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COTREATMENT ENHANCED ANAEROBIC DIGESTION OF LIGNOCELLULOSIC BIOMASS

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Abstract

Anaerobic digestion generates storable renewable energy from organic matter. In this process, organic compounds are degraded in the absence of oxygen by a mixed microbial community into carbon dioxide and methane, which can be burned as an energy source. Lignocellulosic feedstocks like grasses and corn stover are cheap and abundant, but without expensive and energy intensive thermochemical pretreatment their recalcitrance to biological decomposition has thus far limited usage in commercial biogas production. A possible solution to this problem is demonstrated by ruminant animals, which degrade lignocellulosic material efficiently by occasionally regurgitating and chewing the plants they consume. The mechanical analog of this strategy is termed cotreatment and involves mechanical milling during fermentation. This study evaluated the potential of intermittent cotreatment through observation of a pilot scale lignocellulosic anaerobic digester, determination of cotreatment energy consumption, and process modeling at industrial scale.

Cotreatment had previously been studied using systems that milled the material only on a single occasion in a batch process, while in this study cotreatment was implemented daily in a continuous process using a recirculating loop design. A pilot scale 80-liter fermenter was coupled with a conical wet disk mill where the mill rotation also provided the pumping force for recirculation. The system was able to tolerate daily milling of 50% of the reactor volume, but a small decrease in biogas production was observed compared to performance without cotreatment. This could be due to inadequate recovery time for the microbes between milling events, poor milling due to an improper adjustment or an inadequate mechanism, a substrate whose recalcitrance was not sufficiently limiting in the base case without cotreatment, or some combination of these and perhaps other factors. Nutrient limitations were unlikely, as nutrient requirements were tested during system setup and supplemented with at least 150% of requirements. The nutrient supplement studies suggested that while nitrogen and mineral additions were necessary for a switchgrass feedstock that had senesced in the field and was stored dry, addition of vitamins was not required for continuous anaerobic digestion.

The energy necessary to mill switchgrass slurries tended to increase when solids content increased or when the radial gap size decreased. Even the most energy intensive conditions

consumed only about 2.3% of the higher heating value of the cellulose and hemicellulose fraction of the switchgrass per pass through the mill. The magnitude of particle size reduction mirrored that of energy consumption. Increased solids loading caused larger magnitude particle size change despite maintaining the same radial gap size, suggesting that interactions between particles play a role in size reduction during milling.

Using a model to simulate cotreatment at industrial scale for a lignocellulosic anaerobic digester fed with switchgrass, varying degrees of milling effectiveness were evaluated in combination with recycling of a portion of water from the digestate waste stream. For a scenario of 40% cotreatment conversion increase and 80% water recycling, the model predicted the breakeven subsidy price would decrease by 31% from the base case without these innovations, and the net energy return on energy invested for the system would be as high as 2.06. However, the net present value of the investment was -$5.3 million for a system processing 12 dry tons of switchgrass per day. Additional subsidies or revenue streams are necessary, in combination with technology innovations like cotreatment and water recycling, for lignocellulosic anaerobic digestion to become economically feasible.

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Section I: Introduction

Given the slow pace of climate mitigation efforts worldwide, it is increasingly important to develop sources of sustainable energy that not only reduce but reverse greenhouse gas emissions. Biomass energy and biofuels have the potential to play a major role in meeting this need through 1) offsetting challenging fossil emissions, such as for heavy transportation and thermal power, 2) increasing terrestrial soil carbon stocks, and 3) generating pure streams of biproduct CO_2 for low-cost geologic carbon sequestration (Cabral & Dowell, 2019; Liebig et al., 2005; Raud et al., 2019). One technology that could contribute to this effort is anaerobic digestion, by which microbes convert carbon-containing compounds into biogas (a mixture of methane and carbon dioxide) in the absence of oxygen. This biogas can be burned directly to create heat and power onsite or be upgraded into renewable natural gas and injected in the existing natural gas grid (Ullah Khan et al., 2017). Renewable natural gas takes advantage of natural gas infrastructure that is widespread in the United States and offers a storable and dispatchable complement to intermittent renewable energy sources like solar and wind. Several biogas separation technologies are already available that produce a byproduct of nearly pure CO_2 which is useful for food manufacturing, can be upgraded to other industrial products, or can be permanently stored in deep geologic formations (Miltner et al., 2017). While anaerobic digestion is already used in many wastewater treatment plants and a growing number of large-scale livestock operations, it can be difficult to achieve economic viability at medium and small scales.

There is opportunity to improve the commercial feasibility of this process and achieve better economies of scale by diversifying the types of organic matter that can be fed into anaerobic digesters. Food security and sustainability concerns can be addressed by focusing on wastes and bioenergy crops that do not interfere with food production systems and maximize greenhouse gas mitigation benefits. Lignocellulosic biomass feedstocks, including agricultural residues and perennial grasses, fit this description but are infrequently utilized in anaerobic digestion due to the limited ability of microbes to degrade recalcitrant plant cell walls. For bioprocessing to achieve high conversion rates with lignocellulosic biomass, it is typically pretreated using thermochemical methods to disrupt its structure, increasing access to the carbohydrates and making it more readily digestible (Hendriks & Zeeman, 2009). Conventional pretreatment methods often require large energy and monetary investments (Lynd et al., 2017), limiting the sustainability and economic feasibility of bioprocessing of lignocellulosic material.

A possible solution to this problem can be found in ruminant mammals, like cows, which convert about 70% of the carbohydrate energy content in their food into the fatty acids used in their metabolism (Weimer et al., 2009). Rather than pretreating their feed before digestion, ruminants regurgitate and chew their cud after it is partially digested, physically disrupting material during fermentation. Mimicking this "cotreatment" strategy in mechanical systems could improve the efficiency of anaerobic digestion of lignocellulose while avoiding the energy and financial investments of thermochemical pretreatment. Estimates are that cows invest less than 2% of the metabolizable energy in their feed in the rumination process (Weimer et al., 2009), whereas effective mechanical pretreatment often requires several times more energy than is returned in increased product yield (Tanjore & Richard, 2015). Decreasing the energy investment for feedstock treatment could help make this process a net energy producer.

Cotreatment of lignocellulose in a pure culture system using *Clostridium thermocellum* has previously been shown to increase carbohydrate solubilization (a major indicator of how digestible material is for microbes) of switchgrass by 43% and 7% compared to untreated and thermochemically pretreated samples respectively (Balch et al., 2017). Previous research suggests that singular cotreatment events are sufficient to increase carbohydrate solubilization and biogas production in mixed culture systems (Bharadwaj, 2020). While these findings are compelling, they were undertaken at the benchtop scale and under conditions that cannot be readily replicated at industrial scale, making the economic significance of these advances difficult to gauge.

This study investigated cotreatment-enhanced lignocellulosic anaerobic digestion from both experimental and modeling perspectives. The first experiment included a pilot-scale lignocellulosic anaerobic digestion system capable of intermittent cotreatment events. During this experiment, the digester contents were subjected to varying intensities of cotreatment while observing biogas production and volatile solids degradation as measures of microbiome robustness to the stress of milling. The energy consumption of the mill used in the experiment at varying solids contents and radial gap sizes was also measured to inform scale-up calculations. The lignocellulosic anaerobic digestion model in this study was created using excel and emphasized ease of modification and understanding when evaluating this system in a variety of scenarios. This research contributes to evaluation of the viability of cotreatment anaerobic digestion of lignocellulosic material as a means of biogas production and its effects when implemented at larger scales.

Section 2: Literature Review

2.1 Overview

Lack of previous decisive action on climate change mitigation will necessitate energy generation solutions that are adaptable, widely implementable, and can result in a net removal of carbon dioxide from the atmosphere. One bioprocessing technology that fits these needs is anaerobic digestion. In this process, microbial communities with a diverse mixture of organisms degrade organic material in the absence of oxygen, creating methane and carbon dioxide as final products. When collected and purified, the methane created from anaerobic digestion can be used as an energy source while the carbon dioxide can be sequestered in geologic formations.

While this technology is commonly used as a means of solids treatment for manure in large farms and wastewater at municipal sewage facilities, the energy generation potential is often viewed as a secondary benefit rather than a primary goal. Without subsidies, digester operations are typically unable to recover the high capital investment by selling biogas or producing electricity by burning the biogas (DeVuyst et al., 2011). These subsidies can be either on the feedstock side for waste disposal, odor control, pathogen management, water quality protection or some other waste treatment benefit or on the product side for renewable energy and further climate mitigation, and are particularly important for small to mid-sized farm digesters as digester technologies have significant economies of scale. Underutilized waste streams like crop residues (corn stover) and dedicated biomass crops (switchgrass, miscanthus) could help to achieve economies of scale; however, these lignocellulosic materials are too recalcitrant to be directly broken down in current biorefineries (Oke, Annuar, & Simarani, 2016).

Ruminants, like cows, sheep, and goats, offer a natural analog to anaerobic digestion and can quickly and efficiently digest lignocellulosic material. One reason for this efficiency is thought to be the habit of chewing cud, during which material is occasionally regurgitated and mechanically disturbed while it is being digested. This can be mimicked in a mechanical system by intermittently milling material during anaerobic digestion in a strategy termed cotreatment. Previous studies on cotreatment have shown increased carbohydrate solubilization of lignocellulosic material in bench-scale reactors (Balch et al., 2017; Bharadwaj, 2020). This thesis seeks build on these findings through both pilot-scale experiments with continuous flow reactors, and by parameterizing a model of a commercial scale anaerobic digester. The outcomes of this effort include estimates of the economic viability of a cotreatment-enhanced anaerobic digestion biorefinery and identification of areas of uncertainty that require further research.

2.2 Anaerobic Digestion

Anaerobic digestion is the process of degrading organic material using microbes in the absence of oxygen. This process can be operated under a range of conditions with diverse organic materials ranging from manure to citrus pulp to lawn clippings (Sukhesh et al., 2019). Regardless of the material being digested, the process tends to take place in four steps. The first is hydrolysis of polymers from the substrate into monomers, like glucose or amino acids. For lignocellulosic feedstock, hydrolytic bacteria create complexes of enzymes called cellulosomes, which are capable of breaking off sugar monomers from the long polymers of the substrate (Laiq Ur Rehman et al., 2019). These subunits are then metabolized, producing organic acids in a process called acidogenesis. These organic acids can include butyric, propionic, and acetic acid and are the desired products when completing acidogenic digestion (Zhou et al., 2018). Further metabolism of the organic acids to acetate occurs during acetogenesis. Lastly, the methanogenic archaea present in the anaerobic digester convert acetate into methane, which exits the solution as a gas (Sillas-Moreno et al., 2019). The resulting mix of carbon dioxide and methane produced is termed biogas.

2.2.1 Anaerobic Digester Microbiome

Anaerobic digestion takes place through the activity of multiple groups of microbes, mostly bacteria and archaea, each of which progresses the organic matter a few steps through the process of its conversion to methane. In common practice the digester is inoculated and operated with microbial communities that are undefined mixed cultures, meaning that it is not known exactly what microbes are present. Microbial communities with the ability to carry out anaerobic digestion can be found in the environment anywhere that organic material is degrading in oxygen-limited conditions like wetland sediments, cow rumens, and manure as well as in sludge extracted from existing digesters (Ghanimeh et al., 2018). While pure culture (single microbial strain) systems for converting organic material to useful products can offer higher conversion efficiency due to careful selection and genetic manipulation, they also introduce additional constraints. One of these is that only a narrow range of substrates can be introduced that the organism is able to degrade. Additionally, the substrate must be sterilized to prevent contamination with outside organisms which could compete with the selected strain (Dionisi, Anderson, Aulenta, Mccue, & Paton, 2015).

By contrast, mixed cultures often allow for greater substrate flexibility and do not require sterilization. Mixed culture fermentations do have disadvantages, including fewer options for end products and the possibility of system failure due to imbalances in the populations of microbes completing each phase of anaerobic digestion. The first step in breaking down lignocellulosic organic material, hydrolysis, is generally regarded as the limiting factor in the process. While in aerobic environments fungi and bacteria often excrete cellulytic enzymes, in anaerobic systems some of the most active hydrolyzing bacteria use an enzymatic complex called the cellulosome. Cellulosomes are commonly membrane bound, meaning the microbe must adhere to the plant material to contact the target substrate, which increases local concentrations of hydrolysis products including sugars and other small molecules. Subsequent bacterial groups seek access to these hydrolyzed small molecules which they further convert. This creates biofilms on the plant particles in which a majority of the microbes participate in interactive communities rather than living planktonically (Mason & Stuckey, 2016a).

In order to determine the identities of the microorganisms in an anaerobic digester, next-gen sequencing methods such as Illumina® can be employed. When it is not necessary to know the exact members present, there is a faster and less expensive way to analyze microbial community dynamics and determine whether or not the system has changed over a time period:

Terminal Restriction Fragment Length Polymorphism (T-RFLP). T-RFLP has been shown to yield similar results to Illumina sequencing in a range of anaerobic digester conditions (De Vrieze, Ijaz, Saunders, & Theuerl, 2018). These techniques offer a mechanism to evaluate both the microbes participating in anaerobic digestion and the stability of that community.

2.2.2 Process Parameters

While anaerobic digestion can take place under a variety of conditions, its efficiency and stability can vary greatly with changes to parameters like pH, temperature, retention time, carbon-to-nitrogen ratio (C:N ratio), and solids loading. Generally, anaerobic digestion proceeds most efficiently near neutral pH (Ponsá et al., 2008). During digestion, the pH tends to decrease as organic acids are produced, necessitating the addition of base to prevent killing the methanogenic archaea, which generally cannot tolerate acidic conditions (Akuzawa et al., 2011). The operating temperature of an anaerobic digester also has a marked effect on the speed at which digestion takes place. The most common temperature choices are mesophilic (37^{o}C) and thermophilic (55^{o}C) with greater methane production rates taking place at higher temperatures. While thermophilic digesters allow greater throughput and greater methane production efficiency (Vanwonterghem et al., 2015), the increased energy demanded to maintain the reactor at a higher temperature can cut into these gains. Additionally, anaerobic digesters with shorter residence times can risk removing material at a rate faster than methanogens can grow, leading to "washout" of methanogens and failure to produce methane.

2.2.3 Biogas Usage and Upgrading

After biogas is produced there are two tracks through which it can be used. The first is direct or nearly direct combustion in systems for combined heat and power, which provides thermal heat and power to machinery in the facility. Alternatively, the biogas can be purified by upgrading. This involves removing carbon dioxide, hydrogen, water vapor, and H_2S gas (which can corrode metals) to meet higher purity standards (Ullah Khan et al., 2017). Upgrading allows for the use of the methane in more diverse machinery, including natural gas vehicles, and can satisfy requirements to be injected into the natural gas grid as a revenue source. While upgrading technologies are advancing, they can be capital intensive and often result in non-trivial losses of a dilute stream of methane (Ullah Khan et al., 2017).

Despite this, biogas and renewable natural gas enriched from it offer a renewable energy source that is attractive for three major reasons. The first is that the organic matter that feeds anaerobic digesters is widely available in various forms in both developed and developing countries. Additionally, it is more consistent and storable than other renewable energy sources like solar or wind power (Gohsen & Allelein, 2015). These other renewable energy sources are intermittent and require expensive battery storage, while biogas is dispatchable. Storing biogas or upgrading it to renewable natural gas allows for supplemental electricity generation whenever the grid requires it, providing peak power to meet surges in demand and guarding against loss of power during unfavorable weather. Lastly, existing natural gas infrastructure in the United States and other countries could be used as means of storage and conveyance, lessening the transition costs of this energy generation technique.

2.2.4 Existing Uses and Limitations

While showing potential for energy generation, anaerobic digestion is used primarily to reduce sludge volume and pathogen load in solids during wastewater or manure treatment (Coyne et al., 2017), with biogas generation as a secondary benefit. During wastewater treatment, solids settle out of the influent and are separated from the supernatant. This material still has a high water content and contains readily degradable organic compounds, making it heavy to transport and potentially damaging to aquatic ecosystems and human health if released. This necessitates reducing the volume and biological activity of sewage, which anaerobic digestion does well (Duan et al., 2012). Because the costs and energy requirement of biogas upgrading equipment is high, these wastewater treatment facilities often cannot achieve a scale to make use of biogas other than through combined heat and power systems. Additionally, the capacity of current candidate facilities for anaerobic digesters, including wastewater treatment plants, large livestock operations, and some food processing plants is limited and cannot generate significant amounts of electricity for public usage. In order to increase the energy generation potential of anaerobic digestion, it is necessary to expand the diversity of feedstocks that can be fed into these systems, allowing smaller facilities to supplement the organic waste they produce with biomass crops or agricultural residues to reach the scale required for profitability.

2.3 Lignocellulosic Material and Pretreatment

2.3.1 Lignocellulose Structure and Switchgrass

To become more profitable and widespread, anaerobic digestion must effectively digest a greater range of organic materials. One option for this is lignocellulose, consisting of cellulose, hemicellulose, and lignin, which makes up the structural components of plants. Cellulose chains are bundled together to form fibers that are sheathed by hemicellulose and lignin (Ayeni et al., 2015). Unused agricultural residues and dedicated biomass crops offer a relatively untapped stream of lignocellulosic material.

Switchgrass is a popular biomass crop for several reasons. It is a native North American grass with varieties well-adapted to growth in many climates (Mitchell et al., 2008). Selecting native grasses affords a level of protection from diseases and pests, while providing seasonal habitat for local wildlife. Switchgrass also grows in a natural monoculture, meaning that after the first few years of growth the grass crowds out competitors and no longer requires herbicide application. There is also evidence that switchgrass can reduce nitrogen runoff and soil erosion (Nelson et al., 2006).

2.3.2 Pretreatment

Lignocellulosic material is very resistant to degradation. While this is beneficial for plants, it is a complication for those seeking to degrade lignocellulose. In order to increase the accessibility of cellulose to microorganisms, a variety of pretreatment strategies can be employed before fermentation. Pretreatment centers around disrupting the lignocellulosic structure that prevents microbes from hydrolyzing cellulose. One pretreatment mechanism is mechanical treatment, which is breaks the plant fibers and exposes a greater surface area to microbial attack (Hendriks & Zeeman, 2009). Mechanical treatment has generally been applied to dry biomass, where the energy requirements of milling usually greatly exceed the additional energy available from the degraded biomass, making this strategy impractical for bioenergy although appropriate for other higher value applications (Tanjore & Richard, 2015). A variety of heating mechanisms of pretreatment are also commonly employed. When heated to $150^{o}C$ hemicellulose begins to solubilize, degrading the lignocellulosic structure. Chemical pretreatment with sulfuric acid and cellulolytic or lignolytic enzymes are also frequently used, both of which focus on degrading the lignocellulosic structure (Hendriks & Zeeman, 2009; Isroi et al., 2011).

Dilute acid pretreatment is a means of increasing biological availability of lignocellulosic materials that combines thermal and chemical pretreatment methods. In this process, dilute sulfuric acid solution is added to the lignocellulosic material which is then heated and washed (C. Li et al., 2010). This causes degradation of hemicellulose and decreased lignin content which allows for greater microbial or enzymatic access to the remaining cellulose. As hydrolysis of the substrate is often the limiting step in anaerobic digestion, the degree to which pretreatment increases access to the structural carbohydrates in lignocellulose is of utmost importance. These pretreatment methods can be capital intensive, energy intensive, difficult to scale, and produce inhibitory compounds, creating a roadblock for adoption of lignocellulosic anaerobic digestion (Lorenci Woiciechowski et al., 2020; Lynd et al., 2017).

