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Comparison Study Between Post-Fermentation Stillage and Pre-Fermentation Mash Utilizing Acidification and Steam Explosion Techniques for Cellulose Saccharification

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COMPARISON STUDY BETWEEN POST-FERMENTATION STILLAGE AND PRE-FERMENTATION MASH UTILIZING ACIDIFICATION AND STEAM EXPLOSION TECHNIQUES FOR CELLULOSE SACCHARIFICATION

by

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ABSTRACT

COMPARISON STUDY BETWEEN POST-FERMENTATION STILLAGE AND PRE-FERMENTATION MASH UTILIZING ACIDIFICATION AND STEAM EXPLOSION TECHNIQUES FOR CELLULOSE SACCHARIFICATION

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Pre-fermentation mash fiber and post-distillation stillage fiber were examined and compared using a variety of preparatory techniques to determine the better source for cellulose fiber saccharification. Once screened, dried, and diluted to a 10% solution, mash fiber and stillage fiber were exposed to increasing temperatures for steam explosion techniques as well as increasing acidification techniques. Both underwent enzymatic saccharification to convert the exposed cellulose to glucose and other sugars. Once the optimum steam explosion technique parameters and acidification parameters were determined to be 2.5% sulfuric acid at 127.8°C for 1 hour, a comparison of the saccharification of pre-fermentation mash fiber and post-distillation stillage fiber under these conditions was conducted. While both are capable sources, post-fermentative stillage provides more fiber (64.18%) that shows approximately 6% greater ability of being degraded than the available fiber content in pre-fermentation mash, which was only 60.92% of the original dried sample.

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ACKNOWLEDGMENTS

I would like to acknowledge everyone who participated in the formation of this work. First of all, I would like to sincerely thank Ergon Biofuels LLC as well as the Ergon Corporation as a whole for allowing me the opportunity the use of their facilities and instrumentation to generate the data and process information included in this work.

Secondly, Dr. Sarah Morgan, who patiently provided advice and guidance throughout the research process.

Lastly, my committee members for their willingness to participate in this step furthering my education and knowledge.

Thank you all for providing your support in this endeavor.

DEDICATION

I would like to dedicate this to my wife Laura, who has provided unwavering support throughout my continued education. These accomplishments would not be possible without your support.

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LIST OF ILLUSTRATIONS

LIST OF ABBREVIATIONS

RFS	Renewable Fuels Standard
DDGS	Dried Distillers Grains with Solubles
DP	Degree of Polymerization
DI	De-ionized Water
HPLC	High Performance Liquid Chromatography
ID	Inner Diameter
NDF	Neutral Detergent Fiber
%wt	Percent, by weight
HMF	*Hydroxymethylfurfural*

CHAPTER I – INTRODUCTION

ORGANIZATION

There are four chapters included in this thesis. Chapter I provides an introduction to cellulosic fiber and how this product is currently utilized in the ethanol industry. Also described are the two sources of cellulosic fiber under investigation in this study and why these two were chosen. Chapter II provides a description of the methods and experiments carried out and how the breakdown of cellulosic fiber was analyzed. Chapter III discusses the results of the data gathered. Lastly, Chapter IV expresses the conclusions of the study and potential opportunities for future research.

LITERATURE REVIEW

Ethanol Industry

The ethanol industry in the United States accounts for over 41% of the ethanol produced worldwide (*1*). The vast majority of the ethanol produced is from starch-based feedstocks such as corn. Current energy sources are heavily dependent on fossil resources, which supply approximately 86% of the energy industry (*2*). As part of the effort to replace some of the fossil fuel resources as the primary source of the energy industry, the EPA through the Renewable Fuel Standard program (Energy Policy Act of 2005) provides mandates and incentives for production of various renewable fuels.

Part of this program is a set of yearly goals of renewable fuels production. The 2017 goal for “Conventional Biofuels” is 15.0 Billion gallons, whereas the cellulosic biofuels goal is 5.5 billion gallons. Over the next five years, the “Conventional Biofuels” goal will remain at 15.0 billion gallons, whereas the cellulosic biofuels goal will be tripled to 16.0 billion gallons. The modern ethanol industry in America is heavily centered on ethanol that is fermented using starch from corn and is considered “Conventional Biofuels”. “Conventional Biofuels” can be considered to be plants typically using corn, milo, sugarcane, or beet as the main feedstock in production, whereas cellulosic biofuels can include biomass from wood pellets, wood cube, wood puck (*2*), corn stover, and other sources.

According to the Renewable Fuels Association 2017 Ethanol Industry Outlook, in 2017 there were 213 operational ethanol plants with production capacity of 15.6 billion gallons, whereas the cellulosic biofuel production is limited to no more than 148 million gallons and only 7 operational plants, making up only 3% of the plants and less than 1% of the production capacity of the industry instead of the projected 27% (*3*). While sugar and starch based ethanol production easily meet the intended goal, cellulosic biofuel production is nearly 37 times lower than the present goal and is not trending to match the goal of 16.0 billion gallon production by 2022. These targets lay the foundation for providing significant reductions of greenhouse gas emissions from the use of renewable fuels, reducing imported petroleum, and for encouraging the development of the US’s renewable fuels sector (*4*).

Current Applications of Cellulose Fiber in the Ethanol Industry

Lignocellulosic biomass has originally been attractive as a source of energy due to its availability, higher potential energy consumption, and the fact that it does not compete with food industries (*2*). One of the main problems with producing cellulosic ethanol is that it can be almost double the cost of producing corn-based ethanol (*2*). There is also increased concern that enzymatic conversion of cellulose to glucose is not yet economically feasible due to necessary pretreatment steps that are time and energy consuming (*5*)(6).

The major products from starch-based ethanol production plants are ethanol, CO_2, and dried distillers grains with solubles (DDGS). DDGS account for approximately 15-25% of the total revenue of typical ethanol plants (*7*). Approximately as much DDGS is produced as ethanol on a mass basis (*8*). Currently, the vast majority of DDGS is used directly for low-value livestock and poultry feed (*9*). Potentially, DDGS can be a source of cellulose with properties suitable for films and absorbents (*10*). While this thesis focuses on the saccharification of the cellulose fiber present in DDGS, there are a number of acidic purification methods to provide high purity cellulose showing greater degree of polymerization (DP) when compared to cellulose gathered directly from corn (*10*). Another potential non-feed use of DDGS is as a filler in polymeric composite materials, where it exhibits advantages due to the low cost, comparatively higher DP value than corn cellulosic fiber, and the ability to use the fiber present to reinforce a matrix polymer (*8*). Converting the fiber in DDGS may provide an avenue to increase the revenue directly from ethanol production as

well as expand the market for the remaining components, potentially increasing its value (*7*).

Cellulose

The production of ethanol is mostly dependent upon providing a source of glucose to an organism that ferments the feedstock into ethanol and various byproducts such as carbon dioxide. The most commonly used source of glucose in the ethanol industry is starch from corn. Starch is mostly composed of α-amylose (Figure 1) and α-amylopectin (Figure 2). The polymer α-amylose is made of thousands of glucose molecules linked by α(1-4) bonds.

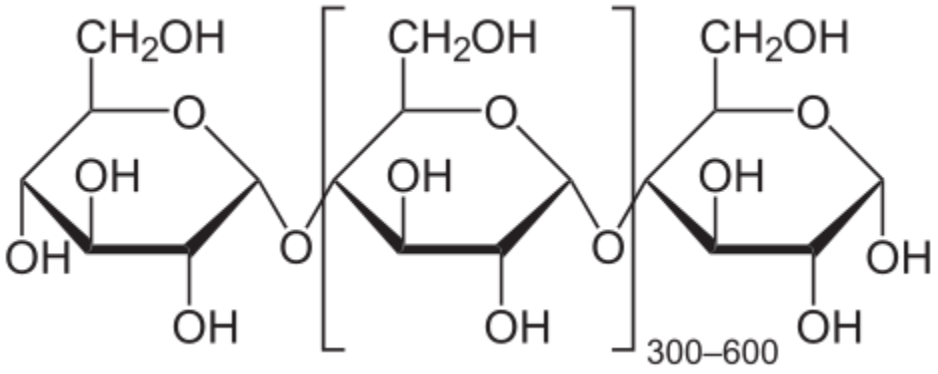

Figure 1. Amylose

Amylopectin is composed of α(1-4) linked glucose molecules as well as branches with α(1-6) linked glucose molecules. These molecules are commonly broken down into glucose through the use of an amylase and α-glucosidase. (*11*)

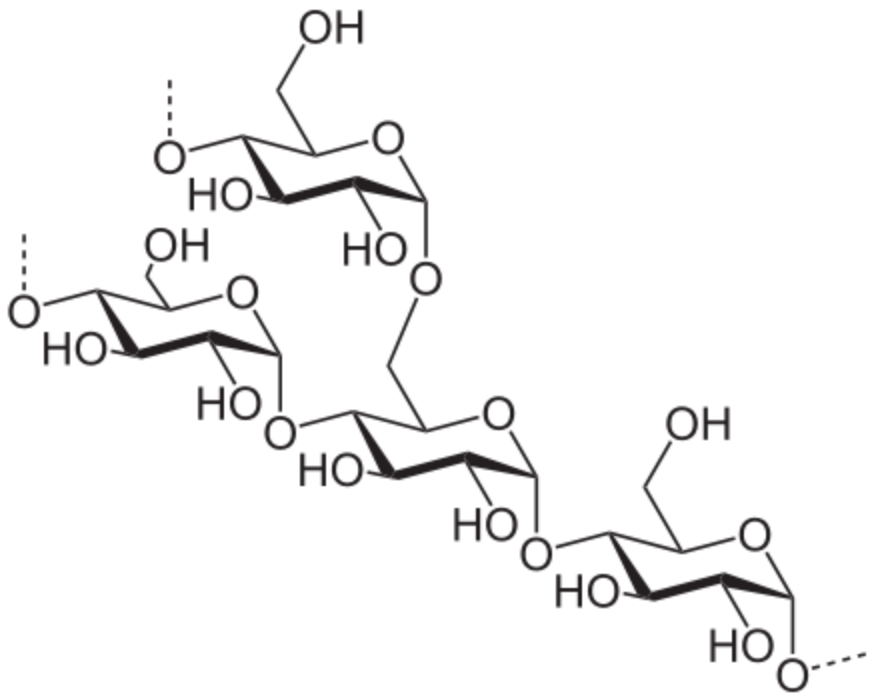

Figure 2. Amylopectin

Cellulose (Figure 3.), however, is composed of glucose molecules bound by β(1-4) glycosidic bonds which are inaccessible to amylase. Also, the hydrogen bonding and van der Waals interactions present between glucose molecules (intra and intermolecular interactions between cellulose molecules) provide increased strength, water insolubility, and evenly distributes stress among reinforcing molecules such as lignin and other polysaccharides (*11*).

