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PRELIMINARY INVESTIGATION INTO SINGLE STAGE ENZYME FREE HYDROLYSIS OF HARDWOOD SAWDUST

By

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PRELIMINARY INVESTIGATION INTO SINGLE STAGE ENZYME FREE HYDROLYSIS OF HARDWOOD SAWDUST

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ABSTRACT

Enzymatic hydrolysis is the most common method of producing fermentable sugars from lignocellulosic biomass. Limitations to this step include the high cost of enzymes, and the time required to process the biomass. To increase the throughput and decrease catalyst cost, the preliminary development of a continuous concentrated sulfuric acid process is explored. The process requires two distinct steps. The first step involves hydrolyzing lignocellulose to fermentable sugars, glucose and xylose. The second step consists of glucose and xylose removal from the concentrated acid stream. The separation of sugars decreases the production of reaction byproducts and allows for the recycle of acid to the process.

Single stage acid hydrolysis was tested at nine different reaction conditions. The optimum reaction condition occurred at 70 weight percent acid and 80°C with 58.2 percent glucose release as a percentage of available glucan and 86.5 percent xylose release as a percentage of available xylan. Detection of xylose and glucose in solution occurred at different times. These peaks are separated by enough time to warrant continuous removal of produced glucose and xylose to limit the production of reaction byproducts. In order to optimize this reaction condition, a limit of 63 percent glucose release as a percentage of available glucan was observed.

A preliminary investigation of Dowex Monosphere 99 ion exchange resin to separate glucose and xylose from 70 weight percent was performed. At the column conditions tested, the sulfuric acid caused shrinkage and deactivation of the ion exchange resin leading to inefficient separation of glucose and xylose.

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NOMECLATURE

E = activation energy
f = conversion of subsystem
k_0 = pre-exponential factor in Arhenius Law
n, m = parameter expressing the catalyst effect in activation energy and entropy
R = gas constant
R_{OH} = severity factor
S = concentration of substrate
S^0 = initial concentration of substrate
t = time, min
T = temperature of reaction, °C
T_{ref} = reference temperature, °C
x = mole fraction
λ = parameter expressing the acid catalyst role in conversion of substrate
ω = temperature role in conversion of the catalysed system
χ = acid loading, weight percent
χ_{ref} = reference acid loading, weight percent
ν = stoichiometric factor

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I. INTRODUCTION

Biomass in its many different forms provides a unique source for the sustainable production of fuels and chemicals to replace traditional petroleum production routes. Petroleum is the single largest energy source in the United States, but the environmental, long-term economic and national security concerns has spurred research to find renewable processes to decrease its use. Biofuels in the form of ethanol from from corn, sugar cane, and other starch rich plants have gained popularity as an alternative transportation fuel through blending into gasoline as an oxygenate. This use has had the untenable effect of decreased food supply and increased cost for both food and energy uses. Cellulosic ethanol from waste materials provides a better production method because it does not utilize the commodities that supply food production worldwide. The production of cellulosic ethanol faces several challenges, most notably the steps involved in saccharification of cellulose to fermentable sugars. High conversion combined with high throughput rates and lower operating costs are key to the implementation of cellulosic ethanol as the standard biofuels resource. Published yields of ethanol of 65-70 gal/ ton dry biomass remain short of the goal of 100 gal/ ton dry biomass set by the government sponsored National Renewable Energy Labs.

To decrease production costs, the yield of fermentable sugars must increase. The best yields of fermentable sugars are currently obtained from enzymatic hydrolysis of lignocellulosic biomass pretreated with dilute acid hydrolysis. Hydrolysis rates decrease quickly as the reaction proceeds leading to long residence times of 10 days or more and increased processing costs. Furthermore, as the solids concentration is increased to

improve throughput and reduce water and energy usage, the overall biomass conversion to sugar falls drastically due to mass transfer limitations and product inhibition.

The other route to extracting sugars from cellulose involves hydrolysis of biomass utilizing acid, usually sulfuric acid. Depending on the reaction conditions, the conversion can take place in a matter of hours or minutes and not days. The largest application of acid hydrolysis is currently in the pretreatment of biomass to remove hemicelluloses and increase enzymatic digestibility of the substrate. Though conditions exist that hydrolyze biomass quickly at low temperatures, they require large amounts of acid, and the highly acidic conditions lead to sugar degradation to byproducts which decrease product yield and inhibit the downstream fermentation. The National Renewable Energy Laboratory (NREL) procedure for determining the structural carbohydrate content of biomass requires a two step acid hydrolysis processes, with a concentrated acid step at room temperature followed by a dilute acid step at 121˚C in an autoclave (Sluiter et. al, 2007). Based on this NREL procedure, it is hypothesized that reaction conditions exist, which will lead to an economical concentrated sulfuric acid process for the production of a high yield sugar stream for fermentation to ethanol. Operating costs associated with acid usage and sugar degradation limits the cost effectiveness of the process. It is desired to find a separation method to remove sugar from the acid-sugar solution and to develop and optimize a single stage process to reach the goal of theoretical sugar yields from biomass.

This thesis presents the preliminary work towards accomplishing this goal. The first objective of this project was to find optimal conditions for a single stage concentrated acid hydrolysis process. Nine experiments were performed with a solids loading of 10 weight percent. The acid concentration was varied from 50 weight percent

to 90 weight percent, and the temperature was varied from 30ºC to 80ºC. The conversion of biomass was tracked by measuring the two main sugars released during sachharification of biomass, xylose and glucose. To monitor the breakdown of the product sugars to the furanic byproducts, a spectrophotomeric method was employed. From this data, optimal conditions for a single stage acid hydrolysis of hardwood sawdust was determined.

The second section of this project details the design of a sugar-acid separation utilizing a commercially available ion exchange resin. The goal of this separation was two-fold: once separated, the sulfuric acid can be recycled back to the process. Secondly, removing the sugars from contact with the acid should allow for an increase in conversion due to decreased degradation.

II. REVIEW OF RELATED LITERATURE

A. Biomass Structure

1. Lignin

Waste feedstocks in the form of lignocellulosic material represent the largest source of feedstocks for biofuels, and they are considered the most feasible biomass materials for production of bioethanol (Wyman, 2007). The plant structural materials are composed mainly of cellulose, hemicellulose, and lignin which interact to form lignocellulose. Lignocellulose is a composite matrix of cross-linked polysaccharide networks, glycosylated proteins, and lignin. This matrix has three main components: cellulose (38–50%), hemicellulose (17–32%), and lignin (15–30%). Lignocellulose plant materials further contain extractives, organic cell wall chemicals which serve a plethora of plant functions (Rowell, 2005). Extractives are removed through thorough washing with water and ethanol before treatment of the biomass.

Lignin, a cross-linked natural polymeric material formed by oxidative coupling of 4-hydroxyphenylpropanoid units, provides the structural stiffness found in lignocellulosic materials (Ralph et al., 2004). The oxidative coupling of the lignin monomers leads to random structure and a variability in composition. The lignin binds to cellulose and hemicelluloses through ester linkages and carbon-carbon bonding (Meshitsuka and Isogai, 1996). These linkages decrease the digestibility of cellulose and hemicelluloses, and they necessitate pretreatment of the biomass in most cases to break the linkages and open up the cellulose structure to chemical or enzymatic hydrolysis (Sarkanen and Ludwig, 1971). When the lignin bonds are broken to access the cellulose, various

phenolic compounds and organic acids are produced which can inhibit fermentation (Bardet and Robert, 1985). The specific nature of the degradation compounds produced depends on the species of biomass.

2. Hemicellulose

Hemicellulose is a complex branched carbohydrate polymer. The complexity arises from the variety of sugar monomers used in the polymer. Each type of monomer is attached to the hemicellulose chain by different linkages. Hemicellulose is largely comprised of aldopentoses (xylose and arabinose). Xylose is the simple sugar most present in the hemicelluloses. Small amounts of aldohexoses such as glucose, mannose, and galatose are also present (Jacobsen, 2000). Hemicellulose displays none of the crystallinity associated with cellulose due to the extensive branching. Its amorphous nature leads to easier breakdown to its constituent units than cellulose due to the lack of hydrogen bonding between chains. Hemicellulose fibers cross-link with cellulose through pectin bound between the two polymers. This provides physical protection to cellulose and decreases cellulose digestibility (Wyman, 1996).

FIGURE 1- Line Structures for Xylose (a) and Arabinose (b)

3. Cellulose

Cellulose is a linear polymer composed of D-anhydroglucopyranose units linked through β-1,4 glycosidic bonds. Two glucose molecules with a water removed, D-

anhydroglucopyranose, form the anhydrocellubiose repeat unit. Each repeat unit is rotated 180° creating a high degree of symmetry since each side has the same number of hydroxyl groups. The symmetry and linear nature of cellulose lead to a high degree of hydrogen bonding between adjacent molecules of glucopyranose creating highly crystalline regions. Figure 2 shows the arrangement of glucose repeat units in cellulose.

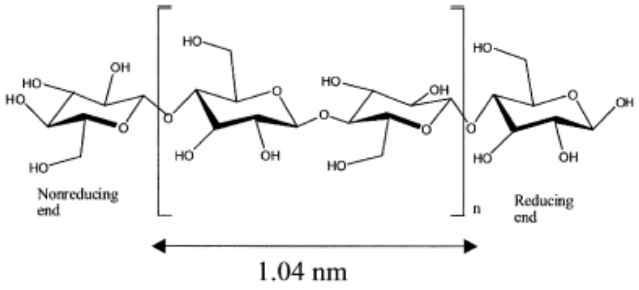


FIGURE 2- Arrangement of Glucose Monomers to form Cellulose Polymers

The crystalline regions are interrupted by amorphous regions which form from the difficulty of arranging the glucopyranose rings in a regular array (Ott, 1954). The crystalline regions pose a barrier to the hydrolysis through the hydrogen bonding and supramolecular structure. The hydrolysis of cellulose is a hetergenous reaction due to the different hydrolysis rates of the crystalline and amorphous regions (Xiang et al. 2003). The hydrolysis of cellulose releases glucose ($C_6H_{12}O_6$). Disrupting these crystalline regions is necessary for efficient hydrolysis of biomass.

B. Conversion of Lignocellulose

1. Enzymatic Hydrolysis

Numerous methods such as enzymatic hydrolysis, acid hydrolysis, and supercritical water extraction have been proposed to convert biomass into fermentable

sugars. Hydrolysis using biologically derived cellulase (enzyme) systems has been the most widely studied biomass conversion technique. This method provides high conversion of the substrate, mild conditions, and the potential for biological engineering to increase efficiency of cellulase systems (Zhang, 2004). Figure 3 presents a typical profile for enzymatic saccharification showing conversion of cellulose to glucose versus time.

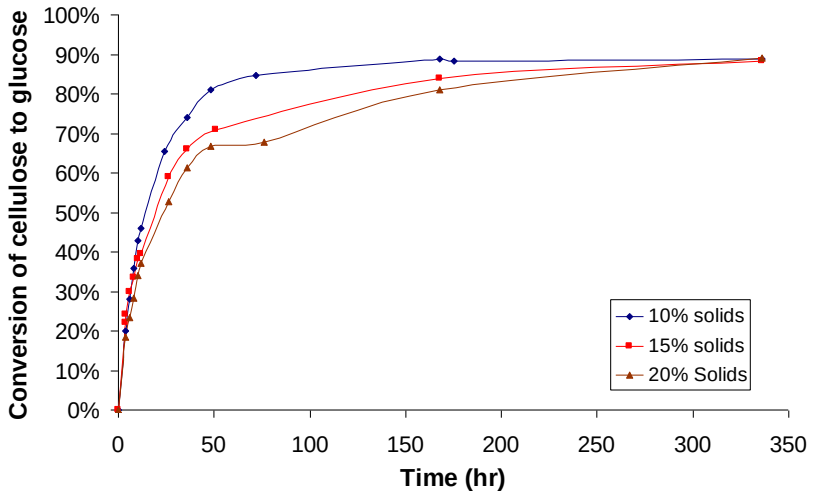


FIGURE 3 – Typical Enzymatic Saccharification Conversion versus Time Curve

Quick initial rates are seen in the first 24 hours with consistent falling rates after that. The falling rates are due to increasing crystallinity of the substrate, product inhibition, and enzyme deactivation (Zhang 2004, Ladish 1983). To achieve peak conversion, 7 to 10 days are needed at solids concentration less than 10 wt%. Increasing solids throughput leads to decreasing overall conversion due to mass transfer limitations from the increasing viscosity of the mixture (Dasari, 2009; Hodge 2005). All enzymatic hydrolysis processes include a pretreatment step to increase the digestibility of cellulose

since without pretreatment enzymatic hydrolysis achieves approximately only 20% conversion (Lynd 2002).