2.4 Cotreatment

2.4.1 Observations from Ruminants

While digestion of lignocellulosic material in engineered systems has had only limited success, there exist efficient natural analogs to this process. Ruminant animals, specifically cows, are excellent examples of this, retaining about 72% of the energy from lignocellulosic material they consume with residence times of about 3 days (Weimer et al., 2009). This rate of digestion is estimated to be about 30 times faster than current anaerobic digesters (Mason & Stuckey, 2016b). For this reason, there has been increased interest in mimicking cows and other ruminants to design more efficient and consolidated lignocellulose bioprocessing systems.

One of the major differences between conventional lignocellulosic bioprocessing methods and bovine digestion is that cows frequently regurgitate and chew small amounts of partially digested grasses rather than extensive treatment before digestion. Chewing the cud is thought to increase degradation rates by exposing previously shielded areas of cellulose to microbial attack. It may also enhance digestion by disrupting the biofilms on particles where the majority of microbes reside, distributing products that have built up in the layers and encouraging new colonization (Mason & Stuckey, 2016b).

Cows introduce oxygen to the cud while chewing it, possibly representing a microaerobic treatment process. A microaerobic pretreatment process has been demonstrated to increase methane production by 16% compared to the control in anaerobic digestion of corn stover (Fu et al., 2015). Cow saliva has also been shown to enhance performance of cellulase and act as a pH buffer, increasing the rate of degradation of lignocellulosic material (Seki et al., 2015). Flow of material through the cow stomach could also play a role in its efficient degradation. After material enters the rumen and has been sufficiently chewed to decrease its particle size, it passes into the omasum. This organ has a large surface area due to many folds in its walls and is cited as a mechanism for liquid recovery before entry into the final stomach (Ehrlich, Codron, Hofmann, Hummel, & Clauss, 2019). Particle entrapment within these folds could give microbes in this organ additional time to degrade the lignocellulosic material, perhaps behaving something like a fixed-film reactor. Distinct microbial communities present in each of the cow's four stomachs suggest differing functionality among the stages of digestion (Peng et al., 2015).

Taken as a whole, the ruminant digestive system functions through a complex collection of pretreatment techniques and microbially- and chemically-enhanced digestion phases. There is not yet a full understanding of which of these mechanisms and catalysts are most important for efficient digestion of lignocellulose in this natural system, making mimicry of the process in engineered systems difficult.

2.4.2 Continuous Ball Milling Cotreatment in Pure Culture

Milling material during digestion is a strategy termed cotreatment. Balch et al. (2017) studied cotreatment in the context of a pure culture fermentation of switchgrass using *Clostridium thermocellum*. In this experiment, a bioreactor was loaded with a bed of stainless-steel ball bearings and agitated during fermentation, which caused constant milling of material. A second reactor had the same conditions without ball bearings. The fermentation proceeded over 5 days while measuring biogas production rates and final carbohydrate solubilization.

In the Balch et al. (2017) study, carbohydrate solubilization with cotreatment averaged 88% while that without cotreatment averaged 45%. The cotreatment fermentation also outperformed the 81% solubilization of switchgrass that had been hydrothermally pretreated (200^{o}C, 15 min). Gas production profiles over the 5-day period showed that while there appeared to be a lag in gas production (an indicator of microbial activity) for the first two days, the rate of production proceeded to sharply increase and overtake the unmilled control. This suggests that cotreatment could be a viable strategy to increase carbohydrate solubilization, and with it, biogas production in a pure culture system. The study, however, used constant milling during the fermentation rather than intermittent milling as practiced by ruminants. Milling energy requirements were not reported, but they likely far exceeded the energy content of the biomass. The reactor contents were also sterilized before inoculation, possibly representing a mild form of thermal pretreatment. Both of these choices increase the energy invested during the cotreatment and fermentation process.

2.4.3 Single Ball Milling Cotreatment in Mixed Culture

Previous mixed culture research has been completed on cotreatment with a single ball milling event during digestion (Bharadwaj, 2020). This experiment used digestate extracted from a mesophilic, switchgrass-fed anaerobic digester, which was then milled using a ball mill for varying lengths of time (0, 0.5, 2, 5, 10 min) and fermented for a further 18 days. While biogas production increased with increasing ball milling, the greatest magnitude change occurred between the unmilled control and 0.5 minutes of milling. The carbohydrate solubilization was about 60% for the 5- and 10-min ball milled samples and 50% for the 0.5 min samples (Bharadwaj, 2020). This shows that the increased biogas production and carbohydrate solubilization rates observed by Balch et al. (2017) can also occur in mixed culture systems, although with much less milling the magnitude of increase is also much less. However, the measurable and significant difference after only a single short milling event relative to the unmilled control suggests that repeating minor cotreatment events could lead to increases in solubilization and biogas production while minimizing energy consumed during milling.

2.4.4 Colloid Milling as an Alternative Milling Strategy

In both of the cotreatment experiments described in the previous sections, a ball mill was used as the mechanism for milling. While solubilization was enhanced, this approach presents two potential problems. First, there is a question as to whether ball milling, or mechanical treatment in general, creates more energy than is consumed by the mill (Lindner et al., 2015). This is generally true for dry milling as a mechanical pretreatment and it is widely recognized that more efficient milling mechanisms are important to progress toward commercial applications. Second, with respect to those mechanisms, the particle size is reduced in ball milling by crushing particles between ball bearings, which is different from the peeling of fibers that tends to take place in ruminant chewing (Weimer et al., 2009). While the energy requirements of ball milling are affordable at a laboratory scale, increasing the scale of this process to the pilot or commercial scale would be difficult. An alternative mill type that might lessen these issues is a colloid mill, in which a conical rotor turns against a stator and forces particles through striations of decreasing width. This milling mechanism more closely resembles the shear grinding of chewing ruminants. While energy consumption of colloid mills at the laboratory scale does not yet meet the necessary threshold for profitability, those at increasing scales suggest that this would change at the size necessary for industrial relevance.

2.5 Anaerobic Digestion Modeling

Due to the large capital costs associated with establishing anaerobic digester systems, it is infeasible to construct pilot or commercial scale facilities for lignocellulosic anaerobic digestion without reasonable expectations for success or profitability. Simulation modeling of these systems is therefore extremely important for recognizing and addressing the problems which prevent widespread utilization of anaerobic digestion. As new anaerobic digestion innovations arise, new models or portions of models must be designed to account for the potential effects of this technology.

Anaerobic Digestion Model No 1 (ADM1) represents one of the most widely accepted models and is based on mathematical representation of the biochemical and physiochemical steps organic matter takes as it is microbially degraded (Batstone & Keller, 2003). While this model is generally accurate and widely adaptable, its complexity limits its applicability to non-standard conditions. This complexity not only makes this model hard for novice users to understand, but it limits the ability to adapt the model to new strategies or to integrate this model into larger biorefinery system models. As anaerobic digestion technologies change and usage diversifies, there is increased need to adapt models to ensure relevance to the target application (Batstone et al., 2015). Increasingly, these models are not only needed to predict mass and energy flows, but also to estimate the costs associated with these systems and how they would react to a range of scenarios.

2.6 Cotreatment Anaerobic Digestion of Switchgrass: State-of-the-Knowledge

Anaerobic digestion is a potentially promising approach to add value to diverse organic matter and generate storable and dispatchable renewable energy in the form of methane. Historically this technology has been used mostly for wastewater solids and manure treatment applications, with energy generation as a secondary benefit to waste management. Increased scale of anaerobic digesters would help to make them more profitable, which is necessary if they are to be used primarily for renewable energy generation. Lignocellulosic feedstocks, including agricultural residues and biomass energy crops, are good candidates to meet this demand for increased organic matter. Switchgrass, a perennial biomass crop, grows well in diverse climates and requires little maintenance. Lignocellulosic material, however, is recalcitrant and cannot be efficiently broken down in anaerobic digesters without pretreatment.

Ruminants, like cows, efficiently digest lignocellulosic material by occasionally chewing regurgitated material (chewing the cud). This process can be mirrored in lignocellulosic anaerobic digestion through cotreatment, during which material is intermittently mechanically milled during fermentation. Previous experiments have established that cotreatment using ball mills in both pure and mixed culture systems can increase biogas production. Further, a single cotreatment event lasting 30 seconds is sufficient to significantly increase biogas production. While these findings are promising, ball mills are unlikely to be energy efficient or adaptable enough to achieve economic feasibility. Colloid mills could offer a shearing method of grinding more similar to that of ruminants while consuming less energy than ball milling.

High capital costs make construction of large-scale testing facilities to study innovations for anaerobic digestion infeasible, and thus require robust modeling to establish the efficacy of cotreatment and other proposed solutions. While accurate biochemical models of anaerobic digestion exist, these have yet to be updated for diverse lignocellulosic feedstocks or for recent innovations proposed for the anaerobic digestion process. Greater analysis of the economics of these systems is necessary to evaluate these innovations, and can be accomplished through simulation of these systems on platforms that allow for easy manipulation to address a range of scenarios.

Section 3: Goal, Objectives, and Hypotheses

While previous work in cotreatment has established that ball and colloid milling during fermentation of switchgrass can increase biogas production in mixed and pure cultures, this work seeks to build on these findings in three major ways. First, prior studies used milling that was either continuous or only a single event, whereas this study implemented intermittent cotreatment events. Second, previous calculations did not consider potential changes in slurry material behavior when solids loading, milling gap size, or level of degradation vary. This study will test the validity of this assumption. Finally, this study seeks to use information from this and previous studies to inform a model of an industrial scale lignocellulosic anaerobic digester with intermittent cotreatment. This knowledge could contribute to the determination of whether cotreatment is an effective strategy for increasing biogas production from lignocellulosic material.

3.1 Research Goals

Demonstrate the mechanical and biological feasibility of implementing a recirculating cotreatment loop in a switchgrass-fed anaerobic digester.

Experimentally determine how key scale-up parameters including solids loading and radial gap size of the colloid mill affect milling energy consumption.

Utilize modeling to determine the impact cotreatment and other process modifications could have on the economic viability of lignocellulosic anaerobic digestion.

3.2 Objectives

Establish a semi-continuous switchgrass-fed anaerobic digester able to complete cotreatment with a colloid mill as a proof-of-concept for a recirculating loop design.

Conduct intermittent cotreatment at increasing intensities to assess whether the microbiome is robust enough to tolerate the stress of milling.

Evaluate energy use by the colloid mill during cotreatment at varying solids contents and radial gap sizes to better predict energy return on energy invested for this process in scale-up.

Create a model for lignocellulosic anaerobic digestion incorporating intermittent cotreatment and evaluate the economic impact this and other strategies could have on the process.

3.3 Hypotheses

- The anaerobic digester microbiome will tolerate repeated daily cotreatment events of up to 50% of the reactor volume.
- Increased solids loading will proportionally increase the energy consumed by the colloid mill.
- Milling energy consumption will be inversely proportional to reductions in radial gap size.
- Cotreatment and water recycling will increase energy return on energy invested when used in combination in a lignocellulosic anaerobic digestion biorefinery to a value greater than one and exceed that of the system without these strategies.
- An industrial-scale lignocellulosic anaerobic digestion facility will become more economically feasible with the addition of intermittent cotreatment and water recycling.

Section 4: Feasibility of Intermittent Cotreatment Recirculation Loop

This experiment sought to establish the feasibility of a recirculation loop for completing intermittent cotreatment and evaluating the ability of the microbiome to tolerate the stress of milling. This system was evaluated during three phases representing degrees of shear stress. Throughout the experiment, biogas production and volatile solids degradation were recorded. Because these relate to the metabolic activity of the microbiome, they served as a comparison between treatments in this experiment and against those previously conducted. A variety of other analyses were necessary to gather a full view of the changes taking place in response to cotreatment. These analyses were not only intended to determine cotreatment's effectiveness from an engineering perspective, but inform explanations of the possible physical and biological processes involved.

4.1 Methods Overview and Flow Chart

To evaluate the feasibility of intermittent cotreatment in lignocellulosic anaerobic digestion, an established reactor was subjected to three treatment phases beginning with a no-cotreatment baseline. The subsequent two phases included cotreatment at increasing intensities, defined by increasing the proportion of digester volume subjected to daily cotreatment. Samples were collected during these phases for analysis of volatile solids, biogas production, and particle size. The data from these analyses were compared across treatments to determine whether significant changes in the parameters of interest had occurred. A flowchart illustrating this methodology can be found in Figure 1.

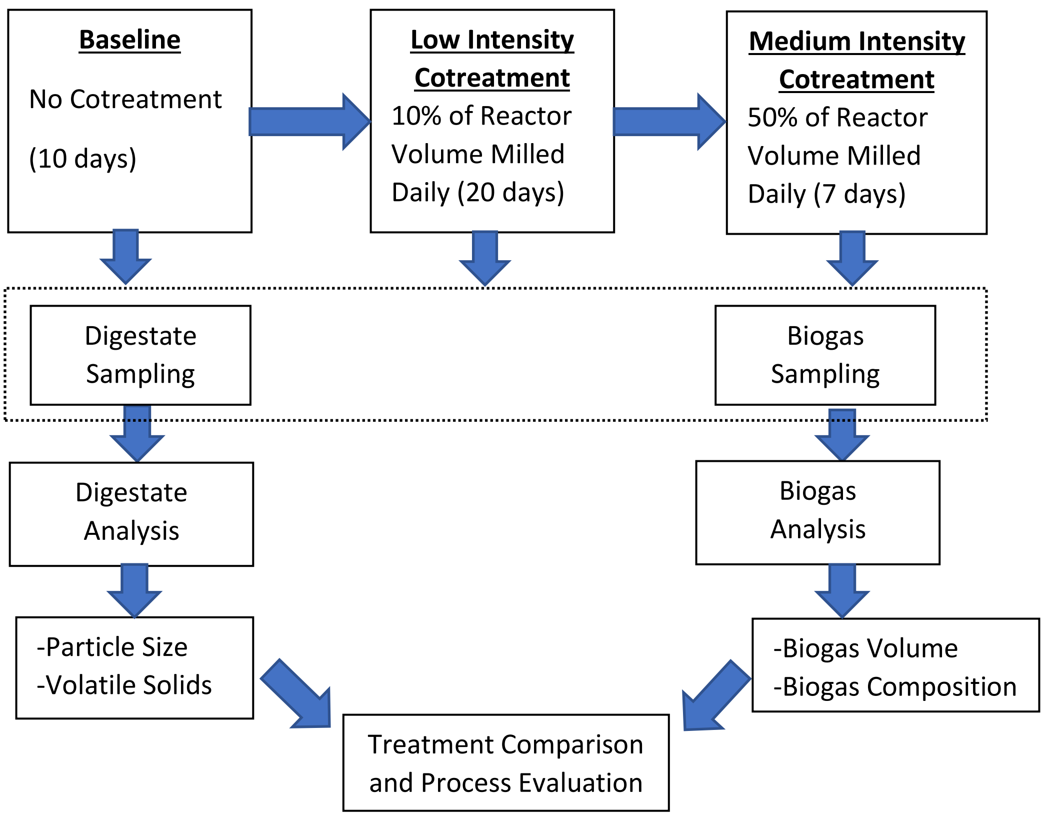


Figure 1: Methodology and Analysis Layout

4.2 Equipment and Configuration

In this experiment an 80 L bioreactor (Mobile Pilot Scale Fermenter, New Brunswick Scientific, Edison, New Jersey) was the main reservoir for material during anaerobic digestion

(Fig. 2). This system included a custom-built data acquisition and control system to autonomously control temperature and pH. Temperature was adjusted by a steam jacket surrounding the body of the system while a peristaltic pump dosed sodium hydroxide into the reactor when the pH dropped below the set point. Because the production of organic acids drove the pH of the reactor down during digestion, acid addition was not necessary. This bioreactor was airtight, maintaining anaerobic conditions in the headspace. Gas produced during digestion escaped through an exhaust port that directed the gas through a Milligas Counter® (Ritter, Boheim, Germany) to record cumulative biogas production. Agitation was completed by a pair of double-pitched, axial-flow impellers, which were well-suited to agitation of viscous material. A peristaltic pump (603S, Watson-Marlow, Falmouth, England) connected to the reactor's drain port served as the transfer mechanism for material extractions and additions.

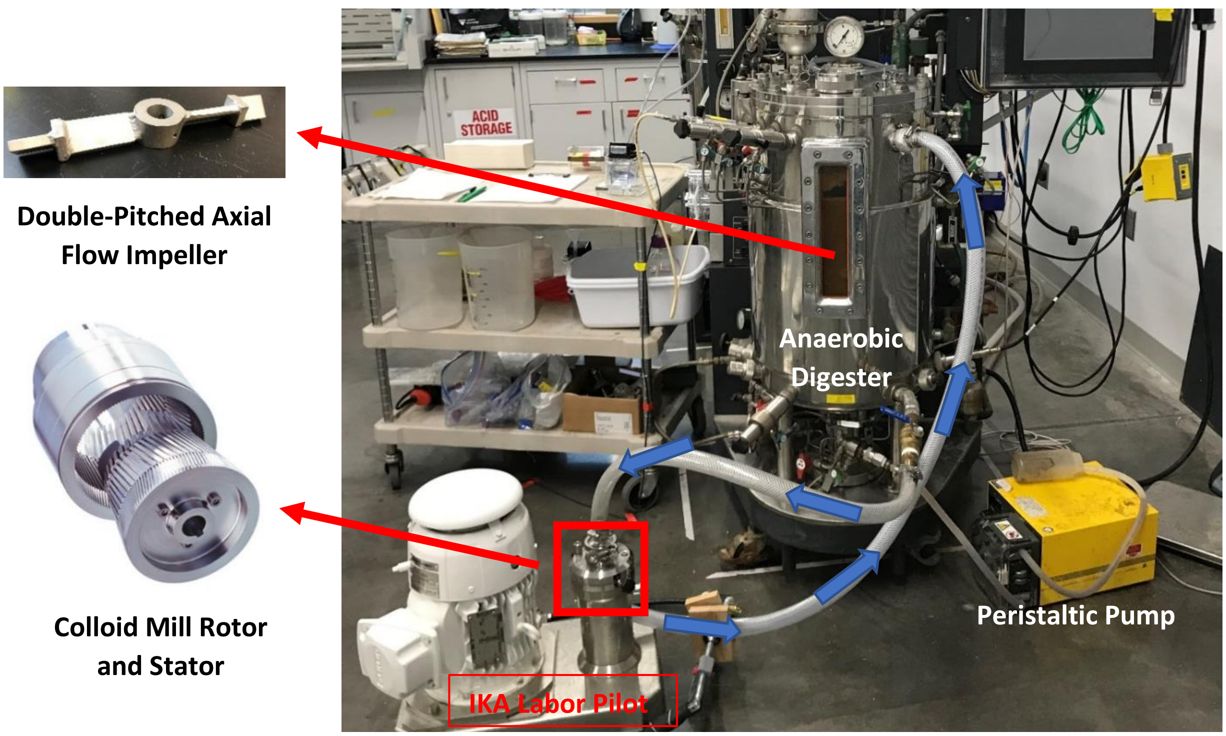


Figure 2: Recirculating Intermittent Cotreatment System Layout

To cotreat reactor contents, the slurry moves from the anaerobic digester to the labor pilot, where milling takes place, then is pumped back into the digester. Extraction and addition of material is completed via the peristaltic pump. The type of agitator used in the digester is shown in the upper right. Colloid mill rotor and stator photo retrieved from https://www.ikaprocess.com/en/Products/Inline-dispersers-Mills-dispersing-machine-high-shear-cph-6/Colloid-Mill-MK-csb-MK/

An IKA Labor Pilot® (2000/4, IKA Works, Wilmington, North Carolina) was used to complete cotreatment milling. This type of colloid mill functions by turning two striated cones against one another with an adjustable gap size between them, shearing material as it passes through. Using a modified sampling port, the bioreactor was connected to the intake and outlet of the labor pilot. This allowed for milling of the material in a contained loop without introduction of oxygen. The layout of major system components is shown in Figure 2.

