THE EFFECT OF GRINDING LIGNOCELLULOSIC BIOMASS AS A MIXED-ACID FERMENTATION COTREATMENT

An Undergraduate Research Scholars Thesis

by

DREW JAMAL SAMUELSON MARKS

Submitted to the Undergraduate Research Scholars program at
Texas A&M University
in partial fulfillment of the requirements for the designation as an

UNDERGRADUATE RESEARCH SCHOLAR

Approved by Research Advisor: Dr. Mark T. Holtzapple

May 2020

Major: Chemical Engineering

TABLE OF CONTENTS

Page

ABSTRACT....1

ACKNOWLEDGMENTS....2

CHAPTER

I. INTRODUCTION....3

Mixed-acid fermentation overview....3
Lignocellulose as a substrate....4

II. METHODS....8

Cotreatment assumptions....8
Fermentation materials....10
Fermentation procedures....12
Cotreatment energy analysis....16

III. RESULTS....18

Cotreatment fermentation yields....18
Cotreatment energy consumption....24

IV. CONCLUSION....27

REFERENCES....28

APPENDIX....30

ABSTRACT

The Effect of Grinding Lignocellulosic Biomass as a Mixed-Acid Fermentation Cotreatment

Drew Jamal Samuelson Marks
Department of Chemical Engineering
Texas A&M University

Research Advisor: Dr. Mark T. Holtzapple
Department of Chemical Engineering
Texas A&M University

In order to prevent the detrimental effects of climate change in the near future, novel energy solutions must be explored and developed today. Lignocellulosic biomass (LCB), a major component in wood-like plant matter, is a primary candidate for the production of biofuels as a carbon-neutral energy alternative due to its abundance and renewable nature. Although lignocellulose is cheap and readily available, the process of converting LCB into valuable products (e.g., fuels, chemicals) can be uneconomical due to the recalcitrance of LCB. Traditionally, in order to improve the economic viability of LCB fermentation, LCB is chemically pre-treated to disrupt its rigid cell structure and achieve greater yields, however, this pretreatment remains expensive and can result in wasted lignin and hemicellulose in the LC biomass (Kucharska, 2018). In contrast to an expensive and wasteful chemical pretreatment of LCB, a mechanical cotreatment approach to mixed-acid fermentation can be key to making the fermentation process economical by achieving similar yields at a reduced cost. In this thesis, the effects of grinding LCB over the course of a mixed-acid fermentation process are explored. Preliminary data suggests a mechanical cotreatment approach on corn stover can yield results similar to chemical pretreatments for $4.69 per grind per tonne of biomass.

ACKNOWLEDGMENTS

First and foremost, I would like to thank my research advisor, Dr. Holtzapple, for giving me the opportunity to explore the world of biofuels hands-on. Being able to work on novel solutions to one of the greatest threats to our planet is nothing short of amazing, and I am grateful for his support throughout the journey.

Next, I would like to thank the graduate students under Dr. Holtzapple for their support in and out of the laboratory. In particular, I want to thank Opeyemi Olokede for his guidance and teachings regarding the finer points of laboratory procedures and etiquette.

Lastly, I would like to thank my colleagues, Tennille Faber and Natalie Marshall, for their efforts in helping perform experiments and making the experience enjoyable day in and day out.

CHAPTER I

INTRODUCTION

For decades, researchers have been developing methods of producing biofuels as a carbon-neutral alternative to consuming traditional fossil-fuels. While progress has been steady in the development of these methods, many remain uneconomical and thus unattractive to investors and consumers alike (Biello, 2011). As the threat of climate change looms near the point of no return, development of economical methods of producing biofuels is essential to attracting investors and driving a global divestment from the fossil-fuel industry.

Although several economical clean energy alternatives exist (e.g., solar, wind), biofuels are uniquely compatible with much of the technology and infrastructure that exists around the world today, particularly in the case of transportation. In addition to the unique compatibility of biofuels with existing technology, biofuel production methods often coincide with chemical synthesis methods for many commonly used chemicals across numerous industries (Den, Sharma, Lee, Nadadur, & Varma, 2018).

Mixed-acid fermentation overview

One promising method of producing biofuels is mixed-acid fermentation, an anaerobic fermentation method that utilizes bacteria to convert glucose into useful carboxylic acids. After the fermentation produces a mixture of acids as an intermediate, the acids produced can then be chemically converted into useful chemicals and fuels. One key trait that defines the mixed-acid fermentation, is the choice of bacterial culture used to convert biomass into the targeted product. This choice of a mixed culture to perform the mixed-acid fermentation is commonly referred to as the ‘carboxylate platform’, and is often beneficial due to broad metabolic capabilities of the

mixed culture. In addition to these broad metabolic capabilities, the mixed culture is expected to be more effective at fermentation than a pure culture under a wider set of temperatures, pressures, and broth conditions (Agler, 2011).

When choosing a biomass for the fermentation, the choice of culture also plays a significant role. In the case of waste treatment, for example, the largely varying composition inherent to wastes like sewage sludge requires a mixed culture capable of handling said variation. For the experiments detailed in this thesis, a mixed culture is used in the fermentation of corn stover, a lignocellulosic biomass (LCB). Due to the nature of LCB, a mixed culture is used for many of the same reasons listed above. Despite using the carboxylate platform to convert LCB into carboxylic acids, the use of LCB presents challenges that greatly limit the yield of carboxylic acids. Unlike other biomass options, such as sewage sludge or food waste, LCB is very resistant to metabolization, due to its rigidity and recalcitrant nature (Kucharska, 2018). As a result, it is fairly difficult for bacteria to hydrolyze significant amounts of the cellulose within the LCB into glucose, the principle substrate of the carboxylate platform.

Lignocellulose as a substrate

In an effort to address the difficulties arising from the rigid structure of LCB (Figure 1), several methods have been developed over the past few decades.

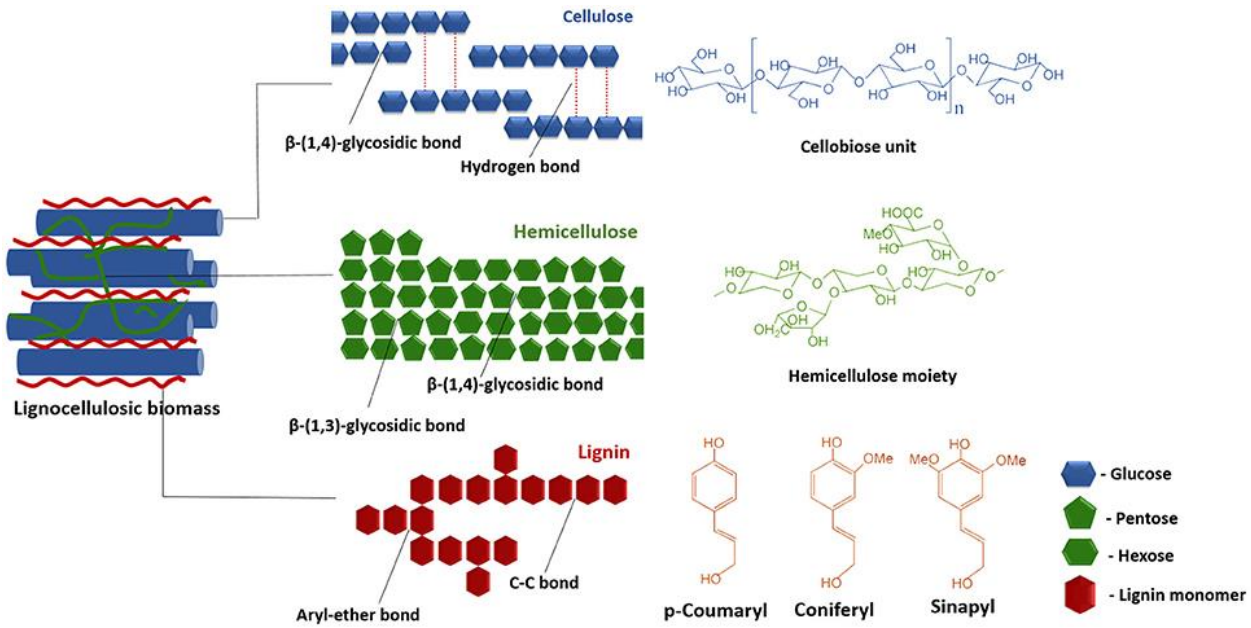

Figure 1. Breakdown of the structure of LCB and its components (Baruah et al., 2018).

