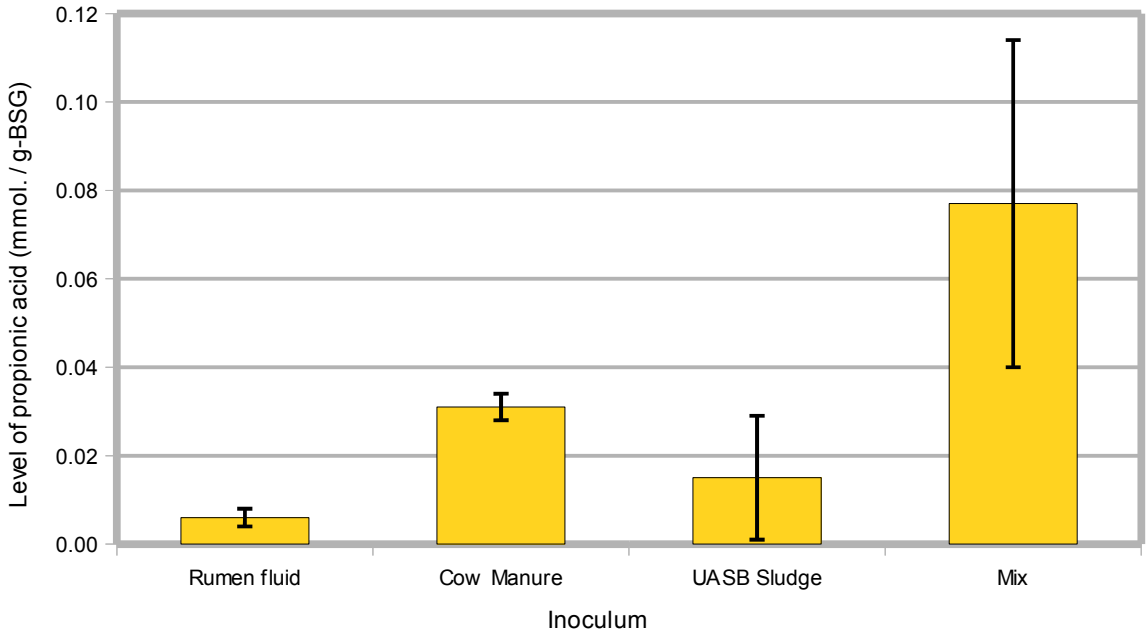

Figure 7. Propionic acid production of different inocula (Day 16)

Increased levels of propionic acid, as mentioned in the literature review, have implications for reactor stability. Generally, as propionic acid increases, the chance of reactor failure increases, as well. Here, it is unclear as to whether the cow manure and mix inocula are generating propionic acid quicker than the other two, or simply contain environments less conducive to the growth of the sensitive propionate-reducers. This remains an unanswered question.

Two percent solids

In the second experiment, the type of inoculum had a very significant effect on the level of VFAs in solution ($p=0.0008$). Of the two most prevalent acids, acetic and butyric, only the acetic acid levels varied between the treatments ($p=0.0262$, figure8). Across all treatments, the butyric acid did not differ and was between 7-8mM (per g BSG).

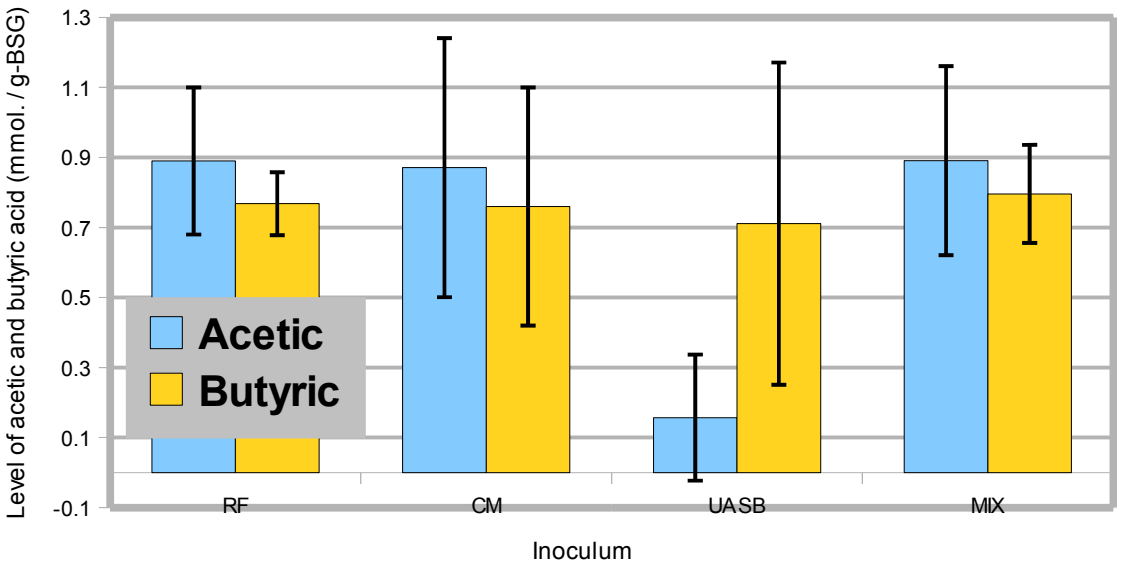

Figure 8. Concentrations of acetic and butyric acids in reactors with different inocula (Day 7)

The reactors inoculated with UASB sludge did not produce nearly the level of acetic acid as did the other two inocula. Because the 'mix' inoculum had similar levels of acetic acid as the other two inocula, it can be concluded that the UASB sludge probably did not contain some sort of compound inhibitory to the production of acetic acid. Rather, the UASB sludge probably lacked the proper balance of the appropriate bacteria to effectively produce acetic acid.

Figure 9 shows the propionic acid in solution after only seven days. Neither the rumen fluid nor the UASB sludge had produced any propionic acid at this point. Both the cow manure and the mixed inoculum possessed propionic acid which suggests that the bacteria that produce propionate are in higher concentrations in the cow manure than in the other inocula.

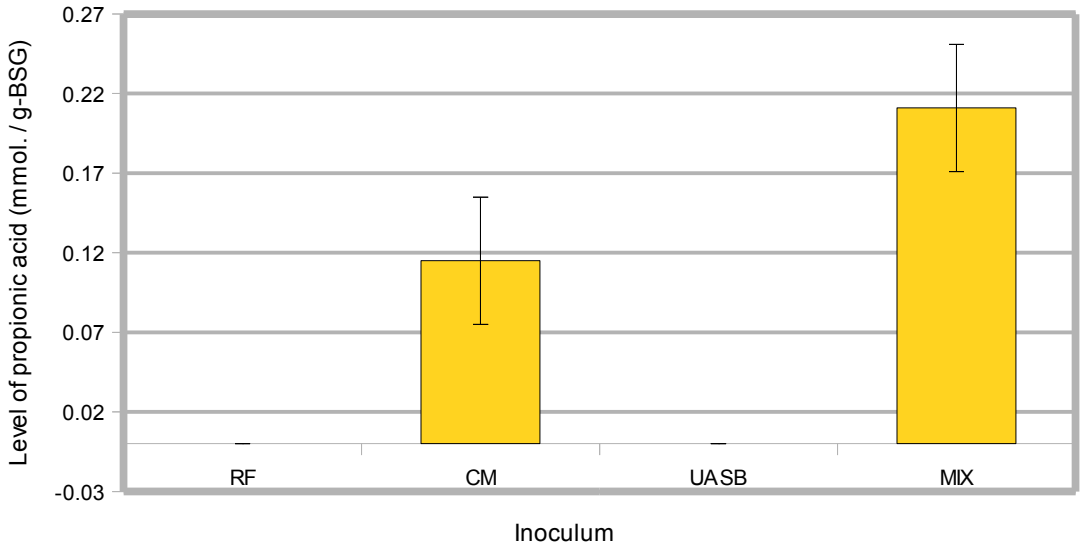

Figure 9. Propionic acid levels of reactors with different inocula (Day 7)

While elevated propionic acid levels can be indicative of reactor imbalance, that does not appear to be the case here. In both experiment 1 and 2, the increases in propionate is correlated with increases in acetic acid. On day seven both the cow manure and the mixed inocula have elevated levels of propionic acid, but on day 22, the two most abundant VFAs, acetic and butyric, are higher than or approximately equivalent to the other inoculum types (figure 10).

