5-2016

Minimizing Ethanol Concentration in Organosolv Pretreatment for the Saccharification of Loblolly Pine

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An Undergraduate Honors College Thesis

in the

College of Engineering

University of Arkansas

Fayetteville, AR

by

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April 29, 2016

Abstract

Organic solvent pretreatment, commonly known as organosolv, is a method used to prepare biomass for enzymatic hydrolysis for the production of biofuels. This method common uses ethanol as the organic solvent. However, this creates an economic issue with the product stream, as ethanol becomes a product and input. This project sought to explore how decreasing the use of ethanol in organosolv pretreatment affected the recoverability of sugars after enzymatic hydrolysis. Loblolly pine (*Pinus taeda L.*) was pretreated at 170 °C for 60 minutes in 1% dilute sulfuric acid and an ethanol concentration varying from 65% to 35%. Compositional analysis was then performed on each pretreated sample to determine composition of glucan, lignin, xylan, and arabinan. The pretreated samples were then enzymatically hydrolyzed and the concentrations of the resulting sugars were analyzed by high pressure liquid chromatography (HPLC). Results from the compositional analysis showed that although lignin composition decreases with increasing ethanol concentration, there is very little delignification happening during the pretreatment process. Results from the enzymatic hydrolysis indicated that ethanol concentration above 35% results in similar recoveries, approximately 70% of glucan available. Overall, results indicated that lignin was not the only limiting factor during enzymatic hydrolysis. Additionally, the results indicated that sufficient sugar recovery could be achieved at lower ethanol concentrations.

1.1 Introduction

With global climate change becoming an increasing concern for the world's population, research into alternative forms of energy production is imperative. At the center of this current research is the study of biofuels created from non-food destined lignocellulosic biomass.

Biofuels like ethanol can be produced from sugar, such as glucose, via fermentation. These sugars can be obtained from food-destined commodities, such as corn, or from non-food-consumable plants, such as pine or switchgrass. The abundance of non-food-consumable plant resources makes the production of ethanol very attractive to industry because their use does not adversely affect food and feed industries. Arkansas is one of the most forested states in the US, with 18.0 million acres of timberland, with Loblolly pine (*Pinus taeda* L.) representing 22% of the total standing tree volume (Graham-Rowe 2011). Therefore in Arkansas, the use of pine as a sugar source for ethanol production is an interesting proposition.

Unfortunately, barriers exist in creating a reliable and economically efficient sugar supply from pine for fermentation processes (Sanderson 2011). Pine, as well as all lignocellulosic biomass, contains cellulose, which is a polymer made from chains of glucose found in cell walls. To extract these glucose molecules for fermentation, biomass must first be pretreated to release the cellulose polymers from lignin, creating space for the hydrolyzing enzymes to successfully cleave sugar monomers from polymers (Frederick et al. 2008). Thusly, pretreatment is a critical step in the recovery of glucose from biomass, as higher sugar recovery yields higher ethanol titers. Figure 1 illustrates the general production process used to create biofuels from biomass.