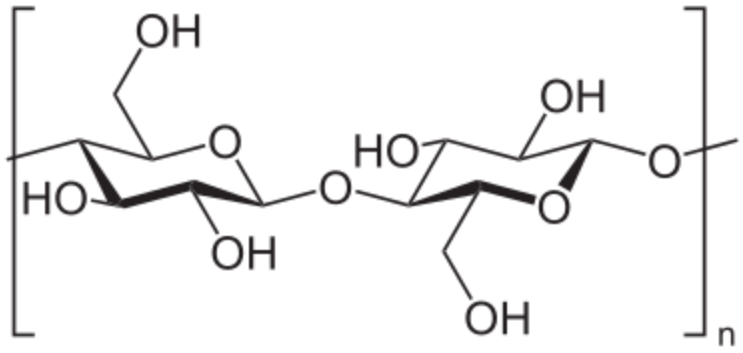

Figure 3. Cellulose

These properties make cellulose resistant to degradation and hydrolysis even when subjected to similar environments that dissolve amylose and amylopectin. Additional enzymes and aggressive pre-treatments are necessary to expose the glucose molecules so that they are capable of being consumed. Pretreatments that increase the surface area are essential in driving the enzymatic accessibility of lignocellulosic biomass (*12*).

Cellulases are enzymes that hydrolyze the β(1-4) glycosidic bond, allowing for degradation of the cellulose structure (*11*). Celluclast® by Novozymes is the cellulase used in this research, and it has a mixture of different activities that will provide a more thorough breakdown of cellulose than a cellulase that functions by hydrolyzing the β(1-4) glycosidic bond alone (Figure 4.). Although cellulase provides a method to break down the available glycosidic bonds, the enzyme may be prevented access to the binding sites due to the presence of hemicellulose and lignin. Cellulases can function to cut at various points in the cellulose structure (see Figure 4). Also, high temperature acid treatment methods are reported to show high recovery of cellulose compounds due to the removal of hemicelluloses (*13*)(*14*). Utilization of dilute acid pre-treatment can

also provide up to 300% improvement to glucose yield when used along with enzymes (*15*).

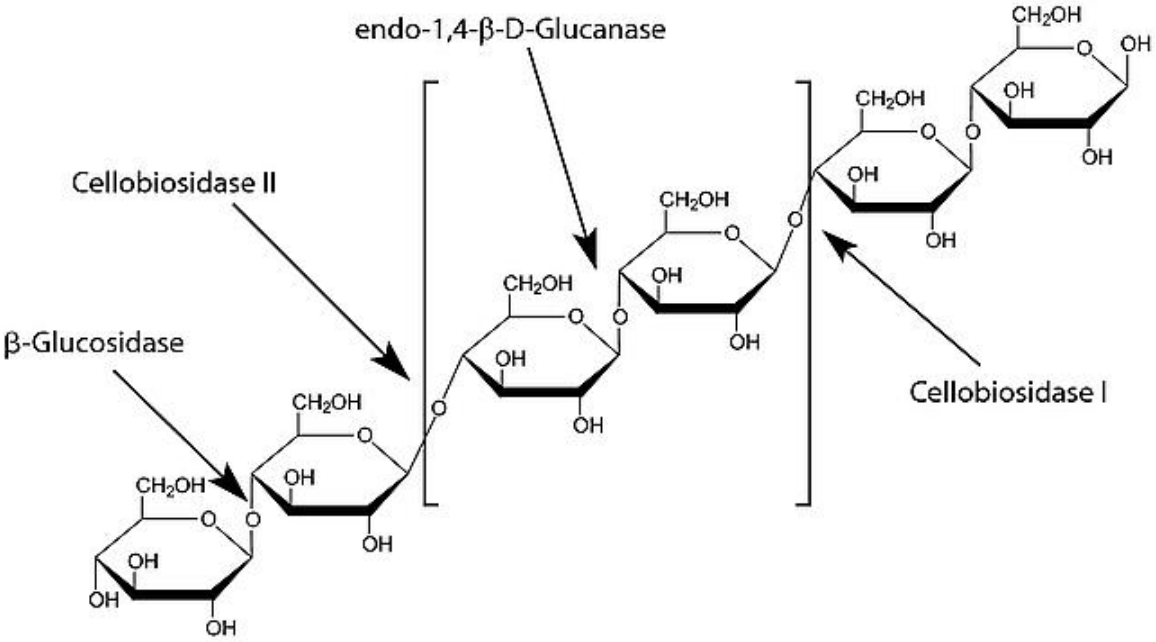

Figure 4. Cellulase activity on Cellulose

Hemicellulose and Lignin

Hemicellulose is a heterogeneous collection of monomeric residues with 5 or 6 carbon rings (Figure 5.). Hemicellulose and lignin bind at lignin-carbohydrate complexes using covalent bonds (*16*). Hemicelluloses are various carbohydrate polymers that are easily fragmented into sugar units, including xylose, mannose, arabinose, glucose, and glucouronic acid (*16*). These saccharides can be extracted using dilute acid pretreatments, alkaline extraction, alkaline peroxide extraction, liquid hot water extraction, steam treatment, microwave treatment, ionic liquid extraction, and other methods (*16*). Also, cellulase activity can

contribute to degrading hemicellulose (Figure 6.). Separation of hemicelluloses and cellulose can be a challenge due to the close association of the hemicellulose with lignin through chemical bonds, possibly preventing full separation (*17*).

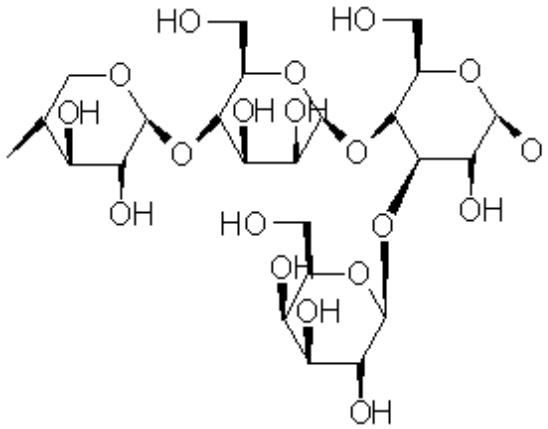

Figure 5. Hemicellulose with various individual sugars.

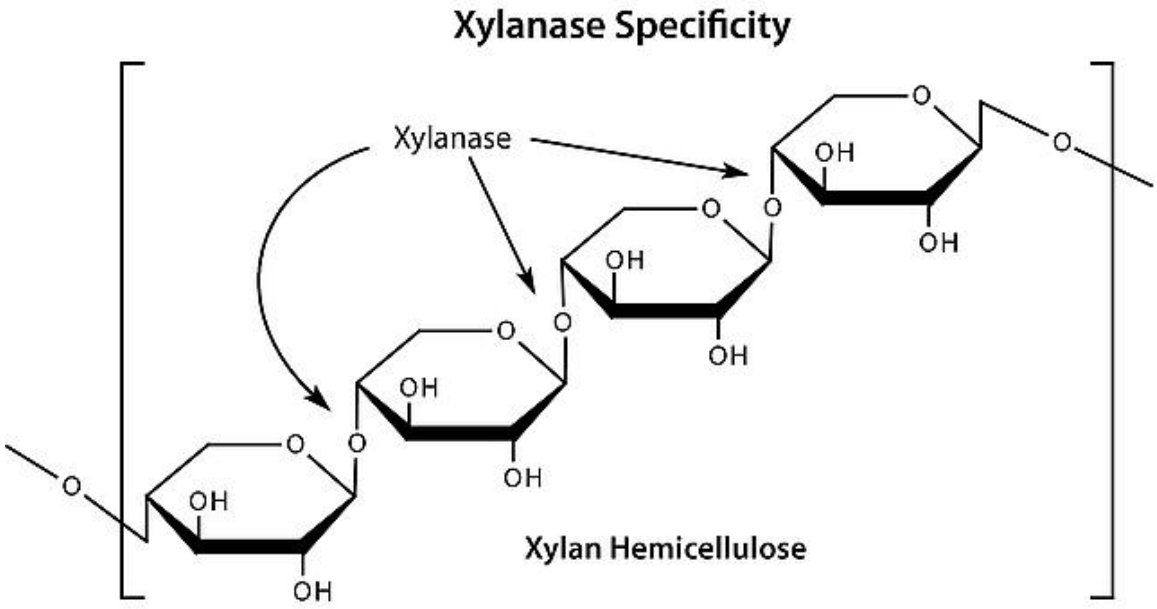

Figure 6. The cellulase being used also has hemicellulose activity, specifically as a xylanase.

Lignin, mixed with hemicellulose, is also present in cellulosic fiber. The structure of lignin varies with the source and the separation method (*18*). Lignin is a natural phenolic polymer with propyl-phenol groups such as guaiacyl, syringyl, and hydroxylphenyl functioning as structural units (see Figure 7 for a possible variation). When exposed to ethanol-water mixtures with sulfuric acid, lignin shows the ability to hydrolyze, especially at elevated temperatures (*18*). Lignin is typically covalently bonded to hemicellulose in varied and complex matrices. This in turn can cause decomposition of hemicellulose that is proportional to lignin's ability to hydrolyze (*18*). Recovery of hemicellulose components may be lower than projected due to incomplete disassociation of the lignin as well as hydrolyzed hemicellulose components forming precipitates with solubilized lignin, particularly with xylose (*18*). Introduction of various solvents such as concentrated phosphoric acid, ionic liquid, and concentrated sulfuric acid can disrupt the hydrogen bonds of cellulose and further linkages among cellulose, hemicellulose, and lignin (*19*), allowing for greater access to cellulose. Lignin is also left over as a by-product of lignocellulosic ethanol production. As much as 1.26-1.85 tons of dry lignin residue can be generated from the production of one metric ton of ethanol fuel from lignocellulose sources (*20*). This can provide an additional fuel source to be sold or utilized in energy production.