2. Pretreatment

Cellulose digestibility is a function of the structural features of cellulose such as crystallinity, degree of polymerization, surface area, and lignin content. Pretreatment methods work to overcome the physical and chemical barriers inherent in lignocelluloses to increase the digestibility of cellulose. Pretreatment methods include physical methods such as milling, chemical pretreatment technologies which disrupt hemicellulose and lignin bonding through the use of ammonia, sulfuric acid, and cellulose solvents, and thermal methods including steam explosion and supercritical water extraction (Mosier et al., 2005). Effective pretreatments avoid the need for size reduction, preserve the pentose released from the hemicelluloses fraction, minimize the degradation products, and limit the overall cost (National Research Council, 1999 and Ladisch 1983). Pretreatment represents the largest processing cost followed by enzymatic hydrolysis (Wooley et al. 1999). Pretreatment methods have not been shown to be universally successful with all substrates. Some pretreatment methods perform better on specific substrates than others. Most pretreatment methods remove a large amount of the hemicellulose fraction and restructure the lignin fraction (Wyman 1996). This increases digestibility through reducing hydrogen bonding to cellulose and increasing the surface area of cellulose available for attack by enzymes.

3. Acid Catalyzed Hydrolysis of Cellulose

Acid catalyzed processes represent the other major cellulose hydrolysis route. It is less favored due to the increased capital cost associated with corrosion resistant materials, the energy cost associated with acid recovery and re-concentration, and the formation of degradation byproducts that reduce yield and inhibit fermentation (Ladisch, 1983). Many acids have been investigated such as maleic acid, phosphoric acid, and hydrochloric, but the most studied has been sulfuric acid due to its commodity chemical status and ability to hydrolyze cellulose (Mosier et al., 2002, Choi and Matthews, 1996, Zhang et al., 2007).

The acid hydrolysis of cellulose begins with the H^+ ion from the acid interacting with the oxygen bond between the two sugar monomers forming a conjugate acid. The C-O bond then splits and the conjugate acid breaks down yielding a carbonium ion. A water molecule is added to the carbonium ion, and then a H^+ ion and a glucose molecule is liberated. This reaction takes place more readily at the end of the cellulose or hemicellulose chain than in the middle of the chain (Xiang, 2003). This reaction scheme holds for pH values under 2.0 because above a pH of 2.0 a hydroxyl reaction mechanism competes with the hydronium reaction mechanism. The reaction kinetics for the conversion of cellulose to glucose was first modeled as a pseudo first order reaction by Saeman (1945).

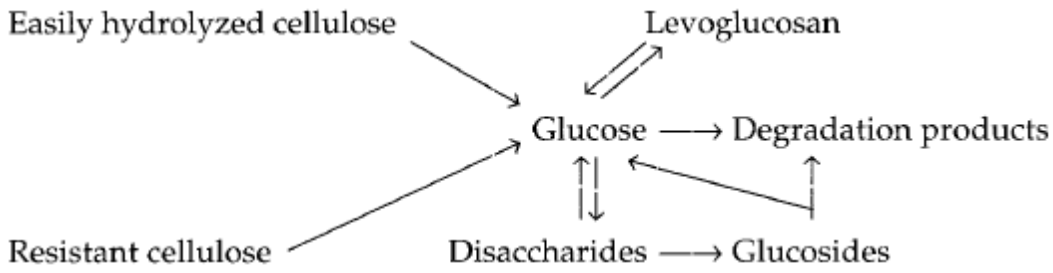
$$\text{Cellulose} \xrightarrow{k_1} \text{Glucose} \xrightarrow{k_2} \text{Degradation products}$$

According to this model the cellulose and glucose hydrolysis rates are described by the following differential equations:

$$\frac{d(Cellulose)}{dt} = -k_1 * C_{Cellulose} \quad (1)$$

$$\frac{d(Glucose)}{dt} = -k_2 * C_{Glucose} + k_1 * C_{Cellulose} \quad (2)$$

The reaction rate constants are assumed to follow an Arrhenius relation. The Saeman model is known to over predict the amount of glucose released. Through research it has been found that this model makes three major simplifications. First, from the previous discussion of cellulose structure and enzymatic hydrolysis, it was seen that the amorphous regions of cellulose hydrolyze at a quicker rate than the crystalline form of cellulose. This heterogeneous nature is also seen in the acid hydrolysis reaction. The product sugars released from the amorphous region are more prone to form degradation products since they are released earlier and subjected to proton attack. Through incorporating the different rates of cellulose hydrolysis and the reversible reactions of glucose, Conner et al. (1985) defined the following detailed model:



This model provides results with a better fit to experimental data than the Saeman model, though several reaction factors are not accounted for in this model. The hydrolysis rate is not only controlled by the reaction conditions but also the physical state of the cellulose such as the degree of polymerization and swelling of the fibrils. Once cellulose is dissolved, the rate of hydrolysis is two orders of magnitude greater. This was shown to

happen in both dilute and concentrated sulfuric acid systems at a specific temperature (Xiang, 2003).

Another simplification of this model is that the cellulose is not hydrolyzed directly to glucose, instead the cellulose forms soluble oligomers which then hydrolyze to the sugar monomers. This has lead to the development of two stage acid hydrolysis processes; in the first stage, cellulose is converted into oligosaccharides, and then in the second stage under less severe conditions the oligomers are converted to glucose. This reaction scheme limits the glucose degradation seen at the high severities needed to quickly break down the cellulose to oligosaccharides.

Lastly, several researchers have found that cellulose conversion during acid catalyzed hydrolysis in batch and percolation reactors is limited to approximately 70% (Mok and Antal, 1992 and Bouchard et al., 1989). The conversion ceiling can not be fully explained by the formation of degradation byproducts from glucose. Therefore, a parasitic pathway has been proposed to exist in the hydrolysis of cellulose. One proposed mechanism involves the oxidative formation of a carbonyl group from the hydrogen and hydroxyl groups (Bouchard, 1989). On the molecular level, water forms a thin boundary layer with the cellulose chains due to hydrogen bonding between the hydrogens of water and the oxygen of the hydroxyl group. This organization of water around the cellulose chains could create a barrier that inhibits diffusion of the oligomer or glucose from the reaction surface. This extra time in contact with the cellulose structure and H^+ ion is hypothesized to promote the parasitic pathway (Xiang, 2003). Viscous effects can also interfere with the transport of the oligosaccharide or glucose away from the cellulose structure. This parasitic pathway has been circumvented through changing the reactor

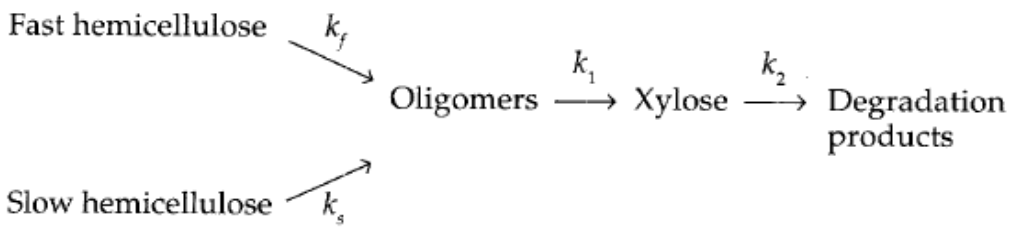
configuration. Torget et al. (2000) achieved a 92% conversion using dilute acid hydrolysis and a novel shrinking bed reactor that keeps a constant pressure on the bed as it shrinks in volume due to the dissolution of the cellulose structure. Keeping the bed of biomass constantly packed leads to a shearing effect on the solid phase which is proposed to assist the transport of the product away from the cellulose structure.

4. Acid Catalyzed Hydrolysis of Xylose

The hemicellulose fraction is more easily reduced to monomeric sugars than the cellulose fraction. This is due to the amorphous nature of hemicellulose and lack of hydrogen bonding in the polymer structure. The hydrolysis proceeds as a random cleavage of polymers. Saeman (1945) also modeled the saccharification of hemicellulose as a pseudo first order reaction as shown below.

$$\text{Hemicellulose} \xrightarrow{k_1} \text{Xylose} \xrightarrow{k_2} \text{Degradation products}$$

As in cellulose hydrolysis it has been found that there exists an easily hydrolyzed fraction and a recalcitrant fraction of hemicellulose. Additionally, the hemicellulose first releases oligomers which then are further broken down to xylose and other product sugars.



5. Dilute Acid Hydrolysis

Dilute acid hydrolysis is the most widely studied non-enzymatic hydrolysis process. Dilute acid hydrolysis is favored over concentrated acid hydrolysis due to low acid consumption. Depending upon reactor configuration, dilute acid processes utilize acid concentrations from 0.07 wt% to 3 wt% and temperatures ranging from 130F to 220F. The higher temperatures required are compensated for with savings in acid consumption and ability to control severity. Any acid hydrolysis processes will degrade the product sugars into the degradation products furfural from xylose and 5-Hydroxymethylfurfural from glucose. These products are known to inhibit fermentation. Glucose yields in dilute acid hydrolysis using plug flow reactors have experimentally peaked at 70% of the theoretical maximum (Torget et al., 2000).

The traditional degradation products of 5-HMF and furfural do not fully explain the lost yield. The majority of the lost yield is attributed to side reactions between the oligomers and lignin. It has been shown that through specific reactor configurations, temperature and acid concentrations can yield higher amounts of glucose than previously seen. For example, Kim et al. (2001) gained upwards of 90% conversion of cellulose to glucose in a novel shrinking bed reactor design. This result demonstrates that the oligosaccharide degradation reactions can be overcome. Due to the perceived limit of substrate conversion in dilute acid hydrolysis, the research has focused on this method as a pretreatment step before enzymatic hydrolysis. It has gained favor as a pretreatment method due to the high conversion of hemicelluloses to monosaccharides, almost 90%, and its ability to disrupt the lignin and cellulose structure to allow for greater digestibility (Mosier et al., 2005).

6. Concentrated Acid Hydrolysis

Concentrated acid hydrolysis has also been investigated for the conversion of biomass to sugars for fermentation to ethanol. Concentrated sulfuric acid hydrolysis operates at temperatures from room temperature to 120 C and acid concentrations from 3 wt% to 70 wt%. The results from this single step method have been mixed, with conversion from 20% to 65% reported. The best conversions are obtained through a two step process utilizing a high acid concentration to solublize the cellulose into oligosaccharides then a second hydrolysis at a more dilute concentration. A reference two step procedure is detailed in the National Renewable Energy's laboratory analytical procedure on determination of carbohydrates in biomass. The first step dissolves cellulose due to the breaking of water cellulose barrier and disruption of van der Waals interactions. The dissolution of cellulose has been noted in both dilute and concentrated processes. For example, dissolution takes place at 225C and 0.07 wt% sulfuric acid in a dilute acid process and in the concentrated acid system at 25C and 65 wt% sulfuric acid (Xiang et al., 2003). This leads to a reaction rate constant jump of 2 orders of magnitude. During cellulose dissolution, the acidic environment disrupts the highly ordered cellulose fibers leaving multiple single glucan chains.

Though the high acid concentration provides almost full digestion of cellulose, the high severity promotes degradation of the product sugars. Therefore, a second step at a lower acid concentration is required to raise the total yield. This two stage process raises process costs due to increased water usage in dilution of the acid and high energy input in re-concentrating the recovered acid. It is desired to develop a single step high severity process which limits the degradation reaction, possesses a quick reaction time, and

operates at a temperature below 100 C. Kamm and Kamm (2004) has suggested the single stage acid hydrolysis process as the best path forward for the biorefinery platform due to the exothermic nature of the reaction, short reaction times, and lower temperatures required when compared to dilute acid hydrolysis. The major hurdle using this process becomes acid recovery technology.

