FRACTIONATION AND CHARACTERIZATION OF SOLUBILIZED BIOPOLYMERS FROM ALKALINE PULPING LIQUORS

By

Ryan Joseph Stoklosa

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Chemical Engineering – Doctor of Philosophy

2014

ABSTRACT

FRACTIONATION AND CHARACTERIZATION OF SOLUBILIZED BIOPOLYMERS FROM ALKALINE PULPING LIQUORS

By

Ryan Joseph Stoklosa

The conversion of lignocellulosic biomass to renewable liquid fuels or chemicals offers one approach to decrease dependence on fossil fuels. Attempts at commercializing the biochemical production of biofuels from lignocellulose has been hindered by the costs associated with pretreatment, enzyme production, and feedstock transportation. The infrastructure established by the forest products industry offers a way to decrease initial capital costs, and to generate new products and revenue from an industry showing declined profits. This work addresses two general themes of applying alkaline pulping chemistries to: (1) extract, separate, and recover chemically altered non-cellulosic biopolymers from woody biomass and (2) evaluate the impact that the removal of non-cellulosic biopolymers have on the yields of monomeric sugars by enzymatic hydrolysis. These general themes were addressed through three studies.

First, bench scale alkali extractions performed on a variety of hardwoods indicated that hemicellulose and lignin extractability was dependent on biomass species, which in turn were related to initial composition and properties of the cell walls. Ethanol precipitation could recover the hemicellulose fraction and following precipitate bleaching the recovered material yielded 65-80% hemicellulose, depending on biomass source. Molecular weight characterization of the recovered hemicellulose by size-exclusion chromatography (SEC) indicated the presence of lignin and carbohydrates eluting at the 3-12 kDa range, and the application of novel reducing end quantification estimated the number average degree of polymerization (DP_N) would reach an asymptotic limit of 25 for switchgrass and 120 for silver birch. The high extraction yields of

hemicellulose identified conditions that could be utilized to remove hemicellulose prior to chemical pulping or during alkaline pretreatments.

Lignin characterization was performed on fractions generated from a softwood kraft black liquor to determine the properties of solubilized lignin that resulted in their phase-partitioning behavior during a novel acidification process using CO_2. A bulk of the lignin precipitated between a pH of 11.6 to 10.0, with fractions obtained at higher pH contaminated by larger quantities of aliphatic extractives and polysaccharides. Lignin fractions were characterized for functional groups by ^{1}H and ^{13}C NMR, molecular weight by gel-permeation chromatography (GPC), and lignin monomer generation by analytical pyrolysis-GC/MS. Select structural and chemical properties of the lignin fractions were correlated based on their linear dependencies to relate phase-partitioning behavior with respect to pH.

Lastly, alkaline pulping trials were conducted in a pressurized reactor vessel to determine hemicellulose and lignin dissolution during a soda pulping process and to understand how pulping conditions may impact hydrolysis yields. Hemicellulose dissolution increased up to 170°C followed by a decrease in the polysaccharide content of the black liquor, an effect of polysaccharide degradation and re-adsorption to the residual wood fibers. Lignin dissolution increased throughout the pulping trials, and at the most severe condition (1 hour at 170°C) only 10% of the lignin remained in the woody biomass. The alkaline pulping trials also produced a digestible substrate that generated over an 80% yield of monomeric glucose.

ACKNOWLEDGEMENTS

I would like to offer a word of thanks to my advisor Dr. David Hodge for all of his guidance and support during my years completing my doctoral research at Michigan State University. Also, a word of thanks goes to my Ph.D. committee for their advice and suggestions towards the development of my research. To all my colleagues in Dr. Hodge's lab, I want to offer my gratitude for the work we have collaborated on over the years, and all the thoughtful discussions that occurred when analyzing experimental results. Lastly, special thanks to former lab manager Kristine Van Winkle for her advice and expertise by diagnosing problems and troubleshooting the analytical equipment located in the lab.

Additionally, I would also like to offer a word of thanks to the people who graciously donated feedstocks for this research: Curt Lindström at Smurfit Kappa AB (Piteå, Sweden), Todd Smith at the Devereaux Sawmill (Pewamo, MI) and Dr. Raymond Miller (MSU) for providing wood chips and Dr. Pascal Kamden (MSU) for use of his Wiley Mill.

Special thanks would also like to be given to Dr. Mark Thies and his graduate student Julian Velez from Clemson University for the opportunity to collaborate on an industrial related research project.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

KEY TO ABBREVIATIONS

4-O-MeGA: 4-O-methyl glucuronic acid

AQ: anthraquinone

Ara*f*: arabinofuranosyl

BCA: bicinchoninic acid

C_1, C_2, or C_3: number of additional carbons on aromatic compounds

CTMP: chemi-thermomechanical pulping

$\overline{DP_N}$: number average degree of polymerization

G: guaiacol monomer

GAGX: glucuronoarabino(galacto)xylan

Gal*p*: galactopyranosyl

Gal*p*A: galactouronopyranosyl acid

GAX: glucuronoarabinoxylan

GGM: galactoglucomannan

Glc*p*: glucopyranosyl

Glc*p*A: glucuronopyranosyl acid

GM: glucomannan

GPC: gel permeation chromatography

GX: glucuronoxylan

H: hydrogen group

HMF: hydroxymethylfurfural

HPLC: high performance liquid chromatography

IFBR: Integrated Forest Bio-Refinery

LCC: lignin-carbohydrate complex

LHW: liquid hot water

Man*p*: mannopyranosyl

MeO: methoxyl group

MW: molecular weight

PDADMAC: polydiallyldimethylammonium chloride

Ph: phenol monomer

PSAQ: polysulfide-anthraquinone

Py-GC/MS: Pyrolysis gas chromatography-mass spectrometry

Rha*p*: rhamnopyranosyl

RI: refractive index

S: dimethoxybenzene and dimethoxyphenol monomers

SEC: size exclusion chromatography

SSF: simultaneous saccharification and fermentation

SPORL: sulfite pretreatment to overcome the recalcitrance of lignocellulose

TMP: thermomechanical pulping

UV: ultraviolet

XOS: xyloolgosaccharides

Xyl*p*: xylopyranosyl

CHAPTER 1. NON-CELLULOSIC CELL WALL BIOPOLYMERS AS A SOURCE FOR CONVERSION TO FUELS AND CHEMICALS

1.1 Introduction

There exists an overall effort to reduce petroleum consumption predicated upon the rising costs of the petroleum supply and increased consciousness of the societal and environmental impacts that accompany petroleum utilization. The supply of petroleum on a global basis has started to level off around 9.0×10^4 thousand barrels per day making the cost of a barrel of oil increase to the point where alternative liquid transportation fuels derived from biomass feedstocks can be competitive (B. Dale, 2008; *International Energy Statistics - Petroleum Production*, 2013). Furthermore, the increase in not only liquid transportation fuel consumption, but also general energy consumption (*i.e.* electricity, natural gas, coal, etc.) will only rise in the coming decades as developing nations improve modes of transportation and quality of life for their citizens (B. Dale, 2008; B. E. Dale & Ong, 2012). Converting biomass feedstocks to renewable liquid fuels or value-added chemicals is one solution to offset petroleum consumption, and to temper the release of greenhouse gases (GHG) that originate from outside the biosphere. In the United States first-generation ethanol production from corn has been successfully commercialized to decrease overall petroleum consumption by passenger vehicles. To successfully displace further petroleum consumption the development of second-generation biofuels and commodity chemicals from cellulosic sources (*i.e.* lignocellulosic biomass) is currently under development.

Much of the United States already has dedicated land and infrastructure to utilize lignocelluloic biomass as a feedstock for biological or catalytic conversion to renewable fuels and chemcials. A recent combined USDA/DOE study assessed the biomass inventory available

from agricultural and forestry required to displace 30% of US petroleum consumption (Perlack & Stokes, 2011) and estimated that 27% of the biomass inventory would come from woody biomass. This greater utilization of woody biomass will push the planting of trees from dedicated forestry areas to more traditional agricultural plots of land. The USDA Forestry Service has indicated that hybrid poplars (*Populus* spp.) planted on agricultural land with a short rotation schedule can produce yields ranging from 37 to 55 dry tons per acre with a 6-year rotation schedule and 900 trees per acre; however, more general planting stocks can yield 5 to 12 dry tons per acre per year under current management systems (White, 2010). Additionally, woody based resources already contribute to a sizable portion of energy usage as reported in 2008 that 2.1 x 10^{15} BTUs are consumed from woody based resources; this includes black liquor from pulping processes (White, 2010). Woody biomass utilization is even more attractive within the United States based on the Renewable Fuel Standard (RFS) stipulated in the Energy Independence and Security Act of 2007 that mandates at least 16 billion gallons of cellulosic ethanol must be produced by 2022. However, the updated RFS places substantial restrictions on the use of woody biomass to reach these mandates including the requirement that only biomass that was grown on land that was actively managed as wood plantations in 2007 is eligible for classification as use as an "advanced biofuel" (EPA, 2013).

Regardless, the research into utilizing woody biomass as a source for renewable fuels and chemicals should continue. Illustrating this potential in 2012 it was reported that the United States produced 5.66 x 10^{7} metric tons (air dried) of wood pulp (*Pulp and Paper Capacities Survery 2011-2016*, 2012). This high capacity of pulp production shows a functioning industry already developed in the United States with dedicated logistical transportation operations that can be adapted to develop forest biorefineries. To this end the primary barriers holding forest

biorefinery development back include species potential, economically competitive and sustainable feedstock supply, and appropriate production technologies (Mayfield, Foster, Smith, Gan, & Fox, 2007). Moreover, the pulp and paper industry is the largest consumer of woody biomass and has the existing infrastructure for both its collection and transportation, and the capital-intensive chemical pulp mills for thermochemical fractionation and conversion. Based upon this it is reasonable to expect that future incremental technology developments for the chemical and thermochemical conversion of woody biomass can be developed within the framework of existing chemical pulping technologies and facilities. Moreover, the breakthroughs in process development associated with pulp mills to generate greater utilization of hemicellulose and lignin fractions from woody biomass will be helpful to further offset petroleum consumption on a larger scale.

One of the necessary steps for the production of biologically generated fuels or chemicals is a restructuring of the pulp and paper industry. Currently, North American pulp mills are in a period of decreased profitability and consolidation due to a number of economic factors such as increased competition from newer pulp mills with higher production capacities located outside of the United States. For the purposes of increasing profitability while also contributing to the development of renewable fuels and chemicals, the adjustment of current pulp mills to Integrated Forest Biorefineries (IFBR) is one way to achieve these goals. The IFBR concept allows for greater utilization of hemicellulose and lignin fractions from woody biomass to develop a new product portfolio of specialty derived fuels and chemicals from woody biomass to accompany cellulosic pulp production (Ragauskas et al., 2006; Van Heiningen, 2006). Technological developments for an IFBR can be quite analogous to current petrochemical refineries. The existing global infrastructure for energy and the many synthetic products from petrochemical

feedstocks is a result of the development of technologies in the last century that allowed for economical extraction and refining (*i.e.* fractionation and chemical upgrading) from the raw materials of crude oil and natural gas. Similarly, biorefining can be considered as the fractionation and conversion of plant biomass to a suite of products and energy that include, for example, stationary power, heat, fuels, chemical intermediates, solvents, polymers, and modified plant fibers such as chemical pulps and cellulose derivatives. In order to develop successful, economic processes for the biorefining of lignocellulosic biomass, similar developments are required for the extraction, fractionation, and catalysis of the raw material to fuels and chemicals. Continuing with this comparison, while the petroleum components consist of miscible liquids and gases, with separations based on liquid-vapor equilibrium such as distillation and adsorption, the biomass fractionation systems are different in that the chemical constituents are typically solutes in an aqueous phase. The properties of hydrocarbons are very well characterized and are used for process design and, in particular, separations. Similarly, the dissolved plant cell wall components in an aqueous phase need further characterization and study to determine the relevant processes that will be implemented to separate and recover the fractions for biological or chemical conversion to renewable products.

For this reason thermochemical pulp mills offer an opportunity for the enhanced utilization of hemicellulose polysaccharides and aromatic lignin biopolymers. The two primary types of pulp mills consist of the kraft process and the sulfite process. Worldwide the kraft process dominates the pulp production field with more than 80% (Sjostrom, 1993) of global pulp production performed through this process. This process is preferred for chemical pulping due to the favorable economics of chemical recovery and the robustness to produce strong pulps from hardwoods or softwoods. Through the kraft process hemicellulose and lignin components are

solubilized into the aqueous alkaline liquid phase by the peeling reaction and nucleophilic aromatic substitution of sulfur by S^{2-} from Na_2S at elevated temperatures, respectively. Dissolved hemicellulose can be stabilized from further depolymerization in the alkaline medium when its aldehyde end groups are oxidized to carboxylic acids, but at the elevated temperature settings the dissolved polysaccharides are degraded to aldonic acids (Johansson & Samuelson, 1977; Sjostrom, 1993). During the chemical recovery process, which is illustrated in Figure 1.1, the dissolved plant cell wall fractions of hemicellulose and lignin are concentrated through successive evaporation followed by combustion to produce heat and power within the pulp mill itself.

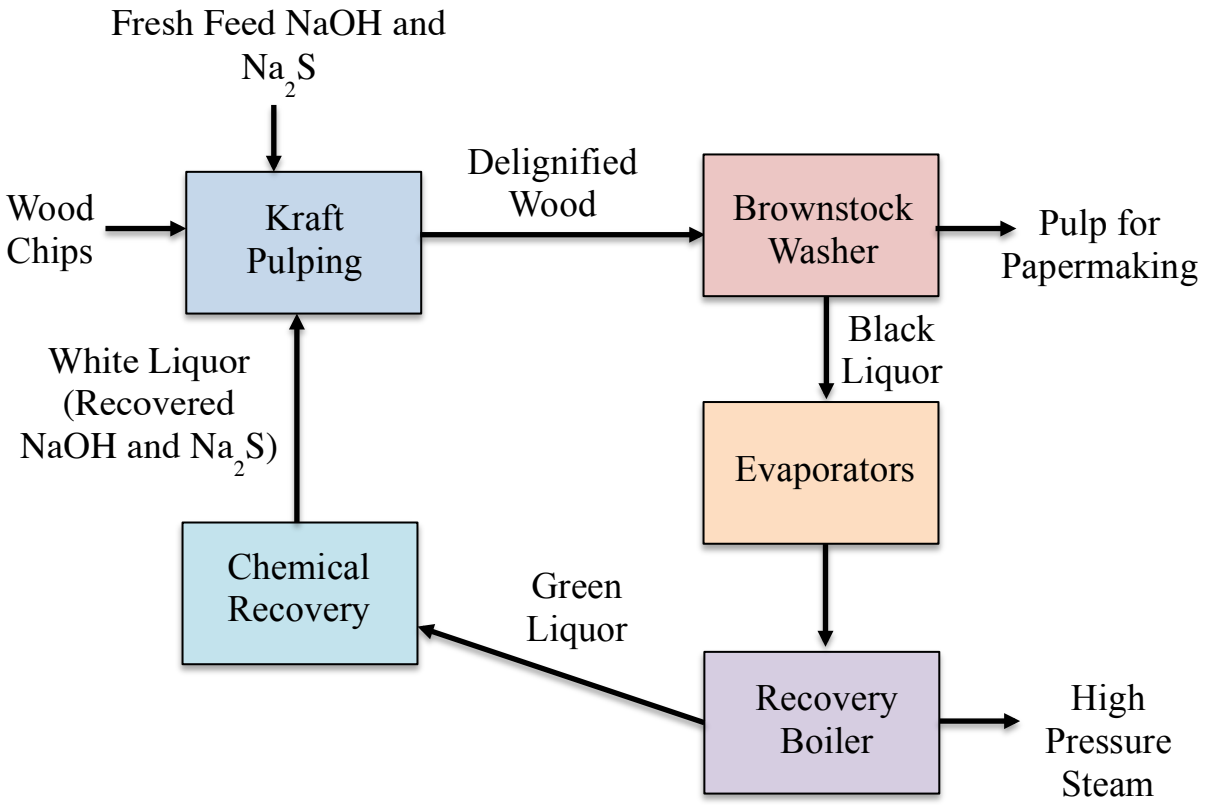

Figure 1.1. An example of the kraft pulping process with chemical recovery.

Lignin recovery through the form of black liquor precipitation is produced at a limited scale for the production of binders, resins, or activated carbons (Vishtal & Kraslawski, 2011), while hemicellulose utilization, as a value-added product, is noticeably absent. On a more limited scale the sulfite process produces cellulosic pulp by the addition of the chemical components sulfite or bisulfite. This process has more variability since it can occur at acid, neutral, or alkaline conditions. All three conditions can affect the lignin structure to some degree through sulfonation, cleavage of certain bond linkages (*e.g.* β-O-4), and lignin softening and solubilization (Sjostrom, 1993). Due to the larger amounts of sulfur present in the process and the option for different balancing metal cations (*e.g.* Na^{+}, Ca^{2+}, Mg^{2+}), the chemical recovery of sulfite black liquor is more challenging and presents greater environmental concerns. The dissolved plant cell wall fractions, however, have been utilized much more than their counterparts in the kraft process. The recovery of lignosulfonates is practiced globally while older sulfite mills would produce bioethanol through the conversion of hemicellulose polysaccharides by the acidic sulfite process that hydrolyzes hemicellulose to monomeric sugars in the spent black liquor (Gargulak & Lebo, 2000; Lawford & Rousseau, 1993).

Understanding the underlying chemistries behind these pulping processes gives great incentives to recover hemicellulose and lignin fractions for greater utilization. Figure 1.2 shows a hypothetical case of plant cell wall fractions that are dissolved from a hardwood species during a pulping stage and the options for recovery. The presented case in Figure 1.2 only applies to alkaline processes. In these processes the pH remains relatively constant, but could slightly decrease due to consumption of OH^{-} as hemicellulose and lignin are solubilized. Notice that the precipitation of dissolved plant cell wall biopolymers will not be exclusively pure hemicellulose or pure lignin. Precipitation techniques (*e.g.* addition of organic solvent or pH adjustment) will

yield fractions consisting of both hemicellulose and lignin fractions due to their crosslinking associations within native plant cell walls (Himmel et al., 2007).

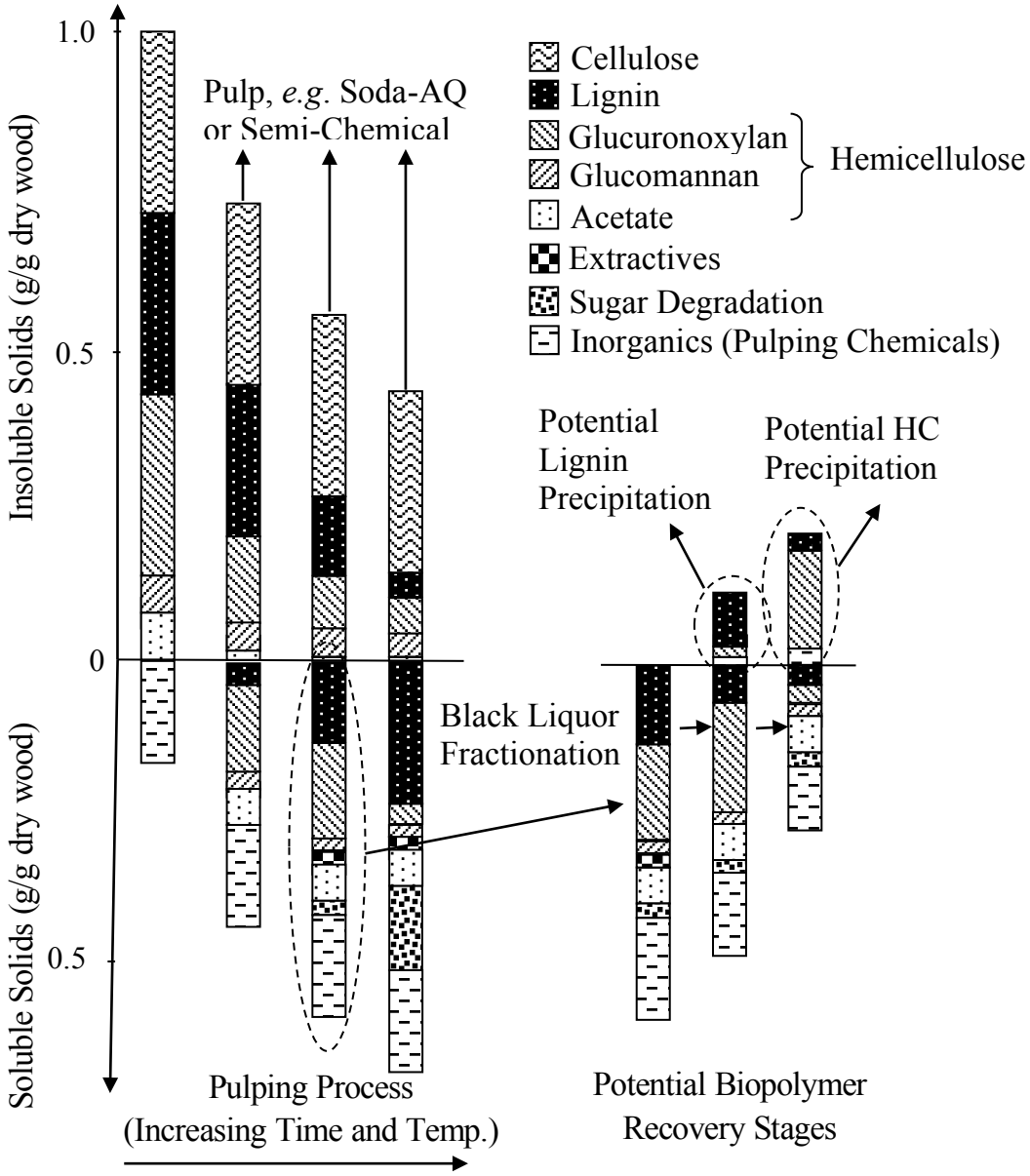

Figure 1.2. Hypothetical example of hardwood components dissolved during alkaline pulping followed by the recovery of hemicellulose and lignin by precipitation.