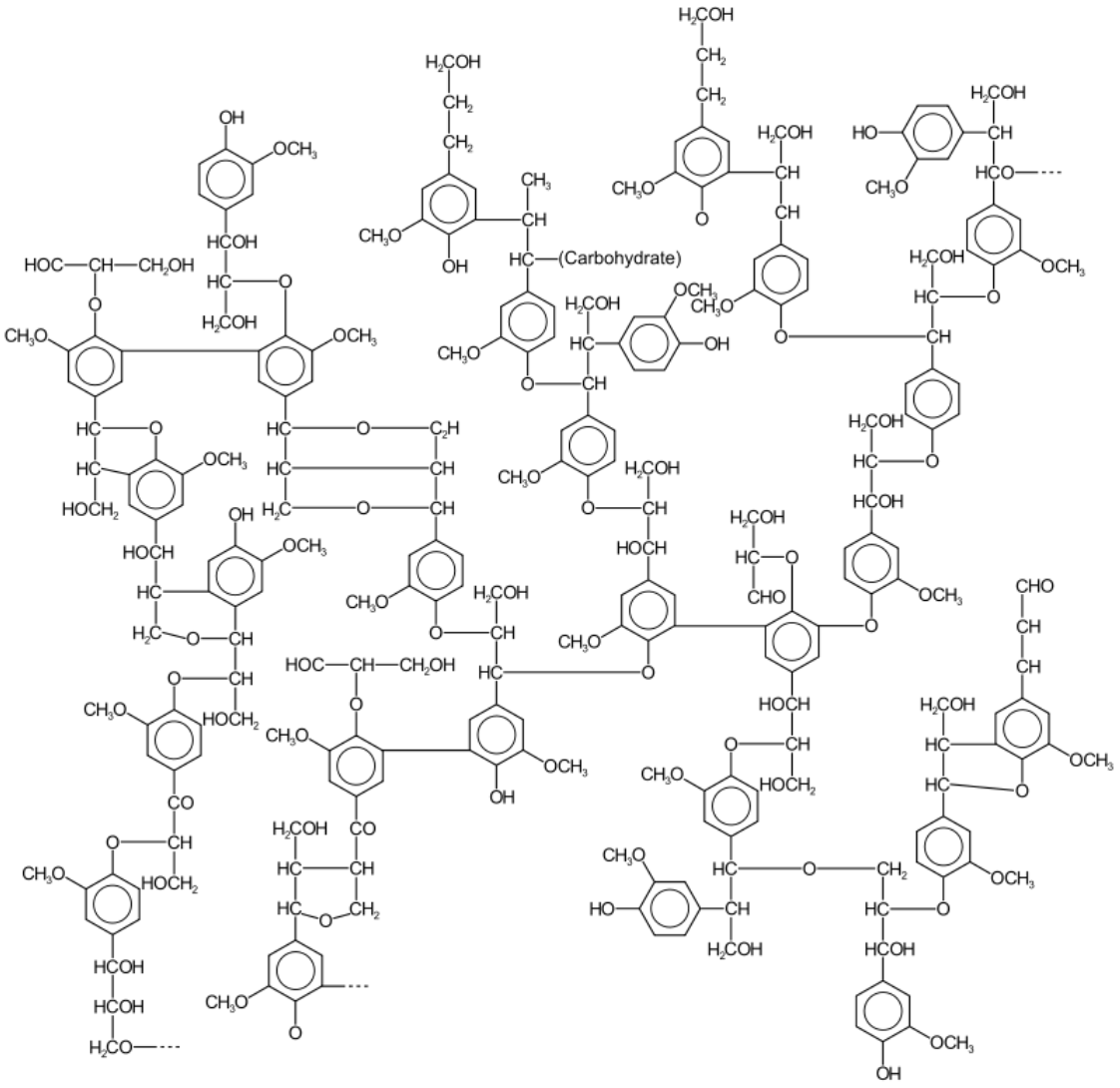

Figure 7. A possible variation of lignin. This structure will be different based on the source and manner of extraction of the molecule.

MOTIVATION FOR RESEARCH

Ergon Biofuels LLC in Vicksburg, MS is an ethanol production plant that uses No. 2 Yellow Dent Corn as the feedstock for the process. According to Hamby, No.2 Yellow Dent Corn is typically 65% starch, 10% moisture, 1.66% ash, 8.9% protein, and 4% fat. This corn will be ground and will be mixed with water and an α-amylase and glucosidase to convert the starch into glucose molecules, which remain in solution. This mixture is described as mash. During fermentation, the dissolved glucose is converted into ethanol and carbon dioxide. The fermentation can cause typical ethanol concentrations of 13-14%, at which time it is considered “beer”. The ethanol in the beer is then distilled and the leftover beer without ethanol is called stillage. Dried stillage is called Dried Distillers Grains with Solubles (DDGS) and is sold as a dry feed.

Once through production, DDGS contain oil (8-11%), proteins (about 25-30%; of which 50% is zein), cellulose (9-16%), and other carbohydrates (*10*). Ergon has seen a residual starch presence of 1-2% in the DDGS produced, which also shows that nearly 99% of the available starch from the corn is successfully saccharified in preparation for fermentation. Currently, fiber can be isolated and sold as a booster for feed or can be dried with protein and oil as DDGS. DDGS being sold as a feed does not currently hold the same value as ethanol, but part of the stillage going into DDGS could be converted into ethanol from the glucose present in cellulose fibers. Cellulosic fermentation processes of wood pulp, corn stover, and various other sources are present within the industry already, but not

many of the current starch-based processes are taking advantage of the alternative source of glucose in animal feed that is readily available. Potentially this new source of cellulosic ethanol can provide additional revenue as well as help the EPA meet their goals for cellulosic ethanol production.

The production of ethanol at Ergon Biofuels currently follows a common pathway. Corn is ground into a meal and mixed with water, recycled stillage with solids removed, acid, and alpha amylase. This combination is mixed continuously at an elevated temperature until all available starch has been saccharified into complex sugars such as maltotriose, maltose, and others. This "mash" is then cooled and dosed with antibiotics, yeast, glucoamylase, and a nitrogen source, usually urea. The glucoamylase further breaks down the complex sugar molecules into glucose, which is then converted by the yeast into ethanol and carbon dioxide. After a period of time (usually 54 hours), all available glucose has been consumed and the ethanol concentration is at its highest. This beer is sent to distillation where ethanol is removed. The stillage is now free from ethanol and is mainly water, fiber, and protein. The stillage then has solids (mostly fiber and some protein) removed through centrifugation. The solids that are centrifuged out of the suspension are sent to a dryer and DDGS are produced, where the liquid is recycled back into the system. In this pathway, there are two sites that are being considered for fiber separation. The first is the mash going to fermentation, and the second is the stillage leaving distillation.

Utilizing mash going to fermentation as the site of fiber separation would allow for increased capacity for each of the fermentation tanks since roughly 14% of

the space would be available for more fermentable solids since all available starch has been saccharified and is in solution. There is the risk that separation at this point will remove some incoming nutrients, and protein bound to the fiber would not be consumed by the yeast, causing lower quality fermentation. The second option, post-distillation stillage, provides a source of fiber that has already undergone additional mechanical and chemical pretreatment. Ethanol present in fermentation acts as a solvent and potentially can cause fibrillation in cellulosic fibers (*21*). In addition, low acid concentrations with a pH of 3-4, increased pump agitation, increased temperatures, and longer time in circulation provide additional stressors that can cause damage to existing cellulose fibers.

The cellulosic fiber present in stillage and DDGS can be somewhat inaccessible to enzymes due to the previously described protective sheath around it and highly ordered crystalline structure of cellulose itself (*9*). This in combination with the low specific activity and cost of current commercial cellulase enzymes has prevented the industry from pursuing cellulosic fibers as a major source of renewable energy (*9*). This hindrance can be overcome with the right enzyme, but the next problem is having the enzyme active on an exposed fiber strand. Cellulosic fiber is tough, and it can be difficult to expose active sites to enzymes. Pretreatment of any lignocellulosic biomass is crucial before enzymatic saccharification (*22*). Commonly used pre-treatment steps include temperature, steam explosion, acidification, alkylation, and solvent treatment. More aggressive treatments provide for better enzymatic saccharification, but may in turn cause inhibitory compounds such as furfuran that can cause

problems in fermentation. Also, with more aggressive treatments, either chemical, thermal, or physical additional risks to operators are present. Past work has shown that higher acid concentration, although providing higher yields (*23*), leads to a higher rate of sugar degradation, higher costs of acid neutralization and/or recovery, as well as higher equipment maintenance costs due to corrosion (*24*). Previous experiments have shown that various low concentrations of sulfuric acid as well as elevated temperatures give higher expected yields. Treatment with 0.75% sulfuric acid at 121°C for 1 hour gave maximum yield of 64% carbohydrates with no detectable quantities of inhibitory compounds (*22*). In another study, optimum levels of treatment were determined to be 3.1% sulfuric acid at 112°C for 84.5 minutes (*9*). Further studies showed that the highest yield of monomeric sugars was observed with the highest concentration of sulfuric acid (1.5%vol) and when the temperature was 140°C, but formation of furfural was significantly lower at 120°C (*25*). Additional chemical treatment can increase recovery of hemicellulose content as well. One study listed the highest yield was obtained using alkaline peroxide pretreatment at 120°C for 90 minutes giving recovery of nearly 51.6% of the available hemicellulose (*26*). Hemicellulose can be a second source of ethanol or additional chemical production from pentose sugars such as xylose and arabinose. A problem associated with dilute acid pretreatments has been the production of inhibitory compounds, such as furfural, formic acid, and hydroxymethylfurfural (HMF), and usually a detoxification step is needed to enhance fermentation (*22*). One such detoxification step to be considered is the

process of overliming, which has shown increased yield and production rate in simultaneous saccharification and fermentation (SSF), but can contribute to sugar loss due to precipitate formation (22). Another hurdle that has prevented cellulosic fermentation from taking hold is the lack of a hearty organism that can ferment both glucose and pentose sugars into ethanol. *S cerevisiae* has now been shown (although not naturally) to reduce xylose into xylitol in the presence of glucose (*27*)(*28*), which can take the place of the pentose sugar fermenting organism. While hemicellulose content represents an easily extractible source of sugars, it is the largest polysaccharide fraction wasted in most cellulosic ethanol plants due to the low fermentability by the most commonly used industrial microbial strains (*29*). Even though common yeast has shown some effectiveness in converting xylose into xylitol, glucose fermentation into ethanol is the preferred pathway. Increased ethanol then can cause stress on the organism which may further inhibit pentose fermentation.

HYPOTHESIS

In modern ethanol plants there are generally two periods in the processing of starch from ground corn into ethanol when a fiber stream can be separated easily. One of these is the mash immediately before fermentation, and the second is the stillage left over after distillation of ethanol from a completed fermentation. The stillage is hypothesized to be a better source for cellulosic saccharification than mash, because it has experienced longer exposure time to dilute acid, greater physical stress due to pumping and recirculation, and

exposure to increased temperature from the distillation column. We hypothesize that this increased exposure to processing will create a greater concentration of available fiber that will more readily be converted to saccharides. The hypothesis will be tested by processing mash and stillage through a variety of pre-treatment techniques and determining the ability to enzymatically convert the available cellulose into glucose once the preparatory steps have been completed.

RESEARCH OBJECTIVES

This project is intended to compare the mash and stillage product streams' ability to undergo various pre-treatment combinations and enzymatic saccharifications with the purpose of determining the best suited site for cellulosic fermentation. It is hypothesized that stillage will perform as a better source for cellulosic saccharification than mash due to its exposure to longer dilute acid treatment times, greater physical stress due to pump and recirculation, and increased temperature from the distillation column. The benefits that Ergon Biofuels would experience due to either of these choices would be primarily increased ethanol production, as well as high quality protein feed production, lowered dryer operation costs, lowered dryer maintenance costs, and increased incentives provided by the EPA for producing cellulosic ethanol. Comparison of pretreatment steps that include dilute acid treatment as well as a variation of "steam explosion" will be simulated by Ergon Biofuels Laboratory's autoclave. Steam explosion is a common thermomechanochemical process where the

breakdown of structural components is aided by heat in the form of steam, shear stresses due to the expansion of moisture, and hydrolysis of glycosidic bonds once the mixture is (self)-catalyzed (*29*). Rapid decompression leads to desegregation of the lignocellulosic matrix, breaking down inter-and intra-molecular linkages (*29*). The various pretreatments should provide additional comparisons for determining the better source of feed, either from mash or stillage.