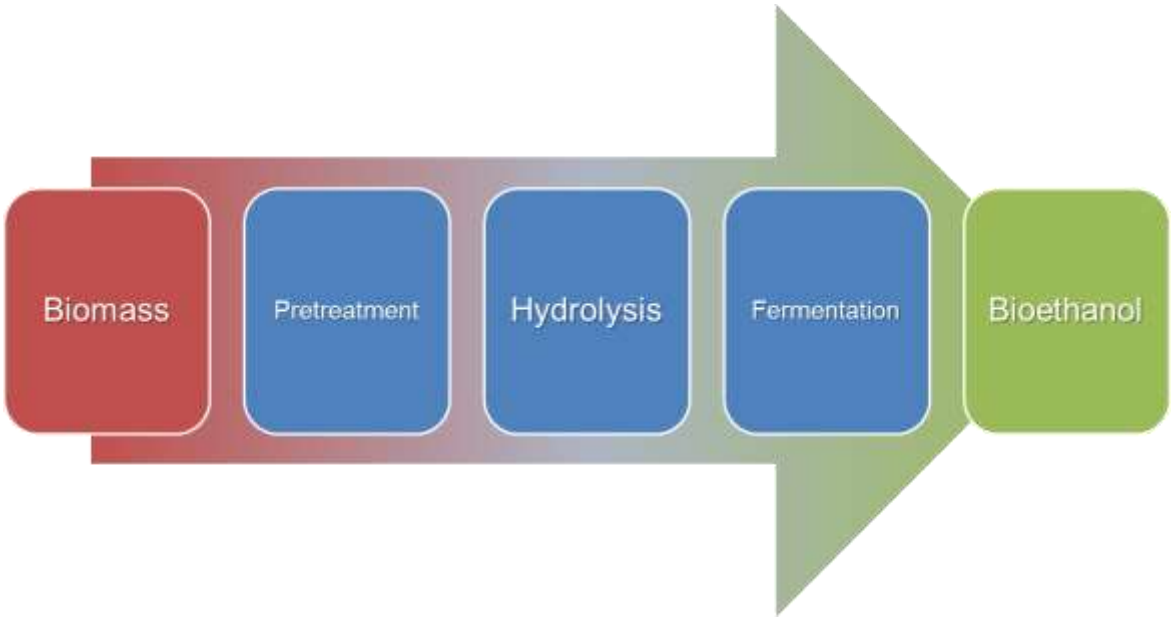

Figure 1: A flowchart presenting the general process used to create bioethanol from lignocellulosic biomass

Pretreatment with organic solvents, commonly known as organosolv, has recently arisen as a potential pretreatment method (Li et al. 2012). Although more expensive than other studied methods, organosolv has the potential to produce several value-added lignin based products along with the biofuels (Pan et al. 2005). This pretreatment method consists of two major steps. First the lignocellulosic biomass is treated with an organic solvent at a specific temperature for a specific period of time, varying from 140 to 200 °C and 30 minutes to 2 hours. During this step, a majority of the hemicellulose and lignin break down into smaller molecules and dissolve into the liquid phase. The second major step involves precipitating the pretreatment liquor into lignin and extracting leftover solvents for reuse in pretreatment (Pan et al. 2007). This lignin precipitation is where the valorization of the organosolv process becomes important. The resulting lignin can be used for specific adhesives and resins for coatings, concrete plasticizers for constructions, and friction materials for high-performance brake products (Arato et al. 2005).

Despite the benefits of organosolv pretreatment processes, there are still several drawbacks. Most notably, the organic solvents are quite expensive, especially considering the relative low cost of the pretreatment materials required for dilute acid pretreatment. Furthermore, more organic solvents are required to wash the pretreated pulp to prevent lignin from precipitating back into the hydrolysate. For this reason, decreasing the volume of solvents required for optimum biomass saccharification is essential for establishing an efficient and profitable organosolv-based process. The goal of this research project was to determine optimum organic solvent concentrations and volumes for the organosolv pretreatment process, decreasing processing costs. Reducing the cost of organosolv pretreatment is critical to establishing the use of this pretreatment, which could lead to higher sugar titers as well as value-added lignin streams.

In order to study the effect of decreasing organic solvents, experiments were performed with pine biomass using ethanol as an organic solvent. Ethanol was chosen for its relatively low toxicity, high boiling point, and low potential for combustion. The experiments were performed in a 1 L Parr reactor with 1% sulfuric acid (w/v) at 170 °C for 60 minutes. Four concentrations of ethanol were tested 65% v/v down to 35% v/v in increments of 10%. Aliquots of the enzyme hydrolysate were saved for testing. Mass balances of carbohydrates were determined and the effect of varying ethanol concentration was studied.