7. Relating Different Reaction Conditions Using a Severity Parameter

To relate complex reaction systems, equations can be devised that describe the conversion as a function of a severity parameter which depends on the reaction type and several dimensionless parameters. Overend and Chornet (1987) applied this method to the steam pretreatment of hardwoods. They obtain a severity parameter which related time and temperature.

$$R_o = exp\left[\frac{T - 100}{14.75}\right] * t \qquad (3)$$

Chum et al. (1990) applied the concept to the organosolv delignification of wood through incorporation of a catalyst term. Abatzoglou et al. (1992) expanded on the idea of a catalyst term applying the combined severity parameter to the acid catalyzed pretreatment of various lignocelluloses. Their method can be applied to other aqueous acid catalyzed systems such as the concentrated acid hydrolysis of hardwood. This reaction is a complex, heterogeneous system which is traditionally modeled as a homogeneous first order reaction. The complex reaction system consists of complicated steps where the exact transfer function or exact conversion steps from substrate to product are unknown. The system is lumped together in one transfer function where the conversion of the entire system is given by:

$$\frac{df}{dt} = \sum_i x_i \left(\frac{df_i}{dt}\right) \quad (4)$$

where the conversion is defined as:

$$f_i = 1 - \frac{S_i}{S_i^0} \quad (5)$$

Now applying this general treatment to the hydrolysis reaction gives the general expression in Equation (6).

$$\frac{df_i}{dt} = \frac{\nu_i}{S_i^0} * k[Temperature, catalyst] F\left[S_i^0(1 - f_i)\right] \quad (6)$$

where k is the reaction rate constant, and F is the unknown function of the reactants.

To incorporate the catalyst effect, it is assumed that the catalyst affects the activation energy and pre-exponential factor as seen in Equations (7) and (8)

$$E_i^* = E_i - nX \quad (7)$$

$$k_0^* = k_0 exp(mX) \quad (8)$$

Plugging theses equations into Equation (6), taking a Taylor series expansion to linearize with respect to catalyst concentration and temperature yields Equation (9):

$$\int_0^{f_{if}} \left(\frac{S_i^0}{F\left[S_i^0(1 - f_i)\right]} df_i\right) = \left[\nu_i k_0 exp(mX_{ref}) exp\left(\frac{E_i - nX_{ref}}{RT_{ref}}\right)\right]\left[\exp\left(\frac{X - X_{ref}}{\lambda X_{ref}}\right) exp\left\{\frac{T - T_{ref}}{\omega}\right\} t_R\right] \quad (9)$$

The severity parameter is extracted from Equation (9).

$$R_{oH} = \exp\left(\frac{X - X_{ref}}{\lambda X_{ref}}\right) exp\left\{\frac{T - T_{ref}}{\omega}\right\} t_R \quad (10)$$

where λ and ω are defined as follows:

$$\lambda = \left[mX_{ref} - \frac{nX_{ref}}{T_{ref}} \right]^{-1} \quad (11)$$

$$\omega = \frac{RT_{ref}^2}{E_i - nX_{ref}} \quad (12)$$

The rigorous calculation of the severity parameter requires conversion versus time data to find the parameters λ, ω, and K which are specific to the type of biomass. Assuming the reaction follows first order kinetics with respect to the substrate, Equation (9) becomes

$$f_{if} = 1 - \exp(KR_{oH}) \quad (13)$$

The parameters then can be found through a non-linear optimization routine in Polymath.

8. Reaction Byproducts

The degradation by-products referenced previously for cellulose and hemicellulose consist of a variety of chemicals. The two main by-products, furfural and 5-Hydroxymethylfurfural, form from the product sugars. Furfural and 5-HMF arise from the fact that the protons not only attack the polymeric sugar chain in the biomass but also the monomeric product sugars. The acid protonates the hydroxyl groups on the glucose and xylose molecules leading to the release of a water molecule and the subsequent degradation to 5-HMF and furfural, respectively. Other degradation products can be formed depending on the specific hydroxyl groups protonated which was shown through molecular dynamics simulations performed by Qian et al. (2005).The overall sugar degradation reactions are shown below:

$$\text{Glucose} \rightarrow \text{5-HMF} + 3H_2O$$

$$\text{Xylose} \rightarrow \text{Furfural} + 3H_2O$$

The reactions due to the acidic condition continue with the H^+ catalyzing side reactions with furfural and 5-HMF. The furfural will form furanic resins, condensation products, and formic acid. 5-HMF will further break down to levulinic acid and formic acid. The rates of both these reactions increase with acid concentration and temperature (Zeitsch, 2000). In addition to these by-products, acetic acid and various phenolic compounds evolve from the decomposition of the hemicellulose and lignin fractions. The exact compounds produced depend on the species of biomass due to the extreme variability in lignin composition and structure (Bardet and Robert, 1985).

In addition to reducing yield of product, these degradation products serve as fermentation inhibitors (Larsson et al., 1999). The by-products are liposoluble and are absorbed into the yeast cell limiting growth and ethanol productivity. Formic acid has the greatest inhibitory effect followed by levulinic acid and lastly acetic acid. Furfural and 5-HMF are metabolized by the yeast and serve mainly to retard cell growth with 5-HMF having the greater effect (Palmqvist and Hanh-Hagerdal, 2000). The phenolic compounds have not been widely studied in model fermentations, but through fermentations with dilute acid hydrolysates of hardwood, the phenolics have shown a great inhibitory effect on the yeast (Jonsson et al., 1998). Detoxification methods such as lime treatment, laccase enzyme treatment, and treatment with ion exchange resins have been proven to increase fermentability of hydrolysates. The removal or retardation of fermentation inhibitors will be necessary for any acid catalyzed processes especially those with high severities.

C. Ion Exchange

1. Structure and Basic Function

Ion exchange applications have been known over the ages; certain soils haqve been used to desalt brackish waters. These soils contain inorganic clays and zeolites which possess the ability to reversibly exchange a metal ion in their structure for another ion as long as electroneutrality is conserved. In 1850 the soil chemists Thompson and Way were the first to describe ion exchange materials using scientific language and methods. The ion exchange reaction involving cations is defined as follows:

$$M^-A^+ + B^+ \rightarrow M^-B^+ + A^+$$

M^- is the solid fixed anionic complement of cation A^+ called the fixed anion. The cations A^+ and B^+ are referred to as counter-ions. Any ions in solution which bear the same charge as the fixed ion are denoted co-ions. An analogous model can be defined in the same way for anion exchange systems. Ion exchange reactions follow several principles. Exchange involves stoichiometrically equivalent amounts of ions trading places with certain ions that display more affinity for the exchanger; this is also called selectivity. An ideal ion exchanger features a hydrophilic structure, controlled and effective ion exchange capacity, rapid rate of exchange, and stability through a range of chemical and physical conditions (Grimshaw and Harland, 1994).

The naturally occurring ion exchangers have little application outside of water purification due to the low capacity for exchange that clays possess; while zeolites have a capacity comparable to modern synthetic exchangers, they are sensitive to acids. Modern organic polymer networks have been developed which display a high capacity for exchange and display a high stability in most environments. These polymers arise from

the copolymerization of styrene and divinylbenzene (DVB) which produces a crosslinked hydrocarbon matrix. The fixed anion or cation groups are then added to the matrix. In the case of a strong acid cation exchange resin, which is employed in this work, the matrix is contacted with concentrated sulfuric acid to sulfonate the benzene rings at one site yielding sulfonic acid fixed anion groups. Other fixed groups include carboxylic acid for weak cation exchangers, amine groups for weak anion exchangers, and quaternary ammonium groups for strong anion exchangers.

In using ion exchange resins to separate monosacharrides from sulfuric acid and degradation products, ion exchange does not take place, but several other properties of ion exchangers are exploited. The ability of the resins to sorb nonelectrolyte solutes and the Donnan potential are used. Nonelectrolytes are sorbed into the pore structure of the matrix; therefore, this type of sorption is strongly dependant on pore structure. London forces weakly bind the solute to the fixed ion groups.

The pore properties of a synthetic ion exchange resin matrix can be controlled by the degree of crosslinking in the resin. The percentage of DVB determines the extent of crosslinking which influences many aspects of the polymer's properties as an ion exchanger. If no crosslinking occurred, the hydrophobic polystyrene with the hydrophilic fixed ion groups such as $-SO_3^-H^+$ would dissolve in water. The resin is made insoluble through the crosslinking between various polystyrene chains because its dissolution would entail the breaking of C—C bonds (Helferich, 1962).

Placement of the resin in water or other polar solvents results in a certain amount of swelling and expansion of the matrix. The ionogenic groups attached to the styrene monomers attract the water molecules through electrostatic interactions, beginning the

process of dissolution. The swelling of resin is an equilibrium process balancing the solvation and stretching of the matrix with the elastic force exerted by the matrix crosslinks, thereby a swelling pressure exists within the matrix. Increased crosslinking reduces the amount of swelling in the resin thereby increasing the pressure within the pore. The increased swelling pressure restricts the amount of solute in the pore, limiting sorption.

2. Donnan Potential

When an ion exchanger is placed in a dilute electrolyte solution, an electrostatic force arises called the Donnan potential. For illustration of this phenomenon, picture a cation exchanger placed in solution with a dilute electrolyte such as NaCl. Considerable concentration differences exist between the two phases. The cation concentration is greater in the resin versus the solution. Further, the mobile anion concentration is greater in the solution. Diffusion would naturally reconcile these concentration differences, but the migration of cations into the solution and anions into the resin phase would violate electroneutrality, creating a positive charge in the solution. A few ions will still initially diffuse leading to a potential difference between the two phases. This difference is referred to as the Donnan potential. This size and effectiveness of this potential decreases as the electrolyte concentration increases because the concentration gradient between the cations in the resin phase and those in solution decreases; this causes fewer cations to migrate into solution from the resin thereby decreasing the potential. As seen in the adsorption of nonelectrolytes, increasing degree of crosslinking affects the Donnan potential. Increasing the amount of DVB leads to a higher fixed anion concentration in the resin which leads to a larger Donnan potential between the resin and solution phases.

The Donnan potential excludes mobile anions in solution from entering the resin. Since electroneutrality must be preserved, the counter-ions in solution are also excluded. Therefore, almost no electrolyte is adsorbed onto the resin. This is called Donnan exclusion or ion exclusion. The separation of monosaccharides (nonelectrolye) from sulfuric acid (electrolyte) utilizes this principle. The sulfuric acid can be thought to exist in solution with the sugars as hydronium ions (H_3O^+) and the conjugate base HSO^-_4. When placed in solution with ion exchange material the conjugate base anions will be excluded from the resin through the Donnan potential whereas the nonelectrolyte sugars will adsorb onto the resin resulting in separation of the sugar and acid. As shown earlier, both ion exclusion and nonelectrolyte adsorption depend on the degree of crosslinking. Consequently, there exits an optimum DVB resin content between 3 and 8 percent for this application (Helfferich, 1962).

3. Application of Ion Exchange Resins to Hydrolysates

Ion exclusion has been applied to separation of sugar-acid streams and to detoxification of hydrolysates for fermentation. Neuman et al. (1987) investigated the separation of sugar from 0.822 N sulfuric acid. They found 94 percent recovery of glucose from the acid using a sulfonic acid cation exchanger with a DVB content of 4.5 percent. The optimal conditions for the separation were found at a temperature of 55°C, flowrate of 3.2 mL/min, and a loading of 10 gram of glucose per liter of external void volume. The effects of temperature and glucose loading were examined. Above and below the optimum temperature, the width of the glucose peak increased which in turn effected the sharpness of the separation. Higher sugar loadings created a tail and shoulder at the beginning of the sugar elution curve, also inhibiting optimal separation. The

authors acknowledge that a resin with a high crosslinking should be investigated due to its effect on Donnan exclusion.

Resins with a higher degree of crosslinking (6% DVB, Dowex 99 H^+ form) have been tested for separation. These resins provide a greater throughput of sugars for a given percent recovery (Xie et al., 2005). Further, the increased structural strength yields less resin volume change when placed in acid (Springfield and Hester, 1999). Xie et al. (2005) additionally studied anion exchange resins made from poly(4-vinyl pyridine) (PVP) for the separation of acid, sugar, and degradation compounds. The PVP resin adsorbs the sulfuric acid, and it allows the sugars to pass through followed by the degradation products. In this case, the resin needs to be regenerated with NaOH to remove the sulfuric acid.

4. Detoxification of Hydrolysate

The fermentability of hydrolyates processed using the two exchangers was also examined. The PVP treated hydrolysate displayed fermentability on par with pure sugar, and the Dowex 99 treated hydrolysate showed 40 percent less fermentabiltiy which is comparable to overlimed hydrolysate. The improved fermentability is attributed to better removal of phenolic compounds by the PVP resin than the Dowex resin (Palmqvist and Hagerdal, 2000). The separation method utilizing PVP provides almost complete separation and better fermentability.