While they vary greatly in their approaches, pretreatment methods employed today all aim to disrupt the structure of LCB (Figure 2) to increase fermentation yields.

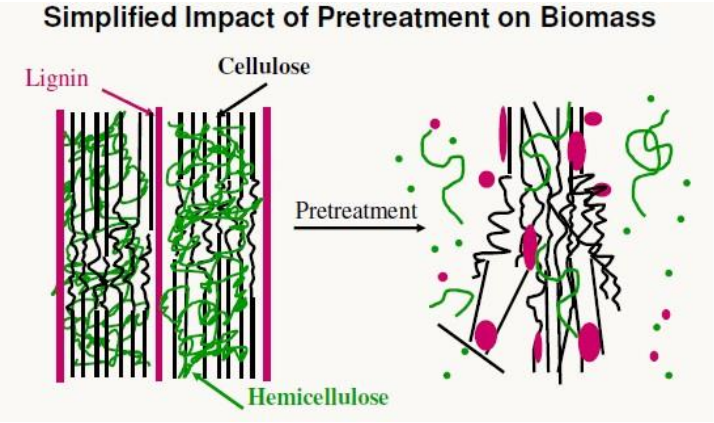

Figure 2. Depiction of the effect of pretreatment on LCB. This disruption in corn stover can be seen in the Appendix in Figure A2. (Mosier et al., 2005, as cited in Liu & Fei, 2013).

Overall, the pretreatment step as it is employed today, regardless of method, is expected to account for at least 20% of total production cost for LCB-based biofuels, and is one of the most promising stages where cost can be reduced, a major motivation for this thesis (Mafe et al., 2015; Seidl and Goulart, 2016, as cited in Baruah et al., 2018).

Chemical pretreatment

The most prominent category of pretreatment methods, chemical pretreatment utilizes chemical pathways to disrupt the structure of the lignocellulose. As one can imagine, this process tends to require harsh chemicals, which can inadvertently destroy valuable celluloses within the structure (Kucharska, 2018). Typically, chemical pretreatment entails the use of either an alkaline substance or an acidic substance to breakdown the biomass and improve digestibility, increasing yields.

For alkaline pretreatments, strong bases made from sodium, potassium, calcium, ammonia, etc., are mixed with LCB at various pressures and temperatures, removing lignin from the structure, and exposing hemicellulose, a compound that is much more digestible (Bensah & Mensah, 2013). The major drawbacks of alkaline pretreatments include recovery of the alkaline substances introduced to the process, loss of significant amounts of lignin, and cost of chemicals (Baruah et al., 2018).

For acidic pretreatments, LCB is exposed to concentrated or dilute acid solutions, at low to high temperatures respectively, in order to breakdown the cellulose and hemicellulose polymers within the structure (Baruah et al., 2018). Acidic pretreatments tend to differ from alkaline pretreatments in that they require corrosion-resistant equipment, and tend to provide less delignification than alkaline pretreatments under similar conditions (Bensah & Mensah, 2013). For these reasons, acidic treatment is preferred for LCB with a low lignin content.

Biological pretreatment

While bacteria tend to have difficulty digesting LCB, white and brown rot fungi are capable of producing lignocellulases and hemicellulases, which breakdown LCB effectively. The major drawback of this action, however, is the relatively slow reaction speed of this enzymatic treatment (Singh & Singh, 2016). As a result of the slow speeds, long residence times are needed to effectively increase fermentation yields, which requires significant CAPEX for large vessels or low throughput.

Mechanical pretreatment

Mechanical pretreatment methods, such as ball-milling, acoustic shock, grinding, and others, aim to disrupt the structure of LCB without the use of chemicals. The largest advantage these mechanical pretreatment methods bring is this absence of chemicals. As a result, less waste is generated from the process, and less processing needs to be done prior to fermentation, such as separation of pretreatment chemicals and the LCB. One major disadvantage, however, of mechanical pretreatments, is the substantial amount of energy required to achieve yields comparable to chemical pretreatment methods.

Cotreatment motivation

Unlike any of the pretreatment methods mentioned above, ‘cotreatment’ employs a distributed-treatment approach to improving digestibility of LCB. That is, instead of a front-loaded treatment of LCB prior to the fermentation stage, the LCB is treated over the course of fermentation. This is performed by using a grinding mechanical treatment to disrupt the LCB. While most similar to a mechanical pretreatment approach, distributing a grinding treatment over the course of fermentation ensures that energy is not wasted treating residual biomass, resulting in reduced energy cost for similar acid yields.

CHAPTER II

METHODS

As mentioned in Chapter I, there are two major components of cotreatment that define its ability to act as a substitute for pretreatment methods commonly employed today; the performance of the fermenters on an acid-production basis, and the cost associated with producing said acids. Therefore, two experimental approaches were taken to fully define the performance of cotreatment mixed-acid fermentation.

Cotreatment assumptions

In order to establish the logic of a cotreatment approach, a few assumptions are made regarding mixed-acid fermentation. First, it is assumed that the specific substrate consumption rate is near max over the duration fermentation when a grinding treatment is applied, barring thermodynamic limitations (Figure 3). This assumption is made on the basis that the digestible LCB concentration in the fermenters is sufficiently high at all points of fermentation. Due to the nature of the mixed culture used in the experiments detailed in this thesis, it is difficult to determine the true substrate uptake rate of the culture, with accurate cell growth kinetics. Despite this limitation, the assumption made above appears well-founded given the results discussed in Chapter III.

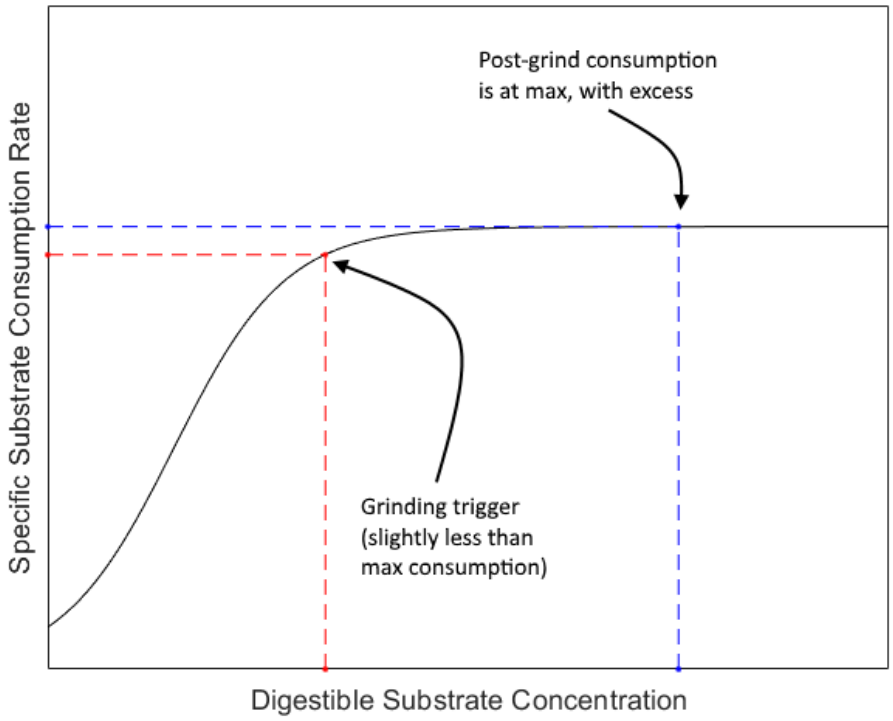

Figure 3. Depiction of the expected effect of grinding on specific substrate consumption in the fermenters based on the assumption that the fermenters remain near the max consumption rate.