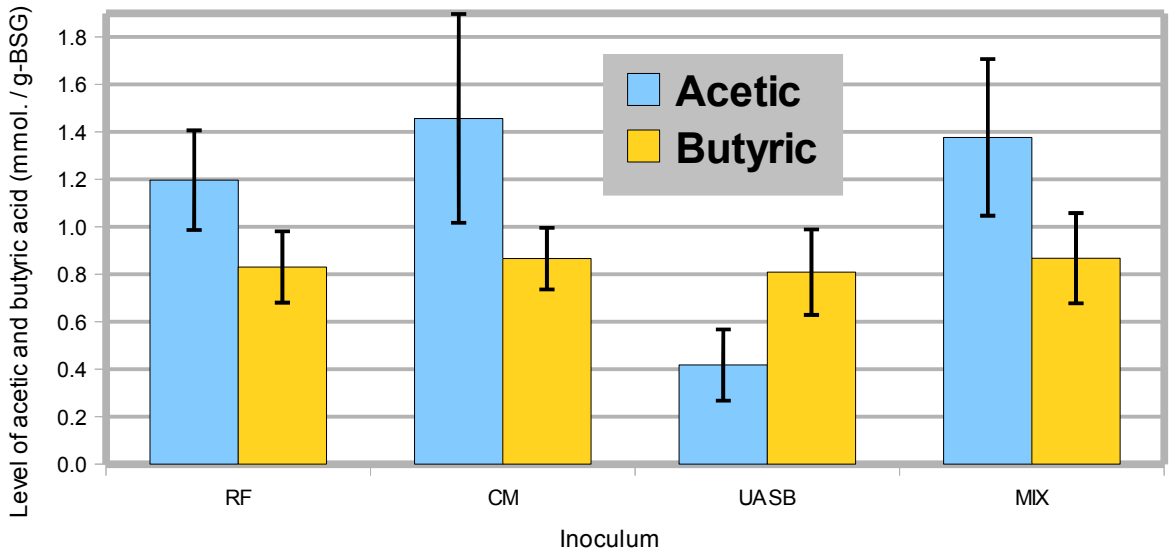

Figure 10. Concentrations of acetic and butyric acids from reactors with different inocula (Day 22)

This result suggests that, in this case, the propionate is not correlated to decreased reactor performance at these low levels. From these graphs and the statistical analysis, the opposite seems to be the case: increasing propionic acid levels are correlated with increased production of acetic acid. This finding corroborates the results from experiment 1.

Grinding

Biogas production as a function of grinding

From the first experiment of the effect of grinding on the AD of BSG, the biogas production is seen in figure 11.

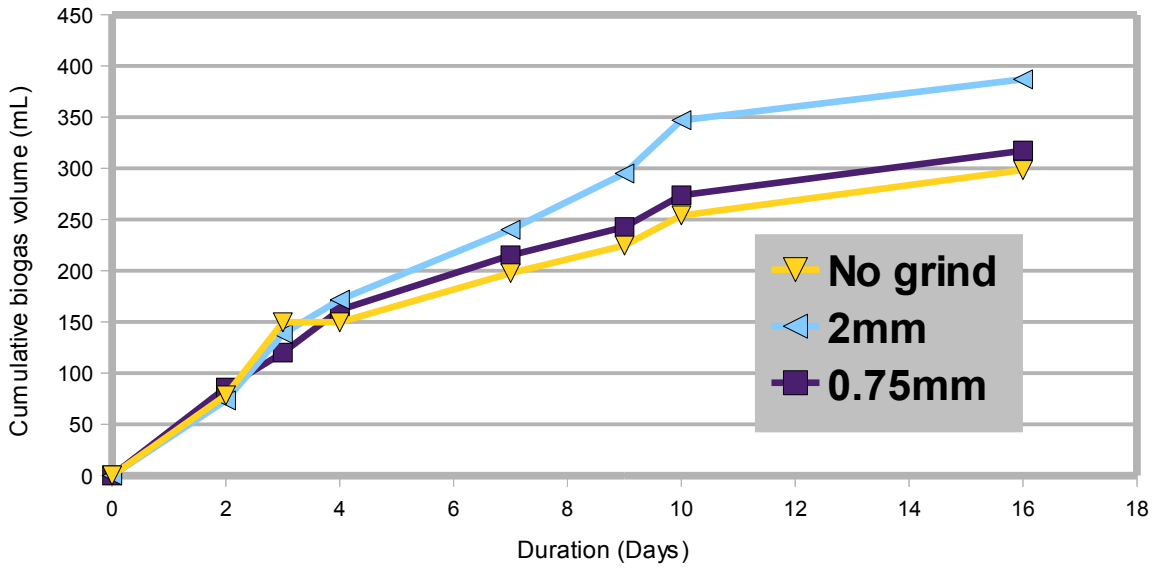

Figure 11. Effect of grinding on cumulative biogas production over time

From this graph, it appears that the biogas production is continuing and has yet to level off. While the moderate grinding level appears to be producing the most gas, the differences between the three groups are not statistically significant.

Biomass reduction as a function of grind size

For the first experiment, grinding of the substrate had no effect on the mass lost in the course of the digestion of the BSG. The effect of grinding in the second experiment yielded significant differences ($p=0.0282$). Counter-intuitively, however, grinding has a negative effect on the anaerobic digestion of spent grain (figure 12). That is, the finer the brewer's spent grain is ground, the lower the overall removal of spent grain from the reactors. Also important to note is that the standard deviations for this experiment were very low which increased the robustness of these results. One obvious possible explanation for these results is that the grinding of the grain

initially increases the digestion rate. Consequently, the rapidly growing fermentative bacteria grow and metabolize the available substrates, and create a very acidic environment. As a result, other bacteria that usually use the acids as a substrate are inhibited, and the acidified environment is not brought back to an equilibrium. Thus, the whole process is functioning at lower than optimal levels.

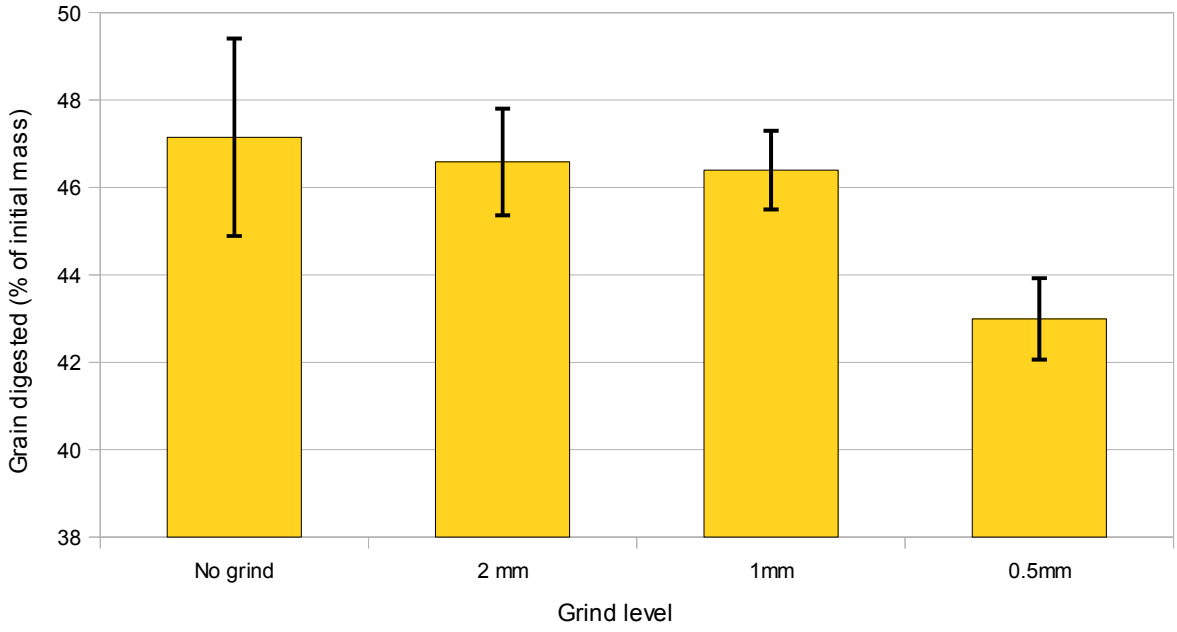

Figure 12. Effect of grinding on biomass reduction

Volatile fatty acid production as a function of grinding

After four days in experiment one, there is already significant difference in the concentration of VFAs across the levels of grinding (figure 13).

