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ALKALINE HYDROGEN PEROXIDE PRETREATMENT FOR ITS USE IN AN ON-FARM BIOPROCESSING FACILITY

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THESIS

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Biosystems and Agricultural Engineering the College of Engineering at the University of Kentucky

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2013

ABSTRACT OF THESIS

ALKALINE HYDROGEN PEROXIDE PRETREATMENT FOR ITS USE IN AN ON-FARM BIOPROCESSING FACILITY

Pretreatment is an essential step in biofuel production from lignocellulose. Disruption of the lignin structure gives enzymes and fermentation organisms access to long chains of cellulose and hemicellulose. For this project's purposes, the pretreatment must work within the framework of an on-farm butanol bioprocessing facility. Alkaline hydrogen peroxide (AHP) is a delignification method that potentially provides several advantages. At the alkaline pH, powerful hydroxyl radicals are formed; which attack lignin. The objectives of this study were to determine if AHP removes substantial lignin for the feedstocks, corn stover, wheat straw, switchgrass and miscanthus, and to determine if AHP acts as a biocide? Compositional analysis determined if lignin was removed and HPLC data were used to determine whether or not *Clostridium thermocellum* hydrolyzed the pretreated material. Sterility was determined by plating the AHP material. All materials showed approximately 10% lignin removal with AHP. AHP increased structural carbohydrate concentrations for wheat straw, switchgrass and miscanthus. Corn stover showed no benefit from adding peroxide to a traditional alkaline pretreatment. AHP appears to suppress visible microbial growth for the first 24 hours after pretreatment. If AHP does not provide the additional hygienic effects, AHP does not provide a significant advantage over sodium hydroxide pretreatment.

KEYWORDS: alkaline hydrogen peroxide, *C. thermocellum*, pretreatment, fermentation, lignin

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September 10, 2013

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Chapter One
INTRODUCTION
LIGNOCELLULOSIC BIOMASS

The major obstacle in the widespread use of lignocellulosic biofuel is the high cost of fuel production. The major difficulties in efficient lignocellulosic conversion and biofuel production are (a) the lignin structure itself and (b) the recalcitrance of crystalline cellulose (Gould 1984). Pretreatment methods are necessary to remove or alter the lignin in order to increase the accessibility of cellulose and hemicellulose during enzymatic hydrolysis. An ideal pretreatment would optimize the amount of sugars released during hydrolysis and limit sugar losses due to inadvertent conversion to fermentation inhibitors (Modenbach and Nokes 2012); similarly an ideal pretreatment is effective, simple, inexpensive, non-inhibitory and compatible with high biomass loadings (Banerjee, Car et al. 2011). A pretreatment is considered effective when the physical barrier of the plant cell wall is disrupted (the interconnections between the lignin, cellulose and hemicellulose are loosened) and the cellulose crystallinity is reduced; (Wyman, Dale et al. 2005). While many pretreatment technologies are available, this research evaluated only the pretreatments that were judged to be transferable to an on-farm large-scale environment.

Lignocellulosic plant matter is a matrix of hemicellulose wrapped around long chains of cellulose encased by lignin (Figure 1-1). Cellulose, a glucose polysaccharide, forms highly dense and ordered groups with a degree of polymerization (DP) in the 10,000s (before any treatment). The degree of polymerization is the number of monomeric units in a large molecule, or specifically for cellulose the DP is equivalent to the number of glucose molecules. Cellulose forms microfibrils; these long interwoven

chains of cellulose (microfibrils) order themselves into a highly dense structure the cellulose is considered to be crystalline. Crystalline cellulose is generally unreactive and insoluble because the structure is too stable. Hemicellulose is a mixture of hexoses and pentoses with a much lower degree of polymerization (100-200) but can physically prohibit enzymatic access to the cellulose. Lignin is the largest non-carbohydrate portion of plant matter. Lignin cannot be broken down enzymatically to its basic components (monomeric alcohol groups) and hence serves to protect and support the plant (Brown 2003).

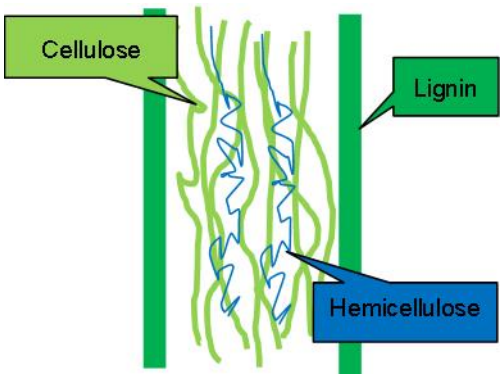

Figure 1-1: General structure of lignocellulosic plant material. Adapted from(Mosier, Wyman et al. 2005)

PRETREATMENT

In order for enzymatic hydrolysis and subsequent fermentation to be successful the lignin structure must be disrupted or removed. Several pretreatment methods have proved adequate including dilute sulfuric acid, steam explosion, lime and pH controlled hot water (Wyman, Dale et al. 2005). The main disadvantages to many of these pretreatments are the high temperatures and pressures required, as well as the potential environmental impacts of harsh chemicals. High temperatures and pressures are costly and difficult to maintain on-farm on a large scale. Researchers have shown that alkaline hydrogen peroxide application at atmospheric pressure and room temperature is an effective pretreatment method (Gould 1985; Saha and Cotta 2006; Banerjee, Car et al. 2011). Banerjee et al. (2011) noted that alkaline hydrogen peroxide is understudied compared to other methods of delignification. Alkaline peroxide pretreatment is more effective at solubilizing lignin and improving digestibility than alkali treatments, another form of mild pretreatment (Karagoz, Rocha et al. 2012). The objective of this research was to evaluate the alkaline hydrogen peroxide pretreatment method for an on-farm biomass processing setting.

ALKALINE HYDROGEN PEROXIDE

Hydrogen peroxide is a clear colorless liquid that is completely miscible with water, highly reactive and an extremely powerful oxidizer. While not a catalyst in the true sense, surrounding reagents give H_2O_2 the ability to produce different hydroxyl radicals, acting similarly to a catalyst. These radicals are what make hydrogen peroxide a versatile reagent. The pulp and paper industry has long used this reactive compound for environmentally-benign bleaching. Numerous industries rely on hydrogen peroxide's chemical purifying ability to separate carboxylic acids from alcohols and oils from

waxes, among others (Jones 1999). The medical community utilizes its sterilizing capabilities for wound treatment and instrument cleaning.

In addition to pretreating lignocellulose for biofuel production, Kerley (1985) showed that agricultural residues can be used as an acceptable energy source for ruminants after the residue has been treated with an alkaline solution of hydrogen peroxide. Other farm-related uses of hydrogen peroxide include a livestock water supply supplement for heard health improvement and sterilization of milking equipment. Potentially harmful organic pollutants found in ground water can be oxidized with the application of H_2O_2 (Jones 1999). Hydrogen peroxide is familiar, safe to handle (at low concentrations) and readily available at large volumes. Because H_2O_2 dissociates into oxygen and water, with no other residues, it poses little to no environmental danger. According to the EPA there are no known adverse effects to humans or the environment from exposure to hydrogen peroxide. However, it is important to note the corrosive and potentially explosive nature of hydrogen peroxide at concentrations greater that 35%, so much so that NASA has considered its use for rocket propellant (Jones 1999).

The same mechanism that increases digestibility of H_2O_2 treated agricultural residues for animal feed translates well into lignocellulosic pretreatments for biofuel production. Early work has shown that dilute alkaline solutions of hydrogen peroxide will remove approximately half of the lignin in materials like wheat straw (Gould 1984). When hydrogen peroxide is raised to an alkaline pH (11.5-11.6) it dissociates into hydrogen and the hydroperoxyl anion (HOO-), as seen in Equation 1. The anion then reacts with remaining peroxide to form highly-reactive hydroxyl radicals which attack the lignin structure (Equation 2).

$$H_2O_2 \rightleftharpoons H^+ + HOO^- \quad \text{Equation 1}$$

$$H_2O_2 + HOO^- \rightarrow HO\cdot + O_2{}^-\cdot + H_2O \quad \text{Equation 2}$$

The reaction between lignin and the hydroxyl radical yields low molecular weight water-soluble oxidation products. During hydrogen peroxide application, plant material has been shown to disintegrate into small, highly dispersed fibers (Gould 1985). The cell wall loses most of its rigid structure and becomes less uniform in texture (Martel and Gould 1990; Selig, Vinzant et al. 2009), providing access points for enzymatic hydrolysis. Alkaline peroxide pretreatment expands the lignocellulosic matrix, providing more access points for enzymes between the lignin, cellulose and hemicellulose.

A large energy consuming step in an on-farm processing facility is reactor sterilization before fermentation. Maintaining the reactor vessel at 121°C and 15 psi for five minutes causes a one log reduction in bacterial population (Shuler and Kargı 1992). This sterilization process would require scaling to the on-farm system so the inoculated organism has the competitive advantage during fermentation. It is our hypothesis that the alkaline peroxide pretreatment may eliminate the need for heat sterilization because hydrogen peroxide acts as a sterilizer. A previous study found no bacterial or fungal growth at any point during pretreatment or hydrolysis after alkaline hydrogen peroxide was applied (Banerjee, Car et al. 2012).

Cellulose crystallinity is commonly used as a metric for measuring pretreatment effectiveness. The effects alkaline peroxide pretreatment on cellulose crystallinity are still not fully understood. Gould's (1984) work determined that crystallinity decreased after AHP pretreatment because the substrate demonstrated an increase in water absorbency

after hydrogen peroxide was applied. Other work confirmed crystallinity was lowered with alkaline hydrogen peroxide because the material swelled as the pretreatment progressed, deconstructing the crystalline regions (Shen, Tao et al. 2011). Yet another study concluded that highly crystalline cellulose is not affected by alkaline hydrogen peroxide and may even increase overall crystallinity (Martel and Gould 1990). These two studies differed in feedstock, hydrogen peroxide concentration and method of crystallinity measurement. The method of hydrolysis evaluated in Chapter Three utilizes the extracellular cellulosome of *Clostridium thermocellum*. Lynd et al. (1987) noted that *C. thermocellum* will saccharify crystalline cellulose at the same rate as commercial enzymes. Therefore the effects on crystallinity may be unnecessary in this context.

OBJECTIVES

The overall goal of this research is to evaluate the alkaline hydrogen peroxide pretreatment for its use in an on-farm bioprocessing facility. The specific objectives of this research were to:

1) Evaluate the effectiveness of alkaline hydrogen peroxide pretreatment in terms of percent lignin removal and residual structural carbohydrates for four feedstocks (corn stover, wheat straw, switchgrass and miscanthus) compared to an alkali treatment.

2) Monitor the microbial activity of alkaline hydrogen peroxide pretreated biomass using fermentablity by *Clostridium thermocellum* and the length of time without microbial growth as metrics.

PRETREATMENT AND FERMENTATION FRAMEWORK

The alkaline hydrogen peroxide pretreatment is being evaluated under the framework of a Biomass Research and Development Initiative (BRDI) project for an On-Farm Bioprocessing facility. The project goals are to limit transportation between harvesting and processing while creating an energy-dense value-added outgoing product stream (Figure 1-2). Four substrates (wheat straw, corn stover, switchgrass and miscanthus) will be harvested, baled and placed in concrete bunker-silos for pretreatment, hydrolysis and fermentation. A co-culture of *Clostridium thermocellum* and *Clostridium beijernickii* will be used for hydrolysis and butanol production. *C. thermocellum* produces the cellulase enzymes responsible for substrate saccharification. Several cycles inoculations will be introduced to ensure complete sugar conversion from the bales. Each bunker is estimated to hold 100 tons biomass and go through six biomass-to-fuel rotations per year.

Figure 1-2: Conceptual modeling of on-farm bioprocessing facility (drawn by Will Adams).

ALKALINE HYDROGEN PEROXIDE PRETREATMENT PARAMETERS

The effectiveness of alkaline hydrogen peroxide (AHP) pretreatment is affected by a number of environmental conditions; including the temperature, pH, reaction time, hydrogen peroxide loading, solids concentration and particle size of the feedstock. Previous studies have investigated the effects of these variables for one feedstock at a time, usually wheat straw or corn stover. Our study included dedicated energy crops like switchgrass and miscanthus for a more complete evaluation of the AHP pretreatment.

Unlike the high temperatures required for steam explosion and acid pretreatments (Modenbach and Nokes 2012) AHP has been successful at normal room temperatures (Gould 1985; Yang, Boussaid et al. 2002; Rabelo, Maciel Filho et al. 2008; Banerjee, Car et al. 2012). Mild pretreatment conditions provide an important advantage for the large-scale on-farm production of biofuels. If little energy is needed to control the temperature during pretreatment, the overall energy balance will be more favorable.

The literature presents mixed results for the effect of temperature on AHP. Sun (2000) and Selig (2009) show increasing sugar yields with increasing temperatures on maize stems and corn stover respectably, while Karagöz (2012) attained higher yields with rapeseed straw at 50°C than 70°C due to H_2O_2 decomposition at higher temperatures. It is unknown whether decreasing the temperature below typical ambient conditions has any effect on the pretreatment effectiveness.

As noted previously, hydrogen peroxide requires an alkaline pH to produce the oxidizing radicals necessary to degrade lignin. NaOH has traditionally been used raise the pH up to 11.5. Gould achieved approximately 50% delignification with an initial pH measurement of 11.5 (1985). The question then becomes whether or not it is necessary to

maintain the initial pH for the duration of the reaction. In Banerjee's (2012) kilogram-scale experiment the pH was adjusted at different times (0, 3, 6, 12, 18, 24 and 36 hours) to compare the effect of pH adjustment throughout the experiment on the efficacy of the pretreatment; a slight increase in glucose yield was found. The pH increases for the duration of alkaline hydrogen peroxide pretreatment; Sun et al (2001) found that the increase of pH increases the hemicelluloses solubilized and regular pH adjustment is unnecessary.

Before enzymatic hydrolysis the pH must be neutralized with concentrated HCl. The hydrogen chloride combines with the sodium hydroxide to form sodium chloride and water, resulting in a relatively high final salt concentration. It is important to limit the amount of salts produced as they are known inhibitors of microorganisms. Sodium chloride changes the osmotic pressure exerted on cells, making cell growth during fermentation impossible (Qureshi, Saha et al. 2008). Therefore, keeping the pH constant throughout AHP pretreatment is harmful to the desired end product and would be difficult to maintain at a farm scale.

Further research showed that a constant pH of 11.5 was not necessary. As the reaction becomes more alkaline an increasing amount of lignin and hemicellulose are solubilized (Fang, Sun et al. 1999; Sun, Tomkinson et al. 2000). With less sodium hydroxide present, there will be less salt formation, resulting in less inhibition during fermentation.

Pretreatment time has a major influence on the amount of lignin removed. On the farm, time will not likely be the limiting factor. Irrespective of substrate or hydrogen

peroxide loading the amount of lignin removed or altered increases with reaction time. Previous work evaluated reaction times as short as three hours to an extended 48 hours; sugar yields tend to increase with the increasing reaction time (Saha and Cotta 2006; Banerjee, Car et al. 2011). Most of the morphological and quantifiable changes occur within the first six hours of pretreatment, yet enzyme digestibility increases with increasing pretreatment time suggesting that there are in fact effects of the treatment that are not measurable by our current technologies (Banerjee, Car et al. 2012). It is likely that after lignin is removed, the remaining portions of cellulose, hemicellulose and lignin get rearranged during the remaining treatment time to provide access points for saccharification.

The two most influential components of the AHP pretreatment are the concentration of solids and the hydrogen peroxide loading. The feedstock solids concentration has major implications in terms of reactor mechanics and vessel design. With higher solids concentrations, the amount of free water in which the reaction will take place is diminished, decreasing the overall reaction efficiency (Modenbach and Nokes 2012). It is desired to use the least amount of hydrogen peroxide with the highest concentration of solids that still provides an effective pretreatment. Table 1-1 summarizes previous studies and their resulting glucose yields (when available).

Gould's (1984) original research demonstrated that hydrogen peroxide could be used as a delignifying agent for corn stover or wheat straw. Approximately 50% of the lignin in a 2% (w/v) solution of corn stover or wheat straw can be removed with a 1% solution of hydrogen peroxide (0.5 g H_2O_2/g biomass or 3.68 g H_2O_2/g lignin) at an initial pH of 11.5. Saha et al. (2006) optimized the AHP method for 8.6% (w/v) wheat straw for

enzymatic saccharification. A series of concentrations from 0-4.3% H_2O_2 were evaluated on the basis of sugar yields after enzymatic hydrolysis, while all other variables were held constant. The total reducing sugars (glucose, xylose and arabinose) doubled (250 to 550 mg/ g wheat straw) with the addition of 2.15% H_2O_2. This suggests the more hydrogen peroxide available; the more radicals are produced resulting in more delignification up to a point. Once a threshold concentration is reached access to the lignin in the plant becomes rate limiting. Saha et al. (2006) found no significant difference between 2.15% and 4.3% so the lower concentration was chosen . The 2.15% is equivalent to 0.3625 gram H_2O_2 per gram wheat straw (4.44 g H_2O_2/g lignin), slightly lower than Gould's loading, and Saha et al. achieved similar sugar yields. Another study further decreased the loading to 0.25 g H_2O_2 / g substrate (79.2 ± 0.2% glucose conversion with optimal enzyme combination, 1.56 g H_2O_2/g lignin using literature based lignin content) which gave similar sugar conversion results as the 0.5 g/g loading (92.1 ± 0.4% glucose conversion, 3.13 g H_2O_2/g lignin using literature based lignin content)(Banerjee, Car et al. 2012); decreasing to 0.125 g/g corn stover (0.78 g H_2O_2/g lignin using literature based lignin content) lowered the glucose yield to 48.9 ± 0.5% . While it is desired to use the least amount of hydrogen peroxide possible, thereby also using less salt and water; life cycle analysis will help determine if it is better to have less conversion with a lower concentration of hydrogen peroxide or higher conversion with more chemical application.

The bunker-silo proposed for this project will be a high-solids environment and its physical size will restrict the amount of free liquid available in the system. Previous studies have used a wide range of solid concentrations, the highest published are from

Gould's (1989) work in a modified extruder at approximately 40% solids. The reactor design allowed "all of the liquid phase to remain absorbed by the straw product." If the per gram amount of H_2O_2 is held constant, there is an increase of AHP effectiveness with higher solids concentration, the reduced water content gave higher molar concentrations of hydrogen peroxide and sodium hydroxide (Banerjee, Car et al. 2011). **Table 1-1** shows glucose conversions of about 75% are possible with a low H_2O_2 applications and high - solids concentrations. Because this pretreatment has not been explored as definitively as others, there is a lot of room for improvement and optimization in terms of high-solids loadings.

Comminution (particle size reduction) is a major energy consuming step in biofuel production. The energy required is a function of regression coefficients based on feedstock, moisture content and milling size (Equation 3) (Miao, Grift et al. 2011).

$$E_T = a\, x^b \qquad \text{Equation 3}$$

E_T Specific Commination Energy Consumption $\left(\frac{kJ}{kg\ DM}\right)$

a, b Regression Coefficients $\left(\frac{kJ}{kg\ DM \cdot mm}\right)$

x Aperture Size (mm)

The BRDI project is developing a single pass harvesting method which bales immediately after cutting; the lack of time between cutting and baling leads to a high moisture content, further increasing the energy required for size reduction. If AHP can work on larger particle sizes the net energy balance will be more favorable. Pretreatment aims to increase the internal surface area available chemically or physically. Gould (1985) has shown that field-chopped straw will respond the same as ground straw to an

alkaline peroxide pretreatment. The fibers lose their structural integrity and become flaccid. The lignocellulosic matrix swells as lignin and hemicelluloses are removed (Thompson, Chen et al. 1992) leaving the material susceptible to enzymatic digestion (Kerley, Fahey et al. 1985). Agitation by stirring will quickly disintegrate the long fibers (Gould 1985) leaving a mixture similar to those that have been ground.

FEEDSTOCKS

The reaction mechanics of AHP pretreatment are determined by the surface Figure 1-3 and Figure 1-4): corn stover, wheat straw, switchgrass and miscanthus, have varying degrees of lignin, cellulose and hemicellulose (Table 1-2) they will likely interact differently with the hydrogen peroxide. It is important to evaluate the conditions of AHP for each of the four substrates to ensure maximum yields (Modenbach and Nokes 2012). Most studies have focused on corn stover and wheat straw. By expanding the investigation to include dedicated energy crops, the alkaline peroxide pretreatment will be further validated.

Corn stover is the residual plant after the corn cob has been removed, mainly husks and stalks (Brown 2003). Wheat straw is a by-product of wheat production, typically 1.3-1.4 pounds of straw are produced per pound of grain (Saha and Cotta 2006). Both corn stover and wheat straw are considered agricultural residues and their use would increase the worth of a traditionally low value product agricultural residue. Miscanthus is a perennial grass considered to be a dedicated energy crop that can be grown in almost any soil and requires minimal nutrients and water (Bin Guo 2009). Switchgrass is a perennial warm season grass that has the potential to produce 540% more energy than it

takes to grow and process into ethanol (Gupta and Lee 2010). This energy gain is due to the higher population densities and potential strides in conversion technologies.

Time of harvest also has significant effects on the composition of each feedstock (Huang, Faulkner et al. 2012). Corn stover and wheat straw are harvested relatively close to the date of grain harvest, corn and wheat. For corn stover harvest typically falls between August and October and occurs sometime in June for wheat straw. Switchgrass and miscanthus are not associated with a traditional harvest date and allowed to reach full maturity before cutting and baling. For the materials used in this study, switchgrass and miscanthus were harvested in March, right before the start of a new growing season. The length of time in the field directly affects crop maturity, and crop maturity affects the compositional analysis (Chen, Mowery et al. 2007). Beale et al. (1997) showed nutrients and minerals in shoots and leaves of perennials decrease after the summer growing season. The minerals are transported to the rhizomes to prepare for new growth. Without these nutrients a higher percentage of the plant is hemicellulose and cellulose, and ultimately C5 and C6 sugars. The later harvest date is likely the reason switchgrass and miscanthus gave higher percent sugar compositions than corn stover and wheat straw in the following chapters.

Figure 1-3: Feedstocks before and during harvest: (a) Corn Stover (b) Wheat Straw (c) Switchgrass (d) Miscanthus. *Photos by Mike Montross.*

Figure 1-4: The four feedstocks being evaluated, from left to right: corn stover, wheat straw, switchgrass and miscanthus. All materials were air dried to approximately 5% moisture and ground to pass a 5 mm screen.

Table 1-1: Summary of Previous AHP Studies

Author	Year	Substrate	H_2O_2 Loading (g /g *x**)	Solids Concentration (%, w/w)	Time (h)	Temperature (°C)	Stirring (rpm)	Glucose Conversion (%)	Initial pH	pH Adjustment	Particle Size
Gould	1984	Crop Residues†	0.500	2.00%	4	Room	Gently	95	11.5	initial	2
Banerjee	2011	Corn Stover	0.125	14.93%	8	23	90	76.8	11.5	6 hours	0.5
Saha	2006	Wheat Straw	0.363	8.60%	4	35	250	96.7	11.5	initial	1.27
Rabelo	2008	Sugarcane Bagasse	1.25	4.00%	4	20	150	69.4	11.5	initial	Milled
Banerjee	2012	Corn Stover	0.125	15.00%	8	24	150, every 6 h	75	11.5	throughout	5
Sun	2000	Maize Stems	0.800	2.50%	2	45	Gently	--	12.6	not needed	1
Selig	2009	Corn Stover	0.250	4.00%		65	Constant Orbital	--	11.5	initial	6.35

* where *x* denotes the substrate
† Wheat Straw, Corn Stalks, Corn Husks and Kenaf

Table 1-2: Organic Components of Lignocellulosic Crops by Weight Percentage on Dry Basis

FEEDSTOCK	CELLULOSE	HEMICELLULOSE	LIGNIN	OTHER*
Corn Stover	53	15	16	16
Wheat Straw	38	36	16	10
Switchgrass	45	30	15	10
Miscanthus	50	25	12	13

* Includes proteins, oils and mineral matter
Adapted from Table 3.1 Biorenewable Resources (Brown 2003)

CLOSTRIDIUM THERMOCELLUM

Clostridium thermocellum is a thermophilic anaerobe with limited ATP production (Demain, Newcomb et al. 2005). It relies on an assortment of cellulases, including several types of endo- and exoglucanases, for conversion of the intricate cellulose matrix into smaller polysaccharides (Lamed, Kenig et al. 1985). These polysaccharides can then be easily transported into the cell for use as a carbon source (Bhat and Wood 1992). The cellulases are part of a high ordered cellulosome; the cellulosome is a very stable macromolecular machine capable of hydrolyzing cellulose in an extremely efficient matter, eliminating the need for enzymatic or acidic hydrolysis (Schwarz 2001). The cellulosome "digests" cellulose chains into glucose or cellobiose intermediates that can easily pass through the bacterial cell walls for metabolism (Figure 1-5). *C. thermocellum* has a fermentation pathway that produces mainly ethanol, lactate, acetate, carbon dioxide and hydrogen (Demain, Newcomb et al. 2005). The cellulosome works independently from the bacteria's metabolism; the intermediates are available for uptake to any bacterial species present. The goal of using this organism is for cellulosome generation rather than the fermentation end-products.

Most of the previous research revolving around *C. thermocellum* has focused on the model celluloses, avicel and filter paper (Shao, Jin et al. 2011). Great strides have been made in ethanol tolerance and determining optimal conditions for cellulosome activity (65°C and pH 7.4) with avicel (Xu, Qin et al. 2010). However avicel and filter paper share very few similarities with lignocellulosic feedstocks other than the presence of cellulose. There is a lack of information about *C. thermocellum*'s behavior on actual lignocellulose. Lynd's (1987) study determined untreated substrates are inaccessible to *C.*

thermocellum's cellulosome. Some method of pretreatment is necessary (Hormeyer, Tailliez et al. 1988). Pretreatment releases a variety of potentially inhibitory compounds for hydrolysis and fermentation (Xu, Qin et al. 2010). At the time of this research there is no published literature examining *C. thermocellum*'s ability to saccharify and ferment alkaline hydrogen peroxide pretreated lignocellulose.

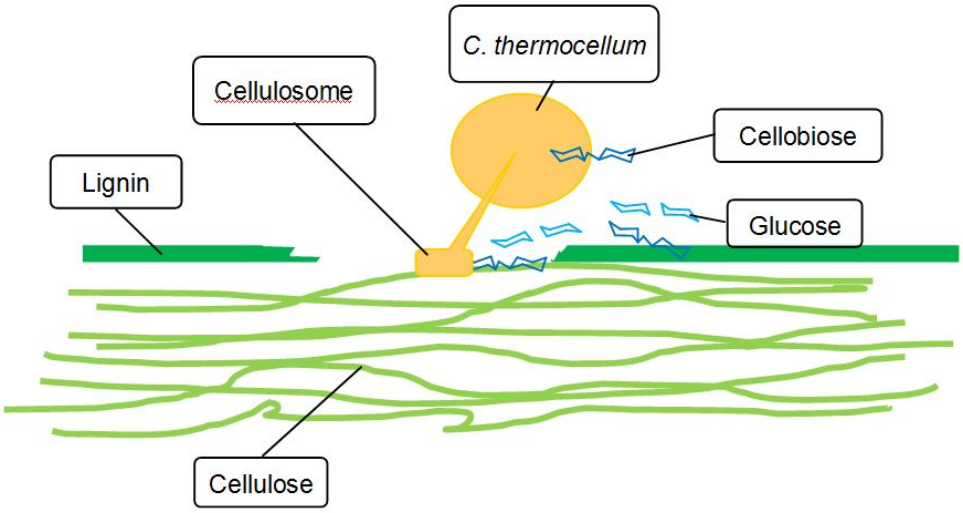

Figure 1-5: Illustration of Clostridium thermocellum's cellulosome acting on a lignocellulosic substrate. Glucose and Cellobiose intermediates are available for transport into the cell for fermentation. Figure adapted from (Gilbert 2007) and (Schwarz 2001). Note: *For illustration purposes only, not drawn to scale.*

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Chapter Two

EVALUATION OF THE ALKALINE HYDROGEN PEROXIDE PRETREATMENT

SUMMARY

Four feedstocks (corn stover, wheat straw, switchgrass and miscanthus) were subjected to alkaline hydrogen peroxide pretreatment to determine the effects pretreatment has on lignin content and structural carbohydrates. All four materials showed significant lignin removal (each percentage by weight decreased approximately 10%) with alkaline hydrogen peroxide pretreatment. Wheat straw, switchgrass and miscanthus treated with peroxide had higher glucose yields after acid hydrolysis than those treated with sodium hydroxide or water. Glucose concentrations of alkaline hydrogen peroxide treated corn stover were statistically equal to those treated with NaOH. Alkaline hydrogen peroxide removes more lignin and increases structural carbohydrate concentrations for wheat straw, switchgrass and miscanthus. Corn stover shows no benefit from adding peroxide to a traditional alkaline pretreatment.

INTRODUCTION

LIGNOCELLULOSIC BIOFUELS

Large scale production of lignocellulosic biofuels requires a major investment in pretreatment. Lignin structure and cellulose crystallinity must be disrupted to create access for sugar conversion and subsequent fuel production (Gould 1984; Banerjee, Car et al. 2012; Karagoz, Rocha et al. 2012). Pretreatment methods are necessary to remove or alter lignin in order to render cellulose and hemicellulose accessible to hydrolysis. All other downstream processing steps (hydrolysis, fermentation, separation, power requirements, etc.) are impacted by the pretreatment (Galbe and Zacchi 2007). Pretreatments should be evaluated on their ability to alter the physical structure of the

substrate to improve saccharification rates and extents and the cost effectiveness of the pretreatment (Thompson, Chen et al. 1992).

Lignin, cellulose and hemicellulose provide structure to lignocellulosic plant matter. Lignin is a heterogeneous aromatic polymer made of stable carbon-carbon and ether linkages (Higuchi 1982) that protect and support the plant (Brown 2003). By removing lignin, molecular-level access can be gained to the cellulose and hemicellulose. Valuable glucose monomers form the long microfibrils that make up cellulose (Brown 2003). Hemicellulose, made of pentoses and hexoses, provides rigidity to the plant through interconnections between cellulose and lignin fibers (Hendriks and Zeeman 2009).

Several methods of lignin removal have been evaluated; most of which require elevated temperatures and pressures. Alkaline conditions have proven successful at removing lignin, while most of the cellulose and hemicellose remain as a solid (Jorgensen, Kristensen et al. 2007). Studies have shown that at room temperature and atmospheric pressure alkaline hydrogen peroxide is an effective pretreatment (Gould 1984; Gould 1985; Saha and Cotta 2006; Banerjee, Car et al. 2011). With this research the pretreatment capabilities of alkaline hydrogen peroxide were evaluated for two categories of lignocellulosic biomass (agricultural residues and dedicated energy crops) in terms of their ability to remove the lignin.

ALKALINE HYDROGEN PEROXIDE PRETREATMENT

Gould's 1984 work illustrated that dilute alkaline solutions of hydrogen peroxide could remove approximately 50% of the lignin in agricultural residues. When the pH reaches 11.5, H_2O_2 dissociates into superoxide and hydroxyl radicals (Selig, Vinzant et

al. 2009). Hydroxyl radicals then attack the lignin structures, creating access points for hydrolysis by whole cells, enzymes or other chemicals. Alkaline hydrogen peroxide (AHP) opens the lignocellulosic structure (Martel and Gould 1990) and collapses the rigid biomass into a group of small highly dispersed fibers (Gould 1985; Selig, Vinzant et al. 2009). Gupta (2010) showed that adding 5% H_2O_2 to a 5% NaOH pretreatment solution kept more of the structural carbohydrates in the solid phase while increasing the material's susceptibility to enzymatic hydrolysis.

AHP has other benefits that would translate well into large scale on-farm processing. H_2O_2 is familiar to producers, and agricultural residues treated with peroxide can be used as animal feed (Kerley, Fahey et al. 1985). At low concentrations peroxide can be added to water supplies to deter microbial growth and improve heard health. The EPA has reported that hydrogen peroxide has no environmental dangers as it dissociates into hydrogen and water. The medical community has long harnessed hydrogen peroxide's sterilizing capabilities (Jones 1999). Banerjee's (2012) experiments observed that these properties translate to biomass, possibly eliminating sterilization prior to hydrolysis and fermentation.

This work evaluates the alkaline hydrogen peroxide pretreatment on four feedstocks at a high-solids concentration. The amount of lignin remaining and sugars available after acid hydrolysis serve as metrics to determine the success of AHP as a pretreatment. Comparing the results of the alkaline hydrogen peroxide pretreatment to the well documented sodium hydroxide pretreatment will determine if the addition of hydrogen peroxide benefits the overall on-farm paradigm.

SODIUM HYDROXIDE PRETREATMENT

Sodium hydroxide is one of the oldest and most thoroughly studied lignocellulosic biomass pretreatments (Mosier, Wyman et al. 2005). NaOH is a widely available commodity, relatively inexpensive and familiar for agricultural applications. As a pretreatment, NaOH utilizes a peeling mechanism that works along the reducing ends of carbohydrates (Gupta and Lee 2010). The biomass swells, which increases the internal surface area and decreases the cellulose crystallinity and degree of polymerization (Galbe and Zacchi 2007). This increase in surface area allows for enzymatic and bacterial access to the cellulose for hydrolysis and fermentation (Hendriks and Zeeman 2009). The peeling mechanism that removes lignin is in competition with the conversion of reducing ends into stable alkali carboxyl groups (Gupta and Lee 2010). NaOH dissolves into hydroxyl ions that attack ether linkages. The ether linkages comprise 50-70% of the lignin structure (Gupta and Lee 2010). These ions sever bonds between lignin and carbohydrates while solubilizing the hemicellulose (Galbe and Zacchi 2007; Hendriks and Zeeman 2009). The cellulose and majority of the hemicellulose remain in the solid phase (Jorgensen, Kristensen et al. 2007). Sodium hydroxide is effective at atmospheric pressure and room temperatures; producing measurable results in either hours or days depending on the conditions (Mosier, Wyman et al. 2005). Major disadvantages to the sodium hydroxide pretreatment are the degradation of carbohydrates (these losses decrease sugar yields) and the potential formation of fermentation inhibitors (Hendriks and Zeeman 2009; Gupta and Lee 2010).

FEEDSTOCKS

Pretreatment reaction mechanics are incredibly reliant on the biomass surface. Each feedstock is composed of varying degrees of lignin and structural carbohydrates. The four feedstocks studied, corn stover, wheat straw, switchgrass and miscanthus, are representative of two main types of lignocellulose, agricultural residues and dedicated energy crops. Table 2-1 summarizes compositions for each biomass reported in the literature.

Table 2-1: Compositional analysis of corn stover, wheat straw, switchgrass and miscanthus.(Lee, University et al. 2007; Brosse, Dufour et al. 2012)

	Corn Stover	**Wheat Straw**	**Switchgrass**	**Miscanthus**
	% by Weight			
Cellulose	**37.5%**	**37.6%**	**37.3%**	**38.2%**
Glucose	37.50	32.1%	34.2%	39.5%
Hemicellulose	**26.1%**	**28.8%**	**28.5%**	**24.3%**
Xylose	21.7%	19.5%	22.8%	19.0%
Arabinose	2.7%	2.8%	3.1%	1.8%
Galactose	1.6%	1.1%	1.4%	0.4%
Mannose	0.6%	0.6%	0.3%	-
Total Lignin	**18.9%**	**14.50%**	**19.1%**	**25.0%**
Acid Soluble	2.9%	2.5%	3.5%	0.9%
Acid Insoluble	16.4%	10.9%	16.2%	24.1%
Crude Protein	**4.7%**	**3.8%**	**3.1%**	**-**
Ash	**6.3%**	**6.4%**	**5.9%**	**2.0%**
Soil	**1.3%**	**0.0%**	**0.0%**	**-**

MATERIALS AND METHODS

BIOMASS

Corn stover, wheat straw, switchgrass and miscanthus were harvested locally and dried before being ground to pass a 5 mm screen with a Retsch Muhle SM1 mill (Nr. 70947). Moisture content was determined to be < 10% for all feedstocks.

ALKALINE HYDROGEN PEROXIDE AND SODIUM HYDROXIDE

Ten grams of each biomass were soaked in 50 mL of pretreatment liquid (20% solids) for 24 hours at 25°C and shaken at 90 rpm (Innova 4200 Incubator Shaker, New Brunswick Scientific). The reactions took place in 500 mL Erlenmeyer flasks to allow for particle expansion. The AHP solution was composed of 16.6 mL 30% ACS grade H_2O_2 (Fisher Scientific, H325, Lot #122122), 20 mL 5M NaOH (Fisher-Scientific, S613-3, Lot #046821) and 13.3 mL DI H_2O. These values are equivalent to 0.5 g H_2O_2 and 0.4 g NaOH per gram biomass. Banerjee's (2011) work showed significant delignification for these concentrations. The NaOH treatments had 20 mL 5M NaOH and 30 mL DI H_2O. Upon completion of pretreatment, the biomass was washed at 6:1 volume with DI water and 3.0 mL of concentrated HCl (12.1 M) for pH adjustment. The material was then dried in a 44°C Thelco Model 6 drying oven for 48 hours and stored in a closed container at room temperature until compositional analysis was performed.

COMPOSITIONAL ANALYSIS

Untreated (raw), AHP, H_2O and NaOH materials were prepared and analyzed according to NREL Laboratory Analytical Procedures (LAP) "Preparation of Samples for Compositional Analysis" (2008) and "Determination of Structural Carbohydrates and Lignin in Biomass" (2011). Samples were analyzed in duplicate by HPLC using Bio-Rad Aminex HP-87P column at 78°C with 0.45 mL min^{-1} flow rate and a water mobile phase.

Chromeleon 7.1 software processed the HPLC data. Glucose, xylose and arabinose were the only structural carbohydrates reported due to low values of mannose and galactose. The concentration values reported represent the concentration of sugar in the hydrolyzed sample after correction for loss on 4% hydrolysis and anhydrous sugars, from sections 11.9 and 11.10 in the LAP. To account for the structural changes that occur during pretreatment, the overall percentages of sugars had to be adjusted, Appendix F explains the calculations. “Determination of Total Solids in Biomass” LAP was followed to determine moisture content of all materials. Sugar standards were based on the values seen in Table 2-1.

STERILIZATION

To simulate possible sterilization techniques that would be required in a processing facility three sterilization treatments were applied in triplicate. Sterilization was considered to be a 30 minute autoclave (Steris Amsco Lab 250, Model # 0333808-21) cycle at 121°C and 15 psi. The three levels were (NS) no sterilization, (SB) sterilization before and after pretreatment and (SA) sterilization after washing following pretreatment.

EXPERIMENTAL DESIGN

AHP and NaOH pretreated materials were subjected to the three types of sterilization in triplicate by feedstock. Therefore each experiment provided a randomized block of 18 experimental units. The H_2O treatment for all four materials was conducted simultaneously in duplicate. All subsequent testing of composition and moisture content were performed in duplicate. Figure 2-1 illustrates the experimental design.

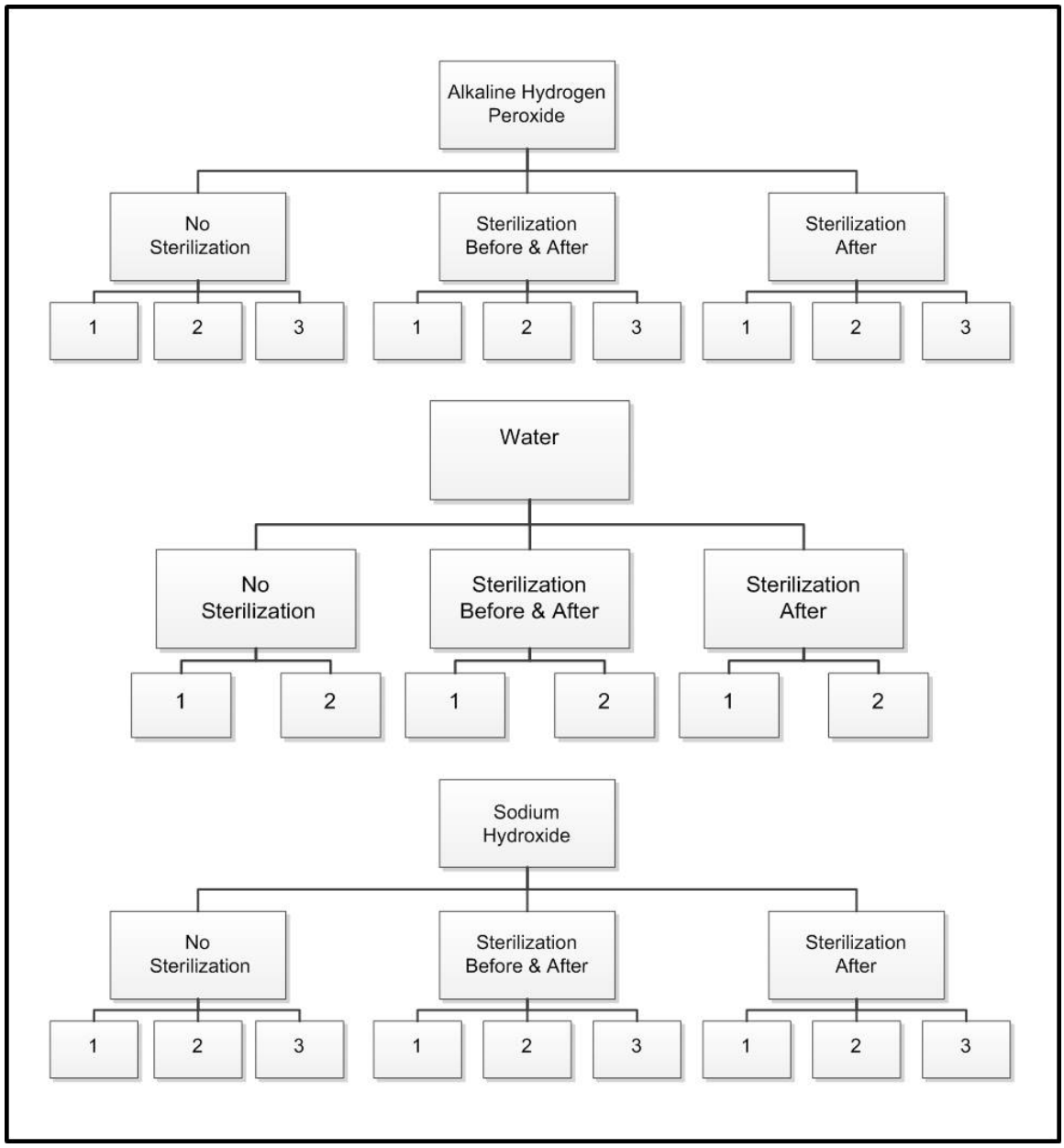

Figure 2-1: Experimental design for pretreatment. Each replication was analyzed in duplicate for NREL (2012) compositional analysis.

STATISTICAL ANALYSIS

The data were analyzed using a completely randomized full-factorial with the PROC GLM function of SAS. Pretreatment, sterilization and their interaction were used as blocks for analysis of lignin content as well as glucose, xylose and arabinose concentrations (APPENDIX B). The least squares means and Tukey's correction were used to evaluate the effects of each block. APPENDIX C contains the full hypothesis testing.