In addition to the assumption made above, another assumption made regarding the cotreatment approach is that the amount of mass passed through the grinder decreases as the bacteria convert LCB into liquid-phase carboxylic acids and gas-phase biogas. As a result, this means the amount of energy expended to achieve 'sufficiently-milled' (according to the first assumption) LCB is less when a cotreatment approach is applied (Figure 4). Overall, the synergistic effects of mechanical shear and bio-digestion disrupt the rigid structure of the corn stover and are expected to enhance yields of carboxylic acids at a decreased cost.

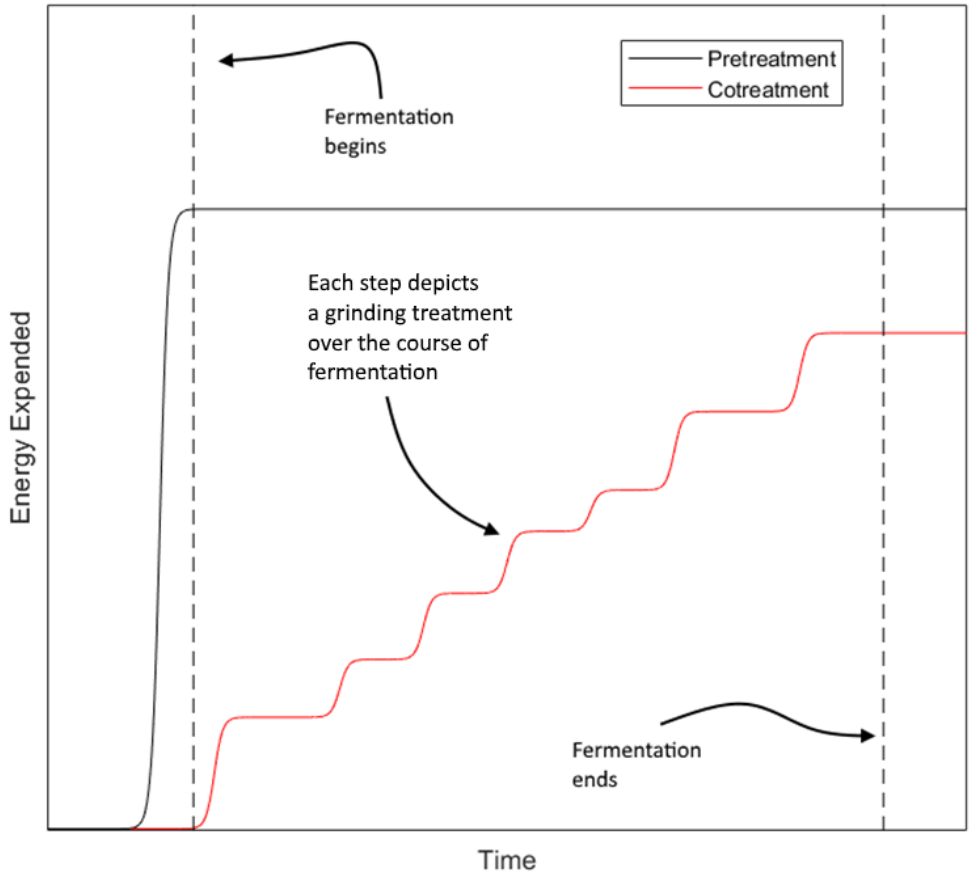

Figure 4. Comparison of the expected energy expenditure of a pretreatment approach versus a cotreatment approach with similar acid yields. As the fermentation progresses, the torque required to sufficiently mill the LCB is expected to increase, resulting in similar amounts of energy expended (step size) despite the solid mass lost over the course of fermentation.

Fermentation materials

The fermenters were created using Nalgene® 1-L high-density polyethylene (HDPE) bottles with rubber stoppers as caps. In each rubber stopper, a glass tube was inserted and capped with a rubber septum and aluminum crimp seal. Two 1/4-inch stainless steel rods were inserted through the rubber stopper to facilitate mixing in the fermenter as it rotated in the incubator (Figure 5).

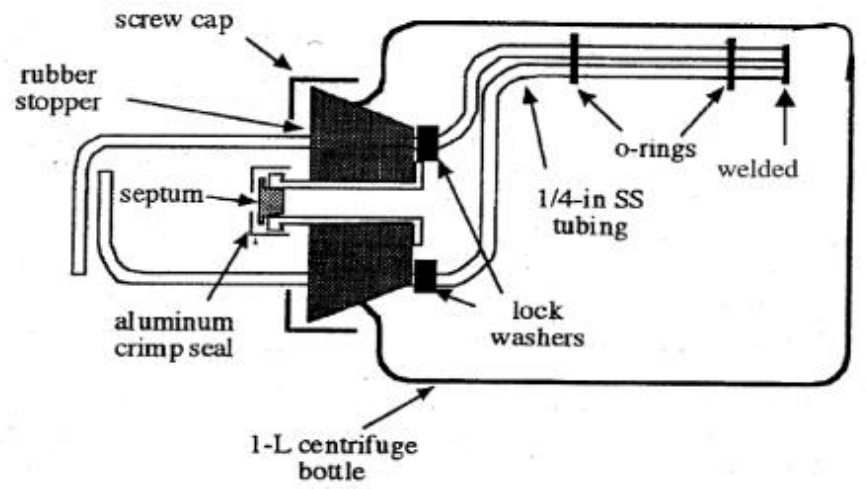

Figure 5. Diagram of plastic fermentation bottle (Golub, 2012).

The components of each fermenter are depicted below in Table 1.

Table 1. Components added during the preparation of each fermenter.

Component
Corn stover
Baked chicken manure
Deoxygenated water
Urea
Inoculum
Iodoform

The inoculum used in the experiments were originally sourced from soils taken from 1-m-deep holes at the beaches of Galveston, Texas, however, for the experiments detailed in this

thesis, the liquid from previous fermenter bottles containing the mixed culture was used as inoculum. This liquid was used because it contained a culture of microorganisms previously adapted to the substrate (corn stover) and the nutrient (chicken manure). In addition to the materials mentioned above, a cast iron manual crank grain mill was used to grind the solids during the cotreatment experiment (Figure 6).