4.3 Reactor Conditions

Maintaining control of reactor conditions was essential for reliable production of biogas, as methanogens could have been disturbed by fluctuation in a variety of parameters. The digester was inoculated with sludge from a farm waste anaerobic digester in a 3:1 volatile solids ratio of digestate to switchgrass. It operated under thermophilic conditions (55^{o}C), a pH of 7.0, a 3 % solids loading, and a working volume of 60 L. A semi-continuous feeding operation was established with a residence time of 10 days. Post-senescent switchgrass (Ernst Biomass, Meadville, Pennsylvania) milled to a 3.2 mm screen size was used as the substrate. The reactor was supplemented with nitrogen, micronutrients, and vitamins to prevent limitation of bacterial growth and activity. Adjustment of pH was accomplished using 5 M sodium hydroxide.

4.4 Feeding and Cotreatment Procedure

The volume of gas produced as indicated by the Milligas Counter® was recorded. A volume of 6 L was extracted from the digester using a peristaltic pump and the mass of the extracted material was recorded. Samples were collected by filling and quickly capping separate containers, maintaining a total of 6 L extracted. A volume of 6.5 L of 3.2 mm switchgrass and water at 3% solids content was prepared and incubated for 24 hours at 55^{o}C. This allowed the switchgrass to soften and become saturated. Supplements were added just before feeding to avoid degradation or consumption by other microbes before addition to the reactor. The peristaltic pump was then reversed to inject material into the reactor while taking care not to introduce air into the system. The mass of the bucket before and after feed addition was recorded.

Cotreatment took place directly after digestate removal but before addition of fresh substrate. The radial gap size of the mill was set to 0.520 mm. The mill was then activated for a predetermined amount of time corresponding to the appropriate volume of reactor contents to be cotreated. This time period was determined by preliminary runs of the colloid mill during which the flow rate was measured by recording the volume of material milled in a given time.

4.5 Experimental Setup

This experiment took place in three phases to compare the biogas production and volatile solids degradation without cotreatment and with increasing intensities (defined as increasing proportions of the reactor volume being milled) of cotreatment. During the initial phase, no cotreatment was completed. This served as the baseline to which the cotreatment phases were compared. After inoculation with a microbiome from a dairy manure digester (see section 4.7.3) followed by several months of continuous operation with the same switchgrass feeding rate and residence time, the observed process variables achieved a quasi-steady-state. After achieving steady state conditions, the “no cotreatment” baseline measurement period covered one residence time (10 days). Biogas production rates and composition were evaluated as well as baseline levels of other process parameters.

During the second phase, 10% of the reactor volume was cotreated each day. This period extended through two residence times (20 days) with sampling conducted to examine the same parameters as in the baseline. The third phase was carried out in the same manner as the second, except with 50% of the reactor volume being milled each day for 7 days. Concerns about COVID-19 caused a truncation of the original planned length of the second and third phases of the experiment.

4.6 Sampling Procedures and Analysis

Because the reactor conditions were not expected to change rapidly other than during shifting cotreatment conditions, sampling was skewed for greater frequency at the beginning and end of each phase. A summary of the samples collected during each phase of the experiment is shown in Table 1. A full breakdown of sample collection by experiment day can be found in Appendix A.

Table 1: Intermittent Cotreatment Sample Collection Summary

Parameter	Baseline	Low Intensity Cotreatment	Intermediate Intensity Cotreatment	Total Samples
Biogas Volume	Daily	Daily	Daily	29
Biogas Composition	Alt. Days	Alt. Days	Alt. Days	15
Particle Size	2	3	2	7
Volatile Solids	3	6	2	11

4.6.1 Biogas Volume and Compositional Analysis

Cumulative biogas production volume was measured by routing the headspace exhaust of the digester through a Milligas Counter® (Ritter, Boheim, Germany) which functioned by counting the number of times a submerged scale tipped as it collected exiting biogas. Biogas samples were collected from the headspace before volume exchange using an airtight syringe through a septum. The gas sample was injected into an SRI 8610C Gas Chromatograph (SRI Instruments, Torrance, CA, USA) and measured for methane, nitrogen, oxygen, hydrogen, and carbon dioxide contents. Known gas composition standards were then used to convert these data into the gas composition of each biogas sample.

4.6.2 Particle Size Analysis

Samples of digestate were collected during volume exchange directly into two 15 mL tubes every 5 days (1/2 residence time). These samples were analyzed in triplicate for particle size distribution at Penn State's Materials Characterization Lab using the Mastersizer 3000® (Malvern Pananalytical, Egham, United Kingdom). This instrument used laser diffraction to measure the size of particles that passed in front of a light source, yielding information on the range of particle sizes in a given sample.

4.6.3 Volatile Solids Measurement

On each sampling day, seven 15 mL digestate subsamples were collected from the reactor during volume exchange and distributed into pre-weighed and dried crucibles. Each crucible and digestate weight were measured before drying for 24 hours at 105°C. Each crucible was reweighed to determine the total solids of the digestate before undergoing ashing (575 °C for 3 hours) and a final round of weighing for determination of volatile solids. The volatile solids loadings of the seven samples were averaged to give a volatile solids loading for that sampling day.

4.7 Results and Discussion

4.7.1 Equipment Design and Performance

The main object of this portion of the study was to assess the mechanical and biological feasibility of cotreatment. There were three mechanical concerns in executing this design, all of which were relieved by the system's performance. The first of these was clogging, which was a frequent issue when running preliminary testing with the colloid mill. During the study, however, the loop and mill never clogged during its 27 days of operation. This was aided by the relatively low 3% solids loading and the softening of switchgrass particles that took place during digestion. The next concern was that the colloid mill would not generate adequate pumping power to convey the digestate back to the reactor, as the return port was located a few inches above the digestate's surface. The colloid mill provided adequate power to overcome this barrier without issue. The final concern was gas intrusion at the reactor ports; however, gaskets, tight fittings, and hose clamps were sufficient to prevent intrusion of oxygen into the headspace. Under these conditions, intermittent cotreatment anaerobic digestion seemed to be mechanically feasible.

While the recirculating loop design performed well at 3% solids content and a 1.247 mm radial gap size in the colloid mill, further research is necessary to characterize its physical limits. During preliminary work, the system successfully operated at 6% solids loading using undigested switchgrass that had been soaked for 48 hours in a refrigerator. This suggested that the design could operate at slightly higher than 6% solids loading with digested material, which was generally more easily milled and conveyed. At 9 to 10% solids content, the switchgrass slurry became considerably less flowable, which would likely lead to clogging at the recirculation loop intake port or the entrance of the milling head. At and above these solids loadings, addition of conveyance mechanisms like augers or screw conveyers may be necessary to prevent clogging and a colloid mill may no longer be a viable milling option. Additionally, the radial gap size in the milling head affected both the flow rate and the force with which material exited the colloid mill. This could lead to failure of the recirculation loop due to clogging or inadequate pumping force for the material to reenter the reactor.

While there were no major mechanical failures in the system, the valves and clamps located near the recirculation loop entrance and exit were helpful when adjustment of the colloid mill was necessary. These were located as close to the reactor ports as possible to minimize the area particles could settle and to keep the port accessible in the case that a clog needed to be cleared. Care was also taken to limit reductions in tube diameter in the loop, as these points were found to be the most likely areas to clog. Rearrangement of the recirculating loop to keep the reentry port below the fluid surface could also help to ensure clogging does not occur due to inadequate pumping power by the colloid mill. In this arrangement, however, it might be more difficult to recognize when clogging has occurred or is about to occur. For this reason, use of clear tubing for the recirculation loop is suggested. Inclusion of these and other mechanisms to avoid and recover from clogging events are advised during experiments at increased solids contents or decreased radial gap sizes.

4.7.2 Microbiome Robustness

Table 2 provides a summary of the baseline and cotreatment process performance results. Given that previous researchers had observed increases in biogas production from the addition of cotreatment to their lignocellulosic anaerobic digestion systems, it was expected that this study would produce similar findings. While some cotreatment intensities previously completed were extreme compared to those undertaken in this study (many passes of all material through the mill compared to fractions of the material), these treatments were completed only once (Paye et al., 2016). For intermittent cotreatment, the question remained of whether the microbiome would benefit from repeated exposure of fresh substrate or be inhibited by frequent disturbance.

Table 2: Process Indicator Values for Baseline and Cotreatment Conditions

Treatment	Baseline (no cotreatment)	Low Intensity (10% contents/day)	Intermediate Intensity (50% contents/day)
Avg Digestate VS Content (%)	2.51[a]	2.59[a]	2.75[a]
VS Content of Total Solids (%)	88.2[a]	87.0[a]	86.8[a]
VS Conversion (%)	14.9[a]	12.2[a]	6.8[a]
Cellulose Conversion (%)	42.4[a]	34.7[a]	19.2[a]
Avg Hourly Biogas Production (mL/L digestate)	21.6[a]	20.5[b]	20.7[b]
Difference from Base (%)	-	-4.98	-4.21
Biogas Composition (% CH_4/CO_2)	56.7/40.0	56.9/40.2	56.9/39.5

*Values with the same letter superscript are not significantly different (ANOVA, α=0.05). Average hourly biogas production is an average of biogas production over the final half residence time (5 days) of the treatment. Biogas composition values reflect the final recorded value during each treatment.

The volatile solids content in the digester was monitored by periodic sampling during the baseline measurement period as well as during low (10% of reactor contents milled daily) and intermediate intensity (50% of reactor contents milled daily) intermittent cotreatment. During this time, the volatile solids content of extracted digestate was 2.51, 2.59, and 2.75% for the baseline, low, and intermediate treatments, respectively. The 10% and 50% cotreatment volatile solids values were not significantly larger than the baseline or one another (ANOVA, α=0.05). Only two sets of volatile solids measurements were taken during the intermediate cotreatment period. The first averaged 2.6% volatile solids, in line with values from the low and baseline phases, while the second averaged 2.9% volatile solids. Given that biogas production data did not reflect a precipitous decline in conversion and that the coefficient of variation for sets of volatile solids measurements ranged from 4 to 9%, it was expected that the 2.9% volatile solids value may be erroneous.

This suggested that throughout the study, the rate of degradation of the switchgrass volatile solids underwent little change despite the addition of cotreatment. Despite the lack of conclusive statistical evidence, the volatile solids content increased with cotreatment intensity. Had the overall volatile solids loading of the reactor been higher or there been increased replication of volatile solids measurements, the differences between the treatments could have been pronounced enough to show conclusive differences. All pipetting of the digestate was avoided during volatile solids measurement, as this was found to be a significant source of error during preliminary measurements. Further experimentation with intermittent cotreatment anaerobic digestion at higher solids loadings could help to determine whether the volatile solids trend observed in this study is significant.

Biogas measurements were a more reliable measure of reactor performance than VS or cellulose content. Biogas composition was constant throughout the observation period with methane and carbon dioxide making up 57 and 40% respectively. Analysis of hourly biogas production rates showed a decline of 4.97% and 4.21% for the last 5 days (0.5 residence time) of the low and intermediate cotreatment periods respectively compared to the baseline. The last 5 days of each treatment were analyzed to observe the biogas production after the microbiome had adjusted to the stress of cotreatment. This reinforced the trend of cotreatment impaired performance displayed in the volatile solids data, except that the poorest biogas production performance took place during the low intensity rather than the intermediate intensity cotreatment phase. The decrease in biogas production rate between the baseline treatment and each cotreatment intensity was statistically significant, however the difference between the low and intermediate cotreatment intensities was not (ANOVA, α=0.05).

While there was a statistically significant decline from the baseline to the cotreatment treatments, the practical significance of this finding could be limited due to drift of biogas production rates even when conditions are constant. For instance, the coefficient of variation for biogas production over the 25 days prior to the start of baseline measurement was 5.37% of the average production rate. When evaluated over the entire duration of the baseline and cotreatment intensities rather than just the last 5 days, the difference between baseline and low intensity cotreatment biogas production rates dropped to 3.19% and was no longer statistically significant.

Though there was a decrease in biogas production when cotreatment was implemented, there was no evidence of a precipitous drop during the 27 days of observation. The relatively minor changes observed in biogas production suggested the microbiome was able to tolerate daily cotreatment under these conditions. More research is necessary to evaluate the robustness of the microbiome to intermittent cotreatment at a greater intensity. This could be achieved by milling more frequently (allowing less recovery time), milling a larger proportion of the material during each cotreatment event, or milling material at a smaller gap size during these events. Each method of intensity increase could also be explored independently, as their effectiveness in increasing conversion of lignocellulosic feedstocks could differ.

The lack of cotreatment benefit to biogas production observed in this study differed from previous findings in similar systems (Balch et al., 2017). There are a few possible explanations for this. Firstly, particle size distribution analysis showed no statistical change between the baseline and cotreatment periods as shown in Figure 3. The lack of particle size evidence of cotreatment effect could also mean that the selected treatments were not intense enough to meaningfully change conditions for the microbial community. Additionally, the material used in some previous studies was considerably more degraded when cotreatment was completed (Bharadwaj, 2020). While biofilms had likely extensively colonized the surfaces of those switchgrass particles and degraded all accessible cellulose and hemicellulose, this may not be true for the larger and less digested material used in this study. Exposure of new surface areas from cotreatment could have had little effect because the microbes were not limited by accessible carbohydrates or surface area before it was implemented.

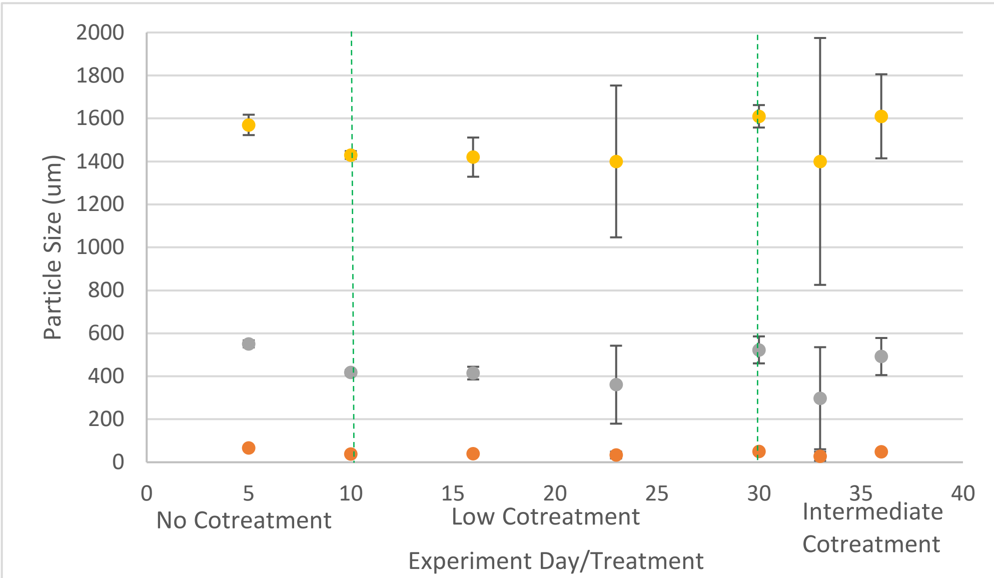


Figure 3: Particle Size Distribution over Measurement Period

Yellow - 90th Percentile Particle Size, Grey - 50th Percentile Particle Size, Orange - 10th Percentile Particle Size. Green vertical dashed lines indicate changes of cotreatment intensity.

Another reason for the difference in performance of cotreatment could be the intermittent nature in which it was implemented. While previous cotreatment strategies lasted for the duration of the fermentation or for only a single treatment, cotreatment was repeated daily in this study. Under the stress of daily milling the microbiome may have had too little time to recover and grow between cotreatment events. Most problematic could be disruption of methanogens, as these microbes grow slowly and can be vulnerable to disturbances. Future studies could focus on intermittent cotreatment that is repeated at longer intervals, like one or two treatments per residence time. Cotreatment could also be considered for implementation between stages of a multi-reactor system to expose material that had not been degraded by the previous fermentation.

4.7.3 Anaerobic Digester Nutrient Supplementation

The anaerobic digester used in this study was run for a total of 183 days prior to the start of cotreatment. The digester was inoculated at a 1:3 volatile solids ratio of digestate from a farm waste digester to switchgrass. Following a 10 day batch period, volume exchange of 10% of digestate with fresh material was completed daily. Throughout this period, the switchgrass content of the feed material was held constant at 3%, while the levels of supplemental nutrient media were varied over time as indicated in Table 3. The concentrations of these media components were based on levels that had been successful in sustaining lignocellulosic anaerobic digesters for long periods during previous studies in the hosting lab as well as calculations of microbial growth requirements and measurements of losses in the digestate, and are detailed in Table 4. For each supplement treatment, the biogas production from the last residence time (10 days) was used for comparison to subsequent treatments, as it represented the microbial community's performance after it had maximally adjusted to the supplement conditions. A figure showing daily biogas production rates for all supplements is located in Appendix A.

Table 3: Anaerobic Digester Performance over Various Nutrient Supplement Conditions

Supplement Mix	Start Day	Avg Hourly Biogas Production Rate (ml/L digestate)	Difference from Previous (%)
None	11	12.5	-
Vitamins*	48	7.8	-37.29
NH_4Cl (1 g/L)	54	19.0	142.60
NH_4Cl (2 g/L)	100	20.7	9.22
NH_4Cl+Minerals	129	22.0	6.20
Anaerobic Minimal Media	142	21.8	-1.10

All treatments are statistically different from one another (One Way ANOVA, α=0.05) except NH4Cl+Nutrients and Anaerobic Minimal Media. All concentrations are based on g/L in volume exchange.

*The vitamins supplement mix was administered after the biogas production in the system had begun sharply declining. The vitamin addition likely did not cause the further decline, but failed to reverse this trend because it was not the limiting nutrient.

Table 4: Nutrient Supplement Concentrations in Volume Exchange

Supplement Mix	Vitamins	NH4Cl (1 g/L)	NH4Cl (2 g/L)	NH4Cl + Minerals	Anaerobic Minimal Media
Compounds	**Nutrient Concentration (g/L in volume exchange)**				
NH_4Cl	0	1	2	2	2
NaCl	0	0	0	1	1
$MgCl_2$ $6H_2O$	0	0	0	1	1
$CaCl_2$ $2H_2O$	0	0	0	0.5	0.5
KH_2PO_4	0	0	0	0.4	0.4
Trace Compounds	**Nutrient Concentration (ug/L in volume exchange)**				
H_3BO_4	0	0	0	50	50
Nitrolotriacetic Acid	0	0	0	1000	1000
$CuCl_2$ $2H_2O$	0	0	0	38	38
$MnCl_2$ $4H_2O$	0	0	0	50	50
$(NH_4)_6Mo_7O_{24}$	0	0	0	50	50
$AlCl_3$	0	0	0	50	50
$CoCl_2$ $6H_2O$	0	0	0	50	50
$NiCl_2$ $6H_2O$	0	0	0	92	92
$FeCl_2$ $4H_2O$	0	0	0	2000	2000
$ZnCl_2$	0	0	0	50	50
Na_2SeO_3 $5H_2O$	0	0	0	100	100
Na_2WO_4 $2H_2O$	0	0	0	100	100
Biotin	0.2	0	0	0	0.2
Folic Acid	0.2	0	0	0	0.2
Pyridoxine hydrochloride	1	0	0	0	1
Riboflavin	0.5	0	0	0	0.5
Thamine hydrochloride	0.5	0	0	0	0.5
Canocobalamin	0.01	0	0	0	0.01
Nicotinic Acid	0.5	0	0	0	0.5
p-aminobenzoic Acid	0.5	0	0	0	0.5
Lipoic Acid	0.5	0	0	0	0.5
Pantothenic Acid	0.5	0	0	0	0.5

When volume exchange of reactor contents with feeding material began on day 11, no nutrient or mineral supplements were added to the feed mix (3% total solids slurry of switchgrass and tap water). On day 35, the biogas production declined steeply from 1061 to 499 mL/h over 14 days. Suspecting the crash was the result of a lack of a necessary supplement, a vitamin mix was added to the feed. Gas decline continued over a 5-day period. This continuation of the downward trend is likely because vitamins were not the limiting nutrient, rather than indicating that the vitamin addition was detrimental to digester performance. On day 54 a nitrogen supplement of 1 g/L ammonium chloride began and vitamin supplementation ended. This concentration was selected to match the ratio of nitrogen supplementation relative to switchgrass as used in other anaerobic digesters in the lab. Gas production subsequently recovered to a rate of 1139 ml/h (average of final residence time before next supplement addition); a 52% increase compared to the rate without nitrogen supplementation.