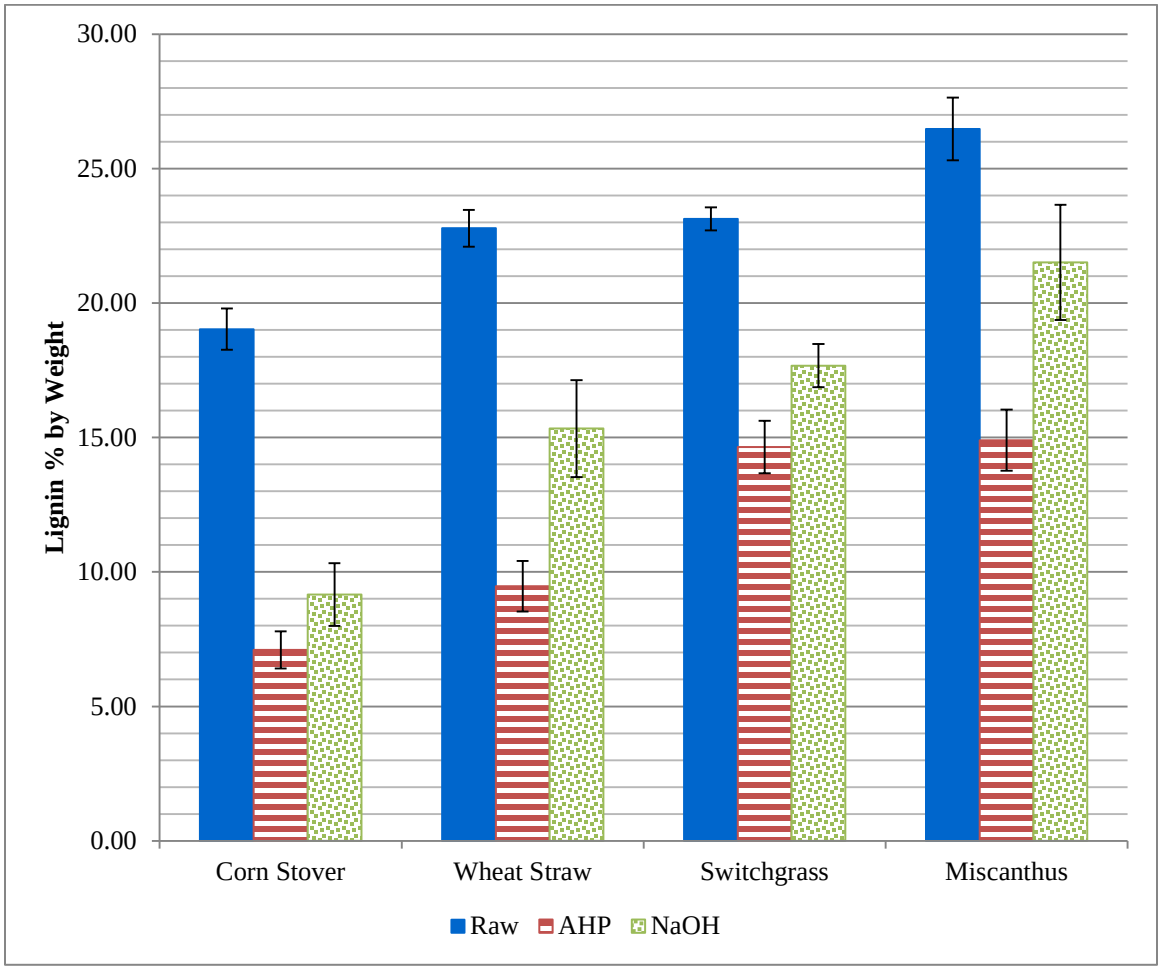

Figure 2-2: Percentage of lignin by weight as determined by NREL Compositional Analysis for the raw, alkaline hydrogen peroxide and sodium hydroxide pretreated material by feedstock.

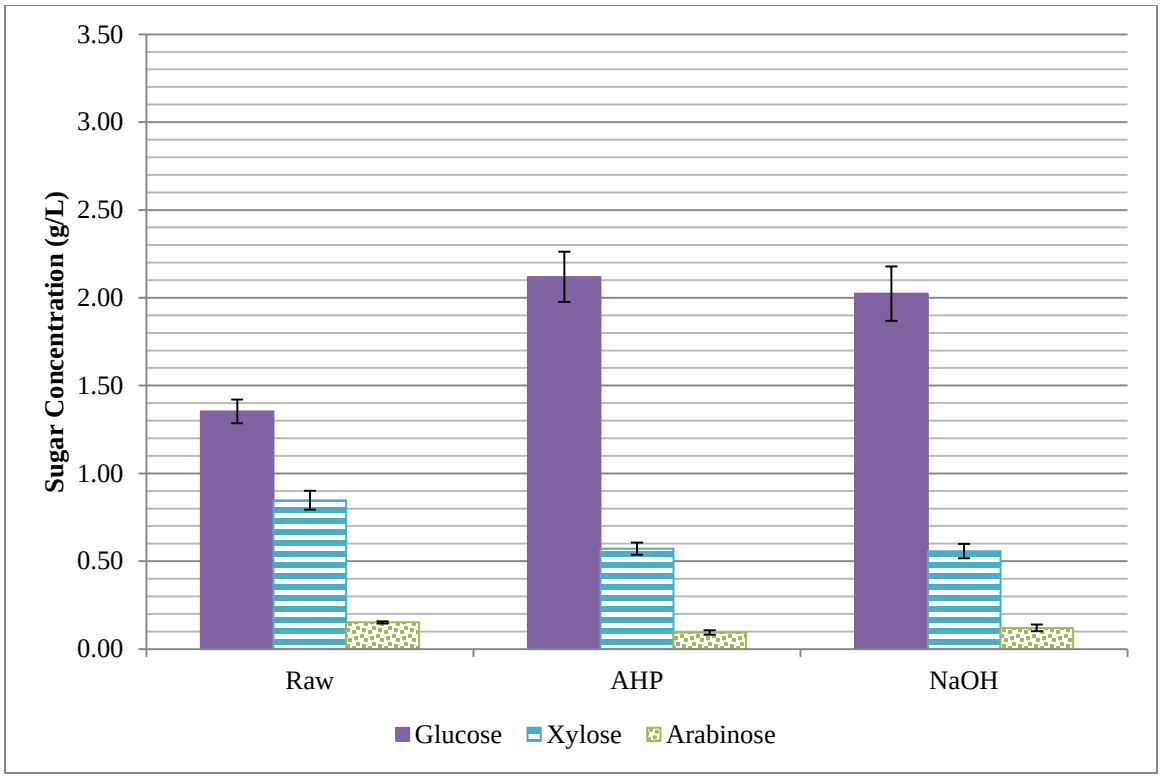

Figure 2-3: Adjusted concentration of structural carbohydrates for corn stover for raw, AHP and NaOH materials. Error bars represent one standard deviation.

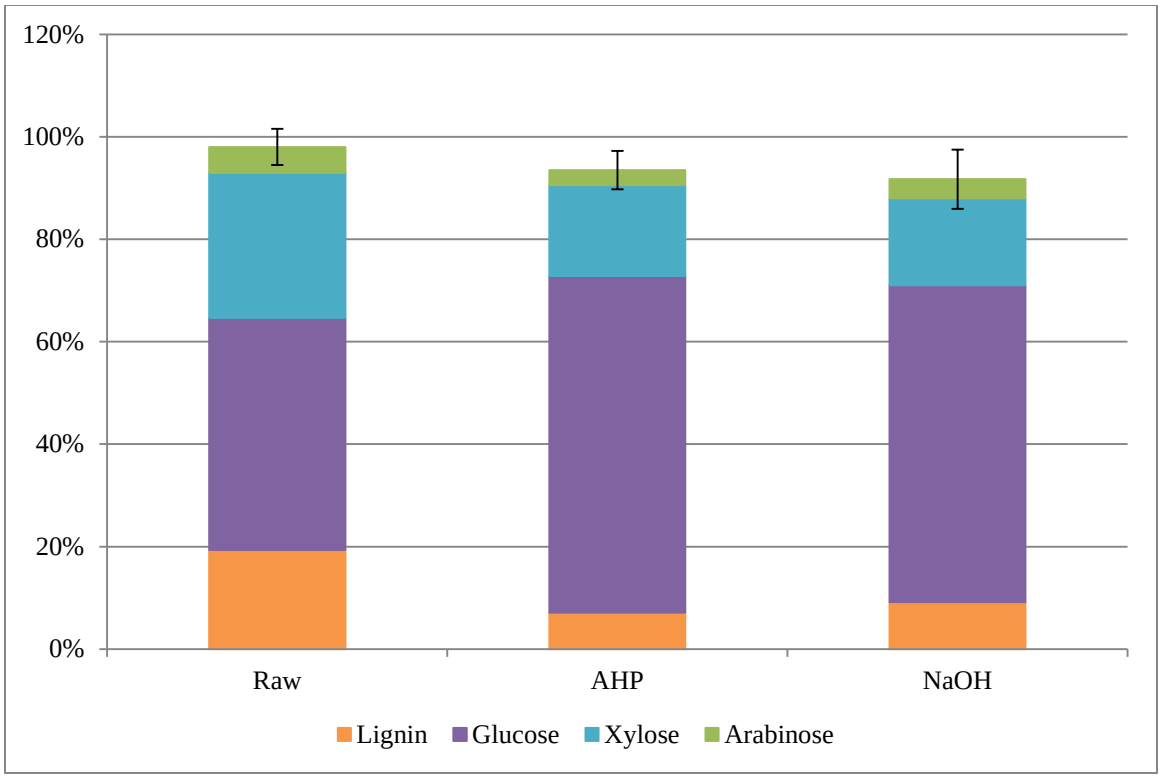

Figure 2-4: Adjusted compositional analysis for raw, AHP and NaOH corn stover. Error bars represent the sum of standard deviations for all individual components.

CORN STOVER

For both lignin content (Figure 2-2) and structural carbohydrate concentrations Figure 2-3), the pretreatment applied had significant effects on the measured results. The AHP treated material exhibited the lowest lignin content and highest concentration of glucose. Sodium Hydroxide pretreated material had significantly lower lignin content and higher glucose yields than the raw. There was no difference in glucose concentration for AHP and NaOH. In most cases evaluated there was no difference in the raw material and those that had been treated with water. The three variations of sterilization did not contribute to any sugar losses/ gains or differences in lignin content. Xylose and Arabinose concentrations are lower for the AHP and NaOH pretreatments. The interaction term between sterilization and pretreatment was not significant for any of the sugars analyzed. Overall compositional analysis for each pretreatment is shown in Figure 2-4.

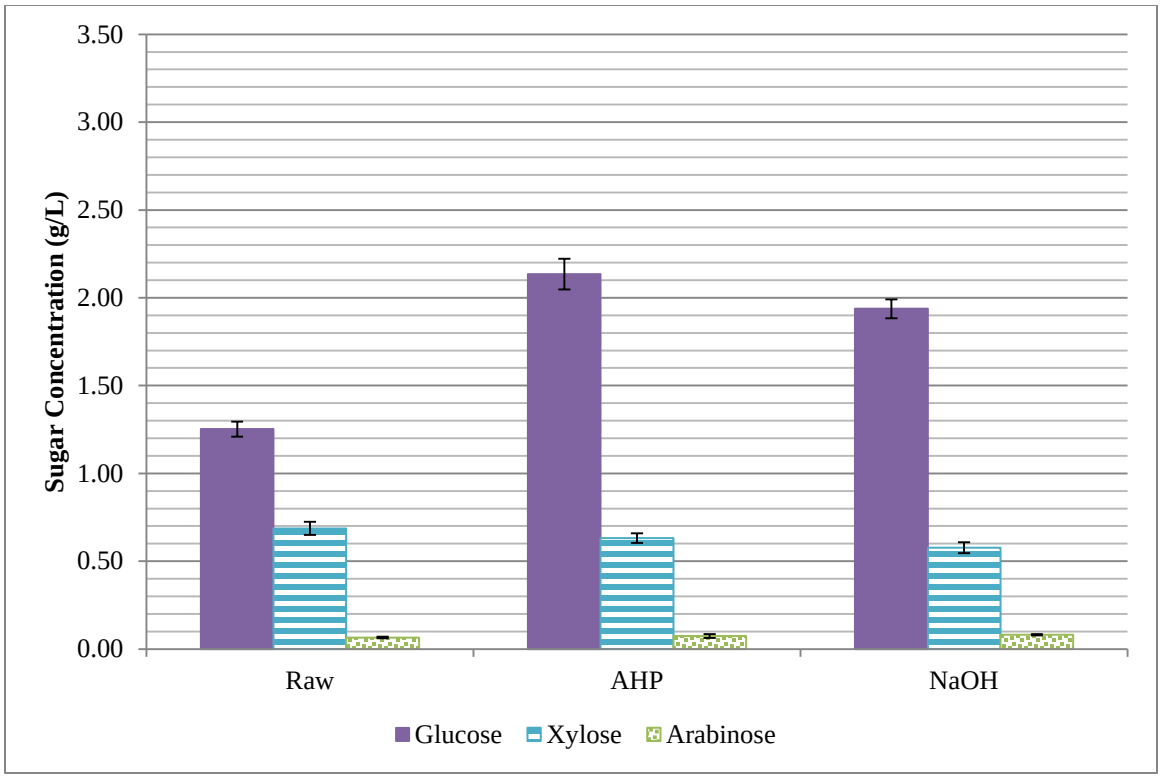

Figure 2-5: Adjusted concentration of structural carbohydrates for wheat straw for raw, AHP and NaOH materials. Error bars represent one standard deviation.

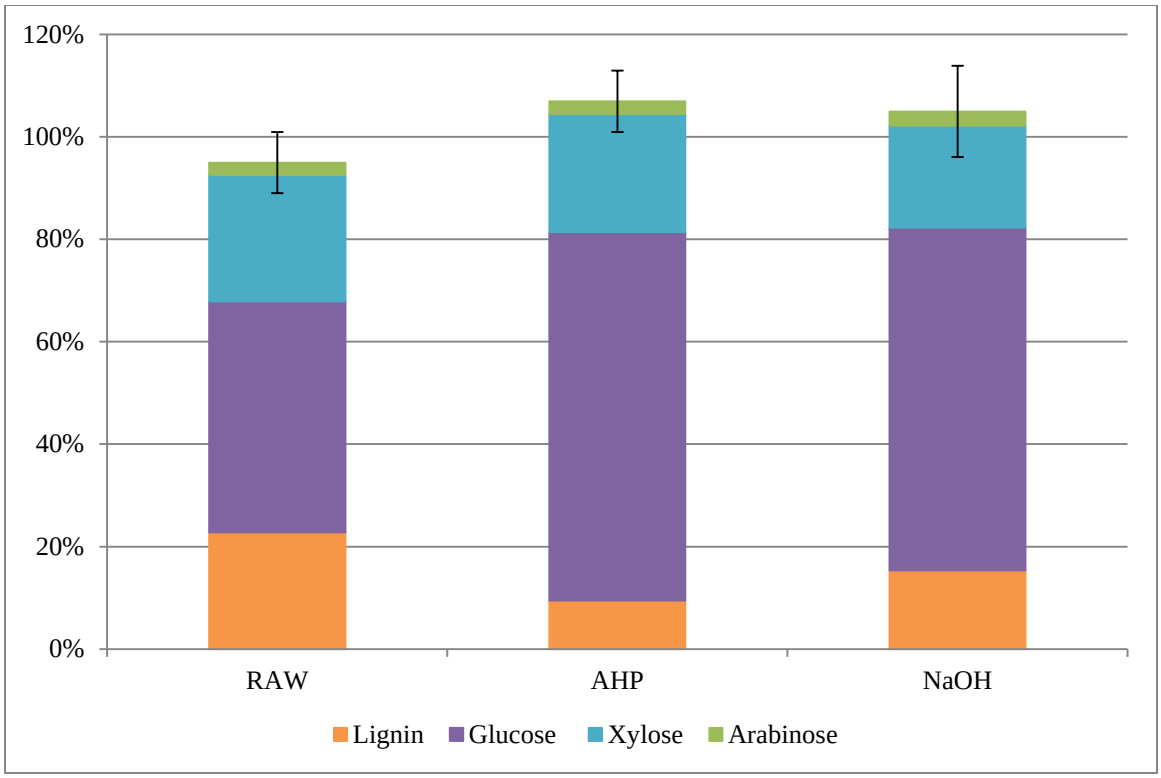

Figure 2-6: Adjusted compositional analysis for raw, AHP and NaOH wheat straw. Error bars represent the sum of standard deviations for all individual components.

WHEAT STRAW

The interaction between sterilization and pretreatment is significant for glucose concentration (Figure 2-5); all AHP and NaOH treated materials are the same with the exception of SBAHP which has the highest concentration (2.2 g/L). All other chemically pretreated material had a glucose concentration of approximately 2.0 g/L. AHP treatment removed more lignin than NaOH (Figure 2-2) but the sugar yields suggest that the extra removal is unnecessary. The interaction is also significant for xylose concentrations; the raw material and all AHP material have the same amount, while SA and NS NaOH have slightly less. Arabinose concentrations did not change with pretreatment or sterilization. Changes in overall composition can be seen in Figure 2-6.

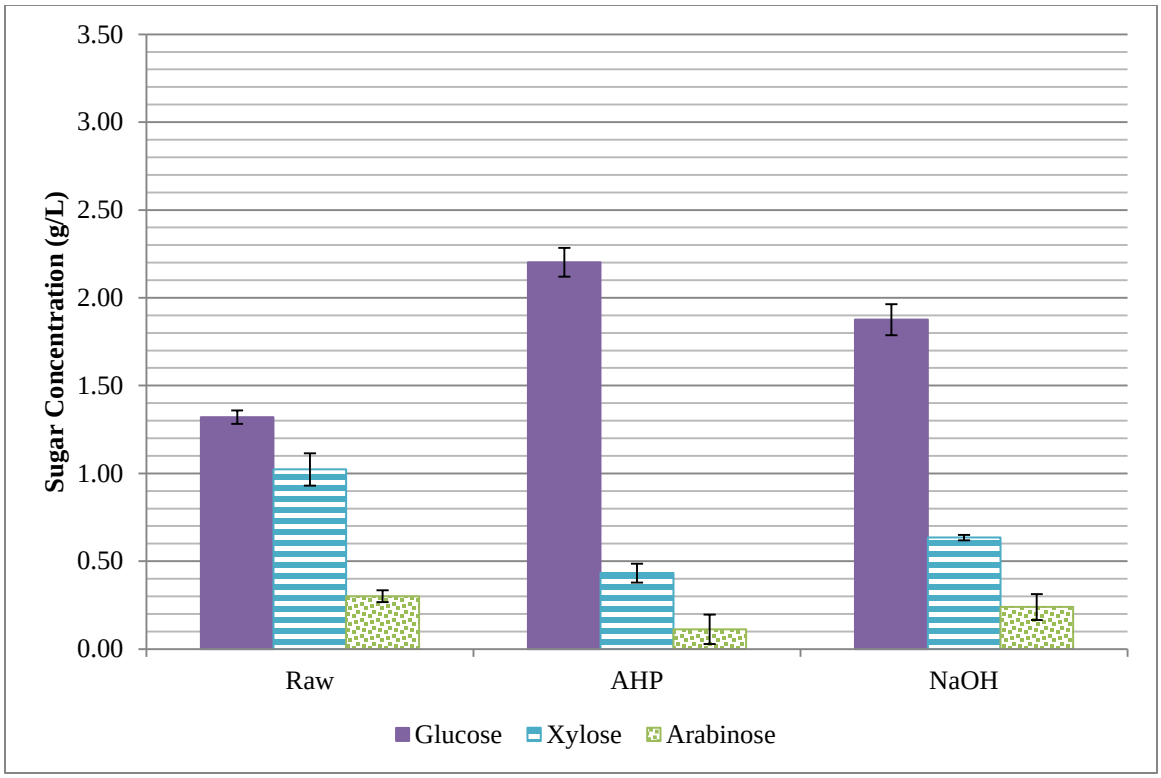

Figure 2-7: Adjusted concentration of structural carbohydrates for switchgrass for raw, AHP and NaOH materials. Error bars represent one standard deviation.

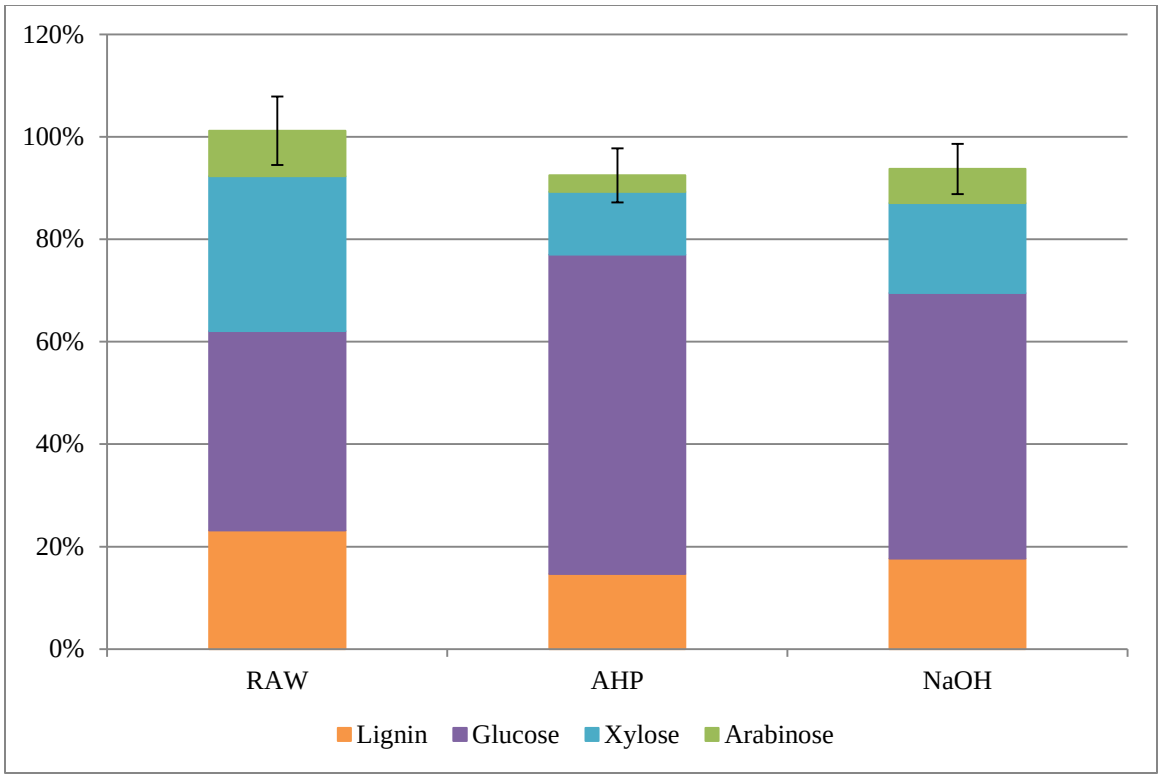

Figure 2-8: Adjusted compositional analysis for raw, AHP and NaOH switchgrass. Error bars represent the sum of standard deviations for all individual components.

SWITCHGRASS

Structural carbohydrates and lignin of switchgrass were significantly affected by pretreatment. AHP pretreated material had the lowest lignin content (Figure 2-2) and highest concentration of glucose (Figure 2-7). NaOH also had a significant impact on lignin content and glucose concentration; lignin is removed and the amount of glucose is greater than the Raw and H_2O treated materials but less than AHP. Xylose yields had the reverse trend from glucose, the more lignin that was removed the lower the concentration of xylose. Arabinose concentration was identical for the raw, H_2O and NaOH, while the AHP material had a significantly lower amount. Figure 2-8 illustrates the overall compositional changes that occur with AHP and NaOH pretreatment.

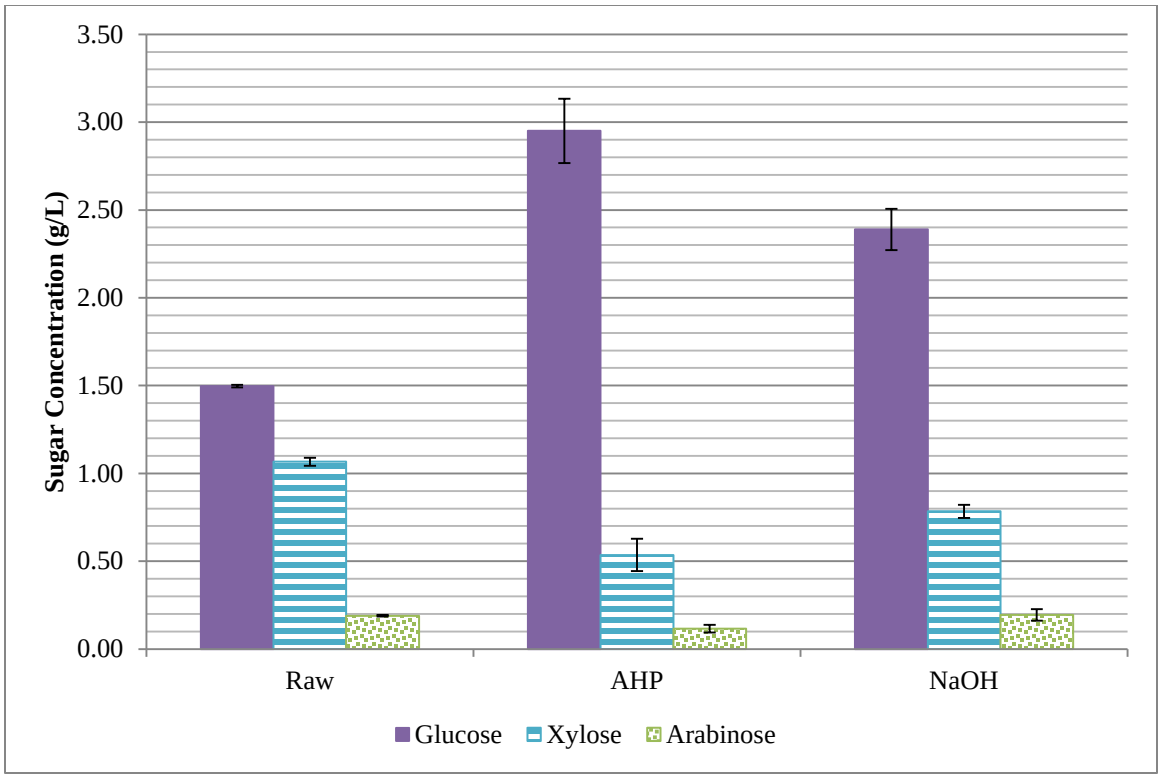

Figure 2-9: Adjusted concentration of structural carbohydrates for miscanthus for raw, AHP and NaOH materials. Error bars represent one standard deviation.

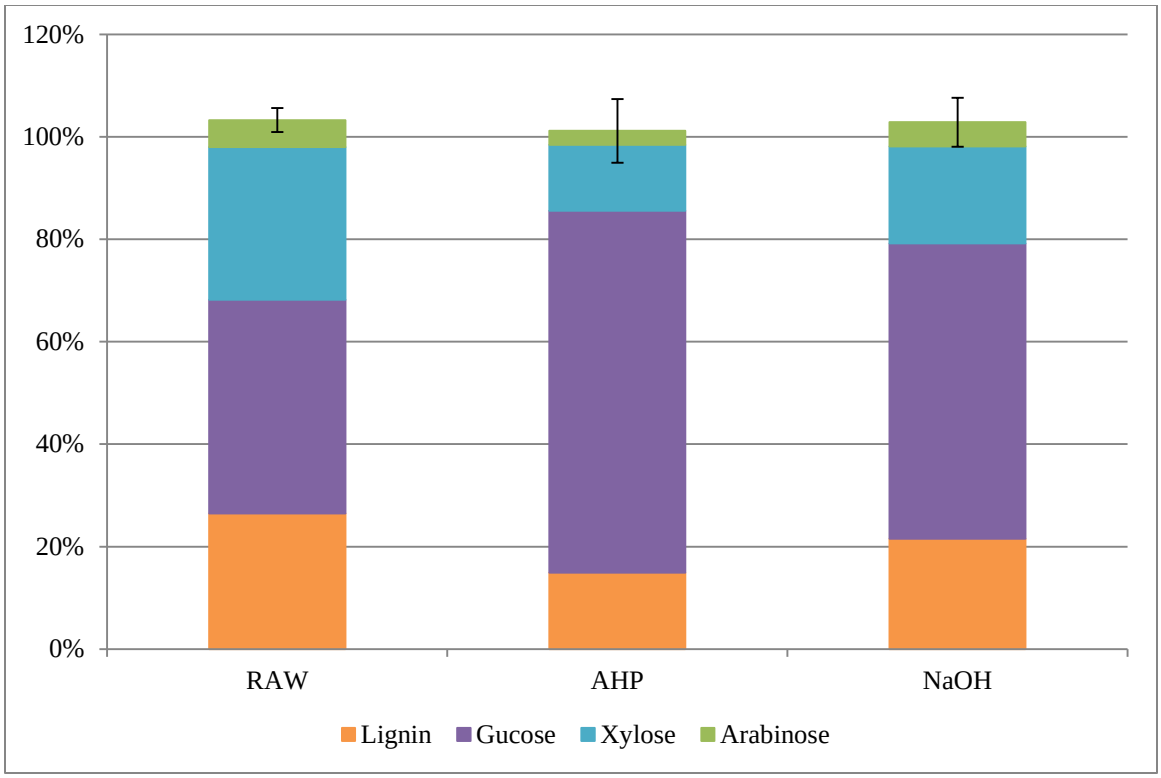

Figure 2-10: Adjusted compositional analysis for raw, AHP and NaOH miscanthus. Error bars represent the sum of standard deviations for all individual components.

MISCANTHUS

The interaction between sterilization and pretreatment was not significant for lignin (Figure 2-2) or the structural carbohydrates (Figure 2-9). AHP treated miscanthus had the lowest lignin content and highest glucose concentration, while pentose sugars xylose and arabinose had lower concentrations. Water pretreated material acted no differently than the raw material when measuring glucose and lignin. NaOH had higher glucose yields than raw material, but lower than AHP, arabinose yields were the same as raw material. Changes in lignin content and percentage of structural carbohydrates are shown in Figure 2-10.

DISCUSSION

For all four feedstocks the amount of lignin was significantly reduced by pretreatment; corn stover and wheat straw had less than half of the lignin that the raw material did. Switchgrass and miscanthus went from approximately 25% lignin down to 15%. In all cases the lignin content of AHP material was significantly lower than the sodium hydroxide treated material. If we were to only using this parameter to evaluate pretreatment effectiveness, the alkaline hydrogen peroxide pretreatment would be considered successful.

Sterilization technique had no effect on lignin content. All four feedstocks' lignin content showed an insignificant response to the three levels of sterilization; the extra heating and pressure have no measurable impact on the lignocellulosic matrix.

Structural carbohydrates behave differently for each feedstock and pretreatment. Each biomass type is approximately 35% cellulose and thereby glucose. The sugar released from AHP and NaOH treated corn stover were statistically the same, while AHP wheat straw, switchgrass and miscanthus had significantly higher glucose yields than both the NaOH pretreated material and the raw material. The majority of hemicellulose is made of xylose and arabinose. There was no difference between AHP and NaOH treated materials for corn stover, but the concentration of C5 sugars released was lower for the untreated material. This loss could be a result of hemicellulose solubilization. As the pretreatment reacts, regions of hemicellulose can be solubilized and washed away, accounting for the reduced concentration. In AHP-treated wheat straw there was no loss of xylose; the amount available was the same as raw material. The NaOH-pretreated material had a slightly lower concentration of C5 sugars. Switchgrass and miscanthus had significantly lower xylose concentrations from both the AHP and NaOH-treated materials. Again this could be the result of hemicellulose solubilization. Arabinose followed the same trend as xylose. Glucose concentrations from miscanthus were higher than those from the other feedstocks, most likely a result of the plant maturity described in Chapter One.

In some cases the interaction between sterilization and pretreatment has significant effects on sugar concentrations. This interaction could partially be due to “cooking” of the biomass. Often hot water is used as a pretreatment. At higher temperatures, hemicellulose is hydrolyzed which forms acids that degrade the lignocellulose (Hendriks and Zeeman 2009). This could lead to the higher glucose yields for those sterilized before and after pretreatment, only sterilized after pretreatment and NaOH materials.

Looking at the combination of lignin content and sugar concentrations it can be determined that AHP is a better pretreatment for wheat straw, switchgrass and miscanthus. Corn stover shows no benefit from adding peroxide to the alkaline solution. There are several other factors that must be considered to determine the overall success of the AHP pretreatment. Fermentability is a major concern which will be further addressed in the next chapter.

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Chapter Three

MICROBIAL ACTIVITY ON ALKALINE HYDROGEN PEROXIDE PRETREATED BIOMASS

SUMMARY

The microbial activity of alkaline hydrogen peroxide (AHP) pretreated corn stover, wheat straw, switchgrass and miscanthus was evaluated. The materials were inoculated with *Clostridium thermocellum* for whole cell hydrolysis and fermentation. For most cases evaluated there were measurable cellulosome and fermentation products, but concentrations were highly variable and therefore inconclusive for each of the feedstocks. The pretreated materials were plated on agar favorable to fungal and bacterial growth to evaluate the sterilizing capabilities of AHP. AHP appears to suppress visible microbial growth for the first 24 hours after pretreatment. If AHP does not provide the additional hygienic effects, AHP does not provide a significant advantage over sodium hydroxide pretreatment.

INTRODUCTION

ALKALINE HYDROGEN PEROXIDE

The alkaline hydrogen peroxide (AHP) pretreatment that was evaluated in Chapter Two successfully removed lignin and increased the amount of glucose after hydrolysis for all four feedstocks. A main concern of any pretreatment is how downstream processes are affected. It is important to keep processing between pretreatment and fermentation to a minimum, because more unit operations increase overall cost. AHP requires a pH of 11.5 to create the powerful lignin-degrading hydroxyl radicals (Gould 1984), which is well outside most fermentation organisms' optimum pH range. When the pH is chemically decreased, a high possibility exists for fermentation inhibitors formation. Sodium chloride is known to prevent clostridium species from

properly metabolizing (Qureshi, Saha et al. 2008). There could also be unknown consequences of AHP in further processing as this method of pretreatment is relatively understudied (Banerjee, Car et al. 2011).

ON-FARM PROCESSING

Large-scale production of biofuels on-farm would require on the order of 100 tons of biomass. Mixing will most likely not be possible in a high-solids environment. The system will have little to no free water, which has a major influence on reaction mechanics (Modenbach and Nokes 2012).

One major concern at the farm scale is bunker sterility. In most commercial fermentations the vessel and all ingredients are heated to the bacterial lethal temperature of 121°C and left there for a calculated amount of time based on contents and volume. The sterilization step kills naturally occurring species giving the introduced microorganism the competitive advantage (Shuler and Kargı 1992). Longer periods of elevated temperature are required to sterilize larger volumes. Therefore the energy required for true sterilization at the farm scale would be impractical. The sterilizing capabilities of hydrogen peroxide may provide a realistic alternative. While not sterilization in the true sense, this chemical pretreatment may create an aseptic environment prior to bacterial inoculation. Plating of pretreated materials will determine whether naturally occurring species are eliminated and therefore whether or not a competitive advantage would be created for the introduced organism.

CLOSTRIDIUM THERMOCELLUM

Clostridium thermocellum is a cellulolytic thermophilic anaerobe that has the ability to hydrolyze cellulose into smaller saccharides that can be easily fermented

(Lamed, Kenig et al. 1985; Demain, Newcomb et al. 2005). This bacterial species relies on an extracellular cellulosome to provide cellulases responsible for saccharification (Bhat and Wood 1992; Schwarz 2001). The cell is then able metabolize the sugars (Bhat and Wood 1992) for production of ethanol, lactate, acetate, carbon dioxide and hydrogen (Demain, Newcomb et al. 2005). Because the enzyme system is extracellular, cellulose hydrolysis and fermentation work essentially independently. Previous studies have shown that the enzyme complex in the cellulosome will break down crystalline cellulose from a variety of feedstocks (Chinn, Nokes et al. 2006; Dharmagadda, Nokes et al. 2010) but there is a lack of information about whether or not alkaline hydrogen peroxide pretreated biomass can support the activity (both hydrolytic and fermentative) of *C. thermocellum*. A main objective of this work was to determine if the cellulosome can hydrolyze cellulose of AHP materials under non-optimal conditions and to quantify subsequent fermentation of the hydrolyzed biomass. A range of sterilization treatments were performed to determine if AHP pretreatment can be used to eliminate sterilization before using *C. thermocellum's* cellulosome and fermentation systems.

MATERIALS AND METHODS

BIOMASS

A Retsch Muhle SM1 mill (Nr. 70947) was used to grind locally harvested corn stover, wheat straw, switchgrass and miscanthus. The biomass was then pretreated according to the specifications described in Chapter Two. A subsample from the same batch of biomass that underwent NREL Compositional Analysis (2012) was directly fermented after washing and three milliliters of concentrated HCl were added for pH

adjustment. A new batch of biomass was pretreated for sterilization testing, using the procedure detailed in Chapter Two.

The previous chapter demonstrated that raw materials (not pretreated) have no significant compositional difference than those treated with water. For the duration of this report the two terms are used interchangeably.

EXPERIMENTAL DESIGN

AHP and NaOH pretreatments were subjected to three sterilization method; each feedstock received each level of each treatment in triplicate. The three levels of sterilization are (NS) no sterilization, (SB) sterilization before pretreatment and after washing and (SA) sterilization after washing. Sterilization is defined as a 30 minute autoclave (Steris Amsco Lab 250, Model # 0333808-21) cycle at 121°C and 15 psi. The H_2O treatment for all four biomass types underwent the three levels of sterilization in duplicate. This experimental design gave 24 samples for each feedstock, two duplicate subsamples were fermented. Figure 3-1illustrates the experimental design.

STATISTICAL ANALYSIS

The data was analyzed using a completely randomized full factorial with the PROC GLM function of SAS (APPENDIX B). Pretreatment, sterilization and their interaction were used as blocks for analysis of cellulosome and fermentation products. Each block was compared to zero with the PROC UNIVARIATE function in SAS. The full hypothesis testing is found in APPENDIX C.

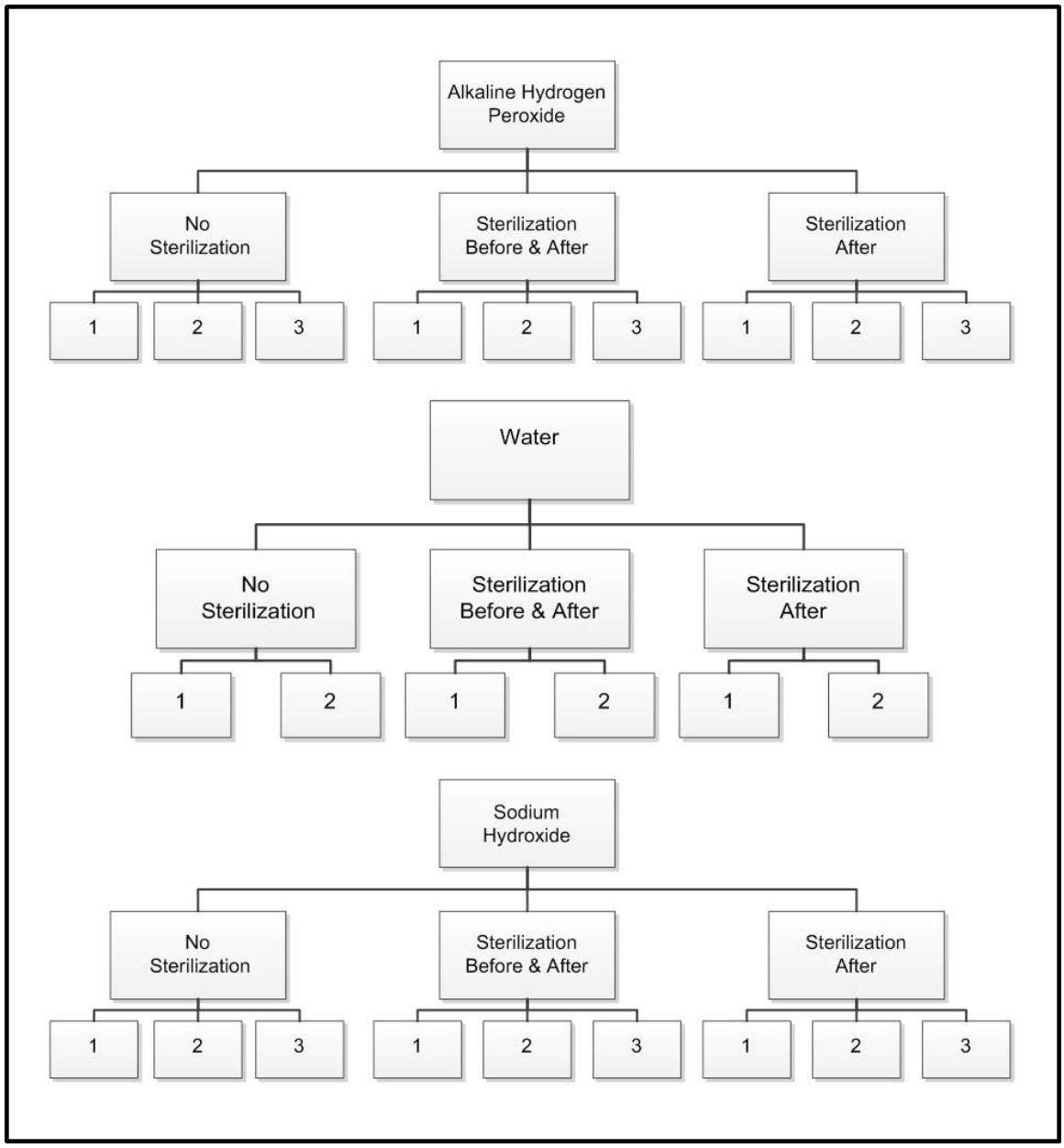

Figure 3-1: Experimental design for pretreatment of AHP, H_2O and NaOH materials. Each of the pretreated replications was fermented in duplicate.

FERMENTATION

The pretreated biomass was fermented in duplicate. Approximately 0.5 bone-dry g of biomass (assuming ~20% solids) were flushed with CO_2 for 15 minutes in test tubes and then capped. The SA and SB samples were autoclaved (Steris Amsco Lab 250, Model #0333808-21) in the fermentation vessel for 30 minutes at 121°C and 15 psi. *C. thermocellum* ATCC 27405 from a -80°C stock was cultivated in 10 mL Thermophilic Media (APPENDIX D) on Whatman filter paper (#1) strips; this process is similar to the cultivation techniques described by Dharmagadda (2010). The 10 mL initial culture was then used to inoculate 50 mL of cellulose-containing (4.4 g/L) media 48 hours before biomass fermentation was started. Approximately 8.5 mL Thermophile Media was added to the anaerobic biomass and then 1.0 mL *C. thermocellum* from the 50 mL stock culture was added. The tubes were incubated at 65°C in a New Brunswick Scientific Innova 4200. All bacterial transfers were performed under a safety hood (NuAire 925-400, SN 18264UN). The fermentation was allowed to progress for 48 hours and then the materials were transferred to 15 mL centrifuge tubes and frozen at -45°C, before HPLC analysis the samples were centrifuged and the supernatant filtered through a 0.45 μm syringe filter.

Fermentation product concentrations were analyzed in duplicate by a Dionex HPLC (Sunnyville, CA) with a refractive index detector (Shodex 101, Kanagawa, Japan). The column (Aminex HP-86H, Bio-Rad, Hercules, CA) was operated at 50°C with a 1.4 mL min^{-1} flow rate and 0.17 N H_2SO_4 mobile phase. Data was processed with Chromeleon 7.1 software.

MICROBIAL ACTIVITY TESTING

To observe the “sterilizing” capabilities of each pretreatment, plates were created by plating approximately one gram of biomass from each treatment replicate on LB Agar (Sigma-Aldrich P6685, Lot #SLBC6869V), which provides a nutrient rich environment favorable for bacterial growth. Potato Dextrose Agar (Potato Dextrose Broth, Sigma Aldrich P6685, Agar granulated, Fisher Scientific BP1423-500) was also plated with biomass from each treatment replicate because PDA supports a wide range of fungal species. The agar plates were then visually observed for growth every 24 hours for 72 hours. The plates were incubated at 35°C in a Thermo Scientific Precision incubator. This temperature was chosen because many bacterial and fungal species prefer mesophilic conditions. Photographs were taken at each time point and can be seen in APPENDIX G.

RESULTS

The following results were separated into two categories: cellulosome products (glucose and cellobiose) and fermentation products (ethanol, lactate and acetate). Table 3-1 shows the microbial activity over time for each four feedstocks. Due to the individualized results each feedstock's results are described independently.

Table 3-1: Microbial activity results for corn stover, wheat straw, switchgrass and miscanthus. "Yes" indicates that growth was visible at the time point.