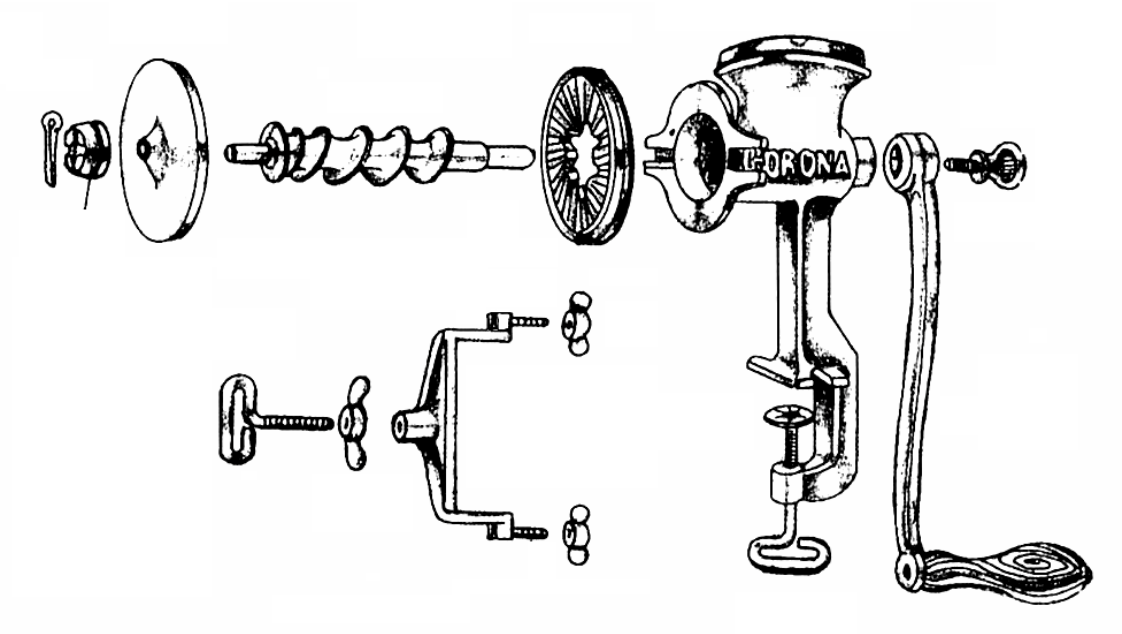

Figure 6. Diagram of the cast iron manual crank grain mill.

Fermentation procedures

Bottle preparation

Six Nalgene® 1-L high-density polyethylene (HDPE) bottles and six stoppers were washed and autoclaved. Then, the weight of each of the empty bottles and the stoppers were recorded. To each bottle, baked chicken manure, corn stover, deoxygenated water, urea, inoculum, and iodoform were added in quantities specified in the materials chapter above. The

urea was added to act as a nitrogen source for the mixed culture and adjust the carbon/nitrogen (C/N) ratio of the fermenter. Once the bottles were closed with the stopper, the final weight was recorded and the bottles were placed inside the incubator. The incubator consists of about 50 continuously rolling pipes maintained at 40°C. The bottles were placed inside one of the rolling pipes and rotated continuously until being removed after approximately 48 hours. The steel bars inside the fermenters mixed the contents while the bottles rotated.

Although six bottles were prepared, three of the bottles were considered to be a single control batch, while the other three were considered to be a single experiment batch. This was done to ensure losses due to grinding and sampling did not significantly disrupt the conditions of the fermentation broth. As is discussed in more detail further below, the contents of each 'batch' were mixed throughout the experiment and mass-balanced to ensure uniformity.

Sampling-only procedure

Every 48 hours, the fermenters were removed from the incubator, allowed to cool, and initial weights were recorded. The biogas inside the fermenter was vented and the volume recorded. The bottles were then taken to the fume hood where the stopper was removed, and the bottles were prepared for centrifuging. Next, pairs of bottles were balanced and centrifuged for 10 min at 4000 rpm. After centrifuging, the liquid from the three control bottles were combined into one tared beaker to be treated as a single batch, and the liquid weight of the control batch was recorded. This procedure was repeated for three experimental bottles. The six liquid-free bottles containing the biomass were left in the fume hood, covered. After the liquid weights for each of the two batches were recorded, stir bars were added to each beaker, and they were set to stir at 500 RPM for approximately 5 minutes.

After sufficient mixing, two 1.0 mL samples were taken from the control batch liquid, and two 1.0 mL samples were taken from the experimental batch liquid. Next, a pH probe was calibrated and used to record the pH of both batch liquids. If the pH was below 6.5 in the measured liquid, sodium bicarbonate was added to raise the pH above 6.5. If the pH was above 6.5, no corrective action was taken. After adjusting the pH in both batch liquids, 360 μL of iodoform were added to each batch liquid to inhibit methanogenesis, and the liquids were then stirred again at 500 RPM for 3 minutes. Finally, the liquid from the control batch was divided into three equal components by mass to be replaced into each of the control fermenter bottles. This procedure was repeated for the experimental batch liquid. Prior to closing and returning the bottles to the incubator, each bottle was purged with nitrogen for 35 seconds and capped with the respective rubber stopper. The final weights of the bottles were recorded, and the bottles were placed back into the incubator until the next sampling period, 48 hours later, unless a grinding session was scheduled.

Grinding day procedure

On days where a grinding session was scheduled, the procedure detailed above was overridden by the grinding procedure detailed below. For this experiment, grinding sessions were set to occur every four days, or 96 hours, from the previous grinding period. As the experiment approached the end of the fermentation period, the increment between grinding sessions was increased. This was done due to the perceived declining effectiveness of grinding as the fermentation progress progressed.

Grinding day sampling

The sampling procedure for the fermenters on grinding days was nearly identical to the procedure detailed above for non-grinding days. One key difference between the two sampling

procedures, however, is that the quantity of solids in each of the fermenters on grinding days was subject to change after grinding and applying a mass balance across all of the bottles.

Assembly of the grinder

The grinder (Figure 6) was securely fastened to a wooden table via the attached screw clamp. Upon being secured to the table, each component was assembled following the manufacturer's instructions. To ensure adequate shear stress was applied to the feedstock, the burrs of the grinder were tightened until a sufficient amount of force was required to operate the grinder. This grinding setting was also confirmed by visually inspecting the size of the corn stover before and after grinding. A plastic rectangular box was placed under the burrs of the grinder to catch the ground solids.

Grinding procedure

Using a small metal spatula, biomass from each of the experimental bottles was incrementally added to the grinder hopper. As the grinder processed the biomass in the hopper, the hopper was refilled until all of the experimental batch solids (all three bottles) were ground. In order to reduce the effects of confounding variables on the experiment, the control batch solids were incrementally emptied into a similar plastic container to simulate the grinding environment, exposing the solids to the atmosphere.

After all of the experimental batch solids were ground, the ground solids were mixed in their holding container until visually uniform. Next, the grinder was carefully disassembled, and all of the parts were cleaned using spatulas and toothpicks to recover as much solid biomass stuck in the burr blades and auger as possible. Once the residual solids were recovered from the grinding apparatus, they were weighed and equally distributed by weight into the three

experimental bottles. Similarly, the control batch solids were weighed and equally distributed by weight into the three control bottles.

Closing the fermenters

Just like the procedure on sample-only days, after each of the bottles were refilled with solids and liquids from their respective batches, they were purged with nitrogen for 35 seconds, capped, and weighed before being replaced into the incubator.

Cotreatment energy analysis

This experiment utilizes data generated across several "grinding" sessions to develop an accurate measurement of the energy required. By understanding this energy requirement, comparisons can be drawn between cotreatment and pretreatment.