Although precipitation to recover non-cellulosic biopolymers from existing liquor streams is convenient, it is not the only option for recovery nor does it make a process scheme

economically advantageous. Some of the techniques for the recovery of dissolved plant cell wall components are listed in Table 1.1 and these techniques will be expanded upon.

Table 1.1. Brief description of recovery techniques and mechanisms

Method for Recovery	**Primary Components Recovered/Mechanism**	**References**
Acidification	Lignin – Aggregation by increasing protonation of hydroxyl groups.	(Gidh, Decker, Vinzant, Himmel, & Williford, 2006; Guerra et al., 2007; Ragnar, Lindgren, & Nilvebrant, 2000; Sarkanen, Teller, Hall, & McCarthy, 1981)
Precipitation with Organic Solvents	Hemicellulose – Polysaccharide insolubility in excess organic solvents.	(Al-Dajani & Tschirner, 2008; Bian, Peng, Peng, Xu, & Sun, 2010; Stoklosa & Hodge, 2012)
Ultrafiltration	Hemicellulose and lignin – Utilizing molecular weight cutoff filters to obtain oligomeric biopolymers.	(Ibn Yaich, Edlund, & Albertsson, 2012; Krawczyk, Oinonen, & Jönsson, 2013; Saadatmand, Edlund, Albertsson, Danielsson, & Dahlman, 2012)
Direct Utilization of Liquor Streams	Hemicellulose – Direct conversion of monomeric or oligomeric hemicellulose after autohydrolysis or sulfite treatment.	(Franco et al., 2012; Helmerius, von Walter, Rova, Berglund, & Hodge, 2010; G. S. Wang, Pan, Zhu, Gleisner, & Rockwood, 2009)

Furthermore, processes to purify recovered streams of hemicellulose or lignin will be needed to generate more pure fractions with high yields. Some of this recovery is already practiced, but to offset further petroleum consumption within the United States a greater commitment to their recovery and conversion to liquid fuels or value-added chemicals should be practiced. Cellulosic pulp is still a desired commodity for paper production that can be sold at $1017 per metric ton for bleached softwood kraft pulp (FOEX Indexes, LTD., 18th March 2014). Converting the cellulosic portion of woody biomass to biobased fuels or chemicals can be accomplished. However, in a pulp mill setting it is more likely to focus on the non-cellulosic fractions to

increase product output and revenue. This can be accomplished with the introduction of new upstream processes to extract and recover the non-cellulosic biopolymers, or new recovery processes after a traditional chemical pulping stage. The choice and availability of feedstocks (*i.e.* hardwood or softwood) will determine extraction and recovery before or after a pulping stage and is best exemplified based on the hemicellulose composition for different feedstocks.

1.2 Hemicellulose Composition in Woody Biomass Feedstocks

In contrast to the cellulose portion within plant cell walls, the composition of hemicellulose can vary extensively between monocots, hardwoods, and softwoods. Cellulose is a homopolysaccharide of β-1-4-linked glucopyranosyl (Glc*p*) units that form microfibrils within the plant cell wall, partly due to hydroxyl groups emanating from one chain and forming hydrogen bonds with other chains in proximity. Figure 1.3 gives a structural representation of cellulose.

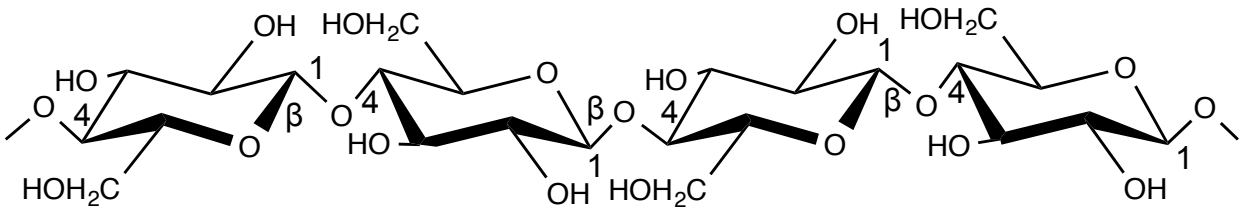

Figure 1.3. Structural representation of a cellulose chain.

Unlike cellulose, hemicelluloses are amorphous heterpolysaccharides that vary in composition and structure with respect to species. The dry mass percentage of hemicellulose in the plant cell wall can vary from 20 to 30%, which represents a significant amount of material that can be isolated and recovered for conversion processes. When considering grasses select species are being investigated as potential bioenergy crops for conversion to liquid transportation fuels

(David & Ragauskas, 2010; Lau, Gunawan, & Dale, 2009; Xu, Cheng, Sharma-Shivappa, & Burns, 2010). The *Poaceae* family, or true grasses, include such varieties of monocots as bamboo, maize, and barley. Essentially, all members of this family contain a form of xylan as the dominant polysaccharide chain (Hampton, Haworth, & Hirst, 1929). Differences amongst hemicellulose distribution in grasses start with side chain substitution on the polysaccharide backbone. These differences are summarized in Table 1.2 by listing the primary polysaccharide backbone and side-chain substitutions for each species.

Table 1.2. Summary of hemicellulose composition in select grass species.

Species Type	**Primary Polysaccharide Backbone**	**Side-Chain Substitutions**	**Reference**
Bamboo (*Arundinaria japonica*)	Heteroxylans	L-arabinofuranose (Ara*f*), O-galactopyranosyl (Gal*p*), and acetyl	(Wilkie & Woo, 1977)
Corn Stover (*Zea mays*)	Glucuronoarabino(galacto)xylan (GAGX)	Ara*f*, acetyl residues, and 4-O-methyl glucuronic acid (4-O-MeGA) residues	(Carpita & Whittern, 1986; Naran, Black, Decker, & Azadi, 2009)
Barley (*Hordeum vulgare* L.)	Glucuronoarabinoxylans (GAX) with linear β-glucans	Ara*f*, and acetyl	(Izydorczyk & MacGregor, 2000)

An important distinguishing feature of the cell walls from this family include the presence of ferulic acid in the primary cell wall (Carpita, 1996). This aromatic acid forms feruloylated oligosaccharides that crosslink to Ara*f* side chains located on the xylan chain (Saulnier, Vigouroux, & Thibault, 1995). This is notable due to the ester linkage of ferulic acid to the hemicellulose polysaccharide that can be broken by mild pretreatment conditions and has been correlated to show improved digestibility of the polysaccharides by enzymes (Li et al., 2012).

These covalently linked structures are important for plant physiological development, but become a hindrance in the deconstruction of biomass for producing biofuels or value-added chemicals. Taken together, grass bioenergy crops have great potential for the development of second generation bioethanol production (Pauly & Keegstra, 2008); however, in this context of non-cellulosic biopolymer recovery in an IFBR setting they will not be discussed because of the characteristic property of non-wood feedstocks containing higher amounts of silicon that make downstream processing in a chemical pulping process difficult (Khiari, Mhenni, Belgacem, & Mauret, 2010; Madakadze, Radiotis, Li, Goel, & Smith, 1999).

Compared to grasses, the composition of hemicelluloses in woody lignocellulosic biomass is considerably different. Hardwoods primarily contain glucuronoxylan (GX) that contains a backbone of β-1,4-linked xylopyranosyl (Xyl*p*) units as shown in Figure 1.4.

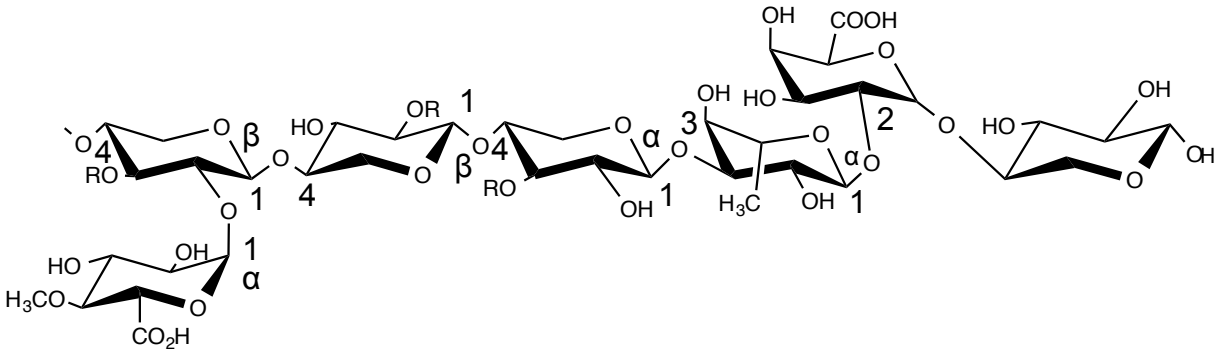

Figure 1.4. Hardwood hemicellulose structure with a 4-O-MeGA side group and an α-L-Rha*p*-1-2-linked-α-D-Gal*p*A at the reducing end of the GX chain.

Substitutions occur frequently along the polysaccharide backbone with either acetyl groups at the C-2 or C-3 position corresponding to 7 acetyl groups per 10 xylose units, or 4-O-MeGA at the C-2 location corresponding to 1 residue for every 10 xylose residues (Sjostrom, 1993; Whistler & Richards, 1970). The presence of 4-O-MeGA units and the lack of Ara*f* side chains distinguishes

hardwood xylans from grass xylans. Previous studies have shown that native GX isolated from various hardwood species have number average degrees of polymerization ($\overline{DP}_N$) ranging in value from 150 to 215 (Goring & Timell, 1960; Koshijima, Timell, & Zinbo, 1965; LeBel, Goring, & Timell, 1963; Timell, 1960). A distinguishing feature for hardwood GX is their ability to become resistant against degradation during a traditional chemical pulping process utilizing alkaline chemistry. At the reducing end of the GX chain a sequence of uniquely linked carbohydrates can stop the alkali peeling reaction. This sequence, shown in Figure 1.3, starts at the reducing end with a xylose unit connected by an α-1,4-linked galacturonic acid (Gal*p*A) residue, which in turn is connected by an α-1,2-linked rhamnopyranosyl (Rha*p*) unit, ending with an α-1,3-linked xylose unit to continue the GX chain (Johansson & Samuelson, 1977; Sjostrom, 1993). The initial reducing end unit of the GX chain is degraded by β-elimination to a saccharinic acid group, which will lead to the exposure of new reducing ends (Whistler & Richards, 1970). The combination of the Rha*p* and Gal*p*A unit can hinder the peeling reaction from progressing further down the GX chain, followed ultimately with a substitution occurring with the 4-O-MeGA to stabilize the chain against further degradation. For this reason hardwoods can be viewed as an attractive feedstock to obtain extended utilization of the hemicellulose portion in pulp mills utilizing soda or kraft pulping processes (Van Heiningen, 2006).

Alternatively, softwood hemicellulose not only has different compositions, but also physical properties when compared to hardwood hemicellulose. The primary hemicellulose polysaccharides in softwoods are glucomannans (GM) or galactoglucomannan (GGM). These polysaccharides contain β-1,4-linked glucopyranosyl (Glc*p*) and mannopyranosyl (Man*p*) units with side chains of α-1,6-linked galactopyranosyl (Gal*p*) units in GGM (Ebringerova, 2006). A partial substitution of acetyl groups at the C-2 and C-3 position still occur for GGM and GM but

at a lower quantity of only one acetyl group per 3-4 hexose units (Sjostrom, 1993). GAX are also present in softwoods at low quantities (5-10% by weight) and are similar to GX from hardwoods through having C-2 substitutions with 4-O-MeGA in a ratio of 2 residues per 10 xylose units, but are dissimilar by having side chains of α-L-Ara*f* at a ratio of 1.3 residues per 10 xylose units (Sjostrom, 1993). Figures 1.5 and 1.6 show the different structures for GGM and GAX.

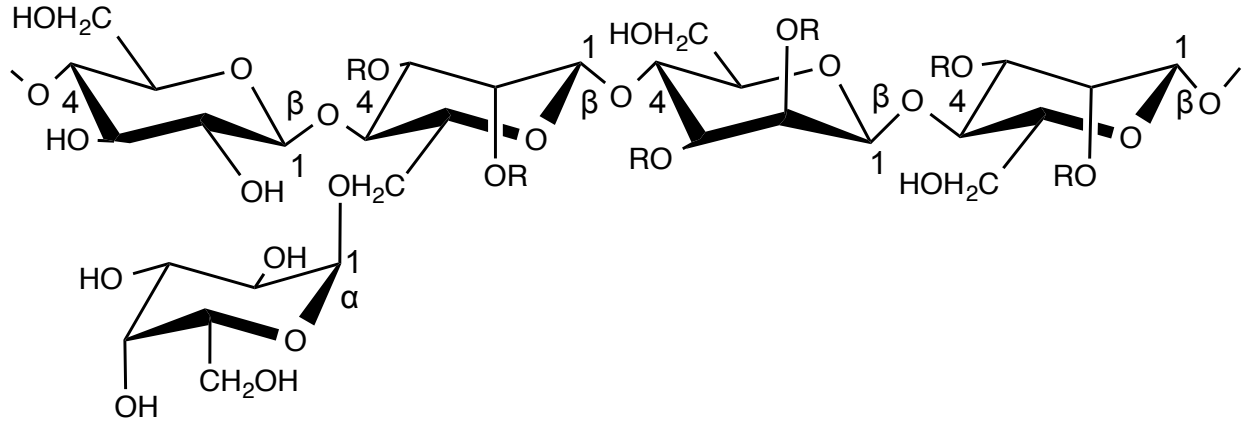

Figure 1.5. Softwood hemicellulose structure of GGM.

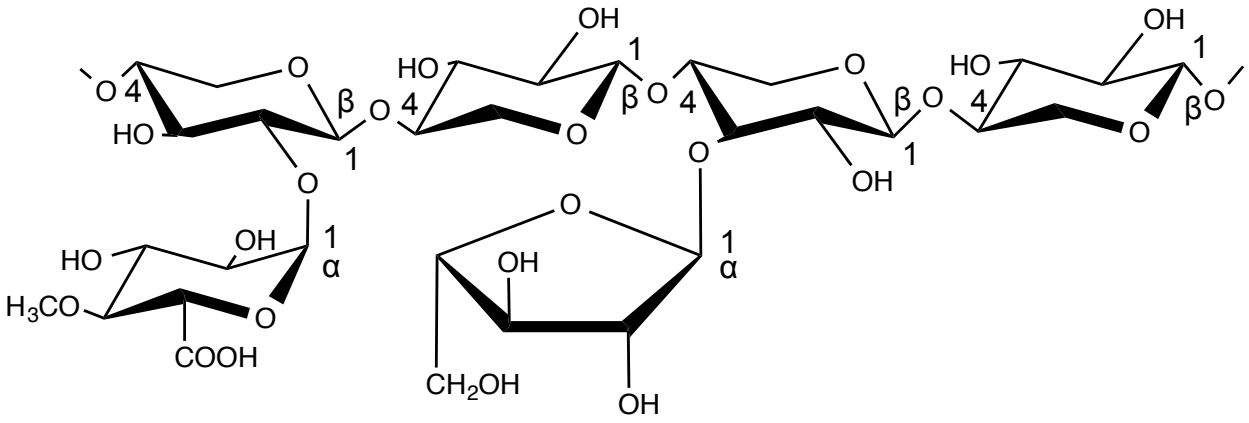

Figure 1.6. Softwood hemicellulose structure of GAX.

Previous extractions of GGM and GAX from softwoods by different chemical pulping techniques yielded characteristic $\overline{DP}_N$ of 90-102 and 89-120, respectively (Jacobs & Dahlman, 2001). By having lower quantities of GAX and lacking the unique Rha*p* to Gal*p*A sequence at

the polysaccharides reducing end, dissolved hemicellulose from softwoods have greater potential towards degradation when exposed to alkali. Consequently, the isolation and recovery of hemicellulose from hardwoods and softwoods as a source for conversion to liquid transportation fuels or value-added chemicals can be developed by the use of different processes. This process integration could include alkali pre-extractions for hardwoods (*i.e.* a less severe soda pulping process) while softwoods would best be utilized in an autohydrolysis or acid sulfite process (Phillips, Jameel, & Chang, 2013; Saadatmand et al., 2012; Stoklosa & Hodge, 2012; Van Heiningen, 2006; Yoon, Tunc, & Van Heiningen, 2011; Zhu et al., 2010).

1.3 Existing Liquor Streams

To improve hemicellulose and lignin utilization from woody biomass, existing black liquor streams produced by the pulping industry may play an important role by recovering these plant cell wall fractions. This section discusses current operations performed in the pulping industry along with the type of hemicellulose or lignin that can be recovered, how they are separated from liquor streams, and their potential conversion to fuels or chemicals.

1.3.1 Chemi-Mechanical Pulping Glucomannan Streams

Although not as prevalent as kraft or sulfite pulping, chemi-thermomechanical pulping (CTMP) or thermomechanical pulping (TMP) is utilized to produce pulps with higher overall yields and higher lignin content. The pulping conditions are less severe in terms of pH, but still offer the potential to recover hemicellulose oligomers that are dissolved during the process. Liberated hemicellulose fractions that contain both sugar monomers and oligomers can be collected from process water used during CTMP or TMP. In the case of using a dilute sulfuric

acid pre-extraction on a hardwood feedstock the hemicellulose fraction in the plant cell wall is hydrolyzed to monomers while the residual wood chips are mechanically refined to produce a pulp that still maintains industrially important strength properties (W. Liu, Hou, Mao, Yuan, & Li, 2012; W. Liu, Yuan, Mao, Hou, & Li, 2012). The hydrolyzed sugars can serve as a feedstock for ethanol fermentation. Alternatively, hemicellulose oligomers have applications as a source for barrier films with low oxygen permeability in the packaging industry. Dissolved oligomers within process water fractions after TMP can be concentrated through the use of ultrafiltration or microfiltration (A. Andersson, Persson, Zacchi, Stalbrand, & Jonsson, 2007; Persson, Krawczyk, Nordin, & Jonsson, 2010). GGM from softwoods have been studied as a potential source for the barrier film production. A CTMP process applied to spruce produced soluble polysaccharides that passed a 10 kDa membrane cutoff recovered 90% of the hemicellulose fraction with a sufficient lignin content to promote oxidation by laccase enzymes for barrier film production (Krawczyk et al., 2013). Comparatively, an economic analysis on spruce hemicellulose recovered from TMP process water fractions at 30 g/L and 80% purity yielded a net product of 4 metric tonnes produced daily at €670 per metric tonne, which is less expensive than the petrochemical ethylene vinyl alcohol (Persson, Nordin, Zacchi, & Jönsson, 2007). Aside from CTMP or TMP, woody biomass prehydrolysis in water prior to pulping can be performed producing liquid side streams of hydrolyzed hemicellulose. Both GGM from softwood hemicellulose and GX from hardwood hemicellulose recovered after prehydrolysis have been evaluated for barrier film production (Ibn Yaich et al., 2012; Saadatmand et al., 2012) by incorporating additives such as carboxymethylcellulose or microfibrillated cellulose for barrier films derived from hemicellulose to show that tensile strength and oxygen permeability properties meet the performance requirements associated with current barrier film production.

From a process point of view current CTMP and TMP mills would have to add separation and recovery stages for process water fractions. Hemicellulose oligomers could be separated through an ultrafiltration step whereby the polysaccharides oligomers are recovered for use as barrier films and the concentrated monomeric sugar could be collected for a fermentation process. Although lignin removal by CTMP or TMP is low, these processes are known to deacetylate biomass, which has implications for the fractions solubilized and the residual solid fractions. The noteworthy trends include diffusion limited release of large hemicellulose polymers (*i.e.* high $\overline{DP}_N$), which can be increased after the refining stage, and GGM insolubility after deacetylation (Konn, Pranovich, & Holmbom, 2006). Furthermore this release of acetyl groups as acetic acid can inhibit downstream fermentation indicating that an additional detoxification process should be added before fermentation (Sun, 2009). Although the production of a liquid fuel can be achieved through CTMP or TMP dissolved hemicellulose, the isolation and recovery of higher molecular weight hemicellulose may provide better utilization as a source for barrier film production.