On day 100, the nitrogen supplementation rate was doubled to 2 g/L ammonium chloride, as free ammonia levels in the digestate were measured at as low as 11 ppm. The subsequent 9.2% increase in biogas production to 1244 mL/h suggests that the culture had been nitrogen limited. Following this addition, free ammonia levels were measured at 380 ppm, which suggested that the microbes were being supplied with adequate nitrogen. Assuming losses of ammonia to headspace gas were negligible, 2 g/L is likely the minimum ammonium chloride supplement concentration that is appropriate. It may be advisable to increase the nitrogen supplement rate further to allow a greater buffer should increased microbial growth occur, however care must be taken not to reach levels of nitrogen toxicity.

Minerals (Ca, Mg, Fe, etc.) were added to the nitrogen supplement beginning on day 129. A further 6.1% increase in biogas production to a rate of 1321 mL/h was observed. This suggests that there was mineral limitation before this point. The most likely limiting components were calcium, magnesium, or phosphorus. Assuming that microbial biomass composition in an anaerobic digester is similar to that of a generalized bacterium, these elements represent the most abundant biomass components which were estimated not to be supplied at sufficient rates by switchgrass alone.

Addition of a vitamin mix to the feed on day 141 did not result in a statistically significant increase in biogas production over the remainder of the observation period. This suggested that for this microbial community, vitamin addition was not necessary to prevent system failure.

While this study suggested that both nitrogen and mineral supplementation are necessary for this switchgrass-fed anaerobic digester and vitamin addition is not, more research is necessary to precisely define the necessary concentrations of each of the supplements. This is especially true concerning the minerals that were added during this study in a group rather than individually, as some of the additions could be unnecessary. Future studies should also consider the diversity of microbes in the inoculum used to start the digestion process, as less diverse microbiomes could lack members capable of certain synthesis or degradation pathways that could change the supplement needs of the community. Likewise, established digesters which were not supplied with a nutrient over a period of time may have lost members which required it and could have increased the speed or efficiency of the conversion process if they were present.

Considering that the diverse nutrients added to the digester are components of microbial biomass rather than biogas, the nutrient need of digesters depends on the microbial biomass concentration as well as the feedstock properties. Biomass concentration has potential to vary depending on many conditions in the digester, making it difficult to estimate. Once this has been established, suggested nutrient loading rates based on microbial biomass composition are available for certain anaerobic digester conditions (Hendriks, Lier, & Kreuk, 2018). Especially for nutrient poor and recalcitrant substrates, like some lignocellulosic materials, nutrient limitation of maximal microbial biomass loading could result in suboptimal conversion rates.

4.8 Conclusions

This study demonstrated the mechanical and biological feasibility of a lignocellulosic anaerobic digester using a recirculating loop design and executing intermittent cotreatment. During this study, no clogs or mechanical problems occurred and safeguards, including valves at reactor entry and exit ports, were sufficient to avoid failure. Measurement of biogas production suggested that while the intermittent cotreatment conditions used in this study did not increase biogas production, the microbiome was able to tolerate daily milling of 50% of the digester volume. Further research focused on the intensity of intermittent cotreatment could help to establish under which conditions cotreatment increases conversion of lignocellulosic feedstocks. Lack of benefit from cotreatment may have resulted from too low an intensity, as evidenced by no change in particle size distribution, or material that was not heavily degraded enough for the microbes to be substrate limited. Monitoring of biogas production while modifying nutrient supplementation suggested that nitrogen and mineral supplementation are necessary for optimal biogas production from switchgrass, but vitamins were not. Further research into microbial biomass composition and concentration in anaerobic digesters could help to establish the supplement formula necessary for lignocellulosic biomass conversion.

Section 5: Mill Energy Consumption under Varying Digestate Conditions

In order to evaluate the efficacy of cotreatment as a process intensification technique, the energy consumption of this process must be defined. While the previously described experiment ran at 3% solids loading, it is likely that for economic feasibility to be achieved, a higher solids loading would be necessary. Previous analyses relied on two major assumptions concerning the energy requirement by colloid mills. The first is that the mill consumes similar amounts of energy regardless of solids content. Second, the role of the radial gap size in relation to the particle size was not considered when estimating milling energy consumption. Both of these assumptions could lead to inaccuracies when increasing the scale of cotreatment and process modeling.

The colloid mill used in this study reduced biomass particle size by shearing between a conical rotor and stator with striations. The stator could be moved up or down relative to the rotor, which allowed for variation in the distance between the surfaces. This distance between the nearest points of contact of the rotor and stator is termed the radial gap size. In addition to changing the shearing distance, the cross-sectional flow area changed with radial gap size. The interplay of these factors along with the particle size distribution and degree of degradation of the slurry material created a complex relationship between power drawn, flow rate, and energy consumption as radial gap size varied.

5.1 Digestate Preparation

This experiment sought to measure the energy consumption of the colloid mill in conditions as similar to those in a functioning digester as possible using the pilot-scale equipment available. To do so, slurries of 1.6 mm screened switchgrass were mixed at the appropriate solids loading (3%-8% by mass for solids loading variation, 6% for gap size and slurry material variation) and incubated for 3 days at 55°C. This allowed for saturation and softening of the particles to better mimic the rheological properties of digestate.

Two slurries were prepared from switchgrass that had been knife milled (Munson SCC-10-MS, Munson Machinery Co., Utica, NY) through 1.6 mm or 3.2 mm screens, referred to as 1.6 mm switchgrass and 3.2 mm switchgrass respectively. The third slurry material was digestate that had been removed from the lignocellulosic anaerobic digester outlined in Section 4 during volume exchange but before the milling treatments were implemented. That digester had been fed switchgrass originally knife milled through a 3.2 mm screen and was subjected to digestion in an 80 L complete mix reactor operated at 55 C with a residence time of 10 days. The material removed from this digester during volume exchange is referred to as 3.2 mm digestate, although the particle size was smaller than for the 3.2 mm switchgrass because of the digestion process.

These materials were selected to allow comparison between particle size distributions (1.6 mm and 3.2 mm switchgrass) and degrees of material degradation (3.2 mm switchgrass and 3.2 mm digestate). The treatments of these slurries reflected the range of radial gap sizes from the smallest possible through which the material could be milled without clogging to larger than the majority of particles in the slurry. A solids loading of 6% was selected to better model solids loadings that could be present in commercial settings and gain greater resolution between treatments.

5.2 Experimental Setup

For this experiment, the colloid mill was outfitted with a feeding reservoir capable of containing the necessary volume of digestate for the experiment (see Figure 4). A two-way valve was installed at the outlet of the milling apparatus, allowing for either recirculation of the milled material or ejection into a drain. A ring stand, movable cart, and drill with a paint mixer attachment were situated to allow for vigorous agitation of the slurry. The milling head was adjusted to an appropriate radial gap size (0.520 mm for solids loading variation, 0.312-1.663 mm for gap size and slurry material variation). The mill was then connected to a device capable of measuring instantaneous voltage and power over time.

Figure 4: Milling Energy Consumption Experimental Setup

5.3 Experimental Procedure

The power measurement device was activated, allowing for adequate measurement of baseline load before activation of other components. The drill was then turned on to agitate the feed and 5 kg of digestate was loaded into the feeding reservoir of the mill. The colloid mill was turned on simultaneously to avoid settling and clogging. Milling periods lasted until the feeding reservoir was empty, which ranged from 10 to 70 seconds. Triplicate energy measurements were conducted for each selected solids loading and gap size. Following conclusion of the measurement, the digestate was purged from the system and rinsed with water to remove residual material. Before each set of measurements, the radial gap size of the mill was examined to check if drift had occurred, however drift was not observed during this experiment. Following measurements, the data for each run was plotted, transient behavior during start-up and shut-down was truncated, and the power required over the middle 50% of the quasi-steady-state period was determined. Multiplying these values by the length of measurement time yielded a total energy consumption for the milling event. The average energy consumption from the triplicate measurements of each treatment was used for comparison.

5.4 Results and Discussion

While previous cotreatment experiments had documented energy consumption during the milling process, these measurements focused on singular solids loadings, slurry material types, and radial gap sizes. Anecdotal evidence from milling freshly prepared slurries as well as heavily degraded digestate suggested that the energy consumed during milling can vary greatly between these conditions. Similar effects on milling energy requirements were observed with changing gap sizes. Accurately predicting energy consumption given certain mill and slurry conditions is essential to evaluating the feasibility of cotreatment as a process intensification technique at increased scales. Accordingly, this study sought to evaluate the effect of slurry materials, solids loading, and mill radial gap size on major cotreatment process parameters. These include flow rate, power requirement, and energy consumed during the milling process. Increased reliability of estimates for energy consumed during cotreatment could help to improve the accuracy of assumptions that are crucial to successful modeling of cotreatment enhanced anaerobic digestion at scale.

5.4.1 Effect of Gap Size Variation for Switchgrass Slurry and Digestate

Power drawn by the mill was broadly inversely proportional to radial gap size as illustrated in Figure 5 and tabulated in Appendix B. This relationship was expected, as the decreased gap size led to both a greater proportion of particles being milled and the milled particles undergoing a greater size reduction. The highest observed power drawn was 1006 W from the 1.6 mm switchgrass slurry at a 0.312 mm gap size. The highest power drawn for the 3.2 mm digestate and 3.2 mm switchgrass was 788 W at 0.624 mm and 540 W at 1.040 mm radial gap sizes respectively. The power for each material dropped until a plateau at about 450 W after the radial gap exceeded 0.728 mm for 1.6 mm switchgrass and 1.040 mm for the 3.2 mm switchgrass and 3.2 mm digestate slurries. This was likely the result of the gap reaching the point where particles in the slurry were mostly being pumped through the mill rather than undergoing a size reduction, and may thus simply represent the pumping power requirements for a wet slurry of 6% solids.

For both the 1.6 mm switchgrass and 3.2 mm digestate slurries, however, it appeared there could be a second plateau at the smallest gap sizes observed. In these areas, the flow rate tended to be slow and failure occurred when the solid portion of the slurry could not pass through the mill as quickly as the liquid portion. Thus, at small gap sizes this apparent second plateau could have been an indication of the mill reaching the mechanical limits of its operation capacity with partial clogging causing decreasing and fluctuating solids concentrations as reflected in the higher standard deviations for these conditions.

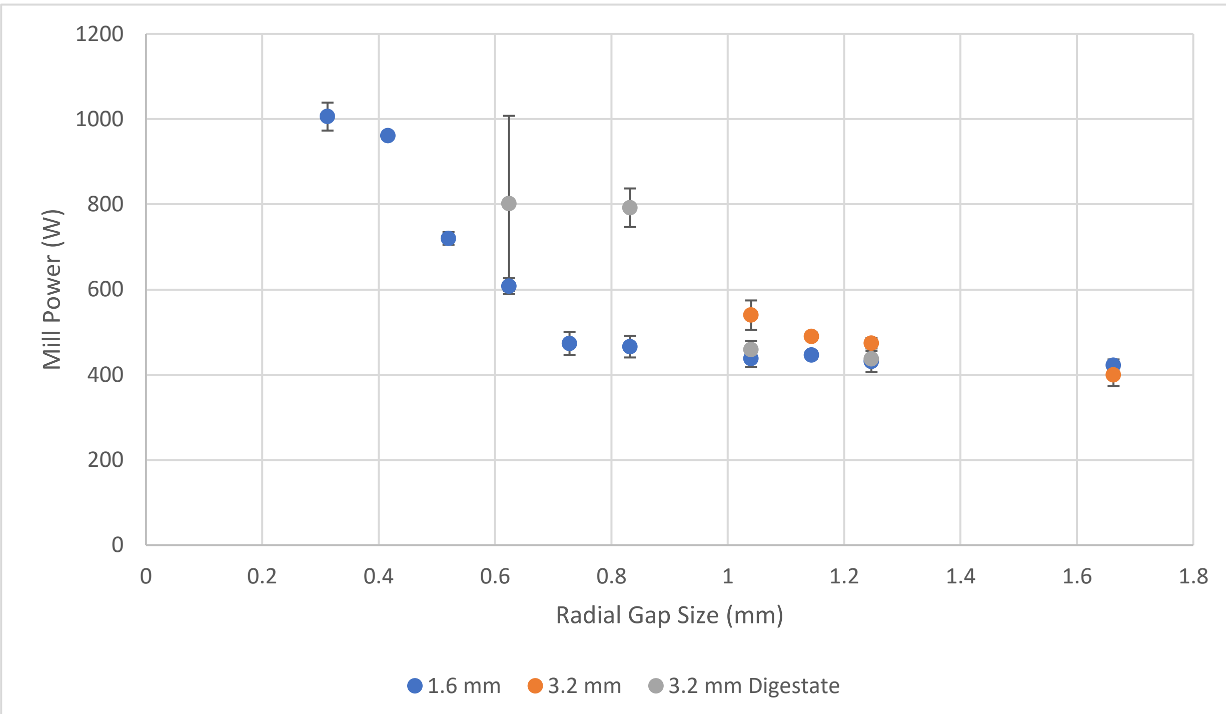


Figure 5: Mill Power vs. Radial Gap Size

For each gap sized at which multiple slurry materials were tested, the 3.2 mm switchgrass tended to consume the most energy, followed by the 3.2 mm digestate. This was likely due to the larger proportion of particles that underwent a large particle size reduction at a given gap size compared to the 1.6 mm switchgrass. The lower energy required for milling the 3.2 mm digestate relative to the 3.2 mm switchgrass slurry could have been due to the greater amount of degradation this material had undergone during digestion, making it softer and more easily sheared.

In order to quantify the effect of changing gap size on power drawn by the mill, a power regression was fitted to the data from each slurry type using Excel (Microsoft Office, Microsoft

Corp, Redmond WA) in the form shown by Equation 1, where *a* and *b* are constants, milling power is in watts, and radial gap size is in millimeters.

Equation 1: $$Milling\ Power = a * radial\ gap\ size^{b}$$

These regression coefficients are provided in Table 5, and were notable due to the similarity of *b* values for the 1.6 mm and 3.2 mm switchgrass and *a* values for the 3.2 mm switchgrass and the 3.2 mm digestate. The 1.6 mm and 3.2 mm switchgrass treatments were prepared from fresh switchgrass that had been incubated, but not digested, before use, suggesting that these treatments could have similar rates of change in power consumption as radial gap size increases (b value). The 3.2 mm switchgrass and 3.2 mm digestate treatments were more similar in particle size distribution, so it was not surprising that they would share a similar magnitude of power consumption at a gap size of 1 mm. Further research would be necessary to estimate these relationships for additional material types to conclusively define the effects of particle size distribution and degree of material degradation on power requirements for a colloid mill.

Table 5: Mill Power Equation Constant Values and Correlation

Material	a value	b value	R^2 value
1.6 mm Switchgrass	478.31	-0.602	0.8732
3.2 mm Switchgrass	543.38	-0.612	0.9832
3.2 mm Digestate	540.65	-1.01	0.8181

While the R^2 values for these power curves indicated a strong correlation with gap size, visual inspection suggests a different form of equation may better represent this relationship with power requirements. For instance, a piecewise function of two straight lines intersecting around the 0.728 mm gap size might better represent the behavior of the 1.6 mm switchgrass slurry. A sigmoidal curve could capture the plateaus at the upper and lower gap sizes as well as the slope at intermediates gaps, possibly leading to a better fit. Regardless of the equation chosen, extrapolation of these equations outside of the demonstrated gap sizes is not recommended, as physical constraints, including clogging for small gap sizes and shifting to a pumping rather than shearing regime for large gaps, could cause deviations from these equations.

As indicated in Figure 6, the observed flow rate at each gap size tended to be highest for the 1.6 mm switchgrass. This slurry material had the smallest particles which likely made it more easily flowable than the other treatments. At larger gap sizes, the flow rates seemed to converge asymptotically at a flow rate of about 0.6 kg/s. This trend could have a similar cause to the plateauing of power consumption, likely occurring at gap sizes where few of the particles were undergoing size reduction. Plate gap variation in a small commercial scale system grinding corn stover experienced a 29% decrease in material throughput when decreasing the plate gap from 1.78 mm to 0.25 mm (Chen et al., 2014). In contrast, in the current study there was a much larger 85% reduction in material throughput observed for the 1.6 mm switchgrass as radial gap size decreased from 1.663 mm to 0.320 mm. The large error bars around the 3.2 mm digestate treatments at the 0.624 and 0.832 mm gap sizes were likely due to the mill running under conditions at which it was on the verge of clogging. This made the flow rate during the run more variable. Tabular flow rate data for all slurry conditions and gap sizes is included in Appendix B.

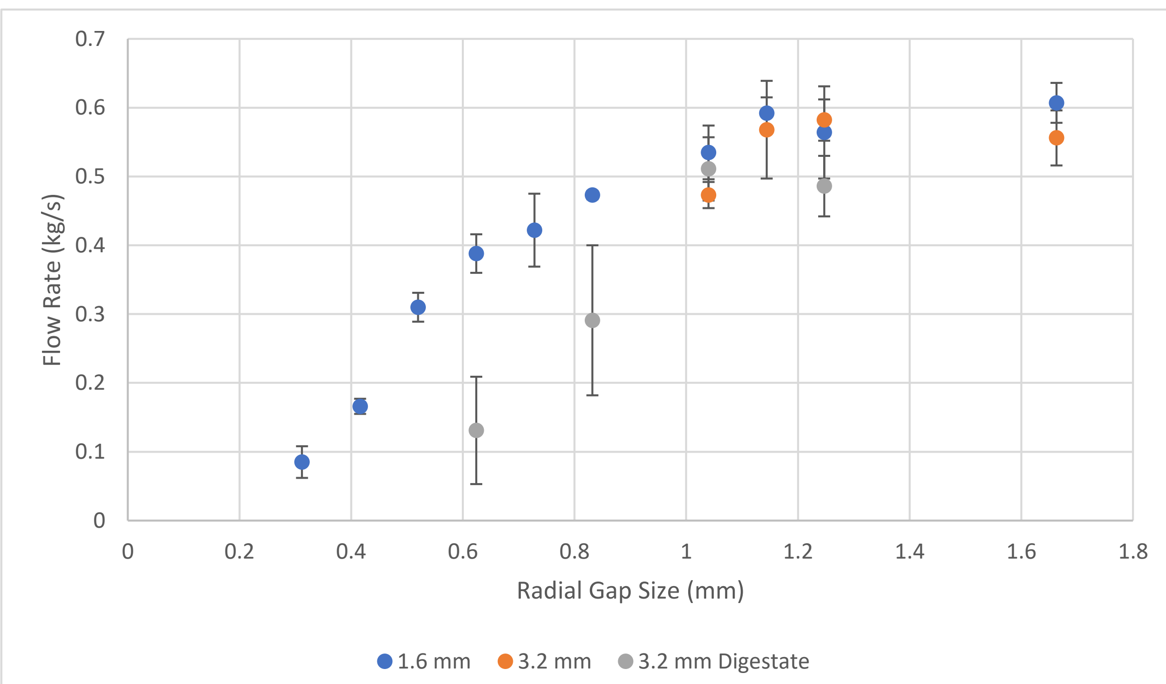


Figure 6: Slurry Flow Rate vs. Radial Gap Size

Logarithmic regression was used to fit curves in Excel and model the flow rate for each of the slurry materials. While the correlation was strong for the 1.6 mm switchgrass and 3.2 mm digestate treatments, the 3.2 mm switchgrass experienced clogging at larger gap sizes so there were fewer data points available for estimating the regression coefficients. This relationship is shown in Equation 2, for which a and b are constants, flow rate is in kilograms per second, and radial gap size is in millimeters. Regression coefficients for these curves are shown in Table 6.