Analysis Procedure

In order to analyze the energy requirement of the grinding process, a weight was placed on grinder handle, which was allowed to rotate freely, until the resistance of the LCB in the auger/burrs of the grinder prevented further motion. At this point, a photograph was taken of the apparatus, and the angle of the shaft relative to the horizontal was determined digitally (Figure 7). After determining the angle of the shaft relative to the horizontal, the static torque was determined using geometric relationships and physical analysis. In addition to torque analysis, the biomass throughput in the grinder was recorded when grinding by placing a scale beneath the bin that collected the biomass as it came out of the burrs of the grinder. This process was repeated for many rotations, and an average torque requirement and throughput was determined.

After performing analysis on the static torque of the apparatus, analysis on the dynamic torque was planned to take place, however, due to the unforeseen events surrounding

the COVID-19 virus in spring 2020, construction of the dynamic torque measurement system was not completed at the time of publication for this URS thesis.

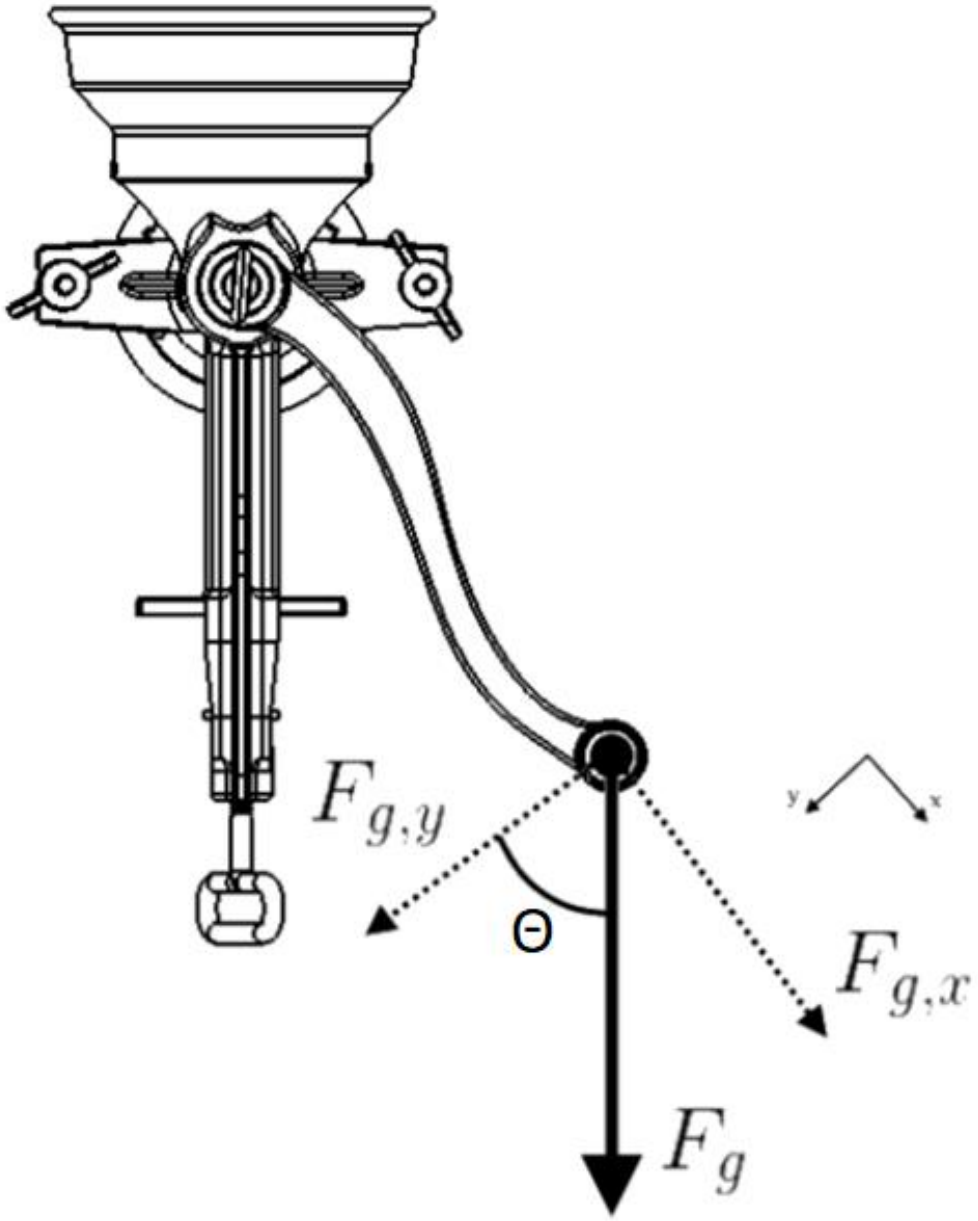

Figure 7. Sample depiction of the energy analysis procedure, where $F_{g,y}$, after coming to a halt, is the effective force needed to induce motion.

CHAPTER III

RESULTS

Due to the events surrounding COVID-19, complete data is unavailable for some portions of the analysis and discussion below. Despite this fact, the analysis does show promise for cotreatment as a cost-reduced approach to LCB mixed-acid fermentation.

Cotreatment fermentation yields

In total, three experiments of cotreatment were performed in accordance with the procedures outlined in Chapter II: a short-term fermentation (Figure 8), a medium-term fermentation (Figure 9), and a long-term fermentation (Figure 10), corresponding to 28 days, 48 days, and 65 days respectively. Across all of these experiments, there is strong evidence that cotreatment results in increased yields of acetic acid equivalents produced.

Short-term fermentation

The quantities of the components used to construct the short-term fermentation are depicted below in Table 2.

Table 2. Short-term fermentation components.

Component	**Quantity**
Corn stover (wet basis)	51.12 g
Baked chicken manure (wet basis)	12.24 g
Deoxygenated water	350 mL
Urea	1.20 g
Inoculum	50 mL
Iodoform	120 µL

Interestingly, the short-term fermentation (Figure 8) outperformed both the medium-term and long-term fermentations shown further below.

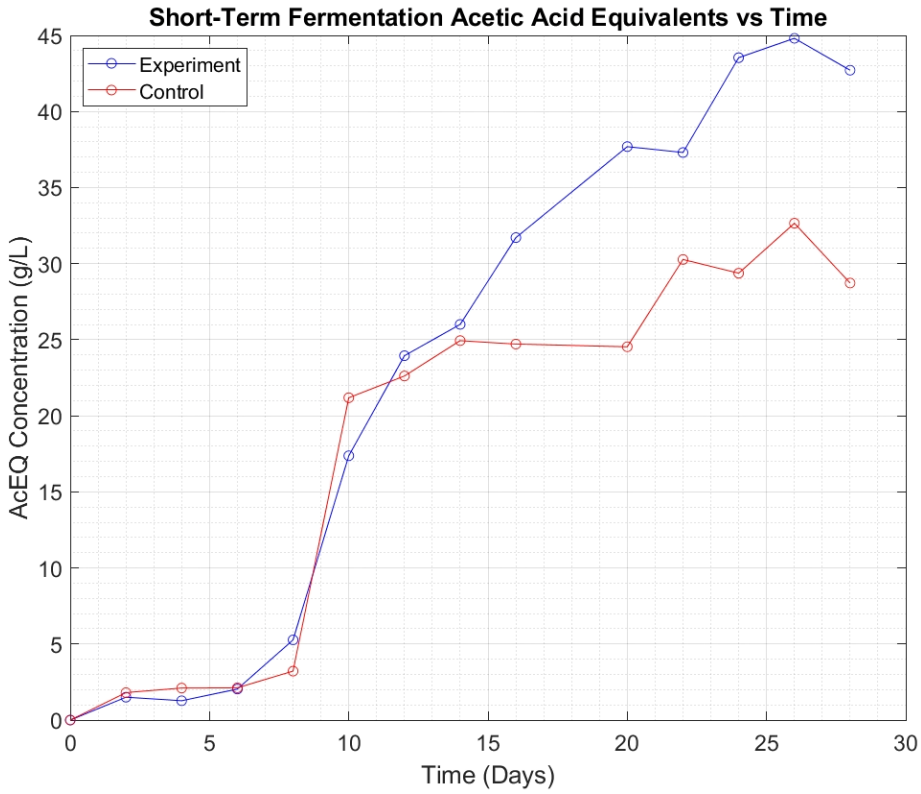

Figure 8. The short-term fermentation acetic acid equivalents produced versus time. Each data point was obtained using a gas chromatograph (GC).