1.2 Objectives

The goal of this investigation was to determine the effect of decreasing ethanol concentration on the recoverability of glucose in pine biomass in the context of an organic solvent pretreatment method.

2. Methodology and Experimental Design

2.1 Biomass

Loblolly pine (*Pinus taeda* L.) from Monticello, Arkansas was used as the lignocellulosic biomass in this experiment. Pine wood was selected for its availability in Arkansas and for its high lignin content (36.2 % as determined by our laboratory's unpublished result), leading to a large potential lignin recovery. The biomass was ground using a Wiley Mill (Thomas Scientific, Swedesboro, NJ) with a 20 mesh filter, maximizing surface area for the pretreatment reaction to take place. The moisture content of the biomass was measured with an Ohaus MB45 Moisture Analyzer (Pine Brook, NJ) to ensure reproducibility.

2.2 Pretreatment

Biomass was loaded into a 1L Parr 4525 Reaction Vessel (Model # 4848, Moline, IL) using a loading adapted from Pan et al. (2007) and an optimized catalyst composition as determined in Park et al. (2010). Twenty grams of biomass was mixed into a 1% (w/v) solution of H_2SO_4 and an ethanol concentration ranging from 35-65% (v/v) for a solids loading of 10% and a total working volume of 200 mL. The ethanol concentration was decreased in increments of 10% for 4 total pretreatment conditions. Pretreatment proceeded as described in Pan et al. (2007). The reaction took place at 170 °C for 60 minutes, with heating taking approximately 60 minutes.

After the residence time was complete, the reactor was immediately cooled with water to prevent formation of known inhibitory compounds (Frederick et al. 2014). The resulting slurry was filtered using a Buchner funnel to separate the black liquor from the pulp. The pulp resulting from the pretreatment was washed in 300 mL of ethanol at 60 °C, using an equivalent concentration from the pretreatment process. The resulting wash was then mixed with the spent

liquor and saved for future analysis. Remaining pulp was washed with 300 mL of water at 60 °C with the washes being discarded.

2.3 Compositional Analysis

Compositional analysis was performed on each pretreatment replicate, as described in NREL LAP/TP-510-42618 (Sluiter et al. 2008). 100 mg of each sample was placed in a glass reaction bottle and mixed with 1 mL of 72% H_2SO_4. Each bottle was then placed in a water bath set to 30 °C and agitated for 1 hour. After agitation, the samples were mixed with 28 mL of distilled water and autoclaved at 121 °C for 55 minutes. All samples were then filtered using a Buchner funnel. The liquid portion was neutralized and analyzed for sugar content using a high pressure liquid chromatography (HPLC) instrument, as described in Frederick et al. (2014). The solid portion was placed in an oven at 105 °C for 12 hours, weighed, and finally placed in a furnace at 1066 °F for 24 hours. The difference in mass between the oven and furnace treatments was considered the total lignin content of the pretreated samples.

2.4 Enzymatic Hydrolysis

After compositional analysis, the pulp samples were enzymatically hydrolyzed to extract glucose molecules from the biomass. The commercial enzyme Accellerase 1500 © (Dupont, Rochester, NY) was used for the hydrolysis process following the procedure described below. Each sample was loaded into a 50 mL amber bottle with an enzyme loading of 60 FPU/gram of glucan. Enzymatic hydrolysis took place for 48 hours at 55 °C to allow for maximum saccharficiation. A portion of the liquid hydrolysate for each hydrolysis time was analyzed using an HPLC instrument for glucose recovery (Frederick et al. 2014). All procedures were completed in duplicate to ensure reproducibility.

2.5 HPLC Analysis

A single HPLC (Waters, Milford, MA) was used to quantify sugar content in both the hydrolysis and compositional analysis samples. All HPLC analysis was performed using 10 microliter samples. The HPLC was equipped with a Shodex column (SP0801, Waters, Milford, MA) and precolumn (SP-G). The concentration of each sugar was calculated by comparing peak area obtained by refractive index detection to calibration curves (Frederick et al. 2014).