	TIME		0 h		24 h		48 h		72 h	
	PLATE		LB	PDA	LB	PDA	LB	PDA	LB	PDA
CORN STOVER	AHP	1	NO	NO	NO	NO	NO	YES	YES	YES
	AHP	2	NO	NO	NO	NO	YES	NO	YES	YES
	H2O	1	NO	NO	YES	NO	YES	YES	YES	YES
	H2O	2	NO	NO	YES	NO	YES	YES	YES	YES
	NaOH	1	NO	NO	NO	NO	NO	YES	YES	YES
	NaOH	2	NO	NO	NO	NO	YES	NO	YES	YES
WHEAT STRAW	AHP	1	NO	NO	NO	NO	NO	NO	NO	NO
	AHP	2	NO	NO	NO	NO	NO	NO	YES	NO
	H2O	1	NO	NO	NO	NO	YES	NO	YES	YES
	H2O	2	NO	NO	NO	NO	YES	NO	YES	YES
	NaOH	1	NO	NO	NO	NO	YES	NO	YES	NO
	NaOH	2	NO	NO	NO	NO	YES	YES	YES	YES
SWITCHGRASS	AHP	1	NO	NO	NO	NO	NO	NO	NO	NO
	AHP	2	NO	NO	NO	NO	YES	NO	YES	YES
	H2O	1	NO	NO	NO	NO	NO	NO	YES	YES
	H2O	2	NO	NO	NO	NO	NO	NO	YES	NO
	NaOH	1	NO	NO	YES	YES	YES	YES	YES	NO
	NaOH	2	NO	NO	YES	YES	YES	YES	YES	YES
MISCANTHUS	AHP	1	NO	NO	YES	NO	YES	NO	YES	YES
	AHP	2	NO	NO	YES	NO	YES	NO	YES	YES
	H2O	1	NO	NO	YES	NO	YES	NO	YES	YES
	H2O	2	NO	NO	YES	NO	YES	YES	YES	YES
	NaOH	1	NO	NO	NO	NO	YES	YES	YES	YES
	NaOH	2	NO	NO	YES	NO	YES	YES	YES	YES

CORN STOVER

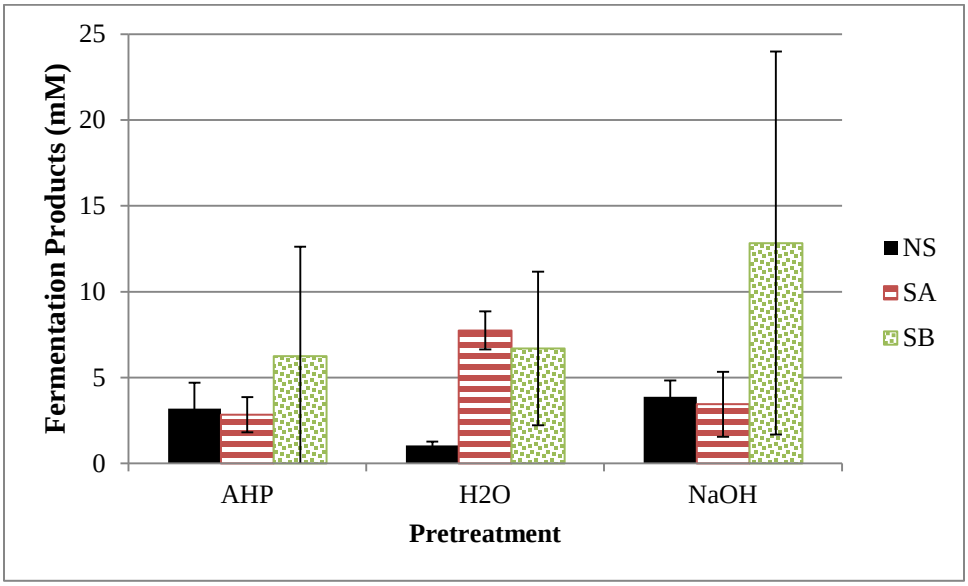

Figure 3-2: Fermentation products (mM) of *C. thermocellum* for corn stover. The interaction between sterilization and pretreatment is shown, however the interaction did not have a statistically significant effect on the amount of fermentation products produced..

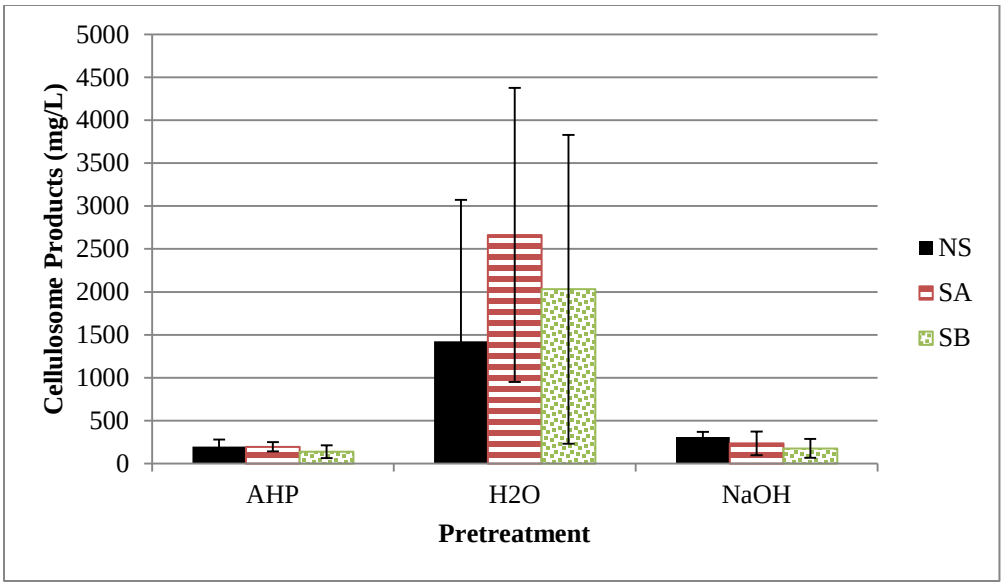

Figure 3-3: Cellulosome products (mg/L) of *C. thermocellum* for corn stover. The interaction between sterilization and pretreatment is shown, however the interaction did not have a statistically significant effect on for the amount of cellulosome products formed.

Comparing each sterilization pretreatment combination to zero determined whether or not there were significant levels of fermentation and cellulosome products. For corn stover the SBH_2O fermentation products (Figure 3-2) were not different that zero and all H_2O (NS, SA, SB) cellulosome products were not detectable. Looking at the main effects for each product category, the concentrations of fermentation products were not statistically affected by pretreatment, but all treatments resulted in measurable amounts of metabolic products, indicating that *C. thermocellum* could metabolize the treated material. Sterilization was not a good indicator variable statistically, as assumptions of the regression model were not met (APPENDIX C). The concentration of cellulosome products were not significantly affected by pretreatment or sterilization (Figure 3-3).

In the microbial activity experiments (Table 3-1**Error! Reference source not found.**) fungal or bacterial growth was visible on AHP and NaOH materials 48 hours after plating. The raw materials showed bacterial colonies after 24 hours and fungal colonies after 48 hours.

WHEAT STRAW

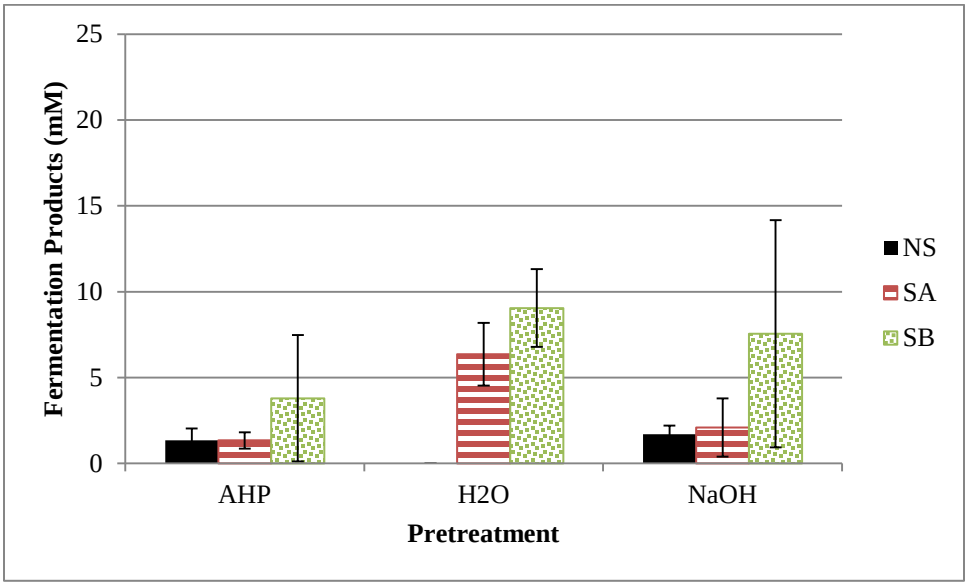

Figure 3-4: Fermentation products (mM) of *C. thermocellum* for wheat straw. The interaction between sterilization and pretreatment is shown, which was statistically significant.

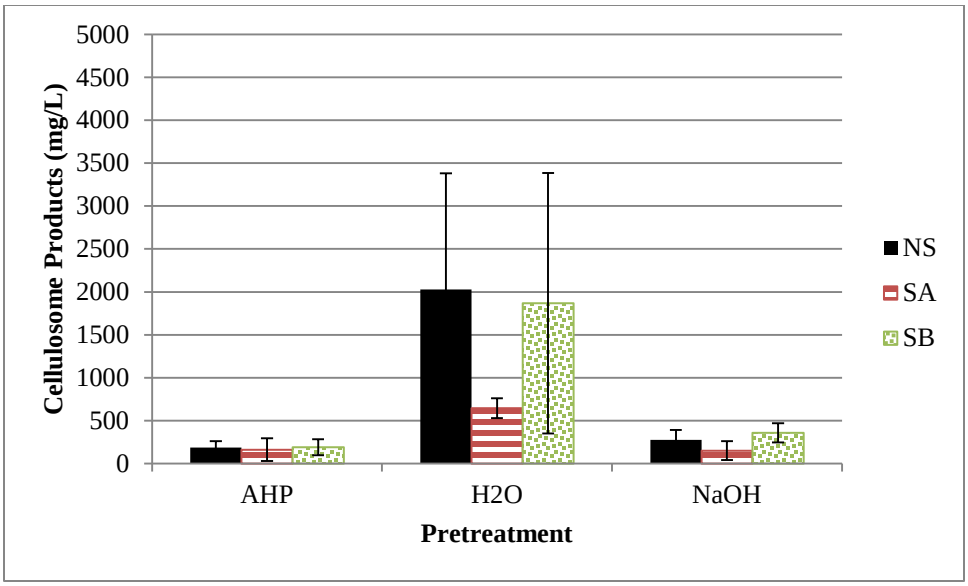

Figure 3-5: Cellulosome products (mg/L) of *C. thermocellum* for wheat straw. The interaction between sterilization and pretreatment is shown, which was statistically significant.

PROC UNIVARIATE results concluded the fermentation and cellulosome products of NSH_2O were not statistically different than zero. The cellulosome products of SBH_2O were not significant either.

Fermentation products were not dependent on pretreatment method for wheat straw. However sterilization did significantly affect the concentrations of acetate, lactate and ethanol: Those sterilized twice (SB) had higher concentrations of end-product metabolites than those sterilized once (SA) or not at all (NS). However in each case all levels of sterilization showed metabolite concentrations greater than zero. The interaction (Figure 3-4) was also significant.

Pretreatment significantly altered the concentrations of glucose and cellobiose saccharified by the cellulosome. In all cases the AHP, H_2O and NaOH sugar concentrations were statistically greater than zero. The effect of sterilization did not have a significant effect on sugar concentrations, but the interaction (Figure 3-5) between sterilization and pretreatment was statistically significant. With the exception of NS and SB H_2O, all other sterilization-pretreatment combinations showed a significant amount of cellulosome product that were statistically the same.

AHP treated materials showed bacterial growth at 72 hours (Table 3-1); however these materials did not show any fungal growth even 72 hours after plating. The H_2O and NaOH pretreatments showed mainly bacterial growth by 48 hours, and both bacterial and fungal growth by 72 hours.

SWITCHGRASS

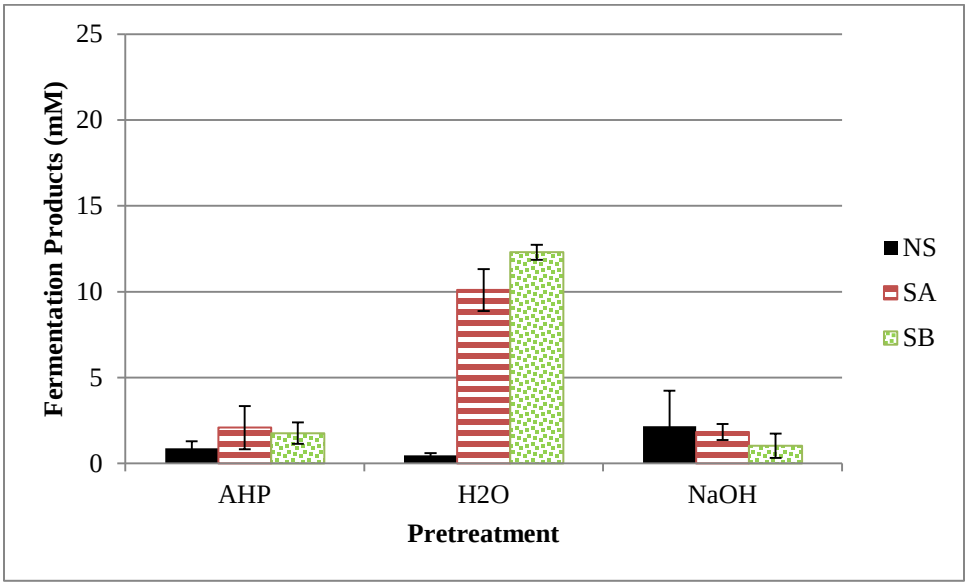

Figure 3-6: Fermentation products (mM) of *C. thermocellum* for switchgrass. The interaction between sterilization and pretreatment is shown, this variable was significant.

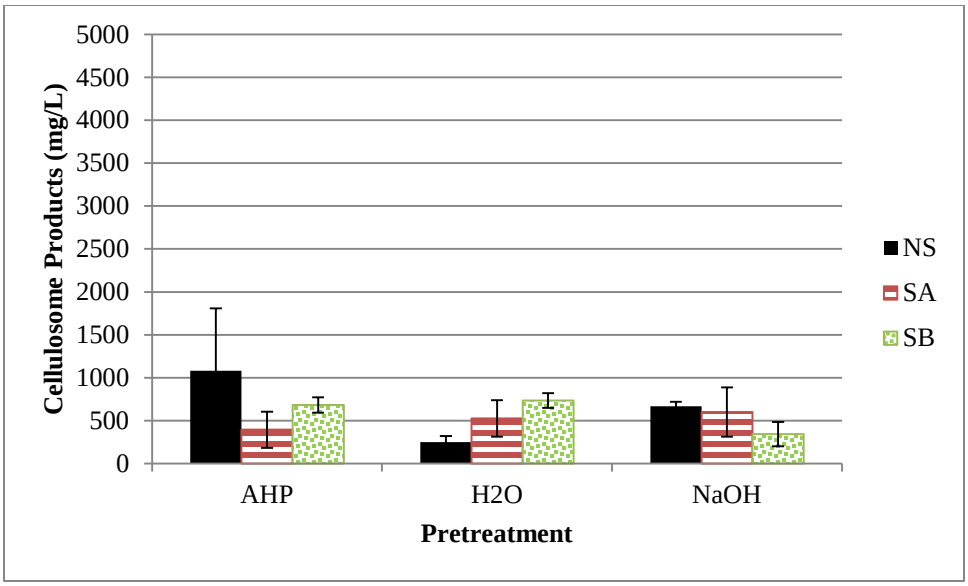

Figure 3-7: Cellulosome products (mg/L) of *C. thermocellum* for switchgrass. The interaction between sterilization and pretreatment is shown; this variable was significant for cellulosome products.

In all cases the product concentrations of the cellulosome and fermentation were statistically greater than zero. Both the metabolism and extracellular enzyme system were active on all types of pretreated and sterilized switchgrass. Fermentation products are not well defined by pretreatment or sterilization; however their interaction was significant (Figure 3-6). For all cases the fermentation products were greater than zero. SA H_2O and SB H_2O were equal but significantly different than the others. There was no statistical difference between the other treatments. All treatments resulted in sugar concentrations significantly greater than zero. Cellulosome activity was not dependent on pretreatment, but sterilization did influence the values. The treatments not subjected to sterilization exhibited the highest sugar concentrations. The interactions between sterilization and pretreatment method were statistically significant (Figure 3-7); NSAHP resulted in the highest sugar concentrations, which was statistically greater than the sugar concentrations from the SAAHP, SANaOH and SBNaOH treatments.

Plate testing (Table 3-1) showed both fungal and bacterial growth on NaOH materials after 24 hours. Bacterial cultures were present after 48 hours on one of the AHP replicates, although on the raw material no fungal or bacterial growth was visible. By 72 hours all plates showed contamination.

MISCANTHUS

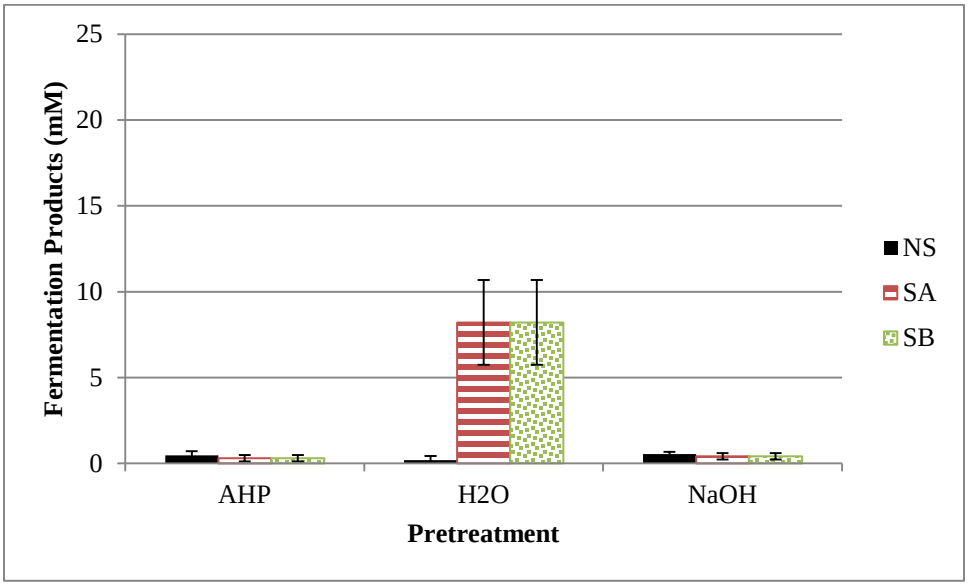

Figure 3-8: Fermentation products (mM) of *C. thermocellum* for miscanthus. The interaction between sterilization and pretreatment is shown; this variable significantly affects product concentration.

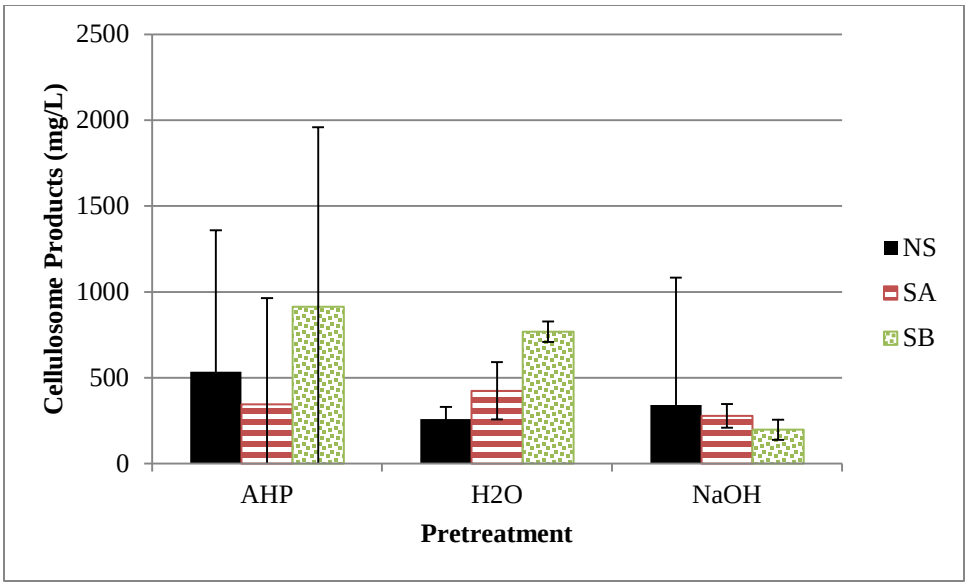

Figure 3-9: Cellulosome products (mg/L) of *C. thermocellum* for miscanthus.

The PROC UNIVARIATE results showed the fermentation products of NSH_2O and the cellulosome products of SAAHP and NSNaOH were not statistically different than zero. None main effects were good indicator variables to predict fermentation or cellulosome product concentrations. Fermentation products show a significant response to the interaction between sterilization and pretreatment (Figure 3-8). From the interaction, all fermentation product values were statistically equal except for the SA and SB H_2O treatments. None of the treatments (Figure 3-9) were significant for cellulosome products.

The microbial activity testing (Table 3-1) showed bacterial growth at 24 hours for all pretreatments; fungal growth did not appear on AHP materials until 72 hours, but was present at 48 hours on H2O and NaOH.

DISCUSSION

Because fermentation data from HPLC was sporadic, it is difficult to make any firm conclusions about treatment effects. The fermentation of *C. thermocellum* was not optimized in any fashion; for example, the pH was only marginally adjusted by adding an equal volume of concentrated HCl to each sample, above the optimal range between 6-8. In addition, a lab stock salt solution was made improperly for the media used for the switchgrass, miscanthus and water treated fermentations. With these issues in mind the only conclusions that can be made are whether or not products are present; meaning that the *C. thermocellum* bacteria were actively growing and metabolizing the materials. APPENDIX C shows the full hypothesis tests for these data.

While the data do not allow for specific quantitative conclusions to be made, overall trends can be deduced from the results. Because the primary reason for using *C.*

thermocellum for the BRDI project is its cellulosome activity, this discussion will focus on the cellulosome product concentrations. For corn stover and wheat straw, the H_2O samples appear to have the highest concentrations; however for AHP and NaOH there are a significant amount of metabolic products. The higher sugar concentrations from H_2O are most likely a result of the elevated pH of AHP and NaOH materials inhibiting *C. thermocellum's* enzyme system on those materials. When samples were inoculated they were still very basic; the addition of HCl and washing steps brought the reading down from ~11.5-12 to ~10-11, still well outside *C. thermocellum's* preferred range. Switchgrass treated with AHP and subjected to no sterilization actually had significantly higher sugar concentrations that all other treatment combinations. This result is encouraging, because we may be able to operate the on-farm system in this manner. Miscanthus data were more difficult to interpret. For miscanthus, AHP appears to have the highest product concentrations, but the sample variability is large.

The microbial activity experiments showed visible microbial growth appears to be suppressed for the first 24 hours using the AHP pretreatment. The H_2O samples showed no contamination in over 50% of the plates 48 hours after plating and NaOH had over 75% with microbial growth, while AHP had approximately 31% with visible colonies. The AHP pretreatment provided an "aseptic-enough" environment for the first 24 hours and simultaneously removed significant lignin (determined in Chapter Two). This switchgrass results also show that *C. thermocellu*m's cellulosome can perform cellulose conversion on AHP-treated material.

The microbial activity results were not conclusive however; no one treatment prevented bacterial or fungal growth for the full 72 hours. AHP appears to suppress

microbial growth, but the same number of plates were contaminated after 24 hours as those treated only with water. If the alkaline hydrogen peroxide does not provide additional hygienic effects, AHP does not have any additional benefits over the proven sodium hydroxide pretreatment method. Further experimentation would be necessary to make a definitive decision.

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Chapter Four
FUTURE WORK

From this initial study of the effects of alkaline hydrogen peroxide pretreatment several opportunities for further research suggested themselves. The focus of the future work could be in several areas including fermentation optimization, introduction of a co-culture, large scale testing and a comprehensive life cycle analysis.

The data presented in Chapter Three showed obvious issues with the *C. thermocellum* fermentation procedures. Adjustments should be made to the experimental procedure so more consistent product yields can be generated. A technique or system could be developed to easily wash and adjust pH to a more favorable range for bacterial growth. There are also alternative hydrolysis methods besides the cellulosome from *C. thermocellum* that could be considered. Commercial enzymes or chemical saccharification may show increased sugar concentrations for AHP materials.

Another aspect to consider is the proposed co-culture of *C. thermocellum* and *C. beijernickii*. There is potential for inhibitor production during pretreatment and washing that could have detrimental consequences on the desired fermentation. There is also the question of *C. thermocellum's* ability to produce sufficient glucose over time for a steady product stream from AHP biomass. A significant amount of lignin was removed and the potential sugar concentrations increase over the raw material, but whether or not the materials are suitable for subsequent butanol production from *C. beijernickii* is yet to be seen.

Because the overall goals of this BRDI project are to develop a production system suitable for farm-scale, studying the reaction mechanics at larger volumes is critical. The reaction between concentrated hydrogen peroxide and sodium hydroxide is extremely exothermic; the ability to capture this heat for further processing or maintenance of *C. thermocellum* would be extremely beneficial to the overall energy balance. When the AHP mixture is applied to the biomass rapid particle expansion occurs, therefore determining the needed volume bunker for a load of biomass could provide an interesting computer model. There are also the traditional scaling concerns like time, agitation, heat transfer and other transport properties to consider. These same issues would be relevant to further downstream processes like fermentation and distillation. At the large volumes required on-farm, how the solutions behave is of considerable interest: Is the AHP solution corrosive to concrete? What volumes can be safely stored? How soon does hydrogen peroxide dissociate (shelf life)?

Life cycle analysis is described by ISO standards as “a compilation and evaluation of the inputs, outputs and potential environmental impacts of a product system throughout its life cycle” (Horne, Grant et al. 2009). By evaluating the energetic and environmental impacts, the results of the AHP pretreatment can be measured accurately. Looking at the manufacturing, travel, recycling and waste management requirements will give the overall BRDI project a basis at which to compare other pretreatment technologies.

Figure 4-1 shows a potential system boundary for AHP, all items inside the boundary are accounted for in terms of energy required and resulting emissions. A functional unit could be defined as the energy and emissions required to remove a certain percentage of lignin; optimizing this unit becomes the engineering problem. This functional unit can be used to compare and contrast various methods of pretreatment proposed by the BRDI project. It is important to remember that pretreatment is only one portion of a very large system. Eventually how the biomass is transported to the bunker and what happens afterward must ultimately be considered.

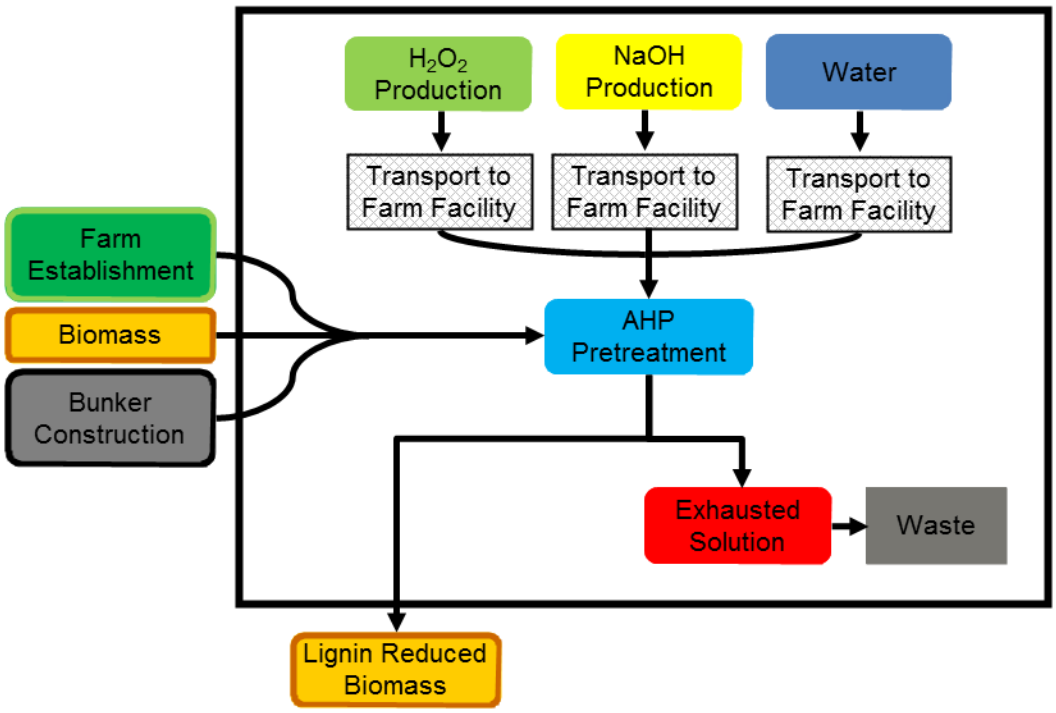


Figure 4-1: System boundary for life cycle analysis of the alkaline hydrogen peroxide pretreatment for an on-farm bioprocessing facility.

These areas are a few of the many directions that future alkaline hydrogen peroxide pretreatment may focus. If the lignin reduction and sterilization methods can be combined for an efficient pretreatment, the on-farm processing paradigm has a high probability for success.

APPENDICES

APPENDIX A: EXPERIMENTAL DATA

CORN STOVER COMPOSITIONAL ANALYSIS – Sterilization technique, pretreatment applied and calculated total lignin content

CORN STOVER		
STERILIZATION	PRETREATMENT	LIGNIN (% DRY MATTER)
SB	NaOH	0.0630
SB	NaOH	0.0860
SB	AHP	0.0715
SB	AHP	0.0635
SB	NaOH	0.0764
SB	NaOH	0.0932
NS	AHP	0.0602
NS	AHP	0.0570
NS	NaOH	0.1099
NS	NaOH	0.1073
NS	AHP	0.0618
NS	AHP	0.0615
SA	NaOH	0.0859
SA	NaOH	0.0877
SA	AHP	0.0781
SA	AHP	0.0694
SA	NaOH	0.0856
SA	NaOH	0.0978
RAW	-	0.2083
SB	AHP	0.0506
SB	AHP	0.0964
SB	NaOH	0.0994
SB	NaOH	0.1197
SB	AHP	0.0738
SB	AHP	0.0781
NS	NaOH	0.0700
NS	NaOH	0.0929
NS	AHP	0.0675
NS	AHP	0.0866
NS	NaOH	0.0938
NS	NaOH	0.0927
SA	AHP	0.0708
SA	AHP	0.0768
SA	NaOH	0.0957
SA	NaOH	0.0914
SA	AHP	0.0819
SA	AHP	0.0725
RAW	-	0.1858
SB	H2O	0.1907
SB	H2O	0.1887
NS	H2O	0.1847
NS	H2O	0.1825
SA	H2O	0.1938
SA	H2O	0.1915
RAW	H2O	0.1864

CORN STOVER COMPOSITIONAL ANALYSIS – Sterilization technique, pretreatment applied and HPLC measured glucose, xylose and arabinose

CORN STOVER				
STERILIZATION	PRETREATMENT	GLUCOSE (mg/L)	XYLOSE (mg/L)	ARABINOSE (mg/L)
SB	AHP	2.2986	0.5977	0.0774
SB	AHP	2.2952	0.6112	0.0799
SB	AHP	1.8503	0.5920	0.0950
SB	AHP	1.8124	0.5589	0.0979
SB	NaOH	2.1832	0.6120	0.1062
SB	NaOH	2.1549	0.6169	0.1193
SB	NaOH	2.0182	0.5528	0.1067
SB	NaOH	2.0220	0.5494	0.1096
SB	AHP	2.1827	0.5520	0.0919
SB	AHP	2.1883	0.5492	0.0869
SB	AHP	2.2196	0.5663	0.0873
SB	AHP	2.2105	0.5668	0.0830
NS	NaOH	1.9753	0.5702	0.1078
NS	NaOH	1.9677	0.5634	0.1250
NS	NaOH	1.9935	0.5737	0.1258
NS	NaOH	1.9900	0.5462	0.1322
NS	AHP	1.9298	0.5541	0.0969
NS	AHP	1.9640	0.5992	0.0975
NS	AHP	1.9150	0.5356	0.0944
NS	AHP	1.9359	0.5382	0.0905
NS	NaOH	1.7572	0.5358	0.1043
NS	NaOH	1.7839	0.5462	0.1202
NS	NaOH	1.8098	0.5412	0.1135
NS	NaOH	1.7976	0.5260	0.1134
SA	AHP	2.2621	0.6112	0.1094
SA	AHP	2.2296	0.5156	0.1094
SA	AHP	2.1141	0.5075	0.0976
SA	AHP	2.1298	0.5793	0.0993
SA	NaOH	2.1413	0.5492	0.1430
SA	NaOH	2.1117	0.5309	0.1377
SA	NaOH	2.2291	0.5700	0.1273
SA	NaOH	2.2213	0.5489	0.1266
SA	AHP	2.3652	0.6731	0.1171
SA	AHP	2.3525	0.7067	0.1142
SA	AHP	2.1508	0.6081	0.1055
SA	AHP	2.1819	0.6139	0.1102
SB	NaOH	1.7471	0.4385	0.0846
SB	NaOH	1.7627	0.4464	0.0848
SB	NaOH	2.2028	0.5298	0.1067
SB	NaOH	2.1779	0.5219	0.1108
SB	AHP	2.1109	0.5767	0.0968
SB	AHP	2.1336	0.5692	0.0987
SB	AHP	2.1635	0.5366	0.1011
SB	AHP	2.1281	0.5302	0.0981
SB	NaOH	2.3742	0.5631	0.1246

SB	NaOH	2.3405	0.5275	0.1227
SB	NaOH	2.1441	0.5557	0.1129
SB	NaOH	2.1314	0.5471	0.1109
NS	AHP	2.2685	0.5883	0.0796
NS	AHP	2.2776	0.5890	0.0837
NS	AHP	2.2784	0.6117	0.0948
NS	AHP	2.2674	0.5894	0.0971
NS	NaOH	1.8556	0.6026	0.1217
NS	NaOH	1.8504	0.6010	0.1252
NS	NaOH	1.8517	0.6296	0.1298
NS	NaOH	1.8684	0.6439	0.1305
NS	AHP	1.8249	0.5511	0.0952
NS	AHP	1.8251	0.5483	0.0906
NS	AHP	1.8725	0.5434	0.0814
NS	AHP	1.8978	0.5497	0.0816
SA	NaOH	2.0818	0.5834	0.1312
SA	NaOH	2.0943	0.5862	0.1199
SA	NaOH	2.0380	0.5172	0.1031
SA	NaOH	2.0178	0.5221	0.1148
SA	AHP	2.0727	0.5400	0.0818
SA	AHP	2.0578	0.5549	0.0874
SA	AHP	2.2885	0.5325	0.0938
SA	AHP	2.2352	0.5313	0.0954
SA	NaOH	1.8240	0.5510	0.1240
SA	NaOH	1.8568	0.5539	0.1164
SA	NaOH	2.0502	0.6686	0.1345
SA	NaOH	2.1003	0.6819	0.1282
RAW	-	1.2768	0.7850	0.1607
RAW	-	1.4000	0.8823	0.1429
RAW	-	1.3833	0.8735	0.1510
SB	H2O	1.7311	0.8212	0.1063
SB	H2O	1.7649	0.7862	0.2093
SB	H2O	1.6564	0.7797	0.1644
SB	H2O	1.6361	0.7341	0.1582
NS	H2O	1.4189	0.7986	0.1486
NS	H2O	1.4025	0.8030	0.1180
NS	H2O	1.3269	0.7519	0.1913
NS	H2O	1.3261	0.7186	0.1937
SA	H2O	1.6863	0.6734	0.1261
SA	H2O	1.7226	0.4893	0.1830
SA	H2O	1.5725	0.8172	0.2442
SA	H2O	1.4957	0.9892	0.2475
SA	H2O	1.2659	0.9483	0.1934
RAW	-	1.2666	0.9152	0.2033

CORN STOVER FERMENTATION PRODUCTS OF *THERMOCELLUM* – Sterilization/Pretreatment, HPLC measured acetate, lactate, ethanol, cellobiose and glucose and calculated fermentation and enzyme products

	CORN STOVER						
INTERACTION	ACETATE (mM)	LACTATE (mM)	ETHANOL (mM)	CELLOBIOSE (mg/L)	GLUCOSE (mg/L)	FERMENTATION (mM)	ENZYME (mg/L)
NSAHP	0.4126	0.4174	0.2051	59.3782	0.0000	1.0352	59.3782
NSAHP	0.4098	0.4561	0.1469	61.5890	0.0000	1.0128	61.5890
NSAHP	0.5813	1.2553	0.5180	181.5767	24.9677	2.3546	206.5444
NSAHP	1.0168	1.2048	0.5470	171.4172	29.6021	2.7686	201.0193
NSAHP	1.1268	0.9122	0.3430	99.5247	26.5486	2.3820	126.0732
NSAHP	1.4094	1.1515	0.0000	126.2605	21.3421	2.5609	147.6026
NSAHP	1.6343	1.4623	0.0000	242.2859	0.0000	3.0966	242.2859
NSAHP	2.3713	1.9753	1.3391	331.4115	10.9679	5.6856	342.3794
NSAHP	1.8881	1.2154	0.5719	225.3064	0.0000	3.6754	225.3064
NSAHP	1.5428	1.4704	0.8357	224.9941	10.5834	3.8489	235.5776
NSAHP	2.4308	1.9481	1.1572	280.7896	1.7882	5.5361	282.5778
NSAHP	1.7903	1.6602	0.9143	235.4971	0.0000	4.3649	235.4971
SBAHP	4.0848	1.0070	1.2508	94.0271	25.2326	6.3427	119.2597
SBAHP	2.1454	0.5422	0.7346	0.0000	22.5354	3.4223	22.5354
SBAHP	0.8932	0.5504	0.0000	87.5404	30.4059	1.4435	117.9463
SBAHP	0.6956	0.5299	0.0000	63.8061	24.9840	1.2256	88.7901
SBAHP	0.8792	1.0641	0.0000	155.8983	31.6091	1.9433	187.5074
SBAHP	0.9920	1.1380	0.0000	172.2035	30.2214	2.1301	202.4249
SBAHP	0.6127	0.5592	0.0000	98.3577	27.3285	1.1719	125.6862
SBAHP	0.5117	0.5620	0.0000	100.1819	0.0000	1.0737	100.1819
SBAHP	10.1056	2.3012	4.3862	209.1125	20.4334	16.7930	229.5459
SBAHP	9.2872	2.2592	7.1652	211.3561	21.8866	18.7115	233.2427
SBAHP	6.4531	1.9297	2.0887	7.8489	15.2789	10.4715	23.1278
SBAHP	6.5093	1.9371	1.8506	189.1824	14.8852	10.2970	204.0676
SAAHP	2.3172	1.6722	1.2974	242.1019	26.4454	5.2867	268.5473
SAAHP	2.0263	1.4744	0.0000	206.7090	31.3692	3.5007	238.0782
SAAHP	1.4954	1.5755	0.5826	269.5283	0.0000	3.6535	269.5283

SAAHP	1.6384	1.6277	0.0000	267.4441	0.0000	3.2661	267.4441
SAAHP	1.2940	1.2421	0.7392	149.9359	35.0817	3.2752	185.0176
SAAHP	1.0875	0.9114	0.5509	111.2956	36.7158	2.5497	148.0114
SAAHP	1.1707	0.9809	0.0000	151.5138	38.9672	2.1516	190.4810
SAAHP	0.8769	0.8503	0.0000	139.7135	42.9396	1.7272	182.6531
SAAHP	1.2279	0.9897	0.0000	161.0142	0.0000	2.2176	161.0142
SAAHP	1.4228	1.0495	0.0000	168.6548	0.0000	2.4723	168.6548
SAAHP	0.8771	0.7557	0.6043	120.7091	41.4945	2.2370	162.2036
SAAHP	0.6953	0.6045	0.3449	103.2919	0.0000	1.6447	103.2919
NSNAOH	1.1345	1.0568	0.5648	242.3105	45.7113	2.7562	288.0218
NSNAOH	1.5470	1.4916	0.7349	339.8151	22.4893	3.7734	362.3045
NSNAOH	1.7062	1.6480	0.9045	276.3582	36.9962	4.2587	313.3544
NSNAOH	1.6812	1.5727	1.1555	256.5051	43.9762	4.4095	300.4813
NSNAOH	1.7353	1.5598	0.8411	253.6288	0.0000	4.1362	253.6288
NSNAOH	1.4250	1.2442	0.0000	201.2583	41.2104	2.6692	242.4688
NSNAOH	2.5792	2.0973	0.0000	387.3716	38.1651	4.6766	425.5367
NSNAOH	2.6135	2.0882	0.8069	384.4200	0.0000	5.5085	384.4200
NSNAOH	1.2420	1.6007	0.2513	294.7784	0.0000	3.0941	294.7784
NSNAOH	1.9057	1.7011	1.2167	303.8956	29.7517	4.8235	333.6473
NSNAOH	1.7844	1.6576	0.5457	311.6396	0.0000	3.9878	311.6396
NSNAOH	1.0496	1.0948	0.3523	216.1157	0.0000	2.4966	216.1157
SBNAOH	21.6460	0.9461	15.5562	292.1713	53.1742	38.1484	345.3455
SBNAOH	19.6158	0.9006	12.2770	264.9634	0.0000	32.7934	264.9634
SBNAOH	8.3191	1.5335	2.8010	126.5197	31.9649	12.6536	158.4846
SBNAOH	6.3265	1.1639	2.7897	94.4025	0.0934	10.2801	94.4959
SBNAOH	1.1222	1.1499	0.4256	217.5802	7.8677	2.6977	225.4479
SBNAOH	0.8848	1.0026	0.0000	177.7484	0.1524	1.8875	177.9008
SBNAOH	4.0950	2.2074	1.0097	292.7210	0.0000	7.3120	292.7210
SBNAOH	4.1272	2.2080	0.7112	295.6920	0.0000	7.0464	295.6920
SBNAOH	4.4810	1.1835	2.5992	106.6802	24.4654	8.2638	131.1456
SBNAOH	5.4166	1.4270	2.6057	0.0000	34.1452	9.4492	34.1452
SBNAOH	7.0347	1.4814	3.2911	0.0000	0.0152	11.8073	0.0152
SBNAOH	7.1064	1.5061	3.0051	100.9832	3.7988	11.6177	104.7820

SANAOH	0.0000	1.1023	0.6935	163.6182	306.5124	1.7958	470.1306
SANAOH	0.9894	0.9532	0.6458	173.9981	289.0068	2.5884	463.0050
SANAOH	0.8598	1.1851	0.6952	224.7360	44.4701	2.7400	269.2062
SANAOH	1.4665	0.5425	0.5116	208.9287	47.4834	2.5206	256.4122
SANAOH	2.7268	0.0000	0.7472	183.2758	46.4799	3.4740	229.7557
SANAOH	0.9449	1.1776	0.0000	184.4228	43.9720	2.1225	228.3948
SANAOH	0.9642	1.3469	0.4584	240.9852	17.5764	2.7694	258.5616
SANAOH	1.0301	1.4858	0.3563	265.0181	16.3550	2.8721	281.3731
SANAOH	1.8798	1.2801	0.6898	104.3814	47.7405	3.8497	152.1219
SANAOH	0.0000	1.2449	0.7593	102.2046	44.7483	2.0042	146.9529
SANAOH	4.7448	0.6403	1.8249	0.0000	32.4200	7.2100	32.4200
SANAOH	4.2122	0.8408	2.3312	0.0000	33.9517	7.3842	33.9517
SBH2O	0.0000	0.0000	0.0000	114.3716	11.4682	0.0000	125.8398
SBH2O	8.6904	0.0000	0.0000	874.7639	0.0000	8.6904	874.7639
SBH2O	8.7866	0.0000	0.0000	0.0000	3418.2460	8.7866	3418.2460
SBH2O	9.2816	0.0000	0.0000	582.5012	3123.5312	9.2816	3706.0324
NSH2O	0.0000	0.7209	0.0000	0.0000	2848.6748	0.7209	2848.6748
NSH2O	0.2487	0.8948	0.0000	0.0000	2853.2663	1.1435	2853.2663
NSH2O	0.4297	0.6106	0.0000	0.0000	0.0000	1.0403	0.0000
NSH2O	0.6070	0.6605	0.0000	0.0000	0.0000	1.2674	0.0000
SAH2O	8.7194	0.0000	0.0000	665.1725	3609.2328	8.7194	4274.4053
SAH2O	8.6779	0.0000	0.0000	664.9385	3345.6885	8.6779	4010.6270
SAH2O	6.6564	0.0000	0.0000	731.6925	553.4095	6.6564	1285.1020
SAH2O	6.9208	0.0000	0.0000	562.3676	522.2589	6.9208	1084.6265

WHEAT STRAW COMPOSITIONAL ANALYSIS – Sterilization technique, pretreatment applied and calculated total lignin content

WHEAT STRAW		
STERILIZATION	PRETREATMENT	LIGNIN (% DRY MATTER)
SB	NaOH	0.1564
SB	NaOH	0.1458
SB	AHP	0.1024
SB	AHP	0.1049
SB	NaOH	0.1663
SB	NaOH	0.1693
NS	AHP	0.0966
NS	AHP	0.0993
NS	NaOH	0.1520
NS	NaOH	0.0376
NS	AHP	0.1176
NS	AHP	0.1090
SA	NaOH	0.1358
SA	NaOH	0.1407
SA	AHP	0.0910
SA	AHP	0.0881
SA	NaOH	0.1491
SA	NaOH	0.1504
RAW		0.2183
SB	AHP	0.0983
SB	AHP	0.0967
SB	NaOH	0.0595
SB	NaOH	0.1797
SB	AHP	0.0888
SB	AHP	0.0911
NS	NaOH	0.1387
NS	NaOH	0.1448
NS	AHP	0.0909
NS	AHP	0.0829
NS	NaOH	0.1462
NS	NaOH	0.1573
SA	AHP	0.0862
SA	AHP	0.0801
SA	NaOH	0.1446
SA	NaOH	0.1873
SA	AHP	0.0895
SA	AHP	0.0908
RAW		0.2161
SB	H2O	0.2491
SB	H2O	0.2468
NS	H2O	0.2245
NS	H2O	0.2355
SA	H2O	0.2328
SA	H2O	0.2306
RAW	-	0.2230

WHEAT STRAW COMPOSITIONAL ANALYSIS – Sterilization technique, pretreatment applied and HPLC measured glucose, xylose and arabinose