The best explanation for the performance of the short-term fermentation likely comes from the given C/N ratio and substrate concentration in the fermenter, of 18.85 and 150 g/L respectively. Finding the 'correct' C/N ratio can be tricky, however one may expect marine microorganisms to exhibit faster growth when the C/N ratio moves closer to 10.2 (Anderson, 1992) rather than 24, which is typically seen as 'optimal' for agricultural soil conditions, despite tending to be slower.

Medium-term fermentation

The quantities of the components used to construct the medium-term fermentation are depicted below in Table 3.

Table 3. Medium-term fermentation components.

Component	**Quantity**
Corn stover (wet basis)	51.51 g
Baked chicken manure (wet basis)	12.75 g
Deoxygenated water	350 mL
Urea	0.448 g
Inoculum	50 mL
Iodoform	120 µL

As can be seen in Figure 9 below, the medium-term fermentation does not reach the acetic acid equivalent level of the short-term fermentation, despite being nearly twice the length. For this fermentation trial, the C/N ratio was 25, and the substrate concentration was 150 g/L. Given the large difference in C/N ratios between the short-term fermentation and the medium-term fermentation while the substrate concentration was held constant, there is greater reason to believe targeting a C/N ratio lower than 25 will result in faster kinetics.

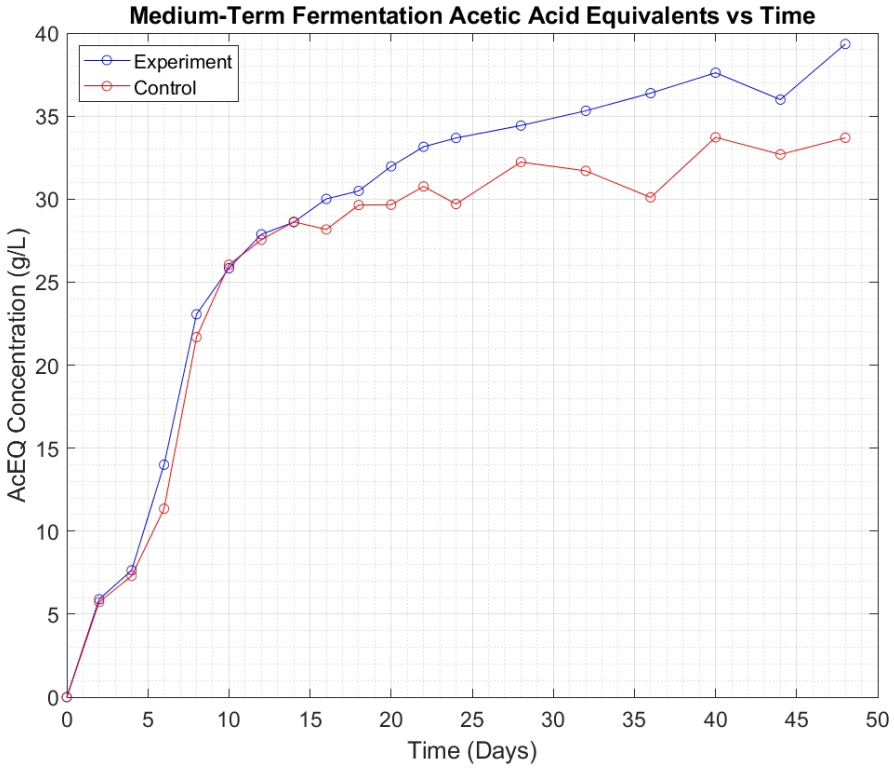

Figure 9. The medium-term fermentation acetic acid equivalents produced versus time. Each data point was obtained using a gas chromatograph (GC).

Long-term fermentation

The quantities of the components used to construct the long-term fermentation are depicted below in Table 4.

Table 4. Long-term fermentation components.

Component	**Quantity**
Corn stover (wet basis)	69.50 g
Baked chicken manure (wet basis)	16.01 g
Deoxygenated water	350 mL
Urea	0.80 g
Inoculum	50 mL
Iodoform	120 µL

Immediately apparent when looking at Table 4 is the large increase in the amount of substrate used in the long-term fermentation. The C/N ratio for this fermentation was 24, with a substrate concentration of 200 g/L. While the goal of this large increase in substrate concentration was to prolong the amount of digestible LCB over the duration of fermentation, it appears that some substrate inhibitory effects took place early on, leading to reduced yields as a whole (Figure 10). Comparing Figure 8 and 9 to Figure 10, short-term and medium-term fermentation exhibited a steeper growth-phase than long-term fermentation. After what appears to be an inhibited growth-phase, the long-term fermentation follows a trajectory similar to that expected of the medium-term fermentation, had the medium-term fermentation been extended by a couple of weeks.

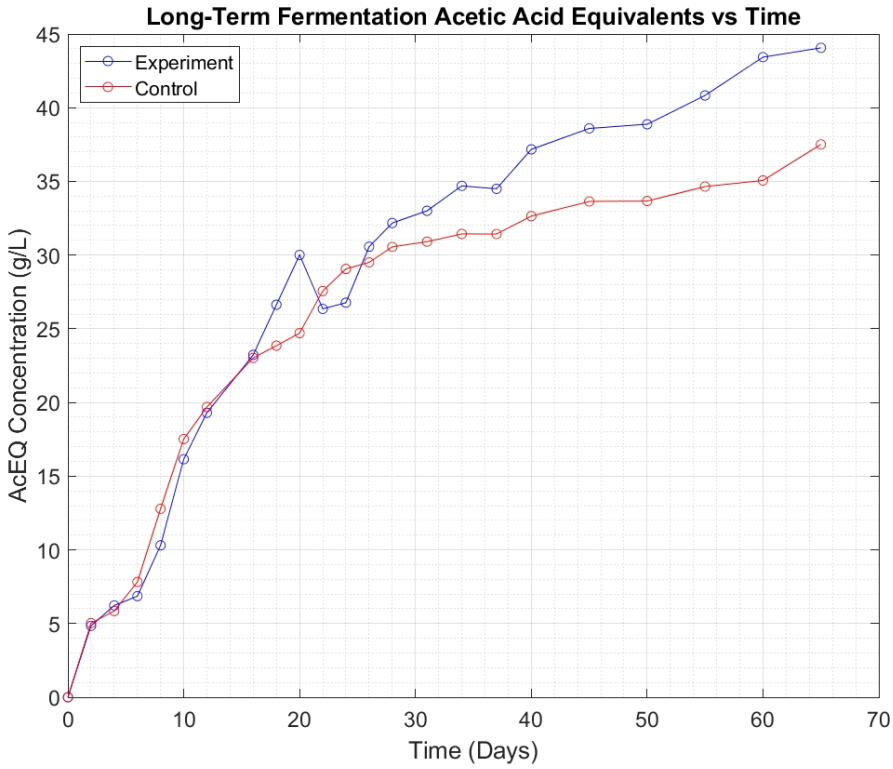

Figure 10. The long-term fermentation acetic acid equivalents produced versus time. Each data point was obtained using a gas chromatograph (GC).