3. Results and Discussion

3.1 Qualitative Results of Pretreatment

After the pretreatment of samples in the Parr reactor, a pattern emerged regarding the visually identifiable composition, i.e. the appearance of the samples. The samples pretreated with the highest concentration of ethanol, 65% and 55%, resembled a paste. There was little to no evidence of any organized structure, implying little presence of undissolved lignin in the samples. On the other hand, the samples treated with lower ethanol concentrations, such as 45% and 35%, exhibited a much more uniform structure, where the general form of the pine samples was preserved. This pattern indicates that the higher ethanol concentrations resulted in increased delignification. A decrease in lignin composition implied higher glucose recoverability. Therefore, qualitative analysis supported that as ethanol concentration increased so did recoverability. The important metric determined in this study, however, is how much the recoverability increases with this increased ethanol concentration.

3.2 Compositional Analysis

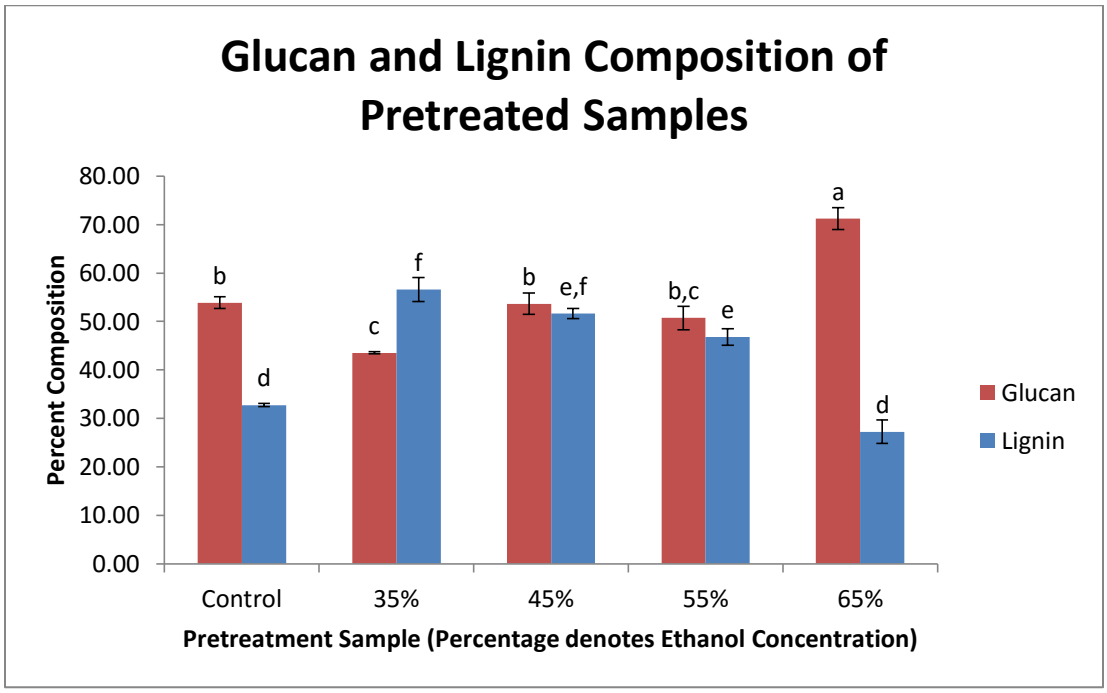

Figure 2: Percent lignin and glucan composition of the biomass samples pretreated at 170 °C, for 60 minutes with 1% H_2SO_4 and varying ethanol concentrations compared to the lignin and glucan composition of the control. Letter data labels denote significant differences determined via Tukey HSD Test ($p < .05$).