1.3.2 Polysaccharides from Viscose Pulp:

Viscose pulping is a process capable of producing derivatized forms of cellulose for the production of semi-synthetic fibers, but has further applications due to the removal of hemicellulose and lignin components. Traditionally, fibers such as rayon or cellophane can be produced from the viscose process which incorporates steeping woody biomass in 18% NaOH solution followed by taking the residual alkali solids and treating them with carbon disulfide to produce a semi-synthetic fiber called cellulose xanthate (Sjostrom, 1993). The high alkali concentration used allows a large fraction of hemicellulose to be dissolved, which can then be

separated and recovered for a source of oligosaccharides. High purity xylan from the alkali extract can be recovered through dialysis followed by precipitation with sulfuric acid to yield oligosaccharides (Griebl, Lange, Weber, Milacher, & Sixta, 2006). Griebl *et al.* (2006) characterized xylan oligosaccharides (XOS) by hydrothermolysis to assess the stability of xylan as a renewable material and found that the soluble fraction of xylan after hydrothemrolysis contained neutral XOS, acidic XOS, and degradation products due to the high temperature pretreatment (Griebl et al., 2006). Additionally, XOS comprised the largest fraction of dissolved plant cell wall material from an autohydrolysis study performed on southern mixed hardwoods at temperatures exceeding 150°C and indicated that up to 70% of the total hemicellulose content dissolved at the highest severity (Tunc & van Heiningen, 2008). Commercially, acid extractions are feasibly performed in pulp mills producing viscose pulps, usually as an acid sulfite stage. Examples of this include the Dömsjö mill in Örnsköldsvik, Sweden where the hemicellulose sugars are fermented to ethanol and the Lenzing mill in Lenzing, Austria where the xylose and acetic acid are recovered and furfural is produced from the xylose. Furthermore, succinic acid production by genetically engineered strains of *Escherichia coli* was successful at the laboratory scale in utilizing all hemicellulosic sugars originating from spruce after a two-stage acid pretreatment (Hodge, Andersson, Berglund, & Rova, 2009). Succinic acid production is not for a biologically derived fuel, but instead as a commodity chemical with applications in food ingredients, pharmaceutical additives, diesel fuel additives, and as a 4-carbon building block for higher value polymers (Song & Lee, 2006; Werpy, Frye, & Holladay, 2006; Zeikus, Jain, & Elankovan, 1999). As with many other commodity chemicals succinic acid is currently produced from petrochemically derived butane through maleic anhydride (Zeikus et al., 1999). This

example of a biologically derived commodity chemical shows one other instance of how petroleum dependence can be decreased through biomass conversion.

Another possibility for recovering hemicellulose fractions from viscose pulps is through the use of sequential extractions with alkali or the use of specific hemicellulases to remove hemicellulose polysaccharides. Bleached hardwood kraft pulp contain appreciable amounts of GX that can be removed in high purity through extractions with alkali or nitren (*i.e.* a metal complex of nickel(II)-hydroxide and tris(2-aminoethyl)amine); both gave similar xylan yields but alkali xylans exhibit a lower DP_N with higher substituent values than nitren xylans (Janzon, Saake, & Puls, 2008). The combination of alkali extractions with a treatment of xylanases can produce a range of xylans from polymeric to XOS. Hakala *et al.* (2013) found that xylan from hardwood kraft pulp could be separated into two fractions that included one with a high yield of original pulp xylan (60%) and one with a high molecular weight (40,000 Da) but with a lower yield (~7%), and that xylan with essentially unaltered molecular weight plus XOS could be obtained after an alkaline extraction (Hakala, Liitia, & Suurnakki, 2013). This also has been shown previously with cold alkali extractions on bleached hardwood kraft pulp and recycled fibers before xylanase treatment to enhance the xylan removal (Jackson, Heitmann, & Joyce, 1998). Viscose pulps, consequently, have the potential to allow an advantage in utilizing the entire plant cell wall fractions since the three main biopolymers of cellulose, hemicellulose, and lignin are separated completely. Through aqueous or biological based separation procedures, followed by recovery of oligomeric or polymeric material if needed, the hemicellulose polysaccharides can be obtained as a renewable substrate for the conversion to other fuels or chemicals.

1.3.3 Sulfite pulping: Lignin and hemicelluloses

Although sulfite pulping is not practiced as much as kraft pulping, the residual black liquor from sulfite pulping processes or similar processes can still contribute to renewable fuel or chemical production. During sulfite pulping lignin is sulfonated under acidic and basic conditions leading to solubilization. Also, the acidic chemistry allows for hemicellulose hydrolysis to lower molecular weight oligomeric and monomeric sugars. Ethanol was once produced at a larger volume scale from sulfite black liquors by fermenting the hexose sugars of softwood GGM. Due to the gradual phase-out of sulfite mills in favor of the kraft process, only a few sulfite mills remain that continue to practice ethanol fermentation of hemicellulose sugars such as the Tembec pulp mill in Temiskaming, Quebec (Williamson, 2013). Even with the decline of sulfite pulping, research continues for producing bioethanol from spent sulfite liquors. A recombinant strain of *Escherichia coli* produced variable results for ethanol production, from 70% to 92% conversion efficiency of sugars for hardwood spent sulfite liquors (Lawford & Rousseau, 1993). Recombinant yeasts from *Candida shehatae, Pichia stipitis,* and *Saccharomyces cerevisiae* were able to ferment spent sulfite liquors originating from softwood feedstocks with ethanol concentrations ranging from 13.0 to 18.8 g/L, and yields corresponding to 0.34 to 0.45 g/g (ethanol/sugar) (Olsson & HahnHagerdal, 1996). While acidic sulfite pulping is more conducive for obtaining a stream of hydrolyzed sugars, alkaline sulfite pulping can be viewed as a pretreatment by removing lignin from the wood cell wall while maintaining a stabilized polysaccharide chain in the wood chips. Franco *et al.* (2012) explored alkaline sulfite pulping followed by disk refiner to produce pulps amenable to fermentation by commercial *Saccharomyces cerevisiae* with a maximum ethanol concentration of 22.0 g/L (Franco et al., 2012).

Additionally, a new subset of research has looked into a process known as sulfite pretreatment to overcome the recalcitrance of lignocellulose (SPORL) (Shuai et al., 2010; G. S. Wang et al., 2009; Zhu, Pan, Wang, & Gleisner, 2009). This process differs from traditional sulfite pulping by operating at different chemical conditions and preparing a feedstock that is readily digestible to monomeric sugars for the production of bioethanol (G. S. Wang et al., 2009). Softwoods have been the common feedstock analyzed because of their perceived recalcitrance by having higher lignin content, and possessing a larger fraction of hexose sugars in the hemicellulose fraction comprised of GGM. High glucan conversion during enzymatic hydrolysis can be achieved only after 48 hours for most of the conditions analyzed; however, some of these high conversions occur with high loadings of sulfuric acid where corrosion can be an issue within the reactor vessel. Though the acid loadings are high, it serves the purpose of maximizing hemicellulose removal and simultaneously hydrolyzing the polysaccharides in the spent liquor to monomeric sugars for fermentation to bioethanol. After pretreatment SPORL residual wood chips are passed through a disk refiner for size reduction followed by enzymatic hydrolysis to yield a counterpart sugar stream for fermentation. Consequently, fermentation can be performed on almost all of the polysaccharides from the woody biomass minus the losses from conversion to furan degradation components. These furans are formed under acidic conditions by dehydration reactions that form furfural from pentose sugars and HMF from hexose sugars. For SPORL pretreatment the spent liquor is conditioned for the removal of these products either by rotary evaporation or adsorption to a polymeric adsorbent (*e.g.* Amberlite XAD-4) (Lan, Gleisner, Zhu, Dien, & Hector, 2013; Zhu et al., 2010). Previous fermentation trials have been completed with engineered strains of *S. cerevisiae* to utilize hexose and pentose sugars for the production of ethanol. A combined fed-batch simultaneous saccharification and

fermentation (SSF) procedure on lodgepole pine achieved a commercially feasible ethanol concentration of 47.4 g/L, corresponding to 285 L/tonne of wood, while independent spent liquor fermentation separate from SSF on pretreated lodgepole pine could produce 276 L/tonne wood, or 72% theoretical yield (Lan et al., 2013; Zhu et al., 2010). With this process being analogous to sulfite pulping, further recovery of lignosulfonates after pretreatment can diversify the product portfolio for increased revenue since lignins can be valuable co-products obtained from woody biomass meeting range of product applications including adsorbents, epoxy resins, adhesives, and pellets for combined heat and power generation (Holladay, Bozell, White, & Johnson, 2007; Lora & Glasser, 2002).

1.3.4 Lignin Recovery by acidification

Tangential to biologically derived liquid fuels, lignin recovery from aqueous black liquors can potentially integrate with existing kraft or soda pulping mills to generate renewable co-products and decrease recovery boiler loads. During alkali pulping a sizable fraction of the lignin from the plant cell wall is dissolved and can be recovered from the aqueous phase by lowering the pH through acidification that will protonate lignin moieties containing ionizable groups to increase aggregation (Gidh et al., 2006; Guerra et al., 2007; Ragnar et al., 2000; Sarkanen et al., 1981). Besides direct addition of acid it is also possible to decrease the pH of black liquor by utilizing CO_2. Nagy et al. (2010) characterized precipitated lignin and residual lignin remaining in the filtrate after pH reduction and found that lignin still dissolved in the filtrate had more carboxylic and phenolic groups while the precipitated lignin had more methoxyl content (Nagy, Kosa, Theliander, & Ragauskas, 2010). Alternatively, Velez and Thies (2013) described obtaining lignin from a softwood kraft black liquor by sparging CO_2 into the

bulk aqueous phase at elevated temperature and pressure (115°C and 6.2 bar) where the precipitated lignin emerges from the black liquor as a highly solvated suspension that is termed "liquid-lignin" (Velez & Thies, 2013). Softening point analysis performed on these "liquid-lignin" fractions showed that the solvated fractions were all below 115°C, but the dried fractions tested up to 375°C for their softening point (Velez & Thies, 2013). This "liquid-lignin" offers the advantage of not requiring extra separation processes, such as microfiltration or nanofiltration, that would be needed to recover soluble lignin (Jönsson, Nordin, & Wallberg, 2008; Mänttäri, Van der Bruggen, & Nyström, 2013). Some process design and costing has been evaluated for the acidification of black liquor by CO_2. The equipment was specified to recover only 9.5% of the lignin residing as a soluble component in the black liquor and 21% of the recovered lignin would be sold off for specialty chemical production and the rest used as a fuel within a pulp mill (Loutfi, Blackwell, & Uloth, 1991).

Today there are small subsets of companies that do practice the recovery of lignin on an industrial scale for renewable materials applications. The "LignoBoost" process utilizes the precipitation of lignin by the addition of CO_2 followed by filtering. After filtering the lignin is re-dispersed in water and acidified further which aids in obtaining higher lignin yields with lower ash and carbohydrate content, and lowers the amount of acidic washing water that is needed (Tomani, 2010). The company Borregaard based in Sarpsborg, Norway also utilizes lignin recovery for the production of a range of chemicals including industrial binders, emulsion stabilizers, and even vanillin. Processes and companies such as the ones discussed show the potential that lignin can have in terms of producing value-added chemicals alongside the liquid fuels derived from hemicellulosic components.

1.4 Pre-Extraction of Hemicellulose Prior to Pulping

1.4.1 Hot Water Pre-Extractions

Existing pulping liquor streams can be utilized for the recovery of dissolved hemicellulose and lignin for use as conversion to liquid fuels or higher value chemicals. Alternatively, the implementation of new processes into pulp mills to pre-extract desired cell wall components will enhance the utilization of hemicellulose and lignin fractions while providing product diversification. These processes could be based around extractions with hot water (*i.e.* autohydrolysis) or alkali prior to pulping. Most pulp mills have on-site water treatment that can allow autohydrolysis processes to make use of closed water loops in the mill. Processes utilizing hot water at elevated temperatures and pressure fall right in line for the production of specialty fiber applications, but the additional removal of hemicellulose components as a soluble side stream gives rise to further applications for the development of renewable products. Specific studies have focused on utilizing autohydrolysis for the production of a soluble sugar stream through the hydrolysis of hemicellulose polysaccharides followed by a chemical pulping stage to obtain cellulosic fibers. Helmerius *et al.* (2010) looked at pre-extraction of *Betula pendula* by autohydrolysis or varying alkali loadings to assess the impact of hemicellulose removal, conversion to value-added chemicals, and downstream pulping on the residual wood chips after extraction (Helmerius et al., 2010). The alkali extractions produced lower recoveries of hemicellulose than compared with the water extraction, most likely due to the degradation of hemicellulose polysaccharides to saccharinic acids, but the alkali extracted wood chips produced favorable kraft pulp yields and strength properties when compared to a control kraft cook (Helmerius et al., 2010). Although water extractions at elevated temperatures do not necessarily connect well with kraft pulping processes due to pH fluctuations, it was found

that a hydrolysable liquor was produced by the autohydrolysis of *Betula pendula* which could produce succinic acid by fermentation (Helmerius et al., 2010). The main adverse consequence of autohydrolysis processes on woody biomass is the decline in pulp yield and quality due to the depolymerization occurring to a small portion of the cellulose microfibrils (Helmerius et al., 2010; Mendes, Rocha, Sousa, & Carvalho, 2011).

Apart from unwanted cellulose microfibril hydrolysis and pulp yield decreases, the hemicellulose fraction removed by autohydrolysis can degrade at the elevated temperatures (*e.g.* 180-240°C) used in the process through dehydration reactions that form furfural (Borrega, Nieminen, & Sixta, 2011). Although these side reactions are unwanted, the autohydrolysis of woody biomass can be implemented in such a way to make them desirable. For example it was found that a hemicellulose content below 3% after autohydrolysis decreased the pulp yield after a soda/anthraquinone (AQ) pulping stage, but the yield of cellulose could be improved by supplementing the pulping stage with sodium borohydride (Borrega, Tolonen, Bardot, Testova, & Sixta, 2012). The degradation of xylose to furfural can also be exploited since furfural is a commodity chemical that is typically produced by the acid hydrolysis of plant substrates (Mamman et al., 2008). While the hydrolysis liquor after autohydrolysis contains many different dissolved organic components (*i.e.*hemicellulose, lignin, furfural), detoxifying can help to remove inhibitory fermentation components by using activated carbon (AC), oxidized AC, or polydiallyldimethylammonium chloride (PDADMAC) to absorb lignin and furfural inhibitors at 83.3% and 100%, respectively, in high quantities (X. Liu, Fatehi, & Ni, 2012). Ultimately, if some decreases in pulp yield and quality can be tolerated within a certain range the autohydrolysis of woody biomass prior to chemical pulping offers many advantages in terms of

producing different liquid fuels or chemicals; especially if refiner energy savings can be correlated to hemicellulose removal (Kenealy, Houtman, Laplaza, Jeffries, & Horn, 2007).

1.4.2 Alkali Pre-Extractions

Alternatively, the pre-extraction of woody biomass with alkali presents a much more favorable option for integration with current alkali based chemical pulping processes. Figure 1.7 presents a potential process that utilizes a soda pulping process (only alkali) for the production of biologically or catalytically derived fuels. Alkali pre-extractions will not only extract hemicellulose polysaccharides but will also pull out more lignin than autohydrolysis processes, and allow current chemical pulping processes to decrease the alkali loading in the actual pulping stage and facilitate improved recovery boiler operation by sending a black liquor stream with lower solids content. Alkali extractions have been executed under low temperature conditions to assess feasibility and at high temperature conditions to prove industrial relevance.

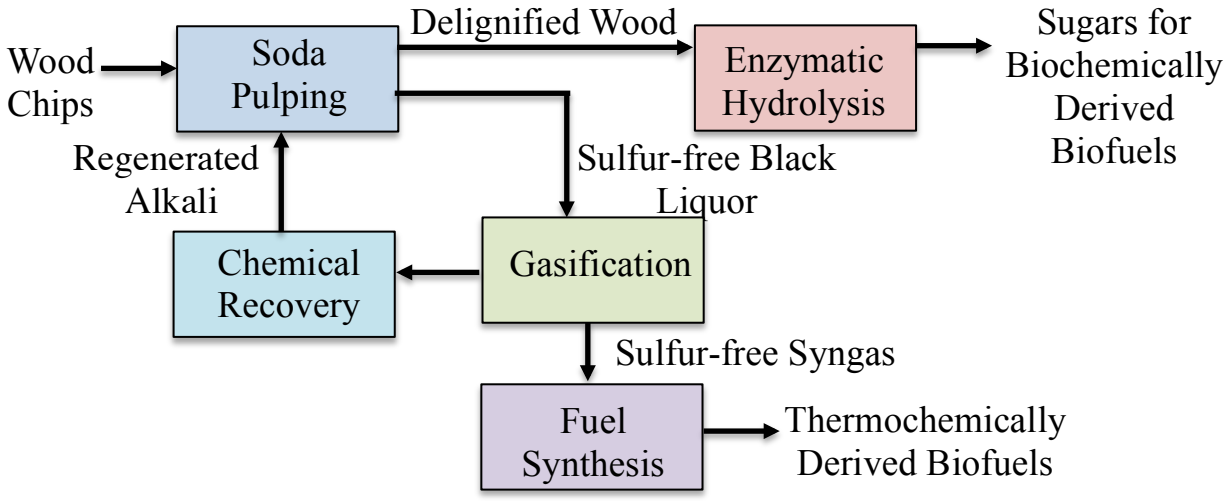

Figure 1.7. Potential process diagram for utilizing soda pulping (or alkali pre-extraction) to generate biofuels.

Low temperature alkali extractions have been performed previously at room temperature and up to 90°C with a multitude of woody biomass feedstocks. Giant bamboo of the species *Bambusa balcooa* cv. Roxburgh had 13.6% of its initial xylan content extracted after exposure to 1 M NaOH at 90°C for 4 hours that resulted in improvements for pulp yields after kraft pulping, no reduction in pulp viscosity, and only a slight decrease in pulp strength properties (Vena, Brienzo, del Prado García-Aparicio, Görgens, & Rypstra). Comparatively, Al-Dajani and Tschirner (2008) investigated mild alkaline extractions at low temperatures (50-90°C) for *Populus tremuloides* (aspen) to find that for one metric ton of wood chips supplied up to 40-50 kg of total wood components can be dissolved by alkali and showed further that kraft pulping on the extracted wood chips needed lower chemical loadings and shorter cooking time (Al-Dajani & Tschirner, 2008). A disadvantage that stands out from this study was a 10% decrease in the tear index and a relatively low yield of extracted hemicellulose; however, the benefits of the alkali extraction prior to pulping still show promise since the overall pulp properties are very comparable to control kraft cooks (Al-Dajani & Tschirner, 2008). While these low temperature studies show promise for hemicellulose extraction and recovery from aqueous alkali liquors, higher temperatures studies are a better indicator for hemicellulose utilization at industrial process parameters.

At higher extraction temperatures (125-160°C) with alkali (NaOH or green liquor) it is possible to remove 5-40% of the total wood components depending on temperature and alkali charge. Yoon *et al.* (2011) found that a 3% green liquor extraction with 0.05% AQ showed improved delignification rates, higher yields, higher tear resistance, lower refiner response, and a 2.5-3% lower effective alkali consumption when compared to traditional kraft cooks (Yoon et al., 2011). Apart from batch alkali pre-extractions, it is also possible to obtain higher value from

hemicellulose in woody biomass by different extraction processes or post-pulping extractions. A modified twin-screw extruder was analyzed for extracting hemicellulose from poplar found that 90% of the initial hemicellulose could be extracted at much lower liquid-to-solid loadings when compared to batch studies and hemicellulose solubilization could be correlated to the specific mechanical energy that was absorbed by the process material (N'diaye, Rigal, Larocque, & Vidal, 1996). Also, it is possible to utilize post-alkali extractions after kraft pulping to extract and later recover hemicellulose. Post-alkali extractions were performed at room temperature to remove hemicellulose from birch kraft pulp as a means to produce xylan films for use in the packaging industry. Alekhina *et al.* (2013) showed that 60% of the xylan present in the pulp could be extracted by alkali at room temperature with the extracted xylan being an essentially pure polysaccharide with no acetyl side units and with a very low content of 4-O-MeGA (Alekhina, Mikkonen, Alén, Tenkanen, & Sixta, 2013). A stable, self-supporting pure xylan film could not be formed, but through carboxymethylation it was shown that with an increase in the degree of substitution the film elongation at break improved along with the reduction in oxygen permeability while water vapor permeability increased; thus showing the potential that xylan has as a biodegradable film for packaging in the food industry (Alekhina et al., 2013). As discussed above hemicellulose recovery and utilization can be varied and diverse. The end usage of hemicellulose will ultimately be determined by the economics associated with industrial chemical facilities and the value of the end product desired.

1.4.3 Renewable Products from an IFBR

To determine end products derived from hemicellulose, researchers have looked at economic studies revolving around coupling pre-extraction processes with current pulp mills in

an IFBR setting. The proposed IFBR model to convert pulp mills has been analyzed mostly from the perspective of producing bioethanol for liquid fuel utilization. An economic model of an IFBR was evaluated on three scenarios: traditional kraft pulping, hemicellulose pre-extraction prior to pulping for ethanol production, and hemicellulose pre-extraction prior to pulping coupled with the conversion of short cellulosic fibers to ethanol (Huang, Lin, Ramaswamy, & Tschirner, 2009; Huang, Ramaswamy, Al-Dajani, & Tschirner, 2010). This study found that with a fixed feed rate of 2000 Mg wood per day a pulp-mill IFBR utilizing alkali pre-extraction, the hemicellulose and short cellulosic fibers converted to ethanol produced 10.04 MM gal (0.038 MM m^3) of ethanol per year with a minimum-selling price of \$1.86/gal (\$491/m^3) (Huang et al., 2010). The economic simulation found with hemicellulose pre-extraction the pulping capacity could be increased by 22%, but both pulp-mill IFBR simulations found that less steam would be produced in the recovery boiler due to a lower solids content in the black liquor; however, this could be offset by a 13% fuel usage reduction in the lime kiln from a lower loading of chemical inorganics (Huang et al., 2010).