Yields summary

Despite variations in the carboxylic acid yields and kinetics across the three experiments, it remains clear that applying cotreatment in each of the three experiments produced better results than the untreated fermenters. Targeting various fermenter conditions would give better insight into the performance of Galveston soil bacteria in LCB mixed-acid fermentation, especially when operating under various C/N ratios.

Cotreatment energy consumption

With evidence of how cotreatment affects the yields of LCB mixed-acid fermentation, the next step is to determine the energy cost associated with grinding the LCB. Using the procedure

outlined in Chapter II, the mean static torque of the grinder was determined to be 65.7 in-lb with a standard deviation of 4.7 in-lb. This value depicts, in a dynamic sense, the stall torque of the grinder under a continuous mode of operation. With this understanding, a dynamic analysis can be performed.

Assuming that the torque analysis accurately describes the torque requirement for grinding the corn stover at early stages of fermentation, and assuming that the torque requirement remains near that value over the course of fermentation, the cost required to grind the corn stover at industrial scales can be estimated by the following:

Given the process, it can be assumed:

- Torque applied is constant for the duration of the grinding process
- The process produces a dry basis mass flow rate of $0.075\frac{g}{s}$
- The motor used for grinding the corn stover has an efficiency $\eta = 0.92$
- The grid price of electricity is $0.05\frac{\$}{kWh}$
- The angle between the applied force from the motor and the arm $\sim 90°$
- The grinder operates at a constant 30 RPM

For this process:

$$dW = Fdl$$

$$dl = rd\theta$$

$$d\theta = \omega dt = 2\pi\frac{dn}{dt}dt = 2\pi N_{RPS}dt = \frac{\pi}{30}N_{RPM}dt$$

$$dl = \frac{\pi}{30}rN_{RPM}dt$$

$$F = \frac{T}{\eta \cdot r \cdot \sin(\theta)} \approx \frac{T}{\eta \cdot r}$$

$$dW = \frac{\pi}{30}\frac{T}{\eta}N_{RPM}dt$$

Upon integrating both sides,

$$W_{GRIND} = \frac{\pi}{30}\frac{T}{\eta}N_{RPM}t$$

$$t = \frac{m}{\dot{m}} = \frac{1\ tonne}{0.075\frac{g}{s}} = \left(\frac{1000000\frac{g}{tonne}}{0.075\ \frac{g}{s}}\right)\left(\frac{1\ hr}{3600\ s}\right) = 3703\frac{hr}{tonne}$$

Finally,

$$C_{GRIND} = W_{GRIND} \cdot C_{POWER}$$

$$= \left(\frac{\pi\ min}{30\ s \cdot rev}\right)\left(\frac{65.7\ lb_f in}{0.92}\right)\left(\frac{1\ ft}{12\ in}\right)\left(\frac{1.356 \times 10^{-3}\ kNm}{1\ lb_f ft}\right)\left(30\frac{rev}{\text{min}}\right)\left(3703\frac{hr}{tonne}\right)\left(0.05\frac{\$}{kWh}\right)$$

$$= 4.69\frac{\$}{tonne\ biomass}\ (per\ grind)$$

With a cost of $4.69 per tonne of biomass per grind, cotreatment looks like a promising option for the future of LCB mixed-acid fermentation. For a typical chemical pretreatment, costs can be expected to run around $50 per tonne of biomass. This means as long as 10 or fewer grinds are performed on the LCB, the cotreatment process is more economical than chemical pretreatment. One important thing to note is that none of the experiments performed and discussed above had more than 10 grinding sessions. While it remains in the design stage due to COVID-19 complications, the concept for a motor-driven grinding apparatus are provided in Figure A1.

CHAPTER IV

CONCLUSION

Given the data generated by the cotreatment experiments presented in this thesis, cotreatment appears to be a promising method of improving the acid yields of mixed-acid fermentation at a reduced cost. Overall, cotreatment was able to increase yields to an acceptable level, making it economically competitive with pretreatment methods, and nearly competitive with fossil fuels.

Moving forward, a few things need to be explored further to better develop a complete picture of the effect of cotreatment on LCB mixed-acid fermentation. First, more work needs to be done exploring the effect of C/N ratio on the Galveston soil bacteria culture used. Next, work needs to be done to determine the optimal number of grinds for the biomass. If this can be determined, the energy cost of grinding can be greatly reduced, as grinding would not occur unnecessarily, as defined by the first assumption made in Chapter II. Furthermore, work needs to be done to explore the possibility of a combinatorial approach to LCB treatment. Exploring how the performance of mixed-acid fermentation changes when chemical treatment is combined mechanical cotreatment could result in yields that justify the associated costs. Lastly, exploring methods of selectively removing acids from solution would likely provide increased yields in accordance with Le Chatelier's principle.

REFERENCES

Agler, M. T., Wrenn, B. A., Zinder, S. H., & Angenent, L. T. (2011). Waste to bioproduct conversion with undefined mixed cultures: the carboxylate platform. *Trends in Biotechnology*, *29*(2), 70–78. doi:10.1016/j.tibtech.2010.11.006

Amin, F. R., Khalid, H., Zhang, H., Rahman, S. U., Zhang, R., Liu, G., & Chen, C. (2017). Pretreatment methods of lignocellulosic biomass for anaerobic digestion. *AMB Express*, *7*(1), 72. doi:10.1186/s13568-017-0375-4

Anderson, T. R. (1992). Modelling the influence of food C:N ratio, and respiration on growth and nitrogen excretion in marine zooplankton and bacteria. *Journal of Plankton Research*, *14*(12), 1645–1671. doi:10.1093/plankt/14.12.1645

Baruah, J., Nath, B. K., Sharma, R., Kumar, S., Deka, R. C., Baruah, D. C., & Kalita, E. (2018). Recent Trends in the Pretreatment of Lignocellulosic Biomass for Value-Added Products. *Frontiers in Energy Research*, *6*. doi:10.3389/fenrg.2018.00141

Bensah, E. C., & Mensah, M. (2013). Chemical Pretreatment Methods for the Production of Cellulosic Ethanol: Technologies and Innovations. *International Journal of Chemical Engineering*, *2013*, 1–21. doi:10.1155/2013/719607

Biello, D. (2011). THE FALSE PROMISE OF BIOFUELS. *Scientific American*, *305*(2), 58-65.

Corona Hand Mill Parts Breakdown [Digital image]. (2013). Retrieved from https://coronamill.com/corona-parts.shtm

Den, W., Sharma, V. K., Lee, M., Nadadur, G., & Varma, R. S. (2018). Lignocellulosic Biomass Transformations via Greener Oxidative Pretreatment Processes: Access to Energy and Value-Added Chemicals. *6*(141). doi:10.3389/fchem.2018.00141

Golub, K. W. (2012). Effect of Bioreactor Mode of Operation on Mixed-Acid Fermentations.