Industrial scale chromatography processes use a continuous system called a simulated moving bed (SMB). Simulated moving beds typically consist of four zones with 2 separation beds per zone arranged in a circle, though other configurations exist. Each zone contains a valve which periodically switches along the ring. This switching

creates the simulation of a moving bed by allowing the port to follow the solute bands as they migrate through the ring. This will perform a binary separation with an extract and a raffinate stream. More advanced methods exist to yield 3 or more separate streams (Chin and Wang, 2004).

Several researchers have designed SMB systems to separate sugars from hydrolysates. Springfield and Hester (1999) demonstrated that a SMB would satisfactorily separate glucose and xylose from a solution of 15 weight percent acid. Wooley et al. (1998) setup a nine zone SMB that separated sugar (xylose and glucose), sulfuric acid, and acetic acid. Xie et al. (2005b) furthered the separation by designing a SMB that would satisfactorily separate six sugars from HMF, furfural, acetic acid, and sulfuric acid. They compared Dowex 99 and PVP for this operation. Both designs had a similar cost, but the PVP was chosen as the process to optimize since the hydrolysate treated with PVP had better fermentability.

Ion exchange resins have been shown to successfully separate sugars, acids, and degradation products. These separations have been performed at acid concentrations below 15 weight percent. In the process considered for this study the acid concentration will be much higher up to 90 weight percent sulfuric acid. The resin response will change due to the decreased Donnan potential at these high electrolyte concentrations.

III. EXPERIMENTAL

A. Experimental Plan

To find the optimal conversion for a single stage acid hydrolysis of hardwood sawdust, nine different reaction conditions were tested. The conditions had to meet the following criteria: optimal conversion of cellulose and xylose reached in less than eight hours and preservation of the xylose fraction since it will be in contact with the acid longer than the glucose fraction. The conditions chosen to study were sulfuric acid concentrations of 50, 70, and 90 weight percent acid at temperatures of 30°C, 50°C, and 80°C. The only solids loading studied was 10 weight percent wood. These acid concentration ranges were chosen as preliminary studies showed that 50 weight percent would give appreciable conversion at the temperature range available. 30 weight percent was tested and found to give less than 15 percent conversion after eight hours. A table of experiments is presented below.

TABLE 1

ACID HYDROLYSIS CONDITIONS

10% Wood		
90 wt% acid, 80°C	90 wt% acid, 50°C	90 wt% acid, 30°C
70 wt% acid, 80°C	70 wt% acid, 50°C	70 wt% acid, 30°C
50 wt% acid, 80°C	50 wt% acid, 50°C	50 wt% acid, 30°C

Each reaction condition was monitored for overall glucose and xylose released and furanic degradation products. Though a full mass balance on the reaction cannot be completed using the analytic methods available, the general trends and optimal reaction time can be observed. From this data, one reaction condition was chosen to further

optimize through the use of ion exclusion to separate product sugars and acid. First, the effect of separating the solids from the acid-sugar mixture, and then reintroducing the solids into fresh acid for a second hydrolysis was studied to hopefully see increased conversion. Any increased conversion will come from the lower degradation of product sugars due to decreased contact time with the acid, and from the longer contact time of re-introduced solids.

Next, the sugar-acid separation step was tested using Dowex 99 K^+ resin beads converted to hydrogen form. The separation was performed with a solution that matched the sugar concentrations and acid concentration seen for the reaction condition that was chosen to be optimized. Previous research has shown this resin to be efficient in separating sugar from acid, but no experiments have been preformed with sulfuric acid above a concentration of 15 weight percent. The acid concentration is known to affect the separation properties through alteration of the resin structure.

B. Materials and Equipment

1. Hydrolysis Procedures

The acid hydrolysis reaction was run in 250 mL Erlenmeyer flasks filled with 100 mL of wood-acid slurry. Stock sulfuric acid was placed in the flask and heated to the reaction condition in a New Brunswick Innova 4230 Incubator/Shaker (Edison, NJ). The flask was covered with parafilm and a double layer of aluminum foil. The stock sulfuric acid solutions of 50, 70, and 90 weight percent were prepared from 98 weight percent reagent grade sulfuric acid (Sigma Aldrich, St. Louis, MO, Lot 08007TE) and deionized water produced in the lab by a Culligan DI system. After sulfuric acid reached the correct temperature, the sawdust hardwood substrate was added to give a solids loading of 10%

(w/v). The wood sawdust substrate was procured from Garrard Wood Products (Lancaster, KY). The mixture of hardwoods had a composition of 39.9 % cellulose and 18.8 % hemicellulose with the balance being lignin, ash, and extractives. The wood-acid slurry was covered and placed in the incubator/shaker at 250 rpm. Sampling occurred in intervals that allowed 6 to 8 samples per reaction conditions. This was not possible for the extremely high severity condition due to the time involved in the sampling. For each sampling interval, 10 mL of the reaction mixture was collected and placed immediately in an ice bath to cool. To stop the reaction, the sulfuric acid was neutralized with 5N Sodium Hydroxide from Ricca Chemical (Lot 1805115). The sodium hydroxide was added until a pH between 4 and 9 was measured with a Denver Instruments UltraBasic Benchtop UB-5**.** At this pH range, catalytic activity was inhibited at room temperature. The sample was then vacuum filtered through Whatman 5 micron filter paper. The filtrate was drawn up into a 5 mL syringe and cooled in an ice bath to facilitate crystallization of $NaSO_4$, which is the neutralization salt of the acid-base titration. The solubility of $NaSO_4$ declines from 497 g/L at 32.4°C to 47 g/L at 0°C. The concentration of the salt had to be lowered because of its interaction with the temperature probe on the YSI 2700 Select (YSI Integrated Systems & Services, Yellow Springs, OH) used to measure glucose. The crystallized salt was removed by passing the mixture through a 0.45 micron syringe filter. The filtered sample is collected in three separate 1.5 ml vials. Each vial was analyzed using the three assay methods described below in the assays section.

2. Procedures Ion Exchange

The resin must be fully hydrated before it could be loaded into the glass chromatography column. The resin swells as water hydrates the resin structure. The

pressure exerted by the hydration could be severe enough to fracture the column. Furthermore, any sites in the resin structure that still contain air will not participate in ion exclusion, reducing efficiency of the resin bed. To hydrate the resin, it was placed under vacuum with distilled and degassed water (DDW) for at least 12 hours. The distilled water came from a Barnsteed FiSteam II (Dubuque, Iowa). The water was degassed under vacuum in a filter flask. This water treatment was used for all subsequent ion exclusion experiments. Once hydrated, the resin slurry was used to fill the 300mm X 25mm chromatographic column from Ace Glass (Vinewood, NJ) to approximately half the column height to allow for bed expansion. Now the resin was converted to hydrogen form by pumping 5 bed volumes of 5% (w/w) hydrochloric acid upward through the colum at 15 mL/min. The hydrochloric acid solution was prepared from 37 weight percent hydrochloric acid (Fisher Chemical, Lot 084711). Two Masterflex 7550-60 peristaltic pumps equipped with size 14 Tygon LFL tubing control the feed to the column and the effluent from the column. The column and pump setup is shown in Figure 4.

FIGURE 4- Ion Exclusion Column Setup

Once the bed was converted to hydrogen form, it must be washed of residual acid and packed according to bead size. Water was pumped up through the column at 40

mL/min to allow the bed to expand nearly double its original volume. After bed equilibration for 30 minutes, the flowrate was decreased in 5 mL/min increments every 10 minutes to allow the denser beads to settle to the bottom of the bed. Once the flow reaches 10 mL/min the wash was stopped, and the bed fully settled by gravity. The water remaining in the column was pumped out at 10 mL/min.

The resin bed temperature was regulated by a heating box. The temperature in the box was controlled by a Love Controls Series 8500 setpoint controller which manipulates a Master Appliance HG-201A heat gun. The water fed to the column as an elutant was heated on a Corning PC-351 Hot Plate. To begin the experiment, a model hydrolysate was slowly added to the top of the resin bed. The liquid sample was loaded into the bed by pumping downward to match the height of the bed. The sample hydrolysate was made with glucose 99%+ (Lot B0122614) and xylose 99%+ (Lot A0241957) from Arcos Organics. The concentrations of sugar and acid matched the concentrations in the optimal hydrolysis case. Once the sample was loaded, the eluting water was fed to the column. The outlet flow was controlled by a Masterflex pump. The outlet flowed to a Gibson FC-80E fraction collector, which collects 7.5 mL fractions. Each fraction was analyzed for glucose, xylose, and sulfuric acid.

3. Assays

a. Xylose Measurement. Analysis of xylose utilizes the orcinol reaction first proposed by Bial (1902). The assay method was adopted from procedures given by Ashwell (1957). In this test, the xylose is degraded to furfural by acid. The furfural then reacts with $FeCl_3$ and orcinol to form a blue-green chromatogen. The glucose is also broken down to 5-HMF, but the reaction proceeds more slowly. The 5-HMF forms a

reddish-brown complex with $FeCl_3$ and orcinol, which is corrected for by taking the absorbance difference between the wavelengths of 660 nm and 600 nm. To begin the test, a solution of one part water and one part hydrochloric acid is mixed, and ACS grade $FeCl_3$ from Ricca Chemical (Lot 1809596) is added to make a 0.1% solution. 3mL of this solution is placed in a reaction tube. Next, orcino from MP Biomedicals (Lot 1764J) is mixed to a 10% concentration in 95% ethanol (Acros Organics, Lot B0514428). This solution must be made fresh daily due to the instability of orcinol in solution. 150 μL of this solution is added to the test tube. Finally, 25 μL of sample is added. The sample is diluted to concentrations of 1 g/L or less. This mixture is placed in a VWR Scientific 1120 water bath at 100°C for exactly 21 minutes. A calibration curve is made for each test due to the instability of the orcinol in solution. The reaction time optimizes the breakdown of xylose to furfural over glucose to 5-HMF.

b. Glucose Measurement. The sample is tested for dissolved sugar content using a YSI 2700 Select Biochemistry Analyzer (Yellow Springs, OH). The liquid is diluted to a glucose concentration between 2 and 10 gm/L, the recommended range of the YSI.

c. Furans Measurement. Martinez et al. (2000) reported that furfural and 5-HMF display similar absorbance patterns between 240 nm and 320 nm. Both compounds have equal absorbance on a weight basis at 284 nm. This wavelength is close to the spectral peaks of furfural and 5-HMF. The absorbance at 284 nm is subtracted from the absorbance at 320 nm to remove any unknown contribution to absorbance from unknown compounds. Each sample is diluted 1:500, placed in a quartz cuvette, and read at 284 nm and 320 nm in a Varian spectrophotometer. Concentration of furans was determined from calibration curves prepared with furfural solutions between 1 g/L and 10 g/L.

d. Sulfuric Acid. The molarity of the sulfuric acid in the elution fraction is determined by titration with 0.5N NaOH. The NaOH is added in constant volume increments. The titration data is entered into an Excel based regression routine. The equivalence point is determined as the peak of the derivative measuring the change in pH over the change in volume of titrant. This can also be defined as the point when the second derivate equals zero.

IV. RESULTS AND DISCUSSION

A. Acid Hydrolysis Results and Analysis

1. Glucose

To find the optimal conditions for concentrated acid hydrolysis of hardwood sawdust, both the acid concentration and temperature were varied. In each test the glucose released, xylose released, and furanic compounds formed were tracked with reaction time. The greatest amount of glucose release occurred for test conditions of 70 weight percent acid, 80 ˚C, and 10% wood which has a peak glucose release of 25.7 g/L glucose which corresponds to a conversion of 58.18% after 45 minutes. The glucose released for all tests are displayed in Table II.