EXPERIMENTAL PLAN

Mash and stillage will both be collected during typical operation of the plant in Vicksburg, MS. Both samples will be screened using a 45µm screen allowing for soluble material to be removed, leaving fiber, protein, and any other non-starch components. The screenings will be dried overnight and ground to pass through a 850µm screen. Samples will be weighed appropriately for the specific test they will undergo. The samples will be subjected to either acid treatments, temperature and pressure treatments, or both.

Stillage and mash samples will be prepared and will be autoclaved for 1 hour at the following temperature settings before having the pressure released:

- Room Temperature (not autoclaved)
- 100°C
- 110.5°C
- 121.1°C

- 127.8°C
- 135°C

Stillage and mash samples will also be prepared and autoclaved at 127.8°C at the following times before having the pressure released:

- 0 minutes (not autoclaved)
- 15 minutes
- 30 minutes
- 45 minutes
- 60 minutes
- 90 minutes
- 120 minutes

Stillage and mash samples will then undergo the following varying sulfuric acid concentration treatments in a 50°C water bath with agitation set to 150RPMs:

- 0% (no sulfuric acid added)
- 0.05%
- 0.10%
- 0.25%
- 0.50%
- 0.75%
- 1.0%
- 2.5%
- 5.0%

Stillage and mash samples will also undergo the following periods of exposure to 2.5% sulfuric acid treatment in a 50°C water bath with agitation set to 150RPMs:

- 0 minutes (no acid added)
- 15 minutes
- 30 minutes
- 45 minutes
- 60 minutes
- 90 minutes
- 120 minutes
- 3 hours
- 6 hours

From these studies, optimal temperature and time for the autoclave and optimal concentration and time of acid treatment will be determined. Samples will also be evaluated with acid treatment preceding the autoclave treatment. All samples that have been acidified will be neutralized and dosed with cellulase to begin to break down any available cellulose and hemicellulose into primary components. Saccharide production will be measured using a High Pressure Liquid Chromatography system with an organic acid column that separates sugars based on the charge and size of the molecule. The total fiber is calculated using the Van Soest method to determine cellulose content (*30*).

Goals

The goals of this research are as follows:

- Compare the degradation of cellulose in mash and stillage when exposed to different temperature ranges.
- Compare the degradation of cellulose in mash and stillage when exposed to different lengths of time at an elevated temperature.
- Compare the degradation of cellulose in mash and stillage when exposed to different sulfuric acid concentration ranges.
- Compare the degradation of cellulose in mash and stillage when exposed to different lengths of time undergoing a sulfuric acid treatment.
- Compare the degradation of cellulose in mash and stillage when exposed to both autoclave and acid treatment.
- Compare the degradation of cellulose in mash and stillage when exposed to acid treatment before autoclave treatment.
- Determine the preferable source of cellulose (mash or stillage) based on the degree of enzymatic degradation when subjected to various temperature and acid treatments.

CHAPTER II – Materials and Methods

Sample Collection

Whole stillage and mash samples were collected form Ergon Biofuels LLC ethanol production plant in Vicksburg, MS. Within the plant, whole stillage was collected from the storage tank for the post-fermentation sample. Ethanol has already been distilled from whole stillage, and is less than 0.05%. All starch-based saccharides have been consumed by this point and residual glucose is less than 0.1%. The mash sample was collected from the tank which feeds fermentation. Enzymatic saccharification of starch has already occurred and all available starch has been converted to soluble sugar compounds. Samples were frozen until further use.

Screening and Drying Fiber from Samples

Whole stillage and mash samples were thawed and filtered using a 45µm screen, allowing for all solubilized sugars, dissolved solids, water, and solids less than 45µm in diameter to be excluded. All remaining solids consist mainly of fiber, protein, and undissociated fats and starch. The samples that were screened were then dried in an oven overnight at 104°C. Dried solids were then ground until they passed through a screen of 850µm in order to mimic grinding abilities of the plant. Dried whole stillage solids were collected and mixed within one container and kept in the freezer until further use. Dried mash solids were collected in a separate container, mixed, and kept in the freezer until further use.

Sample Preparation

Moisture content was first determined using a Halogen Lamp Moisture Analyzer for whole stillage and mash samples. Once moisture was determined for the samples, solids were diluted to a concentration of 10% by weight with de-ionized water. Weights of the sample, water, and any additional components used later in the experiment were recorded in order to determine the exact %solids content.

Autoclave Temperature Variation

Mash and stillage samples were autoclaved for 1 hour at the following temperature settings: room temperature (not autoclaved), 100°C, 110.5°C, 121.1°C, and 127.8°C. Autoclaving at 135°C was also attempted but equipment restrictions prevented completion of this setting. After one hour, the pressure was released quickly and the sample cooled to room temperature.

Saccharification (Enzymes)

Samples after undergoing autoclave temperature variation were allowed to cool to room temperature before dosing with enzymes. The pH of samples was adjusted to 4.5-6.5 with the amounts of Sulfuric Acid or Sodium Hydroxide recorded to account for the change in solid content. Celluclast® by Novozymes was used as the cellulase and was dosed at 2% by weight of the solids for both the stillage and mash samples. This dosing was completed by first diluting the

cellulase by 20g into 100mL DI water in a volumetric flask. 1mL of the dilute cellulase was then added to the pH-adjusted sample. Once the cellulase was added, the sample was shaken in a 50°C water bath with agitation of 150RPM for two hours.

Sigma Aldrich G4511-250UN, β-Glucosidase from almonds was the second enzyme used. It was stored in a refrigerator (2-4°C) until use. β-Glucosidase was diluted and dosed so that each sample received 19units of the enzyme. Samples were then shaken in a 37°C water bath at 150RPM for 48 hours.

Determination of Saccharification (Comparison)

High Performance Liquid Chromatography (HPLC) was used to determine the amount and type of sugars and organic acids present at the end of enzymatic saccharification. The HPLC used was an Agilent 1200 Series with Rezex ROA-Organic Acid H+ (8%) LC 150x7.8mm column. Pump speed was set to 0.6ml/min, column temperature set to 60°C, and the detector (1260 RID Refractive Index Detector) was set to 40°C. The mobile phase used was 0.005N sulfuric acid. In addition to this, a security guard column with Security Guard Cartridges Carbo-H 4x3.0mm ID was used to filter incoming sample and mobile phase prior to the column.

After enzymatic saccharification, the samples were centrifuged at 5000RPM for 10 minutes and the centrate was filtered using a syringe and 0.45µm filter. Of the filtered sample, 1mL of filtrate was added to a 2mL vial. 9µl of 0.555N

sulfuric acid was added to the 1mL of sample. Sample vials were capped, shaken to mix, and added to the autosampler in a specific location determined by the run file. Once run, ChemStation version C.03.05 was used to integrate, determine, and report the data gathered.

Autoclave Time Variation

Once analysis of enzymatic saccharification was completed, the sample showing the greatest degradation was selected in order to use this temperature setting for the autoclave time variation. For both mash and whole stillage the temperature was determined to be 127.8°C. Samples were prepared as before, diluting to 10% solids content. Once the optimum temperature was selected and the samples prepared, both mash and stillage samples were held at this temperature for varying amounts of time. The time period was started once the target temperature was reached within the autoclave. The time variations selected were: 0 minutes (not autoclaved), 15 minutes, 30 minutes, 45 minutes, 60 minutes, and 90 minutes. Autoclaving for 120 minutes was attempted but the experiment failed to maintain pressure due to equipment restraints. Once the hold time was completed, the samples were allowed to cool to room temperature. Once all samples in the time variation step completed the allotted autoclaving time and were cooled to room temperature, the samples were subjected to the enzymatic saccharification steps using cellulase and β-glucosidase as described previously. After enzymatic saccharification was completed, HPLC analysis was performed as described previously.

Sulfuric Acid Concentration Variation

Mash and stillage samples were prepared using varying amounts of concentrated sulfuric acid and water. The concentrations were adjusted for a target amount of 10% solids for each sample. The variations in sulfuric acid for samples were as follows: 0% (no sulfuric acid added), 0.05%, 0.10%, 0.25%, 0.50%, 0.75%, 1.0%, 2.5%, and 5.0%. After the addition of acid to the sample, it was shaken in a 50°C water bath set to 150RPM for 4 hours. After 4 hours, the pH of each sample was adjusted to 4.5-6.5 in order to prevent denaturing of the enzymes. The samples then were subjected to enzymatic saccharification and HPLC analysis. The results of the acid variation study helped determine the acid concentration of the next treatment.

Sulfuric Acid Treatment with Time Variation

Although significantly greater degradation of fiber was shown using 2.5% and 5.0% concentrated sulfuric acid, 2.5% concentrated sulfuric acid was chosen in order to be most compatible with the process settings within the plant. Samples were prepared as before to yield 10% solids content with 2.5% sulfuric acid. Samples were then shaken in a 50°C water bath set to 150RPM for varied times. The amount of time for each round of samples was: 0 minutes (no acid added), 15 minutes, 30 minutes, 45 minutes, 60 minutes, 90 minutes, 120 minutes, 3 hours, and 6 hours. After the specified time, samples were removed from the bath and the pH was adjusted to 4.5-6.5. Samples then were exposed

to enzymatic saccharification and HPLC analysis to determine the most effective acid treatment time.

Combined Autoclave and Sulfuric Acid Treatment

After completion of the autoclave temperature variation treatment, autoclave time variation treatment, acid concentration variation treatment, and acid concentration time variation treatment, the samples were subjected to combined treatments of autoclave and acidification steps. Three samples each of mash and stillage were prepared and received autoclave treatment of 127.8°C for 1 hour and then underwent acidification for 1 hour with concentrations of 2.5% and 0.5% sulfuric acid. A second set of samples was subjected to the acidification step first followed by the autoclave treatment. Samples were pH adjusted to 4.5-6.5 once the acidification treatment was completed. All of the temperatures, times, and concentrations were kept the same and only the order was reversed. Once all treatments were complete and samples were pH adjusted to 4.5-6.5, enzymatic saccharification and HPLC analysis were performed.

Fiber Presence Determination

Once the preferred treatment settings were determined and the combined treatment steps of autoclave and acidification were completed, fiber analysis was completed on each of the original dried, ground samples of mash and stillage as well as the samples that underwent the treatments. The fiber content was determined using the Van-Soest Procedure so that results for neutral detergent

solution fiber (NDF) displayed the presence of cellulose, hemicellulose, and lignin left in the sample. (19)

CHAPTER III – Results

Autoclave Temperature Variation

Identification and quantification of the various sugars available allow for comparison of mash and stillage cellulose and hemicellulose saccharification. Increases in cellobiose and glucose are indicators of saccharification of cellulose. Increases in xylose, arabinose, and glucose are indicators of saccharification of hemicellulose. Graphs are shown throughout this chapter that show the results of saccharification of mash and stillage cellulose and hemicellulose. The results are discussed with the purpose of determining the preferable source of the sugar being produced. Cellulosic content (cellobiose and glucose) is expected to make up roughly 9-16%. Hemicellulosic (xylose and arabinose) content is expected to make up an additional 16%. Most of the remaining material is assumed to be protein, fat, and lignin. Lignin may interferewith the saccharification of sugars by preventing access to binding sites for the enzymes.