Aside from ethanol production, value-added chemicals also have industrialization potential in a biorefinery. A cultivar of larch, *Larix sibirica* Lebed, was analyzed for use in a biorefinery utilizing hot water extractions to dissolve hemicellulose at optimized conditions (140-150°C for 60-90 minutes) followed by acid hydrolysis and fermentation or SSF with *Bacillus coagulans* MXL-9 for the production of lactic acid (Horhammer, Walton, & van Heiningen, 2011). Fermentation after acid hydrolysis produced favorable results by converting 36 g/L of monomeric sugars to 28 g/L of lactic acid (78% yield), but SSF did not produce optimal results, which was attributed to a lack of enzymes that were specific to the larch species and not to degradation products (*e.g.* furfural, acetate, etc.) since they constituted less than 1% of

the hot water extract (Horhammer et al., 2011). After hot water extraction kraft pulp yields were lower by 4-5%, but this yield could be improved through polysulfide-anthraquinone (PSAQ) pulping to produce a good papermaking pulp with similar yields (Horhammer et al., 2011).

1.5 Further Investigating the Direct Fractionation of Black Liquors

The highest volume of liquor streams generated from pulping processes, and therefore containing the most dissolved hemicellulose and lignin, originate in kraft and sulfite pulping mills. Although recovery of dissolved non-cellulosic plant cell wall fractions are possible from these streams, negative side effects still remain. At the temperatures and chemical loadings traditionally used in those pulping processes it is expected that a sizeable portion of the hemicellulose would be degraded to saccharinic acids, ultimately giving low yields of hemicellulose. Lignin would be dissolved in high quantities, but most of the lignin recovered would be sulfonated. A market does exist for sulfonated lignin to be used as a binding agent, however, if other materials are more desirable the lignin needs to be in a more pure form. For example, lignin can be used as a precursor to generate carbon fibers as long as it is free from contaminants, such as inorganics, that can contribute to undesirable broad melting point temperatures (Compere, Griffith, Leitten, & Pickel, 2005). Additionally, the sulfur compounds in the cooking chemicals for these pulping processes still present environmental concerns for downstream processing. Black liquor gasification presents a thermal catalytic conversion route for lignin to produce drop-in fuels, but residual sulfur components diminish the potential benefits. After gasification H_2S is formed which will require the use of a scrubber to remove the component from the stack gas, thus increasing capital cost. Also, any residual sulfur remaining in the syngas will decrease the yield of drop-in fuel produced since sulfur is known to contaminate

catalysts. Kraft and sulfite pulping processes should not be required to remove sulfurous cooking chemicals from their processes, instead, the options to extract, recover, and separate non-cellulosic biopolymers from wood biomass can be achieved from alkali pre-extractions prior to pulping, or an alkali only (*i.e.* soda) pulping process.

The research presented in this dissertation investigated how alkali pre-extractions, kraft pulping, and soda pulping liquors can be fractionated to recover chemically altered non-cellulosic biopolymer fractions that have potential to be used in biological or catalytic conversion processes. In chapter 2 alkali pre-extractions were performed on a variety of hardwoods and a single species of grass to determine the ability of alkali to preferentially extract hemicellulose or lignin, followed by the recovery and characterization of the altered biopolymers. The lone monocot analyzed and a species of birch produced the highest yields of hemicellulose recovered, thus indicating high extractability in alkali prior to pulping. For chapter 3 a detailed characterization was carried out on seven fractions of lignin obtained from softwood kraft black liquor at discrete pH levels. This research explored how each fraction was similar or different in terms of characteristics such as contaminating polysaccharides, functional group content, molecular weight, thermal degradation, and lignin monomers generated by analytical pyrolysis. Correlations were made on several properties to understand how the pH at fractionation can produce distinct fractions of lignin to be used for select conversion processes. Lastly, chapter 4 investigates how a soda pulping process influences hemicellulose and lignin dissolution during the process, and producing a digestible cellulosic substrate after pulping. Dissolved hemicellulose and lignin were quantified at specific time points during the heat up and cook phase of the soda pulping process to determine the mass quantities obtained in the aqueous solution. After pulping the residual wood chips were enzymatically hydrolyzed to determine

yields of soluble sugars. The goal here was to identify if soda pulping is an effective process to generate soluble sugars from cellulose as opposed to generating pulp for papermaking. Taken altogether the research evaluated in this dissertation attempts to understand more thoroughly how altered cellulosic and non-cellulosic plant cell wall components affected by alkali treatment processes can contribute to the advancement of renewable fuels, chemicals, and materials.

CHAPTER 2. EXTRACTION, RECOVERY, AND CHARACTERIZATION OF HARDWOODAND GRASS HEMICELLULOSES FOR INTEGRATION INTO BIOREFINING PROCESSES

This work has been published as original research on 3rd August 2012, in *Industrial and Engineering Chemistry Research*, Volume 51, and Pages 11045 – 11053.

2.1 Introduction

Hemicelluloses are the second most abundant class of polysaccharides after cellulose in the biosphere, comprising up to 40% by mass of plant cell walls. Xylans are the predominant hemicelluloses in the secondary cell walls of angiosperms and represent an immense, underutilized resource. Xylans have many potential commercial applications with increasing recent interest in their use for the production of green chemicals (C. Andersson, Hodge, Berglund, & Rova, 2007), renewable biofuels (Girio et al., 2010), and green polymers (Hansen & Plackett, 2008), among others. However, the current utilization of xylans is very limited due to the challenges of economic extraction, recovery, and conversion to these higher value products. One approach for improving the economic utilization of xylans is through integration with other processes that fractionate and convert plant cell wall biopolymers to fuels and chemicals in a "biorefinery" approach. Biorefining approaches utilizing xylan can be envisioned to be as large as current pulp mill capacity utilizing woody based feedstocks to extract xylan prior to chemical pulping (Helmerius et al., 2010; Ragauskas et al., 2006; Van Heiningen, 2006) or with a cellulosic biofuels process utilizing chemical pretreatment and enzymatic hydrolysis of polysaccharides (Wyman et al., 2005).

Angiosperm xylans consist of a β-1,4-linked Xyl*p* backbone with the possibility for substitutions along this backbone that may include O-acetyl subunits at the C-2 and/or C-3 position of the Xyl*p* backbone (Carpita & Whittern, 1986; Rosell & Svensson, 1975; Teleman, Tenkanen, Jacobs, & Dahlman, 2002; Timell, 1960), and Ara*f* subunits attached with α-1,2 and/or α-1,3 linkages (Ebringerova, 2006). As a proposed cross-linking glycan, xylans are known to form strong associations with cellulose microfibrils or to each other and their ability to associate with other glycans is thought to be through H-bonding and van der Waals forces between unsubstituted regions along the backbone (Kohnke, Ostlund, & Brelid, 2011; Linder, Bergman, Bodin, & Gatenholm, 2003). Glucuronoarabinoxylans (GAXs), along with mixed-linkage β-glucans, are known to be the dominant cross-linking glycans in the primary cell walls of cereals while glucuronoxylans (GXs) are the principal hemicelluloses in the secondary walls of all angiosperms. The glucomannans (GMs) and galactoglucomannans (GGMs) consist of a backbone of β-1,4-linked Glc*p* and Man*p* units with α-1,6-linked Gal*p* subunits present in GGMs (Ebringerova, 2006). GGMs comprise only a small fraction in the cell walls of angiosperms, but are the most abundant hemicellulose in gymnosperms.

A defining characteristic of the hemicelluloses is that these are soluble in alkali and can be extracted from the cell wall with minimal polymer depolymerization (Carpita, 1983) apart from deacetylation due to saponification of acetyl esters. This improved solubility is presumably due to deprotonation of hydroxyl groups on hemicelluloses at high pH. As a consequence of this, alkaline processes specifically for extraction of xylans have been a topic of ongoing research for woody dicots (Al-Dajani & Tschirner, 2008; Karlsson, Roubroeks, Glasser, & Gatenholm, 2006; N'diaye et al., 1996) and agricultural residues such as corn stover (Cheng, Zhan, Fu, & Lucia, 2010). As mentioned previously, integration with chemical pulping or cellulosic biofuels

processes in a biorefinery approach, in particular using alkaline chemistries for the extraction, fractionation, or pretreatment, opens up new possibilities for utilizing all the cell wall fractions in an integrated manner to generate higher value fuels and chemicals.

The kraft process has developed as the dominant chemical pulping process due to the favorable economics of chemical recovery and the robustness of the process to produce strong pulps from a wide range of feedstocks, with over 80% of the chemical pulp produced globally utilizing the this process (Sjostrom, 1993). During any alkaline pulping (*i.e.* kraft, soda, soda/AQ, *etc*.) the lignin is significantly depolymerized and solubilized while a large proportion of the hemicellulose is degraded to carboxylic acids and ultimately concentrated and burned in a recovery boiler. Due to this, hemicellulose extraction prior to pulping has been proposed for integration into kraft pulp mills (Helmerius et al., 2010; Van Heiningen, 2006; Yoon, van Heiningen, & Krishnagopalan, 2008) using methods that include, among others, hot water pretreatment, dilute acid hydrolysis, neutral sulfite, and mild alkaline extraction steps. Commercially, acid sulfite or steam hemicellulose hydrolysis is feasibly performed in pulp mills producing viscose (dissolving) pulps using sulfite or kraft pulping processes, respectively. However, integrating a hemicellulose pre-extraction step into a kraft process can be challenging for applications where fiber strength is a requirement. Solubilized hemicellulose re-deposited on fibers provides one component of the strength characteristics of the resulting pulps (Molin & Teder, 2002) and this has recently been shown through hot water xylan extraction that significantly decrease the strength of the resulting kraft fibers cooked to similar Kappa numbers and refined to equivalent freeness values relative to alkaline extractions (Helmerius et al., 2010). Additionally, acid extractions prior to alkali pulping can result in incomplete impregnation of alkali into chips resulting in uneven cooks and increased alkali requirements. For this reason we

propose that alkaline hemicellulose extractions can be performed prior to pulping in a manner that result in high hemicellulose yields without significant degradation and integrates well with existing alkaline chemical pulping.

Apart from alkaline chemical pulping, another area of interest for the effects of alkaline treatment on the biopolymers contained in plant cell walls is chemical pretreatments for cellulosic biofuels processes utilizing a chemical pretreatment coupled to an enzymatic depolymerization of the polysaccharides to generate fermentable sugars. A wide range of reaction chemistries are possible and these pretreatments generally share common features that may involve chemically modifying, depolymerizing, solubilizing, and/or physically redistributing a portion of the hemicelluloses and lignins in the cell wall. For acidic pretreatments (Wyman et al., 2005) where much of the recent research and nascent commercialization attempts have been focused, the hemicelluloses are hydrolyzed to low MW oligomers, monomers, or sugar dehydration products and when oligomers are present, the deacetylation is often incomplete (unpublished observations). Alkaline pretreatments such as NaOH (Sills & Gossett, 2011; Xu et al., 2010), $Ca(OH)_2$ (Chang, Kaar, Burr, & Holtzapple, 2001), and anhydrous (Wyman et al., 2005) or aqueous ammonia (T. H. Kim, Kim, Sunwoo, & Lee, 2003) or alkaline oxidative pretreatments such as Na_2CO_3-O_2 (Klinke, Ahring, Schmidt, & Thomsen, 2002), $Ca(OH)_2$-O_2 (Chang, Nagwani, Kim, & Holtzapple, 2001), and NaOH-H_2O_2 (Banerjee, Car, Scott-Craig, Hodge, & Walton, 2011) impact the hemicellulose (and lignin) fraction in a significantly different ways than acidic pretreatments.

There is still a need to better understand the fundamental behavior of plant cell wall biopolymers during alkaline biorefining processes and how their properties are affected by the

biorefining processing conditions. Laboratory analytical methods for hemicellulose isolation involve, for example, alkali dissolution followed by alcohol precipitation (2-propanol, methanol, or ethanol) with oxidative bleaching of contaminating lignin in the precipitate (Glasser, Kaar, Jain, & Sealey, 2000; Puls, Schroder, Stein, Janzon, & Saake, 2006), while existing approaches to lignin recovery from alkaline black liquors are based on lowering of the pH with an acidifying reagent such as CO_2, acetic acid, or H_2SO_4 (Ohman, 2006) to decrease the solubility of lignin by increasing aggregation. Sugar separation may be desirable for the for the purpose of removing sugars from the liquors which may be ultimately combusted and recovered for reuse in the process.

This work proposes to better characterize the alkaline extractability, solubility, and properties of the biopolymers (particularly the hemicelluloses) solubilized by alkaline conditions that may ultimately integrate this extraction into either hardwood alkaline pulping, or grass or hardwood alkaline pretreatments to convert polysaccharides through a biochemical platform. Within this scope, this work has three specific aims. These are to: (1) determine the extractability of cell wall polymers from diverse hardwoods and a representative grass by increasing alkali, (2) determine the recoverability of these cell wall polymers by solubility-based separations, and (3) quantify the recovered biopolymer fractions in terms of composition and molecular weight distributions. In support of the third aim, analytical methods are adapted and compared for estimating molecular weight distributions of alkali-extracted hemicelluloses.

2.2 Materials and Methods

2.2.1 Materials and Biomass Composition

Four species of hardwoods were tested along with one cultivar of switchgrass (*Panicum virgatum*, cv. Cave-In-Rock) obtained from Dr. Farzaneh Teymouri (Michigan Biotechnology Institute, Lansing, MI). The hardwood feedstocks were *Acer saccharum* (sugar maple) obtained from Todd Smith (Devereux Sawmill, Pewamo, MI), *Betula pendula* (silver birch) obtained from Curt Lindström (Smurfit-Kappa Kraftliner AB, Piteå, Sweden) and hybrid *Populus* spp. including *Populus tremula x tremuloides* (hybrid aspen) and *Populus nigra x maximowiczii* cv. NM6 (hybrid poplar) obtained from Dr. Raymond Miller (Michigan State University Extension). Debarked wood chips were dried to approximately 5% moisture, milled (Wiley Mini-Mill, Thomas Scientific) to pass a 2 mm screen, and stored sealed at 4°C until use.

Biomass composition was determined according to NREL/TP 510-42618 (Sluiter et al., 2008) except that the procedure was scaled down by a factor of three and that HPLC quantification of monosaccharides was performed with a Bio-Rad Aminex HPX-87H column using 5 mM H_2SO_4 as the mobile phase and RI detection. Ash and extractives were quantified according to NREL/TP 510-42622 (Sluiter, Hames, et al., 2005) and NREL/TP 510-42619 (Sluiter, Ruiz, Scarlata, Sluiter, & Templeton, 2005), respectively. Commercial GXs (Sigma-Aldrich #X4252 and X0502) which are derived from alkali-extracted, bleached *Fagus sylvatica*) (European beech) and *Betula pendula* (silver birch) were utilized as model xylans for testing SEC and the reducing end assay.

2.2.2 NaOH Extraction and Analysis of Extracts

Alkaline extractions were performed using, 1 g of biomass (dry basis) at NaOH concentrations ranging from 0 g/L to 75 g/L at a total liquid volume of 20 mL (L:W of 20) in 50 mL disposable centrifuge tubes. These were incubated in a water bath at 85˚C for one hour with this temperature chosen as a compromise between potential polysaccharide degradation versus improved extraction. Immediately after extraction the centrifuge tubes were cooled in ice water for 10 minutes followed by centrifugation (Eppendorf 5804R) at 4,500 x *g* for 10 minutes. The liquid extract was decanted and saved for further analysis. The insoluble solids were resuspended in water and washed by repeated centrifugation and decanting until neutral. The samples were then transferred to pre-dried aluminum dishes, followed by drying overnight at 105˚C to determine the total amount of material removed by the extraction.

Analysis of the liquid phase consisted of two parts. First the procedure NREL/TP 510-42623 was implemented to assess the amount of dissolved polysaccharide material in the alkaline extract (Sluiter et al., 2006). A small sample of extract was withdrawn and placed into a pressure tube followed by the addition of 72% (w/w) H_2SO_4 to yield a 4% H_2SO_4 solution for hydrolysis. The calculation provided by the procedure to yield a 4% acid solution was designed for extracts from a dilute acid pretreatment of biomass. This calculation was modified slightly to account for the high concentration of OH^- in solution. After the hydrolysis procedure was complete the liquid hydrolysate was separated from precipitated insoluble solids by centrifugation. The hydrolysate was syringed filtered and analyzed for sugars by HPLC as outlined in section 2.2.1. The precipitated solids were washed until neutral, dried overnight at 105°C, and weighed to determine Klason lignin content. The second analysis performed on the alkaline liquor extract was a 2:1 (v:v) ethanol precipitation for the recovery of the dissolved

biopolymers. Two parts ethanol were added to one part of sample in a centrifuge tube and allowed to precipitate for two hours at -4°C. The precipitate that was formed was washed with 95% (v/v) ethanol by repeated cycles of centrifugation at 2,885 x *g* for 10 minutes followed by decanting. Gravimetric analysis was performed on the precipitate after washing to account for the total amount of polymeric material that could be recovered from a given extraction.

Acid precipitation was performed on the liquid phase samples after NaOH extraction with a 1 M H_2SO_4 solution for pH adjustment. Samples of the liquid extract were titrated to either a pH of 2 or 6. After titrating the samples were centrifuged at 2,885 x *g* for 10 minutes followed by decanting the supernatant. The precipitated material was centrifuged washed twice with 15 mL of 5 mM H_2SO_4 followed by two washes with 15 mL of ethanol. These solvents were selected for washing instead of water so as to not lose any material due to dissolution. After washing samples were transferred to pre-dried and weighed aluminum weigh dishes and dried overnight at 105°C. Gravimetric analysis followed to determine the amount of material recovered by pH adjustment.

2.2.3 Characterization of Biopolymers

After washing, the precipitate was dried in an incubator at 65°C to drive off most of the remaining ethanol without complete drying since the solubility properties were found to be significantly reduced upon complete drying. The solids content of the dried precipitates were determined and samples were suspended in a known volume of water and heated in a water bath at 50°C until re-solubilized (approximately 3 hours).

NREL/TP 510-42623 was used to quantify the amount of polysaccharides associated with the recovered biopolymer precipitates (Sluiter et al., 2006). The pH of the solution was measured

so the proper amount of 72% (w/w) H_2SO_4 could be added to yield a 4% (w/w) H_2SO_4 solution for hydrolysis. After completion of the hydrolysis procedure samples were allowed to cool down, and then centrifuged to remove any insoluble material that could have precipitated out of solution upon addition of the acid. The liquid hydrolysate was decanted and syringed filtered prior to analysis by HPLC. If any insoluble material was present the solid material was washed and dried for gravimetric analysis.

Size exclusion chromatography (SEC) was performed using a Waters (Milford, MA) Ultrahydrogel 250 column with elution in an aqueous mobile phase containing 0.1 M $NaNO_3$ and 0.01 M NaOH with detection by RI and UV at 280 nm. Prior to analysis the re-solubilized samples were mixed in a 1:1 with 0.2 M $NaNO_3$ and 0.02 M NaOH. The column was calibrated with dextran standards (Sigma-Aldrich) consisting of molecular weights of 1,000, 5,000, 12,000, 25,000, 50,000, and 80,000 g/mol. For the final comparisons concerning the size of the polymers the molecular weights of the dextran standards were converted to degree of polymerization (DP) using the conversion factor of 132 g/mol monomer subunit for a xylan polymer.

The number average degree of polymerization (DP_N) of the recovered material was determined using a bicinchoninic acid (BCA) assay, which was performed according to Zhang and Lynd (Zhang & Lynd, 2005) only using xylose in the range of 5-60 μM as the standard. The monomer concentration of xylose was determined by the same 4% acid hydrolysis method discussed when determining the composition of the liquid phase extract and precipitate, including correction for xylose dehydration. The ratio of the xylose monomer concentration and reducing end concentration give an estimate of the apparent DP_N of the recovered polysaccharides assuming no interference by other polymers.

2.2.4 Precipitate Bleaching

For the bleaching procedure, 10 g/L of the precipitate was suspended in a solution of 2% (w/v) H_2O_2 and 1.0 mM NaOH solution. This was incubated for one day at room temperature, after which ethanol was added in a 2:1 ratio the flask was placed in a freezer (-4°C) for 2 hours to precipitate the bleached polymers. The bleached material was washed with ethanol by centrifugation at 2,885 x *g* for five minutes during each cycle. After washing the bleached material was dried in the similar manner stated above for the original precipitated material. After drying the bleached material was resuspended in water and the previously described analyses were performed for composition by 4% acid hydrolysis, apparent $\overline{DP}_N$ by BCA, and molecular weight distribution by SEC.

2.3 Results and Discussion

2.3.1 Biomass Composition

The original composition of the biomass used in this work is presented in Table 2.1. Standard deviations were lower than 5% after triplicate measurements for the sugar and lignin content. Arabinan was not detected (N/D). This table shows that the glucan fraction (overwhelmingly cellulose) represents the major component by weight and is relatively unaltered by the extraction. A small extractable fraction of this glucan may derive from starch, sucrose, mixed-linkage β-glucans, xyloglucans, or glucomannans. The hemicellulose fraction is characterized as arabinan and xylan + mannan + galactan since the analytical method does distinguish between these sugars. The majority of the hemicellulose sugars should be xylose since it is well established that the xylans are the predominant hemicelluloses in woody dicots

and grasses. The term "hemicellulose" will be used throughout this text as up to 10% of this fraction may include GMs and GGMs**.**

Table 2.1. Composition of biomass as a percentage of the dry weight; N/D = not detected, NQ = not quantified.