TABLE II

SUMMARY OF PEAK GLUCOSE RELEASED AND GLUCOSE RELEASED AS A PERCENTAGE OF AVAILABLE GLUCAN FOR ALL HYDROLYSIS TESTS

Acid wt%	Temperature (C)	Time (min)	Peak Glucose (g/L)	Peak % Glucose Released	Severity
70	80	45	25.8	58.2%	3.5
70	50	90	25.5	57.6%	3.1
70	30	810	23.3	52.8%	3.3
90	30	40	22.2	50.2%	3.7
50	80	180	6.4	14.3%	2.3
90	80	5	5.4	12.2%	4.3
90	50	20	4.9	11.0%	4.5
50	50	150	2.6	5.8%	1.8
50	30	60	2.3	5.3%	1.7

Three other tests, 70 weight percent acid at 50˚C, 70 weight percent acid at 30˚C and 90 weight percent acid at 30˚C, achieve cellulose conversions above 50%. These four tests had severity parameters between 3.1 and 3.7. The reaction conditions in these tests yield severe enough conditions such that the hydrogen bonding between the crystalline

cellulose regions becomes disrupted by the sulfuric acid. The now amorphous regions become open to proton attack and generation of oligosaccharides followed by further breakdown to glucose.

It should be noted that the fitted parameters used to calculate the severity parameter had a low r^2 value of 0.72. It could be expected that the two other 90 weight percent acid tests yielded higher conversions also, but this was not the case with both cases displaying conversions below 15%. This low conversion is attributed to the high severity parameter of 4.3 and 4.5, due to the higher temperatures, which leads to breakdown of the product sugars to degradation products. The glucose degrades to 5-HMF almost as fast as it is liberated from the cellulose. In addition, the parasitic pathway proposed by Bouchard et al. (1989) and Mok and Antal (1992) could have an increased effect with the higher severity. This will be illustrated in the discussion on furans.

The wood used in this study is mixed hardwoods with the majority of the wood being red oak. Red oak is known to have a good yield in enzymatic hydrolysis due to the open pore structure. Vinzant et al. (1994) reported 87 percent of theoretical conversion during enzymatic hydrolysis, whereas for white ash, which possesses a more closed pore structure, the subsequent conversion is only 43 percent of theoretical. The pore structure is not the only determining factor in the conversion during enzymatic saccharification; the lignin structure also plays a major role. Concentrated acid hydrolysis of red oak needs improvement to meet the published values of conversion. Though without any optimization, it could provide better conversion for woods such as white ash and pine which provide lower yields in enzymatic hydrolysis because concentrated acid hydrolysis is not highly affected by the lignin and pore structure.

2. Xylose

The xylose released from hemicellulose was also tracked with time for the nine reaction conditions. Xylose release from hemicellulose occurs much sooner from the wood biomass than the liberation of glucose from cellulose. This happens since hemicellulose has an amorphous branched structure in contrast to the structured crystalline state of the majority of the cellulose configuration. The greatest hemicellulose to xylose conversion of 89.07 % after 10 minutes occurs in the 90 weight percent acid, 30°C case. All cases appear to possess high enough catalytic activity to hydrolyze hemicellulose to xylose. Results for all cases are presented in Table III. The less than 50% conversion seen in the other two 90 weight percent acid cases is attributed to the extremely severe conditions. The xylose is liberated into solution, but it is degraded to furfural almost as quickly as it is created, leading to the low peak xylose concentrations seen.

The 70 weight percent acid cases all display conversion above 70% after 15 minutes. The 80°C case displays the highest peak of xylose at 17.18 g/L, followed by the 50°C case at 14.5 g/L, and then the 30°C run at 14.04 g/L. The correlation of higher conversion with increasing temperature is attributed to the influence of degradation on the product sugar. It is thought that the higher temperature leads to a greater increase in the xylose release rate than the xylose degradation rate. Further samples need to be taken before 15 minutes since all three cases display the peak at 15 minutes. Sampling at earlier reaction times could lead to even higher xylose concentrations due to the ongoing degradation.

The 50 weight percent acid displays the best xylose release at reaction conditions of 50°C and 180 minutes with a xylose concentration of 16.7 g/L. The 30°C case displays the second highest xylose peak at 16.52 g/L after 240 minutes, and lastly the 80°C test shows a xylose concentration of 13.92 g/l after 180 minutes. This set of tests does not follow the same pattern seen for the 70 weight percent acid tests.

TABLE III

SUMMARY OF PEAK XYLOSE RELEASED AND XLYOSE RELEASED AS A PERCENTAGE OF AVAILABLE XYLAN FOR ALL HYDROLYSIS TESTS

Acid wt%	Temperature (C)	Time (min)	Peak Xylose (g/L)	Peak % Xylose Released
90	30	10	17.7	89.1%
70	80	15	17.2	86.5%
50	50	180	16.7	80.2%
50	30	240	16.5	79.2%
70	50	15	14.5	73.3%
70	30	15	14.0	70.7%
50	80	150	13.9	66.7%
90	80	15	9.4	44.9%
90	50	20	7.1	34.0%

3. Degradation of Glucose and Xylose to Furans

The third parameter monitored to characterize the reaction is the concentration of furans in solution, which is a combination of the two main furanic compounds, furfural and 5-HMF. To quantify the creation of furan degradation products, a spectrophotomeric method was used. The downside to this method is that the degradation compounds cannot be separated into those formed from glucose and those form from xylose, which would allow more precise determination of the rate at which each product is being decomposed to byproducts. Further, as can be seen in Figures 5-7, especially for the 90 weight percent case at 80°C, the furans are seen to decrease as the reaction proceeds. 5-HMF further reacts to levulinic acid, formic acid, and humic condensation products from interaction of

5-HMF and glucose (Chang et. al, 2006). The rate of decomposition of 5-HMF is largely dependent on the acid concentration; it increases with higher acidity. Furfural is lost through resinification and condensation reactions, which dominate at lower temperatures (Zeitsch, 2000). It is not known if these resins and condensation products have similar UV absorbance patterns to furfural. It is possible since certain products have structures close to furfural.

The 90 weight percent acid cases show the highest decomposition of the product sugars with approximately 6 times more furans produced in these cases than the 70 and 50 weight percent acid cases. The other two sets of acid concentrations appear to follow more of a temperature dependence than an acidity dependence. The 50 weight percent acid, 80°C case is the only test at this acid concentration to show appreciable production and accumulation of furans. This shows that degradation is responsible for the lower yield of xylose for this case than the others at this acid concentration. The 70 weight percent cases do not show a large change in furan production as temperature is varied. The lack of change in furans production is ascribed to a combination of the measurement method and the decay of the furans in acidic mediums.