Figures 8 through 10 show production of sugars from cellulose and hemicellulose as a function of temperature at constant time (one hour) in autoclave studies. One process condition (135°C) was not included due to equipment restrictions. Each set of graphs include data points that are the average of three mash samples and three stillage samples, with error bars representing one standard deviation, at each temperature listed.

Figure 8 shows cellobiose production as the temperature is increasing. The mash samples show wide variation in cellobiose production, while the

stillage samples show narrow standard deviations. Average cellobiose production is higher for stillage than mash, with a general trend of increase with increasing temperature. Recovery of cellobiose (2.7%) from cellulose was lower than the total available from cellulose (9-16%), which indicates inefficient treatment allowing fewer exposed binding sites for enzymatic saccharification. Figure 9 shows xylose production as the temperature is being increased. Neither sample showed a clear trend in xylose production as a function of temperature, with large variation for the mash samples. Average production was greater for the mash. Higher xylose recovery was seen in mash (24%) than was expected (16%). Mash has not experienced the increased exposure time to dilute acid (3.5pH for 60hours) that the stillage has experienced. The higher levels of xylose are attributed to the hemicellulose that is present in mash that is normally dissolved and removed from the stillage stream.

Figure 10 shows glucose production in mash and stillage samples as temperature is increased. Stillage shows higher average glucose production at all temperatures evaluated, although there is significant overlap of standard deviation at moderate temperatures. Figure 11 shows the production of arabinose as temperature is increased. There is a slight increase in arabinose production with temperature for both samples, with no statistical difference between production levels.

Stillage samples showed higher production levels of cellobiose and glucose, no difference in arabinose, and lower levels of xylose in the autoclave

temperature studies. Production levels generally increased with increasing temperature up to 121 °C. Variation was generally higher for mash samples. While mash samples yielded higher average levels of xylose production, no clear trend with increasing temperature was observed. Combined glucose and cellobiose values (3.5%) remain less than the values expected from the available cellulose (9-16%). This can be due to the decreased efficiency of the autoclave treatment or due to lignin preventing access to cellulose for saccharification.

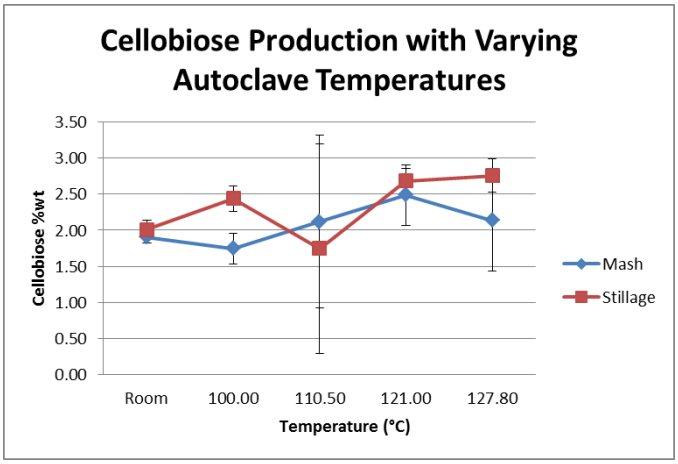

Figure 8. Cellobiose production using temperature as a variation. Results are shown in %wt. Stillage shows increased production as treatment progresses.

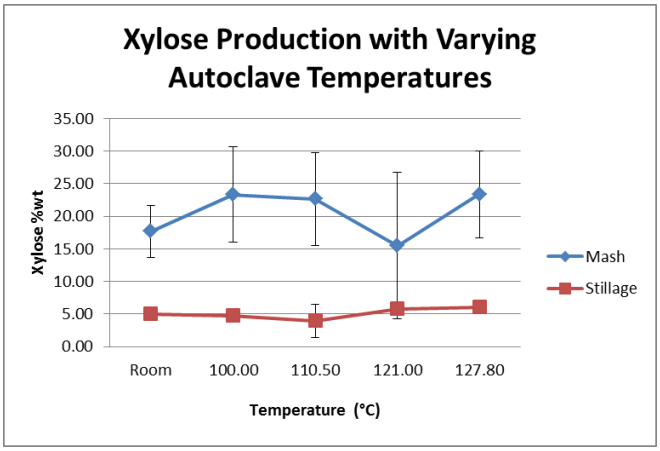

Figure 9. Xylose production using temperature as a variation. Results are shown in %wt.

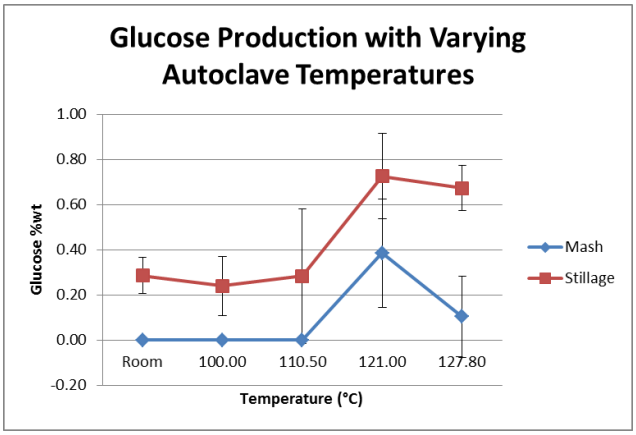

Figure 10. Glucose production using temperature as a variation. Results are shown in %wt. Stillage shows increased production compared to mash.

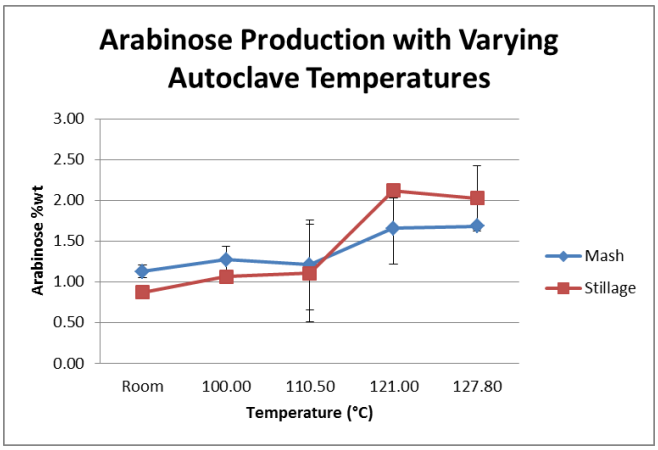

Figure 11. Arabinose production using temperature as a variation. Results are shown in %wt. Stillage shows increased production at higher temperatures.

Autoclave Time Variation

Figures 12 – 15 show the production of sugars from cellulose and hemicellulose as a function of time in autoclave at constant temperature (127.8 °C). One testing condition (120 minutes) was not included due to equipment

restrictions. Each set of graphs include data points that are the average of three mash samples and three stillage samples at each temperature listed (error bars represent one standard deviation).

Figure 12 shows cellobiose production as the time is increased in autoclave. Variation is high for the mash samples, with considerable overlap of the standard deviations of the two distributions. Average cellobiose production is higher for stillage, variation is lower, and production levels increase with time. Cellobiose results (3.3%) remain lower than the available cellulosic content of 9-16%. Figure 13 shows xylose production as the time is increased while in autoclave. As observed in the temperature autoclave study, xylose production is greater for the mash than the stillage samples. No clear trend in xylose production is observed with time, and it remains higher than the expected value (23.4% actual compared to 16% expected).

Figure 14 graphs the production of glucose from mash and stillage samples as time increases in autoclave held at 127.8°C. For both samples no distinguishable difference in glucose production occurs until 45 minutes, after which stillage shows higher recovery. Actual glucose values remain lower than expected values from cellulose, which are being attributed to the less aggressive treatment.

Figure 15 shows the production of arabinose in mash and stillage samples as time increases in autoclave. For both samples, production increases with time greater than 30 minutes. Stillage samples give overall higher yields than mash.

Actual results (2.1%) remain lower than expected values, however mash showed higher than expected results when combined with xylose to represent hemicellulose content (16% expected compared to 24% actual values).

Cellobiose, glucose and arabinose showed increased production in stillage samples in comparison to mash as time increased in autoclave set to 127.8°C. Xylose production is higher for mash samples, but no increase in production as a function of time is observed.

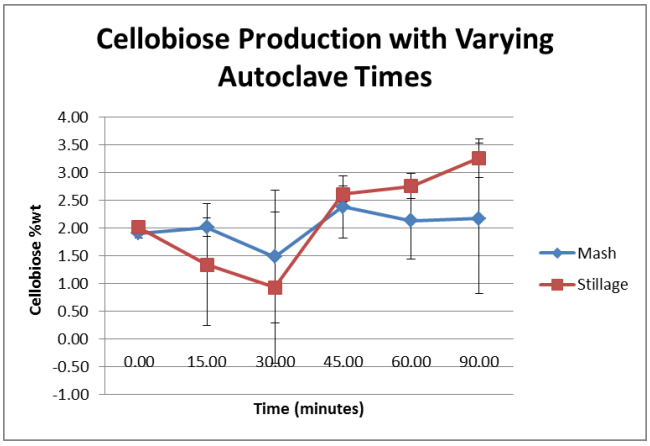

Figure 12. Cellobiose production over changing time. Results are shown in %wt. Stillage shows increased production at higher time requirements.

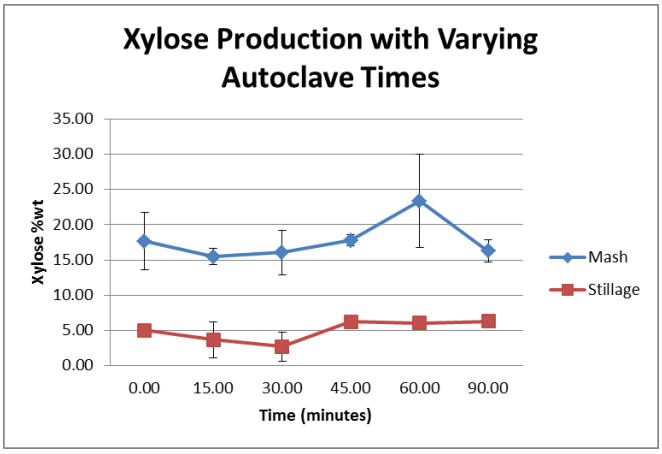

Figure 13. Xylose production over changing time. Results are shown in %wt. Mash shows higher production throughout all time requirements.