	Glucan	**Xyl/Man/Gal**	**Ara**	**Klason Lignin**	**Acetyl**	**Ash**	**Extractives**	**NQ**
Sugar Maple	42.4	20.7	N/D	24.6	3.4	0.5	3.5	4.9
Silver Birch	39.3	25.3	N/D	16.6	4.6	0.2	2.8	11.2
Hybrid Aspen	31.9	16.9	1.3	26.0	4.8	2.9	12.0	4.2
Hybrid Poplar	42.3	18.3	N/D	23.8	3.0	1.7	3.5	7.5
Switchgrass	35.0	26.6	2.0	16.7	2.5	4.3	5.3	7.6

The birch and switchgrass had the highest fraction of hemicellulose sugars, while the birch and aspen had the most acetylated xylans. The maple and aspen had the highest fraction of Klason lignin in their cell walls and the switchgrass was particularly high in ash, perhaps due to the incorporation of silica into the cell walls of grasses rather than calcium in the case of dicots (Carpita, 1996). The birch and aspen had more than 14% total extractives plus "not quantified" material that would likely be extracted by alkali. Comparatively switchgrass contains a large fraction of "not quantified" material, which includes uronic acids originating in pectin or as glucuronsyl substitutions on the xylan and possibly protein.

2.3.2 Extraction and Recovery

After the series of NaOH extractions were performed, the alkali extraction was quantified by total material lost from the cell walls (Figure 2.1) and the total recoverable plant cell wall polymers were quantified by the mass recovered by alcohol precipitation (Figure 2.2). Data are

not presented on an extractives-free basis and error bars indicate data range from duplicate extractions. The data plotted in Figure 2.1 show that increasing NaOH loadings on the biomass had the obvious effect of increasing the amount of material solubilized from the cell walls and that the most material is removed from switchgrass and aspen while maple is the most resistant to alkali extraction. More material is removed from the aspen and switchgrass, which may be a consequence of the higher extractive content in the biomass, 12% and 5.3%, respectively, along with the contribution from the unquantified fraction.

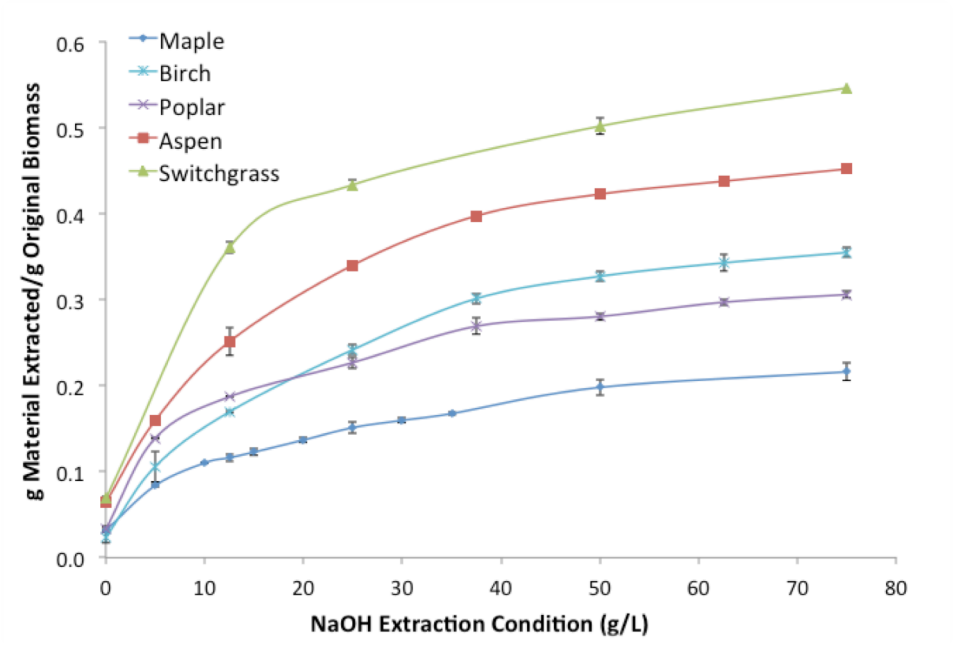

Figure 2.1. Total mass extractability for the five biomass sources.

Specifically for switchgrass, it is well-established that grass cell wall xylans and lignins are particularly susceptible to extraction by alkali with more than 50% typically extractable (Hartley, 1983) while higher temperature alkali pretreatments solubilize a comparable portion of the cell wall (Xu et al., 2010). Factors that contribute to this are that alkali-labile ferulate-mediated ester cross-links between lignin and GAX are a feature of the secondary cell walls of commelinid monocots (Iiyama, Lam, & Stone, 1990).

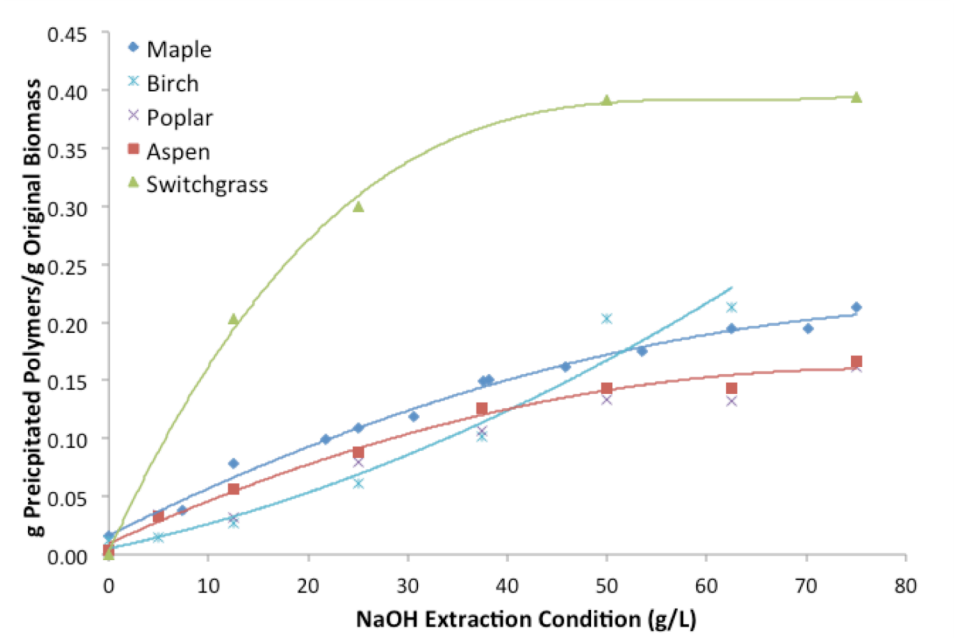

Figure 2.2. Recoverability of the extracted polymers with a 2:1 (v:v) ethanol precipitation for the five biomass sources.

Furthermore, grass lignins have significantly higher free phenolic contents than dicot lignins which gives them a higher solubility in alkali (Lapierre, Jouin, & Monties, 1989). Contributing factors to cell wall polymer extractability of the woody dicots tested include differences in the extractives abundance, secondary cell wall thickness, and vascular structure density between aspens and poplars that have bulk densities 50% lower than maples and birches (Simpson & TenWolde, 2007), although these are not measured for this work.

The data plotted in Figure 2.2 show the recovery profiles for the selected biomass feedstocks. Because the alcohol-precipitated material is presumed to represent primarily solubilized cell wall polymers, this is used to set an upper limit on polymer recoverability. On a mass basis, switchgrass generates the largest amount of recoverable material, while the hardwood samples produce less, albeit, with a similar trend amongst the four. On a percentage basis sugar maple gives close to full recovery of the material removed by NaOH extraction

indicating that the material extracted is primarily polymeric. The other feedstocks perform lower on an overall amount recovered basis with switchgrass recoveries of around 80%, birch 67%, poplar 60%, and aspen yielding the lowest recovery of extracted material at 37.5%. Differences in the recovery can be attributed to differences in the abundance of low MW organics solubilized during alkali extraction (*e.g.* low DP polymers, extractives, acetic acid).

The total amount of the hemicelluloses solubilized by NaOH is plotted in Figure 2.3, which shows that increasing alkali concentration increases hemicellulose extraction.

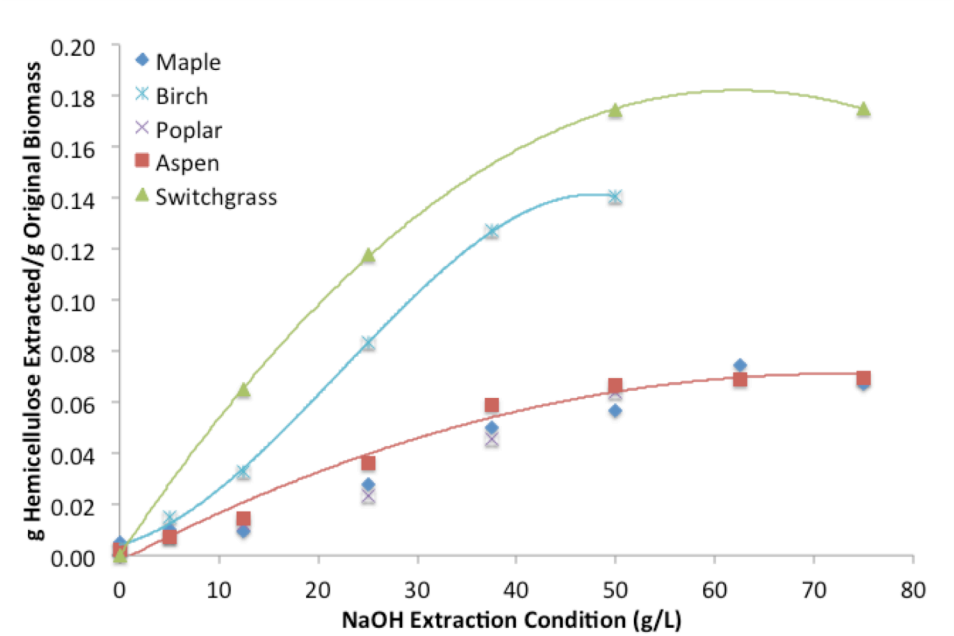

Figure 2.3. Hemicellulose extractability by alkali for the five biomass sources.

The amount of hemicellulose extracted from sugar maple, hybrid poplar, and aspen show very similar trends, while the hemicellulose extracted from birch and switchgrass are significantly higher. This is potentially due to combination of factors including the higher content of overall hemicellulose in these two plants (Table 2.1), the lower lignin content which may make for a more accessibly secondary cell wall, and the aforementioned properties of grass cell wall polymers that increase their alkali extractability. Once again the switchgrass outperforms the other feedstocks by removing up to 60% of the hemicellulose present based on the composition

in Table 2.1. Interestingly the birch sample produces the second greatest fraction of hemicellulose removed. As previously discussed the aspen feedstock produced the most material solubilized from the hardwood samples as shown in Figure 2.1, however, this does not specifically indicate that hemicellulose components are extracted in the greatest quantity from aspen. The larger component of extractives in the aspen sample, 12% as shown in Table 2.1, can presumably explain this factor.

Overall, important properties that may affect plant cell wall polysaccharides in solution could be proposed to include **(1)** the polymer size, **(2)** both the degree of substitution of the polymer backbone and the properties (*e.g.* ionizable groups) of the substituent (hydroxyl, acetyl, glucuronosyl, or arabinosyl), **(3)** the properties of the solvent (including the pH), and **(4)** the potential for aggregation with itself or with other polymers. It is known that gradient extraction at increasing alkali concentrations (Carpita, 1983; Carpita & Whittern, 1986; Peng et al., 2010) or gradient recovery with solvents of at different strengths or with different properties (Bian et al., 2010) can yield hemicelluloses with different properties. For example, graded alkali extraction of maize coleoptiles (Carpita & Whittern, 1986) identified 3 GAX fractions that differed in the level of substitution and composition, with more substituted GAX going into solution first, and mixed-linkage β-glucans going into solution at higher alkali (2-3 M KOH) due to closer association with cellulose microfibrils. Successive extractions with alkali on isolated holocellulose have shown decreases in molecular weight starting at concentration levels of 1M KOH (Peng et al., 2010).

Data from Figures 2.1, 2.2, and 2.3 are replotted in Figures 2.4 and 2.5 to show the amount of hemicellulose extracted versus the overall amount of material removed (Figure 2.4) or the yield of hemicellulose in the precipitated polymers versus NaOH loading (Figure 2.5). The

offset in Figure 2.4 is presumably due to differences in amount of "extractives" that are likely to be easily solubilized during alkali extraction. In Figure 2.5 the hemicellulose content of the precipitated polymers shows a comparatively high fraction, around 80%, present in the birch precipitate while the other feedstocks all have a similarly lower composition.

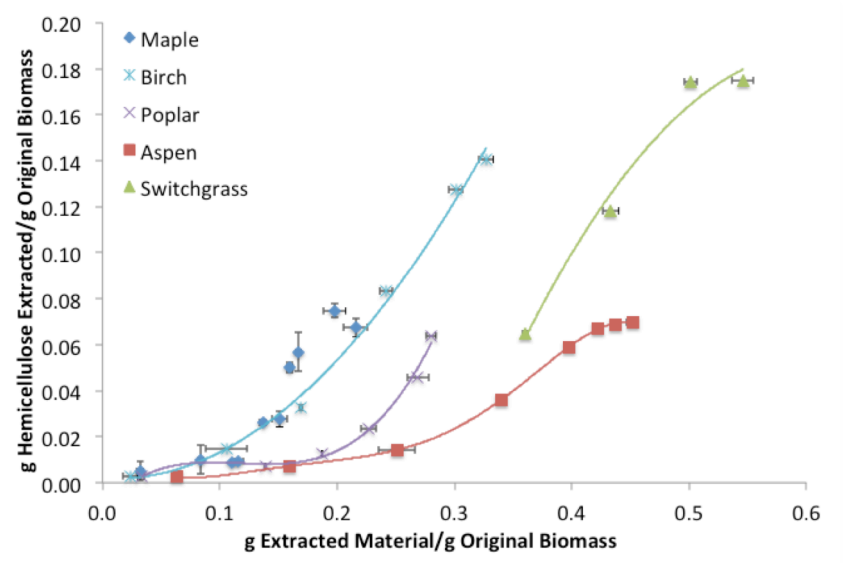

Figure 2.4. Relationship between hemicellulose extracted to the total amount of alkali extracted material.

A higher fraction of hemicellulose can be obtained after a bleaching of the maple precipitate as indicated in Figure 2.5. Accordingly this shows that although the precipitates are not comprised of pure polysaccharides, they can undergo further processing to obtain a relatively pure polysaccharide component.

Figures 2.6, 2.7, and 2.8 compare the recovery of plant cell wall polymers as a function of extraction condition for the sugar maple, aspen, and switchgrass, repspectively, based on precipitation with 2:1 (v:v) ethanol, acidification to pH 6, or acidification to pH 2. The obvious trend is that alcohol precipitation yields the highest recovery of material, followed by precipitation at pH 6 and pH 2. Another clear observation from this plot is that the maple extracts (Figure 2.6) were significantly more soluble at acidic pH than extracts from the other biomass

types which may be due to differences in the degree of substitution by glucuronic acid, although this was not quantified.

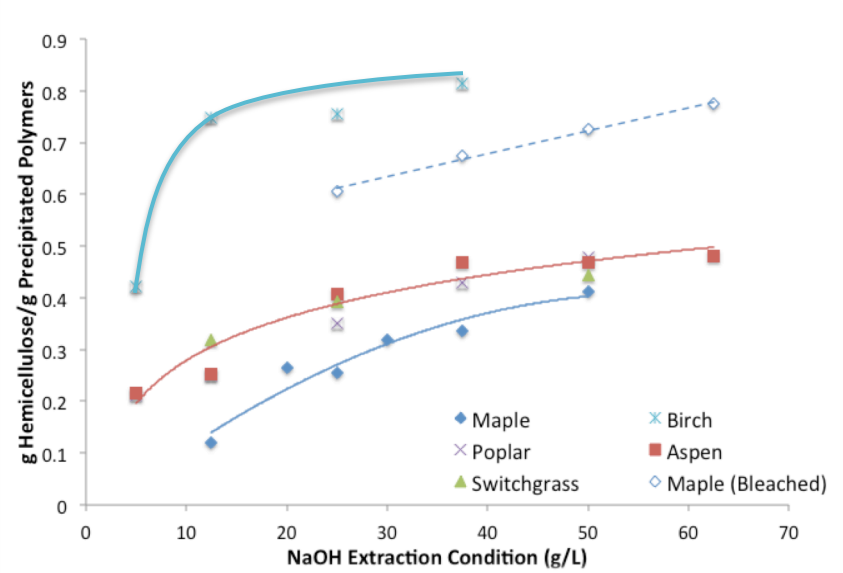

Figure 2.5. Quantified hemicellulose content of the cell wall polymers recoverable by ethanol precipitation.

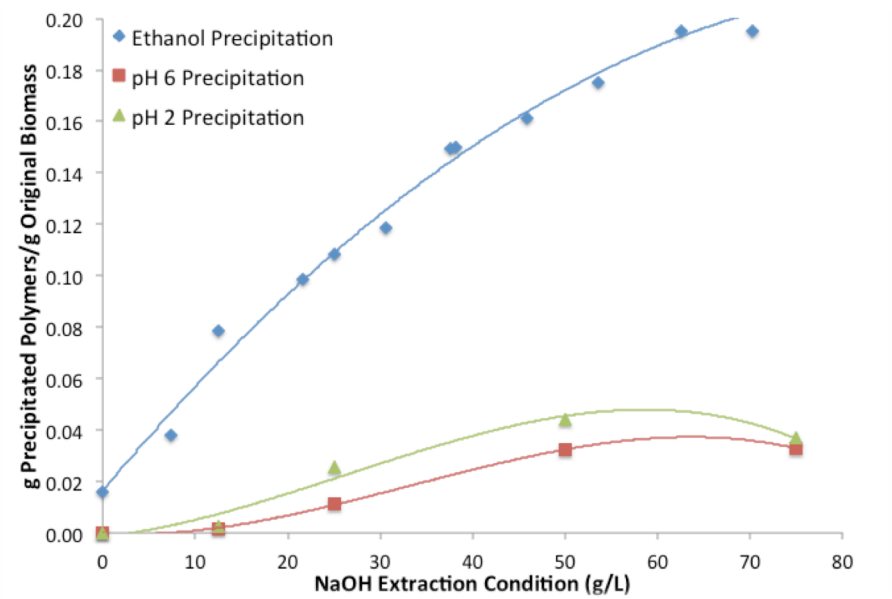

Figure 2.6. Recovery of plant cell wall polymers from sugar maple by ethanol or pH precipitation.

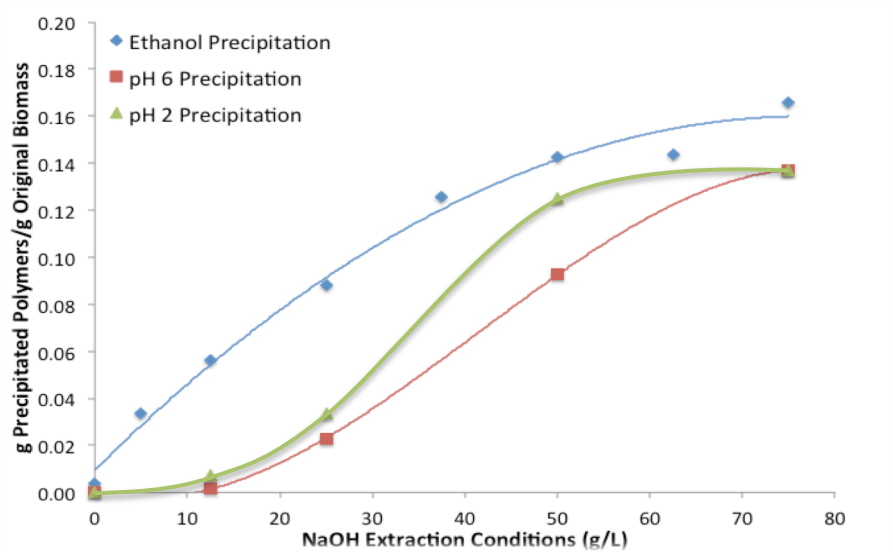

Figure 2.7. Recovery of plant cell wall polymers from aspen by ethanol or pH precipitation.