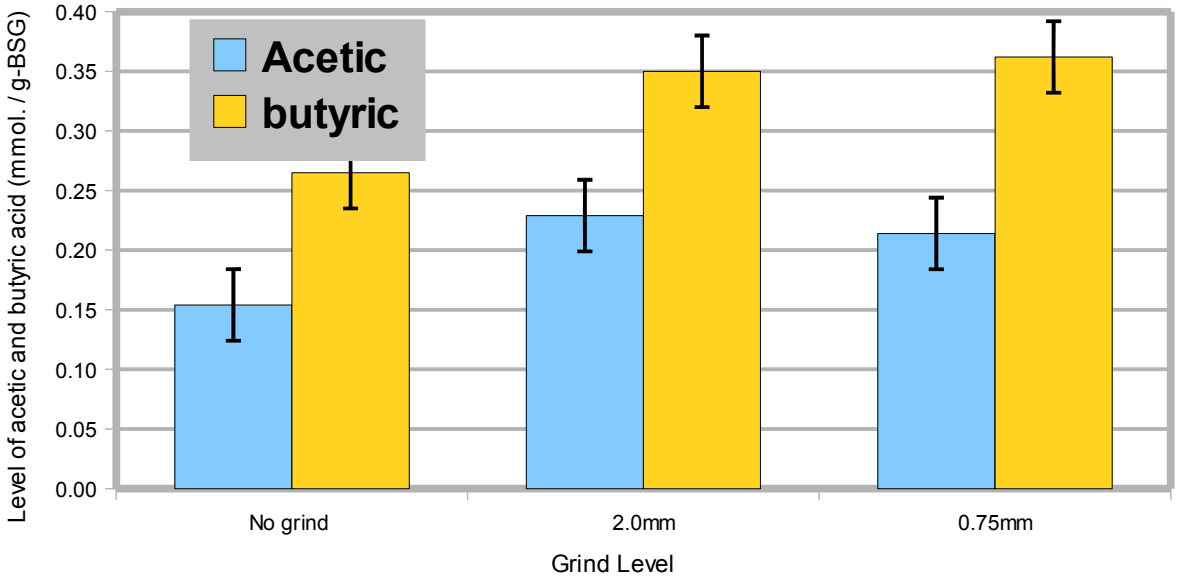

Figure 13. Concentrations of acetic and butyric acids for different levels of grinding (Day 4)

The two grinding treatments had increased levels of butyric and acetic acids compared to the "no grind" control. Grinding increases the surface area to volume ratio of substrates and subsequently has an effect on the progress of digestion. Interestingly, while the types and concentrations of acids produced varied with the different grind sizes, the mass loss (i.e. – the amount of BSG digested) was not significantly different. This result shows that grinding has an effect that could be potentially very important in larger-scale digestions of not only BSG but of all lignocellulosic material. The grind size could be modified and tailored to fit a specific need: if more VFAs are desired in the aqueous phase, grind to a certain level. The day 16 results show a similar trend, each group having approximately 2-3 times the levels of each acid.

Propionic acid in this first experiment increased with increasing grinding. Both the day 4 and day 16 results show this trend (figure 14).

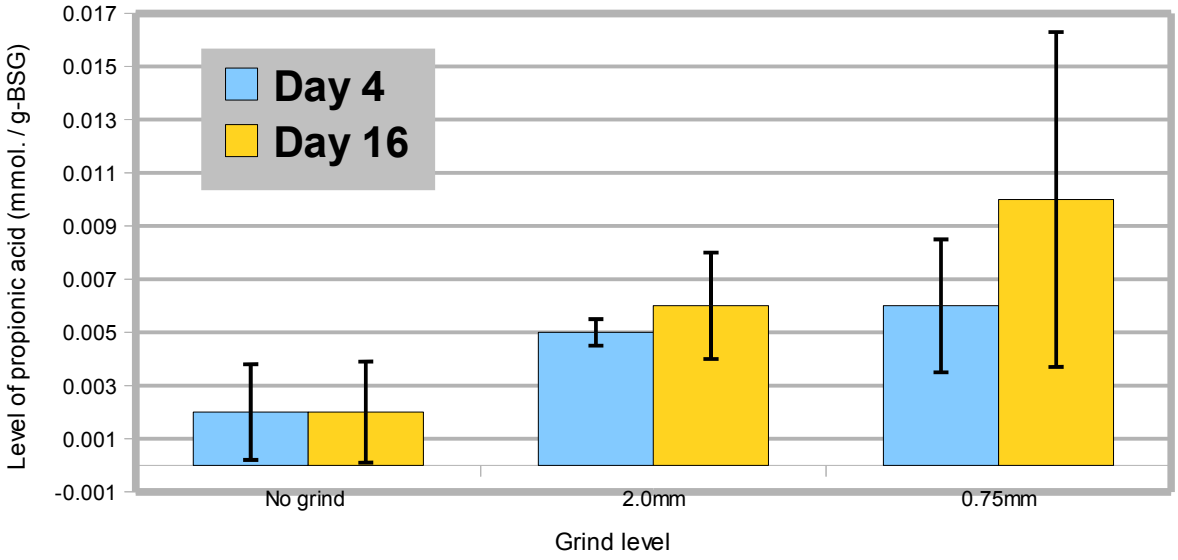

Figure 14. Concentration of propionate for different levels of grinding

In this case, increased propionate levels may be an indicator of reactor stress. This hypothesis is corroborated by the fact that the variability of the VFA production also increases with increasing levels of grinding.

Two percent solids

The second time the grinding experiment was performed, different results were obtained. After five days, there was no significant difference in the level of VFAs ($p=0.8050$). After almost three weeks, the levels of acetic acid decrease with decreasing particle size which differs from the findings of the set 1 experiment (figure 15). However, this result is not statistically significant ($p=0.1548$) because the intra-grind variability was too high. Further experimentation is necessary to confirm the previous finding that increased surface area to volume ratio leads to increases in VFA production.

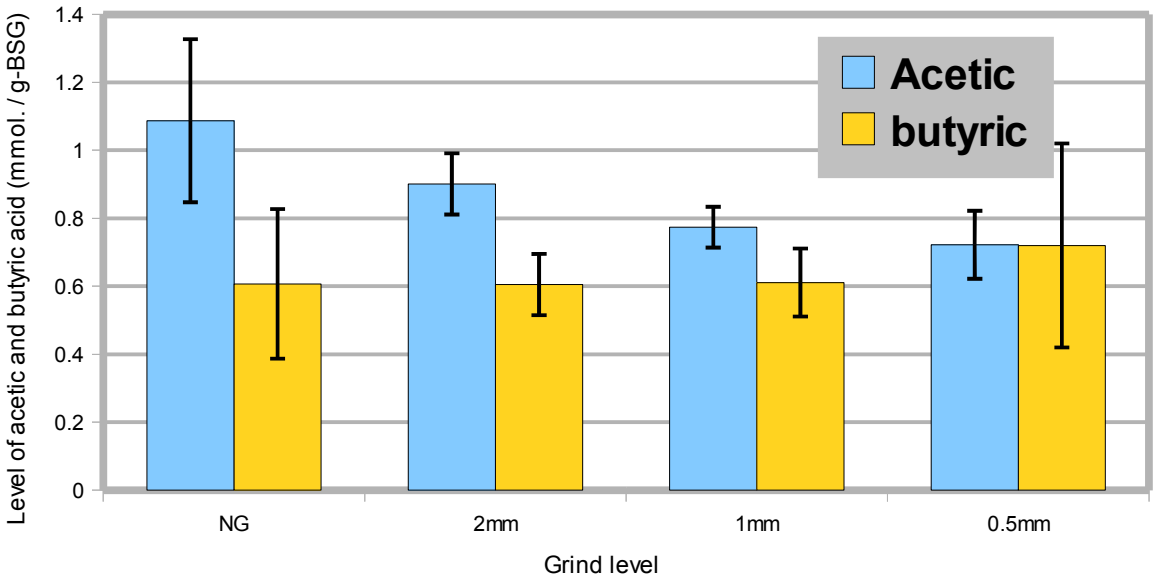

Figure 15. Concentration of acetic and butyric acids over different levels of grinding (Day 20)

Another interesting finding from this experiment is that the level of propionate in the reactors decreases with more grinding (figure 16).

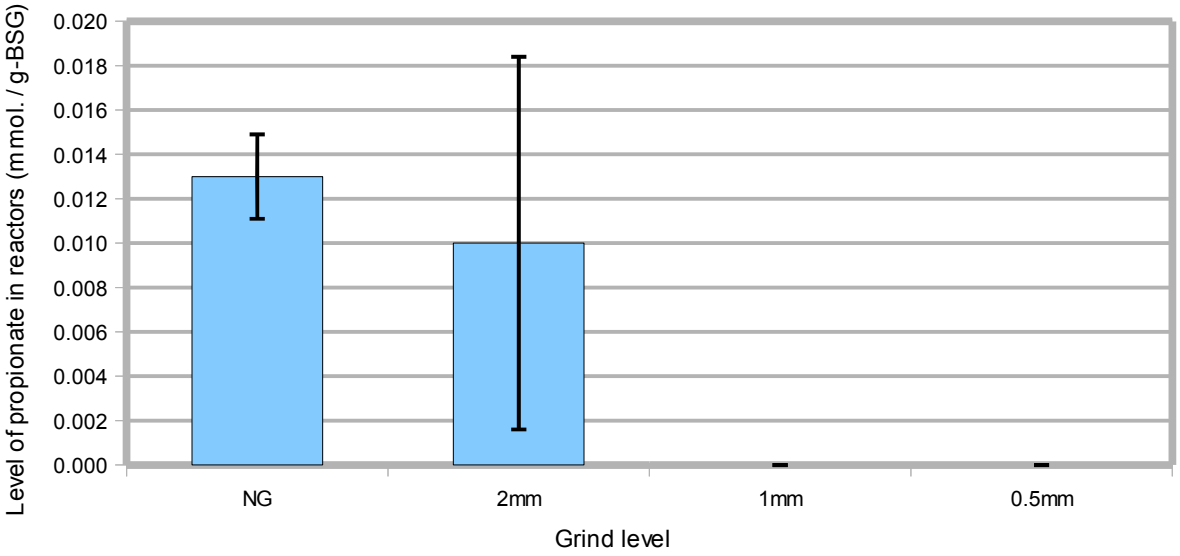

Figure 16. Concentration of propionate for different levels of grinding (Day 20)

This finding is statistically significant (p=0.0115) and offers contradictory evidence to the findings of the first experiment. One reason for this apparently contradictory finding is the differing levels of total grain solids. The first experiment had 10% solids while the second only had 2%. Since the experiments are very similar in all respects except percent solids, it is hypothesized that lowering the percent solids causes an interaction effect with grinding. Further testing would be necessary to make a more reliable conclusion as to the interrelationship between percent solids and grinding and their combined effect on VFA production.