Equation 2: $$Flow\ Rate = a * \ln(Radial\ Gap\ Size) + b$$

Table 6: Mill Flow Rate Equation Constant Values and Correlation

Material	a value	b value	R^2 value
1.6 mm Switchgrass	0.3349	0.5068	0.9535
3.2 mm Switchgrass	0.1158	0.5186	0.2292
3.2 mm Digestate	0.5698	0.4111	0.9064

One of the most important parameters when considering the feasibility of cotreatment is the amount of energy that must be invested per unit of biomass. This value compared to the additional energy generated by the same amount of biomass would allow evaluation of whether the technique was energetically beneficial. Figure 7 illustrates the expected inversely proportional trend between radial gap size and energy consumed by the mill. Milling energy generally steeply increased at the smallest possible gap sizes before clogging of each material. This was not true for the 3.2 mm switchgrass, which could only be milled to a 1.040 mm gap size before clogging occurred. It is expected that for this slurry material, the mechanical limit of the material was reached before the sharp increase in energy consumption occurred. As with the other measurements, the unit energy consumption for each of the slurry materials plateaued at about 25 kJ per kilogram of dry switchgrass after the 1.040 mm gap size. This reflected that little of the material was undergoing size reduction above this gap size, but similar amounts of energy were consumed to pump the material through the colloid mill.

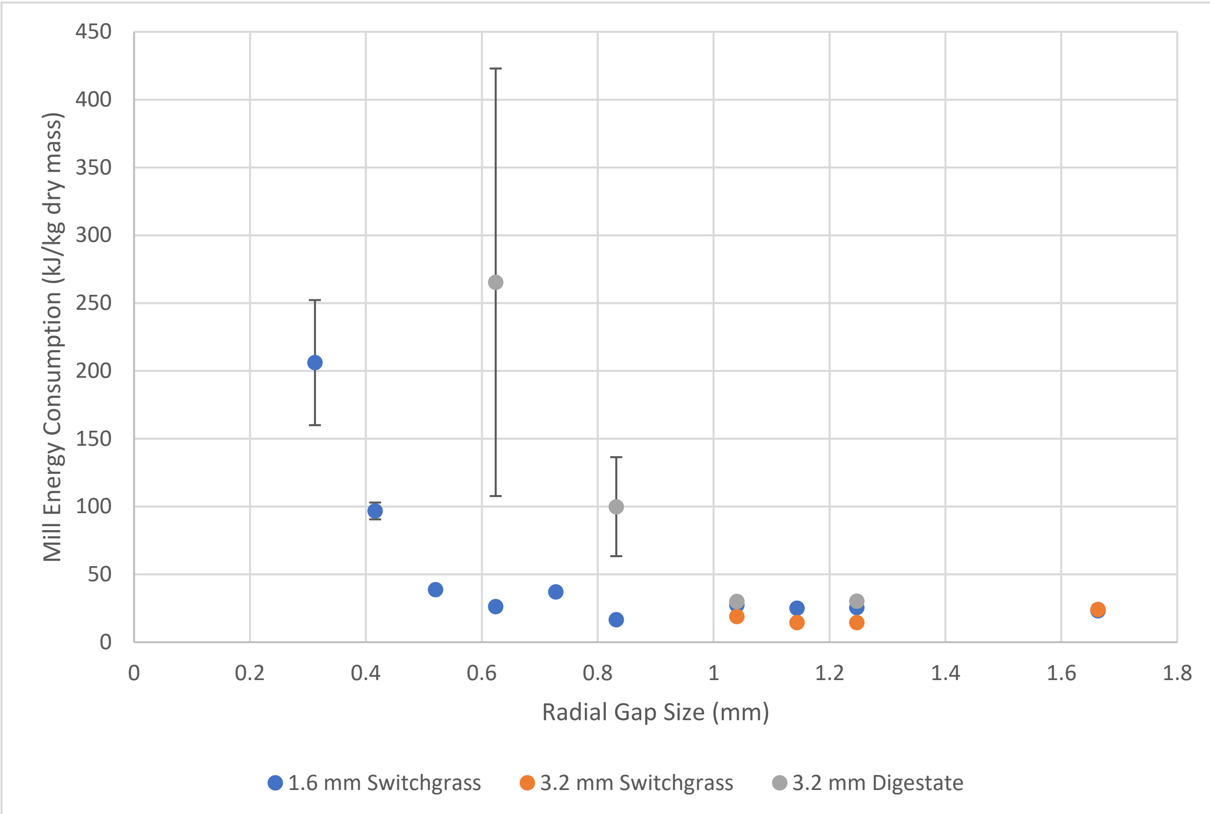


Figure 7: Mill Energy Consumption vs. Gap Size

The large error bars for the 1.6 mm switchgrass and the 3.2 mm digestate at their lowest gap size treatments are likely due to the partial clogging that occurred during these measurements. In these treatments, the mill was operating near the physical limits of its ability to pump the switchgrass slurry without clogging, and intermittently slowed as buildups formed and dislodged at the mill entrance. The differences in the frequency that this occurred between replicates caused variation in flow rates and power drawn, and with it the milling energy consumption.

Given that switchgrass contains about 11000 kJ of energy per kilogram dry matter just from the higher heating value of the cellulose and hemicellulose fractions (Demirbas, 2002), the energy invested during cotreatment only accounted for a small portion of the overall total. Even for the most energy intensive cotreatment conditions, the 250 kJ/kg biomass consumed represented only about 2.3% of the available energy. This energy consumption by the colloid mill even at pilot scale was similar to or less than that consumed by hammer mills (99 kJ/kg) that are commonly used for size reduction of dry materials (Jacobson, Roni, Lamers, & Cafferty, 2014; Mani, Tabil, & Sokhansanj, 2004). Colloid milling is very efficient compared to the energy estimated to be consumed in a dilute acid pretreatment process for olive tree pruning waste, which requires 2410 kJ/kg of dry mass treated (Solarte-Toro et al., 2019), which would represent 22% of the higher heating value of cellulose and hemicellulose contained per kilogram of switchgrass. Another dilute acid pretreatment process for corn stover was estimated to require 666 kJ/kg dry mass (Eggeman & Elander, 2005).

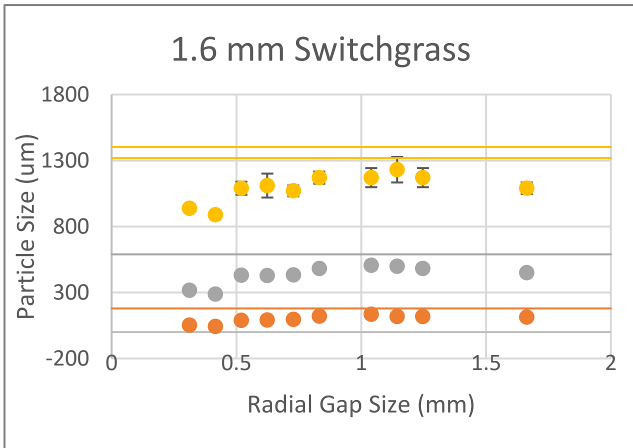
The energy consumption data for each slurry material was fitted using power curves, as shown in Equation 3, in which *a* and *b* are constants, milling energy consumption is given in kilojoules per kilogram of dry switchgrass, and radial gap size is given in millimeters. These correlations were not as strong as those for flow rate and mill power prediction. Regression coefficients for these curves are shown in Table 7.

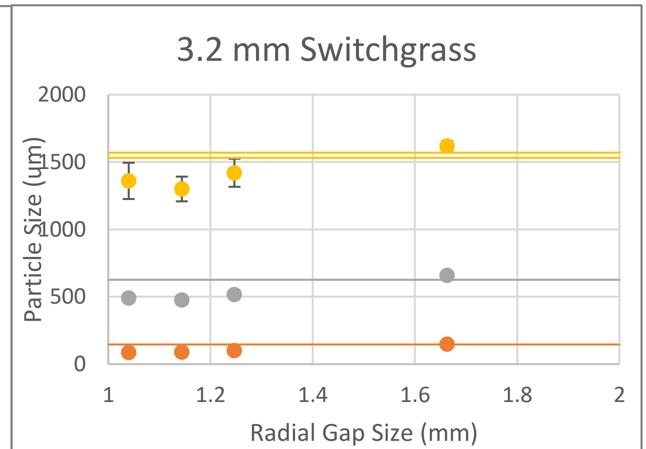
Equation 3: $$Milling\ Energy\ Consumption = a * radial\ gap\ size^{b}$$

Table 7: Mill Energy Consumption Equation Constant Values and Correlation

Material	a value	b value	R^2 value
1.6 mm Switchgrass	26.818	-1.186	0.6654
3.2 mm Switchgrass	19.373	-1.498	0.7597
3.2 mm Digestate	49.969	-3.413	0.9369

While the link between degree of particle size reduction and cotreatment effectiveness has yet to be fully explored, it is likely that larger magnitude changes in particle size will result in increased biogas production rates by exposing more undigested surface area. For this reason, examination of how effectively particle size distributions were reduced in each treatment will be an important step toward implementation of cotreatment. These measurements were displayed using the 10^{th}, 50^{th} and 90^{th} percentile particle sizes in Figure 8, which concisely displays the relative sizes of particles throughout the distribution. In all measurements, the error found in the 90^{th} percentile particle size likely had two major contributions. The first is that these particles were approaching the size limit of the measurement device (Malvern, Mastersizer 3000). A second factor is that the particles have a high aspect ratio, being rectangular fibers rather than spheres. Both of these factors could have led to greater error in the laser diffraction analysis of these particles.





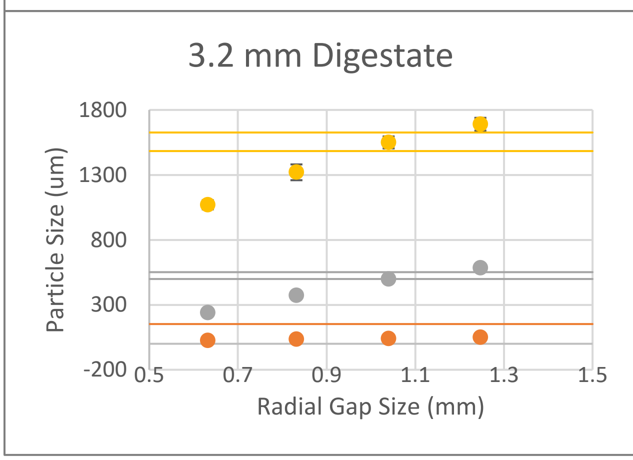


Figure 8: Particle Size Distribution for Varying Gap Size Milling Events
Yellow - 90th Percentile Particle Size, Grey - 50th Percentile Particle Size, Orange - 10th Percentile Particle Size, Lines indicate the starting size of the percentile with the corresponding color (± 1 Standard Deviation)

For the 1.6 mm switchgrass, milling caused a particle size reduction for all treatments at each of the particle size percentiles. The magnitude of these decreases seemed to separate into three groupings with the smallest gap sizes (0.312 and 0.416 mm) causing the largest change. Within these groupings, there was little change in particle size distribution. This meant that there could be an opportunity to achieve the same physical effect with decreased energy consumption by selecting the largest gap size within these groupings. The similar particle size reduction for the 1.6 mm switchgrass at larger radial gap sizes, including even the 1.663 mm gap, was unexpected since the majority of particles would not be large enough to contact the rotor and stator simultaneously. However, even at larger gap sizes the particles would still be subjected to turbulence and shear forces from the counter-rotating surfaces. Several additional mechanisms contributed to particle size reduction, including not just shear forces as the material is pumped, but also collisions and friction between particles.

The 3.2 mm switchgrass slurry experienced a significant particle size reduction in the observed percentiles for all but the largest radial gap size (1.663 mm). However, while the 3.2 mm digestate treatments experienced a decrease in the 10^{th} percentile particle size for all gap sizes, those treatments did not have significant changes in the 50^{th} or 90^{th} percentile for the larger two gap sizes (1.040 and 1.247 mm). One possible interpretation is that small aggregations of particles were being disturbed by the shear forces of milling in all of the 3.2 mm digestate treatments, while larger particles, likely consisting of less heavily degraded switchgrass, were more resistant. More research is necessary to determine whether size reduction in larger particles or smaller aggregates is more important to increasing biogas production. A study of lime pretreatment of switchgrass found no further increase in sugar yield when the material was milled to a sieve size smaller than 420 um (Chang, Burr, & Holtzapple, 1997), however the behavior may be different for biological rather than chemical degradation mechanisms.

5.4.2 Effect of Solids Loading on Cotreatment Process Parameters

In anaerobic digestion, as with other bioproduction techniques, it is often advantageous to increase substrate loading and maximize yield of the desired product. When combining increasing solids loading with a process intensification technique like cotreatment, it must be determined whether the process remains energetically favorable. For instance, if doubling the solids loading quadrupled the energy consumed during cotreatment and counteracted the gains of additional substrate, combination of these strategies would be ineffective. For this reason, the milling power, flow rate, and mill energy consumption were determined for 1.6 mm switchgrass slurry at 3 to 8% solids loadings at a constant radial gap size of 0.520 mm. Milling power results from this trial are illustrated in Figure 9 and tabulated in Appendix B.

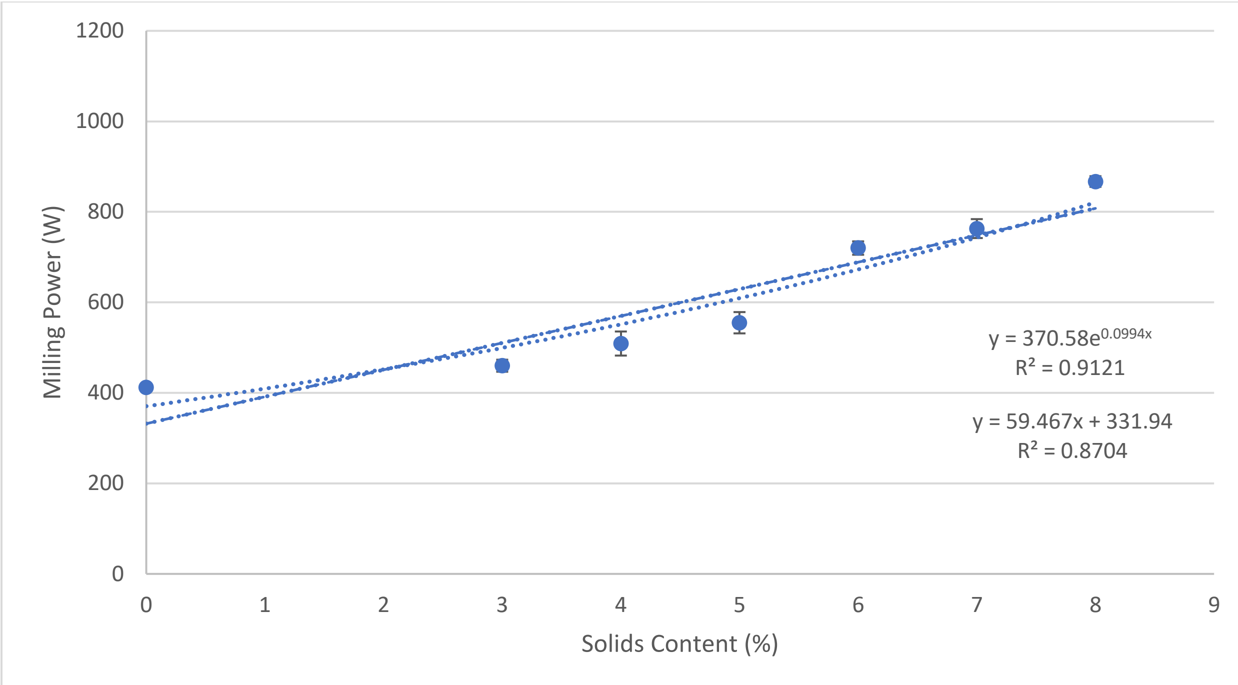


Figure 9: Mill Power vs. Solids Content

While the milling power requirements increased with increased solids loading, the magnitude of this increase was not proportional to the solids loading increase. For instance, milling of 8% solids slurry required only 1.7 times more power than at 4% solids. This trend could be modeled using either exponential or linear trendlines, as both have R^2 values of greater than 0.85, however an exponential curve could be more physically relevant when considering the increasing number of particles that must be milled as well as the material becoming less flowable as the solids loading increases. Extrapolation of these trends beyond 8% solids loading is not recommended, as clogging occurred beyond this tipping point. Switchgrass slurries begin to behave more like saturated solids than slurries at greater than 10% solids loading, which would cause deviations from this trendline. It is important to note that these tipping points are expected to be much higher for digestate as illustrated in the previous milling trials. However, there was not sufficient digestate available to include that as a feedstock for this solids content trial.

Figure 10 illustrates the flow rates of the varying solids content slurries, which decreased from a plateau of about 0.5 kg/s to about 0.15 kg/s as solids increased. The full range of this data was best modeled using the subtraction of an exponential function from the asymptote value, however a straight line was also suitable if the 0% solids treatment (water) was ignored. Clearly, extrapolation of these regression equations beyond the experimental boundaries quickly strays from physical relevance, as both trendlines would produce negative flow rates for switchgrass slurries above 9 to 10% solids.

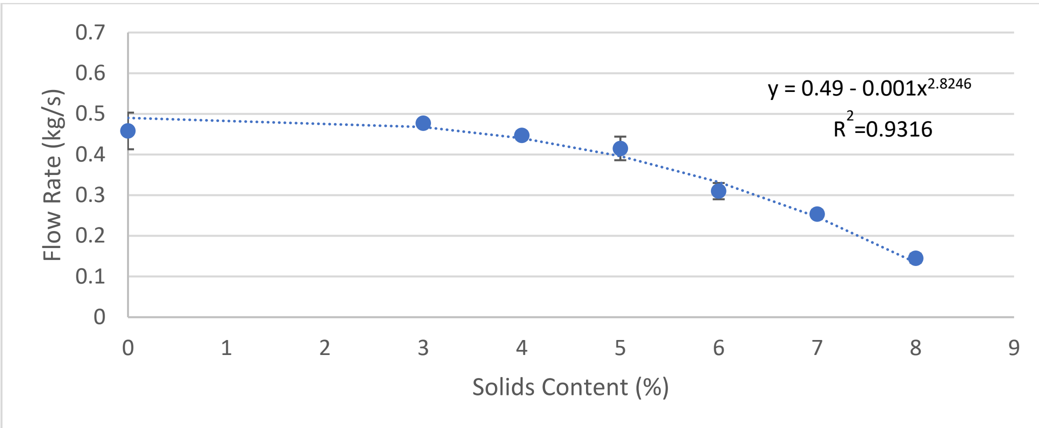


Figure 10: Flow Rate vs. Solids Content

Energy consumption per kg of dry switchgrass decreased from 3% to 5% solids with a minimum value of 26.85 kJ/kg, as illustrated in Figure 11. Above 5% solids, the energy consumption increased to a maximum of 74.81 kJ/kg dry switchgrass at 8% solids. This trend in energy consumption as solids content increased appeared to be parabolic with a minimum between 4 and 5% solids. Fitting a second order polynomial regression to the data using Excel yielded a 0.9527 R^2 value, indicating strong correlation. This relationship was likely caused by the tradeoff that arose as solids loading increased. Increased solids loading meant that a greater amount of material passed through the system, however it also caused the flow rate to decline and the mill power requirements to increase. This created a minimum value at which the slurry has the largest loading of solids that can be achieved without significant rheological changes that inhibit material flow. The trend toward increasing viscosity with solids loading was also found in a study of high-solids slurries of corn stover (Ghosh, Holwerda, Worthen, Lynd, & Epps, 2018). Increased energy consumption as solids content increases was observed for these high-moisture wet biomass slurries. Interestingly, this is contrary to what is commonly observed in milling of relatively dry, high solids lignocellulosic biomass, where milling energy requirements increase as solids content decreases (Mayer-Laigle, Blanc, Rajaonarivony, & Rouau, 2018; Miao, Grift, Hansen, & Ting, 2011). At these much higher solids contents, adding moisture to dry, brittle biomass may impart greater flexibility and therefore resilience relative to milling and particle size reduction.