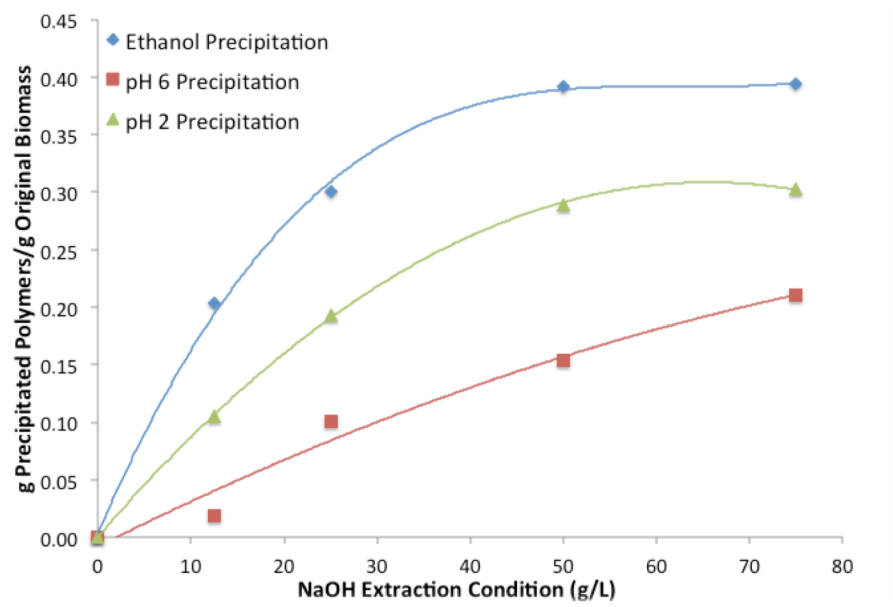

Figure 2.8. Recovery of plant cell wall polymers from switchgrass by ethanol or pH precipitation.

2.3.3 Chemical and Physical Characterization

Subsequent polymer size characterization of the extracted and recovered biopolymers was performed using size exclusion chromatography (SEC) in addition to a method based on quantification of polysaccharide reducing ends. The use of SEC presents challenges for quantifying the molar mass distributions of mixtures of non-cellulosic heteropolysaccharides from the cell wall. Qualitative analysis of MW distributions by SEC requires the implicit assumptions that all polymers will yield a signal that is proportional to the polymer mass, that the dextran calibration standards translate to hemicellulose polymer sizes, that size effects are the only contribution to the separation, and that the polymers do not interact. Illustrating this, Saake et al. (Saake, Kruse, & Puls, 2001) determined that calibration of SEC separations with pullulan standards gives a higher apparent molar masses of xylans than when an in-line viscometer is used. The negative charges on glucuronosyl substitutions may be strongly repelled by the anionic stationary phase (hydroxylated methacrylate) in the column resulting in these polyanionic polymers eluting first. However, it has been shown that 0.1 M $NaNO_3$ used in the mobile phase is sufficient to overcome these effects in aqueous SEC of hemicelluloses (Ponder & Richards, 1997a).

Summaries of some of the SEC results are presented in the following figures. Figure 2.9 shows an SEC elution profile for an alkali-extracted, bleached birch GX (Sigma-Aldrich X0502) with the elution times for dextran MW standards plotted for reference. Without sonication a broad polymer distribution was apparent between approximately 11-14 minutes elution time which corresponds to a range of degrees of polymerization of between 70-1000 assuming primarily β-1,4 linked Xyl*p* subunits (132 g/mol monomer) and an additional large peak eluted at

approximately 10 minutes representing all the polymers above the exclusion limit for the column (which is approximately 100 kDa according to the manufacturer).

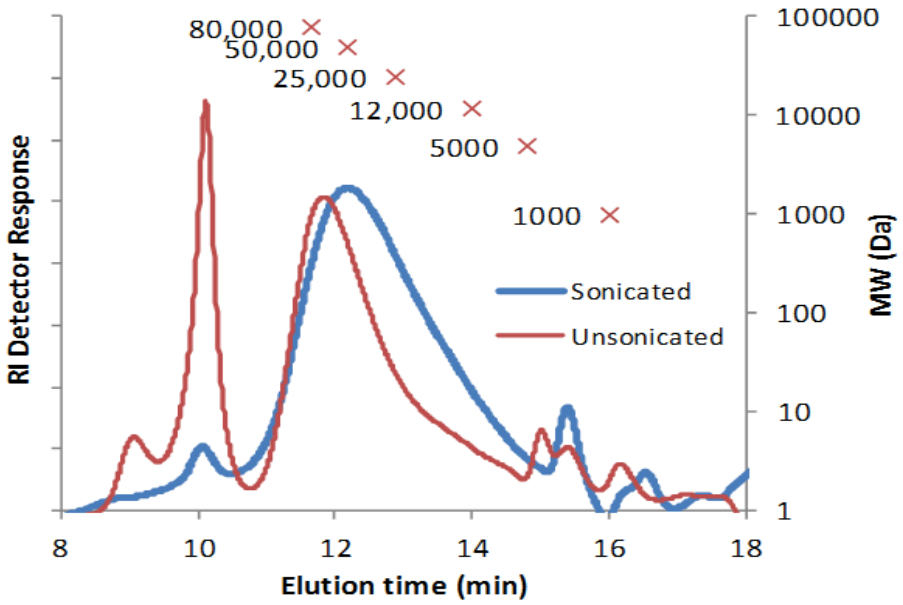

Figure 2.9. Size exclusion chromatography elution profiles for commercial birch glucuronoxylan with elution times for dextran standards plotted for reference.

When multiple columns were run in series (Waters Ultrahydrogel 500 and 250), increasing the exclusion limit to approximately 500 kDa, a second large peak with an apparent DP of greater than 1000 was still present as shown in the Appendix on page 60, Figure A.1. This peak is proposed to represent aggregates of hemicellulose, which are known to form tight hydrogen bonding networks or junction zones between deacetylated chains which are especially resistant to decrystallization (Ebringerova, Hromadkova, Burchard, Dolega, & Vorwerg, 1994). Saake et al. (Saake et al., 2001) found that KOH extraction of hardwood GX resulted in SEC chromatograms with a similar bimodal distribution with the higher MW fraction possibly corresponding to aggregates of deacetylated polymers in an aqueous mobile phase. They also found that DMSO

extraction of xylan (which does not deacetylate the polymer) did not result in the high MW peak. Although DMSO may help in reducing aggregation, the chemical was not used as a solvent for SEC analysis because the column set chosen could only handle up to a 20% composition (v:v) of an organic solvent. In the work by Saake et al. (Saake et al., 2001) an organic phase composition of 90% (v:v) was needed to disrupt the peak of high molecular weight components while a 20% (v:v) organic solvent composition did not suffice. Sonication of samples prior to analysis was found to disrupt these aggregates somewhat and allow for better separation (Figure 9); although, it is anticipated that controlling aggregation may remain a problem and inflating the apparent molecular weight. Moreover, Figure A.2 in the Appendix on page 60 shows the sonication of beech and birch GX followed by the reducing end analysis indicating that the concentration of reducing ends did not increase with sonication time; thus, sonication did not contribute to depolymerization and instead only to aggregate disruption.

The elution behavior of ethanol-precipitated polymers from the maple extracted with varying levels of alkali is presented in Figure 2.10. The chromatogram is bounded on either side by a low MW peak eluting at approximately 15.5 minutes representing salts, solvent, or other material below the permeation limit and a high MW peak eluting at 10 minutes representing polymer aggregates above the exclusion limit. Between these two bounds there are two distinct peaks present. The peak at 14 minutes can be removed by H_2O_2 bleaching (Figure 2.10) and is the only peak giving a strong UV absorbance at 280 nm as shown in the Appendix on page 61 , Figure A.3. This peak decreases with respect to the peak at 11.5 minutes with increasing alkali extraction.

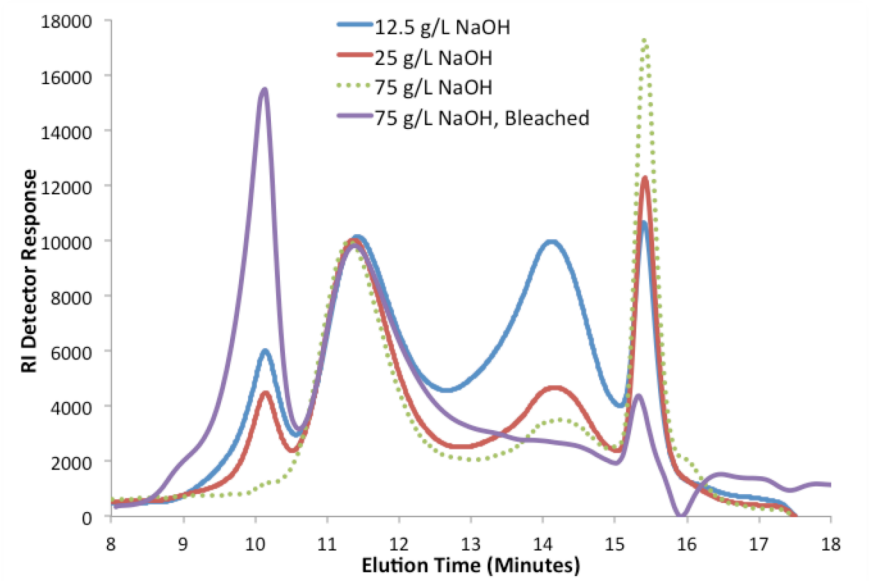

Figure 2.10. Size exclusion chromatography elution profiles of alkali-extracted, ethanol precipitated biopolymers from sugar maple with increasing alkalinity and bleaching.

In the Appendix on page 61, Figure A.4, the lignin to hemicellulose ratio of the precipitates estimated from the composition analysis correlated very well to the ratio of the peak heights of the peak at 14 minutes to the peak at 11.5 minutes.

Bleaching was found to increase the fraction of neutral sugars in recovered precipitates from 20-50% (w/w) to 60-80% for a range of biomass types and extraction conditions (Figure 2.5). All unbleached extracts (aspen, birch, poplar, and switchgrass) gave the same elution behavior as the maple with two major peaks at the same times representing presumably hemicellulose or hemicellulose aggregates and lignin or LCCs and showing the trend that the polysaccharide peak increases relative to the aromatic peak with increasing alkalinity during the extraction.

In order to provide an additional independent estimate of the recovered polysacchide DP_N, an assay was adapted to estimate the number of free anomeric carbons on a polysaccharide chain as a function of extraction condition and biomass source. Analytical methods that quantify

the anomeric or "reducing ends" of the polysaccharide chain have been used for estimating the DP_N of hardwood glucuronoxylans (Johansson & Samuelson, 1977; Sturgeon, 1973), cellulose (Kongruang, Han, Breton, & Penner, 2004; Zhang & Lynd, 2005), cellulose derivatives (Vlasenko, Ryan, Shoemaker, & Shoemaker, 1998), and pectins (Barford, Magidman, Phillips, & Fishman, 1986; Pitifer, McLellan, & Vanburen, 1994), among others. Since the (4-O-Me)-GlcA*p* subunits are known to be substituted onto the polymer backbone in GX and GAX as an α-1,2 glycosidic bond (Teleman et al., 2002) and the Ara*f* subunits are attached to GAX and AX with α-1,2 and/or α-1,3 linkages (Ebringerova, 2006), there should only be one unique anomeric carbon per polymer chain in the xylans. The results for the DP_N estimated with this method are plotted in Figure 2.11 with all of the recovered cell wall biopolymer precipitates showing similar profiles where the DP_N increased until a threshold value was reached. This increase in the apparent degree of polymerization with increasing alkali could be interpreted as increasing extraction of higher MW hemicelluloses due to improved solubility at increasing pH.

A subsequent alkaline-H_2O_2 bleaching stage was performed on the maple precipitates in order to decrease possible interference in the assay from non-polysaccharides in the recovered biopolymer precipitates. This bleaching was found to significantly increase the measured DP_N (Figure 2.11) from a range of 50-60 for unbleached maple to 120-150 for the higher NaOH extraction conditions. Commercial alkali-extracted and bleached GXs from beech (Sigma-Aldrich X4252) and birch GXs (Sigma-Aldrich X0502) were also analyzed this method and gave DP_N values of 156 and 123, respectively.

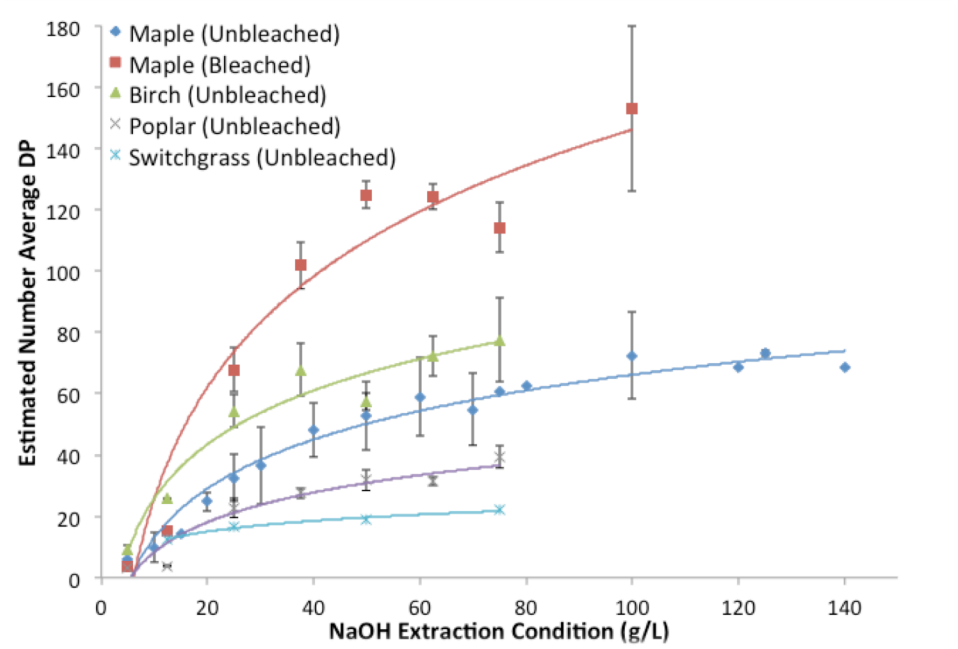

Figure 2.11. Apparent number average DP of recovered precipitates estimated using the BCA reducing end assay.

DP_N values of alkali-extracted hardwood GX in the range of 100-150 have been determined by SEC MALDI-MS (Jacobs & Dahlman, 2001), by terminal reducing end analysis (Sturgeon, 1973), by a number of end group analysis methods (Johansson & Samuelson, 1977), viscometry and sedimentation (LeBel et al., 1963) in the range of 180-215 using viscometry for birch (Goring & Timell, 1960) and aspen (Koshijima et al., 1965) indicating that this method may provide a more accurate estimate for actual polymer size.

Possible reasons that the bleaching step increases the apparent DP_N include any one or combination of proposed mechanisms. One mechanism may be due to interference non-glycans in the precipitates. Since the BCA assay is based on quantifying the stoichiometric reduction of Cu^{2+} to Cu^{+} by the carbonyl group in a terminal aldose on a polysaccharide, polymers like proteins (which are also known to participate in this reduction) and aldehydes present in lignin

may also contribute to this reduction. Other Cu-reducing compounds would have the net effect of increasing the apparent number of polymer chains quantified and decrease the quantified value for the DP_N. If this is the case, then the observed trend in Figure 2.11, which shows the apparent DP_N increasing with increasing alkali, may be due to the increasing hemicellulose-to-lignin ratio whereby the impact of non-polysaccharides would be less at higher alkali extraction conditions. Another possibility is that the bleaching stage results in destruction of the reducing capacity of the polysaccharide end group due to modification by the oxidant (Potthast, Rosenau, & Kosma, 2006) or by alkali (Johansson & Samuelson, 1977) which would also have the net effect of decreasing the quantifiable reducing ends and increasing the apparent DP_N.

2.4 Conclusions

Overall, the quantified amount of lignin and hemicellulose extractability was dependent on the biomass source, and that increasing alkali solubilized an increasing fraction of the hemicellulose. The alcohol precipitation recovered most of the extracted hemicellulose, while acidification removed significantly less and performed particularly poorly for the maple. Precipitates for most of the biomass sources and extraction conditions contained 30-50% (w/w) hemicellulose, which was increased to 65-80% with alkaline-H_2O_2 bleaching. A notable exception was the birch extracts, which contained more than 70% hemicellulose without bleaching.

Size exclusion chromatography characterization of the recovered biopolymers indicated a low-MW lignin or lignin carbohydrate complex fraction estimated in the range of 3-12 kDa as calibrated by dextran molar mass standards. This peak could be removed by alkaline-H_2O_2

bleaching. The SEC separation of the polysaccharides exhibited a bimodal distribution with one peak in the DP range of 100-1000 and a very high MW peak at estimated DP values of greater than 1000 representing xylan aggregates. This aggregate peak could be removed by sonication. The results from the reducing end assay estimated apparent DP_N values that were significantly lower than these obtained by SEC and showed increasing DP_N values with increasing alkali that approached asymptotic limits ranging from about 25 for the switchgrass to 120 for the birch. It was found that bleaching these precipitates increased the asymptotic DP_N value from approximately 80 for the maple to 160 indicating potential interference due to other non-polysaccharides or due to oxidation of the anomeric end of the polysaccharide.

The significance of these results is that it was demonstrated the extractability and recoverability of alkali-solubilized hemicelulloses from plant cell walls which may have important implications for identifying high-yielding conditions for hemicellulose extraction prior to pulping or total sugar recovery for alkaline pretreatments. One important finding was the identification that the hemicelluloses in silver birch (*Betula pendula*), which is the most important commercial hardwood in Scandinavia and Russia for papermaking, were exceptionally well suited for extraction and yielded high recoveries and precipitates that contained a low content of contaminating lignin. It was affirmed that monocot hemicelluloses (and lignins) are highly susceptible to alkaline extraction due to the unique properties and features of their cell walls, their lignins, and potentially differences in cell wall polymer crosslinking. Additionally, this work demonstrated the potential for quantifying the aggregation of alkali-extracted hemicelluloses. This understanding of aggregation should be useful for identifying conditions that encourage polymer self-association and could be important for developing solubility-based

recovery processes to separate polysaccharides (and lignins) from alkali pulping or pretreatment liquors.

APPENDIX

APPENDIX

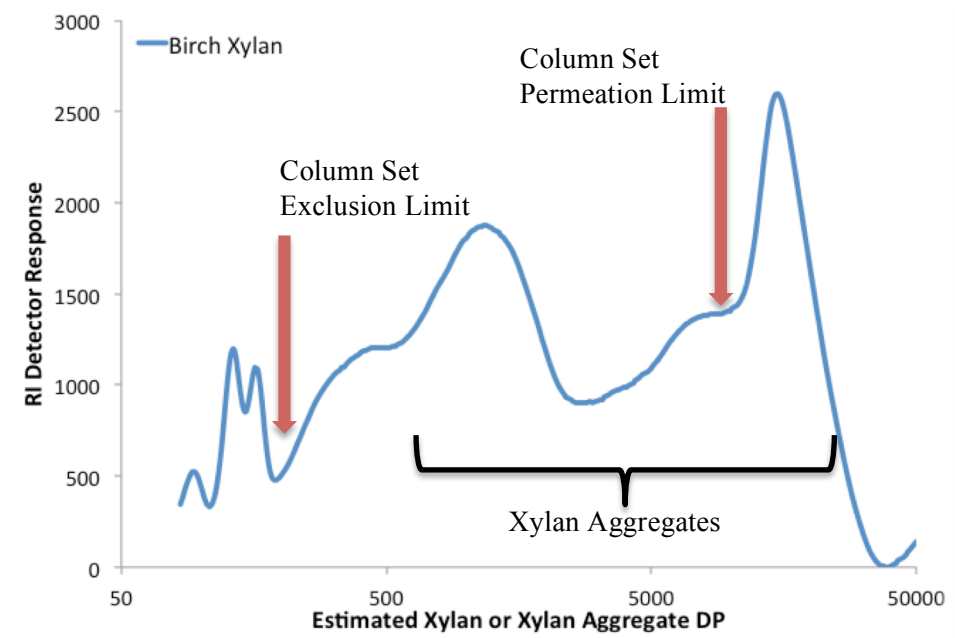

Figure A.1. Estimated degree of polymerization distribution of unsonicated Sigma-Aldrich birch xylan using Waters Ultrahydrogel 250+500 column set in series.

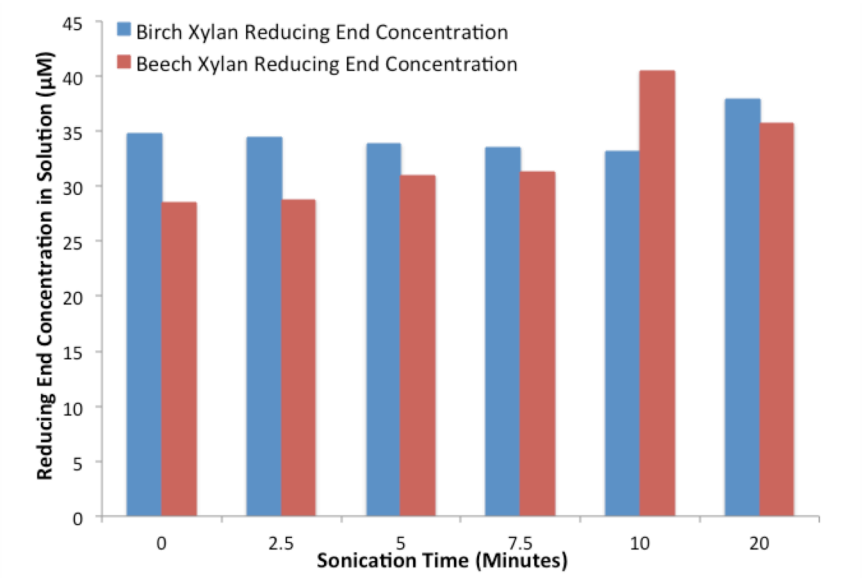

Figure A.2. Concentration of reducing ends in solution from Sigma-Aldrich beech and birch xylan sonicated for up to 20 minutes.