This possible interaction effect of percent solids and grinding has important implications for the second phase of experimentation. At lower percent solids levels, grinding may actually have an inhibitory effect on the production of VFAs. Consequently, not grinding the substrate would yield a higher level of VFAs which is economically beneficial for the economic analysis of anaerobic digestion of BSG because grinding BSG would require a drying stage followed by grinding in a mill, both of which are energy intensive and would cut into the economic benefit of this technology.

Water volume

Biogas production as a function of water volume

The goal of the final experiment of phase I is to test the effect of by-product inhibition. This was tested by using a constant inoculum, size and mass of grain, but a varying amount of water. The hypothesis was that after a given amount of BSG digestion, the by-products (acids, alcohols, etc.) will be in much lower concentrations in the reactors with higher levels of water; consequently the bacteria will experience less inhibition.

In this experiment, the 500mL biogas volumes have not been measured because the caps for this flask size were not the same as the red caps. Also, the in the 200mL series, one outlier produced hardly any gas, and consequently, it is bringing down the average of the whole series. When the outlier is removed, the gas production for 200mL is comparable to the 100mL. Furthermore, gas volume production measurements are taken by removing any over-pressure in the flasks. The 50mL and 100mL trials are in 250mL Erlenmeyer flasks, where as the 200mL replicates are in 500mL flasks and the 500mL reactors are 1000mL flasks. The head-space in the various experiments may be causing inconsistencies from group to group. The important measure in this (and all experiments) will be the total amount of grain "consumed" by the bacteria (i.e. – removed from the solids BSG and converted into energy or soluble compounds).

Biomass reduction at various water volumes

Ten percent solids

The water to grain ratio experiment had results that showed significant difference between the treatments ($p<0.0001$) with no obvious outlying data points. As water volume increased, the amount of spent grain digested increased as well (figure 17).

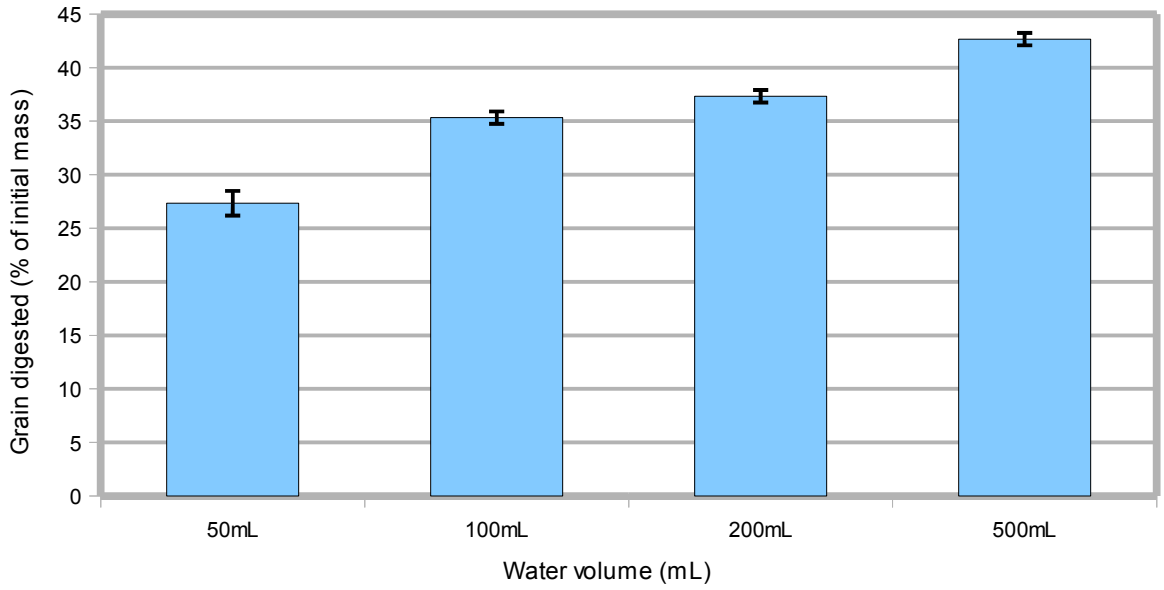

Figure 17. Effect of the volume of water on the biomass digested (Ten percent solids)

A few notes must be made in this analysis. Firstly, the batch reactors with only 50mL volume were at 20% solids, approximately the level of moisture that spent grain comes from the brewery. Further, there was moisture but hardly any "free" water in the reaction vessel which certainly reduced the motility of the bacteria. This decrease in motility and thus decrease in availability of substrate could represent a confounding factor in the level of degradation of the spend grain. Furthermore, 20% solids is well above what is generally regarded as practical in any set-up other than currently experimental "high solids" AD, which had yielded mixed results, at best (Abouelenien et al, 2009a; Abouelenien et al, 2009b; Guendouz et al, 2008).

With respect to the largest volume reactors which had 500mL water (2% solids), black rubber stoppers were used instead of the red rubber seals that were used to cap the flasks of the other water levels. This represents a deviation in the standardization of the process. The black

rubber stoppers could have let in oxygen-rich air which could have caused aerobic, or at least micro-aerobic, degradation of the BSG which may be more complete than anaerobic digestion.

In the first experiment at 20% solids, approximately 27% of the solids were removed from the dry matter during the course of the digestion. While this is a much greater mass removal than the control, it is a much lower removal than the other experimental groups.

Two percent solids

The results for the second experiment on the effect of water:grain ratio on mass reduction were not statistically significant ($p=.1079$). While this is almost significant at the alpha=0.1 level, the intra-trial variability was large which lead to a failure to reject the null hypothesis of no significant difference between the means of the different water levels. However, there does appear to be a trend in the mass digested: as the water volume increases, the amount of grain digested increases up to a point and then appears to level off (figure 18). The least amount of mass solubilized is 36% at the 10% solids level, the highest solids level in this experiment. Consistent in both experiment 1 and 2 is a modest jump in mass digested when moving from 10% to 5% solids. In both the first and second set of experiments, a ceiling around 40% mass loss is reached for reactors at or below 2.5% solids.

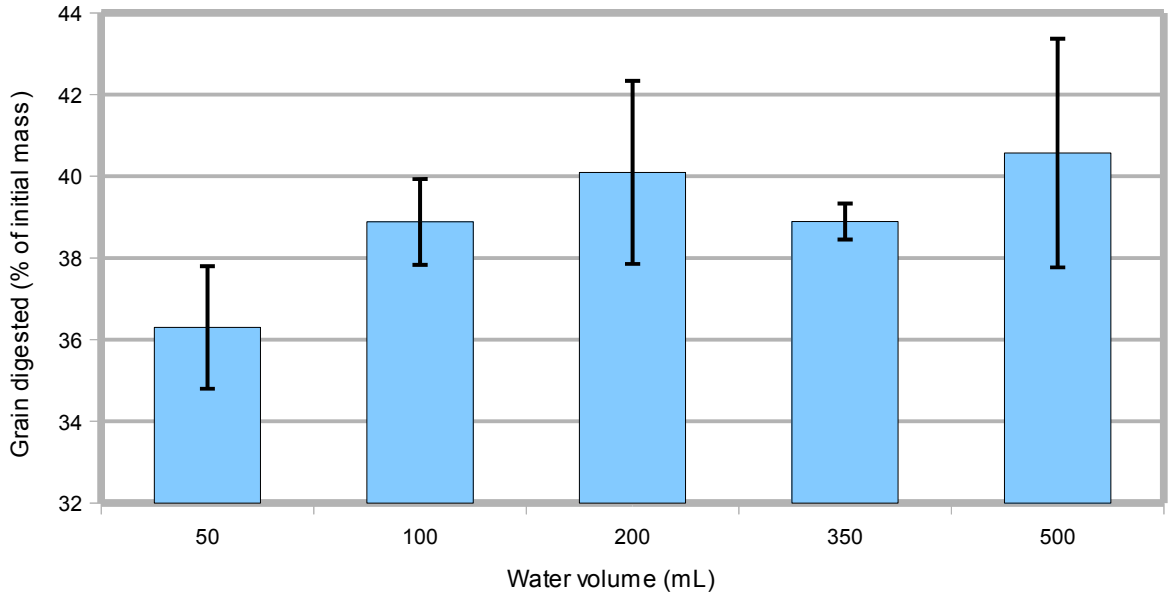

Figure 18. Effect of water volume level on biomass digested (Two percent solids)

Volatile fatty acids production at different water volumes

Ten percent solids

For the first set of water volume experiments, four different percent solids levels were used: 2, 5, 10 and 20%. Because by-product inhibition is expected to cause its effects on a logarithmic scale (similar to that of pH), the X-axis for the following graphs will be the log base 10 of the percent solids. VFA levels were measured for day 16 and day 44 for set one. Both show a similar graph for acetic and butyric acids (figure 19).