WHEAT STRAW				
STERILIZATION	PRETREATMENT	GLUCOSE (mg/L)	XYLOSE (mg/L)	ARABINOSE (mg/L)
SB	AHP	2.1170	0.6515	0.0522
SB	AHP	2.1205	0.6404	0.0502
SB	AHP	2.2039	0.6937	0.0595
SB	AHP	2.2052	0.6907	0.0616
SB	NaOH	2.0536	0.6482	0.1665
SB	NaOH	2.0511	0.6584	0.1136
SB	NaOH	1.8110	0.5996	0.0630
SB	NaOH	1.8177	0.5852	0.0643
SB	AHP	2.3698	0.7677	0.0790
SB	AHP	2.3755	0.7767	0.0785
SB	AHP	2.1794	0.6613	0.0705
SB	AHP	2.1647	0.6392	0.0733
NS	NaOH	1.9702	0.5500	0.0801
NS	NaOH	1.9681	0.5467	0.0803
NS	NaOH	2.0408	0.5598	0.0882
NS	NaOH	2.0324	0.5609	0.0879
NS	AHP	2.1251	0.6828	0.0847
NS	AHP	2.0958	0.6765	0.0814
NS	AHP	2.1968	0.6824	0.0845
NS	AHP	2.1918	0.6750	0.0820
NS	NaOH	1.9823	0.5800	0.0862
NS	NaOH	1.9770	0.5749	0.0904
NS	NaOH	1.8831	0.5572	0.0828
NS	NaOH	1.8794	0.5524	0.0816
SA	AHP	2.2292	0.7075	0.0794
SA	AHP	2.2171	0.7531	0.0864
SA	AHP	2.0406	0.6606	0.0753
SA	AHP	2.0327	0.6474	0.0717
SA	NaOH	1.8550	0.6264	0.0798
SA	NaOH	1.8587	0.5952	0.0859
SA	NaOH	1.9002	0.5621	0.0822
SA	NaOH	1.8733	0.5417	0.0816
SA	AHP	1.9506	0.6423	0.0692
SA	AHP	1.9466	0.6646	0.0796
SA	AHP	2.1153	0.7556	0.0766
SA	AHP	2.1035	0.7321	0.0774
SB	NaOH	1.9145	0.5485	0.0818
SB	NaOH	1.9027	0.5286	0.0758
SB	NaOH	1.9897	0.5442	0.0810
SB	NaOH	1.9329	0.5475	0.0774
SB	AHP	2.3446	0.6640	0.0636
SB	AHP	2.3348	0.6648	0.0623
SB	AHP	2.1593	0.6270	0.0577
SB	AHP	2.1611	0.6287	0.0629
SB	NaOH	2.0464	0.6675	0.0841

SB	NaOH	2.0566	0.6825	0.0808
SB	NaOH	1.8822	0.6286	0.0606
SB	NaOH	1.8908	0.6347	0.1443
NS	AHP	2.1466	0.6888	0.0833
NS	AHP	2.1048	0.6776	0.0873
NS	AHP	2.1418	0.6789	0.0787
NS	AHP	2.1172	0.6695	0.0736
NS	NaOH	1.9782	0.6093	0.0772
NS	NaOH	1.9519	0.5955	0.0774
NS	NaOH	1.9507	0.5499	0.0753
NS	NaOH	1.9536	0.5515	0.0761
NS	AHP	1.9993	0.7262	0.0808
NS	AHP	2.0076	0.7408	0.0820
NS	AHP	2.0706	0.7394	0.0836
NS	AHP	2.0666	0.7381	0.0826
SA	NaOH	2.0961	0.6043	0.0861
SA	NaOH	2.0846	0.5965	0.0916
SA	NaOH	1.9384	0.5552	0.0786
SA	NaOH	1.9230	0.5432	0.0750
SA	AHP	1.9701	0.6403	0.0732
SA	AHP	2.0400	0.6537	0.0729
SA	AHP	2.0943	0.6750	0.0758
SA	AHP	2.1141	0.6822	0.0761
SA	NaOH	1.9824	0.6048	0.0796
SA	NaOH	1.9143	0.5810	0.0768
SA	NaOH	1.8172	0.5381	0.0751
SA	NaOH	1.8237	0.5398	0.0494
RAW	-	1.3019	0.7300	0.0763
RAW	-	1.3065	0.7193	0.0794
RAW	-	1.2246	0.6643	0.0643
RAW	-	1.2316	0.6663	0.0571
SB	H2O	1.5067	0.7245	0.0875
SB	H2O	1.4915	0.6627	0.0699
SB	H2O	1.5084	0.5885	0.1071
SB	H2O	1.4836	0.7088	0.0000
NS	H2O	1.3962	0.8759	0.0693
NS	H2O	1.3957	0.8307	0.0871
NS	H2O	1.3371	0.7884	0.0820
NS	H2O	1.3353	0.7570	0.0889
SA	H2O	1.2534	0.5540	0.0745
SA	H2O	0.2661	0.7512	0.0815
SA	H2O	1.4569	0.5663	0.0794
SA	H2O	1.4364	0.5510	0.0601
SA	H2O	1.2824	0.5984	0.0546
RAW	-	1.2576	0.5481	0.0699

WHEAT STRAW FERMENTATION PRODUCTS OF *C. THERMOCELLUM* – Sterilization/Pretreatment, HPLC measured acetate, lactate, ethanol, cellobiose and glucose and calculated fermentation and enzyme products

	WHEAT STRAW						
INTERACTION	ACETATE (mM)	LACTATE (mM)	ETHANOL (mM)	CELLOBIOSE (mg/L)	GLUCOSE (mg/L)	FERMENTATION (mM)	ENZYME (mg/L)
NSAHP	0.4126	0.0000	0.0000	209.1960	91.7402	0.4126	300.9362
NSAHP	0.4098	0.0000	0.0000	191.0053	0.0000	0.4098	191.0053
NSAHP	0.5813	0.0000	0.0000	125.5581	0.0000	0.5813	125.5581
NSAHP	1.0168	0.0000	0.0000	137.3327	34.5487	1.0168	171.8813
NSAHP	1.1268	0.0000	0.0000	0.0000	0.0000	1.1268	0.0000
NSAHP	1.4094	0.0000	0.0000	144.8352	0.0000	1.4094	144.8352
NSAHP	1.6343	0.0000	0.0000	210.3557	0.0000	1.6343	210.3557
NSAHP	2.3713	0.0000	0.0000	205.5833	0.0000	2.3713	205.5833
NSAHP	1.8881	0.0000	0.0000	246.2703	0.0000	1.8881	246.2703
NSAHP	1.5428	0.0000	0.0000	242.1342	0.0000	1.5428	242.1342
NSAHP	2.4308	0.0000	0.0000	193.4473	0.0000	2.4308	193.4473
NSAHP	1.7903	0.0000	0.0000	201.4866	0.0000	1.7903	201.4866
SBAHP	4.0848	0.0000	0.0000	97.6657	85.2476	4.0848	182.9133
SBAHP	2.1454	0.0000	0.0000	129.4367	79.4319	2.1454	208.8686
SBAHP	0.8932	0.0000	0.0000	160.5389	83.1401	0.8932	243.6790
SBAHP	0.6956	0.0778	0.0000	153.8755	46.1745	0.7734	200.0500
SBAHP	0.8792	0.0140	0.0000	102.3584	35.3936	0.8932	137.7520
SBAHP	0.9920	0.0000	0.0000	0.0000	47.1346	0.9920	47.1346
SBAHP	0.6127	0.0000	0.3512	129.0111	59.5526	0.9639	188.5637
SBAHP	0.5117	0.0000	0.5151	141.1401	65.2300	1.0268	206.3701
SBAHP	10.1056	0.0000	0.3915	0.0000	34.9784	10.4971	34.9784
SBAHP	9.2872	0.0000	0.2669	102.2169	58.1068	9.5540	160.3236
SBAHP	6.4531	0.0000	0.2900	211.0587	142.2902	6.7431	353.3489
SBAHP	6.5093	0.0000	0.4383	289.3679	37.7847	6.9476	327.1526
SAAHP	2.3172	0.0000	0.0000	276.7062	135.9520	2.3172	412.6582
SAAHP	2.0263	0.0000	0.0000	286.9038	0.0000	2.0263	286.9038
SAAHP	1.4954	0.0000	0.0000	173.9774	0.0000	1.4954	173.9774

SAAHP	1.6384	0.0000	0.0000	152.5943	0.0000	1.6384	152.5943
SAAHP	1.2940	0.0000	0.0000	46.8148	0.0000	1.2940	46.8148
SAAHP	1.0875	0.0000	0.0000	47.9884	0.0000	1.0875	47.9884
SAAHP	1.1707	0.0000	0.0000	94.6227	0.0000	1.1707	94.6227
SAAHP	0.8769	0.0000	0.0000	0.0000	0.0000	0.8769	0.0000
SAAHP	1.2279	0.0000	0.0000	142.0934	141.5634	1.2279	283.6568
SAAHP	1.4228	0.0000	0.0000	145.9208	139.6175	1.4228	285.5383
SAAHP	0.8771	0.0000	0.0000	0.0000	0.0000	0.8771	0.0000
SAAHP	0.6953	0.0000	0.0000	90.8322	65.7800	0.6953	156.6123
NSNAOH	1.1345	0.0000	0.0000	0.0000	0.0000	1.1345	0.0000
NSNAOH	1.5470	0.0000	0.0000	0.0000	225.1671	1.5470	225.1671
NSNAOH	1.7062	0.0000	0.0000	83.5584	198.8225	1.7062	282.3809
NSNAOH	1.6812	0.0000	0.0000	0.0000	199.0752	1.6812	199.0752
NSNAOH	1.7353	0.0000	0.0000	139.4727	245.9679	1.7353	385.4406
NSNAOH	1.4250	0.0000	0.0000	136.9883	233.4599	1.4250	370.4482
NSNAOH	2.5792	0.0000	0.0000	0.0000	247.6095	2.5792	247.6095
NSNAOH	2.6135	0.0574	0.0000	0.0000	260.6019	2.6708	260.6019
NSNAOH	1.2420	0.0000	0.0000	243.1069	180.4367	1.2420	423.5437
NSNAOH	1.9057	0.0000	0.0000	216.4445	204.7633	1.9057	421.2078
NSNAOH	1.7844	0.0000	0.0000	238.0967	0.0000	1.7844	238.0967
NSNAOH	1.0496	0.0000	0.0000	256.1104	0.0000	1.0496	256.1104
SBNAOH	21.6460	0.0000	0.0000	240.4643	46.8023	21.6460	287.2666
SBNAOH	19.6158	0.0000	0.4395	256.0148	53.0950	20.0553	309.1098
SBNAOH	8.3191	0.0000	0.0016	375.4732	75.7312	8.3207	451.2044
SBNAOH	6.3265	0.0000	0.0000	402.7698	51.1717	6.3265	453.9415
SBNAOH	1.1222	0.0000	0.0000	368.1438	0.0000	1.1222	368.1438
SBNAOH	0.8848	0.0000	0.0000	347.9588	0.0000	0.8848	347.9588
SBNAOH	4.0950	0.0000	0.0000	230.5762	0.0000	4.0950	230.5762
SBNAOH	4.1272	0.0000	0.0000	269.3199	0.0000	4.1272	269.3199
SBNAOH	4.4810	0.0000	0.0000	233.0624	0.0000	4.4810	233.0624
SBNAOH	5.4166	0.0000	0.0000	263.2828	0.0000	5.4166	263.2828
SBNAOH	7.0347	0.0000	0.0000	548.3284	0.0000	7.0347	548.3284
SBNAOH	7.1064	0.0000	0.0000	524.7291	0.0000	7.1064	524.7291

SANAOH	0.0000	0.0000	0.0000	138.4732	133.0762	0.0000	271.5494
SANAOH	0.9894	0.0000	0.0000	128.3156	0.0000	0.9894	128.3156
SANAOH	0.8598	0.0000	2.6369	0.0000	97.5306	3.4966	97.5306
SANAOH	1.4665	0.0000	2.6037	0.0000	0.0000	4.0702	0.0000
SANAOH	2.7268	0.0000	0.0000	162.3735	0.0000	2.7268	162.3735
SANAOH	0.9449	0.0000	0.0000	189.3174	0.0000	0.9449	189.3174
SANAOH	0.9642	0.0000	0.0000	231.5247	0.0000	0.9642	231.5247
SANAOH	1.0301	0.0000	0.0000	220.1676	116.6020	1.0301	336.7696
SANAOH	1.8798	0.0000	0.0992	0.0000	0.0000	1.9789	0.0000
SANAOH	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
SANAOH	4.7448	0.0000	0.0000	140.8811	85.5370	4.7448	226.4181
SANAOH	4.2122	0.0000	0.0000	133.1347	39.9026	4.2122	173.0373
SBH2O	10.3157	0.0000	0.0000	588.1881	3500.9934	10.3157	4089.1815
SBH2O	11.5897	0.0000	0.0000	735.1208	850.3932	11.5897	1585.5140
SBH2O	7.1601	0.0000	0.0000	373.3163	494.9911	7.1601	868.3074
SBH2O	7.1254	0.0000	0.0000	442.8638	487.0090	7.1254	929.8729
NSH2O	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
NSH2O	0.0000	0.0000	0.0000	0.0000	2761.2965	0.0000	2761.2965
NSH2O	0.0000	0.0000	0.0000	0.0000	2681.6565	0.0000	2681.6565
NSH2O	0.0000	0.0000	0.0000	0.0000	2671.9387	0.0000	2671.9387
SAH2O	8.0202	0.0000	0.0000	295.8734	427.3234	8.0202	723.1968
SAH2O	7.8454	0.0000	0.0000	474.9000	0.0000	7.8454	474.9000
SAH2O	4.7043	0.0000	0.0000	330.6712	376.0809	4.7043	706.7521
SAH2O	4.8575	0.0000	0.0000	282.8669	390.8669	4.8575	673.7339

SWITCHGRASS COMPOSITIONAL ANALYSIS – Sterilization technique, pretreatment applied and calculated total lignin content

SWITCHGRASS		
STERILIZATION	PRETREATMENT	LIGNIN (% DRY MATTER)
SB	NaOH	0.1826
SB	NaOH	0.2194
SB	AHP	0.1543
SB	AHP	0.1740
SB	NaOH	0.1737
SB	NaOH	0.1675
NS	AHP	0.1827
NS	AHP	0.1814
NS	NaOH	0.2074
NS	NaOH	0.1844
NS	AHP	0.1618
NS	AHP	0.1626
SA	NaOH	0.1567
SA	NaOH	0.1602
SA	AHP	0.1371
SA	AHP	0.1370
SA	NaOH	0.1530
SA	NaOH	0.1688
RAW		0.2283
SB	AHP	0.1604
SB	AHP	0.1493
SB	NaOH	0.1758
SB	NaOH	0.1712
SB	AHP	0.1244
SB	AHP	0.1246
NS	NaOH	0.1800
NS	NaOH	0.2046
NS	AHP	0.1530
NS	AHP	0.1542
NS	NaOH	0.1772
NS	NaOH	0.1951
SA	AHP	0.1248
SA	AHP	0.1173
SA	NaOH	0.1485
SA	NaOH	0.1545
SA	AHP	0.1222
SA	AHP	0.1141
RAW		0.2293
SB	H2O	0.2275
SB	H2O	0.2229
NS	H2O	0.2212
NS	H2O	0.2194
SA	H2O	0.2185
SA	H2O	0.2199
RAW	-	0.2361

SWITCHGRASS COMPOSITIONAL ANALYSIS – Sterilization technique, pretreatment applied and HPLC measured glucose, xylose and arabinose

SWITCHGRASS				
STERILIZATION	PRETREATMENT	GLUCOSE (mg/L)	XYLOSE (mg/L)	ARABINOSE (mg/L)
SB	AHP	2.1165	0.4848	0.0000
SB	AHP	2.1334	0.4428	0.0000
SB	AHP	2.2938	0.5307	0.0000
SB	AHP	2.3158	0.5359	0.0000
SB	NaOH	1.8700	0.6080	0.1270
SB	NaOH	1.8750	0.7926	0.2871
SB	NaOH	2.0148	0.6238	0.1265
SB	NaOH	2.0400	0.6899	0.0000
SB	AHP	2.0932	0.4660	0.1742
SB	AHP	2.0848	0.4223	0.1893
SB	AHP	2.0073	0.2955	0.0994
SB	AHP	2.0184	0.4240	0.1776
NS	NaOH	1.7880	0.5928	0.1553
NS	NaOH	1.8034	0.5922	0.0000
NS	NaOH	1.8820	0.6776	0.2030
NS	NaOH	1.8844	0.6196	0.2972
NS	AHP	2.1685	0.4716	0.2275
NS	AHP	2.1909	0.4740	0.3029
NS	AHP	2.3229	0.4823	0.0000
NS	AHP	2.3146	0.4716	0.0000
NS	NaOH	1.7174	0.6417	0.2406
NS	NaOH	1.6909	0.6350	0.3502
NS	NaOH	1.6529	0.6045	0.1978
NS	NaOH	1.6907	0.6216	0.3749
SA	AHP	2.1180	0.4727	0.0000
SA	AHP	2.1382	0.5574	0.2239
SA	AHP	2.1555	0.4838	0.2859
SA	AHP	2.1597	0.4702	0.0000
SA	NaOH	1.9106	0.6964	0.3501
SA	NaOH	1.9050	0.7051	0.3437
SA	NaOH	1.9511	0.6441	0.3475
SA	NaOH	1.9097	0.5670	0.2544
SA	AHP	2.2449	0.4630	0.2099
SA	AHP	2.2289	0.4195	0.2748
SA	AHP	2.0723	0.3770	0.0000
SA	AHP	2.1258	0.4484	0.2417
SB	NaOH	1.8904	0.6692	0.2548
SB	NaOH	1.8553	0.6264	0.2603
SB	NaOH	2.0400	0.6408	0.2370
SB	NaOH	1.9930	0.6116	0.1846
SB	AHP	2.2960	0.4288	0.1522
SB	AHP	2.2881	0.4680	0.0000
SB	AHP	2.3294	0.4765	0.2351
SB	AHP	2.2944	0.3918	0.1356
SB	NaOH	1.9710	0.5803	0.2510

SB	NaOH	1.9294	0.6923	0.1850
SB	NaOH	2.0012	0.6540	0.1938
SB	NaOH	2.0036	0.6964	0.1645
NS	AHP	2.2320	0.4428	0.0748
NS	AHP	2.2091	0.3999	0.1181
NS	AHP	2.2947	0.3422	0.1536
NS	AHP	2.2453	0.4232	0.0834
NS	NaOH	1.8402	0.5978	0.2218
NS	NaOH	1.7878	0.6909	0.1727
NS	NaOH	1.7705	0.6379	0.2316
NS	NaOH	1.8017	0.5847	0.1540
NS	AHP	2.1626	0.3623	0.0876
NS	AHP	2.2091	0.3514	0.1483
NS	AHP	2.0793	0.3099	0.1178
NS	AHP	2.1397	0.3008	0.0925
SA	NaOH	1.8793	0.5831	0.1826
SA	NaOH	1.8984	0.6480	0.1647
SA	NaOH	1.8903	0.5993	0.1097
SA	NaOH	1.8728	0.6414	0.1623
SA	AHP	2.1658	0.3483	0.0909
SA	AHP	2.2224	0.3785	0.1108
SA	AHP	2.3814	0.4863	0.0860
SA	AHP	2.4129	0.4623	0.0661
SA	NaOH	1.8696	0.5962	0.1471
SA	NaOH	1.8801	0.6078	0.1402
SA	NaOH	1.9413	0.6171	0.1748
SA	NaOH	1.9380	0.6713	0.1652
RAW	-	1.2780	0.9340	0.2562
RAW	-	1.2837	0.8583	0.3553
RAW	-	1.3500	1.0174	0.3467
RAW	-	1.3337	1.1171	0.2988
SB	H2O	1.4010	1.1612	0.3270
SB	H2O	1.3838	1.2413	0.3993
SB	H2O	1.3848	1.2286	0.4118
SB	H2O	1.4444	1.2833	0.4411
NS	H2O	1.3243	1.1452	0.5228
NS	H2O	1.3203	1.1337	0.4657
NS	H2O	1.3465	1.1687	0.4317
NS	H2O	1.3431	1.0984	0.4968
SA	H2O	1.3927	0.9923	0.1832
SA	H2O	1.3595	1.1867	0.2537
SA	H2O	1.4546	1.0914	0.3491
SA	H2O	1.4204	1.2216	0.3579
SA	H2O	1.2284	1.3530	0.2805
RAW	-	1.2371	1.2657	0.2973

SWITCHGRASS FERMENTATION PRODUCTS OF *C. THERMOCELLUM* – Sterilization/Pretreatment, HPLC measured acetate, lactate, ethanol, cellobiose and glucose and calculated fermentation and enzyme products

	SWITCHGRASS						
INTERACTION	ACETATE (mM)	LACTATE (mM)	ETHANOL (mM)	CELLOBIOSE (mg/L)	GLUCOSE (mg/L)	FERMENTATION (mM)	ENZYME (mg/L)
NSAHP	1.2512	0.0000	0.0000	772.5393	2472.0125	1.2512	3244.5518
NSAHP	1.1393	0.0000	0.0000	1098.0097	0.0000	1.1393	1098.0097
NSAHP	0.9832	0.0000	0.0000	1108.4148	0.0000	0.9832	1108.4148
NSAHP	0.1569	0.0000	0.0000	983.0078	0.0000	0.1569	983.0078
NSAHP	0.4348	0.0000	0.0000	1302.2969	0.0000	0.4348	1302.2969
NSAHP	0.6484	0.0000	0.0000	1066.5258	0.0000	0.6484	1066.5258
NSAHP	0.5229	0.0000	0.0000	648.2019	0.0000	0.5229	648.2019
NSAHP	0.5873	0.0000	0.0000	646.2484	273.6017	0.5873	919.8501
NSAHP	1.2014	0.0000	0.0000	346.3100	350.3260	1.2014	696.6360
NSAHP	1.1078	0.0000	0.0000	393.1929	0.0000	1.1078	393.1929
NSAHP	0.5797	0.0000	0.6292	647.2478	0.0000	1.2090	647.2478
NSAHP	1.0334	0.0000	0.3533	874.9933	0.0000	1.3867	874.9933
SBAHP	1.8393	0.0000	0.9091	575.5226	84.1478	2.7484	659.6704
SBAHP	1.5367	0.0000	0.0000	652.7468	55.6140	1.5367	708.3609
SBAHP	1.1044	0.0000	0.0000	648.5159	0.0000	1.1044	648.5159
SBAHP	0.9638	0.0000	0.4267	581.5082	0.0000	1.3905	581.5082
SBAHP	1.0707	0.0000	0.4988	690.5810	0.0000	1.5695	690.5810
SBAHP	1.2120	0.0000	0.4441	674.3908	0.0000	1.6561	674.3908
SBAHP	0.8176	0.0000	0.3446	692.2648	0.0000	1.1622	692.2648
SBAHP	0.9194	0.0000	0.0000	651.8581	0.0000	0.9194	651.8581
SBAHP	1.9380	0.0000	0.8832	876.7493	0.0000	2.8212	876.7493
SBAHP	1.4106	0.0000	0.4815	831.7262	0.0000	1.8921	831.7262
SBAHP	1.5594	0.0429	0.4709	606.3432	0.0000	2.0732	606.3432
SBAHP	1.5364	0.0678	0.6862	582.1385	0.0000	2.2903	582.1385
SAAHP	1.0444	0.0000	0.1152	266.5626	0.0000	1.1597	266.5626
SAAHP	0.8486	0.0000	0.0000	212.9984	0.0000	0.8486	212.9984
SAAHP	2.4347	0.0000	0.8090	361.7724	0.0000	3.2436	361.7724

SAAHP	2.0425	0.0000	1.0592	225.5165	0.0000	3.1017	225.5165
SAAHP	3.2958	0.0764	1.1411	232.1554	0.0000	4.5132	232.1554
SAAHP	2.8130	0.0394	0.9113	235.8389	0.0000	3.7636	235.8389
SAAHP	1.4748	0.0000	0.5576	407.7058	0.0000	2.0323	407.7058
SAAHP	1.5642	0.0000	0.3303	380.3254	0.0000	1.8945	380.3254
SAAHP	1.2614	0.0000	0.0000	729.2365	0.0000	1.2614	729.2365
SAAHP	1.1605	0.0000	0.0000	889.7112	0.0000	1.1605	889.7112
SAAHP	1.2713	0.0000	0.0000	427.3942	0.0000	1.2713	427.3942
SAAHP	0.7342	0.0000	0.0000	365.4256	0.0000	0.7342	365.4256
NSNAOH	1.2401	0.0000	0.3520	683.7757	0.0000	1.5921	683.7757
NSNAOH	1.6076	0.0000	0.5773	659.9897	0.0000	2.1849	659.9897
NSNAOH	0.6527	0.0000	0.0000	651.0518	0.0000	0.6527	651.0518
NSNAOH	0.8935	0.0094	0.0000	556.7012	0.0000	0.9029	556.7012
NSNAOH	0.8356	0.0000	0.0000	618.3373	0.0000	0.8356	618.3373
NSNAOH	1.1683	0.0000	0.0000	599.4993	0.0000	1.1683	599.4993
NSNAOH	1.3876	0.0000	0.0000	650.6521	41.9955	1.3876	692.6476
NSNAOH	1.2239	0.0183	0.1128	698.4058	0.0000	1.3550	698.4058
NSNAOH	1.1523	0.0000	0.4216	759.1319	0.0000	1.5738	759.1319
NSNAOH	1.3797	0.0000	0.0000	679.3689	0.0000	1.3797	679.3689
NSNAOH	0.9097	5.1997	0.0000	710.4031	0.0000	6.1094	710.4031
NSNAOH	1.2649	5.4600	0.1195	687.5890	0.0000	6.8444	687.5890
SBNAOH	1.1808	0.0000	0.0000	134.7062	0.0000	1.1808	134.7062
SBNAOH	0.5627	0.0000	0.0000	153.9396	0.0000	0.5627	153.9396
SBNAOH	0.4986	0.0000	0.0000	322.3132	0.0000	0.4986	322.3132
SBNAOH	0.6387	0.0000	0.0000	240.8104	0.0000	0.6387	240.8104
SBNAOH	0.4572	0.0012	0.0000	315.8597	170.7809	0.4584	486.6406
SBNAOH	0.8407	0.0000	0.0000	315.7028	97.4356	0.8407	413.1384
SBNAOH	1.0741	0.0000	0.1417	181.8093	139.5051	1.2158	321.3144
SBNAOH	1.2453	0.0000	0.0000	148.9134	195.4095	1.2453	344.3229
SBNAOH	2.0668	0.6206	0.0000	73.4517	147.9386	2.6874	221.3903
SBNAOH	1.6485	0.0217	0.0856	257.3947	265.2345	1.7557	522.6292
SBNAOH	0.0000	0.0000	0.0000	345.8797	234.2682	0.0000	580.1479
SBNAOH	1.2819	0.0000	0.0000	165.9234	220.3205	1.2819	386.2439

SANAOH	1.6049	0.0035	0.3737	160.0474	248.0243	1.9822	408.0717
SANAOH	1.4951	0.0241	0.0000	589.1447	0.0000	1.5192	589.1447
SANAOH	1.8478	0.1155	0.0000	633.9664	274.6112	1.9633	908.5776
SANAOH	1.8310	0.1940	0.5569	478.9312	263.4758	2.5819	742.4069
SANAOH	1.5148	0.0000	0.5724	646.0054	353.2567	2.0872	999.2621
SANAOH	1.2993	0.0000	0.4523	773.2257	271.6888	1.7515	1044.9145
SANAOH	1.6498	0.1325	0.6013	122.7189	204.1781	2.3835	326.8970
SANAOH	1.1848	0.0000	0.2014	447.7485	136.0989	1.3862	583.8473
SANAOH	1.7564	0.2770	0.2230	525.4024	120.6193	2.2564	646.0217
SANAOH	1.8205	0.0000	0.0000	496.8998	0.0000	1.8205	496.8998
SANAOH	0.8920	0.0187	0.3207	400.8335	0.0000	1.2314	400.8335
SANAOH	1.0336	0.0167	0.0283	81.7746	0.0000	1.0786	81.7746
SBH2O	12.4372	0.0000	0.0000	291.7861	400.7527	12.4372	692.5388
SBH2O	12.4495	0.0000	0.0000	392.6975	422.2892	12.4495	814.9867
SBH2O	12.6435	0.0000	0.0000	287.8536	348.5940	12.6435	636.4477
SBH2O	11.6516	0.0000	0.0000	267.7806	527.3077	11.6516	795.0883
NSH2O	0.5776	0.0000	0.0000	0.0000	201.5371	0.5776	201.5371
NSH2O	0.5643	0.0000	0.0000	0.0000	334.2143	0.5643	334.2143
NSH2O	0.3349	0.0000	0.0000	0.0000	281.6985	0.3349	281.6985
NSH2O	0.4232	0.0000	0.0000	0.0000	184.6922	0.4232	184.6922
SAH2O	10.8768	0.0000	0.0000	208.9434	0.0000	10.8768	208.9434
SAH2O	11.2713	0.0000	0.0000	246.8819	405.6673	11.2713	652.5492
SAH2O	9.6531	0.0000	0.0000	242.0916	397.7133	9.6531	639.8050
SAH2O	8.5892	0.0000	0.0000	239.6435	363.4392	8.5892	603.0827

MISCANTHUS COMPOSITIONAL ANALYSIS – Sterilization technique, pretreatment applied and calculated total lignin content

MISCANTHUS		
STERILIZATION	PRETREATMENT	LIGNIN (% DRY MATTER)
SB	NaOH	0.2204
SB	NaOH	0.2366
SB	AHP	0.1611
SB	AHP	0.1558
SB	NaOH	0.2560
SB	NaOH	0.2350
NS	AHP	0.2093
NS	AHP	0.1725
NS	NaOH	0.2311
NS	NaOH	0.2235
NS	AHP	0.1798
NS	AHP	0.1666
SA	NaOH	0.1905
SA	NaOH	0.1967
SA	AHP	0.1564
SA	AHP	0.1384
SA	NaOH	0.1704
SA	NaOH	0.2185
RAW	-	0.2703
SB	AHP	0.1381
SB	AHP	0.1273
SB	NaOH	0.2183
SB	NaOH	0.2123
SB	AHP	0.1217
SB	AHP	0.1281
NS	NaOH	0.2102
NS	NaOH	0.2164
NS	AHP	0.1480
NS	AHP	0.1419
NS	NaOH	0.2344
NS	NaOH	0.2351
SA	AHP	0.1528
SA	AHP	0.1225
SA	NaOH	0.1787
SA	NaOH	0.1882
SA	AHP	0.1379
SA	AHP	0.1227
RAW	-	0.2514
SB	H2O	0.2654
SB	H2O	0.2581
NS	H2O	0.2555
NS	H2O	0.2646
SA	H2O	0.2449
SA	H2O	0.2502
RAW	-	0.2725

MISCANTHUS COMPOSITIONAL ANALYSIS – Sterilization technique, pretreatment applied and HPLC measured glucose, xylose and arabinose

MISCANTHUS				
STERILIZATION	PRETREATMENT	GLUCOSE (mg/L)	XYLOSE (mg/L)	ARABINOSE (mg/L)
SB	AHP	3.0512	0.6013	0.0770
SB	AHP	2.8319	1.2722	0.0982
SB	AHP	3.2163	0.6870	0.1476
SB	AHP	3.3693	0.5124	0.1662
SB	NaOH	2.8964	0.8639	0.2211
SB	NaOH	2.9159	0.8674	0.2318
SB	NaOH	2.6999	0.8186	0.2014
SB	NaOH	2.7054	0.7997	0.2001
SB	AHP	3.7787	0.5039	0.1317
SB	AHP	3.7589	0.4519	0.1293
SB	AHP	3.2885	0.6080	0.1622
SB	AHP	3.3051	0.5400	0.1458
NS	NaOH	2.5708	0.7731	0.1999
NS	NaOH	2.5491	0.7689	0.1522
NS	NaOH	2.5698	0.8364	0.2229
NS	NaOH	2.5739	0.8066	0.2202
NS	AHP	3.2433	0.4721	0.1085
NS	AHP	3.2016	0.5112	0.1002
NS	AHP	3.2893	0.4599	0.1028
NS	AHP	3.2933	0.4601	0.1048
NS	NaOH	2.5642	0.7694	0.1595
NS	NaOH	2.5711	0.7759	0.1652
NS	NaOH	2.6689	0.8359	0.2013
NS	NaOH	2.6691	0.8525	0.1958
SA	AHP	3.4092	0.5269	0.0895
SA	AHP	3.3876	0.5330	0.0962
SA	AHP	3.6005	0.4953	0.1104
SA	AHP	3.5856	0.4976	0.1228
SA	NaOH	2.4486	0.6746	0.1652
SA	NaOH	2.4624	0.6582	0.1611
SA	NaOH	3.0022	0.8500	0.1869
SA	NaOH	2.9798	0.8635	0.1862
SA	AHP	3.4924	0.6175	0.1395
SA	AHP	3.5445	0.5262	0.1411
SA	AHP	3.4769	0.5066	0.1364
SA	AHP	3.4812	0.5203	0.1409
SB	NaOH	2.5385	0.7023	0.1941
SB	NaOH	2.5207	0.7230	0.1877
SB	NaOH	2.5602	0.7935	0.2028
SB	NaOH	2.6179	0.7098	0.2015
SB	AHP	3.2891	0.4562	0.1184
SB	AHP	3.2438	0.4422	0.1149
SB	AHP	3.4730	0.4499	0.1266
SB	AHP	3.4809	0.4513	0.1171
SB	NaOH	2.5849	0.8134	0.2178

SB	NaOH	2.6076	0.7717	0.2292
SB	NaOH	2.6210	0.7204	0.2238
SB	NaOH	2.5771	0.7595	0.2252
NS	AHP	2.9332	0.6004	0.1246
NS	AHP	2.9899	0.4789	0.1253
NS	AHP	3.1180	0.5712	0.1226
NS	AHP	3.1271	0.4794	0.1298
NS	NaOH	2.3686	0.8120	0.1804
NS	NaOH	2.3853	0.7734	0.1895
NS	NaOH	2.5265	0.8906	0.2427
NS	NaOH	2.5106	0.8739	0.2605
NS	AHP	2.9934	0.5642	0.1396
NS	AHP	2.9924	0.6072	0.1445
NS	AHP	3.0399	0.4696	0.1363
NS	AHP	3.0332	0.4720	0.1318
SA	NaOH	2.8928	0.7645	0.2002
SA	NaOH	2.8878	0.7693	0.1872
SA	NaOH	2.7704	0.7366	0.1531
SA	NaOH	2.7766	0.7502	0.1655
SA	AHP	3.0778	0.5046	0.1085
SA	AHP	3.0848	0.4822	0.1361
SA	AHP	3.2737	0.4776	0.1325
SA	AHP	3.2847	0.4726	0.1311
SA	NaOH	2.6809	0.7620	0.2665
SA	NaOH	2.7387	0.8170	0.2144
SA	NaOH	2.9260	0.8905	0.2783
SA	NaOH	2.9660	0.8827	0.2060
RAW	-	1.6704	1.0901	0.1997
RAW	-	1.6870	1.0938	0.1970
RAW	-	1.6546	1.0650	0.1881
RAW	-	1.6645	1.0446	0.1813
SB	H2O	1.4704	0.8823	0.0740
SB	H2O	1.4994	0.7992	0.0463
SB	H2O	1.5891	0.8797	0.0606
SB	H2O	1.6338	0.9506	0.0807
NS	H2O	1.4413	0.8014	0.0692
NS	H2O	1.4960	0.9584	0.0644
NS	H2O	1.5424	0.6652	0.0828
NS	H2O	1.5125	0.9261	0.1564
SA	H2O	1.5696	1.0128	0.0997
SA	H2O	1.5426	0.9851	0.0843
SA	H2O	1.5486	0.6127	0.0637
SA	H2O	1.5607	1.0119	0.1013
SA	H2O	1.3935	0.9355	0.1050
RAW	-	1.4205	0.8601	0.1360

MISCANTHUS FERMENTATION PRODUCTS OF *C. THERMOCELLUM* – Sterilization/Pretreatment, HPLC measured acetate, lactate, ethanol, cellobiose and glucose and calculated fermentation and enzyme products

MISCANTHUS							
INTERACTION	ACETATE (mM)	LACTATE (mM)	ETHANOL (mM)	CELLOBIOSE (mg/L)	GLUCOSE (mg/L)	FERMENTATION (mM)	ENZYME (mg/L)
NSAHP	0.4263	0.0000	0.0000	74.7542	55.9073	0.4263	130.6615
NSAHP	0.3901	0.0000	0.0000	26.6720	0.0000	0.3901	26.6720
NSAHP	0.3371	0.0000	0.0000	36.8297	1660.5324	0.3371	1697.3621
NSAHP	0.3795	0.0000	0.0000	89.3444	0.0000	0.3795	89.3444
NSAHP	0.4700	0.0000	0.0000	116.1873	0.0000	0.4700	116.1873
NSAHP	0.4520	0.0000	0.7546	99.0419	0.0000	1.2066	99.0419
NSAHP	0.3736	0.0000	0.0000	51.5299	1867.0077	0.3736	1918.5377
NSAHP	0.4460	0.0000	0.0000	82.1922	1980.2209	0.4460	2062.4132
NSAHP	0.2799	0.0000	0.0000	57.6730	0.0000	0.2799	57.6730
NSAHP	0.3348	0.0000	0.0687	99.5664	0.0000	0.4035	99.5664
NSAHP	0.4543	0.0000	0.0000	70.5639	0.0000	0.4543	70.5639
NSAHP	0.4717	0.0255	0.0000	56.7069	0.0000	0.4972	56.7069
SBAHP	0.5905	0.0322	0.0000	153.9869	2151.1831	0.6227	2305.1701
SBAHP	0.5787	0.0191	0.0000	237.5866	2208.3537	0.5978	2445.9403
SBAHP	0.2892	0.0138	0.0000	134.9987	0.0000	0.3030	134.9987
SBAHP	0.3410	0.0203	0.0000	106.3442	0.0000	0.3613	106.3442
SBAHP	0.3806	0.0222	0.0000	209.1719	0.0000	0.4027	209.1719
SBAHP	0.4159	0.0223	0.0000	88.5139	0.0000	0.4383	88.5139
SBAHP	0.3148	0.0211	0.0000	151.3037	0.0000	0.3359	151.3037
SBAHP	0.5091	0.0228	0.0000	169.6841	75.6835	0.5319	245.3676
SBAHP	0.0000	0.0000	0.0000	200.4242	143.2366	0.0000	343.6608
SBAHP	0.4024	0.0000	0.0000	264.6843	125.1087	0.4024	389.7930
SBAHP	0.3735	0.0000	0.0000	175.9387	2124.4950	0.3735	2300.4337
SBAHP	0.3079	0.0000	0.0000	87.2293	2157.0485	0.3079	2244.2778
SAAHP	0.3451	0.0000	0.0000	63.7850	0.0000	0.3451	63.7850
SAAHP	0.2914	0.0000	0.0000	172.7606	94.3031	0.2914	267.0637
SAAHP	0.4423	0.0000	0.0000	104.8845	2198.2417	0.4423	2303.1262

SAAHP	0.2528	0.0000	0.0000	152.0193	0.0000	0.2528	152.0193
SAAHP	0.2431	0.0000	0.0000	107.8250	0.0000	0.2431	107.8250
SAAHP	0.1927	0.0000	0.0000	138.5798	0.0000	0.1927	138.5798
SAAHP	0.2191	0.0000	0.0000	123.9268	93.7434	0.2191	217.6702
SAAHP	0.1308	0.0000	0.0000	127.0347	75.0353	0.1308	202.0700
SAAHP	0.8169	0.0000	0.0000	137.2817	0.0000	0.8169	137.2817
SAAHP	0.2425	0.0000	0.0000	151.4182	70.1548	0.2425	221.5730
SAAHP	0.1589	0.0000	0.0000	183.6426	0.0000	0.1589	183.6426
SAAHP	0.2447	0.0000	0.0000	155.7731	0.0000	0.2447	155.7731
NSNAOH	0.4226	0.0000	0.0000	22.0100	63.0623	0.4226	85.0722
NSNAOH	0.8035	0.0000	0.0000	62.3707	38.2144	0.8035	100.5851
NSNAOH	0.4447	0.0000	0.0000	54.8786	50.6821	0.4447	105.5606
NSNAOH	0.4356	0.0000	0.0000	65.2012	33.3143	0.4356	98.5155
NSNAOH	0.6320	0.0000	0.0000	72.0183	34.3377	0.6320	106.3560
NSNAOH	0.6355	0.0000	0.0000	61.1140	30.3950	0.6355	91.5089
NSNAOH	0.5988	0.0000	0.0000	76.0317	42.0872	0.5988	118.1190
NSNAOH	0.6364	0.0000	0.0000	85.7589	58.3729	0.6364	144.1318
NSNAOH	0.5339	0.0000	0.0000	86.1467	63.2208	0.5339	149.3675
NSNAOH	0.4905	0.0000	0.0000	87.0102	2606.4886	0.4905	2693.4988
NSNAOH	0.3913	0.0000	0.0000	90.9036	106.2359	0.3913	197.1395
NSNAOH	0.5492	0.0000	0.0000	109.1730	89.4477	0.5492	198.6207
SBNAOH	0.5795	0.0000	0.0000	81.9303	0.0000	0.5795	81.9303
SBNAOH	0.7072	0.0000	0.0000	105.2556	111.0228	0.7072	216.2785
SBNAOH	0.4758	0.0000	0.0000	82.4133	92.2778	0.4758	174.6911
SBNAOH	0.5406	0.0000	0.0000	75.0908	97.2096	0.5406	172.3004
SBNAOH	0.5199	0.0000	0.0000	47.4908	236.5426	0.5199	284.0334
SBNAOH	0.4128	0.0000	0.0000	40.2540	126.5678	0.4128	166.8218
SBNAOH	0.3842	0.0000	0.0000	70.8021	221.4915	0.3842	292.2936
SBNAOH	0.3535	0.0000	0.0000	84.2536	163.7748	0.3535	248.0283
SBNAOH	0.3376	0.0000	0.0000	98.7600	102.3321	0.3376	201.0921
SBNAOH	0.3073	0.0000	0.0000	54.0907	91.2549	0.3073	145.3456
SBNAOH	0.3676	0.0000	0.0000	65.4190	114.2549	0.3676	179.6739
SBNAOH	0.3430	0.0000	0.0000	75.7862	127.5371	0.3430	203.3233

SANAOH	0.1461	0.0000	0.0000	248.5321	0.0000	0.1461	248.5321
SANAOH	0.3200	0.0000	0.0000	330.7299	0.0000	0.3200	330.7299
SANAOH	0.2924	0.0000	0.0000	277.8843	0.0000	0.2924	277.8843
SANAOH	0.3518	0.0000	0.0000	278.1534	0.0000	0.3518	278.1534
SANAOH	0.3848	0.0000	0.0000	257.3443	143.5175	0.3848	400.8618
SANAOH	0.4045	0.0000	0.0000	212.8672	0.0000	0.4045	212.8672
SANAOH	0.4958	0.0000	0.0000	230.9081	0.0000	0.4958	230.9081
SANAOH	0.5188	0.0000	0.0000	232.6552	0.0000	0.5188	232.6552
SANAOH	0.3431	0.0000	0.0000	154.7862	0.0000	0.3431	154.7862
SANAOH	0.3025	0.0000	0.0000	133.7458	139.2155	0.3025	272.9613
SANAOH	0.8953	0.0000	0.0000	270.9596	97.5032	0.8953	368.4628
SANAOH	0.5395	0.0000	0.0000	240.5364	81.0503	0.5395	321.5867
SBH2O	11.9412	0.0000	0.0000	392.8348	338.2425	11.9412	731.0773
SBH2O	11.9953	0.0000	0.0000	379.3766	372.2258	11.9953	751.6025
SBH2O	11.7079	0.0000	0.0000	315.8539	418.3808	11.7079	734.2348
SBH2O	11.8389	0.0000	0.0000	430.3609	426.6483	11.8389	857.0092
NSH2O	0.5077	0.0000	0.0000	0.0000	238.0638	0.5077	238.0638
NSH2O	0.2246	0.0000	0.0000	0.0000	246.2456	0.2246	246.2456
NSH2O	0.0000	0.0000	0.0000	0.0000	359.6401	0.0000	359.6401
NSH2O	0.0000	0.0000	0.0000	0.0000	197.0700	0.0000	197.0700
SAH2O	5.7719	0.0000	0.0000	101.0385	296.3791	5.7719	397.4176
SAH2O	6.4011	0.0000	0.0000	258.9792	405.7337	6.4011	664.7129
SAH2O	10.1085	0.0000	0.0000	283.6351	0.0000	10.1085	283.6351
SAH2O	10.5568	0.0000	0.0000	349.8034	0.0000	10.5568	349.8034