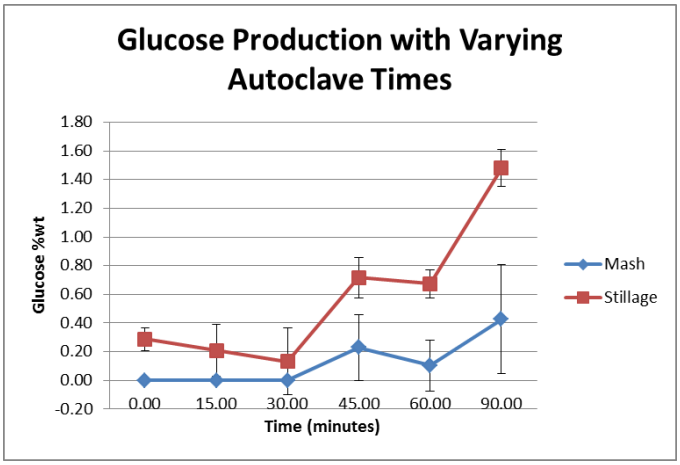

Figure 14. Glucose production over changing time. Results are shown in %wt. Stillage shows increasing production at longer time treatments.

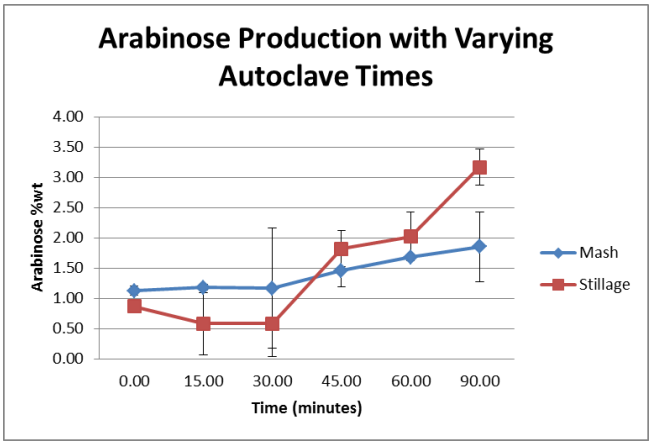

Figure 15. Arabinose production over changing time. Results are shown in %wt. Stillage shows higher production after 45 minutes.

Sulfuric Acid Concentration Variation

Figures 16 through 19 show the recovery of sugars after mash and stillage had been treated with increasing sulfuric acid concentration over 60 minutes.

Each set of graphs include data points and error ranges that are the average of three mash samples and three stillage samples at each concentration listed.

Figure 16 shows cellobiose production as sulfuric acid concentration is increased in both mash and stillage samples. Due to variations in results, no significant increase in either mash or stillage was noticed until 2.50% concentration is reached. The expected cellulosic content (9-16%) is higher than the actual value of cellobiose recovered (2.7%) recovered at 5.00% sulfuric acid. While cellobiose recovered from both mash and stillage increased as sulfuric acid was increased, the results remain close and within error of the other's results.

Figure 17 shows xylose production as sulfuric acid concentration is increased in both mash and stillage samples. Stillage shows a slight trend increasing in xylose production as the acid concentration is increased. Mash xylose production remains higher than stillage but does not exhibit a noticeable trend.

Figure 18 shows glucose production as sulfuric acid concentration is increased in both mash and stillage samples. Stillage shows higher average glucose production at each concentration and significant increases in glucose production at the 5.00% treatment. Mash does not show any noticeable production until the 2.5% treatment and increases to the highest glucose value at the 5.00% treatment. There is significant variation in the mash samples.

Figure 19 shows arabinose production as sulfuric acid concentration is increased in both mash and stillage samples. Both mash and stillage show similar production through all treatments, increasing production with increasing acid concentration.

Cellobiose, glucose, and arabinose were produced at greater percentages as acid concentration increased for both stillage and mash. There was no statistically significant difference observed within the sample sets. Xylose production was higher in mash samples than in stillage, as observed in the autoclave studies.

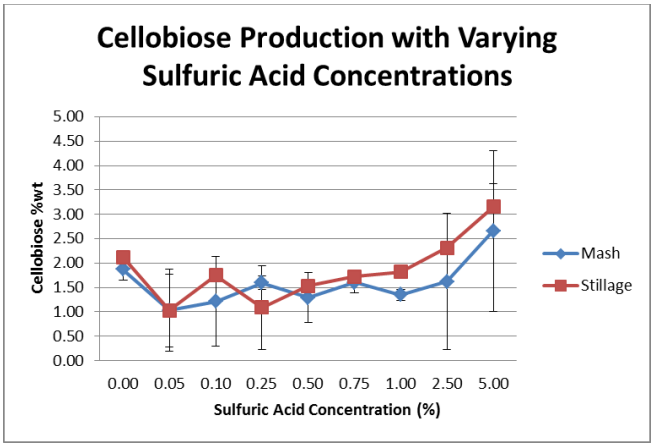

Figure 16. Cellobiose production during increasing sulfuric acid concentration. Results are in %wt. Results remain similar for mash and stillage.

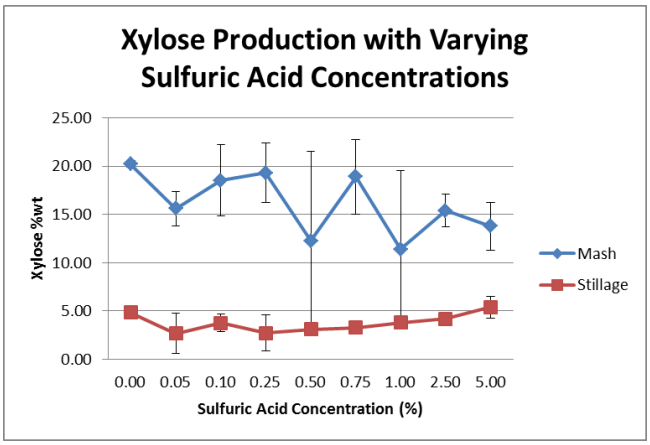

Figure 17. Xylose production during increasing sulfuric acid concentration. Results are in %wt. Mash shows increased average production at each concentration, but higher variation.

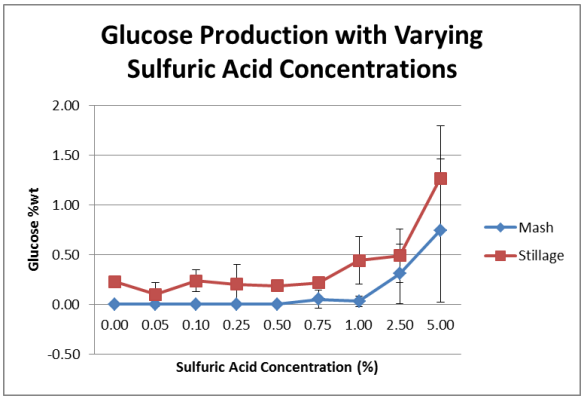

Figure 18. Glucose production during increasing sulfuric acid concentration. Results are in %wt. Stillage showed higher average glucose production at each concentration, but there is significant overlap in standard deviations.

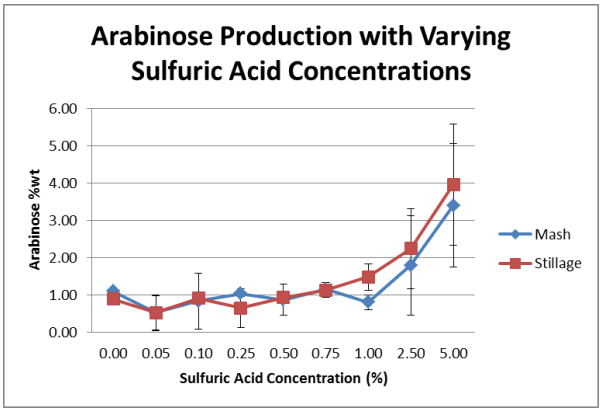

Figure 19. Arabinose production during increasing sulfuric acid concentration. Results are shown in %wt. Stillage and mash both showed similar increased production with increasing acid concentration.

Sulfuric Acid Treatment with Time Variation

Figures 20 through 23 show the production of sugars from stillage and mash after 2.50% sulfuric acid treatment with increasing time. Each set of graphs include data points that are the average of three mash samples and three stillage samples at each concentration listed, with error bars representing one standard deviation.

Figure 20 shows the cellobiose production in mash and stillage as time increases while under acidification. No noticeable difference between mash and stillage is observed as time increases. Figure 21 shows xylose production in mash and stillage as time increases while under acidification. Mash remains at elevated production, while stillage did not show any noticeable trend in production as time increases.

Figure 22 shows increased glucose production in both mash and stillage as time increases while under acidification. Both mash and stillage show an overall trend of increasing glucose production as time is increased and cannot be

determined to be greater than the other due to error values. Figure 23 shows arabinose production in mash and stillage as time increases while undergoing treatment of 2.50% sulfuric acid. While both mash and stillage showed a trend of increasing arabinose production as time increases, one cannot be determined greater than the other with respect to experimental error.

Cellobiose, glucose, and arabinose did not show statistically significant differences in production between mash and stillage as more aggressive acid treatments were completed, however production for both showed a general increase with acid treatment time. Xylose production remains higher in mash than in stillage at all acid treatment times.

See the graphs below for the determination of the various sugars produced.

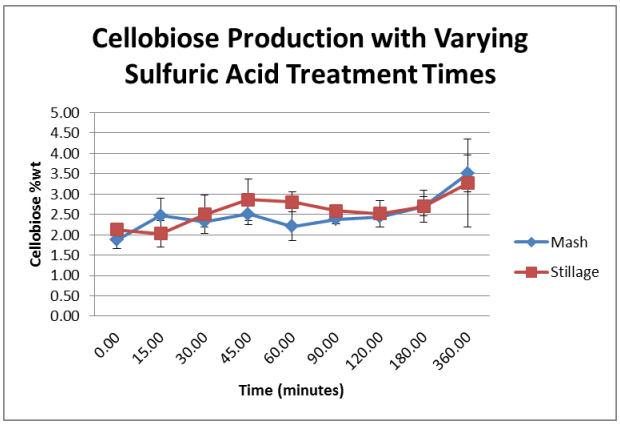

Figure 20. Cellobiose production with increasing time undergoing sulfuric acid treatment. No statistically significant difference between stillage and mash production is observed.

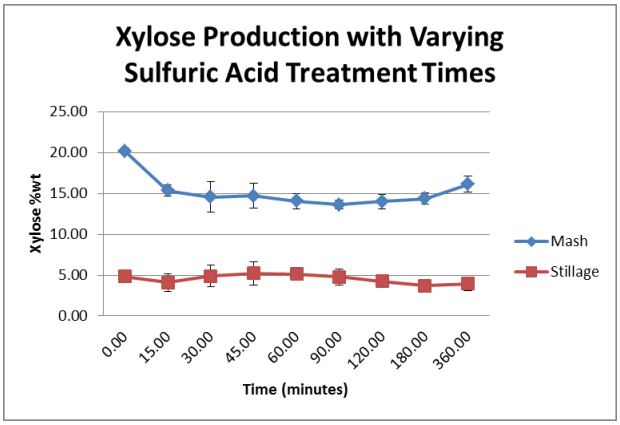

Figure 21. Xylose production with increasing time undergoing sulfuric acid treatment. Mash production is higher than that of stillage. .