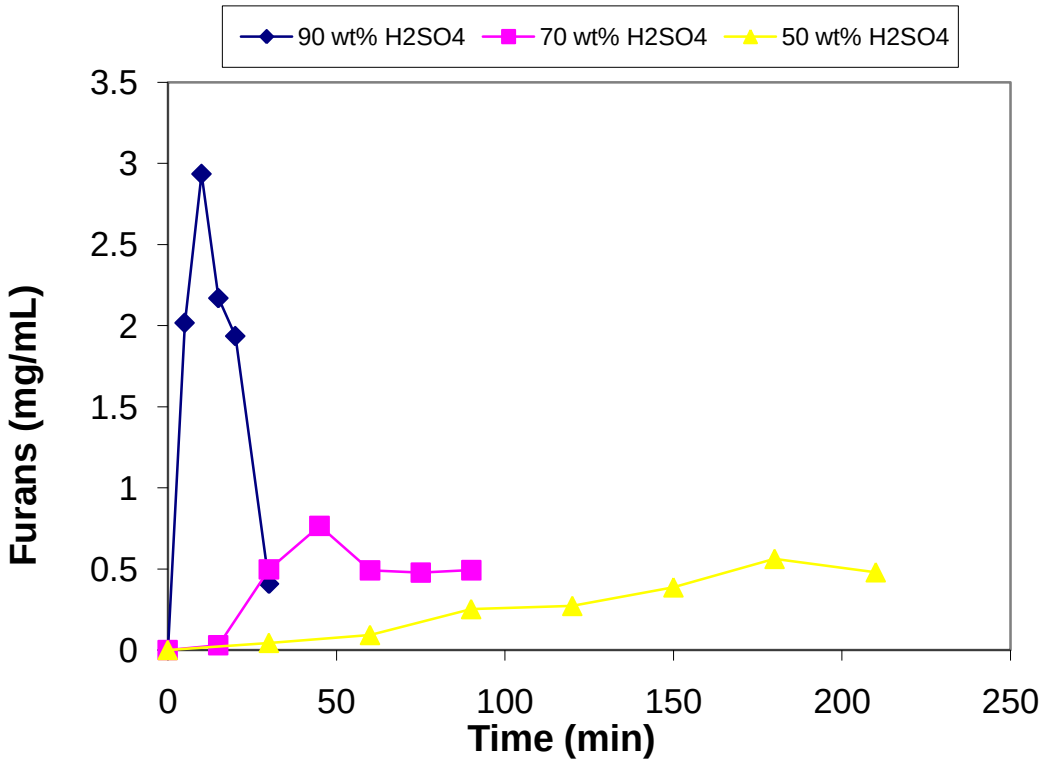


FIGURE 5- Furans over Time at 80°C and Varying Acid Concentrations

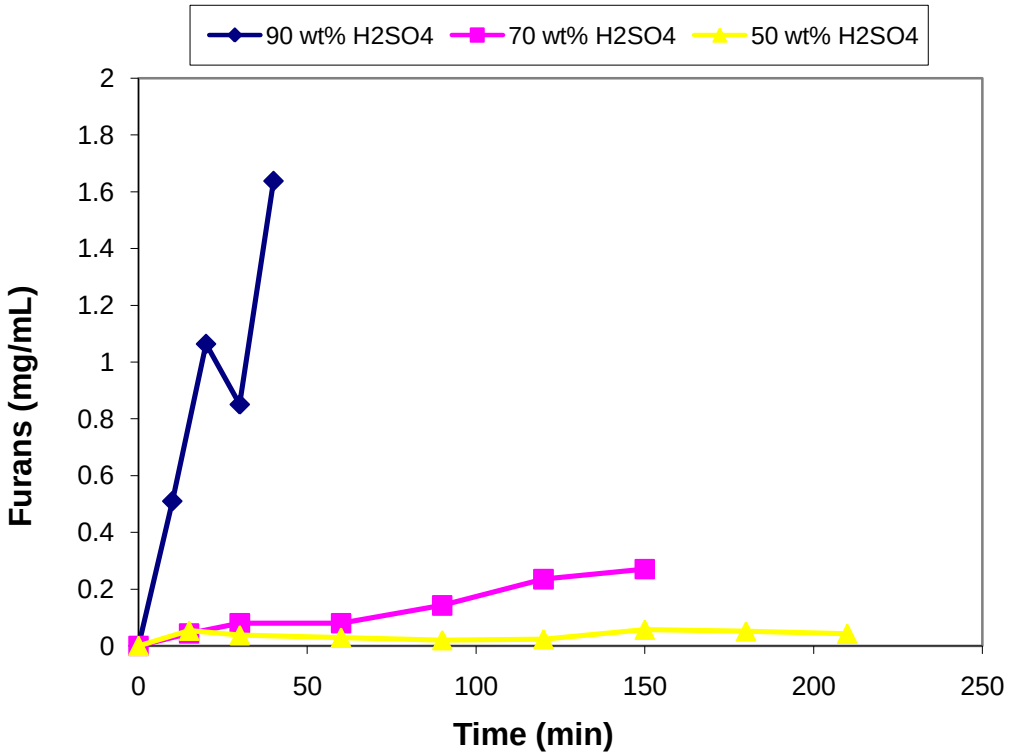


FIGURE 6- Furans over Time at 50°C and Varying Acid Concentrations

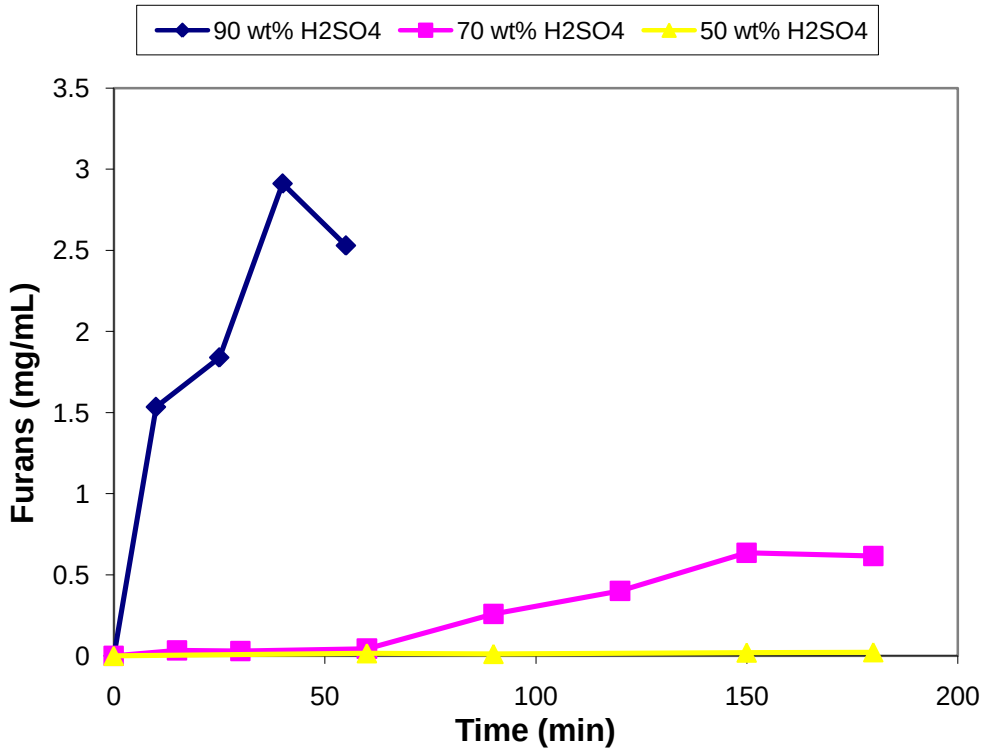


FIGURE 7- Furans over Time at 30°C and Varying Acid Concentrations

4. Comparison of Optimum Reaction Times for Cellulose and Hemicellulose Hydrolysis

The discussion up until now has focused on glucose and xylose as separate entities, though they exist in solution together during the reaction. The conversion peaks for xylose and glucose for most cases occur at different times due to the more easily accessible nature of hemicellulose. For example, in the 90 weight percent acid, 30°C case, the xylose in solution decreases by 46.8% from the xylose peak at 10 minutes of reaction time to the time when the peak glucose concentration occurs at 40 minutes (Figure 10). This is due to the high severity of the reaction which quickly degrades the xylose released. In Figures 8-10 the release of xylose and glucose as a percentage of available xylan and glucan are plotted versus time. This illustrates the time dependence of

the xylose and glucose peaks at the three reaction temperatures and varying acid concentrations.

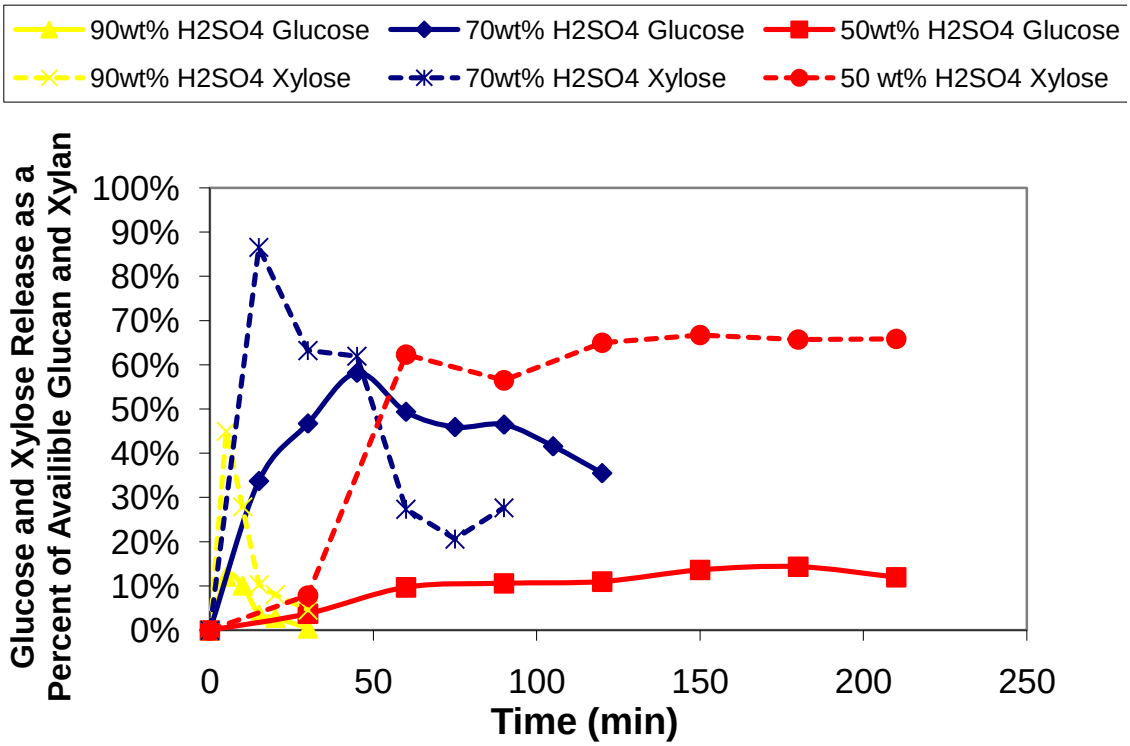


FIGURE 8- Glucose and Xylose Release Versus Time for Varying Acid Concentrations at 80°C

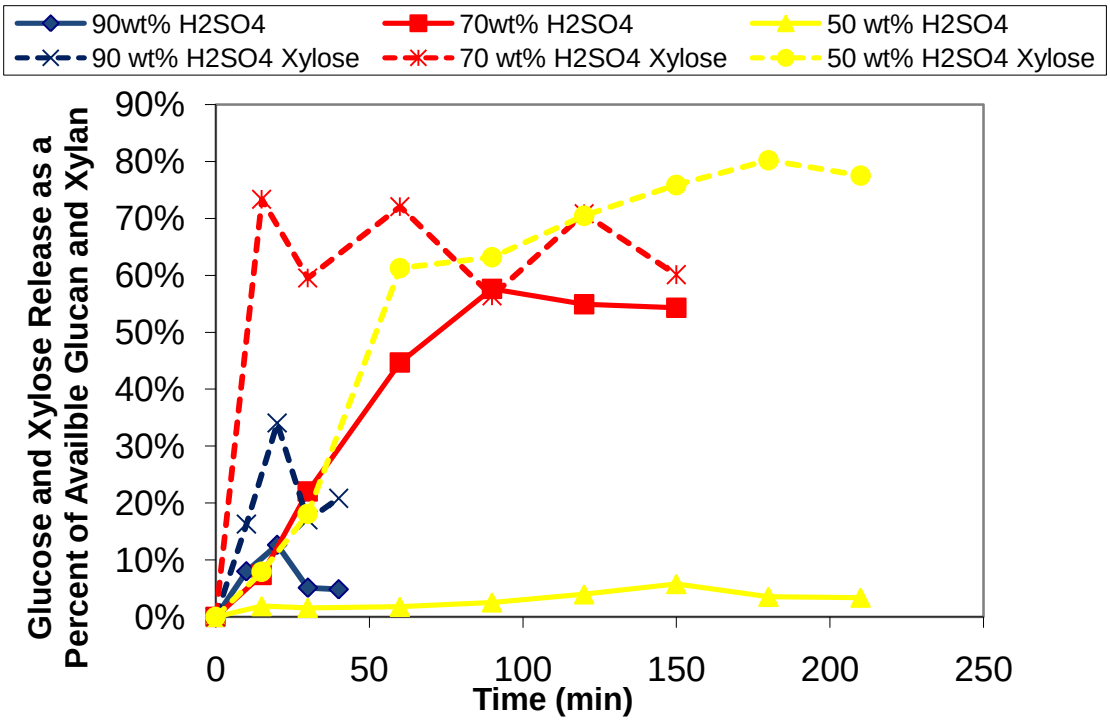


FIGURE 9- Glucose and Xylose Release versus Time for Varying Acid Concentrations at 50°C

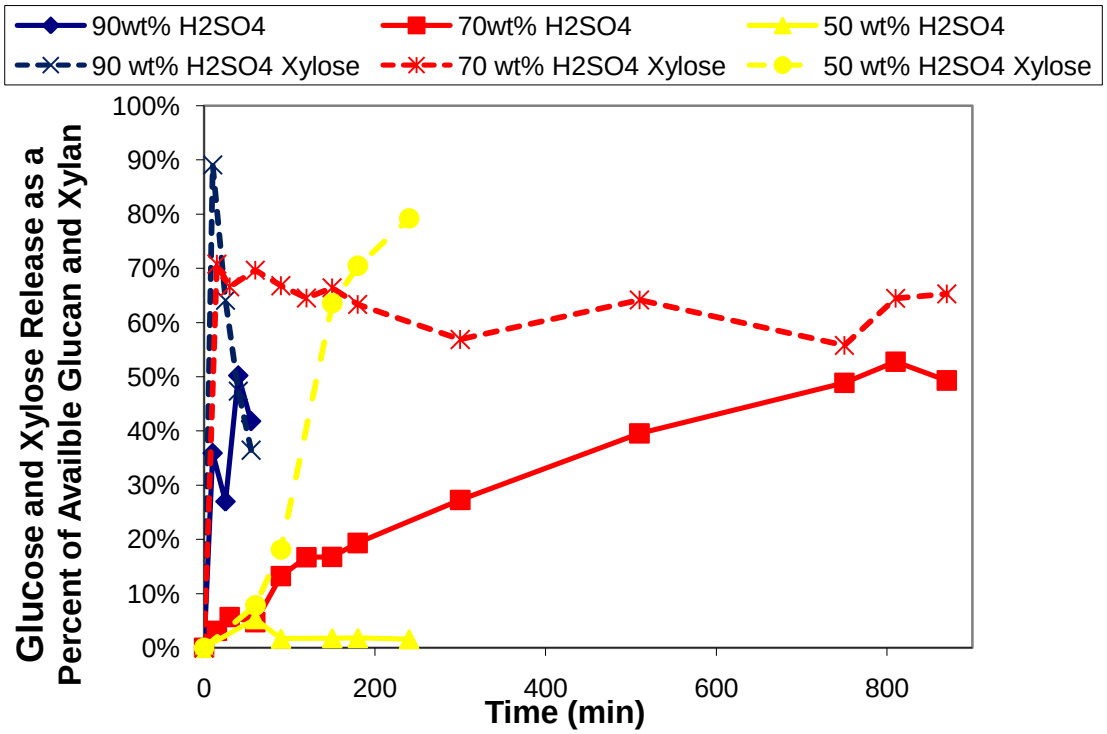


FIGURE 10- Glucose and Xylose Release versus Time for Varying Acid Concentrations at 30°C

For the next set of experiments it is desired to find an optimal reaction condition and time to separate the product sugars from the acid. From the previous figures it is apparent that the xylose and glucose peaks at different reaction times. The 90 weight percent acid cases prove too severe because in the 50°C and 80°C cases the glucose and xylose are degraded at a high rate, limiting overall conversion. The 30°C test has a glucose conversion of 50.2%, but the xylose in solution is rapidly destroyed before the glucose peak is reached as seen in Figure 10. The 70 weight percent acid cases provide conditions severe enough to hydrolyze cellulose, but not too harsh as to cause sizeable degradation of the xylose product. In all the cases the hemicellulose fraction is close to fully broken down in the first fifteen minutes, but only in the 80°C case shown in Figure 8 is xylose degraded at an appreciable rate. 28% of the peak xylose degrades to furfural by the time glucose reaches the peak conversion. The other two cases in Figures 9 and 10 do not show much decomposition between the two peaks, but the values of xylose concentration are seen to fluctuate, which is ascribed to the measurement method. Due to the inaccuracies in the method, the xylose measurement is meant more for a general trend than exact concentration measurements. The 50 weight percent cases all show high hemicellulose conversion, but these conditions do not hydrolyze the crystalline portion of the biomass cellulose.

Next, the energy used to keep the top two glucose yielding cases at reaction temperature is analyzed for another metric to compare. An idealized batch reaction system was modeled as three parts: conduction through a cylinder with free convection at the cylinder wall, conduction through the bottom wall with free convection at the wall, and free convection from the slurry surface. The energy required to heat the slurry from

20°C to reaction temperature is as follows: 242 kJ/kg for the 80°C condition and 121 kJ/kg for the 50°C condition. The heat required per unit surface area to keep the flask at reaction temperature in a 20°C environment was calculated. The 70 weight percent acid case at 50°C displayed heat losses of 2279 kJ/m^2 over the duration of the reaction. For the 45 minutes require to reach peak conversion, the 70 weight percent acid 80°C requires 2649 kJ/m^2 to keep the slurry at the reaction temperature. In comparision, to keep the enzymatic hydrolysis reaction at temperature for one week in this system requires a calculated heat input of 255,309 kJ/m^2. These results apply only to the non-insulated Erlenmeyer flask system and ignore the exothermic nature of the reaction.