Kucharska, K., Rybarczyk, P., Hołowacz, I., Łukajtis, R., Glinka, M., & Kamiński, M. (2018). Pretreatment of Lignocellulosic Materials as Substrates for Fermentation

Processes. *Molecules (Basel, Switzerland)*, *23*(11), 2937. doi:10.3390/molecules23112937

Liu, Z., & Fei, B. (2013). Characteristics of Moso Bamboo with Chemical Pretreatment. *Sustainable Degradation of Lignocellulosic Biomass - Techniques, Applications and Commercialization*. doi:10.5772/55379

Mafe, O. A., Davies, S. M., Hancock, J., and Du, C. (2015). Development of an estimation model for the evaluation of the energy requirement of dilute acid pretreatments of biomass. *Biomass Bioenergy.* 72, 28–38. doi:10.1016/j.biombioe.2014.11.024

Mosier, N., Wyman, C., Dale, B., Elander, R., Lee, Y. Y., Holtzapple, M., & Ladisch, M. (2005). Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresource Technology*, *96*(6), 673–686. doi:10.1016/j.biortech.2004.06.025

Seidl, P. R., and Goulart, A. K. (2016). Pretreatment processes for lignocellulosic biomass conversion to biofuels and bioproducts. *Curr Opin Green Sustain Chem.* 2, 48–53. doi:10.1016/j.cogsc.2016.09.003

Singh, T., & Singh, A. P. (2016). White and Brown Rot Fungi as Decomposers of Lignocellulosic Materials and Their Role in Waste and Pollution Control. *Fungal Applications in Sustainable Environmental Biotechnology Fungal Biology*, 233–247. doi:10.1007/978-3-319-42852-9_9

APPENDIX

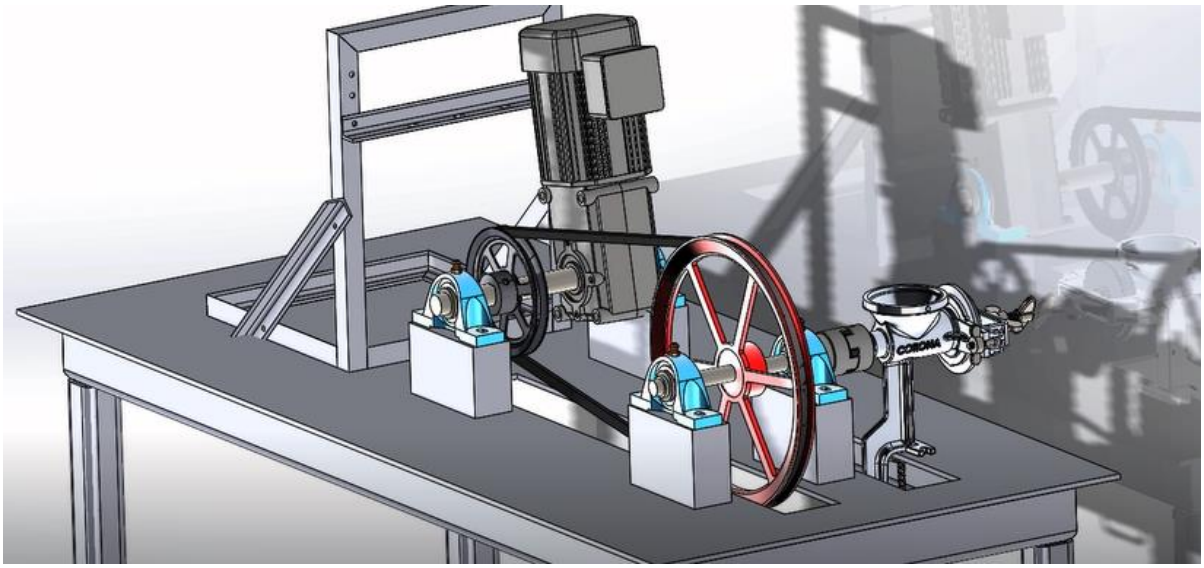

Figure A1. Motor-driven grinding apparatus concept. Here, the motor is used drive a pulley system connected to the grinder. As the motor attempts to rotate about its shaft, it applies tension on a fish scale that connects the motor to a support beam. This force measurement is then collected over time and used to calculate the dynamic toque applied by the motor. In addition to the direct calculation of the forces applied, electronic measurement through the use of a VFD would be expected to provide information about electrical consumption.

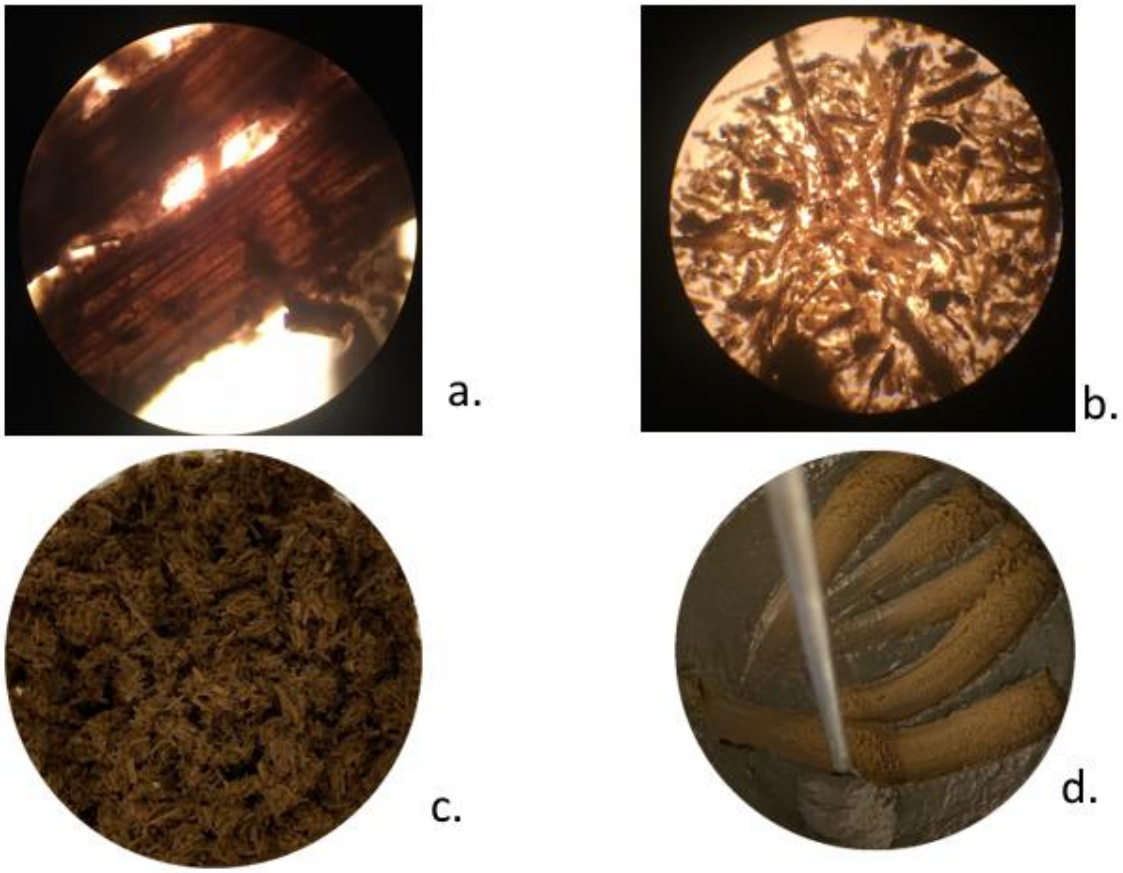

Figure A2. (a) Microscopic image of corn stover from a control fermenter. (b) Microscopic image of corn stover from an experimental fermenter. (c) Image of the contents of a control fermenter. (d) Image of the contents of an experimental fermenter.