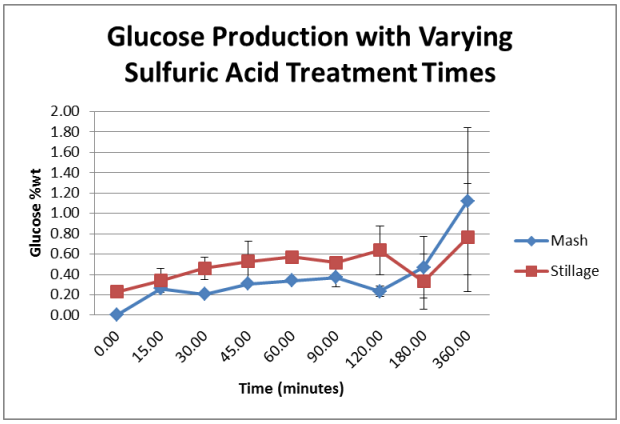

Figure 22. Glucose production with increasing time undergoing sulfuric acid treatment. No statistical difference is observed in the behavior of the two samples. .

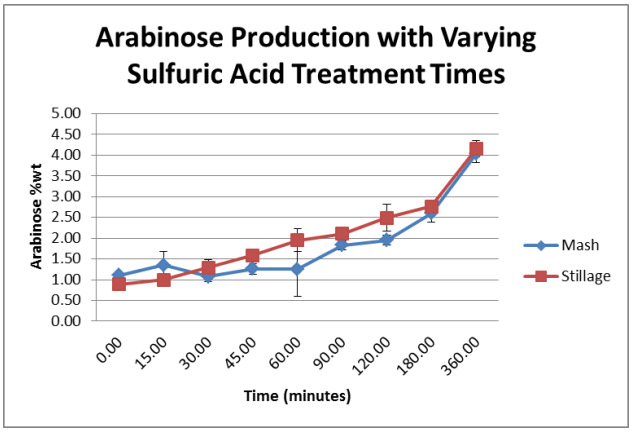

Figure 23. Arabinose production with increasing time undergoing sulfuric acid treatment. Stillage and mash both increase in production but remain similar throughout.

Combined Autoclave and Sulfuric Acid Treatment

Figures 24 through 27 show graphs comparing sugar recoveries from mash and stillage samples after undergoing various combinations of treatments. “Autoclave First 2.5% Acid” and “Autoclave First 0.5% Acid” are treatments with the autoclave treatment performed before the acid treatment. “Acid First 2.5%” and “Acid First 0.5%” are treatments with the acid treatment before the autoclave treatment. The 0.5% acid treatments were included in order to provide a less aggressive acid treatment step than the 2.5% acid treatment step. Each of the sample points listed was run individually 3 different times and the average and error of the three results are presented in Figures 24 through 27.

Figure 24 shows cellobiose production with autoclave treatments followed by acid treatments as well as acid treatments followed by autoclave treatments. In all situations, stillage showed higher average cellobiose production than mash, however in the case of the 2.5% acid treatment prior to autoclave, there was no statistically significant difference between the samples. The highest production was seen for both protocols when 2.5% acid was used. The highest level of cellobiose recovered (3.1%) was lower than the expected amount of cellulose material (9 – 16%).

Figure 25 shows xylose production with autoclave treatments followed by acid treatments as well as acid treatments followed by autoclave treatments. Mash samples exhibited significantly higher xylose production regardless of acid

concentration or order of treatment. The production of xylose from mash (54%) was significantly higher than the expected hemicellulose content (16%), but the production from stillage (9.1%) was closer to that expected.

Figure 26 shows glucose production with autoclave treatments followed by acid treatments as well as acid treatments followed by autoclave treatments. The highest amount of glucose produced was with stillage in which the autoclave was used first followed by 2.5% acidification. The next highest glucose production is 2.5% acidification followed by autoclave treatment. Both 0.5% acid treatments showed decreased glucose production with the autoclave being first having slightly higher glucose production. Mash did not produce any meaningful values of glucose at these setpoints, which is suspected to be due to the decreased treatment time and decreased acid concentration when compared to previously discussed treatment experiments. While acid concentration of 2.5% was shown in a previous section to produce glucose after 4 hours of treatment, in the case of a 1-hour treatment no glucose was obtained from mash. .

Figure 27 shows arabinose production with autoclave treatments followed by acid treatments as well as acid treatments followed by autoclave treatments. Within sample error, no clear differences between mash and stillage samples are observed. However, autoclave first with 2.5% acid showed greatest production for both mash and stillage,

Cellobiose and glucose both show higher production from stillage than mash in the combined treatments, regardless of the order of treatment of the autoclave

and acidification. The available cellulosic material (9-16%) remains higher than the combined results of cellobiose and glucose that was seen. Xylose shows higher production in mash than in stillage in the combined treatments, regardless of the order of treatment of the autoclave and acidification. Arabinose showsno clear difference in production from mass or stillage regardless of treatment. . Hemicellulose content was expected to remain around 16%, but was observed to be higher than that with mash samples and lower than expected in stillage samples. The difference in hemicellulose content is attributed to the reduced exposure of mash to acid treatment in comparison to that of stillage. Stillage has experienced the lowered pH conditions for extended periods of time during the plant process. The dilute acid treatment as well as increased stress from agitation and pumps allow for portions of the hemicellulose to be solubilized and removed from the stillage samples.

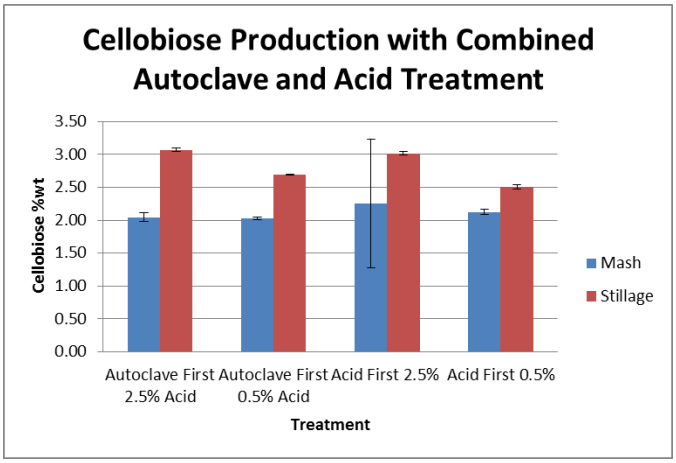

Figure 24. Cellobiose production with first autoclave (simulating steam explosion) treatment at 127.8°C for one hour followed by 2.50% sulfuric acid treatment for one hour. This was repeated with 0.5% sulfuric acid concentration. Both combined treatments were repeated using the acid treatment first followed by the autoclave treatment.

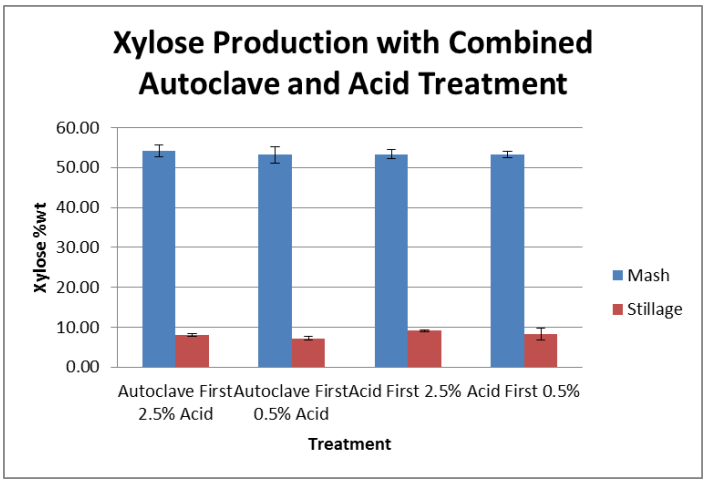

Figure 25. Xylose production with first autoclave (simulating steam explosion) treatment at 127.8°C for one hour followed by 2.50% sulfuric acid treatment for one hour. This was repeated with 0.5% sulfuric acid concentration. Both combined treatments were repeated using the acid treatment first followed by the autoclave treatment.

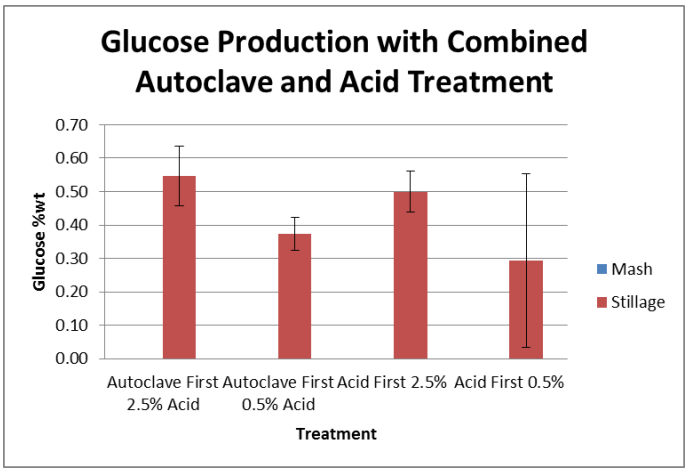

Figure 26. Glucose production with first autoclave (simulating steam explosion) treatment at 127.8°C for one hour followed by 2.50% sulfuric acid treatment for one hour. This was repeated with 0.5% sulfuric acid concentration. Both combined treatments were repeated using the acid treatment first followed by the autoclave treatment.Note that at this time of treatment (1 hour), no glucose production is observed for mash samples.

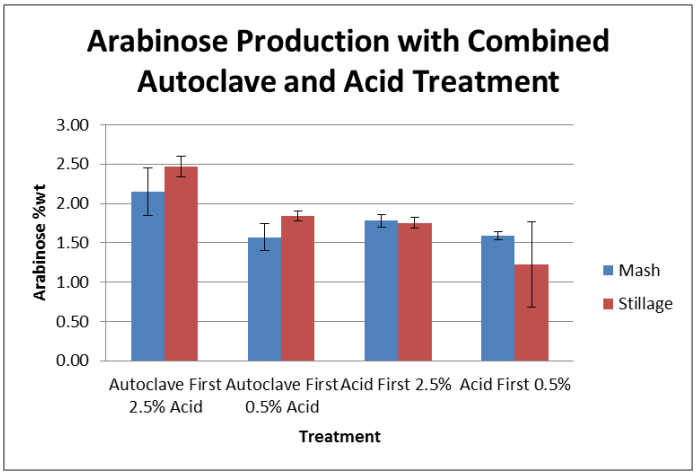

Figure 27. Arabinose production with first autoclave (simulating steam explosion) treatment at 127.8°C for one hour followed by 2.50% sulfuric acid treatment for one hour. This was repeated with 0.5% sulfuric acid concentration. Both combined treatments were repeated using the acid treatment first followed by the autoclave treatment..