For an optimal condition and time for the next set of experiments, the 70 weight percent acid, 80°C case is chosen for the quick reaction time and general preservation of the xylose. The 70 weight percent, 50°C condition could also be chosen although it takes slightly longer to reach peak conversion, but it conserves the xylose in solution better and requires less energy to keep the Erlenmeyer flask at the reaction temperature.

B. Increasing Glucose and Xylose Yield through Separation

1. Re-Introduction of Solids after Sugar-Acid Removal

The discussion above shows that picking a single condition or time for hydrolysis has limitations. Though this research focuses only on a single stage, it is desired to present ideas for further research into a continuous hydrolysis system using ion exchange separation. The difference in optimal times for hemicellulose and cellulose hydrolysis lends itself to two stages. Two stages could be realized through having a hemicellulose reactor and a cellulose reactor, which operate at the same acid concentration but different temperatures. For example, 70 weight percent acid at 30°C hydrolyzes hemicellulose in

under 15 minutes, but this condition takes 810 minutes to reach peak cellulose conversion. So the hemicellulose fraction is removed in the first reactor while the sugar-acid effulent is continuously separated with ion exchange. This effluent would contain a mixture of xylose, oligosaccharides derived from hemicellulose, and a small amount of glucose. The oligosaccharides are separated from the product xylose and glucose through size exclusion, and they are recycled back to the reactor. The solids are then moved to the cellulose reactor where the temperature is raised to 80°C to hydrolyze the cellulose fraction, and the effluent sent to a second ion exchange to remove the glucose and recycle the acid. The effluent from the cellulose reactor would be composed of glucose and oligosaccharides from cellulose. The oligosaccharides would be separated from the glucose in the same manner and recycled back to the cellulose reactor for conversion to glucose. This system would require monitoring for furanic degradation products due to the recycling of oligosaccharides which leads to possible long contact times between the glucose and acid.

In the single stage acid hydrolysis studied in this work, it is hypothesized that conversion can be increased through separating the sugar-acid solution from the solids and re-introducing the solids to fresh acid. In a continuous process, the fresh acid will come from recycle off of the ion exchange column. The effluent acid will need to be re-concentrated due to dilution from the water employed in the ion exchange process before being reintroduced to the hydrolysis reactor. It is expected that this process will increase sugar yield by decreasing contact time between the product sugars and the acid in order to prevent degradation. In this work, the glucose and xylose will not be separated from the acid. For the purpose of proof of concept here, the solids will be re-introduced to

fresh acid. The focus of this experiment is to find the optimum point for sugar-acid separation in the ion exclusion step that follows. The first test is performed at 70 weight percent acid, 80°C. The solids are removed at 45 minutes and then re-introduced into 25 mL of fresh 70 weight percent acid.

When the solids are separated 45 minutes into the reaction, the glucose release as percent of available glucan is 56.8% (25.18 g/L) and the xylose release as percent of available xylan is 52.8%(11.01 g/L). The sugars released from the re-introduced solids are low with 4.0% glucose released (1.77 g/L) and 4.1% xylose released (0.841 g/L). The glucose measurement could be inaccurate because the sugar content was below the recommended operating range of 2 g/L of the YSI 2700. These results displayed in Table IV suggest that almost all of the cellulose and hemicellulose have been reacted before the solids were removed. The second hydrolysis produces limited release of glucose and xylose after 40 minutes leading to the conclusion that minimal hydrolysable cellulose and hemicellulose remain after the first reaction. Most the hemicellulose appeared to be reacted after 15 minutes since a xylose release of 90.9% (19.0 g/L) was measured. The xylose yield loss occurred from degradation between 15 and 45 minutes. The missing glucose cannot be fully explained by degradation to 5-HMF alone. The parasitic pathway described by Bouchard (1989) that converts cellulose to non-reactive polymeric products plays a large role in the reduced yield.

TABLE IV

RESULTS FROM RE-INTRODUCTION OF SOLIDS AFTER 45 MINUTES

Time	Glucose (g/L)	Peak % Glucose Released	Xylose (g/L)	Peak % Xylose Released
0	0.0	0.0%	0.0	0.0%
15	14.8	33.4%	19.0	91.0%
45	25.2	56.8%	11.0	52.8%
Sugar-acid Stream Separated and Solids Reintroduced				
10	1.8	4.0%	0.5	2.4%
20	1.0	2.2%	0.2	0.8%
30	1.5	3.4%	0.8	4.0%
40	1.3	3.0%	0.5	2.5%

The same test is run with the solids now removed at 30 minutes to possibly increase glucose release through decreasing glucose and xylose contact time with the acid. When the solids are separated, the glucose release measures 63.9% (28.3 g/L) and the xylose release measures 83.7% (17.4 g/L). This test, as well, has low levels of sugars released from the re-introduced solids. The glucose peaks at 0.80 g/L and the xylose peaks at 0.55 g/L. The results are reported below in Table V. These two tests indicate that almost all hydrolysable cellulose and hemicellulose are broken down to oligosaccharides or monosaccharides within 30 minutes at 70 weight percent acid and 80°C. In this reaction system, there exists an upper limit on glucan conversion of approximately 60%. This yield can be increased slightly through the reduction of furanic degradation products. The majority of lost yield arises from the production of non-reactive polymeric products. . This unwanted reaction can be controlled through the use of reactors which aid the transport of oligomers and monomers away from the biomass surface and into the bulk solution (Torget et al.,2001).

TABLE V

RESULTS FROM RE-INTRODUCTION OF SOLIDS AFTER 30 MINUTES

Time (min)	Glucose (g/L)	Cellulose Conversion	Xylose (g/L)	Xylose Conversion
0	0.00	0.00%	0	0.00%
30	28.31	63.93%	17.47	83.73%
Sugar-acid Stream Separated and Solids Reintroduced				
10	0.80	1.80%	0.49	2.34%
20	0.52	1.18%	0.55	2.62%

The next test examines the extent that the hydrolysable cellulose and hemicellulose are broken down to monomeric and oligomeric sugars in 15 minutes. This test is run at the same conditions, but the solids are removed from the sugar-acid mixture after 15 minutes. The sugar-acid solution is then allowed to continue to react at the previous conditions. The percent glucose and xylose released as a percentage of available glucan and xylan are plotted in Figure 11. The peak conversion of the mixture is 53.1% after 40 minutes for cellulose and 87.6% after 15 minutes for hemicellulose. When the biomass is removed after 15 minutes, the glucose release is only 29.7% of available glucan. The rise in conversion without solids means that oligomers are present in solution in fairly large quantities. The conversion in this test is 90% of the average value observed for tests at 70 weight percent acid, 80°C. This means that after 15 minutes at this condition, most of sugars are already released from the biomass in the form of soluble oligomers and monomers. Little extra conversion would be gained from separating and re-introducing solids in this case.

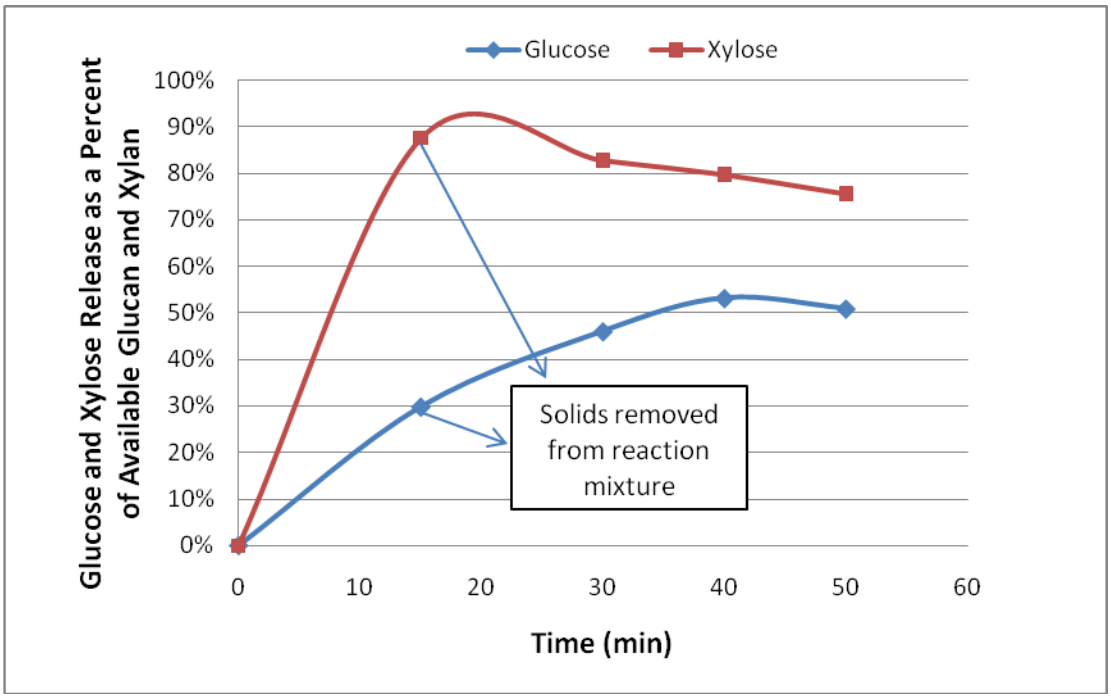


FIGURE 11- Glucose and Xylose Release as a Percentage of Available Glucan or Xylan with Removal of Solids after 15 Minutes

This result suggests a different approach in order to limit degradation from prolonged acid contact with glucose and xylose. In a continuous process the product sugars would still be constantly fed to a separation unit, but the presence of large quantities of oligosaccharides requires a second separation. The acid-free stream from the ion exclusion separation will contain a mixture of oligomers, glucose, and xylose. The oligomers need to be removed from this stream and re-contacted with acid to produce glucose and xylose. This separation could be accomplished using a molecular sieve.

C. Ion Exchange

Separation of glucose and xylose from sulfuric acid using cation ion exchange resins has been shown to work in several studies. Neuman et. al (1987) recovered 94% of glucose from 7.7 weight percent sulfuric acid. Wooley et. al (1998) and Hester and

Springfield (1999) independently developed simulated moving bed processes for the continuous removal of product sugars from sulfuric acid. Hester and Springfield showed that the separation will work for up to 15 weight percent acid. The concentration of sulfuric acid to be separated is much greater than previous experiments. This could pose problems due to the changes in resin properties and principles of the Donnan Exclusion Principle. The ability of ion exchange resin to effectively separate 70 weight percent sulfuric acid and the product sugars of hydrolysis needs to be explored.

The concentrated sulfuric exerts two major changes on the resin which affect the separation properties. First, the ion exchange resin pores will shrink in size. This happens due to greater concentration of ions in solution versus ions in the resin. This concentration gradient draws the water which hydrates resin into the solution. The migration of water out of the resin leads to pore shrinkage caused by the pressure exerted from the DVB crosslinks. This reduction in pore size could be such that the glucose and xylose molecules cannot fit into the pores (Personal Communication Dow Chemical).

Secondly, the Donnan ion exclusion effect decreases as the concentration of the electrolyte (sulfuric acid in this case) increases. This relation arises from the decreased difference in ions between the resin phase and the solution, which leads to a smaller electrochemical difference between the two phases. To test the effectiveness of a sugar-acid separation under the concentrated acid condition, the separation of a model sugar-acid sample in a chromatography column filled with Dowex Monosphere resin under different sample sizes and elutant flow rates will be examined. This is just a preliminary study into the ability of ion exchange resin to separate the glucose and xylose from highly

concentrated sulfuric acid to enhance the sugar yield from the concentrated acid experiments.

Previous studies on sugar-acid separation in literature were consulted to establish beginning operating conditions (Neuman et. al,1987 and Xie et. al, 2005a). For the base operating conditions the column is equilibrated at 55°C. 25 mL of the sample hydrolysate is loaded onto the resin bed, and the elution water flows at 9.5 ml/min. The sample hydrolysate consists of 25 g/L of glucose and xylose in 70 weight percent sulfuric acid. This is used rather than the actual reaction mixture to ensure repeatability. The concentrations of glucose and xylose cannot be controlled in the reaction. In addition, some measurement error is added from the tests required to determine the sugar concentrations. As the sample was added to the bed, sizeable contraction of the resin occurred. The now denser resin on the top of the bed began to sink down the column. This action destabilized the bed as the less dense resin was thrown above the original bed height. The bed was allowed to settle before the sample was loaded into the resin bed. The turnover of the resin bed disrupted the orderly packing present before sample addition. This mixing of the resin and sample is unwanted because it leads spreading of the band throughout the bed. It is desired for the sample to move as a uniform slug through the resin with the glucose and xylose retained in the resin. Further, some resin beads were observed to crack under the stress of the extreme shrinking and swelling cycle. The cracked beads float in water due to the reduced density from shorter polymer chain length. These beads will no longer be active in the separation.