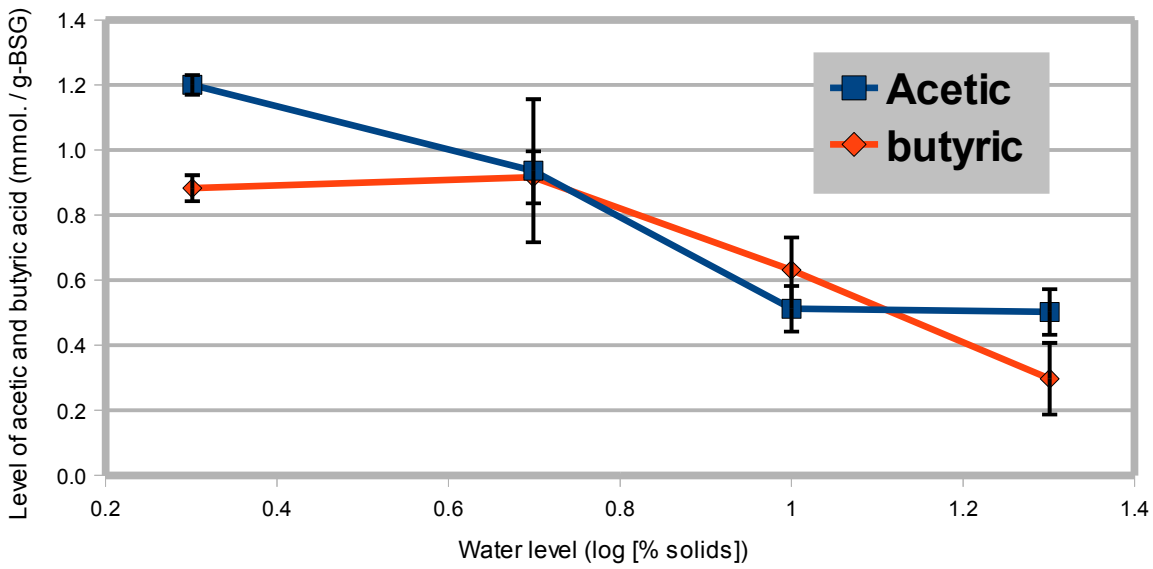

Figure 19. Levels of acetic and butyric acid production for different levels of reactor total solids

As the percent solids increases, the amount of butyric and acetic acids decrease. This is exactly what would be expected if the hypothesis of by-product inhibition is correct. The graph for day 44 has a very similar trend (not shown). Propionic acid also yields important results (figure 20).

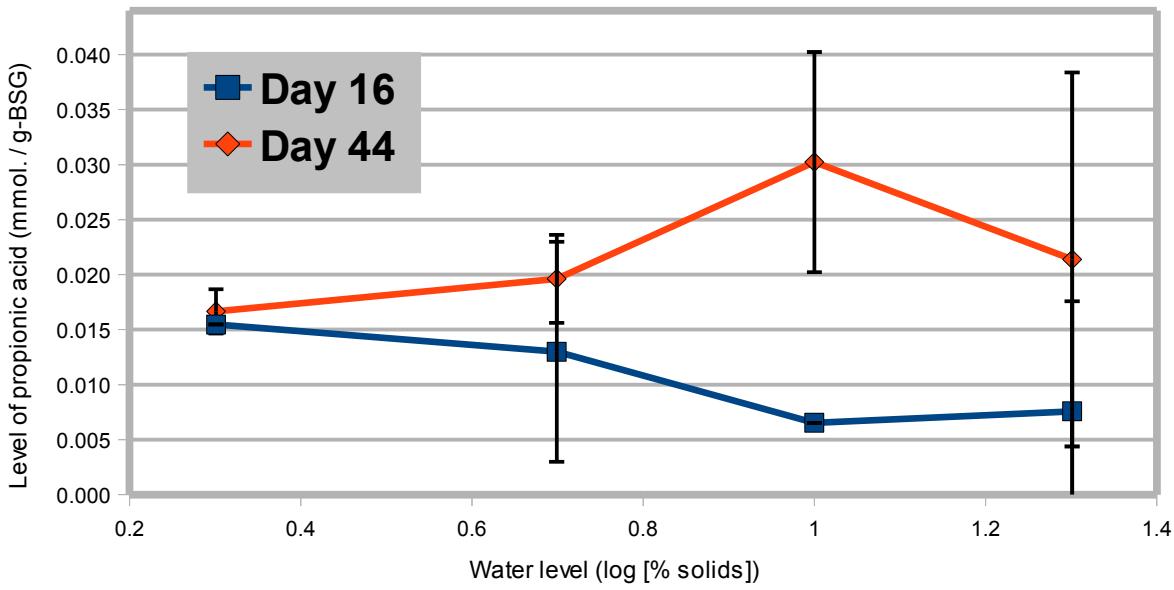

Figure 20. Propionate concentrations at different percent solids levels for two time points

For the day 16 measurements, the data corroborate the hypothesis of by-product inhibition. The day 44 line appears to be a mirror image and would suggest that the hypothesis might not be correct. One alternate interpretation is that the lowest percent solids reactors maintained a constant level of propionate because they were able to utilize all of the newly-produced propionate, thus maintaining a homeostasis. Whereas the next two higher levels of percent solids experienced some amount of by-product inhibition that prevented the propionate-utilizers from adequately converting the propionate into acetate. The highest level did not show as much of a jump in propionate from day 16 to day 44. This could be because the 20% solids level had reached such a low pH that even the acidogens were somewhat inhibited.

Two percent solids

The second set of water volume to grain ratio experiments yielded significant results ($p=0.0476$) after two outliers were removed. The acetic acid levels were significantly different over the different water volumes ($p=0.0184$) but the butyric acid were similar (figure 21).

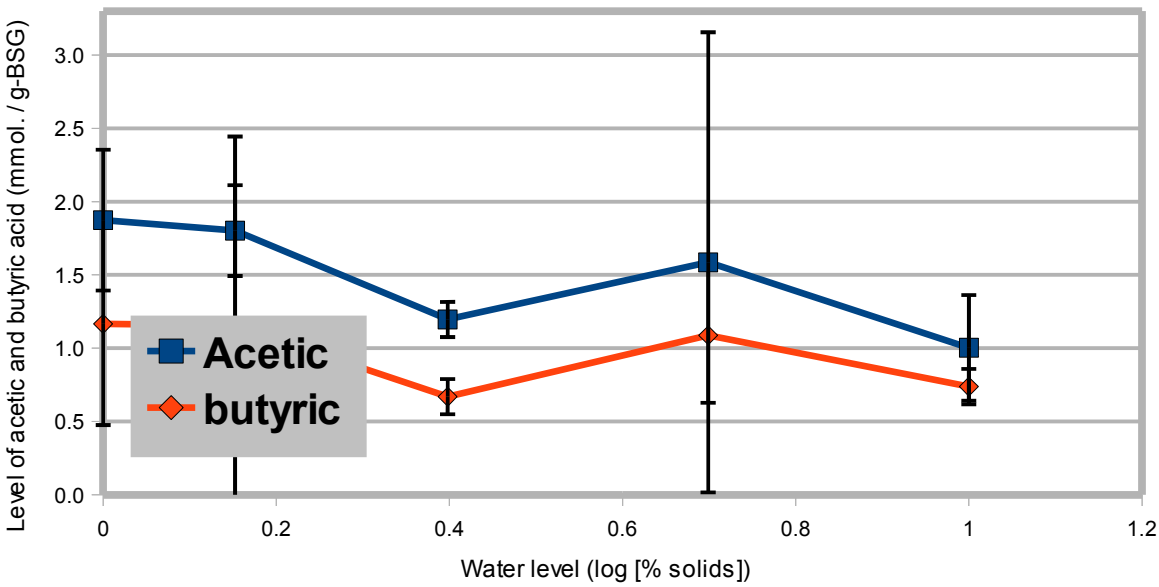

Figure 21. Acetic and butyric acid concentrations for different percent solids levels (Day 20)

The results of this second experiment, while significant, are more difficult to interpret than the easily seen correlation of the first water volume experiment. Some unknown factor may have affected the 2.5% solids level (the middle points in the graph above) which caused the levels of acids to be reduced. The other four solids percentages follow a curve reaching an asymptote. Once again, propionate may be a predictor of reactor stress; after 20 days, only the 5% and 10% solids reactors have any detectable propionate. These experimental data generally

corroborate the hypothesis that lower solids percentages prevent by-product inhibition and lead to higher yields of VFAs.