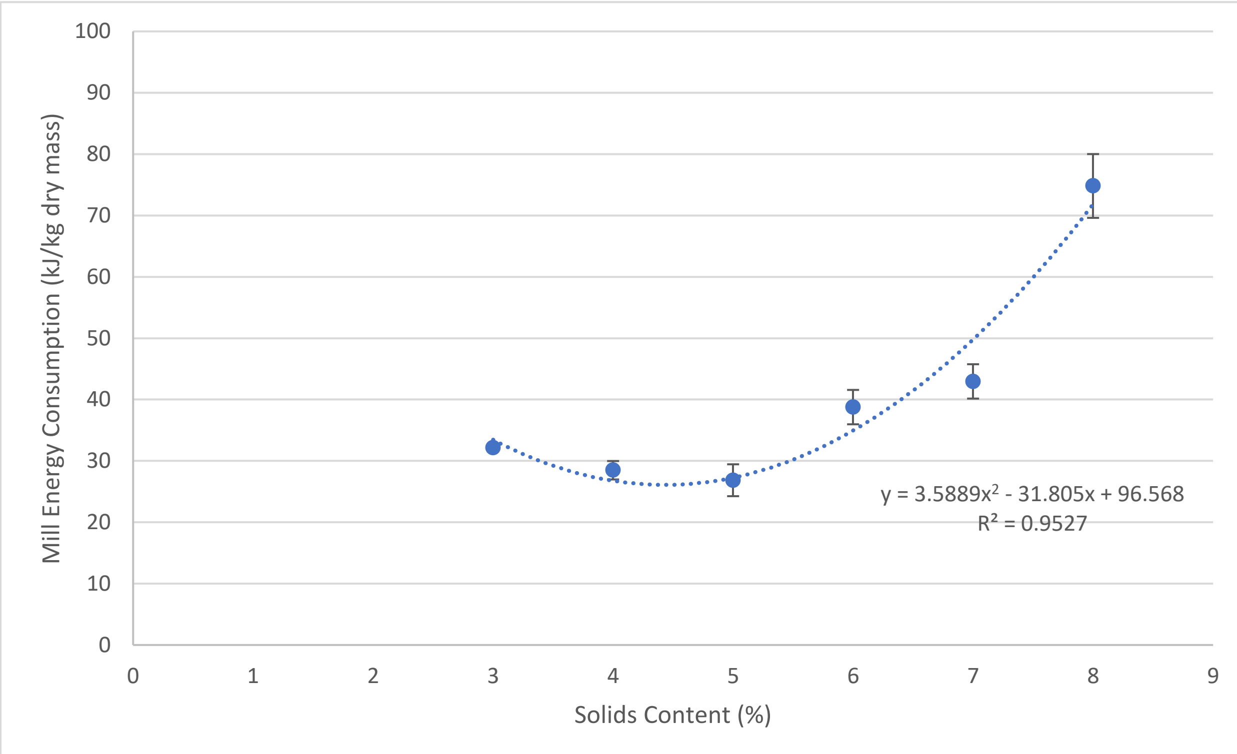


Figure 11: Energy Consumption vs. Solids Content

Increasing solids loadings beyond 5% in this system will require evaluation of whether the biogas production gains from increased substrate concentrations and the cost of savings in terms of reactor size for a given biomass solids loading rate are counteracted by the climbing energetic cost of increasing solids content. Further research in this area using digestate could aid in determining the solids loading at which the milling energy minimum exists for degraded material rather than undigested material. This minimum would likely be at a higher solids loading than fresh material, as digestate tended to flow and be milled more easily.

Figure 12 illustrates particle size reduction as a function of solids loading, with the data underlying this figure presented in Table 8. All treatments along the solids loading spectrum showed differences in 10th, 50th, and 90th percentile particle size compared to the starting material. The magnitude of these changes increased with solids loading, as the size of particles at 8% solids were reduced by over twice the percentage achieved in the 3% solids treatment. This suggests that interactions between particles were a significant contributor to size reduction during milling and that these interactions increased with solids loading. A study in mineral slurry particle size reduction via rod milling also found variation in particle size by moisture content, however the largest magnitude reduction occurred at an intermediate moisture content rather than the highest solids loading (Ghassa, Gharabaghi, Azadmehr, & Nasrabadi, 2016). This was attributed to the tradeoff between reduced slurry flow rate and increased contact between particles as the solids loading increased.

Table 8: Percentage Particle Size Reduction Compared to Start Size

Solids Loading	Particle Size Percentile		
	10th	50th	90th
3%	30.2	18.7	12.5
4%	35.2	20.9	16.9
5%	45.1	28.0	19.9
6%	49.8	27.0	19.9
7%	62.3	37.4	25.7
8%	73.8	43.5	27.5

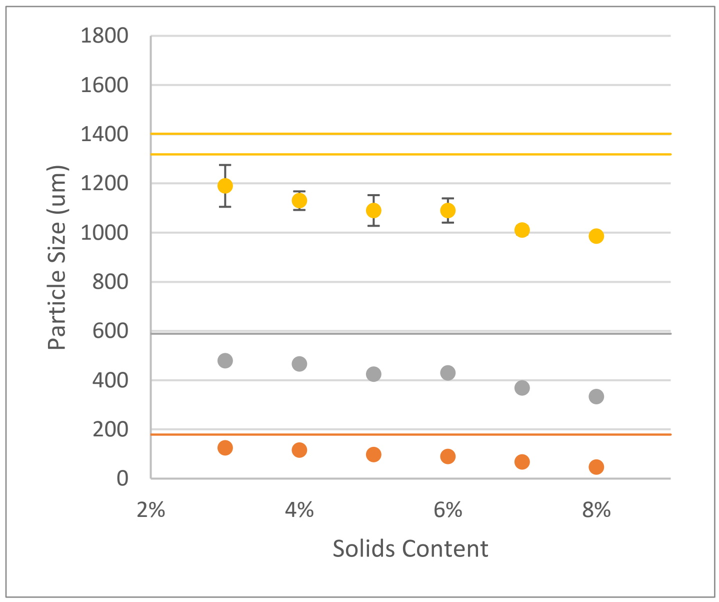


Figure 12: Particle Size Reduction by Solids Loading
Yellow - 90th Percentile Particle Size, Grey - 50th Percentile Particle Size, Orange - 10th Percentile Particle Size, Lines indicate the starting size of the percentile with the corresponding color (± 1 Standard Deviation)

Comparisons of the magnitude of size reduction to the energy invested will be important for determining the solids loadings that are energetically beneficial. For instance, moving from a solids loading of 6 to 7% requires a 10.8% increase in energy investment per kg of dry switchgrass, but the 50th percentile particle size decreases by 14%. Relating the decrease in particle size to the effectiveness of cotreatment in accelerating biogas production will be important for comprehensive evaluation of cotreatment in an anaerobic digestion system. Replication of this study using digestate from anaerobic digesters at different solids loadings is necessary to determine if the trend in increased particle size reduction with increased solids loading is reflected in other slurry materials.

5.4.3 Physical Limitations and Scale-Up Considerations

Throughout this study, treatments at low gap sizes and high solids contents were limited by clogging. Rather than occurring within the milling head, these blockages tended to happen in the piping between the milling vessel and milling head. This suggested that the size of the pipe connecting the vessel to the milling head played a role in creating clogging events, likely due to the rapid change in diameter as particles were pulled toward the mill. Because the size of the particles compared to the conveyance pipe was relatively large, a buildup in particles can block movement through the pipe. While it was a major concern in this study, the likelihood of clogging could decline as cotreatment increases in scale. This is because sizes of pipes and mills will increase compared to the lab or pilot scale setups currently in use, alleviating one of the foremost risks for clogging. While biomass particle sizes entering commercial systems are expected to be as large or larger than those used in this study, they are unlikely to increase as much as the pipe diameters and other reactor components necessary for cotreatment-enhanced anaerobic digester systems to reach industrial relevance.

5.5 Conclusions

This study showed that as gap size decreased more energy was consumed during milling of all slurry materials. At a given gap size, the 1.6 mm switchgrass tended to consume the least energy followed by 3.2 mm digestate, while undigested 3.2 mm switchgrass consumed the most energy. For an individual pass through the colloid mill, even at the most energy intensive conditions, this milling energy accounted for only 2.3% of the higher heating value of the cellulose and hemicellulose contained in the switchgrass per pass. This data from pilot scale trials is comparable to the energy consumed per kg of dry biomass during other industrial particle size reduction techniques at commercial scale, which is important because the milling energy efficiency is expected to improve as processes scale up making the cotreatment approach even more favorable. The magnitude of particle side reduction change tended to increase as radial gap size decreased for all slurry materials. Energy consumption per unit mass of switchgrass showed a parabolic trend, with a minimum at 5% solids. This was likely due to the tradeoff between the increasing amount of material and increasing energy consumption during milling due to rheological changes. Particle size reduction increased in magnitude with increasing solids loading, suggesting that interactions between particles during milling also played a role in particle size reduction. While clogging was problematic at high solids loadings and small gap sizes, this problem is expected to become less prominent at scale when pipe diameters will likely be larger compared to particle sizes.

Section 6: Anaerobic Digestion Scale-up Model with Cotreatment and Water Recycling

Because lignocellulosic anaerobic digestion is an immature technology and capital costs for pilot- or commercial-scale testing facilities are high, modeling is an important mechanism for evaluating the efficacy of potential innovations and solutions to various challenges. Modeling can be especially powerful when implemented on a platform which allows for robust and easily manipulated economic and market scenarios. Excel offers an easily manipulable and understandable tool for preliminary modeling of anaerobic digestion innovations. Even simple models of such systems can be sufficient to predict major trends and the effects of assumptions, both of which provide valuable information for the development of models with increasing complexity.

6.1 Flow Diagram and Explanation of Model

Figure 13 illustrates the flow of material through the proposed process for cotreatment-enhanced anaerobic digestion of lignocellulosic material. Feedstock first enters the system and is processed (chopped or milled) to the specifications required for addition to the anaerobic digester. Biogas produced in the anaerobic digester is removed from the system, while digestate can take two paths. The first is the cotreatment loop, which subjects the material to milling before reentering the main digester. The second is the recovery loop, which separates digested solids from the liquid. The digested solids are removed while a portion of the fluid can be recycled into digester. Excess wastewater is removed from the system.

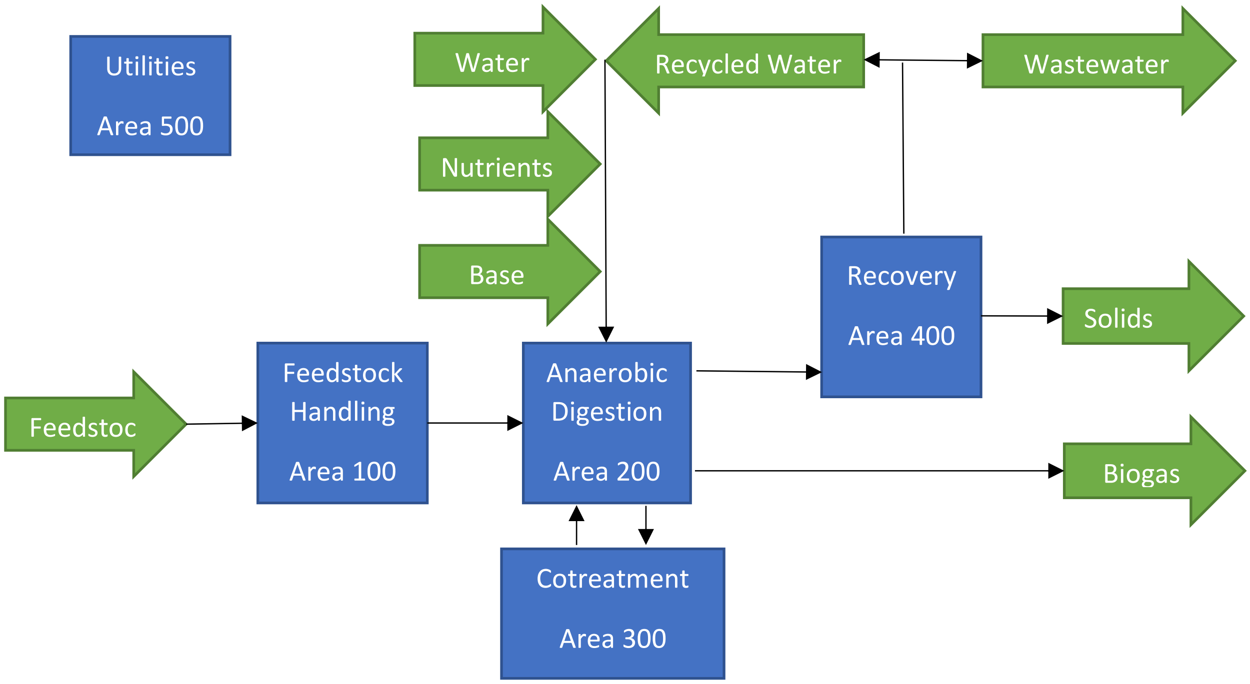


Figure 13: Flow Diagram of Lignocellulosic Anaerobic Digestion Model with Cotreatment

6.1.1 Area 100-Feedstock Handling

The first process unit in this model was feedstock handling, during which incoming lignocellulosic biomass is be manipulated to meet the requirements for addition into the digester. The equipment necessary for this area could vary with the identity of the feedstock and the size of that material upon receipt, but includes at least a milling or shredding mechanism. Hammer mills and knife mills are commonly used for size reduction of agricultural products. Feedstock characteristics, like moisture content and composition are also recorded within this unit.

6.1.2 Area 200-Anaerobic Digestion

The anaerobic digestion process in this model consists of a unit which degrades incoming organic compounds at proportions determined by the reactor conditions (temperature, pH, residence time) and the assumed effectiveness of cotreatment. The baseline conversion values are based on those from previously completed experimental work (Liang et al., 2018; Paye et al., 2016). This approach differs from anaerobic digestion units in previous process models that make the assumption that any insoluble plant cell wall constituents, including cellulose and hemicellulose, do not degrade during digestion (Humbird et al., 2011). While this prior assumption may have been appropriate for models designed for highly soluble feedstocks or systems that filter out most solids before digestion, it is clearly inaccurate and consequential for direct anaerobic digestion of lignocellulosic feedstocks.

6.1.3 Area 300-Cotreatment

Cotreatment is the major unit process differentiating this model from those that have previously been created. While the equipment necessary to complete cotreatment has been previously determined (IKA Colloid Mill), there have been limited scale up analyses of this equipment with respect to economic costs or energy consumption under varying conditions (Amador-Diaz, 2019; Bharadwaj, 2020; Lynd et al., 2017). A range of values for the increase in conversion due to cotreatment was applied in this model, as a definitive relationship between cotreatment intensity and resultant degradation rates has yet to be established. Equations generated earlier in this thesis were used to calculate power consumption and flow rate of digestate through the colloid mill.

6.1.4 Area 400-Recovery

Heating of digester contents is one of the most energy intensive aspects of anaerobic digestion. One mechanism for retaining thermal energy is to recycle water from the effluent digestate back into the system, as has been demonstrated for a variety of systems including a dairy manure digester (Andriamanohiarisoamanana et al., 2018; Larsson, Galbe, & Zacchi, 1997). This strategy could also recover nutrients that would otherwise be discharged. While monitoring of concentration of key nutrients and total salinity would be necessary to execute recirculation without accumulated inhibitory compounds, modern control systems would aid in this endeavor. Because the proportion of removed water that could be recycled back into the system is unknown, this parameter was varied over the full range of possible values.

In order to separate the solid and liquid components of removed digestate, a dewatering mechanism, such as a screw press or centrifugal dewatering machine, must be employed. Excess process water and solids from this process would be discharged from the system. Considering that dewatering mechanisms are common in manure management processes, there could be opportunity to leverage the commercial equipment and expertise developed for existing manure digester facilities to reduce heating energy consumed.

6.1.5 Area 500-Utilities

This system requires water, steam, and electricity for operation. The utilities unit compiles the consumption of these utilities as well as their purchase prices. These resources were modeled as if they must be purchased rather than generated on the facility, allowing greater access to government subsidies for renewable fuel production for transportation, which are considerably greater than the incentives available for on-site use for heat and electricity. Heating is assumed to take place via a natural gas boiler. Additionally, irrigation-grade agricultural water is also assumed to be suitable for use in the anaerobic digester, as it costs an order of magnitude less than drinking water purchased in Pennsylvania (Doran, 2020).

6.1.6 Scale and Boundaries

Definition of the scale at which a process will operate as well as the boundaries for the system being considered can greatly affect the apparent feasibility of the process. To capture this effect, the model was implemented over a range of digester sizes. This model only includes costs incurred and energy used from the time the feedstock is unloaded at the facility to the point that biogas, digested solids, and wastewater exit the facility. Because biogas usage and upgrading can be so diverse, inclusion of these scenarios is considered outside the scope of this model. Some of the market price scenarios would require additional processing, such as biogas separation for Renewable Natural Gas, which would reduce the estimated net present value of these scenarios.

6.1.7 Assumptions

A variety of assumptions had to be made concerning the conditions under which the hypothetical anaerobic digester would run. A residence time of 10 days and thermophilic temperature were chosen since these were shown to be stable in this thesis. Additionally, a solids loading of 8% was selected. While cotreatment anaerobic digestion has not been demonstrated at this solids loading, the considerably larger pipes and other components would be less prone to clogging than those used in pilot scale reactors and are expected to be feasible. Increasing material throughput by increasing solids loading would also be desirable to increase the quantity of biogas produced. The proportion of digester contents cotreated daily was assumed to be 50% regardless of the effect it produced. While a greater or lesser amount of cotreatment may be necessary for optimal increases in conversion, the ideal value is unknown. The digester volume was assumed to be 1200 cubic meters, which roughly corresponds with the digester size required to handle manure from a 1000 cow dairy farm (ASABE, 2000). An internal rate of return of 10% and a 20-year usable life was assumed for the system. A 23-year loan for capital costs was assumed to have a 2% interest rate and equal monthly payments throughout the payback period. A D3 RIN price of $1.25 per gallon of gasoline equivalent was included in most scenarios (US EPA, 2017). Low Carbon Fuel Standards credits (California Air Resources Board, 2020) equal to $2.27 per gallon of gasoline equivalent and a D3 RIN price of $2.50 are also included in some scenarios. A full listing of assumptions is included in Appendix C.

6.2 Results and Discussion

All scenarios modeled in this study resulted in a negative net present value over the 20-year life of the digester. These losses ranged from $1.9 to $7.4 million in 2020 dollars. The projects required large capital investments of $1.2 to $4.3 million, making the financial barrier to implementing this technology large even if it were profitable long term. The majority of this investment stemmed from the digester, which represented about 70% of the construction cost. The most significant operational cost in running the lignocellulosic anaerobic digester was purchasing switchgrass, making up about half of the yearly operation costs. Operation and maintenance accounted for another 30% of costs with the remaining portion consisting of heating, electricity, and water.