After pretreatment, compositional analysis was performed as described in the methodology. This procedure allowed for the determination of a percent composition of both glucan and lignin, which are key components during enzymatic hydrolysis. Percent composition expresses the mass of target components as a function of biomass dry weight. These later values enable the direct comparison of each sample's composition. Figure 2 presents the results of the composition analysis. Lignin composition differed significantly between the pretreatment ethanol concentrations (Figure 2; ANOVA: $F_{4,5} = 96.29$ $p < 0.0001$). Interesting to note are the significant differences between 65% ethanol and the remaining samples. This large discrepancy indicates that 65% ethanol approaches an optimized concentration of ethanol for organosolv pretreatment in terms of delignification compared to the other samples. Furthermore the data

indicated a slight positive correlation between ethanol concentration and lignin composition indicated by the significant difference between 55% ethanol and 35% ethanol (Tukey HSD $p<0.01$). Also of interest in the compositional analysis data is the effect of ethanol concentration on glucan composition. Again, the glucan concentration differed significantly between the different pretreatment samples (Figure 2; ANOVA: $F_{4,5} = 60.31$ $p < 0.001$). Mirrored in the glucan composition is the large discrepancy between 65% ethanol and the remaining samples. This again supports the notion that 65% was an optimum ethanol concentration which was conducive to the release of glucan during enzymatic hydrolysis. The data also showed a slight positive correlation between ethanol concentration and glucan composition indicated by the significant difference between 55% and 35% ethanol (Tukey HSD $p<.05$). Based on the lignin and glucan concentrations alone, the data supported the idea that increasing ethanol concentration allowed for a higher recovery of glucan during enzymatic hydrolysis.

Although these results indicate that increased ethanol concentration allowed for increased delignification during the pretreatment process, a comparison to the untreated control samples raised questions. The pretreatment samples did not have significantly lower lignin composition than the control samples. In fact, the lignin composition of the low ethanol concentration samples (35% - 55%) was significantly higher than that of the control samples, as can be seen in Figure 2 (Tukey HSD $p < .01$). This indicated that, at lower ethanol concentrations, very little actual delignification occurred, and most of the mass loss stemmed from the dissolution of hemicelluloses, such as arabinan and xylan. This result is supported by the composition of hemicelluloses in the pretreated samples, shown in Figure 3. The pretreated samples had only trace amounts of arabinan and xylan (a noticeable decrease from the 1.4% and 7.9%, respectively, found in the untreated control samples). Important to note, however, is that these

values are only a percent composition. Therefore, there could be overall lignin loss in the sample, but the ratio of lignin to glucan did not significantly decrease between the pretreatment samples and the control. Overall the compositional analysis results indicated that 65% ethanol will have the highest glucan recoverability due to high glucan and low lignin compositions.