Another finding that supports the by-product inhibition hypothesis is the changing of the standard deviation with water volume (figure 22). The majority of graphs follow this general trend.

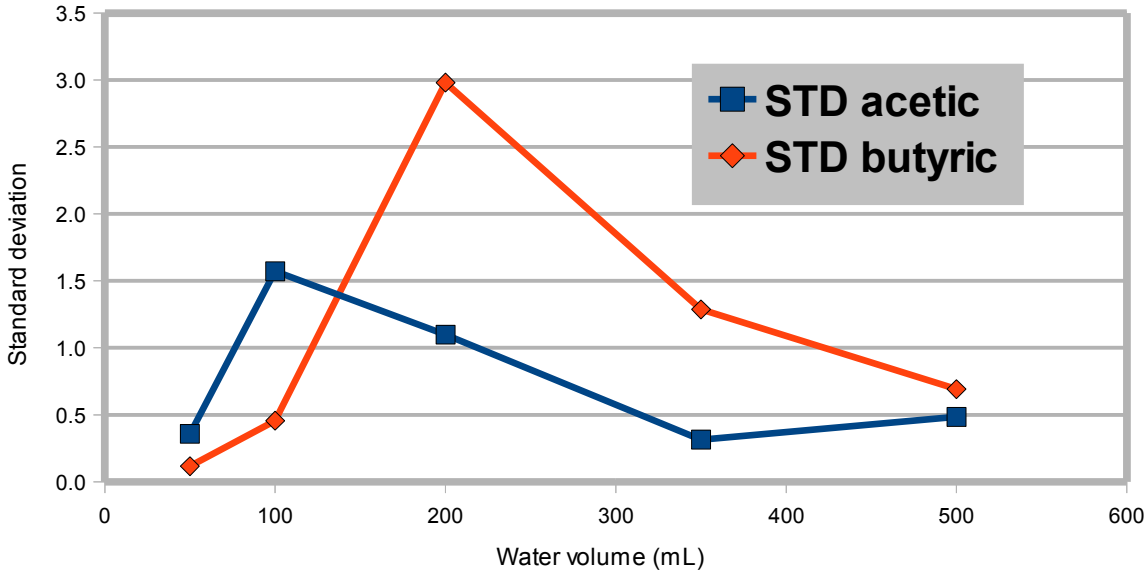

Figure 22. Standard deviation of acid production for differing levels of water volume

One possible explanation for this parabolic shape is that by product inhibition is very high, at the lower volumes of water like 50mL and 100mL. At the 200mL level (5% solids), variation might be high because of stochastic events like the presence of sensitive bacteria in one reactor and less sensitive bacteria in others. As the water level is increased, the amount of variability among reactors of the same treatment goes down. This translates to a greater process stability which overcomes one of the most difficult hurdles in anaerobic digestion systems.

Phase II

In this second phase investigating the anaerobic digestion of brewers' spent grain, two different levels of water flow (differing by a magnitude of approximate three times) are used for two different types of water flow: drip-irrigation, and submerge and purge.

Biomass reduced

The mass losses between all of the various treatments for phase II were not significantly different. The mass loss averages for the drip flow reactors were not significantly different from one another. Phase I showed that after a certain level of water:grain ratio, the grain mass lost reached an upper plateau. Considering that the amount of water used in both treatments of this experiment is as high or higher than the maximum of the phase I, it is concluded that both levels of water in this drip experiment may have been in the plateau region. Furthermore, the levels of water in these experiments may have been excessive, and the process is most likely not optimized. A more sparing use of water could be achieved with a lower flow rate. This lower flow rate would probably yield an equivalent level of grain degradation and a smaller volume of water for treatment in the UASB.

The submerge and purge method for grain digestion yielded similar results to those of the drip flow reactors: there was no significant difference in the mass lost between the reactors with one liter and the reactors with three liters of water. Similar to the previous analysis, this process is probably not optimized for water volume. Both of these experiments probably lie in the plateauing region, and water addition at this rate is not necessary and may be decreased as needed according to the law of diminishing marginal returns.

In the analysis of the mass consumed or converted by anaerobic digestion, no significant difference was detected over the two different water delivery methods (submerge and purge, and drip) or between the different levels of water added. While this does not support the theory of by-product inhibition, it does not weaken it as an important acting force in AD of BSG. With this result in mind, the importance of the VFA profiles is even more important.

Volatile fatty acid production

The production of acetic and butyric acids follow a very similar pattern. There is not too much production in the first two days, but it picks up with a production of about 8mMoles per day, on average (figures 23 and 24). While the rates of VFA production are slowing, they are still producing some VFAs. Based on the rate of change of the slopes of the graphs, the experiments would have offered more information about the complete conversion of biomass to VFAs if the experiments would have been run for three weeks instead of two.

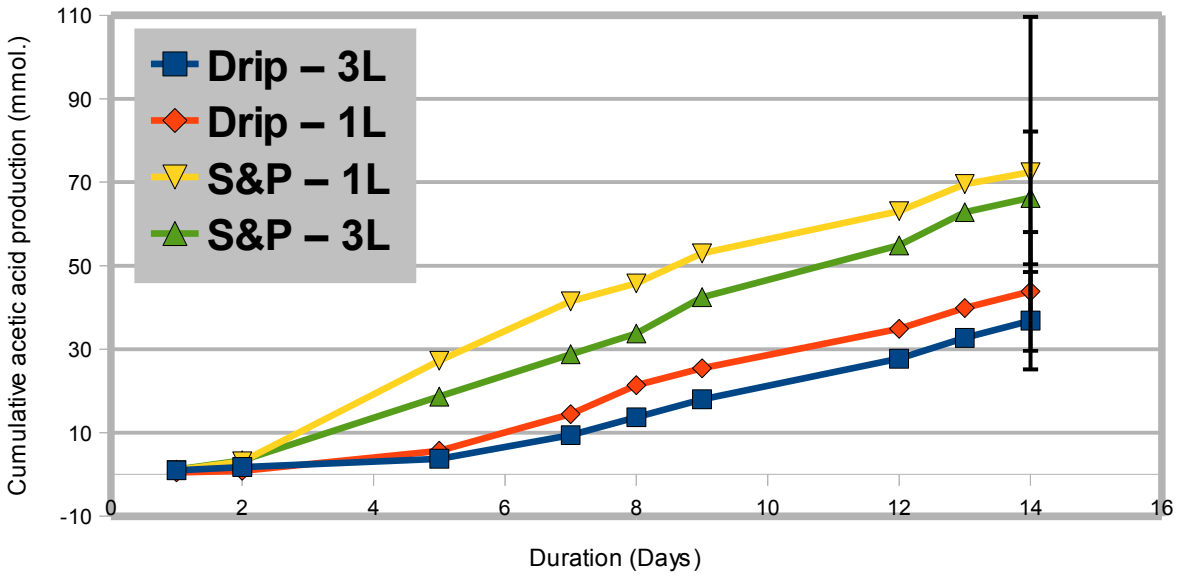

Figure 23. Cumulative acetic acid production over time for phase II

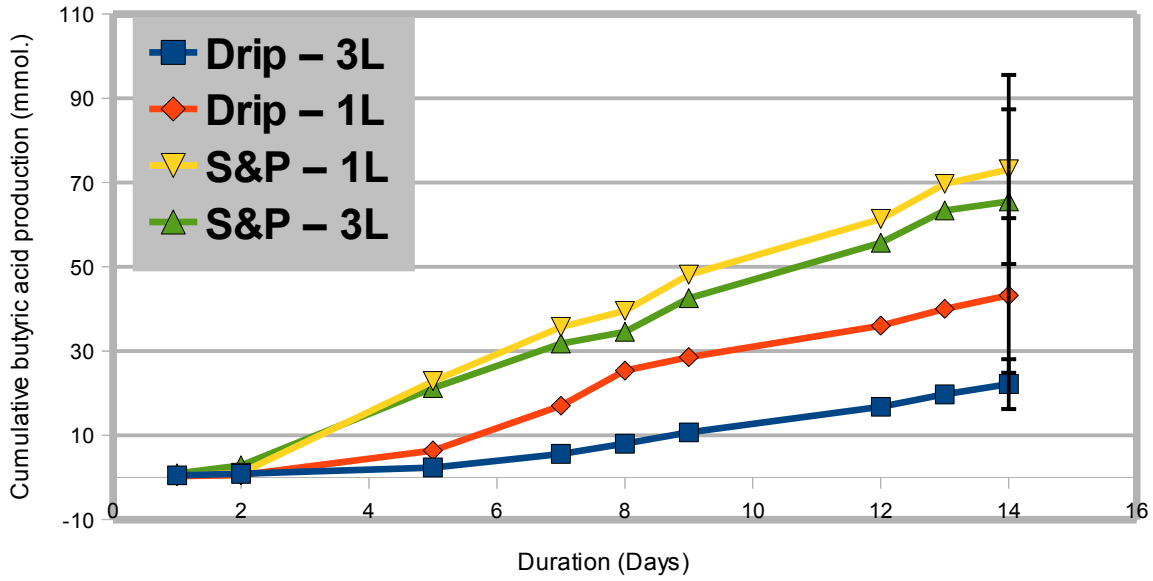

Figure 24. Cumulative butyric acid production over time for phase II

Interestingly, while the high volumes of water going through the reactors may have prevented some by-product inhibition, the 1% solids reactors in the phase one reactors produced more VFAs per gram BSG. This result may be explained by the fact that the water used in the phase one reactors was degassed, while levels of oxygen in the water of the phase two reactors probably led to some aerobic digestion of substrates (including VFAs) which would reduce there levels in solution. This possible explanation is supported by the levels of VFAs in the reactors (previous figures). For all the VFAs tested, the 1L submerge and purge method produced the highest levels of each. Since previous experiments showed that by-product inhibition played a significant role on production of VFAs, it may have been expected that the 3L submerge and purge method should have performed better than the 1L submerge and purge. However, the 3L submerge and purge did not generate as much acid as the 1L, so it is assumed that there is some