APPENDIX B: SAS CODE

The procedures that follow were performed on the four sets of feedstock data seen in Chapter Two and Chapter Three, the data sets were omitted from the code.

```
ods PDF file="d:\CORNSTOVER.pdf";

DATA CORNSTOVER;
        INPUT STERILIZATION $ PRETREATMENT $ SAMPLE $ LIGNIN;
        CARDS;
        …
RUN;

PROC PRINT DATA = CORNSTOVER;
        TITLE "Corn Stover Lignin Content";
RUN;

PROC GLM DATA = CORNSTOVER PLOTS=(DIAGNOSTICS);
        TITLE "Lignin Content with Pretreatment, Tukey's Correction";
        CLASS PRETREATMENT ;
        MODEL LIGNIN = PRETREATMENT;
        LSMEANS PRETREATMENT / PDIFF= ALL;
        RUN;

PROC GLM DATA = CORNSTOVER PLOTS=(DIAGNOSTICS);
        TITLE "Lignin Content with Sterilization, Tukey's Correction";
        CLASS STERILIZATION;
        MODEL LIGNIN = STERILIZATION;
        LSMEANS STERILIZATION / PDIFF = ALL;
RUN;


PROC GLM DATA = CORNSTOVER ;
        TITLE "Lignin Content with Pretreatment Sterilization Interaction, Tukey's Correction";
        CLASS LIGNIN PRETREATMENT STERILIZATION;
        MODEL LIGNIN = PRETREATMENT STERILIZATION
PRETREATMENT*STERILIZATION;
        LSMEANS PRETREATMENT*STERILIZATION / PDIFF=ALL;
RUN;

ods pdf close;


ods PDF file="d:\SUGAR-CS.pdf";

DATA CS_SUGAR;
        INPUT STERILIZATION $ PRETREATMENT $ GLUCOSE XYLOSE  ARABINOSE;
        CARDS;
…
RUN;
```

```
PROC PRINT DATA = CS_SUGAR;
	TITLE "Corn Stover Sugar Concentration";
RUN;

PROC GLM DATA = CS_SUGAR PLOTS=(DIAGNOSTICS);
	TITLE "Glucose Concentration with Pretreatment, Tukey's Correction";
	CLASS PRETREATMENT ;
	MODEL GLUCOSE = PRETREATMENT;
	LSMEANS PRETREATMENT / PDIFF= ALL;
	RUN;

PROC GLM DATA = CS_SUGAR PLOTS=(DIAGNOSTICS);
	TITLE "Glucose Concentration with Sterilization, Tukey's Correction";
	CLASS STERILIZATION;
	MODEL GLUCOSE = STERILIZATION;
	LSMEANS STERILIZATION / PDIFF = ALL;
RUN;

PROC GLM DATA = CS_SUGAR ;
	TITLE "Glucose Concentration with Pretreatment Sterilization Interaction, Tukey's
Correction";
	CLASS GLUCOSE PRETREATMENT STERILIZATION;
	MODEL GLUCOSE = PRETREATMENT STERILIZATION
PRETREATMENT*STERILIZATION;
	LSMEANS PRETREATMENT*STERILIZATION / PDIFF=ALL;
RUN;


PROC GLM DATA = CS_SUGAR PLOTS=(DIAGNOSTICS);
	TITLE "Xylose Concentration with Pretreatment, Tukey's Correction";
	CLASS PRETREATMENT ;
	MODEL XYLOSE = PRETREATMENT;
	LSMEANS PRETREATMENT / PDIFF= ALL;
	RUN;

PROC GLM DATA = CS_SUGAR PLOTS=(DIAGNOSTICS);
	TITLE "XYLOSE Concentration with Sterilization, Tukey's Correction";
	CLASS STERILIZATION;
	MODEL XYLOSE = STERILIZATION;
	LSMEANS STERILIZATION / PDIFF = ALL;
RUN;

PROC GLM DATA = CS_SUGAR ;
	TITLE "Xylose Concentration with Pretreatment Sterilization Interaction, Tukey's
Correction";
	CLASS XYLOSE PRETREATMENT STERILIZATION;
	MODEL XYLOSE = PRETREATMENT STERILIZATION
PRETREATMENT*STERILIZATION;
	LSMEANS PRETREATMENT*STERILIZATION / PDIFF=ALL;
RUN;
```

```
PROC GLM DATA = CS_SUGAR PLOTS=(DIAGNOSTICS);
        TITLE "Arabinose Concentration with Pretreatment, Tukey's Correction";
        CLASS PRETREATMENT ;
        MODEL ARABINOSE = PRETREATMENT;
        LSMEANS PRETREATMENT / PDIFF= ALL;
        RUN;

PROC GLM DATA = CS_SUGAR PLOTS=(DIAGNOSTICS);
        TITLE "ARABINOSE Concentration with Sterilization, Tukey's Correction";
        CLASS STERILIZATION;
        MODEL ARABINOSE = STERILIZATION;
        LSMEANS STERILIZATION / PDIFF = ALL;
RUN;


PROC GLM DATA = CS_SUGAR ;
        TITLE "Arabinose Concentration with Pretreatment Sterilization Interaction, Tukey's
Correction";
        CLASS ARABINOSE PRETREATMENT STERILIZATION;
        MODEL ARABINOSE = PRETREATMENT STERILIZATION
PRETREATMENT*STERILIZATION;
        LSMEANS PRETREATMENT*STERILIZATION / PDIFF=ALL;
RUN;

ods pdf close;


ods PDF file="d:\THERMOCELLUM-CS.pdf";

DATA CS_FERM;
        INPUT INTERACTION $ STERILIZATION $ PRETREATMENT $
FERMENTATION ENZYME;
        CARDS;
…

RUN;

PROC PRINT DATA = CS_FERM;
        TITLE "Corn Stover SIMPLIFIED FERMENTATION PRODUCTS";
RUN;

PROC GLM DATA = CS_FERM PLOTS=(DIAGNOSTICS);
        TITLE "FERMENTATION Products Concentration (mM) with Pretreatment, Tukey's
Correction";
        CLASS PRETREATMENT ;
        MODEL FERMENTATION = PRETREATMENT;
        LSMEANS PRETREATMENT / CL PDIFF=ALL;
        RUN;
```

```
PROC GLM DATA = CS_FERM PLOTS=(DIAGNOSTICS);
	TITLE "Fermentation Products Concentration (mM) with Sterilization, Tukey's
Correction";
	CLASS STERILIZATION;
	MODEL FERMENTATION = STERILIZATION;
	LSMEANS STERILIZATION / CL PDIFF = ALL;
RUN;


PROC GLM DATA = CS_FERM PLOTS=(DIAGNOSTICS) ;
	TITLE "FERMENTATION PRODUCTS Concentration (mM) with Pretreatment
Sterilization Interaction, Tukey's Correction";
	CLASS PRETREATMENT STERILIZATION;
	MODEL FERMENTATION = PRETREATMENT STERILIZATION
PRETREATMENT*STERILIZATION;
	LSMEANS PRETREATMENT*STERILIZATION / CL PDIFF=ALL;
RUN;


PROC GLM DATA = CS_FERM PLOTS=(DIAGNOSTICS);
	TITLE "ENZYME PRODUCTS Concentration (mg/L) with Pretreatment, Tukey's
Correction";
	CLASS PRETREATMENT ;
	MODEL ENZYME = PRETREATMENT;
	LSMEANS PRETREATMENT / CL PDIFF= ALL;
	RUN;

PROC GLM DATA = CS_FERM PLOTS=(DIAGNOSTICS);
	TITLE "ENZYME PRODUCTS Concentration (mg/L) with Sterilization, Tukey's
Correction";
	CLASS STERILIZATION;
	MODEL ENZYME = STERILIZATION;
	LSMEANS STERILIZATION /CL PDIFF = ALL;
RUN;


PROC GLM DATA = CS_FERM PLOTS=(DIAGNOSTICS);
	TITLE "ENZYME PRODUCTS Concentration (mg/L) with Pretreatment Sterilization
Interaction, Tukey's Correction";
	CLASS PRETREATMENT STERILIZATION;
	MODEL ENZYME = PRETREATMENT STERILIZATION
PRETREATMENT*STERILIZATION;
	LSMEANS PRETREATMENT*STERILIZATION /CL PDIFF=ALL;
RUN;

ods pdf close;

ods PDF file="d:\SIMPLE-CS.pdf";
```

```
PROC UNIVARIATE DATA = CS_FERM MU0 = 0;
        TITLE 'CORN STOVER';
        CLASS PRETREATMENT;
RUN;

PROC UNIVARIATE DATA = CS_FERM MU0 = 0;
        TITLE 'CORN STOVER';
        CLASS STERILIZATION;
RUN;

PROC UNIVARIATE DATA=CS_FERM MU0=0;
        TITLE 'CORN STOVER';
        CLASS INTERACTION;
RUN;

ODS PDF CLOSE;
```

APPENDIX C: HYPOTHESIS TESTS

CORN STOVER

LIGNIN CONTENT AS A FUNCTION OF PRETREATMENT

$H_0: L_{AHP} = L_{NaOH} = L_{H_2O} = L_{RAW}$

H_A: At least two of the Lignin Contents $\neq$ each other

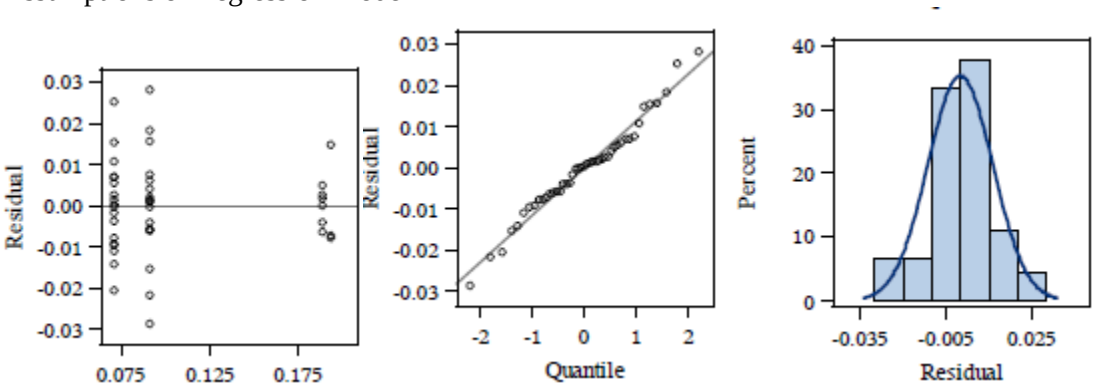
Level of Significance $\alpha = 0.05$, for two-tailed test use $^{\alpha}/_{2}$

Reject H_0 if $p < {}^{\alpha}/_{2}$

SOURCE	DF	Type I SS	Mean Square	F Value	Pr > F
PRETREATMENT	2	0.08936637	0.02978879	215.18	< 0.0001

Therefore, reject the null hypothesis and conclude that at least one of the pretreatments has a significant effect on lignin content.

Assumptions of Regression Model



Mean Zero (Linearity) - Same number of points above and below line

Homoscedasticity (Equal Variance) – All points lie close to Q-Q plot line

Independence – Little overlap in residual plot

Normality – Population distribution follows bell shaped curve

The assumptions of the regression model are met for lignin as a function of pretreatment

Tukey's Correction

Pretreatment	RAW	AHP	H2O	NaOH
RAW		< 0.0001	0.9367	< 0.0001
AHP	< 0.0001		< 0.0001	< 0.0001
H2O	0.9367	< 0.0001		< 0.0001
NaOH	< 0.0001	< 0.0001	< 0.0001	

Lignin content of RAW material is significantly different than AHP and NaOH pretreated material, there is no difference between the H2O Control and RAW and the lignin contents of AHP and NaOH are significantly different.

LIGNIN CONTENT AS A FUNCTION OF STERILIZATION

$H_0: L_{NS} = L_{RAW} = L_{SA} = L_{SB}$

H_A: At least two of the Lignin Contents $\neq$ each other

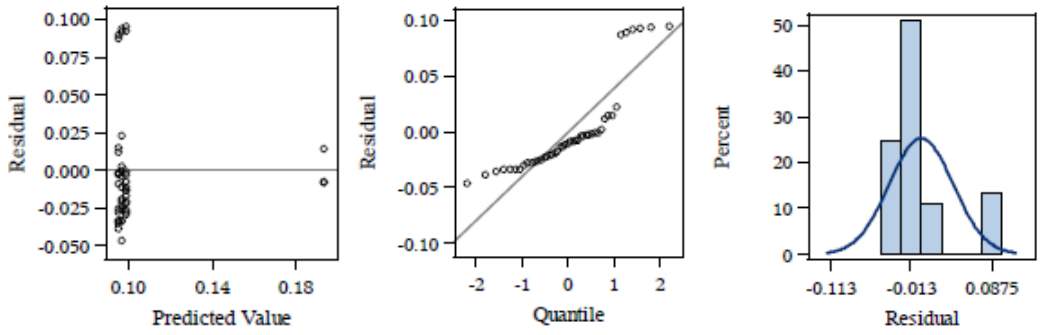
Level of Significance $\alpha = 0.05$, for two-tailed test use $\alpha/2$

Reject H_0 if $p < \alpha/2$

SOURCE	DF	Type I SS	Mean Square	F Value	Pr > F
STERILIZATION	2	0.02636811	0.00878937	5.25	< 0.0001

Therefore, reject the null hypothesis and conclude that at least one of the sterilization methods has a significant effect on lignin content.

Assumptions of Regression Model



Mean Zero (Linearity) – There are an unequal number of points above and below line

Homoscedasticity (Equal Variance) – Does not follow Q-Q Plot

Independence – Lots of overlap in residual plot

Normality – No semblance of normal distribution

The assumptions of the regression model are not met for lignin content as a function of sterilization.

Tukey's Correction

Pretreatment	NS	RAW	SA	SB
NS		0.0027	0.9955	0.9996
RAW	0.0027		0.0040	0.0032
SA	0.9955	0.0040		0.9992
SB	0.9996	0.0032	0.9992	

Lignin content of RAW material is significantly different than NS, SA & SB sterilized material, but not significantly different from each other, so lignin losses cannot be a result of sterilization technique.

LIGNIN CONTENT AS A FUNCTION OF PT STERILIZATION INTERACTION

$H_0: L_{NSAHP} = \cdots = L_{SBNaOH}$

H_A: At least 2 $\neq$ each other

Level of Significance $\alpha = 0.05$, for two-tailed test use $\alpha/2$

Reject H_0 if $p < \alpha$

SOURCE	DF	Type I SS	Mean Square	F Value	Pr > F
PRETREATMENT	3	0.08936637	0.02978879	198.70	<0.0001
STERILIZATION	2	0.00009141	0.00004571	0.30	0.7392
PT*STERILIZATION	4	0.00033737	0.00008434	0.56	0.6913

SOURCE	DF	Type III SS	Mean Square	F Value	Pr > F
PRETREATMENT	3	0.06308968	0.03154484	210.41	<0.0001
STERILIZATION	2	0.00012558	0.00004571	0.42	0.6611
PT*STERILIZATION	4	0.00033737	0.00008434	0.56	0.6913

The null hypothesis would be rejected, and the interaction between pretreatment and sterilization is not significant, so the main effects must be evaluated. It is either pretreatment or sterilization that has significant effects on lignin content.

GLUCOSE CONCENTRATION AS A FUNCTION OF PRETREATMENT

H_0: $G_{AHP} = G_{NaOH} = G_{H_2O} = G_{RAW}$

H_A: At least two of the glucose concentrations $\neq$ each other

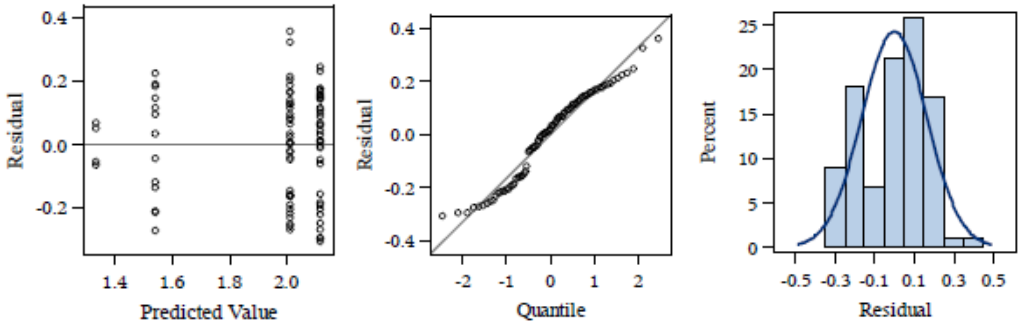
Level of Significance $\alpha = 0.05$, for two-tailed test use $\alpha/2$

Reject H_0 if $p < \alpha/2$

SOURCE	DF	Type I SS	Mean Square	F Value	Pr > F
PRETREATMENT	3	4.9036036295	1.63434432	58.49	< 0.0001

Therefore, reject the null hypothesis and conclude that at least one of the pretreatments has a significant effect on glucose concentration.

Assumptions of Regression Model



Mean Zero (Linearity) - Same number of points above and below line

Homoscedasticity (Equal Variance) – All points lie close to Q-Q plot line

Independence – Little overlap in residual plot

Normality – Population distribution follows bell shaped curve

The assumptions of the regression model are met for glucose concentration as a function of pretreatment.

Tukey's Correction

Pretreatment	Raw	AHP	H2O	NaOH	Average
RAW		< 0.0001	0.1405	< 0.0001	1.331
AHP	< 0.0001		< 0.0001	0.0460	2.119
H2O	0.1405	< 0.0001		< 0.0001	1.539
NaOH	< 0.0001	0.0460	< 0.0001		2.015

$$G_{Raw} = G_{H2O}$$
$$G_{AHP} = G_{NaOH}$$
$$G_{Treated} > G_{Untreated}$$

GLUCOSE CONCENTRATION AS A FUNCTION OF STERILIZATION

H_0: $G_{NS} = G_{RAW} = G_{SA} = G_{SB}$

H_A: At least two of the glucose concentrations $\neq$ each other

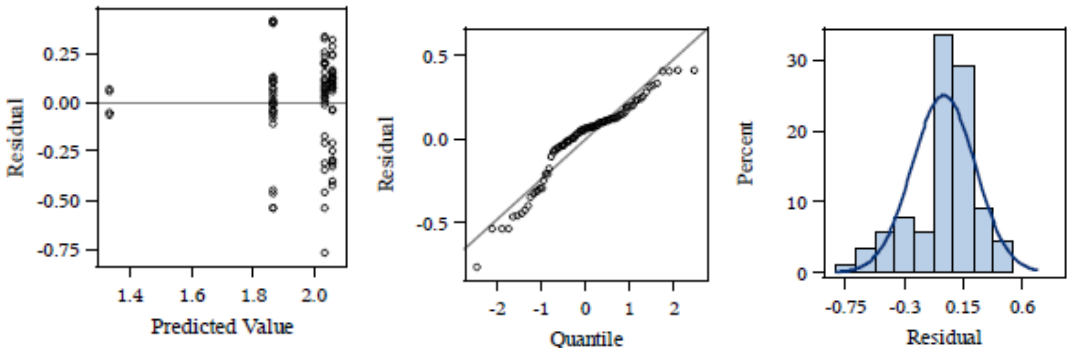
Level of Significance $\alpha = 0.05$, for two-tailed test use $^{\alpha}/_{2}$

Reject H_0 if $p < {}^{\alpha}/_{2}$

SOURCE	DF	Type I SS	Mean Square	F Value	Pr > F
PRETREATMENT	3	2.25437256	0.75145752	12.71	< 0.0001

Therefore, reject the null hypothesis and conclude that at least one of the sterilization techniques has a significant effect on glucose concentration.

Assumptions of Regression Model



Mean Zero – unequal number of points above & below

Homoscedasticity (Equal Variance) – curved Q-Q Plot

Independence – lots of overlap in residual plot

Normality – Population distribution does not follows bell shaped curve

The assumptions of the regression model are NOT met for glucose concentration as a function of pretreatment.

Tukey's Correction

Pretreatment	NS	RAW	SA	SB	Average
NS		0.0005	0.0529	0.0197	1.865
RAW	0.0005		< 0.0001	< 0.0001	1.332
SA	0.0529	< 0.0001		0.9780	2.033
SB	0.0197	< 0.0001	0.9780		2.059

$$G_{NS} = G_{SA} = G_{SB}$$

Because all sterilization methods have statistically the same glucose concentration, any differences in glucose cannot be attributed to sterilization.

XYLOSE CONCENTRATION AS A FUNCTION OF PRETREATMENT

H_0: $X_{AHP} = X_{NaOH} = X_{H_2O} = X_{RAW}$

H_A: At least two of the xylose concentrations $\neq$ each other

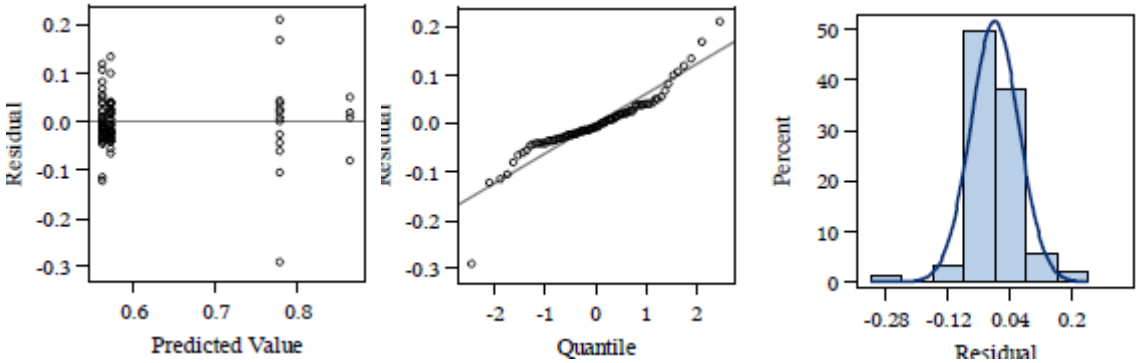
Level of Significance $\alpha = 0.05$, for two-tailed test use $^{\alpha}/_{2}$

Reject H_0 if $p < {}^{\alpha}/_{2}$

SOURCE	DF	Type I SS	Mean Square	F Value	Pr > F
PRETREATMENT	3	0.76239111	0.25413037	63.34	< 0.0001

Therefore, reject the null hypothesis and conclude that at least one of the pretreatments has a significant effect on xylose concentration.

Assumptions of Regression Model



Mean Zero – approximately same number of points above and below line

Homoscedasticity (Equal Variance) – All points lie close to Q-Q plot line

Independence – overlap in residual plot

Normality – Population distribution follows bell shaped curve

The assumptions of the regression model are met for xylose concentration as a function of pretreatment.

Tukey's Correction

Pretreatment	**Raw**	**AHP**	**H2O**	**NaOH**	**Average**
RAW		< 0.0001	0.0847	< 0.0001	0.864
AHP	< 0.0001		< 0.0001	0.8956	0.572
H2O	0.0847	< 0.0001		< 0.0001	0.778
NaOH	< 0.0001	0. 8956	< 0.0001		0.561

$$X_{Raw} = X_{H2O}$$
$$X_{AHP} = X_{NaOH}$$
$$X_{Treated} < X_{Untreated}$$

XYLOSE CONCENTRATION AS A FUNCTION OF STERILIZATION

H_0: $X_{NS} = X_{RAW} = X_{SA} = X_{SB}$

H_A: At least two of the xylose concentrations $\neq$ each other

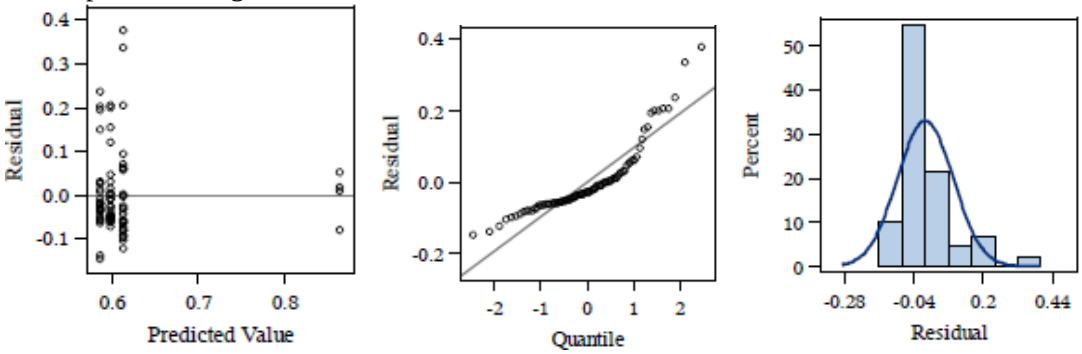
Level of Significance $\alpha = 0.05$, for two-tailed test use $^{\alpha}/_{2}$

Reject H_0 if $p < {}^{\alpha}/_{2}$

SOURCE	**DF**	**Type I SS**	**Mean Square**	**F Value**	**Pr > F**
PRETREATMENT	3	0.27910285	0.09303428	9.66	< 0.0001

Therefore, reject the null hypothesis and conclude that at least one of the sterilization techniques has a significant effect on xylose concentration.

Assumptions of Regression Model



Mean Zero – unequal number of points above & below
Homoscedasticity (Equal Variance) – curved Q-Q Plot
Independence – overlap in residual plot
Normality – Population distribution does not follows bell shaped curve

The assumptions of the regression model are NOT met for xylose concentration as a function of pretreatment.

Tukey's Correction

Pretreatment	NS	RAW	SA	SB	Average
NS		< 0.0001	0.9492	0.09609	0.598
RAW	< 0.0001		< 0.0001	< 0.0001	0.864
SA	0.9492	< 0.0001		0.7296	0.612
SB	0.9609	< 0.0001	0.7296		0.585

$$X_{NS} = X_{SA} = X_{SB}$$

Because all sterilization methods have statistically the same xylose concentration, any differences in xylose cannot be attributed to sterilization.

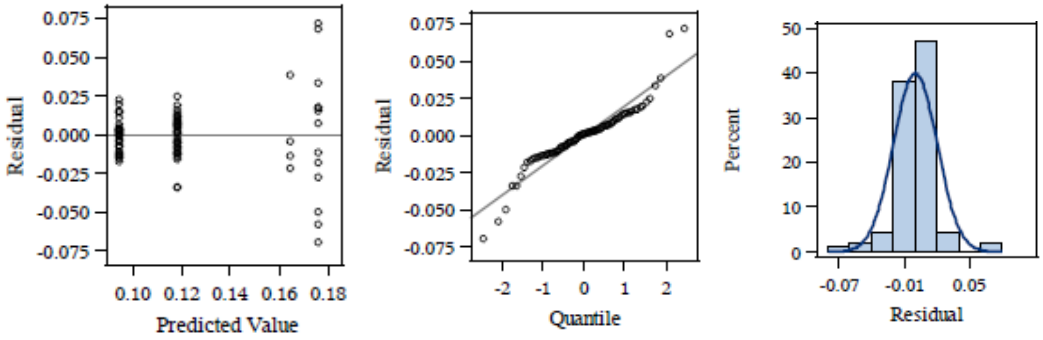
ARABINOSE CONCENTRATION AS A FUNCTION OF PRETREATMENT

H_0: $A_{AHP} = A_{NaOH} = A_{H_2O} = A_{RAW}$
H_A: At least two of the arabinose concentrations $\neq$ each other
Level of Significance $\alpha = 0.05$, for two-tailed test use $^{\alpha}/_{2}$
Reject H_0 if $p < {}^{\alpha}/_{2}$

SOURCE	DF	Type I SS	Mean Square	F Value	Pr > F
PRETREATMENT	3	0.07185614	0.02395205	57.72	< 0.0001

Therefore, reject the null hypothesis and conclude that at least one of the pretreatments has a significant effect on xylose concentration.

Assumptions of Regression Model



Mean Zero – approximately same number of points above and below line

Homoscedasticity (Equal Variance) – All points lie close to Q-Q plot line

Independence – overlap in residual plot

Normality – Population distribution follows bell shaped curve

The assumptions of the regression model are met for arabinose concentration as a function of pretreatment.

Tukey's Correction

Pretreatment	Raw	AHP	H2O	NaOH	Average
RAW		< 0.0001	0.7712	0.0003	0.164
AHP	< 0.0001		< 0.0001	< 0.0001	0.094
H2O	0.7712	< 0.0001		< 0.0001	0.176
NaOH	< 0.0001	0. 8956	< 0.0001		0.118

$$A_{Raw} = A_{H2O}$$
$$A_{AHP} \neq A_{NaOH}$$
$$A_{RAW} > A_{NaOH} > A_{AHP}$$

ARABINOSE CONCENTRATION AS A FUNCTION OF STERILIZATION

H_0: $A_{NS} = A_{RAW} = A_{SA} = A_{SB}$

H_A: At least two of the arabinose concentrations $\neq$ each other

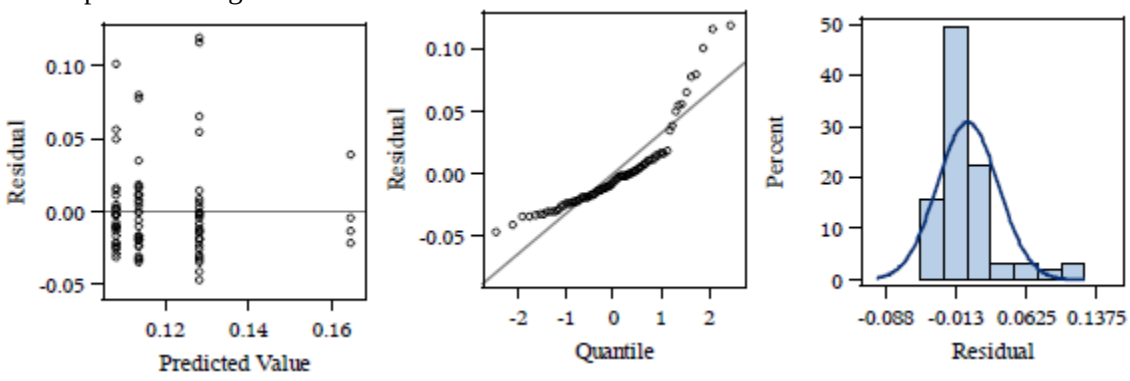
Level of Significance $\alpha = 0.05$, for two-tailed test use $^{\alpha}/_{2}$

Reject H_0 if $p < {}^{\alpha}/_{2}$

SOURCE	DF	Type I SS	Mean Square	F Value	Pr > F
PRETREATMENT	3	0.01480375	0.00493458	4.54	0.0053

Therefore, reject the null hypothesis and conclude that at least one of the sterilization techniques has a significant effect on arabinose concentration.

Assumptions of Regression Model



Mean Zero – unequal number of points above & below
Homoscedasticity (Equal Variance) – curved Q-Q Plot
Independence – overlap in residual plot
Normality – Population distribution does not follow bell shaped curve

The assumptions of the regression model are NOT met for arabinose concentration as a function of pretreatment.

Tukey's Correction

Pretreatment	NS	RAW	SA	SB	Average
NS		0.0253	0.3433	0.9262	0.114
RAW	0.0253		0.1763	0.0105	0.164
SA	0.3433	0.1763		0.1066	0.128
SB	0.9262	0.0105	0.1066		0.108

$$X_{NS} = X_{SA} = X_{SB}$$

Because all sterilization methods have statistically the same arabinose concentration, any differences in arabinose cannot be attributed to sterilization.

FERMENTATION PRODUCTS AS A FUNCTION OF PRETREATMENT

$H_0: F_{AHP} = F_{H2O} = F_{NaOH}$
H_A: At least two $\neq$ each other
$\alpha/_2 = 0.025$
$p - value = 0.1736$

Fail to reject, Pretreatment is not significant when measuring *C. thermocellum's* fermentation products. The assumptions of the regression model ($b_1 \sim Normal\left(\beta_1, \frac{\sigma_e^2}{(n-1)s_x^2}\right)$ are not met either.

$$\therefore F_{AHP} = F_{H2O} = F_{NaOH}$$

In order to determine if the fermentation pathways are functional in the pretreated materials individual hypothesis tests were performed on the levels of the class variables.

$H_0: \mu_x = 0$
Where x = AHP, H2O or NaOH
$H_A: \mu_x > 0$
$\alpha = 0.05$ one sided test

If $(\Pr > |t|) < 0.05$, reject H_0 and conclude with 95% confidence the pretreatment mean is significantly greater than zero. If $(\Pr > |t|) > 0.05$, fail to reject H_0 and conclude with 95% confidence that the pretreatment mean is not significantly different than zero. The results are summarized in the following table.

PRETREATMENT	MEAN	STDEV	N	Pr > |t|	H_0: μ_X=0	CONCLUSION
AHP	4.092	4.023	36	0.0001	REJECT	$\mu > 0$
H2O	5.159	3.903	12	0.0008	REJECT	$\mu > 0$
NaOH	6.719	7.727	36	0.0001	REJECT	$\mu > 0$

All pretreatments have a concentration of fermentation products statistically greater than zero. From this we can conclude that corn stover treated with AHP or NaOH will support *C. thermocellum's* fermentation pathways.

FERMENTATION PRODUCTS AS A FUNCTION OF STERILIZATION

$H_0: F_{NS} = F_{SA} = F_{SB}$
H_A: At least two $\neq$ each other
$\alpha/_2 = 0.025$
$p - value < 0.0001$

Reject the null hypothesis, sterilization is significant when measuring *C. thermocellum's* fermentation products. Although the variable is significant, the assumptions of the regression model are not met.

$$\therefore F_{NS} = F_{SA} = F_{SB}$$

In order to determine if the fermentation pathways are functional in the pretreated materials individual hypothesis tests were performed on the levels of the class variables.

$H_0: \mu_x = 0$
Where x = NS, SA or SB
$H_A: \mu_x > 0$
$\alpha = 0.05$ one sided test

If $(\Pr > |t|) < 0.05$, reject H_0 and conclude with 95% confidence the sterilization mean is significantly greater than zero. If $(\Pr > |t|) > 0.05$, fail to reject H_0 and conclude with 95% confidence that the sterilization mean is not significantly different than zero. The results are summarized in the following table.

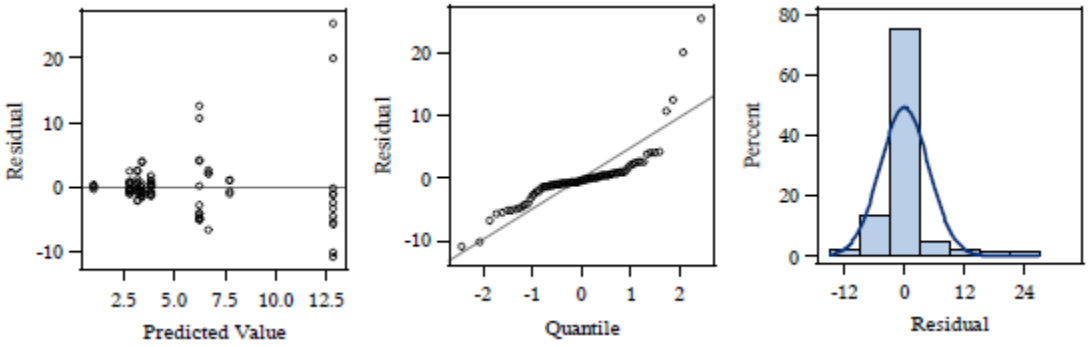
STERILIZATION	MEAN	STDEV	N	Pr > |t|	H_0: μ_X=0	CONCLUSION
NS	3.182	1.483	28	0.0001	REJECT	$\mu > 0$
SA	3.796	2.190	28	0.0001	REJECT	$\mu > 0$
SB	9.134	8.943	28	0.0001	REJECT	$\mu > 0$

All sterilization methods have a concentration of fermentation products statistically greater than zero. From this we can conclude that corn stover treated with AHP or NaOH will support *C. thermocellum's* fermentation pathway.

FERMENTATION PRODUCTS AS A FUNCTION OF PT STERILIZATION INTERACTION

$H_0: F_{NSAHP} = \ldots = F_{SBNaOH}$
H_A: At least two $\neq$ each other
$\alpha/_2 = 0.025$
$p - value = 0.0494$

Fail to Reject the null hypothesis, the interaction is not significant when measuring *C. thermocellum's* fermentation products. Although the variable is not significant, the assumptions of the regression model are met ($b_1 \sim Normal\left(\beta_1, \frac{\sigma_e^2}{(n-1)s_x^2}\right)$.



$\therefore NSAHP = SAAHP = SBAHP = NSH2O = SAH2O = SBH20 = NSNaOH = SANaOH = SBNaOH$

In order to determine if the fermentation pathways are functional in the pretreated materials individual hypothesis tests were performed on the levels of the class variables.

$H_0: \mu_x = 0$

Where x = NSAHP, SAAHP, SBAHP, NSH2O, SAH2O SBH2O, NSNaOH, SANaOH or SBNaOH

$H_A: \mu_x > 0$

$\alpha = 0.05$ one sided test

If $(\Pr > |t|)$ < 0.05, reject H_0 and conclude with 95% confidence the interaction mean is significantly greater than zero. If $(\Pr > |t|)$ > 0.05, fail to reject H_0 and conclude with 95% confidence that the interaction mean is not significantly different than zero. The results are summarized in the following table.

	MEAN	**STDEV**	**N**	**Pr > |t|**	**$H_0: \mu_X=0$**	**CONCLUSION**
NSAHP	3.193	1.511	12	0.0001	REJECT	$\mu > 0$
SAAHP	2.832	1.025	12	0.0001	REJECT	$\mu > 0$
SBAHP	6.252	6.361	12	0.0059	REJECT	$\mu > 0$
NSH2O	1.043	0.234	4	0.003	REJECT	$\mu > 0$
SAH2O	7.744	1.108	4	0.0008	REJECT	$\mu > 0$
SBH2O	6.690	4.467	4	0.0579	FAIL TO REJECT	$\mu_X=0$
NSNaOH	3.883	0.951	12	0.0001	REJECT	$\mu > 0$
SANaOH	3.444	1.889	12	0.0001	REJECT	$\mu > 0$
SBNaOH	12.830	11.149	12	0.0021	REJECT	$\mu > 0$

All pretreatments but SBH2O have a concentration of fermentation products statistically greater than zero. From this we can conclude that corn stover treated with AHP or NaOH will support *C. thermocellum*'s fermentation pathways.

ENZYME PRODUCTS AS A FUNCTION OF PRETREATMENT

$H_0: E_{AHP} = E_{H2O} = E_{NaOH}$
H_A: At least two $\neq$ each other
$\alpha/2 = 0.025$
$p - value < 0.0001$

Reject H_0, Pretreatment is significant when measuring *C. thermocellum's* enzyme products. However, the assumptions of the regression model are not met for this variable.

$$\therefore E_{AHP} = E_{H2O} = E_{NaOH}$$

In order to determine if the enzyme system is functional for the pretreated materials individual hypothesis tests were performed on the levels of the class variables.

$H_0: \mu_x = 0$
Where x = AHP, H2O or NaOH
$H_A: \mu_x > 0$
$\alpha = 0.05$ one sided test

If $(\Pr > |t|) < 0.05$, reject H_0 and conclude with 95% confidence the pretreatment mean is significantly greater than zero. If $(\Pr > |t|) > 0.05$, fail to reject H_0 and conclude with 95% confidence that the pretreatment mean is not significantly different than zero. The results are summarized in the following table.

PRETREATMENT	MEAN	STDEV	N	Pr > |t|	$H_0: \mu_X=0$	CONCLUSION
AHP	176.808	75.222	36	0.0001	REJECT	$\mu > 0$
H2O	2040.132	1643.000	12	0.0013	REJECT	$\mu > 0$
NaOH	240.94	117.940	36	0.0001	REJECT	$\mu > 0$

All pretreatments have a concentration of enzyme products statistically greater than zero. From this we can conclude that corn stover treated with AHP or NaOH will support *C. thermocellum's* enzyme systems.

ENZYME PRODUCTS AS A FUNCTION OF STERILIZATION

$H_0: E_{NS} = E_{SA} = E_{SB}$
H_A: At least two $\neq$ each other
$\alpha/2 = 0.025$
$p - value = 0.7905$

Fail to reject the null hypothesis, sterilization is not significant when measuring *C. thermocellum's* enzyme products. The assumptions of the regression model $(b_1 \sim Normal\left(\beta_1, \frac{\sigma_e^2}{(n-1)s_x^2}\right))$ are not met either.

$$\therefore E_{NS} = E_{SA} = E_{SB}$$

In order to determine if the enzyme system is functional in the pretreated materials individual hypothesis tests were performed on the levels of the class variables.

$H_0: \mu_x = 0$
Where x = NS, SA or SB
$H_A: \mu_x > 0$
$\alpha = 0.05$ one sided test

If $(\Pr > |t|) < 0.05$, reject H_0 and conclude with 95% confidence the sterilization mean is significantly greater than zero. If $(\Pr > |t|) > 0.05$, fail to reject H_0 and conclude with 95% confidence that the sterilization mean is not significantly different than zero. The results are summarized in the following table.

STERILIZATION	MEAN	STDEV	N	Pr > |t|	H_0: μ_X=0	CONCLUSION
NS	421.220	694.700	28	0.0034	REJECT	$\mu > 0$
SA	565.070	1047.000	28	0.0082	REJECT	$\mu > 0$
SB	425.155	901.340	28	0.019	REJECT	$\mu > 0$

All sterilization methods have a concentration of enzyme products statistically greater than zero. From this we can conclude that corn stover treated with AHP or NaOH will support *C. thermocellum's* enzyme system.

ENZYME PRODUCTS AS A FUNCTION OF PT STERILIZATION INTERACTION

$H_0: E_{NSAHP} = \ldots = E_{SBNaOH}$

H_A: At least two $\neq$ each other

$\alpha/2 = 0.025$

$p - value = 0.1110$

Fail to reject the null hypothesis, the interaction is not significant when measuring products of *C. thermocellum's* enzyme system. The assumptions of the regression model are not met ($b_1 \sim Normal\left(\beta_1, \frac{\sigma_e^2}{(n-1)s_x^2}\right)$ for the interaction.

$\therefore NSAHP = SAAHP = SBAHP = NSH2O = SAH2O = SBH20 = NSNaOH = SANaOH = SBNaOH$

In order to determine if the fermentation pathways are functional in the pretreated materials individual hypothesis tests were performed on the levels of the class variables.

$H_0: \mu_x = 0$

Where x = NSAHP, SAAHP, SBAHP, NSH2O, SAH2O SBH2O, NSNaOH, SANaOH or SBNaOH

$H_A: \mu_x > 0$

$\alpha = 0.05$ one sided test

If $(\Pr > |t|) < 0.05$, reject H_0 and conclude with 95% confidence the interaction mean is significantly greater than zero. If $(\Pr > |t|) > 0.05$, fail to reject H_0 and conclude with 95% confidence that the interaction mean is not significantly different than zero. The results are summarized in the following table.

INTERACTION	MEAN	STDEV	N	Pr > |t|	H_0: μ_X=0	CONCLUSION
NSAHP	197.153	84.818	12	0.0001	REJECT	$\mu > 0$
SAAHP	195.410	53.836	12	0.0001	REJECT	$\mu > 0$
SBAHP	137.860	73.672	12	0.0001	REJECT	$\mu > 0$
NSH2O	1425.485	1646.000	4	0.1817	FAIL TO REJECT	μ_X=0
SAH2O	2663.690	1713.000	4	0.0529	FAIL TO REJECT	μ_X=0
SBH2O	2031.221	1798.000	4	0.1090	FAIL TO REJECT	μ_X=0
NSNaOH	310.533	59.962	12	0.0001	REJECT	$\mu > 0$
SANaOH	235.190	137.430	12	0.0001	REJECT	$\mu > 0$
SBNaOH	177.095	109.588	12	0.0002	REJECT	$\mu > 0$

All interactions between sterilization and pretreatment methods have a concentration of enzyme products statistically greater than zero, except those treated with water. From this we can conclude that corn stover treated with AHP or NaOH will support *C. thermocellum's* enzyme system

WHEAT STRAW

LIGNIN CONTENT AS A FUNCTION OF PRETREATMENT

$H_0: L_{AHP} = L_{NaOH} = L_{H_2O} = L_{RAW}$

H_A: At least two of the Lignin Contents $\neq$ each other

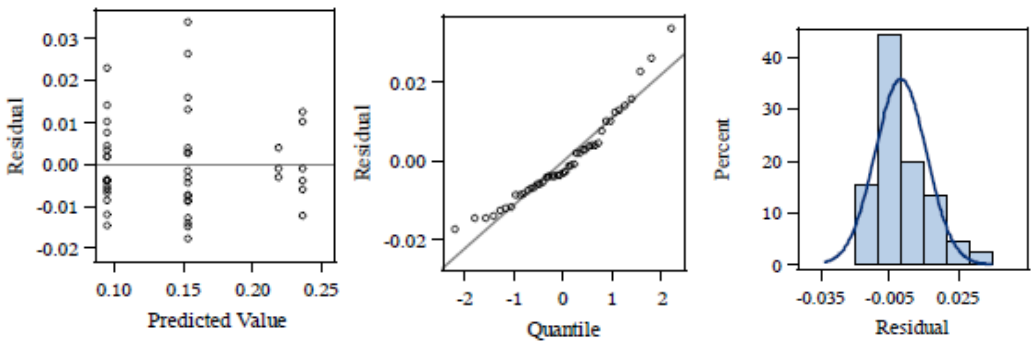
Level of Significance $\alpha = 0.05$, for two-tailed test use $^{\alpha}/_{2}$

Reject H_0 if $p < {}^{\alpha}/_{2}$

SOURCE	DF	Type I SS	Mean Square	F Value	Pr > F
PRETREATMENT	3	0.11359181	0.03786394	283.35	< 0.0001

Therefore, reject the null hypothesis and conclude that at least one of the pretreatments has a significant effect on lignin content.