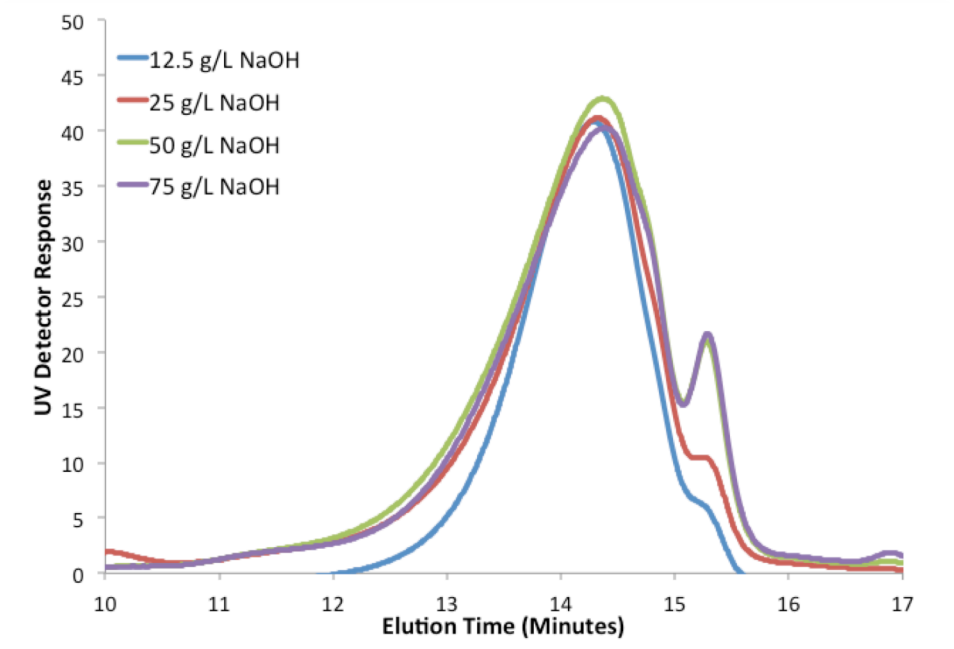

Figure A.3. UV absorbance at 280 nm of alkali-extracted, ethanol precipitated biopolymers for aspen following elution by size exclusion chromatography.

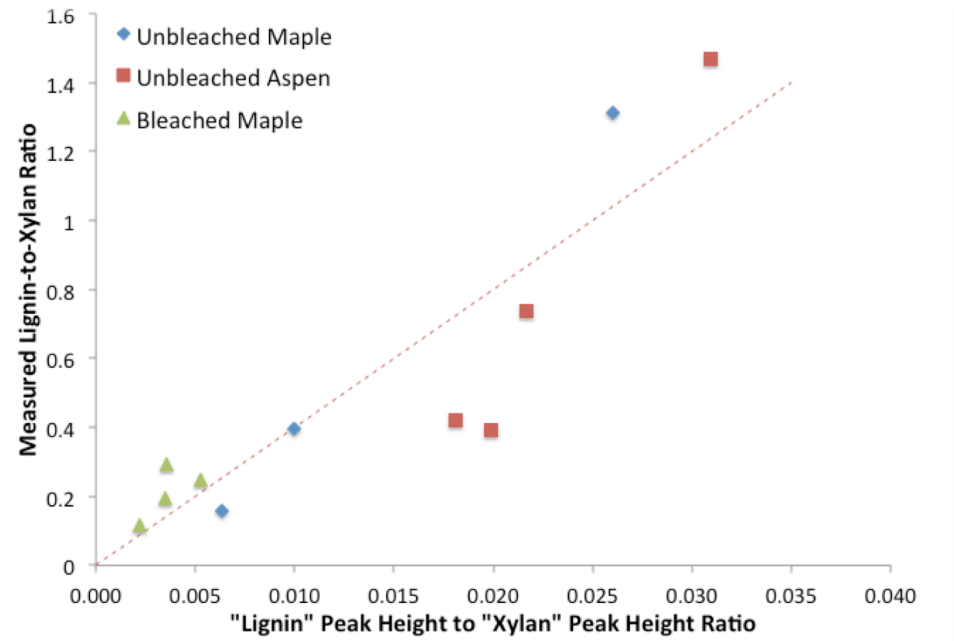

Figure A.4. Correlation of quantified lignin-to-hemicellulose ratio to the ratio of the two SEC peaks (UV Peak Height to RI Peak Height) proposed to represent aromatics and polysaccharides.

CHAPTER 3. CORRELATING LIGNIN STRUCTURAL FEATURES TO PHASE PARTITIONING BEHAVIOR IN A NOVEAL AQUEOUS FRACTIONATION OF SOFTWOOD KRAFT BLACK LIQUOR

This work has been published as original research on 16th August 2013, in *Green Chemistry*, Volume 15, and Pages 2904 – 2912.

3.1 Introduction

It is generally accepted that the sustainable production of heat, power, fuels, and chemicals is imperative for the long-term economic stability and overall human welfare (B. E. Dale & Ong, 2012). The production of fuels and chemicals from renewable feedstocks such as plant biomass is one such route for achieving this goal of long-term sustainability. One such renewable feedstock, kraft lignin, is currently produced at the scale of over $6.3x10^8$ kg of kraft lignin annually as a co-product of the chemical pulping of wood (Vishtal & Kraslawski, 2011) with the majority of this lignin burned in recovery boilers for the production of process heat and power. Lignin offers the potential to be a renewable feedstock for solid and liquid fuels, chemicals, and materials, and while the recovery of lignosulfonates for products from sulfite pulping is widely practiced, the recovery of lignin from kraft black liquors is practiced only on a limited scale. Currently, products from polymeric lignin in kraft black liquors are limited to the DMS, DMSO, $DMSO_2$ family of solvents (Calvo-Flores & Dobado, 2010), lignin precipitated by acidification for use as a solid fuel (Loutfi et al., 1991; Nagy et al., 2010; Tomani, 2010), and lignin as an emulsifier to improve the dispersion of fillers in industrial emulsions such as cement and asphalt (Lora & Glasser, 2002; Wei, Yang, Yang, & Wang, 2012). Additional proposed

products from kraft lignin include synthesis gas for the production of biofuels (Francis, Shin, Omori, Amidon, & Blain, 2006), phenolic resins, epoxy resins, and adhesives for wood composites (Stewart, 2008), sequestrants, adsorbents, aromatic monomers (Holladay et al., 2007; Lora & Glasser, 2002), and low-cost lignin-synthetic polymer blend fibers to produce carbon fibers (Suhas, Carrott, & Riberio Carrott, 2007). In addition to lignins from sulfite and kraft lignins, lignin streams will become available from the deployment of cellulosic biofuels technologies. With their diverse properties, lignins may have value for applications other than heat and power, including materials, fuels, and chemicals.

As a consequence of the random nature of polymerization to form lignin, the characterization of polymeric lignin is challenging, as individual lignin polymers may exhibit a diverse distribution of properties, structures, and chemical functionalities, with quantified properties representing average bulk values. Although recent work has been directed at characterizing individual polymers (Morreel et al., 2010), solvent and pH-based fractionation of lignins, by differences in these properties, is often applied in order to characterize the abundance and distribution of structural features in fractionated subsets of lignin polymers (Villaverde, Li, Ek, Ligero, & de Vega, 2009). Besides fractionation for the purpose of characterization, industrially it may also be desirable to generate lignin fractions that are more suitable for a given application, with properties within a desired tolerance. Important lignin properties for polymer applications include the phenolic hydroxyl content in non-syringyl monomers for phenolic resins (Froass, Ragauskas, & Jiang, 1996; Robert, Bardet, Gellerstedt, & Lindfors, 1984), thermal properties such as a narrow melting-point range for carbon-fiber applications (H. Chum et al., 1989; Elder & Soltes, 1981; Monteil-Rivera, Phuong, Ye, Halasz, & Hawari, 2013), and aliphatic hydroxyl groups and even phenolic hydroxyl groups that under certain reaction conditions have

been identified as carbamylation sites to produce polyurethanes from lignin (Yelle, Ralph, & Frihart, 2011).

Alkali-solubilized lignins are known to undergo increasing aggregation with decreasing pH due to the increasing protonation of ionizable groups in lignin (Gidh et al., 2006; Guerra et al., 2007; Ragnar et al., 2000; Sarkanen et al., 1981). This phenomenon has been exploited by a number of approaches to lignin recovery from alkaline black liquors, in which the pH is decreased with an acidifying reagent such as CO_2, acetic acid, or H_2SO_4 (Loutfi et al., 1991; Ohman, 2006) in order to effect a precipitation of lignin from solution. The resultant lignin precipitate is then recovered by filtration (Jönsson et al., 2008; Mänttäri et al., 2013). This acidification-filtration approach is currently being performed on a limited commercial scale to generate both conventional lignins from the kraft black liquors of hardwoods and softwoods (Nagy et al., 2010; Tomani, 2010), as well as sulfur-free lignins from the black liquors of soda-pulped flax (Lora & Glasser, 2002) and sugar cane bagasse (Nowakowski, Bridgwater, Elliott, Meier, & de Wild, 2010). Besides precipitation, ultrafiltration and nanofiltration have also been investigated as a strategy for the recovery of soluble lignin directly from the black liquor (Keyoumu et al., 2004).

Researchers have recently developed and investigated a novel black liquor lignin recovery technology whereby the black liquors are acidified with CO_2 under elevated temperatures and pressures and a portion of the lignin phase-separates from the bulk black liquor solution as a suspension of solvated lignin, referred to as “liquid lignin”, which can be separated without filtration (Lake & Blackburn, 2011; Velez & Thies, 2013). This lignin recovery technology is proposed to have a number of positive process attributes including improved energy economy and processability (Lake & Blackburn, 2011). To facilitate the characterization

of this liquid lignin phase, in previous work Velez and Thies (Velez & Thies, 2013) acidified an alkaline kraft black liquor with CO_2 as described above, but for their investigation the acidification was done incrementally in pH units of ~0.5, such that the fraction of the liquid lignin that separated out over a given pH range was isolated and quantified. The water content and softening point of each fraction in its "hydrated" state at process operating conditions was previously determined, as was the ash content of each fraction. However, no structural properties of the fractions isolated from the liquid-lignin phase, such as molecular weight and the frequency of ionizable groups on the lignin polymer such as aromatic hydroxyl (free phenolic), were determined. This, then, was the subject of this study. In particular, the goal of this work was to (1) characterize a number of the molecular properties for narrow pH-based fractions of softwood lignin and (2) to relate the properties of these liquid-lignin fractions to their pH-driven, phase-partitioning behavior.

3.2 Material and Methods

3.2.1 Materials

As described above and reported in detail elsewhere (Velez & Thies, 2013), "liquid lignin" fractions were phase-separated from an industrial softwood alkaline black liquor via a unique pH-based fractionation technique, using CO_2 as the acidifying reagent at elevated temperatures and pressures (115°C and 6.2 bar). Upon acidification, these lignin fractions phase-separated and were collected as either a continuous "liquid lignin" phase or a hydrated lignin precipitate containing between 30-50% water by weight. Information about each of these fractions, including the pH at the end of each fraction collection and the mass percentage of each fraction relative to the total lignin recovery, is given in Table 3.1.

Table 3.1. Liquid-lignin fractions obtained by the unique pH-based fractionation process (Velez & Thies, 2013) and used for this study.[a]

		Mass Percentage of Lignin Recovered	
Liquid-Lignin Fraction	Final pH Obtained Per Fraction	Per Fraction (%)	Cumulative Recovery (%)
F1	12.8	0.8	0.8
F2	12.1	0.6	1.4
F3	11.6	3.4	4.8
F4	11.1	31.8	36.6
F5	10.6	36.2	72.8
F6	10.0	22.6	95.4
F7	9.5	4.6	100
[a]Softwood Kraft black liquor with pH of 13.6 served as feed to the acidification process			

These fractions were subsequently further acidified with 1N H_2SO_4 to a pH of 2.5 as suggested by Compere and Griffith (Compere & Griffith, 2009). A schematic of this procedure is given in Figure 3.1.

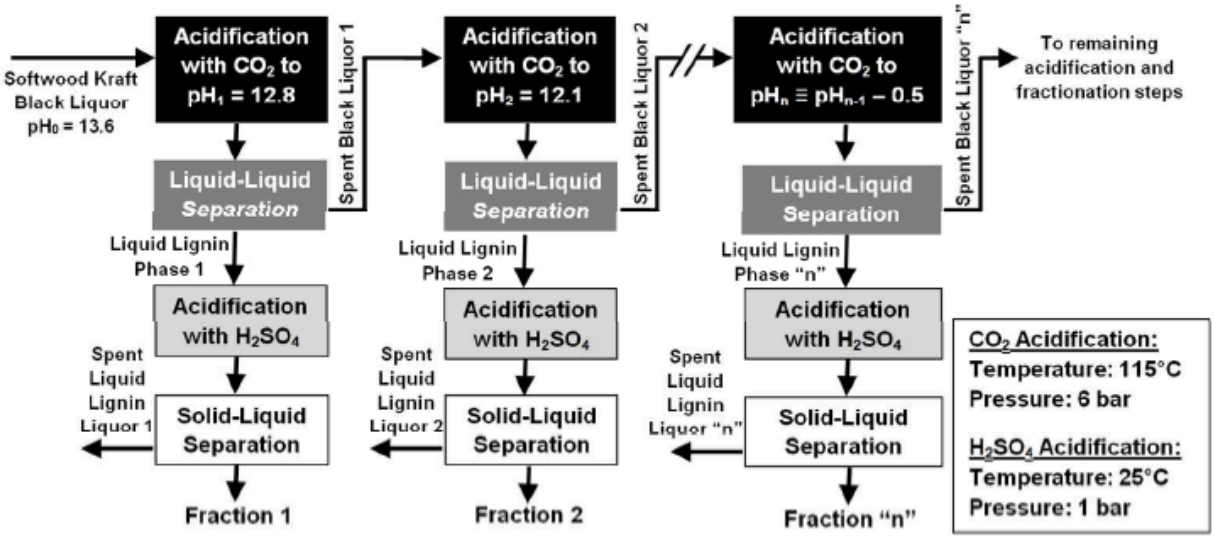

Figure 3.1. Schematic of the pH-based fractionation process for separation of liquid-lignin fractions within narrow (~0.5) pH bands from kraft black liquor via acidification with CO_2 at elevated temperatures and pressures.

Note that fresh softwood kraft black liquor was used as the feed only for the first fractionation. The partially spent black liquor from the first fractionation (*i.e.*, the un-precipitated top phase designated as Spent Black Liquor 1) served as the feed to the second fractionation, Spent Black Liquor 2 from the second fractionation served as the feed to the third fractionation, *etc*. This procedure was repeated to yield fractions at approximately every 0.5 pH increments, with early fractions collected over a wider pH band due to the lower recovery yields. After vacuum-filtering the fractions from the spent acid solution, the samples were air-dried and their ash content was determined using NREL/TP-510-42622 (Sluiter, Hames, et al., 2005). Klason lignin and neutral polysaccharide content were quantified by NREL/TP-510-42618 (Sluiter et al., 2008), and the uronic acid content was assayed enzymatically with a D-Glucronic/D-Galacturonic Kit (Cat. No. K-URONIC, Megazyme Intl. Ireland, Bray, IE).

3.2.2 Lignin acetylation

The lignin fractions were acetylated for analysis by ^{1}H NMR and Gel Permeation Chromatography (GPC) according to the acetylation procedure used by Gosselink et al. (Gosselink et al., 2004), except that ethanol was used in place of methanol. First, approximately 400 mg of a lignin fraction was suspended in 8 mL of a 1:1 (v/v) solution of acetic anhydride and pyridine. For lignin fractions F1 and F2 (see Table 3.1), only 80 mg of material was suspended in 1.5 mL of the acetylating mixture due to the limited availability of sample. The acetylation was allowed to proceed for 24 hours at room temperature in a shaking incubator at 180 rpm. Then 30 mL of ethanol was added to each sample, followed by brief stirring and incubation for an additional thirty minutes. Solvents were then removed by drying under nitrogen. This ethanol washing and nitrogen-drying procedure was repeated three times to ensure a complete removal

of the acetic acid and pyridine. The remaining material was dissolved in 25 mL of $CHCl_3$ and then washed twice with 250 mL of deionized water using a separatory funnel. The $CHCl_3$ solution was then added slowly to 75 mL of diethyl ether to precipitate out the acetylated lignin fraction.

3.2.3 Proton and quantitative ^{13}C NMR Analysis

Quantitative ^{1}H NMR was performed with an Agilent 500/54 Premium Shielded 500 MHz NMR equipped with a Varian 7600 Autosampler. A sample of lignin was dissolved at a concentration of 44 mg/mL in deuterated chloroform containing pentaflurorbenzaldehyde (PFB) as an internal standard and a trace amount of tetratmethylsilane (TMS) as a reference peak. Samples were run using a 30° pulse with a 4 s pulse delay and a total of 800 scans. Integration was performed using VnmrJ v. 3.2 software (Agilent Technologies, Santa Clara, CA) using the integration regions for protons in acetylated aliphatic hydroxyl, acetylated phenolic hydroxyl, and methoxyl groups with peak assignments according to Ralph et al (Ralph, Ralph, & Landucci, 2004). Figure B.1 in the Appendix on page 88 gives a reference to the peaks identified.

Quantitative ^{13}C NMR was performed on fractions F4-F7 in DMSO-d_6 at a sample concentration of 300 mg/mL using a 30° pulse angle with a recycle delay time of 2 s, an acquisition time of 1.36 s, and an observed pulse of 3.4 μs using a minimum of 5000 scans. The analyses included a decoupling mode to reduce the Nuclear Overhauser Enhancement (NOE). Integration was done on the regions showing methoxyl functionality (δ = 54 – 57 ppm) and C_γ in the β-O-4 structure (δ = 59 – 61 ppm), with the relative abundance of a β-O-4 taken as the ratio of this peak to the methoxyl content based on the peak assignments of Ralph et al (Ralph et al.,

2004). Figure B.2 in the Appendix on page 88 gives a representative spectrum used to quantify β-O-4 content. Other structures that contained a hydroxylated C_γ were presumed to have chemical shifts far enough downfield (δ = 61.8 - 64.4 ppm) from the C_γ in a β-O-4 (δ = 60.0 ppm) that they would not overlap.

3.2.4 Gel permeation chromatography

Gel permeation chromatography (GPC) was performed on an Agilent 1100 series HPLC equipped with a Waters Styragel HR 4 (Milford, MA, USA) column, using either a system consisting of tetrahydrofuran (THF) at a 0.5 mL/ min flow rate and 40°C for acetylated lignin samples or a system consisting of dimethylformamide (DMF) and 0.05 M LiCl at a flow rate of 0.5 mL/min and 80°C with detection by UV absorbance at 280 nm. Lignin samples were dissolved in the mobile phase and syringe-filtered (0.45 μm PTFE membrane, Whatman Puradisc Syringe Filters, GE Healthcare, Waukesha, WI) prior to analysis. Monodisperse polystyrene standards (1.0, 10, 50, 100, 200, 600 kDa, Sigma-Aldrich, St. Louis, MO) were run on each system for reference. Based on the reference elution times, number and weight average molecular weights of the lignin fractions were obtained per the procedure given by Malawer and Senak (Malawer & Senak, 2004).

3.2.5 Thermogravimetric analysis

Thermogravimetric analysis (CH-8603 model, Mettler-Toledo, Schwerzenbach, CH) was employed to determine the mass loss during slow pyrolysis using nitrogen as the purge gas with a cell and furnace flow rate of 20 mL/min and a temperature profile of 10°C/min. For each sample, the furnace was initially purged with nitrogen for 10 min; then the sample was dried for

20 minutes at 105°C. Masses were normalized to the weight remaining after complete water volatilization at 115°C. The normalized mass loss data was plotted as differential thermo-gravimetric curves from 115°C – 800°C.

3.2.6 Analytical pyrolysis

Analytical pyrolysis was performed as reported previously (Li et al., 2012) using a microscale pyrolysis unit (CDS Pyroprobe 5250, CDS Analytical, Inc., Oxford, PA) interfaced to a Shimadzu QP-5050A gas chromatograph/mass spectrometer (GC/MS; Shimadzu Corp, Columbia, MD). Approximately 1 mg of lignin sample was packed between quartz wool in a quartz tube with a filler rod. Three replicates of each sample were run. The pyroprobe was set at a filament temperature of 650°C at a heating rate of 1000°C/s and a hold time of 20 s. The GC was equipped with a Restek Rtx-1701 column (Restek, Bellefonte, PA), 60 m x 0.25 mm with a 0.25 μm film thickness. The column gas (helium) flow was 1 cm/s with a split ratio of 1:100 so as to not overwhelm the mass spectrometer. The GC oven temperature program began with a 1-min hold at 40°C followed by a heating rate of 8°C/min to 270°C. The injector and detector temperatures were set to 280°C. The mass spectra were recorded in the electron ionization mode for *m/z* from 33 to 400. Identification of compounds was performed by comparing the mass spectra of the peaks with standard spectra of other compounds from the NIST library to obtain the most probable matches. Pure compounds (Sigma-Aldrich Co., St Louis, MO) were then used to confirm the identities of certain peaks based on matching of retention times and mass spectra.