sort of trade-off. That is, adding more water decreases by-product inhibition which can lead to higher VFA production, but some other mechanism decreases production of VFAs with increasing water added. Only two different levels of water flows were tested in phase two. Subsequent testing is necessary to determine the optimal flow rate. Using the knowledge gained in phase one, the optimal percent solids level is lower than 1% when the water is anoxic because the VFA levels are still increasing with increasing water levels.

The "percent solids," which is more aptly referred to as the grain to water ratio for the phase two experiments is calculated by dividing the mass of the grain added by the total volume of water that passed through the reactor. The breakdown of the percent solids for the phase one reactors is given in table 4.

Table 4. Reactor type and water flow characteristics

Reactor	Ave. Vol. Per day (L)	Total water volume (L)	Percent solids (%)
3L – Drip	2.6	36.3	0.28
1L – Drip	0.9	12.4	0.81
1L – S&P	0.64	9	1.11
3L – S&P	1.9	27	0.37

However, degassing water requires either a large input of energy to heat the water or a large volume of oxygen-free gas, both of which are costly in terms of energy input and would have a large negative impact on the feasibility of this method of producing VFAs. Important to note, however, is that in a large-scale implementation, one of the most important benefits of this reactor system design is the recycling of water. After the water had passed through the BSG, the VFA-rich water would then be processed using a UASB. The "scrubbed" water that comes out

of the UASB would then be recycled to the plug-flow BSG reactor. The process water would already be anaerobic, and simply preventing exposure to air would maintain its anoxic state. It was not feasible to make the water used in these experiments anoxic. However, in a pilot-scale reactor system, the water would be anoxic because of the recycling from the UASB.

On day eight, the production of iso- and n-valeric acids began in the submerge and purge reactors (figures 25 and 26). This may have been indicative of a shifting of the bacterial consortium in the reactors or a change in functionality due to a depletion of one type of substrate.

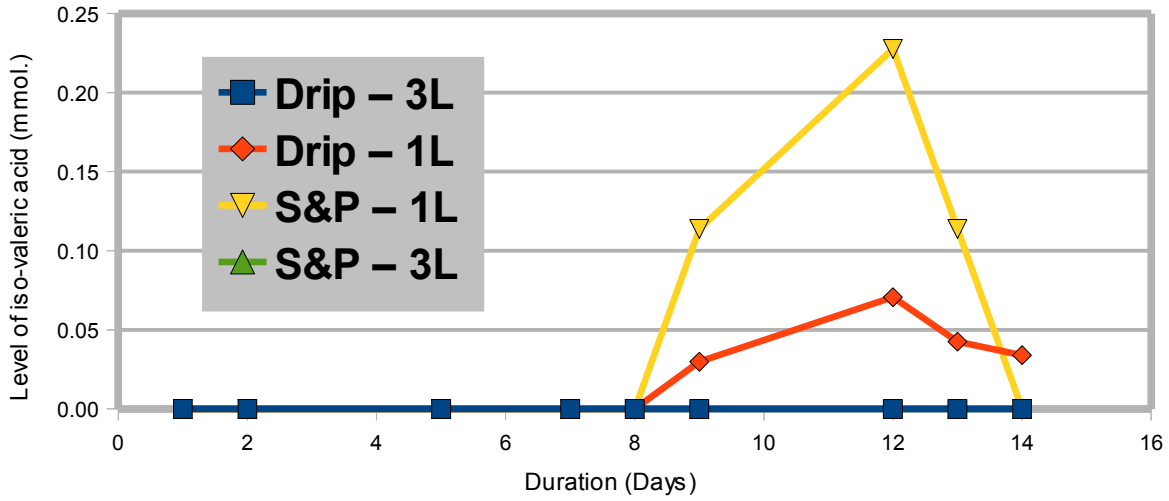

Figure 25. Daily iso-valeric acid production for phase II

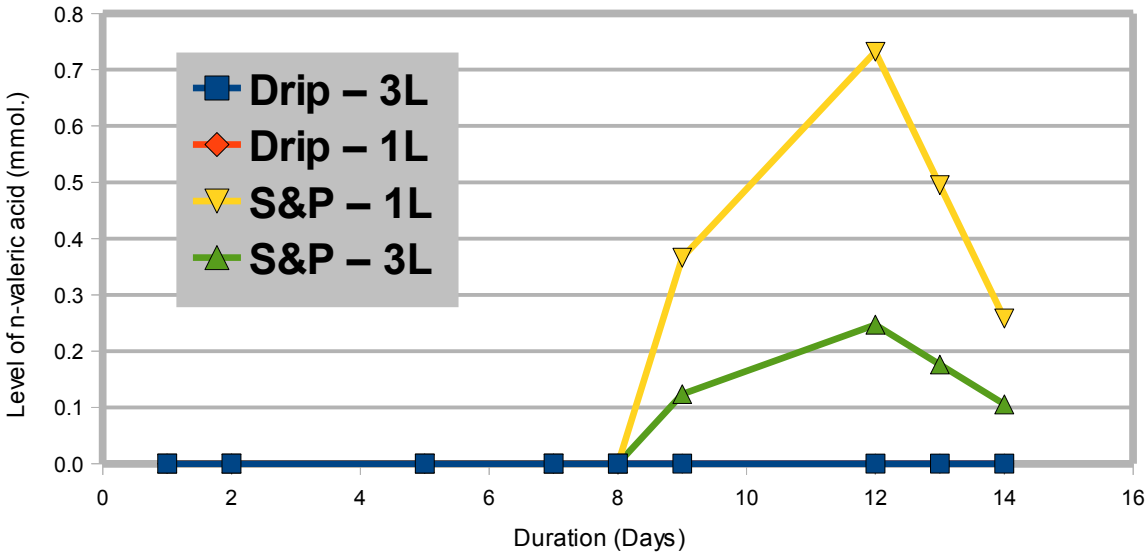

Figure 26. Daily n-valeric acid production for phase II

In a large-scale reactor, 1mMole, 0.1mMole and 1.0mMole of acetic, propionic, and butyric acids would be produced per gram of BSG. Using this conservative estimate of the level of acids produced per gram of BSG, it is possible to estimate the amount of VFAs produced per ton, and further, to approximate the methane yield. There are 1 million grams in a ton. So, one dry ton of BSG would produce 1 thousand moles of both acetic and butyric acids, and 100 moles of propionic acid. To calculate the amount of methane produced from one wet ton, the methane production from the dry ton is divided by 5 because the wet spent grain is approximately 80% moisture. The other fatty acids occur at levels that make them insignificant in the calculation of methane yield.

From table 5 below, one mole of acetate, propionate, and butyrate produce 1, 1.75, and 2.5 moles of methane. A total of 735 moles of methane would be produced from one wet ton of brewers' spent grain.

Table 5. Conversion of acetate, propionate and butyrate to methane (Ozturk, 1993)

Reactants	**Products**
Butyrate + $2H_2O$	2Acetate + $2H_2$ + H^+
$2H_2$ + ½ CO2	½CH_4 + H_2O
2Acetate + H^+	$2CH_4$ + $2CO_2$
Propionate + $2H_2O$	Acetate + $3H_2$ + CO_2
$3H_2$ + ¾CO_2	¾CH_4 + 3/2H_2O

Using the equation PV=nRT, where P, pressure, is 1 atmosphere; V, volume, is 1000 cubic feet, or 28300L; R, the gas constant, is 0.082057 L*atm/(mol*K); and T, temperature, at 75 degrees Celsius, or 303.8K. Solving for n, number of moles of methane to fill 1000 cubic feet is 1138 moles. So, 735 moles of methane, the moderate yields from these experiments, is equivalent to 646 cubic feet. In the U.S., methane is commonly sold in increments of 1000 cubic feet for a residential price of about $11.