Assumptions of Regression Model



Mean Zero (Linearity) - Same number of points above and below line

Homoscedasticity (Equal Variance) – All points lie close to Q-Q plot line

Independence – Little overlap in residual plot

Normality – Population distribution follows bell shaped curve

The assumptions of the regression model are met for lignin as a function of pretreatment

Tukey's Correction

Pretreatment	Raw	AHP	H2O	NaOH
RAW		< 0.0001	0.1611	< 0.0001
AHP	< 0.0001		< 0.0001	< 0.0001
H2O	0.1611	< 0.0001		< 0.0001
NaOH	< 0.0001	< 0.0001	< 0.0001	

Lignin content of RAW material is significantly different than AHP and NaOH pretreated material, there is no difference between the H2O Control and RAW and the lignin contents of AHP and NaOH are significantly different.

LIGNIN CONTENT AS A FUNCTION OF STERILIZATION

H_0: $L_{NS} = L_{RAW} = L_{SA} = L_{SB}$
H_A: At least two of the Lignin Contents $\neq$ each other
Level of Significance $\alpha = 0.05$, for two-tailed test use $\alpha/2$
Reject H_0 if $p < \alpha/2$

SOURCE	DF	Type I SS	Mean Square	F Value	Pr > F
STERILIZATION	3	0.01842915	0.00614305	2.5	.0727

Therefore, we fail to reject the null hypothesis and conclude that sterilization method does not have a significant effect on lignin content.

LIGNIN CONTENT AS A FUNCTION OF PT STERILIZATION INTERACTION

H_0: $L_{NSAHP} = \cdots = L_{SBNaOH}$
H_A: $At\ least\ 2\ \neq each\ other$
Level of Significance $\alpha = 0.05$
Reject H_0 if $p < \frac{\alpha}{2}$

SOURCE	DF	Type I SS	Mean Square	F Value	Pr > F
PRETREATMENT	3	0.11359181	0.03786394	346.21	<0.0001
STERILIZATION	2	0.00092996	0.00046498	4.25	0.0222
PT*STERILIZATION	4	0.00072100	0.00018025	1.65	0.1842
SOURCE	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr > F**
PRETREATMENT	3	0.09609261	0.04804631	439.31	<0.0001
STERILIZATION	2	0.00094377	0.00047189	4.31	0.0211
PT*STERILIZATION	4	0.00072100	0.00018025	1.65	0.1842

The null hypothesis would be rejected, and the interaction between pretreatment and sterilization is not significant, so the main effects must be evaluated. It is either pretreatment or sterilization that has significant effects on lignin content.

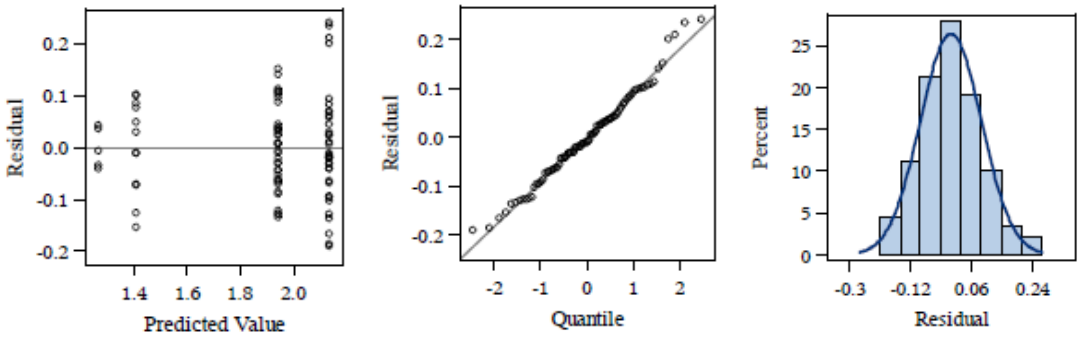
GLUCOSE CONCENTRATION AS A FUNCTION OF PRETREATMENT

H_0: $G_{AHP} = G_{NaOH} = G_{H_2O} = G_{RAW}$
H_A: At least two of the glucose concentrations $\neq$ each other
Level of Significance $\alpha = 0.05$, for two-tailed test use $\alpha/2$
Reject H_0 if $p < \alpha/2$

SOURCE	DF	Type I SS	Mean Square	F Value	Pr > F
PRETREATMENT	3	6.98149846	2.32716615	274.32	< 0.0001

Therefore, reject the null hypothesis and conclude that at least one of the pretreatments has a significant effect on glucose concentration.

Assumptions of Regression Model



Mean Zero - Same number of points above and below line

Homoscedasticity (Equal Variance) – All points lie close to Q-Q plot line

Independence – Little overlap in residual plot

Normality – Population distribution follows bell shaped curve

The assumptions of the regression model are met for glucose concentration as a function of pretreatment.

Tukey's Correction

Pretreatment	Raw	AHP	H2O	NaOH	Average
RAW		< 0.0001	0.0236	< 0.0001	1.264
AHP	< 0.0001		< 0.0001	< 0.0001	2.135
H2O	0.0236	< 0.0001		< 0.0001	1.407
NaOH	< 0.0001	< 0.0001	< 0.0001		1.944

$$G_{Raw} = G_{H2O}$$
$$G_{AHP} > G_{NaOH} > G_{Raw}$$

GLUCOSE CONCENTRATION AS A FUNCTION OF STERILIZATION

H_0: $G_{NS} = G_{RAW} = G_{SA} = G_{SB}$

H_A: At least two of the glucose concentrations $\neq$ each other

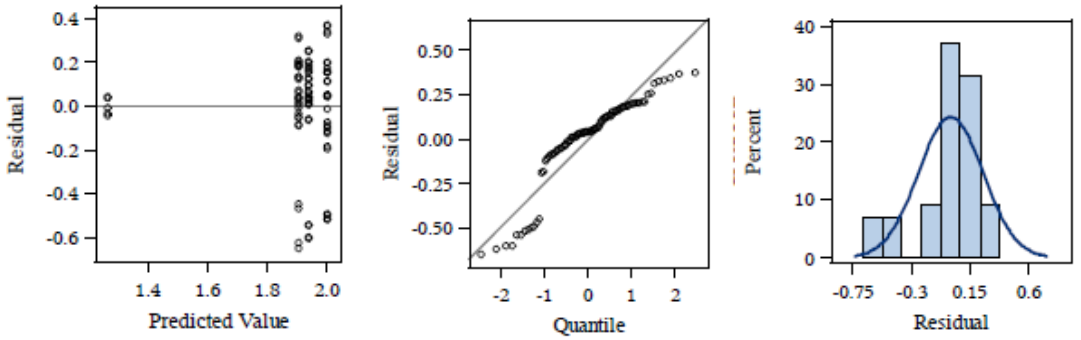
Level of Significance $\alpha = 0.05$, for two-tailed test use $^{\alpha}/_{2}$

Reject H_0 if $p < {}^{\alpha}/_{2}$

SOURCE	DF	Type I SS	Mean Square	F Value	Pr > F
PRETREATMENT	3	2.34864881	0.78288294	12.43	< 0.0001

Therefore, reject the null hypothesis and conclude that at least one of the sterilization techniques has a significant effect on glucose concentration.

Assumptions of Regression Model



Mean Zero – unequal number of points above & below

Homoscedasticity (Equal Variance) – curved Q-Q Plot

Independence – overlap in residual plot

Normality – uneven distribution

The assumptions of the regression model are NOT met for glucose concentration as a function of pretreatment.

Tukey's Correction

Pretreatment	NS	RAW	SA	SB	Average
NS		< 0.0001	0.9580	0.7793	1.939
RAW	< 0.0001		< 0.0001	< 0.0001	1.264
SA	0.9580	< 0.0001		0.4715	1.905
SB	0.7793	< 0.0001	0.4715		2.003

$$G_{NS} = G_{SA} = G_{SB}$$

Because all sterilization methods have statistically the same glucose concentration, any differences in glucose cannot be attributed to sterilization.

GLUCOSE CONCENTRATION AS A FUNCTION OF PT STERILIZATION INTERACTION

H_0: $G_{NSAHP} = \cdots = G_{SBNaOH}$

H_A: $At\ least\ 2\ \neq each\ other$

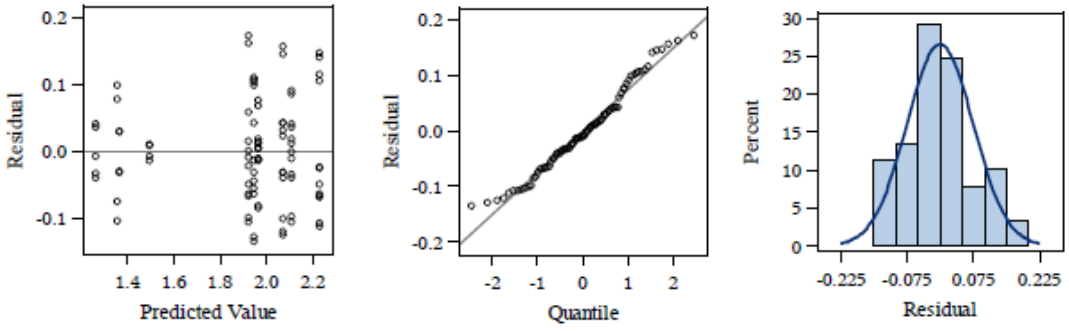
Level of Significance $\alpha = 0.05$

Reject H_0if $p < \alpha/2$

SOURCE	DF	Type I SS	Mean Square	F Value	Pr > F
PRETREATMENT	3	6.98149846	2.32716615	274.32	< 0.0001
STERILIZATION	2	0.13677309	0.06828655	10.85	< 0.0001
PT*STERILIZATION	4	0.08633962	0.02158490	3.42	0.0124

Therefore, reject the null hypothesis and conclude that the interaction between pretreatment and sterilization has a significant impact on glucose concentration

Assumptions of Regression Model



Mean Zero - Same number of points above and below line

Homoscedasticity (Equal Variance) – All points lie close to Q-Q plot line

Independence – Little overlap in residual plot

Normality – Population distribution follows bell shaped curve

The assumptions of the regression model are met for glucose concentration the interaction between pretreatment and sterilization.

Tukey's Correction

Least Squares Means for effect PRETREATM*STERILIZAT
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: GLUCOSE

i/j	1	2	3	4	5	6	7	8	9	10
1		<.0001	<.0001	<.0001	0.6635	0.7675	0.0014	<.0001	<.0001	<.0001
2	<.0001		0.9876	0.0105	<.0001	<.0001	<.0001	0.0015	<.0001	0.0002
3	<.0001	0.9876		0.0003	<.0001	<.0001	<.0001	0.0435	0.0007	0.0080
4	<.0001	0.0105	0.0003		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
5	0.6635	<.0001	<.0001	<.0001		1.0000	0.3748	<.0001	<.0001	<.0001
6	0.7675	<.0001	<.0001	<.0001	1.0000		0.2854	<.0001	<.0001	<.0001
7	0.0014	<.0001	<.0001	<.0001	0.3748	0.2854		<.0001	<.0001	<.0001
8	<.0001	0.0015	0.0435	<.0001	<.0001	<.0001	<.0001		0.9536	0.9999
9	<.0001	<.0001	0.0007	<.0001	<.0001	<.0001	<.0001	0.9536		0.9993
10	<.0001	0.0002	0.0080	<.0001	<.0001	<.0001	<.0001	0.9999	0.9993	

$$G_{Raw} = G_{H2O}$$
$$G_{NSAHP} = G_{SAAHP} = G_{NSNaOH} = G_{SANaOH} = G_{SBNaOH}$$
$$G_{SBAHP} > G_{NaOH} > G_{RAW}$$

XYLOSE CONCENTRATION AS A FUNCTION OF PRETREATMENT

H_0: $X_{AHP} = X_{NaOH} = X_{H_2O} = X_{RAW}$

H_A: At least two of the xylose concentrations $\neq$ each other

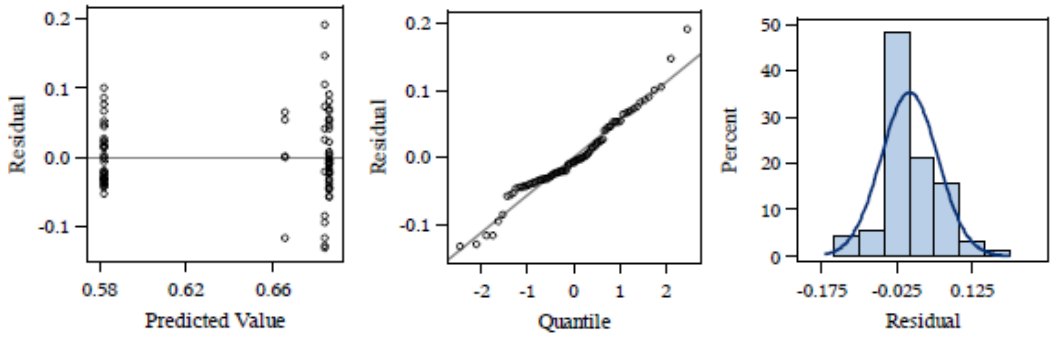
Level of Significance $\alpha = 0.05$, for two-tailed test use $\alpha/2$

Reject H_0 if $p < \alpha/2$

SOURCE	DF	Type I SS	Mean Square	F Value	Pr > F
PRETREATMENT	3	0.22336717	0.07445572	22.54	< 0.0001

Therefore, reject the null hypothesis and conclude that at least one of the pretreatments has a significant effect on xylose concentration.

Assumptions of Regression Model



Mean Zero – approximately same number of points above and below line

Homoscedasticity (Equal Variance) – All points lie close to Q-Q plot line

Independence – overlap in residual plot

Normality – Population distribution follows bell shaped curve

The assumptions of the regression model are mostly met for xylose concentration as a function of pretreatment.

Tukey's Correction

Pretreatment	Raw	AHP	H2O	NaOH	Average
RAW		0.8789	0.9329	0.0158	0.666
AHP	0.8789		0.9995	< 0.0001	0.686
H2O	0.9329	0.9995		< 0.0001	0.684
NaOH	0.0158	< 0.0001	< 0.0001		0.582

$$X_{Raw} = X_{H2O} = X_{AHP}$$
$$X_{RAW|AHP|H2O} > X_{NaOH}$$

XYLOSE CONCENTRATION AS A FUNCTION OF STERILIZATION

H_0: $X_{NS} = X_{RAW} = X_{SA} = X_{SB}$
H_A: At least two of the xylose concentrations $\neq$ each other
Level of Significance $\alpha = 0.05$, for two-tailed test use $\alpha/2$
Reject H_0 if $p < \alpha/2$

SOURCE	DF	Type I SS	Mean Square	F Value	Pr > F
PRETREATMENT	3	0.02296053	0.00765351	1.35	0.2630

Fail to reject the null hypothesis and conclude that sterilization technique does not have a significant effect on xylose concentration.

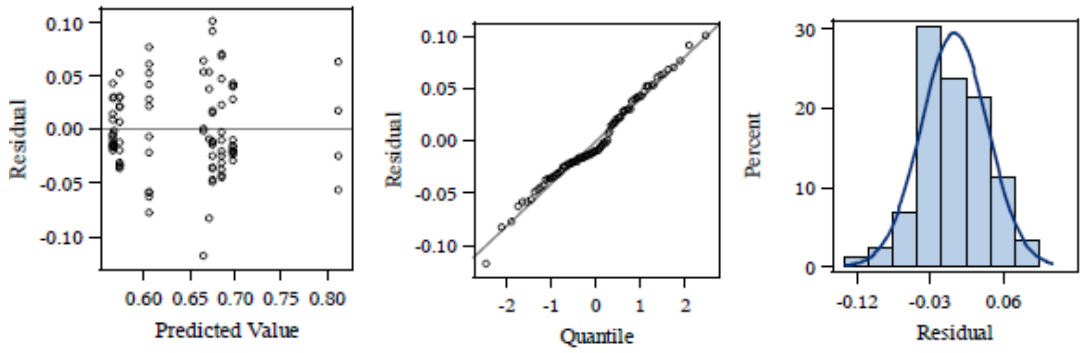
XYLOSE CONCENTRATION AS A FUNCTION OF PT STERILIZATION INTERACTION

H_0: $X_{NSAHP} = \cdots = X_{SBNaOH}$
H_A: $At\ least\ 2\ \neq each\ other$
Level of Significance $\alpha = 0.05$
Reject H_0 if $p < \alpha/2$

SOURCE	DF	Type III SS	Mean Square	F Value	Pr > F
PRETREATMENT	3	0.22053449	0.11026725	60.02	< 0.0001
STERILIZATION	2	0.07540572	0.01006393	50.52	< 0.0001
PT*STERILIZATION	4	0.11548635	0.02887159	15.72	< 0.0001

Therefore, reject the null hypothesis and conclude that the interaction between pretreatment and sterilization has a significant impact on xylose concentration.

Assumptions of Regression Model



Mean Zero - Same number of points above and below line
Homoscedasticity (Equal Variance) – All points lie close to Q-Q plot line
Independence – Little overlap in residual plot
Normality – Population distribution follows bell shaped curve

The assumptions of the regression model are met for xylose concentration the interaction between pretreatment and sterilization.

Tukey's Correction

Least Squares Means for effect PRETREATM*STERILIZAT Pr > |t| for H0: LSMean(i)=LSMean(j) Dependent Variable: XYLOSE										
i/j	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1		0.9171	0.9979	1.0000	<.0001	0.0321	1.0000	0.0014	0.0050	0.2320
2	0.9171		0.9988	0.9535	0.0005	<.0001	0.9847	<.0001	<.0001	<.0001
3	0.9979	0.9988		1.0000	<.0001	0.0004	0.9999	<.0001	<.0001	0.0010
4	1.0000	0.9535	1.0000		<.0001	0.0015	1.0000	<.0001	<.0001	0.0059
5	<.0001	0.0005	<.0001	<.0001		<.0001	0.0005	<.0001	<.0001	<.0001
6	0.0321	<.0001	0.0004	0.0015	<.0001		0.0315	1.0000	1.0000	0.8610
7	1.0000	0.9847	0.9999	1.0000	0.0005	0.0315		0.0022	0.0067	0.2233
8	0.0014	<.0001	<.0001	<.0001	<.0001	1.0000	0.0022		1.0000	0.3930
9	0.0050	<.0001	<.0001	<.0001	<.0001	1.0000	0.0067	1.0000		0.7115
10	0.2320	<.0001	0.0010	0.0059	<.0001	0.8610	0.2233	0.3930	0.7115	

$$X_{Raw} = X_{AHP} = X_{SBH2O} = X_{SBNaOH}$$
$$X_{SBH2O} > X_{AHP|RAW} > X_{SA(B)H2O|NS(SA)NaOH}$$

ARABINOSE CONCENTRATION AS A FUNCTION OF PRETREATMENT

H_0: $A_{AHP} = A_{NaOH} = A_{H_2O} = A_{RAW}$
H_A: At least two of the arabinose concentrations $\neq$ each other
Level of Significance $\alpha = 0.05$, for two-tailed test use $^{\alpha}/_{2}$
Reject H_0 if $p < {}^{\alpha}/_{2}$

SOURCE	DF	Type I SS	Mean Square	F Value	Pr > F
PRETREATMENT	3	0.00258604	0.00086201	2.81	0.0444

Fail to reject the null hypothesis and conclude that pretreatment does not have a significant effect on xylose concentration.

ARABINOSE CONCENTRATION AS A FUNCTION OF STERILIZATION

H_0: $A_{NS} = A_{RAW} = A_{SA} = A_{SB}$
H_A: At least two of the arabinose concentrations $\neq$ each other
Level of Significance $\alpha = 0.05$, for two-tailed test use $^{\alpha}/_{2}$
Reject H_0 if $p < {}^{\alpha}/_{2}$

SOURCE	DF	Type I SS	Mean Square	F Value	Pr > F
PRETREATMENT	3	0.00102411	0.00034137	1.05	0.3752

Therefore, reject the null hypothesis and conclude that at least one of the sterilization techniques has a significant effect on arabinose concentration.

FERMENTATION PRODUCTS AS A FUNCTION OF PRETREATMENT

$H_0: F_{AHP} = F_{H2O} = F_{NaOH}$
H_A: At least two $\neq$ each other
$\alpha/2 = 0.025$
$p - value = 0.0435$

Fail to reject, Pretreatment is not significant when measuring *C. thermocellum's* fermentation products. The assumptions of the regression model ($b_1 \sim Normal\left(\beta_1, \frac{\sigma_e^2}{(n-1)s_x^2}\right)$ are not met either.

$$\therefore F_{AHP} = F_{H2O} = F_{NaOH}$$

In order to determine if the fermentation pathways are functional in the pretreated materials individual hypothesis tests were performed on the levels of the class variables.

$H_0: \mu_x = 0$
Where x = AHP, H2O or NaOH
$H_A: \mu_x > 0$
$\alpha = 0.05$ one sided test

If $(\Pr > |t|)$ < 0.05, reject H_0 and conclude with 95% confidence the pretreatment mean is significantly greater than zero. If $(\Pr > |t|)$ > 0.05, fail to reject H_0 and conclude with 95% confidence that the pretreatment mean is not significantly different than zero. The results are summarized in the following table.

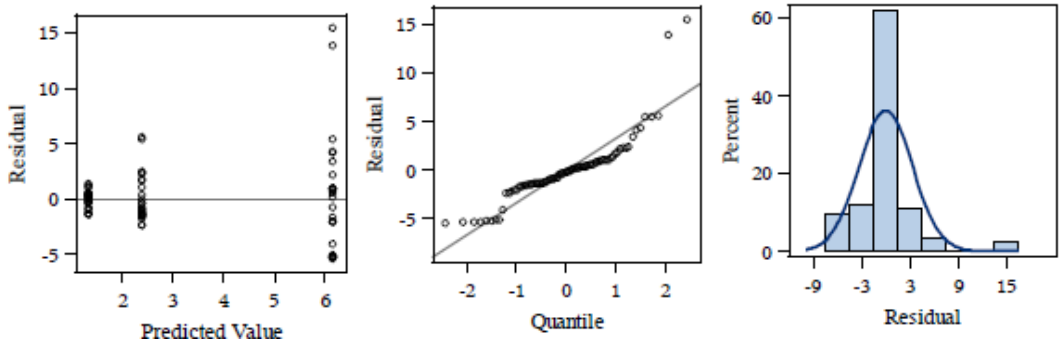
PRETREATMENT	MEAN	STDEV	N	Pr > |t|	H_0: μ_X=0	CONCLUSION
AHP	2.174	2.414	36	0.0001	REJECT	$\mu > 0$
H2O	5.135	4.242	12	0.0015	REJECT	$\mu > 0$
NaOH	3.784	4.696	36	0.0001	REJECT	$\mu > 0$

All pretreatments have a concentration of fermentation products statistically greater than zero. From this we can conclude that corn stover treated with AHP or NaOH will support *C. thermocellum* fermentation pathways.

FERMENTATION PRODUCTS AS A FUNCTION OF STERILIZATION

$H_0: F_{NS} = F_{SA} = F_{SB}$
H_A: At least two $\neq$ each other
$\alpha/2 = 0.025$
$p - value < 0.0001$

Reject the null hypothesis, sterilization is significant when measuring *C. thermocellum's* fermentation products. The assumptions of the regression model ($b_1 \sim Normal\left(\beta_1, \frac{\sigma_e^2}{(n-1)s_x^2}\right)$ are met for fermentation products as a function of sterilization.



$$\therefore F_{NS} = F_{SA} \; and \; F_{SB} > F_{NS} | F_{SA}$$

In order to determine if the fermentation pathways are functional in the pretreated materials individual hypothesis tests were performed on the levels of the class variables.

$H_0: \mu_x = 0$
Where x = NS, SA or SB
$H_A: \mu_x > 0$
$\alpha = 0.05$ one sided test

If $(Pr > |t|) < 0.05$, reject H_0 and conclude with 95% confidence the sterilization mean is significantly greater than zero. If $(Pr > |t|) > 0.05$, fail to reject H_0 and conclude with 95% confidence that the sterilization mean is not significantly different than zero. The results are summarized in the following table.

STERILIZATION	MEAN	STDEV	N	Pr > |t|	$H_0: \mu_X=0$	CONCLUSION
NS	1.321	0.792	28	0.0001	REJECT	$\mu > 0$
SA	2.383	2.118	28	0.0001	REJECT	$\mu > 0$
SB	6.154	5.337	28	0.0001	REJECT	$\mu > 0$

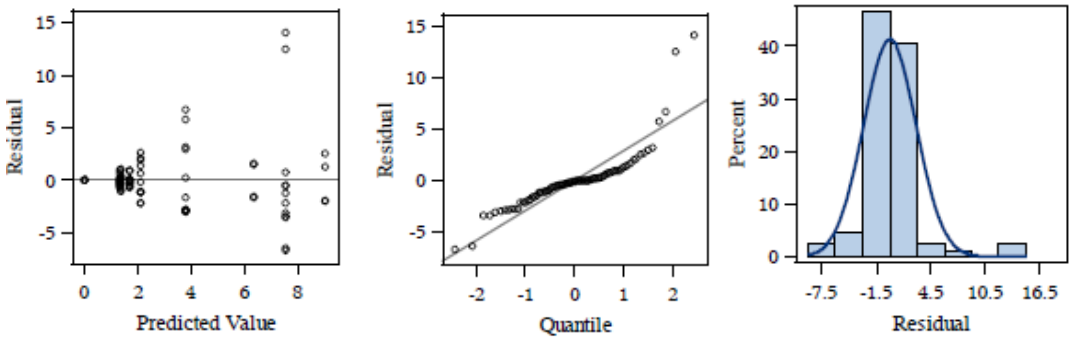
All sterilization methods have a concentration of fermentation products statistically greater than zero. From this we can conclude that corn stover all sterilization techniques will support *C. thermocellum's* fermentation pathways.

FERMENTATION PRODUCTS AS A FUNCTION OF PT STERILIZATION INTERACTION

$H_0: F_{NSAHP} = \ldots = F_{SBNaOH}$
H_A: At least two $\neq$ each other
$\alpha/2 = 0.025$
$p - value = 0.0204$

Reject the null hypothesis, the interaction is significant when measuring *C. thermocellum's* fermentation products. The assumptions of the regression model are met

$(b_1 \sim Normal\left(\beta_1, \frac{\sigma_e^2}{(n-1)s_x^2}\right)$ for the interaction.



From the Tukey's correction p-values, it can be concluded

$$NSH2O \neq SBH2O \; \& \; SBNaOH$$
$$SBH2O \neq NSAHP, SAAHP, NSH2O, NSNaOH \; \& \; SANaOH$$
$$NSNaOH \neq SBH2O \; \& \; SBNaOH$$
$$SANaOH \neq SBH2O \; \& \; SBNaOH$$
$$SBNaOH \neq NSAHP, SAAHP, NSH20, NSNaOH \; \& \; SANaOH.$$

There is not a significant difference in any of the other interaction fermentation means.

In order to determine if the fermentation pathways are functional in the pretreated materials individual hypothesis tests were performed on the levels of the class variables.

$H_0: \mu_x = 0$
x = NSAHP, SAAHP, SBAHP, NSH2O, SAH2O SBH2O, NSNaOH, SANaOH or SBNaOH
$H_A: \mu_x > 0$
$\alpha = 0.05$ one sided test

If $(\Pr > |t|) < 0.05$, reject H_0 and conclude with 95% confidence the interaction mean is significantly greater than zero. If $(\Pr > |t|) > 0.05$, fail to reject H_0 and conclude with 95% confidence that the interaction mean is not significantly different than zero. The results are summarized in the following table.

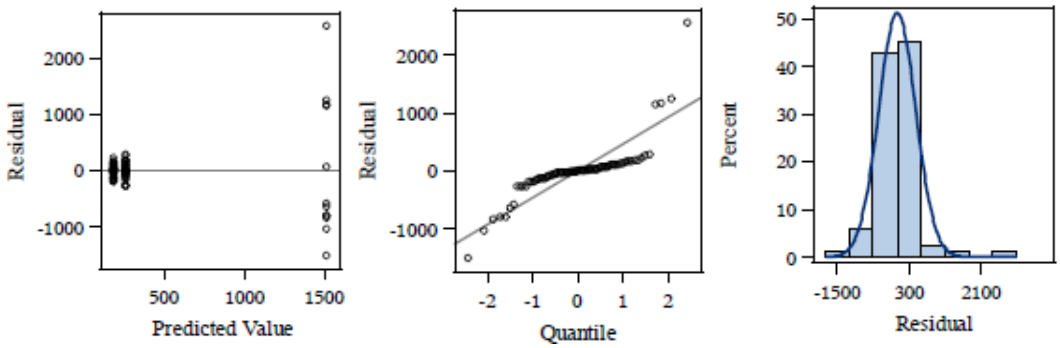
INTERACTION	MEAN	STDEV	N	Pr > |t|	H_0: μ_X=0	CONCLUSION
NSAHP	1.342	0.695	12	0.0001	REJECT	$\mu > 0$
SAAHP	1.344	0.476	12	0.0001	REJECT	$\mu > 0$
SBAHP	3.793	3.681	12	0.0044	REJECT	$\mu > 0$
NSH2O	0.000	0.000	4	1.0000	FAIL TO REJECT	μ_x=0
SAH2O	6.357	1.822	4	0.0060	REJECT	$\mu > 0$
SBH2O	9.048	2.260	4	0.0041	REJECT	$\mu > 0$
NSNAOH	1.705	0.506	12	0.0001	REJECT	$\mu > 0$
SANAOH	2.097	1.693	12	0.0013	REJECT	$\mu > 0$
SBNAOH	7.551	6.613	12	0.0022	REJECT	$\mu > 0$

All pretreatments but NSH2O have a concentration of fermentation products statistically greater than zero. From this we can conclude that corn stover treated with AHP or NaOH will support *C. thermocellum*'s fermentation pathway.

ENZYME PRODUCTS AS A FUNCTION OF PRETREATMENT

$H_0: E_{AHP} = E_{H2O} = E_{NaOH}$
H_A: At least two $\neq$ each other
$\alpha/2 = 0.025$
$p - value < 0.0001$

Reject H_0, Pretreatment is significant when measuring *C. thermocellum's* enzyme products. The assumptions of the regression model $b_1 \sim Normal\left(\beta_1, \frac{\sigma_e^2}{(n-1)s_x^2}\right)$ are mostly met for this variable.



$\therefore E_{AHP} = E_{NaOH}$ and $E_{H2O} > E_{AHP} | E_{NaOH}$

In order to determine if the enzyme system is functional for the pretreated materials individual hypothesis tests were performed on the levels of the class variables.

$H_0: \mu_x = 0$
Where x = AHP, H2O or NaOH
$H_A: \mu_x > 0$
$\alpha = 0.05$ one sided test

If $(Pr > |t|) < 0.05$, reject H_0 and conclude with 95% confidence the pretreatment mean is significantly greater than zero. If $(Pr > |t|) > 0.05$, fail to reject H_0 and conclude with 95% confidence that the pretreatment mean is not significantly different than zero. The results are summarized in the following table.

PRETREATMENT	MEAN	STDEV	N	Pr > |t|	H_0: μ_X=0	CONCLUSION
AHP	179.611	100.929	36	0.0010	REJECT	$\mu > 0$
H2O	1513.863	1244.000	12	0.0014	REJECT	$\mu > 0$
NaOH	264.485	139.644	36	0.0001	REJECT	$\mu > 0$

All pretreatments have a concentration of enzyme products statistically greater than zero. From this we can conclude that corn stover treated with AHP or NaOH will support *C. thermocellum*'s enzyme system.

ENZYME PRODUCTS AS A FUNCTION OF STERILIZATION

$H_0: E_{NS} = E_{SA} = E_{SB}$
H_A: At least two $\neq$ each other
$\alpha/_2 = 0.025$
$p - value = 0.2072$

Fail to reject the null hypothesis, sterilization is not significant when measuring *C. thermocellum's* enzyme products. The assumptions of the regression model $b_1 \sim Normal\left(\beta_1, \frac{\sigma_e^2}{(n-1)s_x^2}\right)$ are not met either.

$$\therefore E_{NS} = E_{SA} = E_{SB}$$

In order to determine if the enzyme system is functional in the pretreated materials individual hypothesis tests were performed on the levels of the class variables.

$H_0: \mu_x = 0$
Where x = NS, SA or SB
$H_A: \mu_x > 0$
$\alpha = 0.05$ one sided test

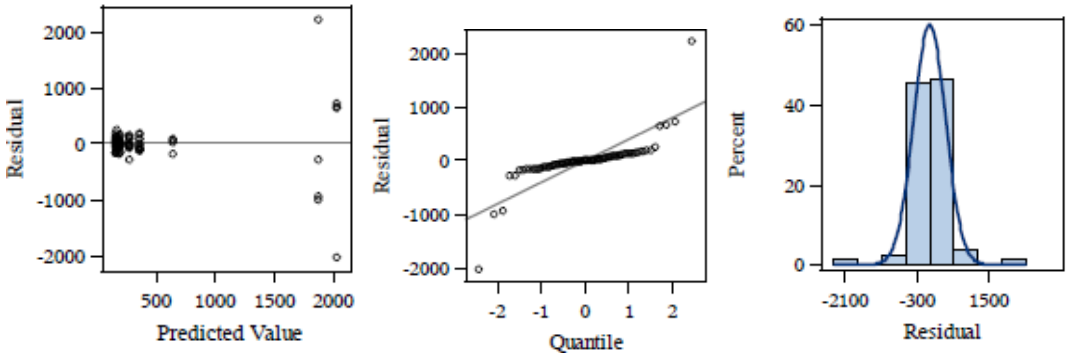
If $(Pr > |t|) < 0.05$, reject H_0 and conclude with 95% confidence the sterilization mean is significantly greater than zero. If $(Pr > |t|) > 0.05$, fail to reject H_0 and conclude with 95% confidence that the sterilization mean is not significantly different than zero. The results are summarized in the following table.

STERILIZATION	MEAN	STDEV	N	Pr > |t|	H_0: μ_X=0	CONCLUSION
NS	487.788	789.617	28	0.0029	REJECT	$\mu > 0$
SA	226.314	209.420	28	0.0001	REJECT	$\mu > 0$
SB	501.819	769.972	28	0.0019	REJECT	$\mu > 0$

All sterilization methods have a concentration of enzyme products statistically greater than zero. From this we can conclude that corn stover treated with AHP or NaOH will support *C. thermocellum*'s enzyme system.

$H_0: E_{NSAHP} = \ldots = E_{SBNaOH}$
H_A: At least two $\neq$ each other
$\alpha/_2 = 0.025$
$p - value = 0.0013$

Reject the null hypothesis, the interaction is significant when measuring products of *C. thermocellum's* enzyme system. The assumptions of the regression model are mostly met ($b_1 \sim Normal\left(\beta_1, \frac{\sigma_e^2}{(n-1)s_x^2}\right)$ for the interaction.



$NSH2O \neq NSAHP, SAAHP, SBAHP, SAH2O, NSNaOH, SANaOH \ \& \ SBNaOH$
$SBH2O \neq NSAHP, SAAHP, SBAHP, SAH2O, NSNaOH, SANaOH \ \& \ SBNaOH$

All other interactions are equal to each other.

In order to determine if the fermentation pathways are functional in the pretreated materials individual hypothesis tests were performed on the levels of the class variables.

$H_0: \mu_x = 0$
Where x = NSAHP, SAAHP, SBAHP, NSH2O, SAH2O SBH2O, NSNaOH, SANaOH or SBNaOH
$H_A: \mu_x > 0$
$\alpha = 0.05$ one sided test

If $(\Pr > |t|)$ < 0.05, reject H_0 and conclude with 95% confidence the interaction mean is significantly greater than zero. If $(\Pr > |t|)$ > 0.05, fail to reject H_0 and conclude with 95% confidence that the interaction mean is not significantly different than zero. The results are summarized in the following table.

INTERACTION	MEAN	STDEV	N	Pr > |t|	H_0: μ_x=0	CONCLUSION
NSAHP	186.124	74.732	12	0.0001	REJECT	$\mu > 0$
SAAHP	161.7806	132.132	12	0.0014	REJECT	$\mu > 0$
SBAHP	190.9279	93.999	12	0.0001	REJECT	$\mu > 0$
NSH2O	2028.229	1353.000	4	0.0577	FAIL TO REJECT	$\mu_x=0$
SAH2O	644.6455	115.018	4	0.0015	REJECT	$\mu > 0$
SBH2O	1868.219	1516.000	4	0.0905	FAIL TO REJECT	$\mu_x=0$
NSNaOH	275.807	117.297	12	0.0001	REJECT	$\mu > 0$
SANaOH	151.403	110.812	12	0.0006	REJECT	$\mu > 0$
SBNaOH	357.144	112.083	12	0.0001	REJECT	$\mu > 0$

All interactions between sterilization and pretreatment methods have a concentration of enzyme products statistically greater than zero, except NSH2O and SBH2O. From this we can conclude that corn stover treated with AHP or NaOH will support *C. thermocellum's* enzyme system.

SWITCHGRASS

LIGNIN CONTENT AS A FUNCTION OF PRETREATMENT

$H_0: L_{AHP} = L_{NaOH} = L_{H_2O} = L_{RAW}$

H_A: At least two of the Lignin Contents $\neq$ each other

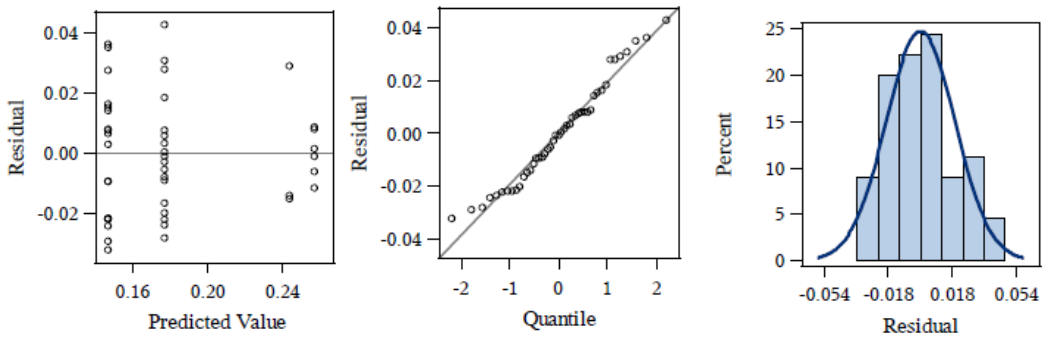
Level of Significance $\alpha = 0.05$, for two-tailed test use $^{\alpha}/_{2}$

Reject H_0 if $p < {}^{\alpha}/_{2}$

SOURCE	DF	Type I SS	Mean Square	F Value	Pr > F
PRETREATMENT	3	0.06766230	0.02255410	55.90	< 0.0001

Therefore, reject the null hypothesis and conclude that at least one of the pretreatments has a significant effect on lignin content.

Assumptions of Regression Model



Mean Zero (Linearity) - Same number of points above and below line

Homoscedasticity (Equal Variance) – All points lie close to Q-Q plot line

Independence – Little overlap in residual plot

Normality – Population distribution follows bell shaped curve

The assumptions of the regression model are met for lignin as a function of pretreatment

Tukey's Correction

Pretreatment	Raw	AHP	H2O	NaOH
RAW		< 0.0001	0.7934	< 0.0001
AHP	< 0.0001		< 0.0001	0.0003
H2O	0.7934	< 0.0001		< 0.0001
NaOH	< 0.0001	0.0003	< 0.0001	

Lignin content of RAW material is significantly different than AHP and NaOH pretreated material, there is no difference between the H2O Control and RAW and the lignin contents of AHP and NaOH are significantly different.

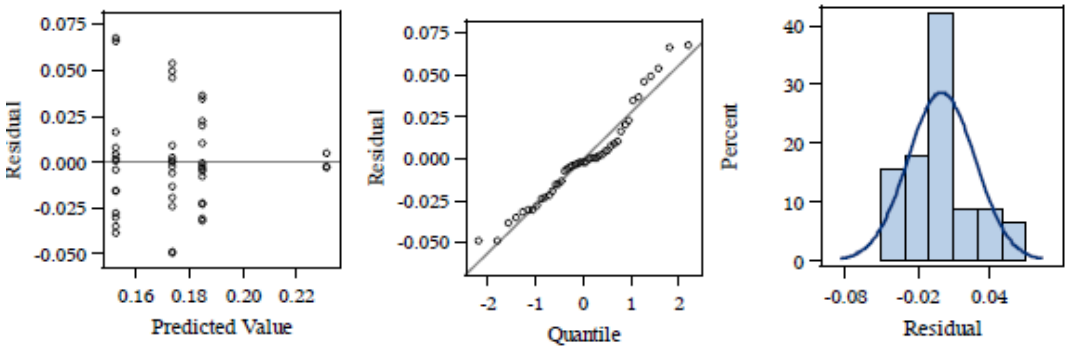
LIGNIN CONTENT AS A FUNCTION OF STERILIZATION

H_0: $L_{NS} = L_{RAW} = L_{SA} = L_{SB}$
H_A: At least two of the Lignin Contents $\neq$ each other
Level of Significance $\alpha = 0.05$, for two-tailed test use $\alpha/2$
Reject H_0 if $p < \alpha/2$

SOURCE	DF	Type I SS	Mean Square	F Value	Pr > F
STERILIZATION	3	0.02137813	0.00712604	4.65	0.0069

Therefore, reject the null hypothesis and conclude that at least one of the sterilization methods has a significant effect on lignin content.

Assumptions of Regression Model



Mean Zero (Linearity) – There are ~ numbers of points above and below line
Homoscedasticity (Equal Variance) – All points fall close to line on Q-Q Plot
Independence – Little overlap in residual plot
Normality – A normal bell shaped curve is found

The assumptions of the regression model are met for lignin content as a function of sterilization.

Tukey's Correction

STERILIZATION	NS	RAW	SA	SB
NS		0.1605	0.1161	0.8589
RAW	0.1605		0.0061	0.0598
SA	0.1161	0.0061		0.4439
SB	0.8589	0.0598	0.4439	

There is no statistical difference between the raw material, sterilization before and after and no sterilization. By sterilizing after pretreatment the lignin content is significantly different than the raw, but there is no difference between that and the other pretreatment methods. It could be then concluded that any lignin loses cannot be a result of sterilization technique.

LIGNIN CONTENT AS A FUNCTION OF PT STERILIZATION INTERACTION

H_0: $L_{NSAHP} = \cdots = L_{SBNaOH}$
H_A: $At\ least\ 2 \neq each\ other$
Level of Significance $\alpha = 0.05$
Reject H_0 if $p < \alpha/2$

SOURCE	DF	Type I SS	Mean Square	F Value	Pr > F
PRETREATMENT	3	0.06766230	0.02255410	104.47	<0.0001
STERILIZATION	2	0.00832615	0.00416307	19.28	<0.0001
PT*STERILIZATION	4	0.00065888	0.00016472	0.76	0.5565

SOURCE	DF	Type III SS	Mean Square	F Value	Pr > F
PRETREATMENT	3	0.05461031	0.04804631	126.48	<0.0001
STERILIZATION	2	0.00484603	0.00047189	11.22	0.0002
PT*STERILIZATION	4	0.00065888	0.00018025	0.73	0.5565

The null hypothesis would be rejected, and the interaction between pretreatment and sterilization is not significant, so the main effects must be evaluated. It is either pretreatment or sterilization that has significant effects on lignin content.

GLUCOSE CONCENTRATION AS A FUNCTION OF PRETREATMENT

H_0: $G_{AHP} = G_{NaOH} = G_{H_2O} = G_{RAW}$

H_A: At least two of the glucose concentrations $\neq$ each other

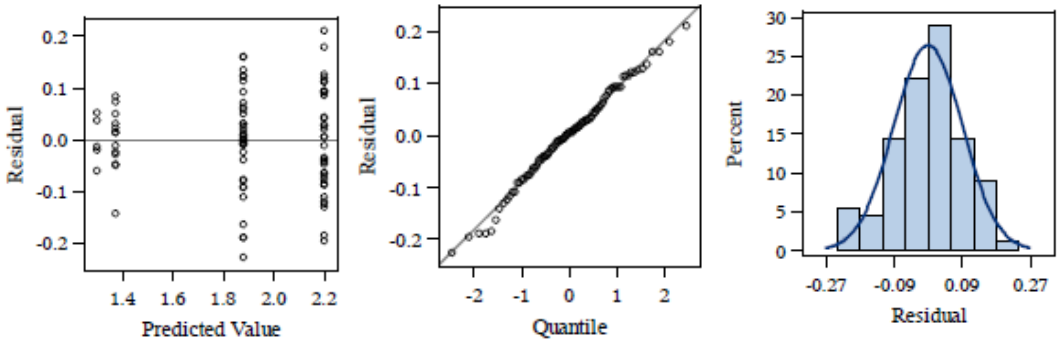
Level of Significance $\alpha = 0.05$, for two-tailed test use $\alpha/2$

Reject H_0 if $p < \alpha/2$

SOURCE	DF	Type I SS	Mean Square	F Value	Pr > F
PRETREATMENT	3	8.77460302	2.92486767	343.10	< 0.0001

Therefore, reject the null hypothesis and conclude that at least one of the pretreatments has a significant effect on glucose concentration.