The elutant from the column was collected in 9.5 mL volume increments. The collected fractions were analyzed for sulfuric acid, xylose, and glucose. The resulting

data displayed little separation between the product sugars and sulfuric acid. Only 6.5% of the glucose and 9.6% of the xylose appeared in acid free fractions. Furthermore, when a mass balance was performed on the collected fractions, only 67% of the sugars in the sample were recovered from the column. The reasons for this are not clear, but could be attributed to degradation of the sugars while in contact with the acid, and inconsistent metering of volume by the fraction collector. Though quantitatively this experiment was not very useful, the results provided information about the problems with the separation and the direction to move the column operating conditions to obtain a more efficient separation.

The concentrations of glucose, xylose, and sulfuric acid in the eluting water is shown Figure 12.

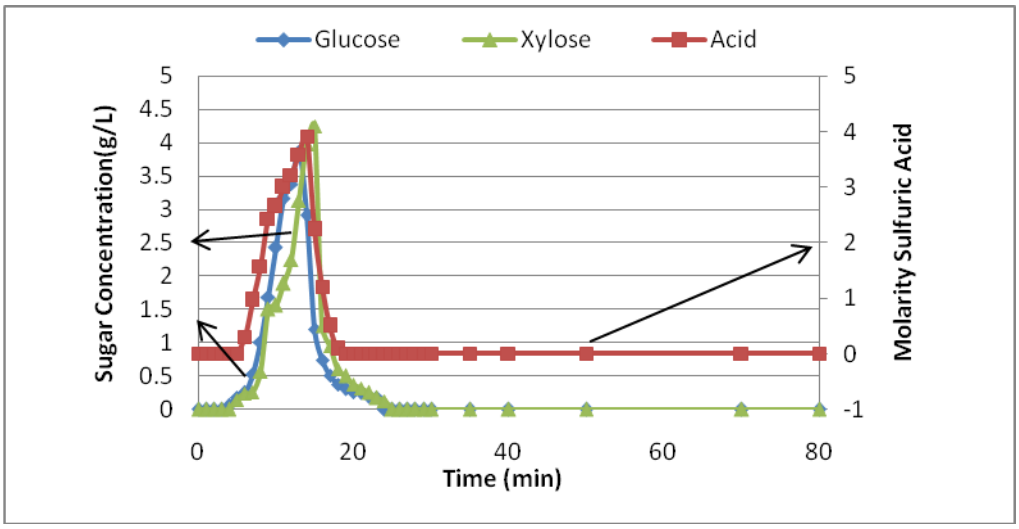


FIGURE 12- Concentration of Glucose, Xylose, and Sulfuric Acid in Elutant, 9.5 mL/min

All three components from the sample appear in the effulent from the resin bed after 6 minutes. All the sulfuric acid in the sample has been collected off the column after 17 minutes, while the glucose and xylose appear in the effulent until 24 minutes after elution has started. These results showed that the ion exchange resin did not retard the

movement of glucose and xylose through the column at the conditions chosen. The sugars had the same affinity for the resin as sulfuric acid which caused the lack of separation between sugars and acid. At the conditions chosen for the first experiment, the Donnon exclusion effect had no appreciable effect on the exclusion of sulfuric acid from the ion exchange resin. The separation seen between the sugars and the acid arose from tailing of the sugar elution profile due to backmixing of the sample with the resin. This backmixing occurred during the contraction of the resin bed while loading the sample. The lack of exculsion of sulfuric acid from the resin phase was utilized to change the operating conditions to possibly provide better separation.

The Donnan exclusion effect was hindered by several variables. The foremost problem was the high concentration of sulfuric acid, since ion exclusion functions best at low concentrations of ions. While the local concentration of the acid cannot be decreased, the overall ion concentration in relation to the whole resin bed could be decreased through loading a smaller sample. The smaller sample size further allows the sugars more pore sites to bind to, slowing the movement of the sugars through the column. Lastly, a lesser flow rate of the eluting water allows for more time for the resin to come into equilibrium with the solution in case the first experiment was diffusion limited. It is possible that the sample moved too fast through the column to allow significant adsorption and separation of sugars. With this in mind a second separation test was run with 10 mL of sample eluted with water at a flowrate of 5 mL/min. The fractions were collected in graduated tubes to verify the volume collected. The column was again kept at 55°C.

The changes made to the operating conditions provided favorable improvements to the separation, but the separation still did not achieve more than 20 percent recovery of the glucose and xylose in the sample from sulfuric acid. The glucose removed from the acid was 13.7% of the sample, and 17% of the xylose was removed from the acid. The mass balance for glucose was 98.8%, and the balance for xylose was 84.7%. The low closure for xylose is attributed to the assay used for quantification. Sulfuric acid appeared in the elutant at 5.8 minutes, and both glucose and xylose were detected not long after at 7.2 minutes. Under the new conditions the sugar elution curves are only slightly shifted as seen in Figure 13. The sharp drop in concentration on the right side of all the curves reflects a density gradient between the sulfuric acid and the water used for elution (Helferrich, 1962). The beginning of the curves are diffuse since the sulfuric acid sits above less dense water. A diffuse boundary was established due to buoyancy effects. The end of peaks become sharp as the boundary is passed, and water now sits on top of sulfuric acid. Since the glucose and xylose curves do not have much separation from the sulfuric acid, they experience the effects of the density gradient.

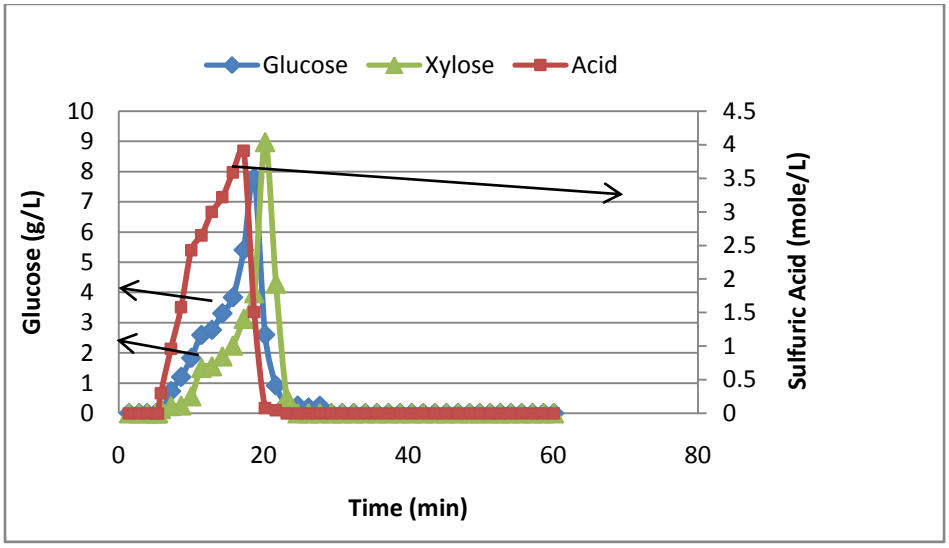


FIGURE 13- Concentration of Glucose, Xylose, and Sulfuric Acid in Elutant, 5 mL/min.

The decreased column loading and elutant flow rate improved the separation slightly, but the high concentration of sulfuric acid lowers the ion exclusion effect, markedly decreasing the efficiency of the separation. In addition, the pore size shrinkage from the concentrated acid eliminates sites for binding of glucose and xylose. The column loading and flow rate of elutant could be decreased further, with some improvement in separation expected. This is not optimal though because any continuous separation using simulated moving bed technology would require a large amount of resin to process a given process flow increasing start-up and operation costs.

Several approaches to increase separation efficiency can be taken. First, a different resin should be chosen for this concentrated acid-sugar separation. Resins with a greater percentage of DVB crosslinking provide a higher Donnan potential and a greater resistance to shrinking. The increase in Donnan potential occurs from the higher concentration of ionic groups in the resin. In such a concentrated system this benefit may be negligible. A resin with more crosslinking has a less elastic structure due to the rigidity imparted by the increased bonds between polymer chains. This will resist contraction when placed in solution with concentrated acid. There will exist an optimum crosslinking percentage because the pore size becomes smaller as the crosslinking increases. Too small a pore size would limit the diffusion of sugars into the resin where the sugars temporarily complex with the resin.

The concentration of the acid could just be too large for ion exchange to efficiently separate the sugar and acid. If this is the case, anionic PVP resin could be used. (Xie et al., 2005a and b) have shown that PVP resins are more cost effective than cation based resins at separating sugar-acid solutions. PVP has a higher selectivity,

requires less equipment, and removes more fermentation inhibiting products than Dowex 99. The PVP resin captures the sulfuric acid through ion retardation while the sugars pass through the resin unhindered. The sulfuric acid is then recovered through regeneration of the resin bed with sodium hydroxide. This resin could be used in a two stage separation system that would not only separate glucose and xylose from sulfuric acid, but also yield a fraction of oligosaccharides to be further hydrolyzed to glucose and xylose. The first resin bed would capture the sulfuric acid while the mixed sugar stream is fed to a second separation column. The second column would consist of a molecular sieve which would separate the sugars into a stream for re-hydrolysis, oligomers, and a product stream to fermentation.

V. CONCLUSIONS

- Continuous concentrated acid hydrolysis of biomass will require continuous separation of sugars from the reaction slurry to reduce byproduct formation. The sugar-acid effluent would be separated, and the acid returned to the process.
- The reactions conditions which produced over 50% conversion of available glucan to glucose had severities of 3.1, 3.3, 3.5 and 3.7.
- The highest release of glucose, 25.7 g/L, occurred at 70 weight percent acid and 80˚C after 45 minutes. This condition corresponded to a severity parameter of 3.7
- Xylose release was fairly consistent across all reaction conditions. The cases with severity lower than 3.0 present the best conditions for hemicellulose hydrolysis because the more severe cases quickly degrade the xylose.
- The optimum condition for single stage concentrated acid hydrolysis is at 70 weight percent acid and 80˚C because it balances glucose release with preservation of the xylose released.
- Most of the hydrolysable sugars were released as oligosaccharides which further breakdown to glucose and xylose. At 70 weight percent acid and 80˚C, yhey were mostly released in 15 minutes
- The use of Dowex Monosphere 99 resin to separate glucose and xylose from 70 weight percent acid resulted in less than 20 percent recovery of the glucose and xylose from the acid. The observed resin shrinkage and fracturing of the resin beads from the concentrated acid lead to the ineffectiveness of the column at the conditions tested.

VI. RECOMMENDATIONS

This research focused on the preliminary steps for designing a continuous concentrated acid hydrolysis process. The hydrolysis reactions which were run in flasks need to be scaled up to a small reactor system. This system should be able to handle varying acid loading and temperatures. It would ideally consist of two reactors at a low and a high severity to optimize the production of both xylose and glucose. The reactor setup should take advantage of the shrinking bed phenomena to increase sugar yields. The hydrolysis tests need to be expanded beyond solids concentrations of 10%. If continuing to measure xylose, a better quantification methods needs to be employed. The current assay test presents high variability, and it worked best for just observing trends in xylose production.

Much work still needs to be done on the sugar-acid separation. First, more column tests at lower flow rates and sample loadings could increase separation efficiency. In this study only one resin was investigated for the separation of glucose and xylose. Resins possessing a DVB content higher than 6% could provide better results. The DVB content needs to be kept low enough so that glucose and xylose can still diffuse into the resin pores. A second resin option to explore is PVP which captures the sulfuric acid and lets the sugars pass through. It would allow for size separation of oligosaccharides from glucose and xylose. This separation will be needed in a continuous process.

So far only batch chromatography has been explored; on a commercial scale simulated moving bed technology will be used. The first step in developing a SMB process for this separation would involve using the batch chromatography experiments

results to simulate a moving bed process. Purdue University has developed a simulator called VERSE that has been used for this purpose.

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VITA

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