Analysis of spent grain

Preparation and analysis of the grain

According to the proximate analysis, the brewers' spent grain had approximately 78% moisture. Of the 22% solids fraction, 77% was volatile compounds, 19% was fixed carbon, and 3.5% was ash. These results are similar to others published in the literature.

Alkalinity of brewer's spent grain

The calculated alkalinity was 112 mg/L $CaCO_3$. This alkalinity level is lower than would be desirable, however, the digestion of proteins can lead to increases in ammonia nitrogen which serves as a buffering agent for the reactors as the digestion proceeds. Furthermore, the pH was considered stabilized after approximately a minute.

CHAPTER 5

CONCLUSION

In both phase I and phase II, important information was gained and will shape the design parameters for future work on anaerobic digestion of brewers' spent grain and other similar substrates. Firstly, even after 26 days, mass was still being lost from the anaerobic digestion reactors and VFA levels were still increasing. This lends further evidence to the hypothesis that hydrolysis of complex substrates is the rate-limiting step in anaerobic digestion of lignocellulosic material. It also suggests that a longer SRT will yield more complete digestion.

Secondly, while the type of inoculum may not affect the total amount of biomass degraded, the types of acids in the effluent will differ with different microbial consortia. Rumen fluid and cow manure inocula produces similar levels of acetate and butyrate, but very different levels of propionate.

Thirdly, the effect of grinding on the anaerobic digestion of BSG varies with initial solids content of the reactors. At a high initial solids concentration, grinding had no perceived effect on the mass degraded. In a lower solids digestion, 2%, the greater the level of grinding, the less grain was digested. Increasing the available surface area of the substrate did not increase digestibility in this work possibly due to a more rapid digestion leading to decreases in pH and reactor inhibition.

Fourthly, by-product inhibition plays an important role in the anaerobic digestion of spent grain. With an increasing water volume to BSG ratio comes increasing levels of grain digestion

and higher levels of VFA production. The water volume to grain ratio that optimizes this digestion was not elucidated in these experiments. From 1.4% to 1% solids, there is still an increase in mass digested and VFAs produced. The optimal level of solids is at or below 1%. Further experimentation is necessary to determine this level.

The phase II results offer an important view of the level of VFAs that are produced at each time point. As suggested by results from phase I, the highest levels of VFAs come out of the solution around day 5. Future work will help reinforce the results from these experiments and offer insight into the anomalous dip in VFA production on day 8.

Also in phase II, while the degradation of the mass of spent grain did not vary between the treatments, significant differences in the amount of VFAs produced did occur. The submerge and purge method of water addition was much better at producing both acetic and butyric acids than the drip irrigation method. The 1L submerge and purge out-performed all other groups in production of acetic, butyric, and n- and iso-valeric acids. While this seemingly contradicts the theory of by-product inhibition, the water used in these experiments was not anoxic which may have partially confounded the results. An important effect to explore is the idea that micro-aerobic conditions may improve anaerobic digestion. Ethanol fermentation was optimized, in one study, under micro-aerobic conditions (Xiros and Christakopoulos, 2009). This finding lends support to the idea that micro-aerobic environments could also be beneficial to the anaerobic production of methane from the same substrate. In a future project, it would be interesting to investigate the effect of micro-aerobic conditions on anaerobic digestion.

As discussed in the results section, enough biogas can be produced to make anaerobic digestion of BSG economically feasible. However, only VFAs were used in that calculation.

Many other products of the fermentation were present in the aqueous phase of the digesters. Many of these metabolites came off of the column between one and two minutes. They were not quantifiable, however, because of the overlapping nature of their peaks. Quantification of these secondary metabolites is possible but requires a longer column, a slower flow rate, and a different carrier gas. These other metabolites consist of alcohols like methanol, ethanol, propanol, butanol, as well as acetaldehyde, acetone, ethyl formate, and diacetyl; many of these compounds can be converted into methane. Consequently, the methane yield will probably exceed the previous estimate of 735 moles per ton BSG, thus increasing the value of AD of BSG.

The research performed herein has laid the groundwork for a pilot-scale reactor set-up. Anaerobic digestion can be used to successfully generate economically viable levels of precursors to methane on a bench-scale. To more completely exploit the resources in BSG, co-products of the digestion like the lignocellulosic digested spent grain, some process water, and excessive sludge from the UASB reactors can be used. The lignocellulosic residues coming out of the modified PFR as well as the excess UASB sludge are both good candidates for soil amendments. Thermophilic anaerobic digestion, one parameter option for the reactor system, has been shown to greatly decrease the levels of pathogenic bacteria in substrates. In conclusion, anaerobic digestion of brewers' spent grain offers one alternative to the current trend in BSG disposal as animal feed. Diversification of usage of this valuable product will allow the creative process to transform wastes into resources.

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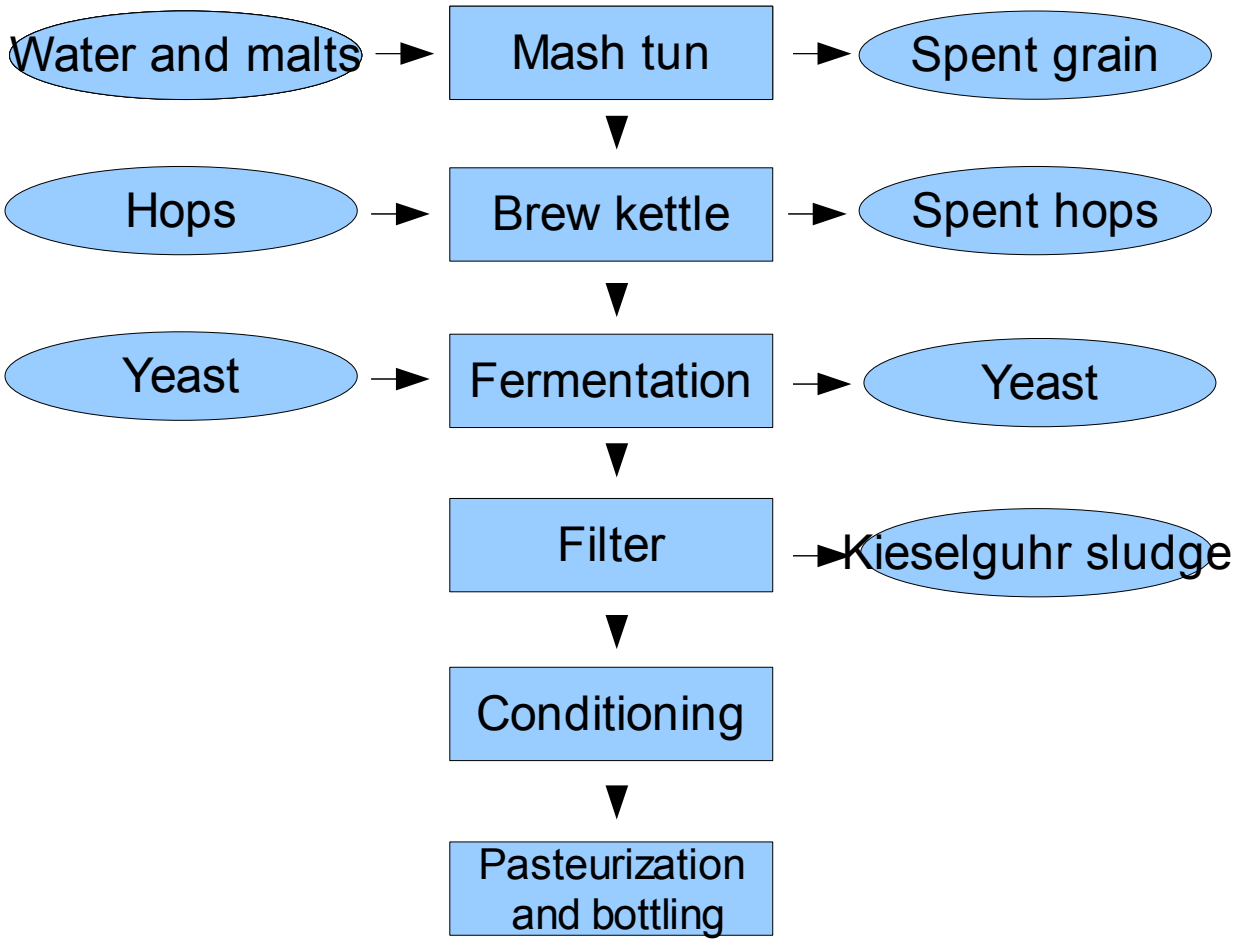
APPENDIX A

BREWING PROCESS

The following schematic of the brewing process shows the processes or locations in the center rectangles. The left and right ellipses contain the inputs and by-products, respectively. In addition to the listed by-products, the cleaning and rinsing of the equipment generates large amounts of low-strength wastewater.



APPENDIX B

PHASES OF ANAEROBIC DIGESTION

The following chart from Ahring, B. K. (2003) outlines the basic stages of anaerobic digestion.