Assumptions of Regression Model



Mean Zero - Same number of points above and below line

Homoscedasticity (Equal Variance) – All points lie close to Q-Q plot line

Independence – Little overlap in residual plot

Normality – Population distribution follows bell shaped curve

The assumptions of the regression model are met for glucose concentration as a function of pretreatment.

Tukey's Correction

Pretreatment	Raw	AHP	H2O	NaOH	Average
RAW		< 0.0001	0.4403	< 0.0001	1.296
AHP	< 0.0001		< 0.0001	< 0.0001	2.202
H2O	0.4403	< 0.0001		< 0.0001	1.370
NaOH	< 0.0001	< 0.0001	< 0.0001		1.879

$$G_{Raw} = G_{H2O}$$
$$G_{AHP} > G_{NaOH} > G_{Raw}$$

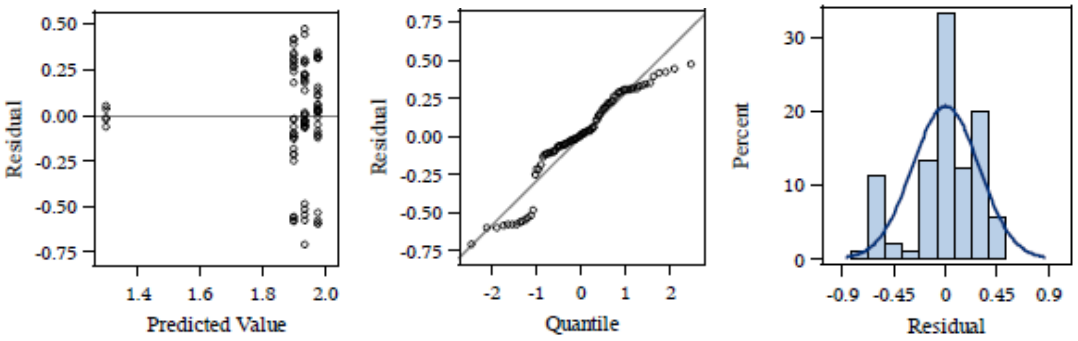
GLUCOSE CONCENTRATION AS A FUNCTION OF STERILIZATION

H_0: $G_{NS} = G_{RAW} = G_{SA} = G_{SB}$
H_A: At least two of the glucose concentrations $\neq$ each other
Level of Significance $\alpha = 0.05$, for two-tailed test use $\alpha/2$
Reject H_0 if $p < \alpha/2$

SOURCE	DF	Type I SS	Mean Square	F Value	Pr > F
PRETREATMENT	3	2.02508793	0.6750231	7.76	0.0001

Therefore, reject the null hypothesis and conclude that at least one of the sterilization techniques has a significant effect on glucose concentration.

Assumptions of Regression Model



Mean Zero – unequal number of points above & below
Homoscedasticity (Equal Variance) – curved Q-Q Plot
Independence – overlap in residual plot
Normality – uneven distribution

The assumptions of the regression model are NOT met for glucose concentration as a function of pretreatment.

Tukey's Correction

Pretreatment	NS	RAW	SA	SB	Average
NS		< 0.0001	0.9580	0.7793	1.939
RAW	< 0.0001		< 0.0001	< 0.0001	1.264
SA	0.9580	< 0.0001		0.4715	1.905
SB	0.7793	< 0.0001	0.4715		2.003

$$G_{NS} = G_{SA} = G_{SB}$$

Because all sterilization methods have statistically the same glucose concentration, any differences in glucose cannot be attributed to sterilization.

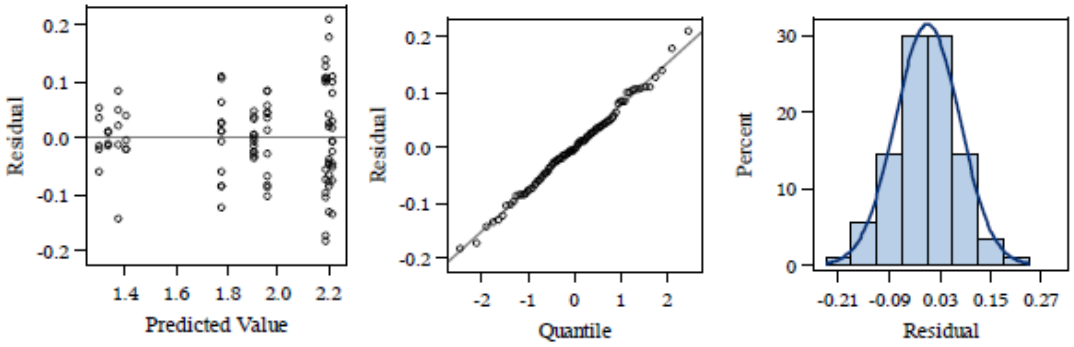
GLUCOSE CONCENTRATION AS A FUNCTION OF PT STERILIZATION INTERACTION

H_0: $G_{NSAHP} = \cdots = G_{SBNaOH}$
H_A: $At\ least\ 2 \neq each\ other$
Level of Significance $\alpha = 0.05$
Reject H_0 if $p < \alpha/2$

SOURCE	DF	Type I SS	Mean Square	F Value	Pr > F
PRETREATMENT	3	6.78850082	3.39425041	530.86	< 0.0001
STERILIZATION	2	0.06430478	0.03215239	5.03	0.0088
PT*STERILIZATION	4	0.13352574	0.03338143	5.22	0.0009

Therefore, reject the null hypothesis and conclude that the interaction between pretreatment and sterilization has a significant impact on glucose concentration

Assumptions of Regression Model



Mean Zero - Same number of points above and below line

Homoscedasticity (Equal Variance) – All points lie close to Q-Q plot line

Independence – Little overlap in residual plot

Normality – Population distribution follows bell shaped curve

The assumptions of the regression model are met for glucose concentration the interaction between pretreatment and sterilization.

Tukey's Correction

Least Squares Means for effect PRETREATM*STERILIZAT Pr > |t| for H0: LSMean(i)=LSMean(j) Dependent Variable: GLUCOSE										
i/j	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1		<.0001	<.0001	<.0001	0.9995	0.8978	0.6053	<.0001	<.0001	<.0001
2	<.0001		1.0000	0.9989	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
3	<.0001	1.0000		1.0000	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
4	<.0001	0.9989	1.0000		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
5	0.9995	<.0001	<.0001	<.0001		0.9994	0.9640	<.0001	<.0001	<.0001
6	0.8978	<.0001	<.0001	<.0001	0.9994		0.9998	<.0001	<.0001	<.0001
7	0.6053	<.0001	<.0001	<.0001	0.9640	0.9998		<.0001	<.0001	<.0001
8	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001		0.0067	<.0001
9	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0067		0.8306
10	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.8306	

$$G_{Raw} = G_{H2O}$$
$$G_{NSAHP} = G_{SAAHP} = G_{SBAHP}$$
$$G_{NSNaOH}$$
$$G_{SANaOH} = G_{SBNaOH}$$
$$G_{AHP} > G_{SA(B)NaOH} > G_{RAW}$$

XYLOSE CONCENTRATION AS A FUNCTION OF PRETREATMENT

H_0: $X_{AHP} = X_{NaOH} = X_{H_2O} = X_{RAW}$

H_A: At least two of the xylose concentrations $\neq$ each other

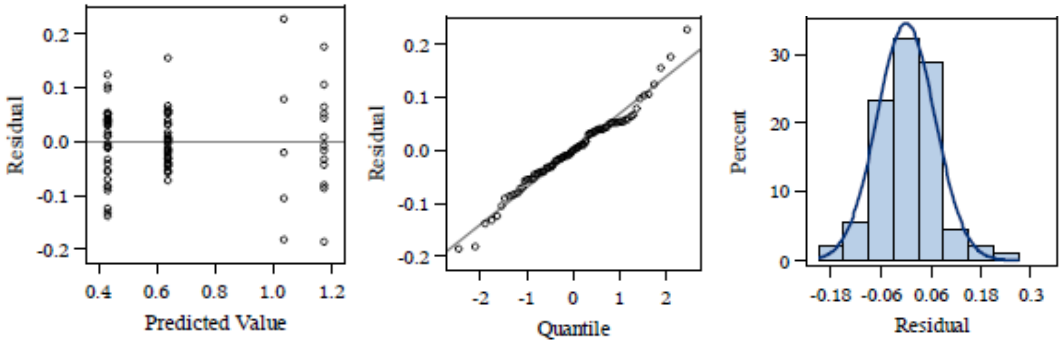
Level of Significance $\alpha = 0.05$, for two-tailed test use $^{\alpha}/_{2}$

Reject H_0 if $p < {}^{\alpha}/_{2}$

SOURCE	DF	Type I SS	Mean Square	F Value	Pr > F
PRETREATMENT	3	6.07668312	2.02556104	407.26	< 0.0001

Therefore, reject the null hypothesis and conclude that at least one of the pretreatments has a significant effect on xylose concentration.

Assumptions of Regression Model



Mean Zero – approximately same number of points above and below line

Homoscedasticity (Equal Variance) – All points lie close to Q-Q plot line

Independence – little overlap in residual plot

Normality – Population distribution follows bell shaped curve

The assumptions of the regression model are mostly met for xylose concentration as a function of pretreatment.

Tukey's Correction

Pretreatment	Raw	AHP	H2O	NaOH	Average
RAW		< 0.0001	0.0018	< 0.0001	1.039
AHP	< 0.0001		< 0.0001	< 0.0001	0.432
H2O	0.0018	< 0.0001		< 0.0001	1.177
NaOH	< 0.0001	< 0.0001	< 0.0001		0.638

$$X_{Raw} = X_{H2O} = X_{AHP}$$
$$X_{H2O} > X_{RAW} > X_{NaOH} > X_{AHP}$$

XYLOSE CONCENTRATION AS A FUNCTION OF STERILIZATION

H_0: $X_{NS} = X_{RAW} = X_{SA} = X_{SB}$

H_A: At least two of the xylose concentrations $\neq$ each other

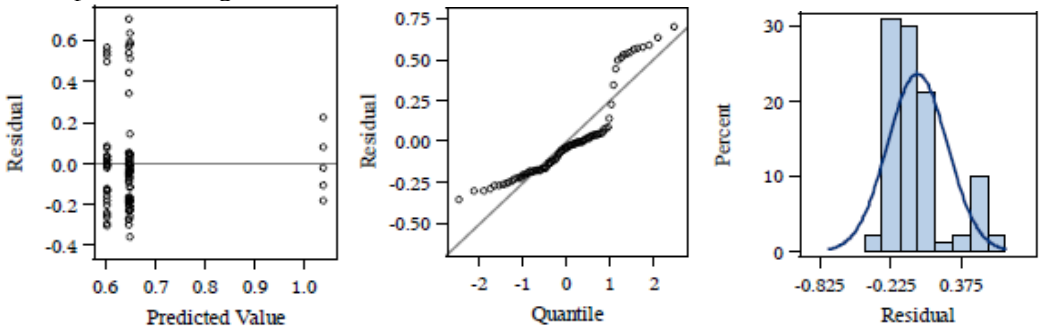
Level of Significance $\alpha = 0.05$, for two-tailed test use $^{\alpha}/_{2}$

Reject H_0 if $p < {}^{\alpha}/_{2}$

SOURCE	DF	Type I SS	Mean Square	F Value	Pr > F
PRETREATMENT	3	0.81458679	0.27152893	4.10	0.0090

Reject the null hypothesis and conclude that sterilization technique has a significant effect on xylose concentration.

Assumptions of Regression Model



Mean Zero – unequal number of points above & below
Homoscedasticity (Equal Variance) – curved Q-Q Plot
Independence – overlap in residual plot
Normality – Population distribution does not follows bell shaped curve

The assumptions of the regression model are NOT met for xylose concentration as a function of pretreatment.

Tukey's Correction

Pretreatment	NS	RAW	SA	SB	Average
NS		0.0042	0.9103	0.9076	0.603
RAW	0.0042		0.0123	0.0123	1.039
SA	0.9103	0.0123		1.0000	0.648
SB	0.9076	0.0129	1.000		0.649

$$G_{NS} = G_{SA} = G_{SB}$$

Because all sterilization methods have statistically the same xylose concentration, any differences in xylose cannot be attributed to sterilization.

XYLOSE CONCENTRATION AS A FUNCTION OF PT STERILIZATION INTERACTION

H_0: $X_{NSAHP} = \cdots = X_{SBNaOH}$
H_A: $At\ least\ 2\ \neq each\ other$
Level of Significance $\alpha = 0.05$
Reject H_0if $p < \alpha/2$

SOURCE	DF	Type III SS	Mean Square	F Value	Pr > F
PRETREATMENT	3	5.26870434	2.63435217	544.21	< 0.0001
STERILIZATION	2	0.03432585	0.01716292	3.55	0.0335
PT*STERILIZATION	4	0.01045208	0.00261302	0.54	0.7069

Fail to reject the null hypothesis and conclude that the interaction between pretreatment and sterilization does not have a significant impact on xylose concentration.

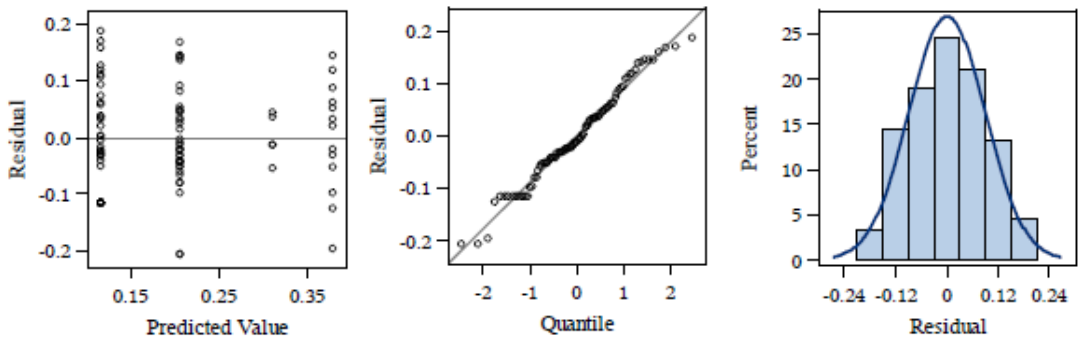
ARABINOSE CONCENTRATION AS A FUNCTION OF PRETREATMENT

H_0: $A_{AHP} = A_{NaOH} = A_{H_2O} = A_{RAW}$
H_A: At least two of the arabinose concentrations $\neq$ each other
Level of Significance $\alpha = 0.05$, for two-tailed test use $^{\alpha}/_{2}$
Reject H_0if $p < {}^{\alpha}/_{2}$

SOURCE	DF	Type I SS	Mean Square	F Value	Pr > F
PRETREATMENT	3	0.73356098	0.24452033	29.90	< 0.0001

Reject the null hypothesis and conclude that pretreatments have a significant effect on xylose concentration.

Assumptions of Regression Model



Mean Zero – approximately same number of points above and below line
Homoscedasticity (Equal Variance) – All points lie close to Q-Q plot line
Independence – overlap in residual plot
Normality – Population distribution follows bell shaped curve

The assumptions of the regression model are met for arabinose concentration as a function of pretreatment.

Tukey's Correction

Pretreatment	Raw	AHP	H2O	NaOH	Average
RAW		0.0001	0.4898	0.0788	0.311
AHP	0.0001		< 0.0001	0.0003	0.116
H2O	0.4898	< 0.0001		< 0.0001	0.378
NaOH	0.0788	0.0003	< 0.0001		0.206

$$A_{Raw} = A_{H2O} = A_{NaOH}$$
$$A_{RAW} > A_{AHP}$$

ARABINOSE CONCENTRATION AS A FUNCTION OF STERILIZATION

H_0: $A_{NS} = A_{RAW} = A_{SA} = A_{SB}$
H_A: At least two of the arabinose concentrations $\neq$ each other
Level of Significance $\alpha = 0.05$, for two-tailed test use $^{\alpha}/_{2}$
Reject H_0 if $p < {}^{\alpha}/_{2}$

SOURCE	DF	Type I SS	Mean Square	F Value	Pr > F
PRETREATMENT	3	0.07946901	0.02647867	1.68	0.1778

Fail to reject the null hypothesis and conclude that sterilization technique does not have a significant effect on arabinose concentration.

FERMENTATION PRODUCTS AS A FUNCTION OF PRETREATMENT

H_0: $F_{AHP} = F_{H2O} = F_{NaOH}$
H_A: At least two $\neq$ each other
${}^{\alpha}/_{2} = 0.025$
$p - value < 0.0001$

Reject H_0, Pretreatment is significant when measuring *C. thermocellum's* fermentation products. The assumptions of the regression model $b_1 \sim Normal\left(\beta_1, \frac{\sigma_e^2}{(n-1)s_x^2}\right)$ are not met for this variable.

$$\therefore F_{AHP} = F_{H2O} = F_{NaOH}$$

In order to determine if the fermentation pathways are functional in the pretreated materials individual hypothesis tests were performed on the levels of the class variables.

$H_0: \mu_x = 0$
Where x = AHP, H2O or NaOH
$H_A: \mu_x > 0$
$\alpha = 0.05$ one sided test

If $(\Pr > |t|) < 0.05$, reject H_0 and conclude with 95% confidence the pretreatment mean is significantly greater than zero. If $(\Pr > |t|) > 0.05$, fail to reject H_0 and conclude with 95% confidence that the pretreatment mean is not significantly different than zero. The results are summarized in the following table.

PRETREATMENT	MEAN	STDEV	N	Pr > |t|	H_0: μ_X=0	CONCLUSION
AHP	1.577	0.967	36	0.0001	REJECT	$\mu > 0$
H2O	7.623	5.404	12	0.0005	REJECT	$\mu > 0$
NaOH	1.678	1.339	36	0.0001	REJECT	$\mu > 0$

All pretreatments have a concentration of fermentation products statistically greater than zero. From this we can conclude that corn stover treated with AHP or NaOH will support *C. thermocellum* fermentation pathway.

FERMENTATION PRODUCTS AS A FUNCTION OF STERILIZATION

$H_0: F_{NS} = F_{SA} = F_{SB}$
H_A: At least two $\neq$ each other
$\alpha/_2 = 0.025$
$p - value < 0.0001$

Reject the null hypothesis, sterilization is significant when measuring *C. thermocellum's* fermentation products. Although the variable is significant, the assumptions of the regression model $b_1 \sim Normal\left(\beta_1, \frac{\sigma_e^2}{(n-1)s_x^2}\right)$ are not met.

$$\therefore F_{NS} = F_{SA} = F_{SB}$$

In order to determine if the fermentation pathways are functional in the pretreated materials individual hypothesis tests were performed on the levels of the class variables.

$H_0: \mu_x = 0$
Where x = NS, SA or SB
$H_A: \mu_x > 0$
$\alpha = 0.05$ one sided test

If $(\Pr > |t|) < 0.05$, reject H_0 and conclude with 95% confidence the sterilization mean is significantly greater than zero. If $(\Pr > |t|) > 0.05$, fail to reject H_0 and conclude with 95% confidence that the sterilization mean is not significantly different than zero. The results are summarized in the following table.

STERILIZATION	MEAN	STDEV	N	Pr > |t|	H_0: μ_X=0	CONCLUSION
NS	1.376	1.516	28	0.0001	REJECT	$\mu > 0$
SA	3.122	3.054	28	0.0001	REJECT	$\mu > 0$
SB	2.954	3.948	28	0.0005	REJECT	$\mu > 0$

All sterilization methods have a concentration of fermentation products statistically greater than zero. From this we can conclude that corn stover treated any sterilization technique will support the fermentation pathway of *C. thermocellum*.

FERMENTATION PRODUCTS AS A FUNCTION OF PT STERILIZATION INTERACTION

$H_0: F_{NSAHP} = \ldots = F_{SBNaOH}$

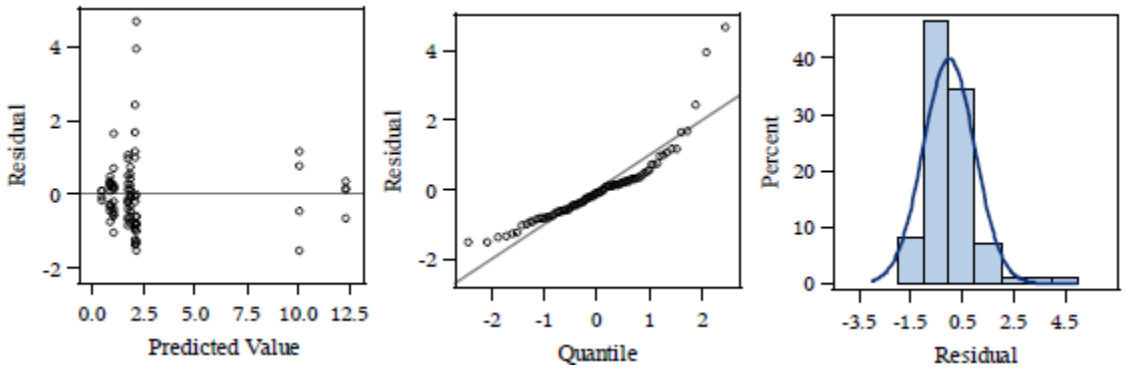
H_A: At least two $\neq$ each other

$\alpha/2 = 0.025$

$p - value < 0.0001$

Reject the null hypothesis, the interaction is significant when measuring *C. thermocellum's* fermentation products. The assumptions of the regression model are met

$(b_1 \sim Normal\left(\beta_1, \frac{\sigma_e^2}{(n-1)s_x^2}\right)$.



In order to determine if the fermentation pathways are functional in the pretreated materials individual hypothesis tests were performed on the levels of the class variables.

$H_0: \mu_x = 0$

Where x = NSAHP, SAAHP, SBAHP, NSH2O, SAH2O SBH2O, NSNaOH, SANaOH or SBNaOH

$H_A: \mu_x > 0$

$\alpha = 0.05$ one sided test

If $(\Pr > |t|) < 0.05$, reject H_0 and conclude with 95% confidence the interaction mean is significantly greater than zero. If $(\Pr > |t|) > 0.05$, fail to reject H_0 and conclude with 95% confidence that the interaction mean is not significantly different than zero. The results are summarized in the following table.

INTERACTION	MEAN	STDEV	N	Pr > |t|	H_0: μ_X=0	CONCLUSION
NSAHP	0.886	0.396	12	0.0001	REJECT	μ > 0
SAAHP	2.082	1.263	12	0.0001	REJECT	μ > 0
SBAHP	1.764	0.620	12	0.0001	REJECT	μ > 0
NSH2O	0.475	0.117	4	0.0039	REJECT	μ > 0
SAH2O	10.098	1.219	4	0.0005	REJECT	μ > 0
SBH2O	12.295	0.440	4	0.0001	REJECT	μ > 0
NSNaOH	2.166	2.060	12	0.0039	REJECT	μ > 0
SANaOH	1.837	0.465	12	0.0001	REJECT	μ > 0
SBNaOH	1.031	0.708	12	0.0004	REJECT	μ > 0

All interactions have a concentration of fermentation products statistically greater than zero. From this we can conclude that corn stover treated with AHP or NaOH will support *C. thermocellum's* fermentation pathway.

ENZYME PRODUCTS AS A FUNCTION OF PRETREATMENT

$H_0: E_{AHP} = E_{H2O} = E_{NaOH}$
H_A: At least two $\neq$ each other
$\alpha/_2 = 0.025$
$p - value = 0.0789$

Fail to reject H_0, pretreatment is not significant when measuring *C. thermocellum's* enzyme products. However, the assumptions of the regression model are met for this variable.

$$\therefore E_{AHP} = E_{H2O} = E_{NaOH}$$

In order to determine if the enzyme system is functional for the pretreated materials individual hypothesis tests were performed on the levels of the class variables.

$H_0: \mu_x = 0$
Where x = AHP, H2O or NaOH
$H_A: \mu_x > 0$
$\alpha = 0.05$ one sided test

If $(\Pr > |t|)$ < 0.05, reject H_0 and conclude with 95% confidence the pretreatment mean is significantly greater than zero. If $(\Pr > |t|)$ > 0.05, fail to reject H_0 and conclude with 95% confidence that the pretreatment mean is not significantly different than zero. The results are summarized in the following table.

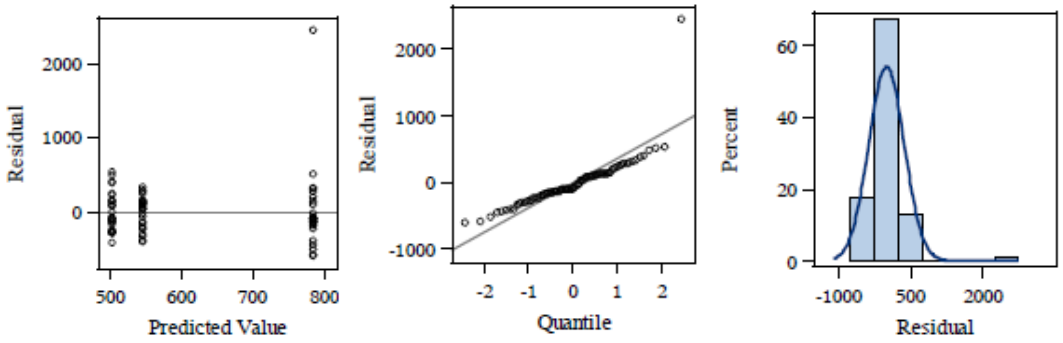
PRETREATMENT	MEAN	STDEV	N	Pr > |t|	H_0: μ_X=0	CONCLUSION
AHP	720.047	513.910	36	0.0001	REJECT	μ > 0
H2O	503.499	241.888	12	0.0001	REJECT	μ > 0
NaOH	537.587	230.428	36	0.0001	REJECT	μ > 0

All pretreatments have a concentration of enzyme products statistically greater than zero. From this we can conclude that corn stover treated with AHP or NaOH will support *C. thermocellum's* enzyme system.

ENZYME PRODUCTS AS A FUNCTION OF STERILIZATION

$H_0: E_{NS} = E_{SA} = E_{SB}$
H_A: At least two $\neq$ each other
$\alpha/_2 = 0.025$
$p - value = 0.0118$

Reject the null hypothesis, sterilization is significant when measuring *C. thermocellum's* enzyme products. The assumptions of the regression model $b_1 \sim Normal\left(\beta_1, \frac{\sigma_e^2}{(n-1)s_x^2}\right)$ are met.



$\therefore E_{NS} > E_{SA} | E_{SB}$

In order to determine if the enzyme system is functional in the pretreated materials individual hypothesis tests were performed on the levels of the class variables.

$H_0: \mu_x = 0$
Where x = NS, SA or SB
$H_A: \mu_x > 0$
$\alpha = 0.05$ one sided test

If $(\Pr > |t|)$ < 0.05, reject H_0 and conclude with 95% confidence the sterilization mean is significantly greater than zero. If $(\Pr > |t|)$ > 0.05, fail to reject H_0 and conclude with 95% confidence that the sterilization mean is not significantly different than zero. The results are summarized in the following table.

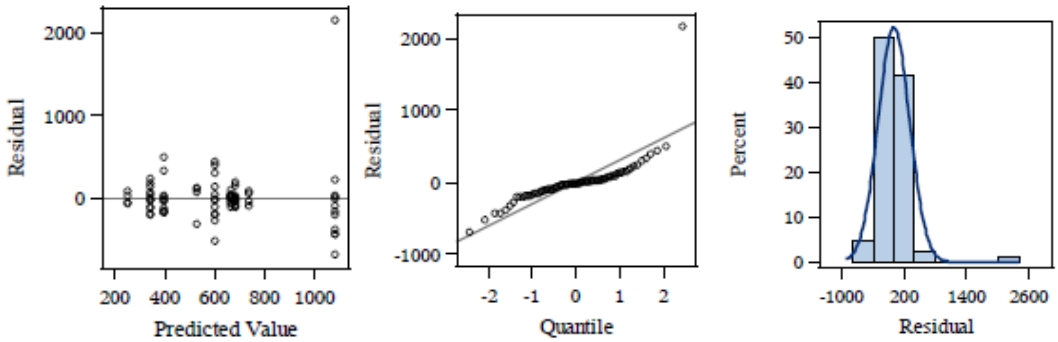
STERILIZATION	MEAN	STDEV	N	Pr > |t|	H_0: μ_X=0	CONCLUSION
NS	785.070	551.821	28	0.0001	REJECT	$\mu > 0$
SA	50.242	257.684	28	0.0001	REJECT	$\mu > 0$
SB	545.384	210.119	28	0.0001	REJECT	$\mu > 0$

All sterilization methods have a concentration of enzyme products statistically greater than zero. From this we can conclude that corn stover treated with AHP or NaOH will support *C. thermocellum's* enzyme system.

ENZYME PRODUCTS AS A FUNCTION OF PT STERILIZATION INTERACTION

$H_0: E_{NSAHP} = \ldots = E_{SBNaOH}$
H_A: At least two $\neq$ each other
$\alpha/_2 = 0.025$
$p - value = 0.0001$

Reject the null hypothesis, the interaction is significant when measuring products of *C. thermocellum's* enzyme system. The assumptions of the regression model are met ($b_1 \sim Normal\left(\beta_1, \frac{\sigma_e^2}{(n-1)s_x^2}\right)$ for the interaction.



In order to determine if the fermentation pathways are functional in the pretreated materials individual hypothesis tests were performed on the levels of the class variables.

$H_0: \mu_x = 0$

Where x = NSAHP, SAAHP, SBAHP, NSH2O, SAH2O SBH2O, NSNaOH, SANaOH or SBNaOH

$H_A: \mu_x > 0$

$\alpha = 0.05$ one sided test

If $(Pr > |t|)$ < 0.05, reject H_0 and conclude with 95% confidence the interaction mean is significantly greater than zero. If $(Pr > |t|)$ > 0.05, fail to reject H_0 and conclude with 95% confidence that the interaction mean is not significantly different than zero. The results are summarized in the following table.

INTERACTION	MEAN	STDEV	N	Pr > |t|	H_0: μ_X=0	CONCLUSION
NSAHP	1081.911	726.477	12	0.0003	REJECT	μ > 0
SAAHP	394.554	211.069	12	0.0001	REJECT	μ > 0
SBAHP	683.676	90.235	12	0.0001	REJECT	μ > 0
NSH2O	250.536	70.023	4	0.0056	REJECT	μ > 0
SAH2O	526.095	212.472	4	0.0158	REJECT	μ > 0
SBH2O	734.765	84.703	4	0.0004	REJECT	μ > 0
NSNaOH	666.408	54.158	12	0.0001	REJECT	μ > 0
SANaOH	602.388	286.922	12	0.0001	REJECT	μ > 0
SBNaOH	343.966	141.800	12	0.0001	REJECT	μ > 0

All interactions between sterilization and pretreatment methods have a concentration of enzyme products statistically greater than zero. From this we can conclude that corn stover treated with AHP or NaOH will support *C. thermocellum's* enzyme system.

MISCANTHUS

LIGNIN CONTENT AS A FUNCTION OF PRETREATMENT

$H_0: L_{AHP} = L_{NaOH} = L_{H_2O} = L_{RAW}$

H_A: At least two of the Lignin Contents $\neq$ each other

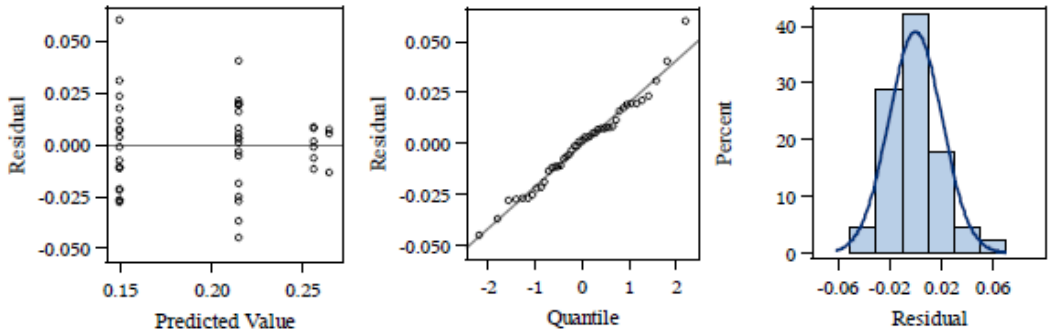
Level of Significance $\alpha = 0.05$, for two-tailed test use $\alpha/2$

Reject H_0 if $p < \alpha/2$

SOURCE	**DF**	**Type I SS**	**Mean Square**	**F Value**	**Pr > F**
PRETREATMENT	3	0.08243329	0.02747776	61.05	< 0.0001

Therefore, reject the null hypothesis and conclude that at least one of the pretreatments has a significant effect on lignin content.

Assumptions of Regression Model



Mean Zero (Linearity) - Same number of points above and below line

Homoscedasticity (Equal Variance) – All points lie close to Q-Q plot line

Independence – Little overlap in residual plot

Normality – Population distribution follows bell shaped curve

The assumptions of the regression model are met for lignin as a function of pretreatment

Tukey's Correction

Pretreatment	**Raw**	**AHP**	**H2O**	**NaOH**
RAW		< 0.0001	0.9460	0.0030
AHP	< 0.0001		< 0.0001	< 0.0001
H2O	0.9460	< 0.0001		0.0010
NaOH	0.0030	< 0.0001	0.0010	

Lignin content of RAW material is significantly different than AHP and NaOH pretreated material, there is no difference between the H2O Control and RAW and the lignin contents of AHP and NaOH are significantly different.

LIGNIN CONTENT AS A FUNCTION OF STERILIZATION

H_0: $L_{NS} = L_{RAW} = L_{SA} = L_{SB}$

H_A: At least two of the Lignin Contents $\neq$ each other

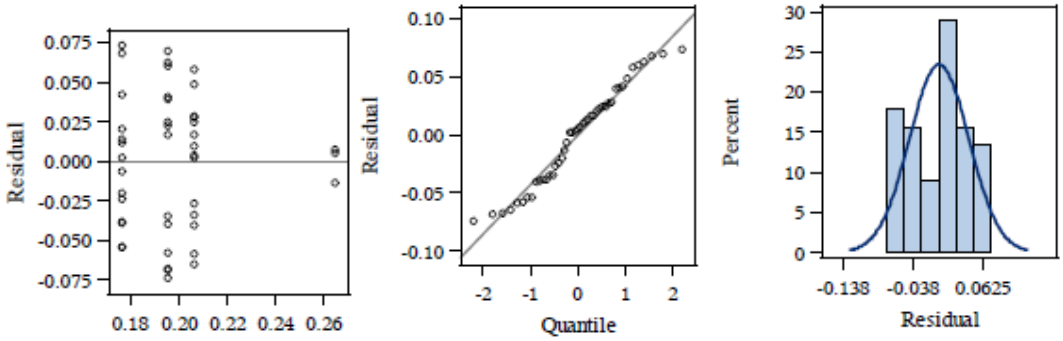
Level of Significance $\alpha = 0.05$, for two-tailed test use $\alpha/2$

Reject H_0 if $p < \alpha/2$

SOURCE	**DF**	**Type I SS**	**Mean Square**	**F Value**	**Pr > F**
STERILIZATION	3	0.02098029	0.00699343	3.59	0.0216

Therefore, reject the null hypothesis and conclude that at least one of the sterilization methods has a significant effect on lignin content.

Assumptions of Regression Model



Mean Zero (Linearity) – Approximately equal number of points above and below line
Homoscedasticity (Equal Variance) – All points fall close to line on Q-Q Plot
Independence – Little overlap in residual plot
Normality – A normal bell shaped curve is found

The assumptions of the regression model are met for lignin content as a function of sterilization.

Tukey's Correction

STERILIZATION	NS	RAW	SA	SB
NS		0.1774	0.2889	0.9102
RAW	0.1774		0.0157	0.0796
SA	0.2889	0.0157		0.6704
SB	0.9102	0.0796	0.6704	

There is no statistical difference between the raw material, sterilization before and after and no sterilization. By sterilizing after pretreatment the lignin content is significantly different than the raw, but there is no difference between that and the other pretreatment methods. It could be then concluded that any lignin loses cannot be a result of sterilization technique.

LIGNIN CONTENT AS A FUNCTION OF PT STERILIZATION INTERACTION

$H_0: L_{NSAHP} = \cdots = L_{SBNaOH}$
$H_A: At\ least\ 2\ \neq each\ other$
Level of Significance $\alpha = 0.05$
Reject H_0 if $p < \alpha/2$

SOURCE	DF	Type I SS	Mean Square	F Value	Pr > F
PRETREATMENT	3	0.08243329	0.02747776	109.05	<0.0001
STERILIZATION	2	0.00644564	0.00322282	12.79	<0.0001
PT*STERILIZATION	4	0.00318793	0.00079698	3.16	0.0255

SOURCE	DF	Type III SS	Mean Square	F Value	Pr > F
PRETREATMENT	3	0.06789863	0.03394931	134.73	<0.0001
STERILIZATION	2	0.00384969	0.00192484	7.64	0.0018
PT*STERILIZATION	4	0.00318793	0.00079698	3.16	0.0255

The null hypothesis would not be rejected, and the interaction between pretreatment and sterilization is not significant.

GLUCOSE CONCENTRATION AS A FUNCTION OF PRETREATMENT

H_0: $G_{AHP} = G_{NaOH} = G_{H_2O} = G_{RAW}$

H_A: At least two of the glucose concentrations $\neq$ each other

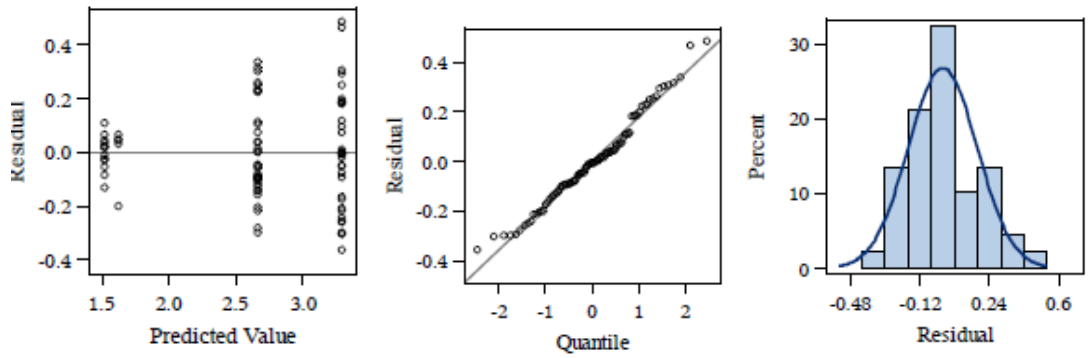
Level of Significance $\alpha = 0.05$, for two-tailed test use $\alpha/2$

Reject H_0 if $p < \alpha/2$

SOURCE	DF	Type I SS	Mean Square	F Value	Pr > F
PRETREATMENT	3	35.94174120	11.98054040	342.08	< 0.0001

Therefore, reject the null hypothesis and conclude that at least one of the pretreatments has a significant effect on glucose concentration.

Assumptions of Regression Model



Mean Zero - Same number of points above and below line

Homoscedasticity (Equal Variance) – All points lie close to Q-Q plot line

Independence – Little overlap in residual plot

Normality – Population distribution follows bell shaped curve

The assumptions of the regression model are met for glucose concentration as a function of pretreatment.

Tukey's Correction

Pretreatment	Raw	AHP	H2O	NaOH	Average
RAW		< 0.0001	0.7621	< 0.0001	1.619
AHP	< 0.0001		< 0.0001	< 0.0001	3.279
H2O	0.7621	< 0.0001		< 0.0001	1.523
NaOH	< 0.0001	< 0.0001	< 0.0001		2.664

$$G_{Raw} = G_{H2O}$$
$$G_{AHP} > G_{NaOH} > G_{Raw}$$

GLUCOSE CONCENTRATION AS A FUNCTION OF STERILIZATION

H_0: $G_{NS} = G_{RAW} = G_{SA} = G_{SB}$

H_A: At least two of the glucose concentrations $\neq$ each other

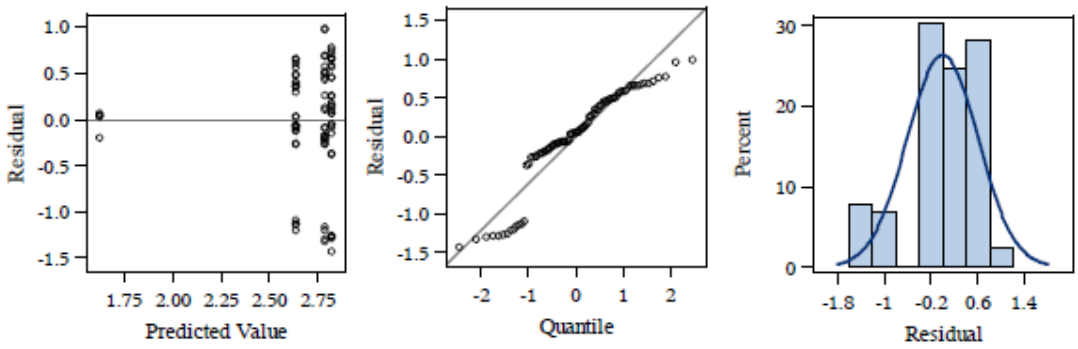
Level of Significance $\alpha = 0.05$, for two-tailed test use $\alpha/2$

Reject H_0 if $p < \alpha/2$

SOURCE	DF	Type I SS	Mean Square	F Value	Pr > F
PRETREATMENT	3	6.60370249	2.20123416	5.85	0.0011

Therefore, reject the null hypothesis and conclude that at least one of the sterilization techniques has a significant effect on glucose concentration.

Assumptions of Regression Model



Mean Zero – unequal number of points above & below
Homoscedasticity (Equal Variance) – curved Q-Q Plot
Independence – overlap in residual plot
Normality – uneven distribution

The assumptions of the regression model are NOT met for glucose concentration as a function of pretreatment.

Tukey's Correction

Pretreatment	NS	RAW	SA	SB	Average
NS		0.0054	0.6575	0.7791	2.635
RAW	0.0054		0.0006	0.0010	1.619
SA	0.6575	0.0006		0.9972	2.822
SB	0.7791	0.0010	0.9972		2.790

$$G_{NS} = G_{SA} = G_{SB}$$

Because all sterilization methods have statistically the same glucose concentration, any differences in glucose cannot be attributed to sterilization.

GLUCOSE CONCENTRATION AS A FUNCTION OF PT STERILIZATION INTERACTION

H_0: $G_{NSAHP} = \cdots = G_{SBNaOH}$
H_A: $At\ least\ 2\ \neq each\ other$
Level of Significance $\alpha = 0.05$
Reject H_0 if $p < \alpha/2$

SOURCE	DF	Type III SS	Mean Square	F Value	Pr > F
PRETREATMENT	3	29.69068396	14.84534198	574.79	< 0.0001
STERILIZATION	2	0.41901410	0.20950705	8.11	0.0006
PT*STERILIZATION	4	0.16222337	0.04055584	1.57	0.1903

Fail to reject the null hypothesis and conclude that the interaction between pretreatment and sterilization does not have a significant impact on glucose concentration.

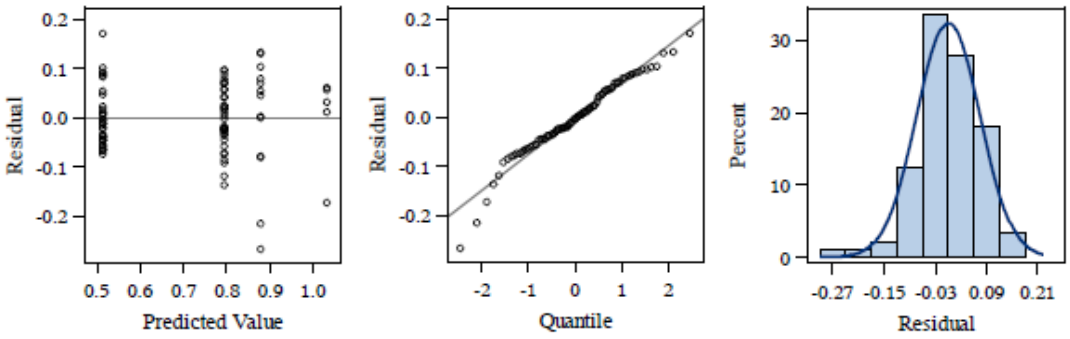
XYLOSE CONCENTRATION AS A FUNCTION OF PRETREATMENT

H_0: $X_{AHP} = X_{NaOH} = X_{H_2O} = X_{RAW}$
H_A: At least two of the xylose concentrations $\neq$ each other
Level of Significance $\alpha = 0.05$, for two-tailed test use $^{\alpha}/_{2}$
Reject H_0 if $p < {}^{\alpha}/_{2}$

SOURCE	DF	Type I SS	Mean Square	F Value	Pr > F
PRETREATMENT	3	2.46512667	0.82170889	145.29	< 0.0001

Therefore, reject the null hypothesis and conclude that at least one of the pretreatments has a significant effect on xylose concentration.