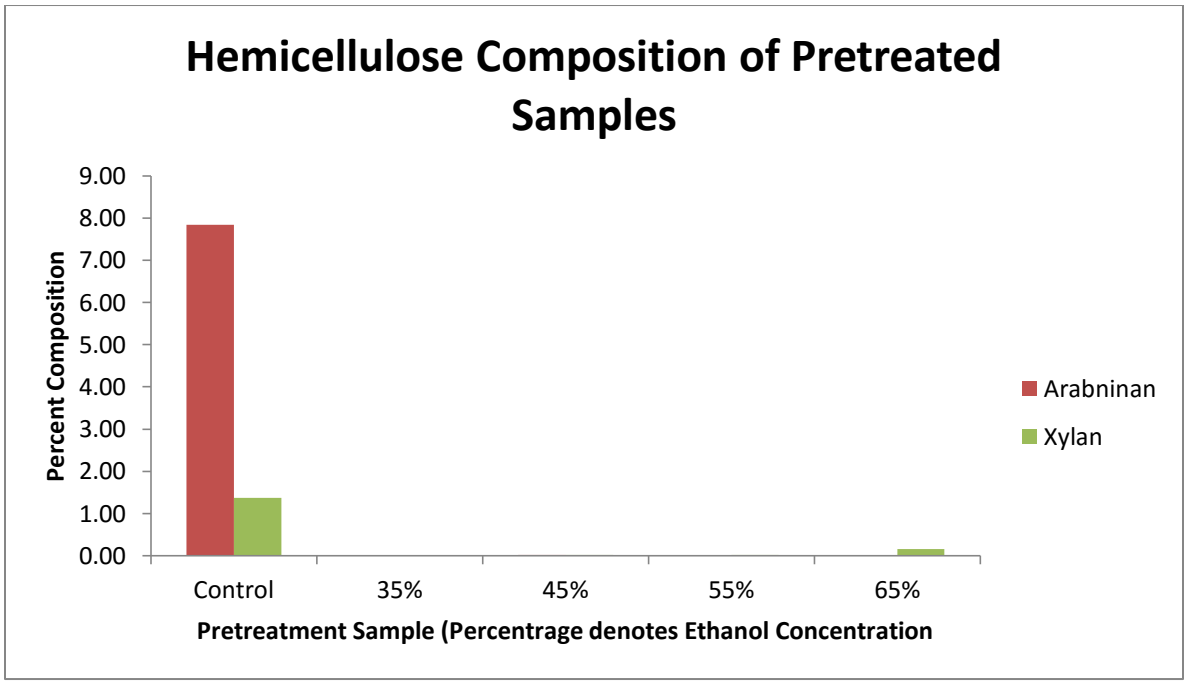

Figure 3: Hemicellulose composition of pretreated samples as determined by the compositional analysis

3.3 Enyzmatic Hydrolysis

As described in the methodology, each sample underwent enzyme hydrolysis to cleave the sugar monomers from polymers. Each hydrolyzed sample was tested for glucose concentration. With a known initial mass of glucan, the mass of glucan recovered was compared to the total mass of glucan present in the sample. This was reported as glucan recovered and was represented by a percentage. Reporting the sample in this manner allows for direct comparison between pretreatment ethanol concentrations. The results of the enzymatic hydrolysis are presented in Figure 4.