For the base scenario of this model, it was assumed that there was no cotreatment and no water recycling taking place. The 1200 cubic meter digester was assumed to run at 8% solids loading with a D3 RIN price of $0.0095 per MJ ($1.25 per gallon gasoline equivalent). Under these conditions, the EROEI was 0.65, meaning that more energy was invested to power and heat the system than was contained in the methane produced. The natural gas price necessary to reach a NPV of zero over the lifespan of the system was $0.0992 per MJ ($108 per 1000 ft^3), compared to a current market value of $0.0027 per MJ ($3 per 1000 ft^3). This scenario is squared in red in Figure 14.

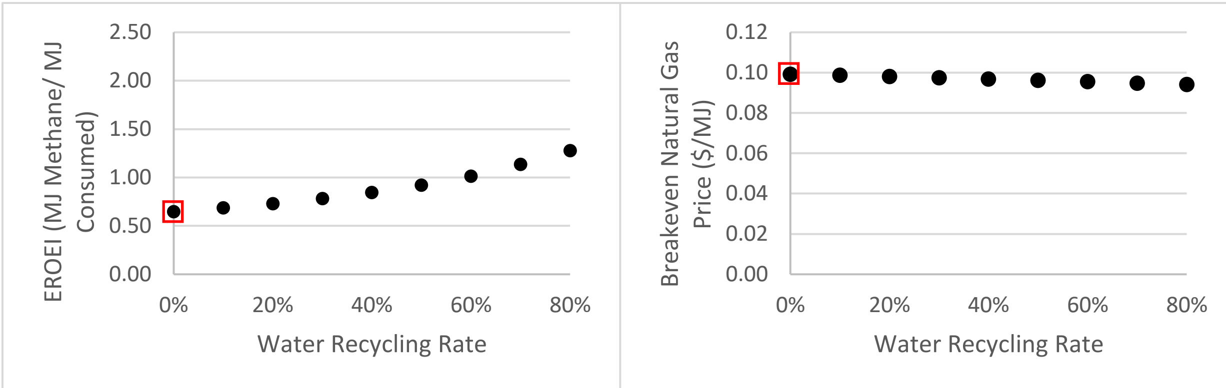


Figure 14: Energy Return on Energy Invested and Breakeven Natural Gas Price for Water Recycling Scenarios

Assumed reactor volume of 1200 m^3 and 8% solids loading for all scenarios. Red square indicates base scenario with no water recycling or cotreatment.

Because the digestate separation processes do not produce a bone-dry solids fraction, a portion of water will always be lost with the digested solids. At an 8% solids loading, the largest water recycling rate possible was estimated at 80%. The effect of water recycling rates at 10% increments on EROEI and the breakeven price of natural gas are shown in Figure 14. The energy returned on energy invested reached a value of 1 at a water recycling rate of 60% and doubled that of the baseline at 80%. The effect of water recycling on the breakeven natural gas price was less significant, with the 80% rate predicted to result in only a 5% reduction.

The cotreatment conversion increases were modeled at increments of 10% to a maximum value of 100%. The original assumed rate of conversion for cellulose and hemicellulose was 0.4 g converted/g available, so a 10% increase in cotreatment conversion this corresponds to an overall increase of 4% of cellulose and hemicellulose converted (0.4 to 0.44 g converted/g available) with a maximum conversion of 0.8 g converted/g available at the 100% increase. As Figure 15 illustrates, while the maximum energy return on energy invested for cotreatment (1.28 MJ Methane/MJ consumed) was the same as that for water recycling, the linear rise to this point meant that modest increases in conversion rate had a more significant impact than modest water recycling rates. Cotreatment also showed potential to reduce the breakeven price of natural gas by nearly half compared to the base scenario of no cotreatment or water recycling, however achieving a 100% cotreatment conversion increase is unlikely to occur in practice.

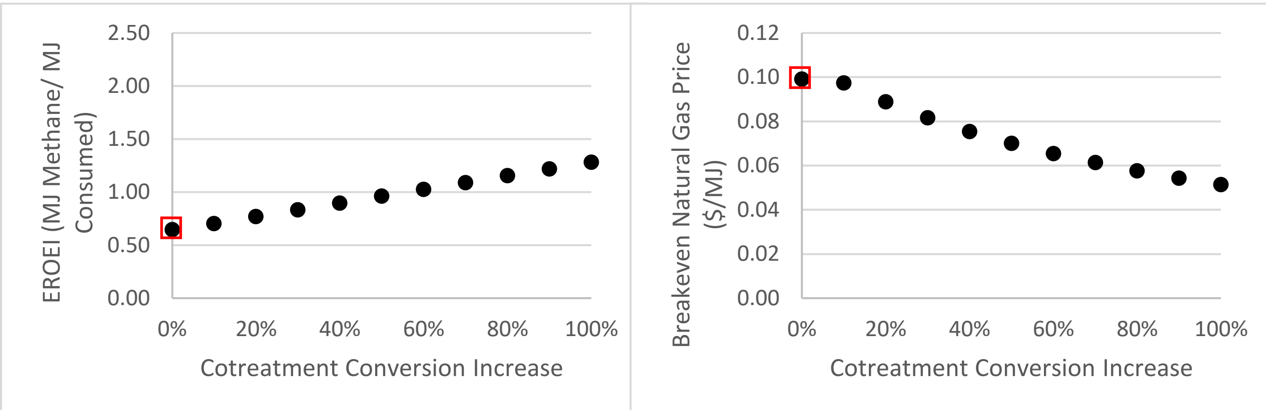


Figure 15: Energy Return on Energy Invested and Breakeven Natural Gas Price for Cotreatment Conversion Increases

Assumed reactor volume of 1200 m^3 and 8% solids loading for all scenarios. Red square indicates base scenario with no water recycling or cotreatment.

The combination of water recycling and cotreatment enhanced the effect on both EROEI and breakeven natural gas price. For these scenarios, the two strategies were modeled simultaneously at 0%, 10%, 20% and 40% cotreatment conversion increases across the range of possible water recycling rates as shown in Figure 16. Individually these strategies did not achieve an EROEI above 1 until a rate of at least 60% was simulated, but when the two strategies were combined they reached this threshold at 40% water recycling. The most optimistic scenario with a 40% cotreatment conversion increase and an 80% water recycling rate raised the baseline EROEI by a factor of three. The combined impact on breakeven natural gas price was less dramatic, however 20 and 40% cotreatment conversion increases with 80% water recycling decreased this value by 21.4% and 32.3% respectively.

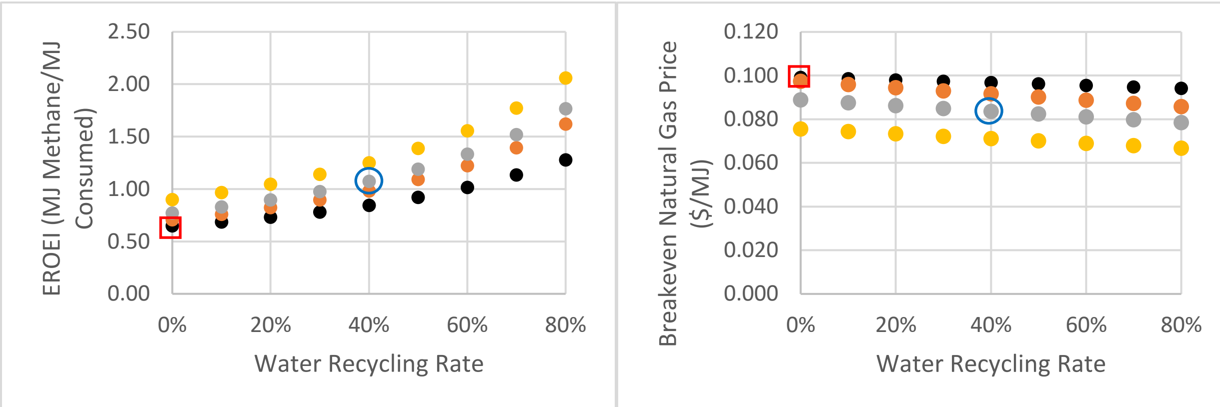


Figure 16: Energy Return on Energy Invested and Breakeven Natural Gas Price for Combined Cotreatment and Water Recycling Scenarios

Black Circle-No Cotreatment, Orange Circle-10% Cotreatment Conversion Increase, Grey Circle-20% Cotreatment Conversion Increase, Yellow Circle-40% Cotreatment Conversion Increase. Assumed reactor volume of 1200 m^3 and 8% solids loading for all scenarios. Red square indicates baseline scenario with no cotreatment or water recycling. Blue circle indicates 20% cotreatment conversion increase and 40% water recycling rate.

Economies of scale often arise in industrial processes, with increased volume of production making it more efficient and less costly to produce an additional unit of product. This effect was also displayed observed in the results. Figure 17 illustrates the impact on EROEI and the breakeven natural gas price when digester size was increased by two-fold or decreased by two- and four-fold compared to the 1200 cubic meter volume assumed in the other scenarios. The 20% cotreatment conversion increase and 40% water recycling rate was used as the base scenario for this comparison. The EROEI for the system decreased by 11% and 26% for the 600 cubic meter and 300 cubic meter digester sizes, while increasing by 9% at 2400 cubic meters. The breakeven natural gas price for these scenarios decreased as volume increased, with the largest drops in price taking place between the 300 and 600 cubic meter digester volumes. While EROEI and breakeven natural gas price are important considerations when selecting the optimal scale of a system, constraints on capital may lead to the selection of a less energetically or financially favorable option. For instance, the total capital investment for the 300 cubic meter digester is \$1.3 million compared to \$7 million for the more efficient 2400 cubic meter size.

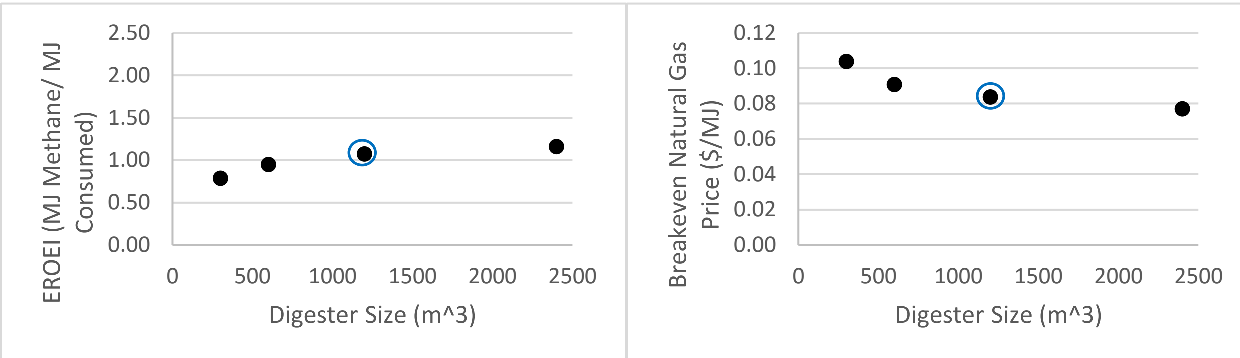


Figure 17: EROEI and Breakeven Natural Gas Price with Varying Digester Size

Assumed 8% solids loading for all scenarios. Blue circle indicates 20% cotreatment conversion increase and 40% water recycling rate at the 1200 cubic meter digester size used as the base scenario for this comparison.

Increasing substrate loading to increase the yield of a target product is another common industrial strategy. To determine its effect on the EROEI and breakeven natural gas price, the solids loading was varied from 6% to 12% as shown in Figure 18. A 40% rate of water recycling and 20% cotreatment conversion increase was assumed for all solids loadings. While EROEI increased linearly from 0.8 to 1.55 MJ methane/MJ energy invested, the breakeven price declined in a non-linear way. This likely resulted from the conflicting trends of greater methane production but larger switchgrass processing units (knife mill and colloid mill) being necessary as the solids loading increased. The lowest breakeven natural gas price was $0.068 per MJ at a solids loading of 12%; a decrease of 31% compared to the 6% solids loading.

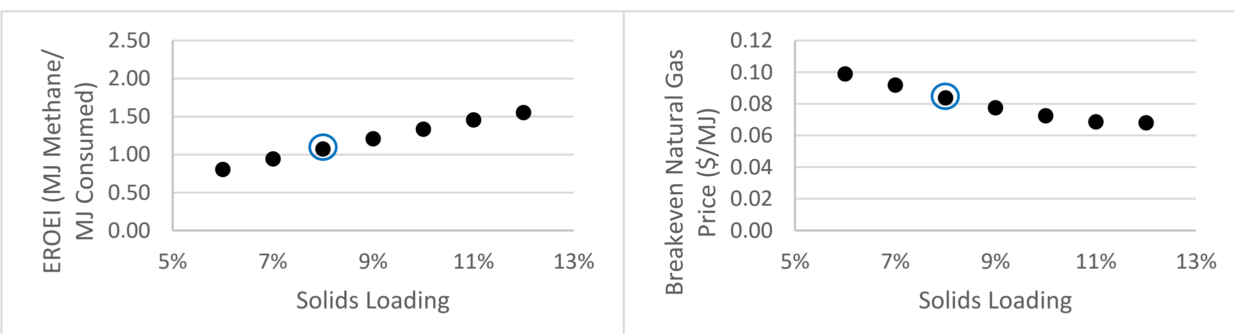


Figure 18: Solids Loading Variation Effect on EROEI and Breakeven Natural Gas Price

Assumed 1200 m^3 digester size for all scenarios. Blue circle indicates 20% cotreatment conversion increase and 40% water recycling rate used as the base scenario for this comparison.

Given the market price of natural gas in 2020 around $0.0027 per MJ, the previously outlined scenarios are not economically feasible without additional revenue streams or cost savings. State and national governments encourage production of renewable fuels, like renewable natural gas, by a variety of market-based incentives. The EPA’s Renewable Fuel Standard requires fossil fuel producers to offset a certain volume of their production with credits, termed Renewable Identification Numbers (RINs), generated by producers of renewable fuel (US EPA, 2017). Different fuel sources produce RINs in varying categories, with cellulosic sources designated as D3. The price of RINs can vary with markets, having peaked at $0.019 per MJ ($2.50 per gallon of gasoline equivalent) in late 2017 before falling to $0.0095 per MJ in 2020. Renewable fuels sold into the California marketplace can also qualify for benefits through the Low Carbon Fuel Standards (LCFS) program, which awards credits based on the amount of carbon released per megajoule of energy created as compared to gasoline (California Air Resources Board, 2020). For this system, the value of the LCFS credit is estimated to be $0.0172 per MJ.

The subsidy value necessary to break even on the investment in a variety of system scenarios is displayed in Figure 19. Cotreatment rate increases had the strongest effect on this price and achieved a 22% decrease in the 40% scenario without water recycling. Combining this with 80% water recycling decreased the breakeven subsidy price by a further 8%. The most optimistic scenario, 40% cotreatment conversion increase and 80% water recycling, produced a breakeven subsidy price of $0.071 per MJ. This was still well above the $0.0362 subsidy available by summation of the value of D3 RINs and the LCFS credits, but halved the difference between the necessary and available subsidies compared to the no cotreatment or water recycling scenario.

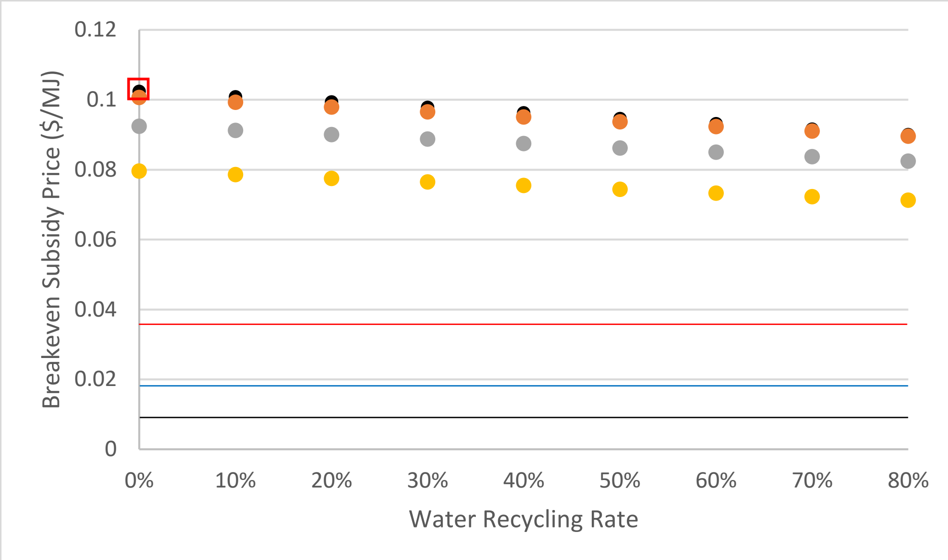


Figure 19: Subsidy Price for Breakeven Net Present Value

Black Circle-No Cotreatment, Orange Circle-10% Cotreatment Conversion Increase, Grey Circle-20% Cotreatment Conversion Increase, Yellow Circle-40% Cotreatment Conversion Increase. Assumed reactor volume of 1200 m^3 and 8% solids loading for all scenarios. Red square indicates baseline scenario with no cotreatment or water recycling. Black line indicates $0.0095 D3 RIN price, Blue line indicates $0.019 D3 RIN price, Red line indicates $0.019 D3 RIN price plus $0.0172 LCFS credit.

Market forces or more beneficial government regulations could increase the price of D3 RINs in the future. While the LCFS credit price is near its cap, anaerobic digestion systems have the opportunity to increase revenue by decreasing the carbon intensity of their production, thus qualifying for a greater number of these credits. Sequestration of the carbon dioxide produced during anaerobic digestion would be an ideal mechanism for doing this, with the opportunity to double the amount of the LCFS credits acquired. An increased subsidy of this magnitude would nearly meet the breakeven price required, and could make the resulting carbon negative renewable natural gas price profitable in some specialty markets.

6.3 Conclusions

While cotreatment and water recycling were not sufficient to make a lignocellulosic anaerobic digester economically feasible, they did improve the process compared to implementation without these strategies. Cotreatment had a greater impact than water recycling and more significantly decreased the breakeven natural gas price. With respected to the energy return on energy invested, cotreatment performed similarly to water recycling and both had a positive impact. Used in combination, these strategies could increase EROEI by up to 300% while reducing the breakeven price of natural gas by 32%. EROEI tended to increase and breakeven price tended to decrease with increasing scales and solids loadings. Government programs like the Renewable Fuel Standards and Low Carbon Fuel Standards offer another revenue source through credits awarded, however at current subsidy prices these were insufficient to achieve economic viability for this system. Market forces could increase the value of D3 RINs, while sequestering the carbon dioxide produced in the anaerobic digester could increase the amount of LCFS credits received, and the combination could push the system nearer to a competitive market price.

Section 7: Conclusions and Future Directions

Anaerobic digestion of lignocellulosic material is a promising, but early-stage technology for achieving carbon negative energy production. While anaerobic digestion has historically been widely used for wastewater and manure solids treatment, in most applications its energy generation potential was considered of secondary importance. Abundant quantities of lignocellulosic material could help these systems reach the economies of scale necessary to achieve profitable production of methane. However, process intensification techniques are necessary to overcome this material's recalcitrance.