Assumptions of Regression Model



Mean Zero – approximately same number of points above and below line

Homoscedasticity (Equal Variance) – All points lie close to Q-Q plot line

Independence – little overlap in residual plot

Normality – Population distribution follows bell shaped curve

The assumptions of the regression model are met for xylose concentration as a function of pretreatment.

Tukey's Correction

Pretreatment	Raw	AHP	H2O	NaOH	Average
RAW		< 0.0001	0.0013	0.0013	1.031
AHP	< 0.0001		< 0.0001	< 0.0001	0.515
H2O	0.0013	< 0.0001		0.0037	0.879
NaOH	< 0.0001	< 0.0001	0.0037		0.793

$$X_{Raw} > X_{H2O} > X_{NaOH} > X_{AHP}$$

XYLOSE CONCENTRATION AS A FUNCTION OF STERILIZATION

H_0: $X_{NS} = X_{RAW} = X_{SA} = X_{SB}$

H_A: At least two of the xylose concentrations $\neq$ each other

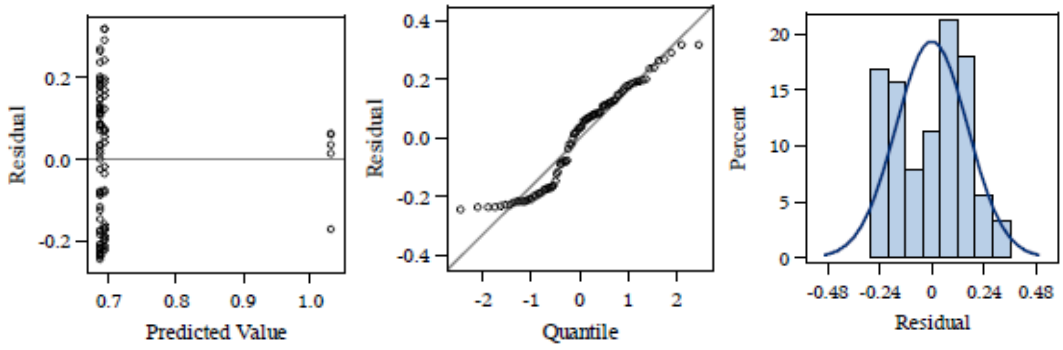
Level of Significance $\alpha = 0.05$, for two-tailed test use $^{\alpha}/_{2}$

Reject H_0 if $p < {}^{\alpha}/_{2}$

SOURCE	DF	Type I SS	Mean Square	F Value	Pr > F
PRETREATMENT	3	0.53226395	0.17742132	5.60	0.0015

Reject the null hypothesis and conclude that sterilization technique has a significant effect on xylose concentration.

Assumptions of Regression Model



Mean Zero – unequal number of points above & below
Homoscedasticity (Equal Variance) – curved Q-Q Plot
Independence – overlap in residual plot
Normality – Population distribution does not follows bell shaped curve

The assumptions of the regression model are NOT met for xylose concentration as a function of pretreatment.

Tukey's Correction

Pretreatment	NS	RAW	SA	SB	Average
NS		0.0009	0.9991	0.9742	0.688
RAW	0.0009		0.0011	0.0019	1.031
SA	0.9991	0.0011		0.9911	0.694
SB	0.9742	0.0019	0.9911		0.708

$$X_{NS} = X_{SA} = X_{SB}$$

Because all sterilization methods have statistically the same xylose concentration, any differences in xylose cannot be attributed to sterilization.

XYLOSE CONCENTRATION AS A FUNCTION OF PT STERILIZATION INTERACTION

H_0: $X_{NSAHP} = \cdots = X_{SBNaOH}$
H_A: $At\ least\ 2\ \neq each\ other$
Level of Significance $\alpha = 0.05$
Reject H_0if $p < \alpha/2$

SOURCE	DF	Type III SS	Mean Square	F Value	Pr > F
PRETREATMENT	3	1.67979174	0.83838087	68.41	< 0.0001
STERILIZATION	2	0.00668954	0.00334477	0.27	0.7619
PT*STERILIZATION	4	0.05066548	0.01266637	1.03	0.3952

Fail to reject the null hypothesis and conclude that the interaction between pretreatment and sterilization does not have a significant impact on xylose concentration.

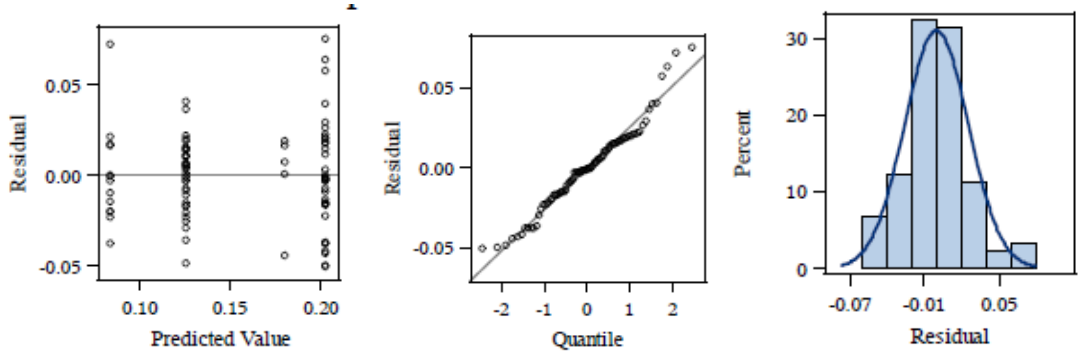
ARABINOSE CONCENTRATION AS A FUNCTION OF PRETREATMENT

H_0: $A_{AHP} = A_{NaOH} = A_{H_2O} = A_{RAW}$
H_A: At least two of the arabinose concentrations $\neq$ each other
Level of Significance $\alpha = 0.05$, for two-tailed test use $^{\alpha}/_{2}$
Reject H_0if $p < {}^{\alpha}/_{2}$

SOURCE	DF	Type I SS	Mean Square	F Value	Pr > F
PRETREATMENT	3	0.18388497	0.06129499	89.66	< 0.0001

Reject the null hypothesis and conclude that pretreatments have a significant effect on arabinose concentration.

Assumptions of Regression Model



Mean Zero – approximately same number of points above and below line

Homoscedasticity (Equal Variance) – All points lie close to Q-Q plot line

Independence – overlap in residual plot

Normality – Population distribution follows bell shaped curve

The assumptions of the regression model are met for arabinose concentration as a function of pretreatment.

Tukey's Correction

Pretreatment	Raw	AHP	H2O	NaOH	Average
RAW		0.0001	< 0.001	0.2867	0.180
AHP	0.0001		< 0.0001	0.0003	0.125
H2O	< 0.0001	< 0.0001		< 0.0001	0.084
NaOH	0.2867	< 0.0001	< 0.0001		0.203

$$A_{Raw} = A_{NaOH}$$
$$A_{RAW} > A_{AHP} > A_{H2O}$$

ARABINOSE CONCENTRATION AS A FUNCTION OF STERILIZATION

H_0: $A_{NS} = A_{RAW} = A_{SA} = A_{SB}$

H_A: At least two of the arabinose concentrations $\neq$ each other

Level of Significance $\alpha = 0.05$, for two-tailed test use $^{\alpha}/_{2}$

Reject H_0 if $p < {}^{\alpha}/_{2}$

SOURCE	DF	Type I SS	Mean Square	F Value	Pr > F
PRETREATMENT	3	0.00449509	0.00149836	0.54	0.6555

Fail to reject the null hypothesis and conclude that sterilization technique does not have a significant effect on arabinose concentration.

FERMENTATION PRODUCTS AS A FUNCTION OF PRETREATMENT

H_0: $F_{AHP} = F_{H2O} = F_{NaOH}$

H_A: At least two $\neq$ each other

${}^{\alpha}/_{2} = 0.025$

$p - value < 0.0001$

Reject H_0, Pretreatment is significant when measuring *C. thermocellum's* fermentation products. The assumptions of the regression model $b_1 \sim Normal\left(\beta_1, \frac{\sigma_e^2}{(n-1)s_x^2}\right)$ are not met for this variable.

$$\therefore F_{AHP} = F_{H2O} = F_{NaOH}$$

In order to determine if the fermentation pathways are functional in the pretreated materials individual hypothesis tests were performed on the levels of the class variables.

$H_0: \mu_x = 0$
Where x = AHP, H2O or NaOH
$H_A: \mu_x > 0$
$\alpha = 0.05$ one sided test

If $(\Pr > |t|) < 0.05$, reject H_0 and conclude with 95% confidence the pretreatment mean is significantly greater than zero. If $(\Pr > |t|) > 0.05$, fail to reject H_0 and conclude with 95% confidence that the pretreatment mean is not significantly different than zero. The results are summarized in the following table.

PRETREATMENT	MEAN	STDEV	N	Pr > |t|	H_0: μ_X=0	CONCLUSION
AHP	0.387	0.205	36	0.0001	REJECT	$\mu > 0$
H2O	6.754	5.261	12	0.0010	REJECT	$\mu > 0$
NaOH	0.469	0.153	36	0.0001	REJECT	$\mu > 0$

All pretreatments have a concentration of fermentation products statistically greater than zero. From this we can conclude that corn stover treated with AHP or NaOH will support *C. thermocellum* fermentation pathway.

FERMENTATION PRODUCTS AS A FUNCTION OF STERILIZATION

$H_0: F_{NS} = F_{SA} = F_{SB}$
H_A: At least two $\neq$ each other
$\alpha/_2 = 0.025$
$p - value = 0.1225$

Fail to reject the null hypothesis, sterilization is not significant when measuring *C. thermocellum's* fermentation products. The assumptions of the regression model $b_1 \sim Normal\left(\beta_1, \frac{\sigma_e^2}{(n-1)s_x^2}\right)$ are not met.

$$\therefore F_{NS} = F_{SA} = F_{SB}$$

In order to determine if the fermentation pathways are functional in the pretreated materials individual hypothesis tests were performed on the levels of the class variables.

$H_0: \mu_x = 0$
Where x = NS, SA or SB
$H_A: \mu_x > 0$
$\alpha = 0.05$ one sided test

If $(\Pr > |t|) < 0.05$, reject H_0 and conclude with 95% confidence the sterilization mean is significantly greater than zero. If $(\Pr > |t|) > 0.05$, fail to reject H_0 and conclude with 95% confidence that the sterilization mean is not significantly different than zero. The results are summarized in the following table.

STERILIZATION	MEAN	STDEV	N	Pr > |t|	H_0: μ_X=0	CONCLUSION
NS	0.463	0.224	28	0.0001	REJECT	$\mu > 0$
SA	1.479	2.922	28	0.0124	REJECT	$\mu > 0$
SB	2.053	4.084	28	0.0130	REJECT	$\mu > 0$

All sterilization methods have a concentration of fermentation products statistically greater than zero. From this we can conclude that corn stover treated any sterilization technique will support the fermentation pathway of *C. thermocellum*.

FERMENTATION PRODUCTS AS A FUNCTION OF PT STERILIZATION INTERACTION

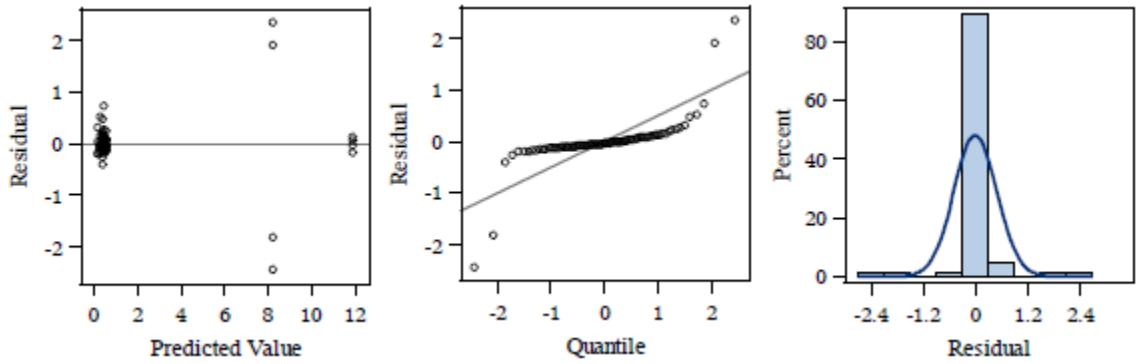
$H_0: F_{NSAHP} = \ldots = F_{SBNaOH}$

H_A: At least two $\neq$ each other

$\alpha/2 = 0.025$

$p - value < 0.0001$

Reject the null hypothesis, the interaction is significant when measuring *C. thermocellum's* fermentation products. The assumptions of the regression model are mostly met ($b_1 \sim Normal\left(\beta_1, \frac{\sigma_e^2}{(n-1)s_x^2}\right)$.



In order to determine if the fermentation pathways are functional in the pretreated materials individual hypothesis tests were performed on the levels of the class variables.

$H_0: \mu_x = 0$

Where x = NSAHP, SAAHP, SBAHP, NSH2O, SAH2O SBH2O, NSNaOH, SANaOH or SBNaOH

$H_A: \mu_x > 0$

$\alpha = 0.05$ one sided test

If $(\Pr > |t|)$ < 0.05, reject H_0 and conclude with 95% confidence the interaction mean is significantly greater than zero. If $(\Pr > |t|)$ > 0.05, fail to reject H_0 and conclude with 95% confidence that the interaction mean is not significantly different than zero. The results are summarized in the following table.

INTERACTION	MEAN	STDEV	N	Pr > |t|	H_0: μ_X=0	CONCLUSION
NSAHP	0.472	0.239	12	0.0001	REJECT	$\mu > 0$
SAAHP	0.298	0.183	12	0.0001	REJECT	$\mu > 0$
SBAHP	0.390	0.163	12	0.0001	REJECT	$\mu > 0$
NSH2O	0.183	0.241	4	0.2259	FAIL TO REJECT	μ_x=0
SAH2O	8.210	2.472	4	0.0070	REJECT	$\mu > 0$
SBH2O	11.871	0.127	4	0.0001	REJECT	$\mu > 0$
NSNaOH	0.548	0.120	12	0.0001	REJECT	$\mu > 0$
SANaOH	0.416	0.187	12	0.0001	REJECT	$\mu > 0$
SBNaOH	0.444	0.122	12	0.0001	REJECT	$\mu > 0$

All interactions, except NSH2O, have a concentration of fermentation products statistically greater than zero. From this we can conclude that corn stover treated with AHP or NaOH will support *C. thermocellum's* fermentation pathway.

ENZYME PRODUCTS AS A FUNCTION OF PRETREATMENT

$H_0: E_{AHP} = E_{H2O} = E_{NaOH}$
H_A: At least two $\neq$ each other
$\alpha/_2 = 0.025$
$p - value = 0.0959$

Fail to reject H_0, pretreatment is not significant when measuring *C. thermocellum's* enzyme products. The assumptions of the regression model are not met for this variable.

$$\therefore E_{AHP} = E_{H2O} = E_{NaOH}$$

In order to determine if the enzyme system is functional for the pretreated materials individual hypothesis tests were performed on the levels of the class variables.

$H_0: \mu_x = 0$
Where x = AHP, H2O or NaOH
$H_A: \mu_x > 0$
$\alpha = 0.05$ one sided test

If $(\Pr > |t|) < 0.05$, reject H_0 and conclude with 95% confidence the pretreatment mean is significantly greater than zero. If $(\Pr > |t|) > 0.05$, fail to reject H_0 and conclude with 95% confidence that the pretreatment mean is not significantly different than zero. The results are summarized in the following table.

PRETREATMENT	MEAN	STDEV	N	Pr > |t|	H_0: μ_X=0	CONCLUSION
AHP	598.337	856.988	36	0.0002	REJECT	$\mu > 0$
H2O	484.209	242.623	12	0.0001	REJECT	$\mu > 0$
NaOH	271.797	423.250	36	0.0005	REJECT	$\mu > 0$

All pretreatments have a concentration of enzyme products statistically greater than zero. From this we can conclude that corn stover treated with AHP or NaOH will support *C. thermocellum's* enzyme system.

ENZYME PRODUCTS AS A FUNCTION OF STERILIZATION

$H_0: E_{NS} = E_{SA} = E_{SB}$
H_A: At least two $\neq$ each other
$\alpha/2 = 0.025$
$p - value = 0.3160$

Fail to reject the null hypothesis, sterilization is not significant when measuring *C. thermocellum's* enzyme products. The assumptions of the regression model $b_1 \sim Normal\left(\beta_1, \frac{\sigma_e^2}{(n-1)s_x^2}\right)$ are not met.

$$\therefore E_{NS} = E_{SA} = E_{SB}$$

In order to determine if the enzyme system is functional in the pretreated materials individual hypothesis tests were performed on the levels of the class variables.

$H_0: \mu_x = 0$
Where x = NS, SA or SB
$H_A: \mu_x > 0$
$\alpha = 0.05$ one sided test

If $(\Pr > |t|) < 0.05$, reject H_0 and conclude with 95% confidence the sterilization mean is significantly greater than zero. If $(\Pr > |t|) > 0.05$, fail to reject H_0 and conclude with 95% confidence that the sterilization mean is not significantly different than zero. The results are summarized in the following table.

STERILIZATION	MEAN	STDEV	N	Pr > |t|	H_0: μ_X=0	CONCLUSION
NS	412.651	716.258	28	0.0051	REJECT	$\mu > 0$
SA	327.727	404.601	28	0.0002	REJECT	$\mu > 0$
SB	585.883	753.400	28	0.0003	REJECT	$\mu > 0$

All sterilization methods have a concentration of enzyme products statistically greater than zero. From this we can conclude that corn stover treated with AHP or NaOH will support *C. thermocellum's* enzyme system.

ENZYME PRODUCTS AS A FUNCTION OF PT STERILIZATION INTERACTION

$H_0: E_{NSAHP} = \ldots = E_{SBNaOH}$
H_A: At least two $\neq$ each other
$\alpha/2 = 0.025$
$p - value = 0.3804$

Fail to reject the null hypothesis, the interaction is not significant when measuring products of *C. thermocellum's* enzyme system. The assumptions of the regression model are not met ($b_1 \sim Normal\left(\beta_1, \frac{\sigma_e^2}{(n-1)s_x^2}\right)$ for the interaction.

$$\therefore NSAHP = SAAHP = SBAHP = NSH2O = SAH2O = SBH20 = NSNaOH = SANaOH = SBNaOH$$

In order to determine if the fermentation pathways are functional in the pretreated materials individual hypothesis tests were performed on the levels of the class variables.

$H_0: \mu_x = 0$
Where x = NSAHP, SAAHP, SBAHP, NSH2O, SAH2O SBH2O, NSNaOH, SANaOH or SBNaOH
$H_A: \mu_x > 0$
$\alpha = 0.05$ one sided test

If $(\Pr > |t|) < 0.05$, reject H_0 and conclude with 95% confidence the interaction mean is significantly greater than zero. If $(\Pr > |t|) > 0.05$, fail to reject H_0 and conclude with 95% confidence that the interaction mean is not significantly different than zero. The results are summarized in the following table.

INTERACTION	MEAN	STDEV	N	Pr > |t|	H_0: μ_X=0	CONCLUSION
NSAHP	535.394	822.751	12	0.0456	REJECT	$\mu > 0$
SAAHP	345.867	618.824	12	0.0790	FAIL TO REJECT	$\mu_x=0$
SBAHP	913.748	1046.000	12	0.0115	REJECT	$\mu > 0$
NSH2O	260.255	69.662	4	0.0050	REJECT	$\mu > 0$
SAH2O	423.892	167.189	4	0.0148	REJECT	$\mu > 0$
SBH2O	768.481	59.705	4	0.0001	REJECT	$\mu > 0$
NSNaOH	340.706	741.939	12	0.1400	FAIL TO REJECT	$\mu_x=0$
SANaOH	277.532	69.116	12	0.0001	REJECT	$\mu > 0$
SBNaOH	197.150	58.710	12	0.0001	REJECT	$\mu > 0$

All interactions between sterilization and pretreatment methods, except SAAHP and NSNaOH, have a concentration of enzyme products statistically greater than zero. From this we can make no conclusion that corn stover treated with AHP or NaOH will support *C. thermocellum's* enzyme system.

APPENDIX D: Thermophile Medium (T Media)

REAGENTS

		per L		per 500 mL	
1	DI Water	850	mL	425	mL
2	Resazurin Stock	1	mL	0.5	mL
3	Salt T1	50	mL	25	mL
4	Salt T2	50	mL	25	mL
5	Yeast Extract	2.0	g	1	g
6	Cysteine	0.5	g	0.25	g
7	Vitamins	10	mL	5	mL
8	Modified Metals	5	mL	2.5	mL

PROCEDURE

1. Start with the water and add Resazurin Stock, Salts T1 & T2, powdered salts (yeast extract and cysteine), vitamins and modified metals. Stir until all ingredients are dissolved , in the same order as above to prevent precipitation.
2. The vitamin and modified metal solutions are stored in the refrigerator.
3. Several color changes are expected because of resazurin indicator solution.
4. Adjust to pH 6.7 with 10% NaOH.
5. Autoclave to degas, LIQUID7 cycle. Make sure that the clamps are tightly closed.
6. Once removed from the autoclave, bubble with CO_2 until medium cools to room temperature.
7. Anaerobically add 50 mL of 8% Na_2CO_3 to the medium.
8. Anaerobically transfer the medium to tubes or serum bottles.
9. Autoclave again on the LIQUID7 cycle.

SODIUM BICARBONATE BUFFER

1. Place 250 mL rounded flask on stand.
2. For every liter of media 50 mL of water is needed.
3. Insert gassing jet and bubble with CO_2.
4. Heat until water starts to evaporate.
5. Cool with CO_2 gas.
6. Prepare 100 mL vial with gassing jet and 4 g Na_2CO_3
7. Cap vial and clamp.
8. Only transfer Buffer when media is cool to the touch.

STANDARD VITAMINS

REAGENTS	per 100ml	per 1L
Pyridoxamine 2HCl	10 mg	100 mg
Riboflavin	20 mg	200 mg
Thiamine HCl	20 mg	200 mg
Nicotinamide	20 mg	200 mg
CaD Pantotheinate	20 mg	200 mg
Lipoic Acid	10 mg	100 mg
P-aminobenzoic acid	1.0 mg	10 mg
Folic Acid	0.5 mg	5.0 mg
Biotin	0.5 mg	5.0 mg
Cobalamin (Co B_{12})	0.5 mg	5.0 mg
Pyridoxal HCl	10 mg	100 mg
Pyridoxine	10 mg	100 mg

Need one of the following:
K_2HPO_4 (MW: 174.18) 0.1 M=1.74g/100ml or 17.4g/L pH 6.0
KH_2PO_4 (MW: 136.09) 0.1 M=1.36g/100ml or 13.6g/L pH 6.0

PROCEDURE:

1. Weigh out vitamins 1 through 12 for the chosen volume.
2. Bring up to volume with 0.1 M K_2HPO_4 or 0.1 M KH_2PO_4 at pH 6.0.

PFENNINGS/MODIFIED METALS SOLUTION

REAGENTS	per L	
Na_4EDTA	500	mg
$FeSO_4 \cdot 7H_2O$	200	mg
$ZnSO_4 \cdot 7H_2O$	10	mg
$MnCl_2 \cdot 4H_2O$	200	mg
H_3BO_3	20	mg
$CoCl_2 \cdot 6H_2O$	20	mg
$CuCl_2 \cdot 2H_2O$	1	mg
$NiCl_2 \cdot 6H_2O$	2	mg
$Na_2MoO_4 \cdot 2H_2O$	3	mg

PROCEDURE:

1. Weight out metals.
2. Bring up to volume with ddH_2O. • Note: The sulfide will precipitate many of the metals.

To make modified metals solution for thermophile media follow the recipe above then add the following:

10mg $Na_2WO_4 \cdot 2H_2O$
1mg Na_2SeO_3

SALT T1

	per L	per 5L	mM [Stock]	mM [Media]
$Na_2HPO_4 \cdot 12H_2O$	84g	420g	235	11.8
--OR--				
Na_2HPO_4	30.60g	153g	250	12.5

SALT T2

	per L	per 5L
KH_2PO_4	30g	150g
NH_4Cl	10g	50g
$(NH_4)_2SO_4$	10g	50g
$MgCl_2 \cdot 6H_2O$	1.8g	9.0g
$CaCl_2$	0.6g	3.0g

APPENDIX E Alkaline Hydrogen Peroxide Pretreatment and *Clostridium thermocellum* Fermentation

210 g	Biomass (5mm Particle Size)	(42)	Large Test Tubes
150 mL	30% ACS Reagent Grade H_2O_2		Aluminum Foil
360 mL	5M NaOH		Coffee Filters
540 mL	H_2O		Parafilm
65 mL	12.1M HCl		CO_2 Gassing Jet
50 mL	Clostridium thermocellum		
400 mL	T-Media		

1. Prepare 500 mL flasks with appropriate amount of biomass, 10 g per flask, record the actual amount. There will be a total of 21 flasks for the three treatments. Seven flasks will be autoclaved for sterilization (this can be done ahead of time).

2. Prepare 5M stock NaOH and concentrated HCl solutions to be used for pH adjustment.

3. In an ice bath mix 150 mL of 30% H_2O_2 with 180 mL 5M NaOH and 120 mL DI H_2O. The pH should be approximately 11.5. Use caution this reaction is highly exothermic. This is the AHP solution.

AHP			
	No Sterilization	No Sterilization	No Sterilization
	Sterilization Before & After PT	Sterilization Before & After PT	Sterilization Before & After PT
	Sterilization After PT	Sterilization After PT	Sterilization After PT

NaOH			
	No Sterilization	No Sterilization	No Sterilization
	Sterilization Before & After PT	Sterilization Before & After PT	Sterilization Before & After PT
	Sterilization After PT	Sterilization After PT	Sterilization After PT

H_2O	
	No Sterilization
	Sterilization Before & After PT
	Sterilization After PT

4. For the NaOH solution, mix 180 mL 5M NaOH with 270 mL H_2O.

5. When all flasks have reached room temperature add 50 mL of the pretreatment solutions to each, stir well. Cover with parafilm and foil. The AHP will expand to fill the entire flask volume, ensure the biomass will not spill.

6. Each flask will contain 10 g biomass and 50 mL liquid

7. The flasks should then be placed in an incubator at 23°C and 90 rpm for 24 hours.

8. At the end of the 24 hours remove the flasks from the incubator. Prepare for washing.

9. The biomass should then be washed at a 6:1 ratio with water. Using the Nalgene vacuum filter set up and commercially available coffee filters, using the first 150 mL of DI water to transfer from pretreatment beaker to filter. All free liquid should be removed and the biomass transferred to 400 mL beaker. Add 50 mL DI water and 3 mL concentrated HCl, stir well. Transfer the pH adjusted biomass to the filter set up with the remaining 100 mL of wash water. Once free liquid has drained move to drying pan.

10. Prepare the fermentation test tubes in duplicate for all samples. Add ~ 0.5 g (assuming 20% solids) to each test tube. Each tube should be flushed with CO_2 for ~ 15 minutes, then capped and clamped. The remaining material of SA & SB samples should be transferred to 250 mL flasks.

11. All SB and SA samples in flasks and test tubes should be sterilized by autoclaving at 121°C and 15 psi for 30 minutes (Labware cycle).

12. Determine moisture content of pretreated material using the NREL LAP: Biomass and Total Dissolved Solids in Liquid Process Samples.

13. Anaerobically add 8.5 mL of T-Media and 1 mL *C. thermocellum* inoculum to the fermentation test tubes. Incubate at 65°C for 48 hours.

14. To stop fermentation, remove caps and stoppers and transfer to 15 mL centrifuge tubes and store in freezer until ready for HPLC analysis.

15. Store dried samples in plastic bags until ready for NREL LAP: Determination of Lignin and Structural Carbohydrates in Biomass can be performed.

APPENDIX F: THEORETICAL GLUCOSE CONCENTRATIONS FOR PRETREATED MATERIALS

Variables

x	Pretreated Material
y	Raw Material
R	Raw Material
PT	Pretreated Material
L	Lignin
G	Glucose
X	Xylose
Ar	Arabinose
As	Ash
T	Total

Mass Balance

$$(yL_R - xL_{PT}) + (yX_R - xX_{PT}) + (yAr_R - xAr_{PT}) + (yAs_R - xAs_{PT}) = (yT_R - xT_{PT})$$

$$y(L_R + X_R + Ar_R + As_R) - x(L_{PT} + X_{PT} + Ar_{PT} + As_{PT}) = yT_R - xT_{PT}$$

$$\frac{x}{y} = \frac{(T_{PT} - L_{PT} + X_{PT} + Ar_{PT} + As_{PT})}{(T_R - L_R + X_R + Ar_R + As_R)}$$

$\frac{x}{y}$ = Ratio of glucose in pretreated sample to the amount of glucose in raw material

Let $y = T_R$to find proportion of material that is glucose in treated material

y =Total amount of raw material accounted for in compositional analysis

x = Total amount of pretreated material accounted for in compositional analysis

$$\%Glucose_{Adjusted} = \frac{G_R}{x} \approx \frac{G_{PT}}{T_R}$$

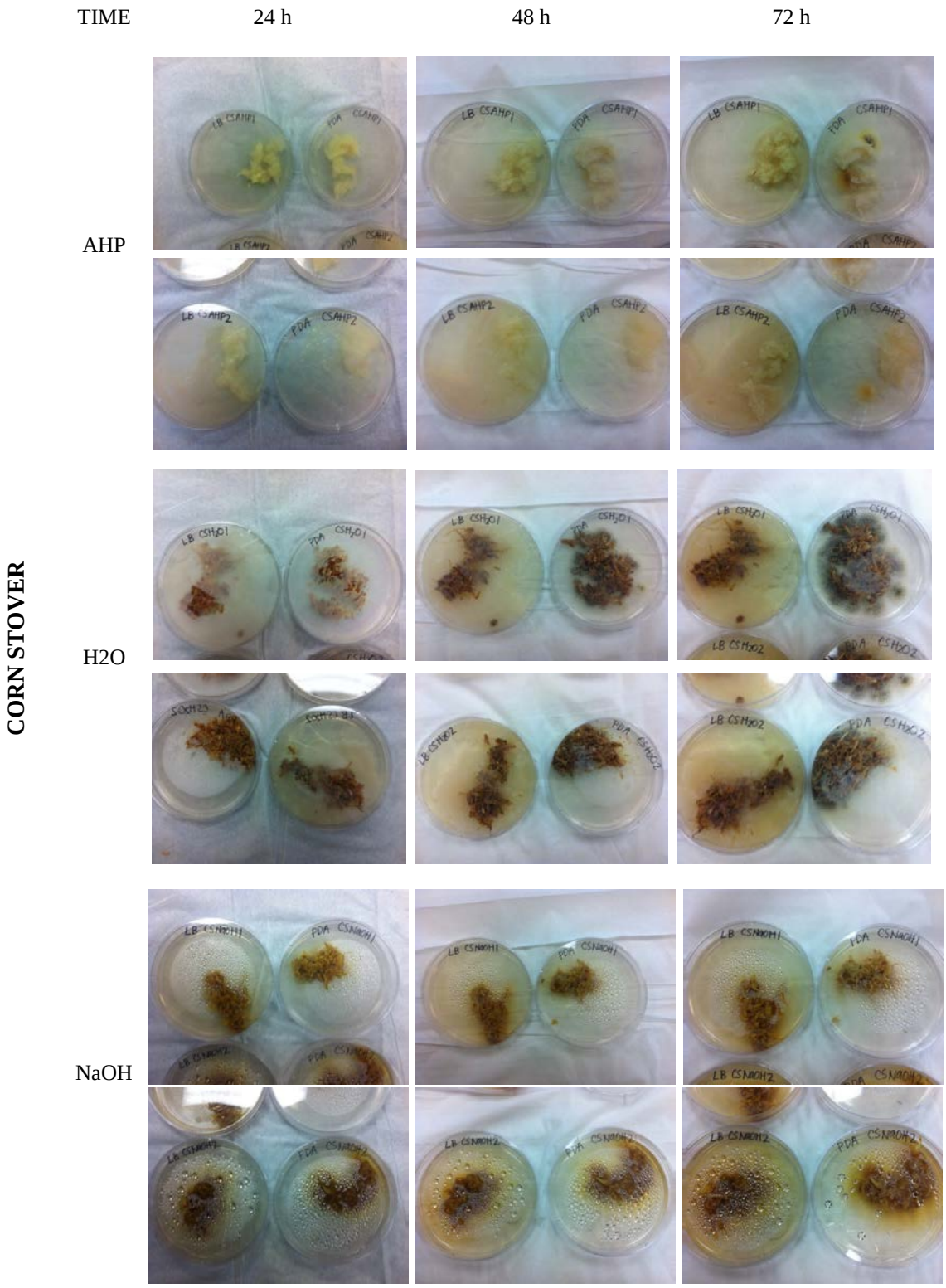
$$Theoretical\ [Glucose] = \frac{(Oven\ Dry\ Weight)(\%Glu_{Adj})(\frac{180}{162})}{Volume}$$

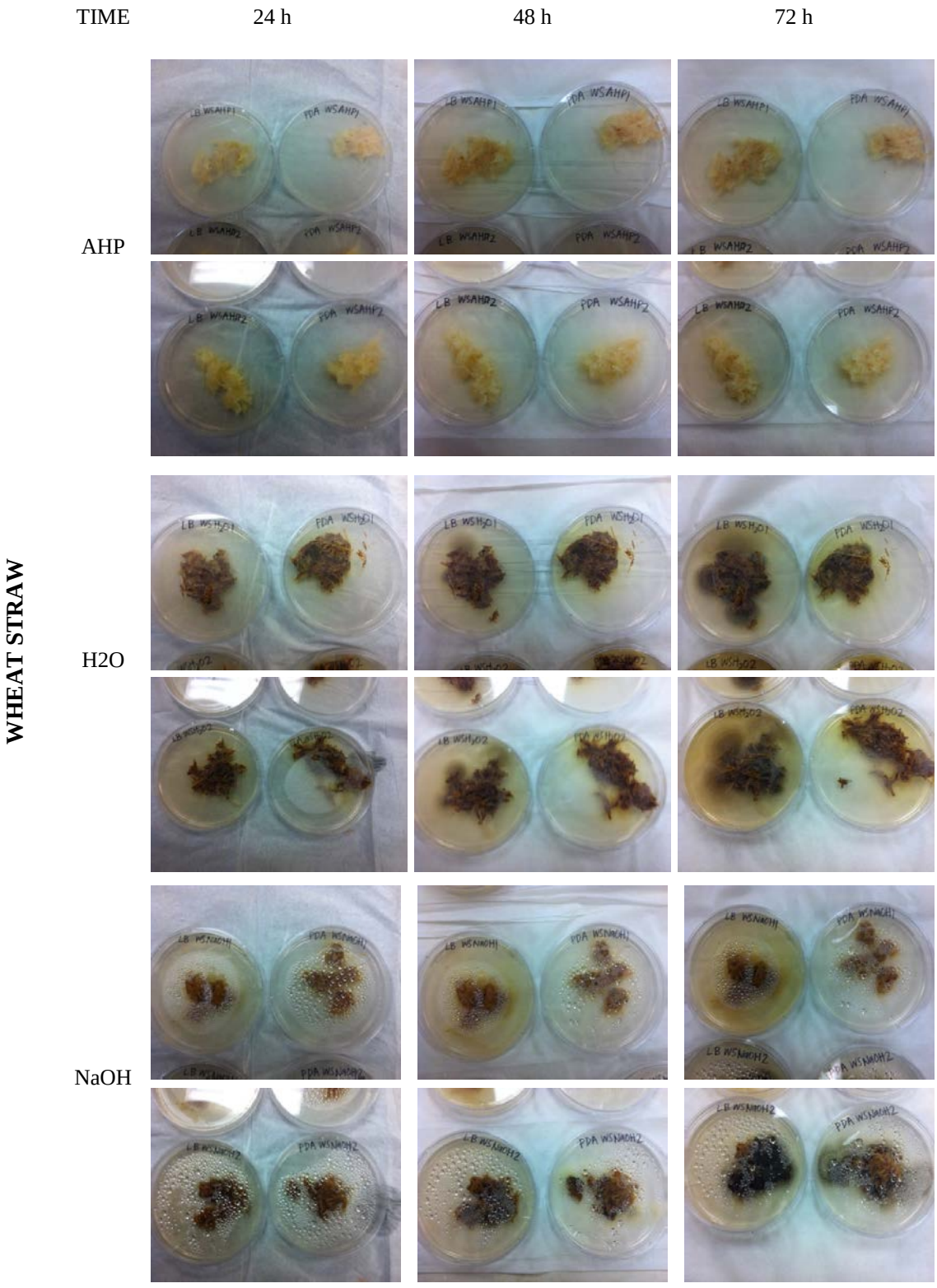
$$Glucose\ Yield = \frac{HPLC}{Theoretical}$$

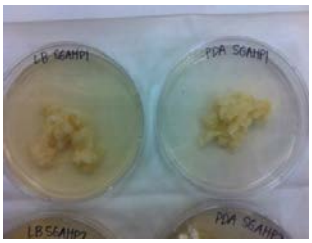
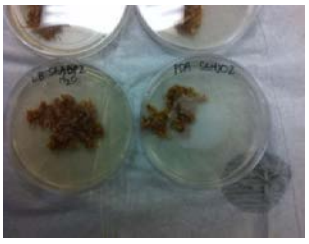
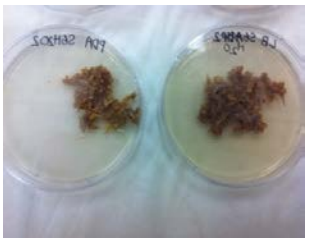
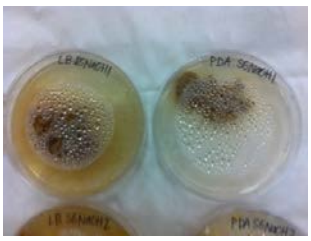
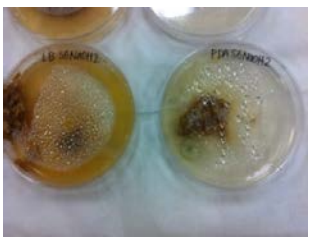
APPENDIX G: STERILIZATION EXPERIMENT RESULTS

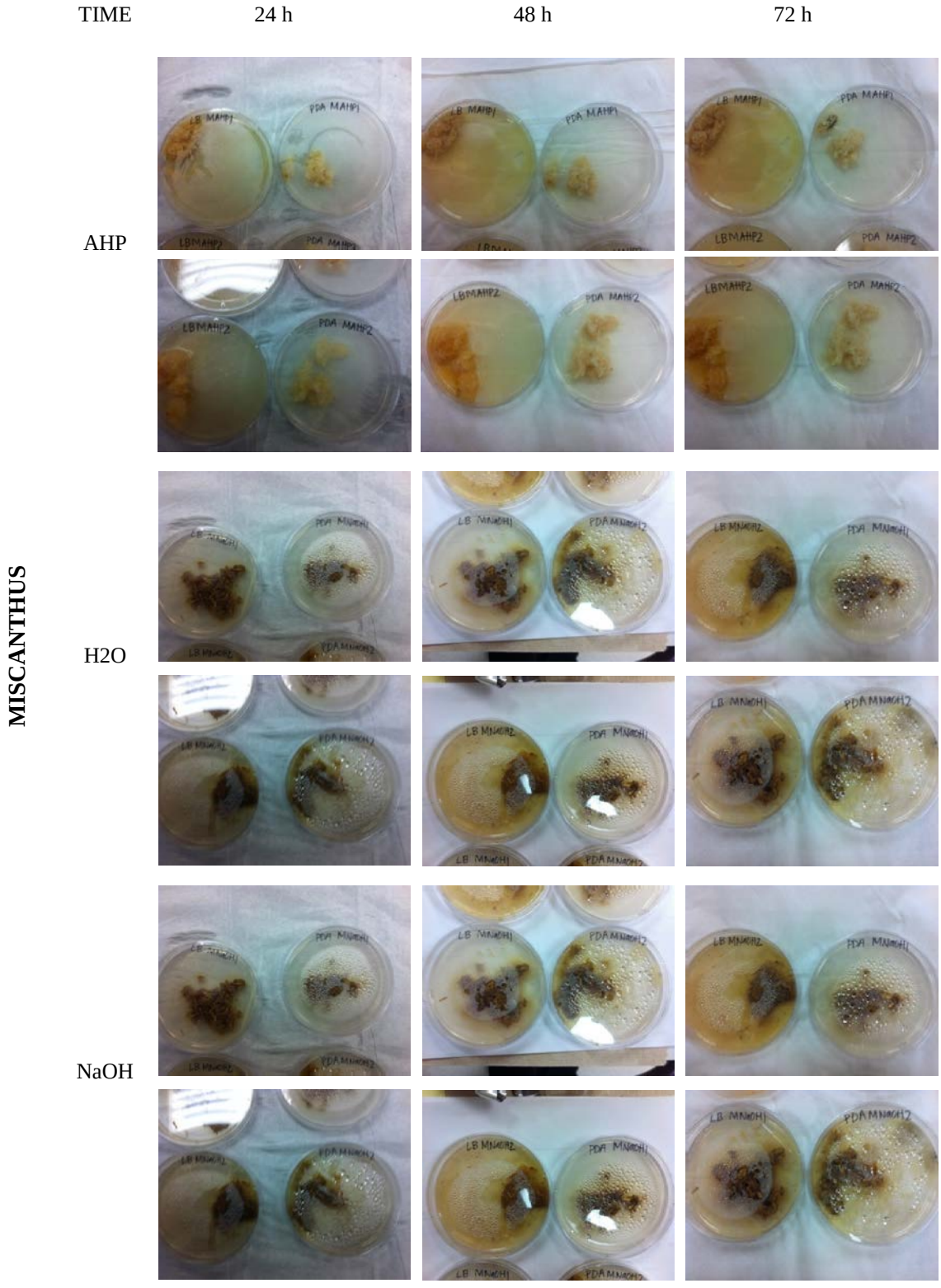
	TIME		0 h		24 h		48 h		72 h	
	PLATE		LB	PDA	LB	PDA	LB	PDA	LB	PDA
CORN STOVER	AHP	1	NO	NO	NO	NO	NO	YES	YES	YES
	AHP	2	NO	NO	NO	NO	YES	NO	YES	YES
	H2O	1	NO	NO	YES	NO	YES	YES	YES	YES
	H2O	2	NO	NO	YES	NO	YES	YES	YES	YES
	NaOH	1	NO	NO	NO	NO	NO	YES	YES	YES
	NaOH	2	NO	NO	NO	NO	YES	NO	YES	YES
WHEAT STRAW	AHP	1	NO	NO	NO	NO	NO	NO	NO	NO
	AHP	2	NO	NO	NO	NO	NO	NO	YES	NO
	H2O	1	NO	NO	NO	NO	YES	NO	YES	YES
	H2O	2	NO	NO	NO	NO	YES	NO	YES	YES
	NaOH	1	NO	NO	NO	NO	YES	NO	YES	NO
	NaOH	2	NO	NO	NO	NO	YES	YES	YES	YES
SWITCHGRASS	AHP	1	NO	NO	NO	NO	NO	NO	NO	NO
	AHP	2	NO	NO	NO	NO	YES	NO	YES	YES
	H2O	1	NO	NO	NO	NO	NO	NO	YES	YES
	H2O	2	NO	NO	NO	NO	NO	NO	YES	NO
	NaOH	1	NO	NO	YES	YES	YES	YES	YES	NO
	NaOH	2	NO	NO	YES	YES	YES	YES	YES	YES
MISCANTHUS	AHP	1	NO	NO	YES	NO	YES	NO	YES	YES
	AHP	2	NO	NO	YES	NO	YES	NO	YES	YES
	H2O	1	NO	NO	YES	NO	YES	NO	YES	YES
	H2O	2	NO	NO	YES	NO	YES	YES	YES	YES
	NaOH	1	NO	NO	NO	NO	YES	YES	YES	YES
	NaOH	2	NO	NO	YES	NO	YES	YES	YES	YES

Plates Showing Visible Growth	**24 h**	**48 h**	**72 h**
AHP	2	5	11
H2O	2	9	15
NaOH	5	13	15


TIME
24 h
48 h
72 h
CORN STOVER
AHP
H2O
NaOH


TIME
24 h
48 h
72 h
WHEAT STRAW
AHP
H2O
NaOH

SWITCHGRASS	TIME	24 h	48 h	72 h
	AHP			
				
	H2O			
				
	NaOH	Photo Not Available		
		Photo Not Available		


TIME
24 h
48 h
72 h
MISCANTHUS
AHP
H2O
NaOH

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