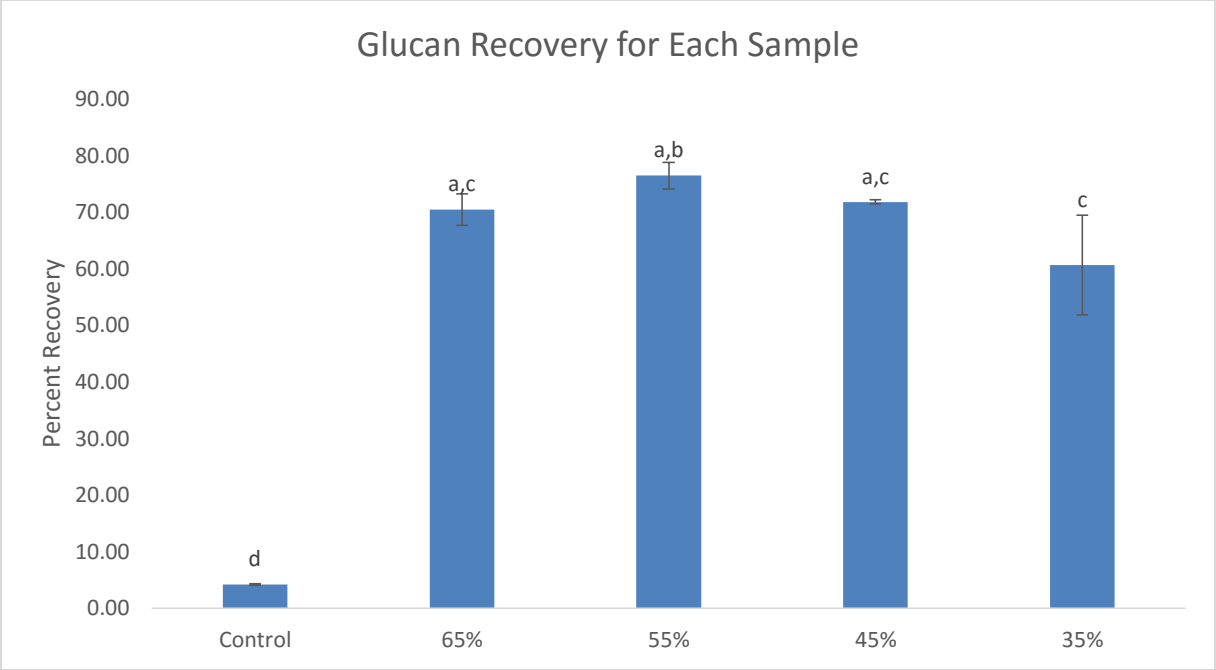

Figure 4: Glucan recovery for each pretreatment sample. Letter data labels indicate significant differences as determined by a Tukey HSD test ($p < .05$).

The data shows that there was a significant difference between the samples pretreated with varying ethanol concentrations and the control (Figure 4; ANOVA: $F_{3,12} = 5.81$ $p < 0.05$). This indicated that the pretreatment process, as a whole, had a desirable effect and increased the recovery of glucan during enzymatic hydrolysis. However, the data also indicated that there was little significant difference between the individual ethanol concentrations (Tukey HSD $p < .01$). In fact, the only significant difference came between the 35 % ethanol treatment and the 55 % ethanol treatment. This is very indicative of diminishing returns from increasing ethanol concentration. The results indicated that above 45 % ethanol during pretreatment will not have a significant impact on the recoverability of glucan.

4. Conclusion

The compositional analysis of the pretreated samples indicated that as ethanol concentration increased, glucan recovery would also increase. This was especially true for the

65% ethanol sample as it had the highest percentage of glucan and lowest percentage of lignin. However, there was no significant difference in glucan recoverability among the highest ethanol concentrations (65%, 55%, and 45%). Several factors could lead to these results. First, the enzymatic hydrolysis could require a longer treatment time for the samples with higher glucan composition. A longer treatment period would allow additional time for the enzymes to cleave the glucose monomers from polymers. This theory is supported when examining the glucan composition. The 55% and 45% samples did not have significantly different glucan compositions. Therefore, it is feasible to assume that 48 hours only allowed enough time to reach a maximum recovery for the 55% and 45%. The 65% samples would then have only reached this same maximum recovery. Another theory to explain the lack of a significant increase is an unknown factor. Perhaps the lignin composition is not the limiting factor in glucan recoverability. All samples, even those with higher lignin composition, had a significantly higher glucose recovery. Therefore, even the data suggests another mechanism is occurring. Further research is required to fully understand the limiting factors during enzymatic hydrolysis.

With the current results, it is unclear exactly how decreasing ethanol concentration affects glucan recovery after enzymatic hydrolysis. However, it is possible to suppose that an ethanol concentration of 45% and above will provide an optimum level of glucan recovery. One important piece to consider is that recoverability of glucan is not the sole valorized product gained from this process. During organosolv, the lignin dissolves into the ethanol solution allowing it to be precipitated out during downstream processing. This allows for another valorized byproduct which greatly increases the economic feasibility. This experiment did not test for the content of lignin in the pretreatment liquor. Therefore, it is certainly possible that higher ethanol concentrations will lead to higher recoverability of solubilized lignin, resulting in

a subsidy of the increased ethanol costs. Such testing would need to be done to fully determine the effects of decreasing ethanol concentration.

5. Acknowledgements

The author would like to thank the University of Arkansas and the Department of Biological Engineering for the use of lab facilities. Additionally, the author thanks the faculty and staff in the Department of Biological Engineering for their support during research and preparation of this report. Finally, the author thanks the Arkansas Department of Higher Education for the Student Undergraduate Research Fellowship (SURF) which provided the financial backing for the materials and time put into this research.

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