Cotreatment had previously been studied using systems that milled the material only on a single occasion in a batch process, while in this study cotreatment was implemented daily in a continuous process using a recirculating loop design. The system was able to tolerate daily milling of 50% of the reactor volume, but at this milling intensity there was a small decrease in biogas production compared to performance without cotreatment. This could be due to inadequate recovery time for the microbes between milling events, poor milling due to an improper adjustment or an inadequate mechanism, a substrate whose recalcitrance was not sufficiently limiting in the base case without cotreatment, or some combination of these and perhaps other factors. Future research could focus on determining the frequency and intensity at which intermittent cotreatment causes biogas production increases. Studies to determine the mechanisms by which cotreatment impacts biogas production as well as studies relating these impacts to physical characteristics of the substrate such as particle size, could also be valuable for evaluating whether cotreatment could be used for process intensification in a given system.

Nutrient limitations were unlikely to have prevented an increase in biogas production during this study, as nutrient requirements were tested during system setup and supplemented with at least 150% of the requirements necessary. The nutrient supplement studies suggested that while nitrogen and mineral additions were necessary for a switchgrass feedstock that had senesced in the field and was stored dry, addition of vitamins were not required for continuous anaerobic digestion. More research is necessary to determine which nutrients are required for growth and what the minimum required supplement levels are, since multiple components were added at once rather than individually. More robust mechanisms for estimating microbial biomass within the system and the composition of that microbial biomass would make these findings more applicable across a range of anaerobic digestion conditions.

The energy necessary to mill switchgrass slurries tended to increase when solids content increased or when the radial gap size decreased, but were low relative to the energy in the biomass. Even the most energy intensive conditions consumed only about 2.3% of the higher heating value of the cellulose and hemicellulose contained in the switchgrass for each pass through the mill, with many treatments consuming less than 1% of this higher heating value. The magnitude of particle size reduction mirrored that of energy consumption. Increased solids improved particle size reduction even with the same radial gap size, suggesting that interactions between particles play a role in size reduction during milling. Completion of a follow-on study testing the energy consumed by milling digested switchgrass slurries of different solids loadings could provide more accurate estimation of the energy that would be consumed during cotreatment in a fully functioning anaerobic digester.

A model was developed to simulate cotreatment at industrial scale for a lignocellulosic anaerobic digester fed with switchgrass. Varying degrees of milling effectiveness were evaluated individually and in combination with recycling of a portion of water from the digestate waste stream. For a scenario of high cotreatment effectiveness with maximum water recycling, the model predicted the breakeven subsidy price would decrease by 32% from the base case without these innovations, and the net energy return on energy invested for the system could be as high as 2.06. The net present value of the investment, however, was -$5.5 million for a system processing 12 dry tons of switchgrass per day. Additional subsidies or revenue streams are necessary, in combination with technology innovations like cotreatment and water recycling, for lignocellulosic anaerobic digestion to become economically feasible. Sequestration of carbon dioxide produced in this process could maximize the value of subsidies provided by California's Low Carbon Fuel Standards program, however more research is necessary to determine the mechanisms and costs required to do so. Translation of this model to other simulation modeling platforms, like BioSTEAM, could allow for more robust technoeconomic analysis and integration of diverse cost saving and process intensification techniques for lignocellulosic anaerobic digestion.

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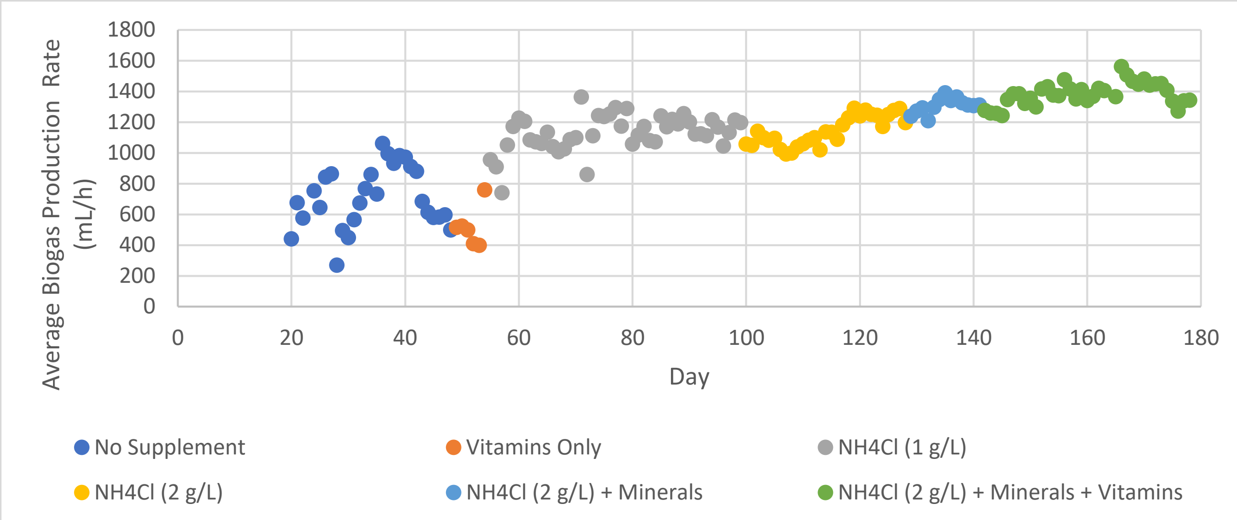
Appendix A: Intermittent Cotreatment Supplementary Data

Intermittent Cotreatment Sampling Timeline

Sample Type	Experiment Day	Biogas Volume	Biogas Composition	Particle Size Distribution	Volatile Solids Loading
No Cotreatment	1				
	2				
	3				
	4				
	5				
	6				
	7				
	8				
	9				
	10				
Low Cotreatment (10% of reactor volume per day)	11				
	12				
	13				
	14				
	15				
	16				
	17				
	18				
	19				
	20				
	21				
	22				
	23				
	24				
	25				
	26				
	27				
	28				
	29				
	30				
Intermediate Cotreatment (50% of reactor volume per day)	31				
	32				
	33				
	34				
	35				
	36				
	37				

Digester Biogas Production Rate History

*Biogas production data was not available and reliable until Day 20.



Appendix B: Milling Experiment Data

Gap Size Variation for 1.6 mm Switchgrass

Solids Loading	Gap (mm) and Rep	Power (W)	Unit Energy (kJ/kg slurry)	Unit Energy (kJ/kg DM)	Flow Rate (kg/s)
6%	0.312 Gap	Power	Unit Energy	DM Unit Energy	Flow Rate
	Rep 1	1028.8	9.349	155.818	0.11
	Rep 2	968	14.777	246.293	0.066
	Rep 3	1019.9	12.977	216.29	0.079
	Avg	1005.567	12.36767	206.1337	0.085
	SD	32.83662	2.764826	46.08465	0.022605
6%	0.416 Gap	Power	Unit Energy	DM Unit Energy	Flow Rate
	Rep 1	963.5	5.404	90.07	0.178
	Rep 2	962.2	5.878	97.97	0.164
	Rep 3	956.2	6.139	102.3	0.156
	Avg	960.6333	5.807	96.78	0.166
	SD	3.894012	0.372608	6.201234	0.011136
6%	0.520 Gap	Power	Unit Energy	DM Unit Energy	Flow Rate
	Rep 1	704.2	2.215	36.911	0.318
	Rep 2	723.2	2.517	41.943	0.287
	Rep 3	733.3	2.248	37.468	0.326
	Avg	720.2333	2.326667	38.774	0.310333
	SD	14.77509	0.165657	2.758529	0.020599
6%	0.624 Gap	Power	Unit Energy	DM Unit Energy	Flow Rate
	Rep 1	614.9	1.484	24.738	0.414
	Rep 2	587.5	1.508	25.125	0.39
	Rep 3	622.7	1.733	28.894	0.359
	Avg	608.3667	1.575	26.25233	0.387667
	SD	18.48711	0.137357	2.295919	0.027574
6%	0.728 Gap	Power	Unit Energy	DM Unit Energy	Flow Rate
	Rep 1	481.8	1.046	34.86	0.46
	Rep 2	436.7	1.209	40.296	0.361
	Rep 3	479.9	1.08	35.991	0.444
	Avg	466.1333	1.111667	37.049	0.421667
	SD	25.50771	0.08599	2.868283	0.053144

6%	0.832 Gap	Power	Unit Energy	DM Unit Energy	Flow Rate
	Rep 1	444.5	0.928	15.462	0.479
	Rep 2	476.7	1.02	16.997	0.467
	Rep 3	498.6	1.053	17.548	0.474
	Avg	473.2667	1.000333	16.669	0.473333
	SD	27.21293	0.064779	1.080989	0.006028
6%	1.040 Gap	Power	Unit Energy	DM Unit Energy	Flow Rate
	Rep 1	460.6	0.824	27.455	0.559
	Rep 2	423.5	0.761	25.378	0.556
	Rep 3	430.2	0.877	29.238	0.49
	Avg	438.1	0.820667	27.357	0.535
	SD	19.77144	0.058072	1.931865	0.039
6%	1.144 Gap	Power	Unit Energy	DM Unit Energy	Flow Rate
	Rep 1	449	0.768	25.612	0.584
	Rep 2	434.8	0.756	25.185	0.575
	Rep 3	453.4	0.733	24.443	0.618
	Avg	445.7333	0.752333	25.08	0.592333
	SD	9.720768	0.017786	0.591531	0.022679
6%	1.247 Gap	Power	Unit Energy	DM Unit Energy	Flow Rate
	Rep 1	411.3	0.799	26.627	0.515
	Rep 2	422.9	0.789	26.298	0.536
	Rep 3	459.5	0.718	23.924	0.64
	Avg	431.2333	0.768667	25.61633	0.563667
	SD	25.15737	0.044163	1.474807	0.066935
6%	1.663 Gap	Power	Unit Energy	DM Unit Energy	Flow Rate
	Rep 1	437.8	0.696	23.197	0.629
	Rep 2	410.7	0.714	23.788	0.575
	Rep 3	418.8	0.618	22.599	0.618
	Avg	422.4333	0.676	23.19467	0.607333
	SD	13.91055	0.051029	0.594503	0.028537

Gap Size Variation for 3.2 mm Switchgrass

Solids Loading	Gap (mm) and Rep	Power (W)	Unit Energy (kJ/kg slurry)	Unit Energy (kJ/kg DM)	Flow Rate (kg/s)
6%	1.040 gap	Power	Unit Energy	DM Unit Energy	Flow Rate
	Rep 1	511.6	1.106	18.425	0.463
	Rep 2	529.1	1.071	17.855	0.494
	Rep 3	578	1.251	20.855	0.461
	Avg	539.5667	1.142667	19.045	0.472667
	SD	34.41516	0.095438	1.593204	0.018502
6%	1.144 gap	Power	Unit Energy	DM Unit Energy	Flow Rate
	Rep 1	499.6	0.769	12.809	0.65
	Rep 2	481.2	0.931	15.522	0.517
	Rep 3	490.5	0.91	15.174	0.539
	Avg	490.4333	0.87	14.50167	0.568667
	SD	9.200181	0.088097	1.476183	0.07129
6%	1.247 gap	Power	Unit Energy	DM Unit Energy	Flow Rate
	Rep 1	461.3	0.831	13.847	0.555
	Rep 2	476.2	0.822	13.699	0.579
	Rep 3	485.6	0.791	16.187	0.614
	Avg	474.3667	0.814667	14.57767	0.582667
	SD	12.2533	0.020984	1.395687	0.02967
6%	1.663	Power	Unit Energy	DM Unit Energy	Flow Rate
	Rep 1	374.4	0.619	20.62	0.605
	Rep 2	396.5	0.742	24.74	0.534
	Rep 3	427.9	0.809	26.971	0.529
	Avg	399.6	0.723333	24.11033	0.556
	SD	26.88438	0.096366	3.221981	0.042509

Gap Size Variation for 3.2 mm Digestate

Solids Loading	Gap (mm) and Rep	Power (W)	Unit Energy (kJ/kg slurry)	Unit Energy (kJ/kg DM)	Flow Rate (kg/s)
6%	0.624 Gap	Power	Unit Energy	DM Unit Energy	Flow Rate
	Rep 1	788.3	3.957	131.895	0.199
	Rep 2	1013.8	6.746	224.872	0.15
	Rep 3	602.9	13.178	439.28	0.046
	Avg	801.6667	7.960333	265.349	0.131667
	SD	205.7759	4.728918	157.6394	0.07813
6%	0.832 Gap	Power	Unit Energy	DM Unit Energy	Flow Rate
	Rep 1	829.4	3.276	109.207	0.253
	Rep 2	741.5	1.79	59.662	0.414
	Rep 3	804.8	3.927	130.884	0.205
	Avg	791.9	2.997667	99.91767	0.290667
	SD	45.34766	1.095351	36.50838	0.109473
6%	1.040 Gap	Power	Unit Energy	DM Unit Energy	Flow Rate
	Rep 1	471.2	0.946	31.523	0.498
	Rep 2	436.5	0.923	30.771	0.472
	Rep 3	470.6	0.838	27.93	0.562
	Avg	459.4333	0.902333	30.07467	0.510667
	SD	19.86311	0.056889	1.895012	0.046318
6%	1.248 Gap	Power	Unit Energy	DM Unit Energy	Flow Rate
	Rep 1	438.8	0.83	27.663	0.529
	Rep 2	434.6	0.986	32.852	0.441
	Rep 3	438.6	0.9	29.985	0.488
	Avg	437.3333	0.905333	30.16667	0.486
	SD	2.369247	0.078137	2.599266	0.044034

Solids Loading Variation for 1.6 mm Switchgrass

Solids Loading	Gap (mm) and Rep	Power (W)	Unit Energy (kJ/kg slurry)	Unit Energy (kJ/kg DM)	Flow Rate (kg/s)
0%	0.520 Gap	Power	Unit Energy	DM Unit Energy	Flow Rate
	Rep 1	405.9	0.891	-	0.456
	Rep 2	416.4	0.776	-	0.537
	Rep 3	414.7	0.896	-	0.462
	Avg	412.3333	0.854333	-	0.485
	SD	5.635897	0.067885	-	0.045133
3%	0.520 Gap	Power	Unit Energy	DM Unit Energy	Flow Rate
	Rep 1	453.6	0.957	31.911	0.474
	Rep 2	452.2	0.953	31.757	0.475
	Rep 3	475.9	0.986	32.851	0.483
	Avg	460.5667	0.965333	32.173	0.477333
	SD	13.29749	0.018009	0.592193	0.004933
4%	0.520 Gap	Power	Unit Energy	DM Unit Energy	Flow Rate
	Rep 1	496.1	1.088	27.209	0.456
	Rep 2	491.1	1.126	28.142	0.436
	Rep 3	539.5	1.203	30.078	0.448
	Avg	508.9	1.139	28.47633	0.446667
	SD	26.61804	0.058592	1.463429	0.010066
5%	0.520 Gap	Power	Unit Energy	DM Unit Energy	Flow Rate
	Rep 1	536.2	1.322	26.442	0.406
	Rep 2	547.1	1.223	24.456	0.447
	Rep 3	581.3	1.483	29.658	0.392
	Avg	554.8667	1.342667	26.852	0.415
	SD	23.53175	0.131226	2.625124	0.028583
6%	0.520 Gap	Power	Unit Energy	DM Unit Energy	Flow Rate
	Rep 1	704.2	2.215	36.911	0.318
	Rep 2	723.2	2.517	41.943	0.287
	Rep 3	733.3	2.248	37.468	0.326
	Avg	720.2333	2.326667	38.774	0.310333
	SD	14.77509	0.165657	2.758529	0.020599

7%	0.520 Gap	Power	Unit Energy	DM Unit Energy	Flow Rate
	Rep 1	740.2	2.835	40.5	0.261
	Rep 2	768.8	2.97	42.42	0.259
	Rep 3	780.9	3.216	45.949	0.243
	Avg	763.3	3.007	42.95633	0.254333
	SD	20.9	0.193176	2.763809	0.009866
8%	0.520 Gap	Power	Unit Energy	DM Unit Energy	Flow Rate
	Rep 1	871	5.5	68.761	0.158
	Rep 2	875.7	6.242	78.02	0.14
	Rep 3	853.3	6.212	77.652	0.137
	Avg	866.6667	5.984667	74.811	0.145
	SD	11.812	0.420002	5.242684	0.011358

Appendix C: Anaerobic Digestion Scale-up Model

Assumptions

Area 100-Feedstock Handling

- Switchgrass is 8% Moisture.
- Switchgrass used is of the Cave-in-Rock variety with a composition of 33.48% cellulose, 26.1% hemicellulose, and 17.35% lignin on a dry mass basis. (David & Ragauskas, 2010)
- Accessible energy in the feedstock is only that contained in cellulose and hemicellulose, which have a higher heating value of 18.57 MJ/kg. (David & Ragauskas, 2010)
- Energy consumption by the hammer mill is 26 kwh/DM ton to reduce feedstock size adequately for digestion regardless of digester size. (Koch, 2002)

Area 200-Anaerobic Digestion

- Volume of digester refers to working volume.
- Base cellulose and hemicellulose conversions are 0.4 g/g available for thermophilic, 10 day RT conditions.
- Methane yield from cellulose is 250 mL/g and from hemicellulose is 200 mL/g. (W. Li et al., 2018)
- Digester agitation and control system requires 20 hp for operation. (TetraTech, 2017)
- Volume exchange pumps require 10 hp during operation. (TetraTech, 2017)

Area 300-Cotreatment

- IKA labor pilot flow rates, power requirements, and prices are based on information contained in the labor pilot user's manual as well as lab-measured values. (Amador-Diaz, 2019; IKAWorks, 2000)
- Cotreatment is assumed to take place at a rate of 50% of reactor volume per day regardless of the cotreatment conversion increase.
- Flow rate decreases by 13% for each 1% increase in solids loading from 6%.
- Power consumption increases by 8.3% for each 1% increase in solids loading from 6%.
- There are not changes in power consumption or flow rate due to particle size differences between IKA colloid mill models.

Area 400-Recovery

- The centrifugal dewatering machine is assumed to require 10 hp to separate 77.5% of solids from the effluent. (O'Connor, 2007)
- Removed solids are assumed to have a moisture content of 80%.
- The water lost during separation is adequate to prevent buildup of salts and inhibitory compounds.

Area 500-Utilities

- Digester shape is assumed to be cylindrical, where the diameter is twice the height.
- Heat transfer coefficients of the wall, floor, and roof are assumed to be 0.39, 2.85, and 0.3 W/m^2C respectively. (Persson, Bartlett, Branding, & Regan, 2016)
- Ambient temperature is assumed to be 10 C.
- A 10% cooling to ambient temperature is assumed to take place during dewatering.
- Digestate has the same specific heat as water (4.186 J/kg-C) at all solids loadings.
- The natural gas boiler has an efficiency of 0.85.
- Natural gas purchased costs $8.05/1000 cubic feet and contains 1038 BTU/ft^3.
- Electricity costs $0.0823/kwh.
- Irrigation water can be used in the digester and costs \$19/1000 m^3. (OECD, 2010)

Capital, Costs, and Revenue

- Component capital costs were gathered via previous designs and online posted costs that were scaled and adjusted to 2020 dollars if necessary.
- Total capital invested is 5.4 times the total purchase cost.
- Maintenance costs are 4% of fixed capital investment.
- Operation costs are calculated from digester size and methane production. (Cowley & Brorsen, 2018)
- Switchgrass costs $80/dry ton.
- Selling price of methane from the facility is $3/1000 cubic feet.

Economics

- Internal rate of return is 10%.
- Rate of loan is 2% over a 23 year period (3 years construction, 20 years operation).

Vita

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Richard Lab, Penn State Department of Agricultural and Biological Engineering

- Designed cotreatment-enhanced anaerobic digestion systems
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- Planned and fabricated headplates for a new bioreactor system

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- Utilized molecular techniques to complete a gene transfer between bacterial strains
- Maintained stocks and samples from 7 strains of bacteria
- College of Ag Undergraduate Research Grant ($2000), 3-time awardee