Fiber Presence Determination

Analysis of mash and stillage samples using neutral detergent fiber (NDF) (Van Soest method) (*19)* provides the amount of cell wall material, which includes cellulose, hemicellulose, and lignin. The NDF values show a dramatic decrease of cellulosic and hemicellulosic material left at the end of the treatment steps using acidification. Less aggressive treatments such as autoclave treatments showed a decrease in cellulosic material, but not as great a decrease as that observed after acidification. Enzymatic saccharification by itself without any pre-treatment also showed a slight decrease in cellulosic content for both mash and stillage. Cellulosic content in the stock samples was shown to have higher NDF content in stillage samples (64.18%) than in mash (60.92%). This continues to be evident with only enzymatic saccharification showing stillage having 59.13% and mash having 53.94% fiber content. Autoclaved samples

show similar results with decreasing fiber content, where stillage displayed 51.04% and mash had 49.75%. When samples receive acidification treatment, the fiber content remains similar between stillage and mash, with the stillage fiber being 16.87% and mash fiber being 16.55%. Figure 28 shows that more fiber is left in the non-treated samples, with stillage having more available NDF content than mash. Each of the acidified samples, regardless of the order of autoclaving, show a low content of NDF when compared to any non-acidified sample, showing that acidification is an aggressive step necessary for the breakdown of cellulose and hemicellulose content in stillage and mash.

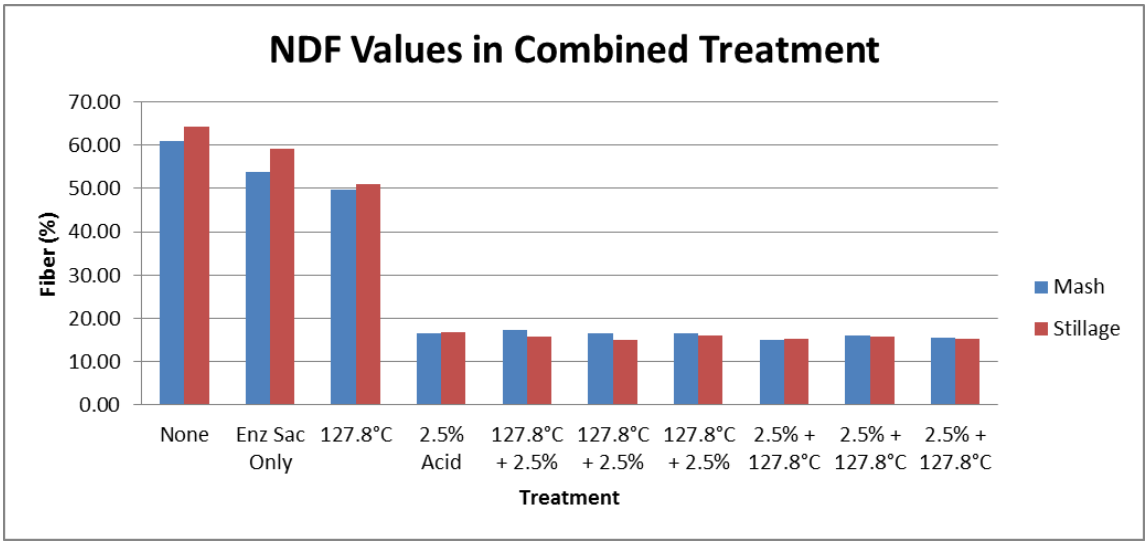

Figure 28. NDF results showing cellulosic material present in each of the samples. Included are non-treated samples, enzymatic saccharification only, autoclave treated only, acid only, then multiples of the combination treatment steps.

Figure 29 shows how much of the available cellulose and hemicellulose content within mash and stillage has been degraded when subjected to various treatments. Mash shows slightly higher consumed cellulosic content than stillage

does with enzyme treatment only. All other treatments show stillage as having greater utilization of cellulosic and hemicellulosic material than mash. Without acidification or autoclave treatment, the better performance of mash can be attributed to multiple factors, including 1) the semi-degraded state that the hemicellulose is in before the treatments occurred and 2) the increased temperature and agitation of the enzymatic saccharification step, which allowed the release of xylose into solution while degrading hemicellulose content. After autoclave treatment, stillage shows a greater level of cellulosic and hemicellulosic material degradation than mash. Once acidified, most of the available cellulosic and hemicellulosic content has been degraded regardless of the source, although stillage has more available material to be degraded than mash.

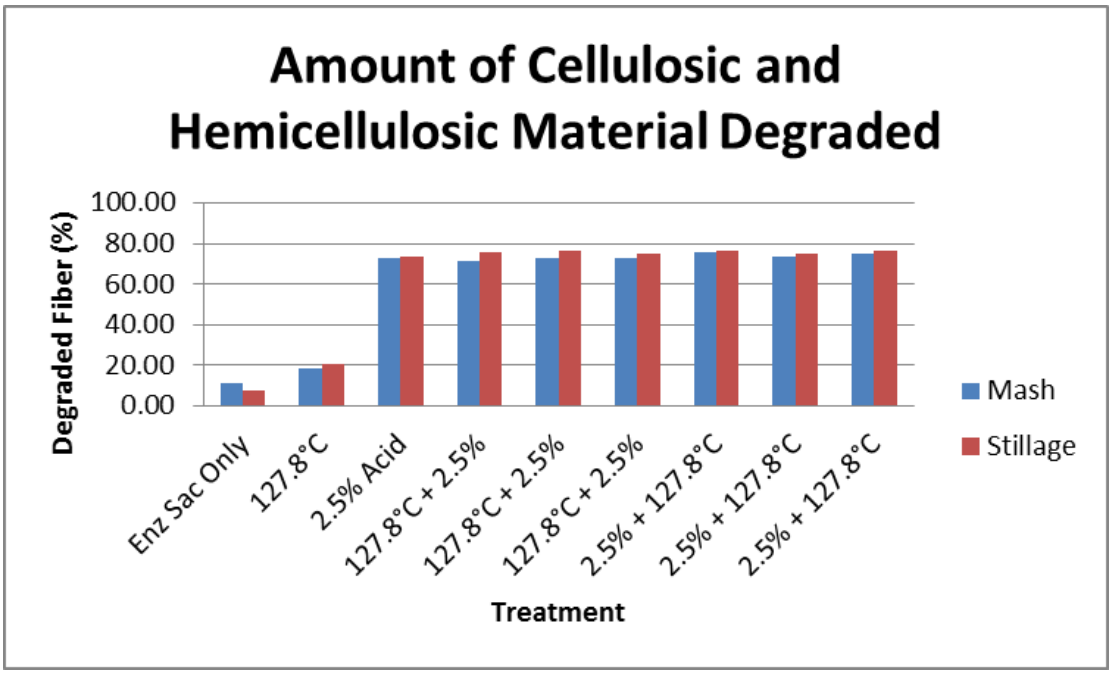

Figure 29. Shows the amount of saccharified cellulosic material in each sample when compared to the cellulosic material originally present in the untreated sample

It is also evident in these results that the stillage samples started with slightly more cellulosic content than the mash samples did, yet showed a greater decrease in cellulosic content in most of the treatment steps (other than enzymatic saccharification treatment alone). With combined pretreatment steps, 75.78% of available stillage fiber (the average of utilized fiber over all combined treatments) was utilized compared to 73.48% mash fiber (the average of utilized fiber over all combined treatments). Of the original samples, there was a greater amount of fiber originally available in stillage (64.18%) than in mash (60.92%). When comparing stillage that is 64.18% available material and 75.78% conversion efficiency against mash that has 60.92% available material with 73.48% conversion efficiency, stillage has the ability to provide 5 – 6 % more cellulosic material to be saccharified into sugars.

CHAPTER IV – Conclusion

The results of this thesis show that both sources of pre-fermentation production material, mash and post-fermentation stillage, are capable of being used as a source of enzymatic saccharification as long as effective pre-treatment steps are utilized. While both are capable sources, post-fermentative stillage provides more fiber (64.18%) and greater efficiency of degradation of cellulosic material (75.8%) than pre-fermentation mash (60.92% available with 73.48% degradation efficiency). However, hemicellulosic material is more readily available in pre-fermentative mash than in stillage because the mash has experienced decreased levels of acid and thermomechanical treatment. Stillage is exposed to stressors such as increased heat, lowered pH values (3.5pH), agitation, shear stress from pumps, and ethanol for extended periods of time. Stillage is exposed over the period of 54 hours to ethanol concentrations that steadily increase to 13.5% and may increase to as much as 14.5% with the current process. This environment provides an additional pre-treatment step that allows better exposure of fiber in the subsequent pre-treatment steps of autoclave and acidification, and results in better enzymatic saccharification of cellulosic fibers. This action on the fibers may also release starch, protein, and other molecules that are bound in the cell wall during fermentation, therefore allowing a slightly elevated fiber content to be gathered in stillage than in mash samples. Fiber generally has a high carbohydrate content (70%), containing

20% residual starch, 15% cellulose and 35% hemicellulose as well as a small lignin content (*9*).

The high xylose content present in mash samples throughout each of the different treatment steps cannot be ignored. If the purpose of this degradation of cellulose and hemicellulose is to provide a glucose stream, stillage would be the best choice. If selection of xylose is preferred, then mash would be the best source for this pentose sugar. The decreased amount of xylose in the stillage can be attributed to the low pH environment previously mentioned that helps pre-treat the stillage. The xylose present in hemicellulose is assumed to enter into solution while undergoing dilute acidification while being exposed to elevated temperatures (85-90°C) during enzymatic saccharification of starch immediately after the corn is ground and mixed into the mash stream. This xylose is assumed to remain in solution and pass through the system without being utilized, and it potentially contributes to increased machinery upkeep costs due to accumulation on equipment in distillation and production of DDGS.

The recommendation for Ergon Biofuels is to utilize the stillage stream as a source of cellulosic enzymatic saccharification as opposed to using the mash stream. The mash stream should, however, be reinvestigated specifically for xylose content, and how this xylose stream can be isolated and utilized.

Future Research Considerations

This project was meant to provide better understanding of the degradative ability of cellulosic and hemicellulosic content of pre-fermentative mash and post-

fermentative stillage specific to this location. However, more research must be done in order to achieve a more thorough understanding of the potential of these two product streams. The experiments listed below may be considered for future research and can provide more insightful information to help drive the cellulosic ethanol industry into a more profitable environment while using their available resources.

1) Evaluate the rate at which xylose is released from pre-fermentative mash into solution while under elevated temperatures and dilute acid treatment. Being able to quantify xylose concentration in mash as well as optimize the available conditions for maximum xylose production can provide another product stream for ethanol plants and also another source of pentose-sugar fermentation if the correct organism is selected.

2) A study to determine the effect of ethanol on cellulose, hemicellulose, and lignin present in post-fermentative stillage. If it is shown that additional solvent steps can provide an increased ability to degrade cellulose and hemicellulose into useable material, optimized systems may allow for even more efficient production of cellulosic ethanol or other cellulose-based products.

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