3.2.7 Data correlation

Correlation coefficients for selected properties of lignin fractions F3 through F7 were determined in MATLAB (MathWorks, Natick, MA), using a hierarchical ordering of correlation coefficients according to the shortest Euclidian distance between clusters. Incomplete data for β-O-4 content were handled by determining the correlations only between fractions F4-F7.

3.3 Results and Discussion

3.3.1 Lignin yields and non-lignin contaminants

Physical properties of the seven fractions isolated by our unique pH-based fractionation process, using the methods briefly described in sections 3.1 and 3.2, and in detail elsewhere (Velez & Thies, 2013) are given in Table 3.1. The pH at which the collection of a given fraction was terminated is shown, and the mass percent of total lignin in each fraction is given on an ash-free basis. Results indicate that the majority of the lignin phase-separates between a pH of 10.0 and 11.6, corresponding to fractions F4, F5, and F6, which comprise more than 90% of the precipitated material.

Non-lignin contaminants such as inorganics, polysaccharides, and extractives (*e.g.,* resin and fatty acids, sterols/terpenoids, and flavonoids/tannins) are detrimental in some lignin applications (Compere & Griffith, 2009); for example, for carbon-fiber applications they can contribute to an undesirably broad melting point (Compere et al., 2005). During black liquor acidification, it is known that proteins, polysaccharides, and some extractives can precipitate out at a higher pH than the bulk of the lignin (Compere & Griffith, 2009; Dai, Jameel, & Chang, 2006). Thus, this pH-based fractionation process could result in high levels of contaminants in the first fractions to phase-separate. However, all samples were found to be comprised primarily

of lignin as determined by ^{1}H NMR, which quantified comparable methoxyl contents (between 3.0 and 6.4 mmol/g lignin, see Appendix page 89, Figure B.3) for all samples. The neutral polysaccharide content of fractions F3 through F7, along with the estimated bulk polysaccharide content, are presented in Figure 3.2.

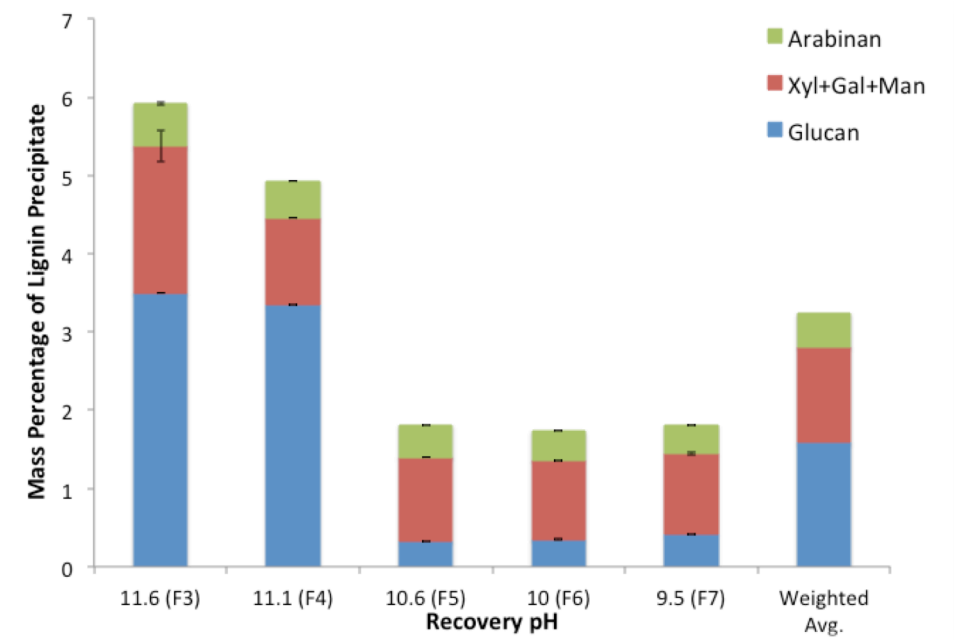

Figure 3.2. Quantified neutral polysaccharide content of fractions F3 through F7 with estimated weight average polysaccharide content.

Sugar composition analysis was not performed on fractions F1 and F2 because of the limited quantities of sample that were available. The weight-averaged content of neutral polysaccharides in the precipitated lignin samples, expressed as a percentage of the total lignin precipitate from the black liquor, was 2.91% by weight and consisted primarily of the polysaccharides contained in GGMs and arabinoxylans. Fractions F3 and F4 are more highly enriched in polysaccharides, particularly in glucan, relative to the overall, weight-averaged polysaccharide content, while fractions F5 through F7 contain significantly less polysaccharides than the bulk. The differences in glucan content between the fractions indicates that the glucomannans are more likely to

partition into the liquid-lignin phase at a higher pH than the xylans. The content of uronic acids in the fractions was below the detection limit, although this may potentially be due to their conversion to hexenuronic acids.

Using ^{1}H NMR, the abundance of the aliphatic extractives (*e.g.,* resin and fatty acids, sterols/terpenoids) relative to lignin was estimated (Figure 3.3) based on integration of the aliphatic -CH_2- and -CH_3 peaks (δ = 0.7-1.4 ppm).

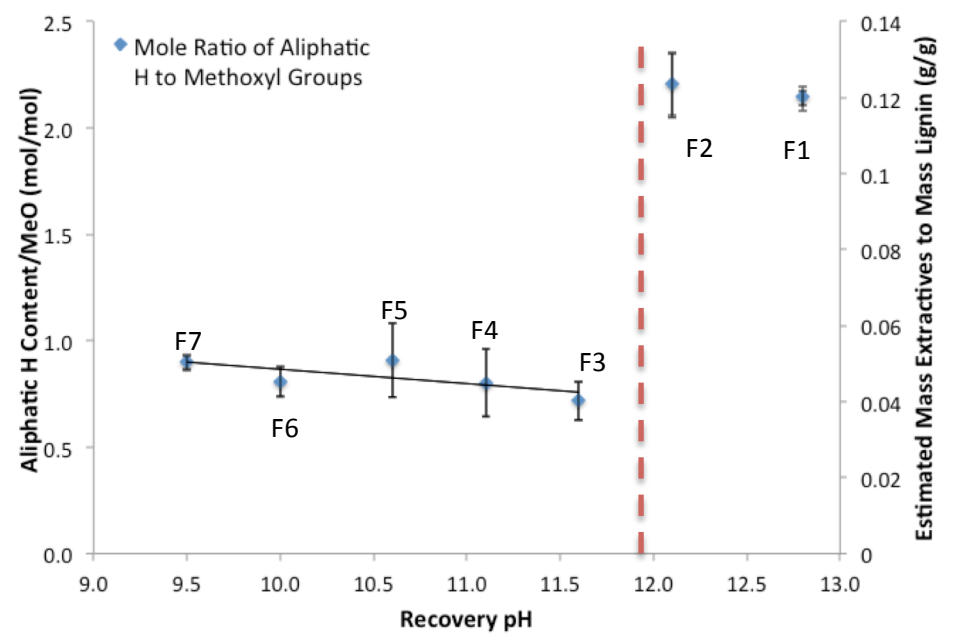

Figure 3.3. Quantified aliphatic H to MeO ratio of fractions F1 through F7 as determined by ^{1}H NMR as a proxy for extractives-to-lignin ratio.

These are presumed to originate primarily from extractives, as polysaccharides and the aliphatic side regions of lignin should not yield signals in this region with the exception of a terminal methyl group. This integration is normalized to the molar methoxyl content based on integration of the methoxyl peak (δ = 3.5-4.0 ppm), which is taken to represent a single lignin monomer because (1) the overwhelming majority of the lignin is presumed to be comprised of guaiacyl

monomers containing a single methoxyl group and (2) demethoxylation is not considered to be significant during kraft delignification (Pinto, Evtuguin, Neto, Silvestre, & Amado, 2002). Assuming that terpenes and fatty acids have a mass-to-proton ratio of approximately 10 g/mol H, while a guaiacyl monomer in lignin will have a molar mass of approximately 180 g/mol monomer, the same data can be presented as an approximation on a mass ratio basis (see secondary y-axis on Figure 3.3). These results show two notable trends: (1) fractions F1 and F2 contain two to three times the extractives than the other fractions (approximately 0.12 g aliphatic extractives per g lignin versus 0.04-0.07 for the other fractions), and (2) the aliphatic extractives-to-lignin ratio in fractions F3 to F7 shows a slight increase with decreasing pH of fractionation.

3.3.2 Structural features of recovered lignin fractions

^{1}H NMR also quantified the relative abundance of aromatic and aliphatic hydroxyls in acetylated lignin fractions (Figure 3.4). These results are presented on a mole hydroxyl per mole methoxyl basis, which can be considered as a proxy for a per mole lignin monomer basis as defined previously. Each of these sources of hydroxyl groups shows a clear trend for fractions F3 through F7, where the bulk of the material separates and contains the least non-lignin contaminants. The trend for aliphatic hydroxyl content exhibits minimal variation between the fractions, with an average value of 1.16 mol of OH mole per monomer, while the phenolic hydroxyl content shows a nearly 50% increase with decreasing pH from 0.47 to 0.69 mol of OH per monomer. This is a significant result, as it demonstrates that the aliphatic hydroxyl content of a lignin species does not contribute (either directly or indirectly) to its tendency to phase-separate from solution during pH-based fraction.

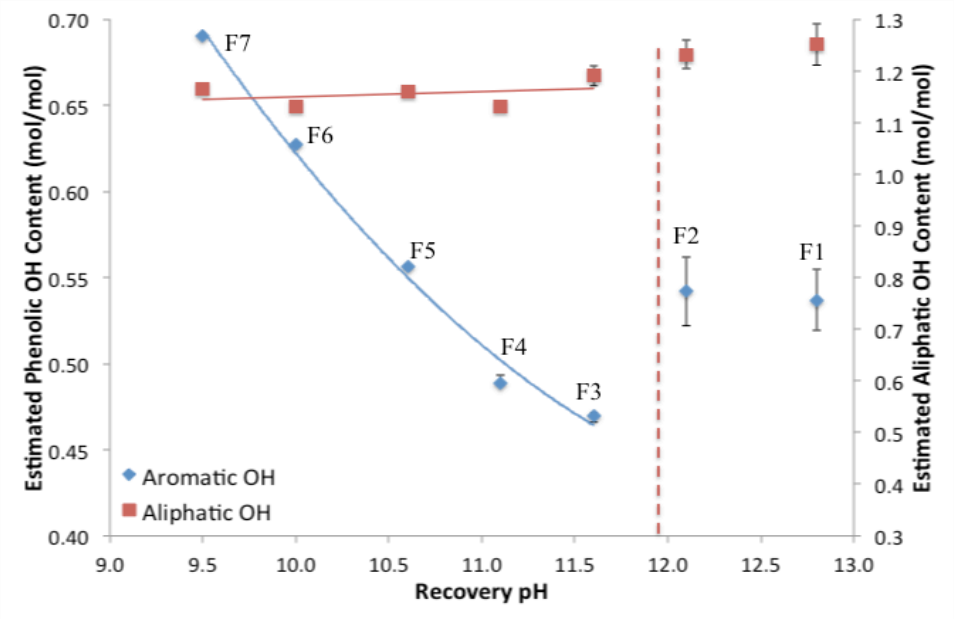

Figure 3.4. Phenolic hydroxyl and aliphatic hydroxyl content of lignin fractions by ^{1}H NMR (mol OH/mol lignin monomer).

Conversely, phenolic hydroxyl content is a strong predictor of the tendency of a lignin to phase-separate from solution as a function of pH, either directly (*i.e.*, by pH-dependent deprotonation) or indirectly (*e.g.,* as it relates to another property such as molecular weight, as phenolic hydroxyl content increases with decreasing lignin molecular weight).

The relative content of C_γ in a β-aryl ether with respect to the methoxyl content, again as a proxy for a mole of lignin monomer, was determined by quantitative ^{13}C NMR (Figure 3.5). This result shows that the carbon content present in this configuration undergoes about a 40% decrease with decreasing pH of fractionation, which may be a consequence of a decrease in the overall molecular weight of the lignin.

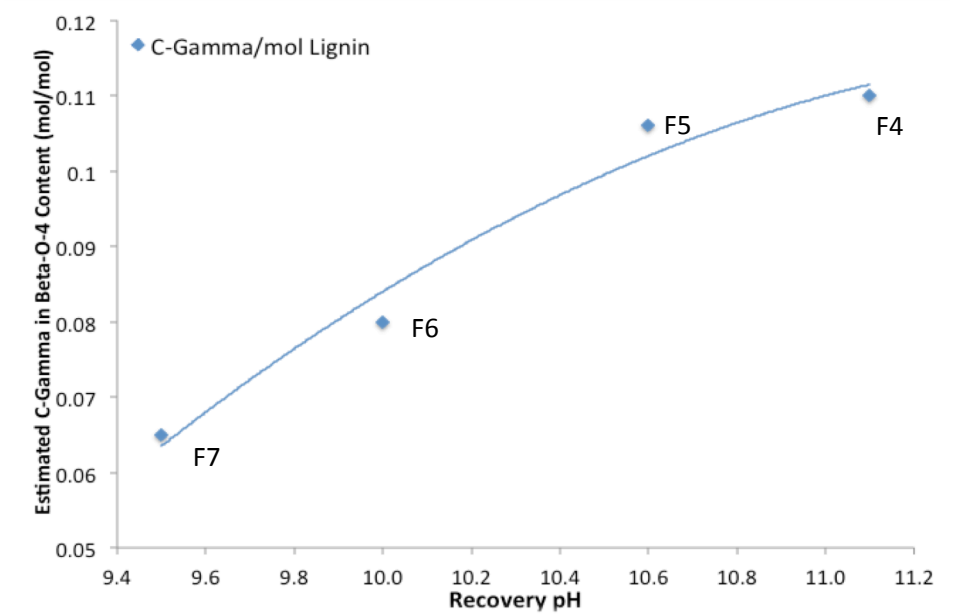

Figure 3.5. Decrease in C_γ (mol C_γ/mol lignin monomer) with decreasing separation pH by ^{13}C NMR.

Interestingly, the total molar aliphatic hydroxyl content as determined by ^{1}H NMR (Figure 3.3) is about an order of magnitude greater than the molar β-O-4 content as determined by ^{13}C NMR (Figure 3.5). This can be interpreted as meaning the aliphatic hydroxyl groups are predominantly contained on other lignin structures (*e.g.*, terminal aliphatic hydroxyl or one of at least four other linkages containing a hydroxylated C_γ).

3.3.3 Relative lignin molecular weight distributions by gel permeation chromatography (GPC)

Lignin self-aggregation is significant in aprotic solvents such as THF (Crestini, Melone, Sette, & Saladino, 2011), necessitating the derivatization of hydroxyl groups, for example, by acetylation before GPC analysis can be undertaken. Using acetylated lignin fractions and a mobile phase of THF, estimates of number and weight average molecular weights were obtained by GPC (Figure 3.6).

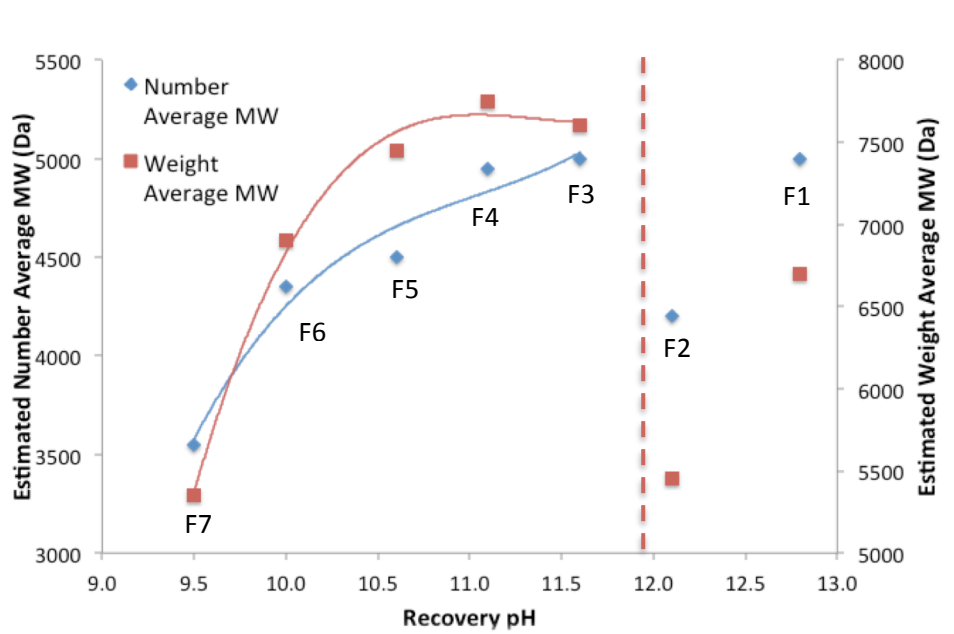

Figure 3.6. Estimated lignin molecular weights by GPC (acetylated lignin, THF mobile phase).

Column retention volumes were calibrated using monodisperse polystyrene standards. The use of GPC as a tool for quantitatively determining lignin molecular weights is problematic in that reported results and are strongly dependent on the solvent properties (*e.g.*, pH, ionic strength, etc.), lignin properties (*e.g.*, the tendency for lignin to self-associate during analysis and interact with the stationary phase in the column), and the detection method (Cathala, Saake, Faix, & Monties, 2003; Contreras, Gaspar, Guerra, Lucia, & Argyropoulos, 2008; D. Dong, 1993; Glasser, Dave, & Frazier, 1993; Gosselink et al., 2004; Guerra et al., 2007; Harton et al., 2012). These results show that with decreasing pH, the molecular weight of the precipitated liquid-lignin fractions decreases. Thus, the lowest MW lignin species are the last to phase-separate from solution during pH-based fractionation.

The use of strong polar solvents such as DMF in conjunction with electrolytes such as LiBr or LiCl can yield effective solubilization of underivatized lignin, as well as effective

separation which can be size-fractionated by GPC (Baumberger et al., 2007). However, it has been noted that polystyrene elutes more slowly than comparably-sized lignin polymers in this system, resulting in unusually high molecular weight estimates for lignin when using polystyrene for the reference standards (H. L. Chum, Johnson, Tucker, & Himmel, 1987). This behavior was confirmed by our GPC results for underivatized lignin in DMF + 0.05 M LiCl (Figure 3.7). It can be observed that fractions F1 through F4 have a high molecular weight tail, while fractions F5 through F7 are less polydisperse. Comparing the lignin elution profiles, a decrease in molecular weight is apparent with decreasing pH of fractionation for fractions F3 through F7 (*i.e.*, the retention times are shifted to higher elution volumes).

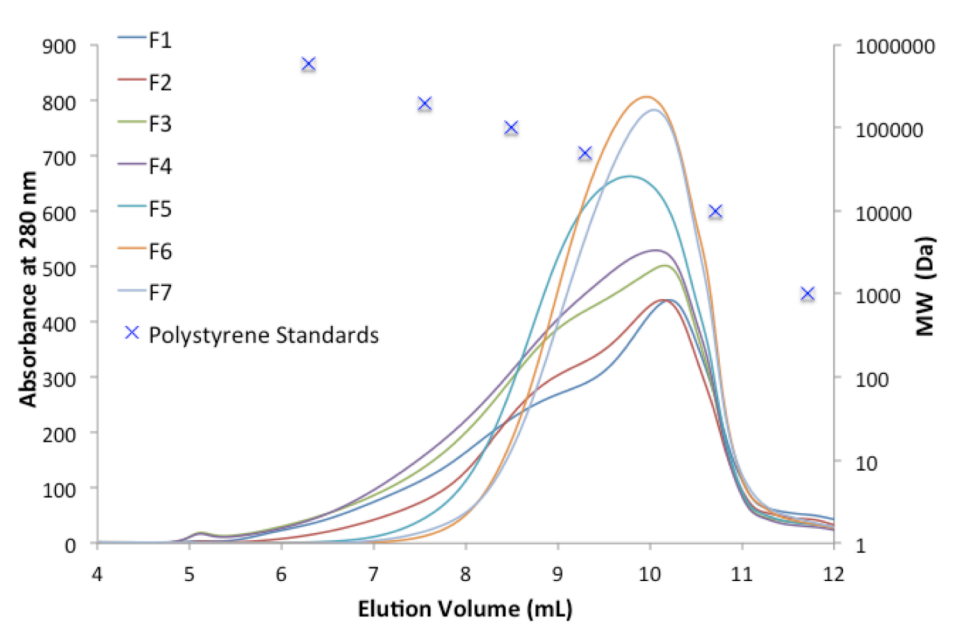

Figure 3.7. GPC elution profiles of nonacetylated lignin fractions in DMF + 0.05 M LiCl mobile phase, referenced to polystyrene standards.

These results provide a second independent validation of the trends (but not the absolute values) for the molecular weights obtained using the THF system (Figure 3.6) of decreasing molecular weight with decreasing fractionation pH.

3.3.4 Thermogravimetric analysis of lignin fractions

Results from the thermogravimetric analysis (TGA) of fractions F3-F7 are presented in Figure 3.8; TGA for the entire liquid-lignin phase (still recovered by acidification with CO_2, but not incrementally so as to produce fractions) is also included as a reference.

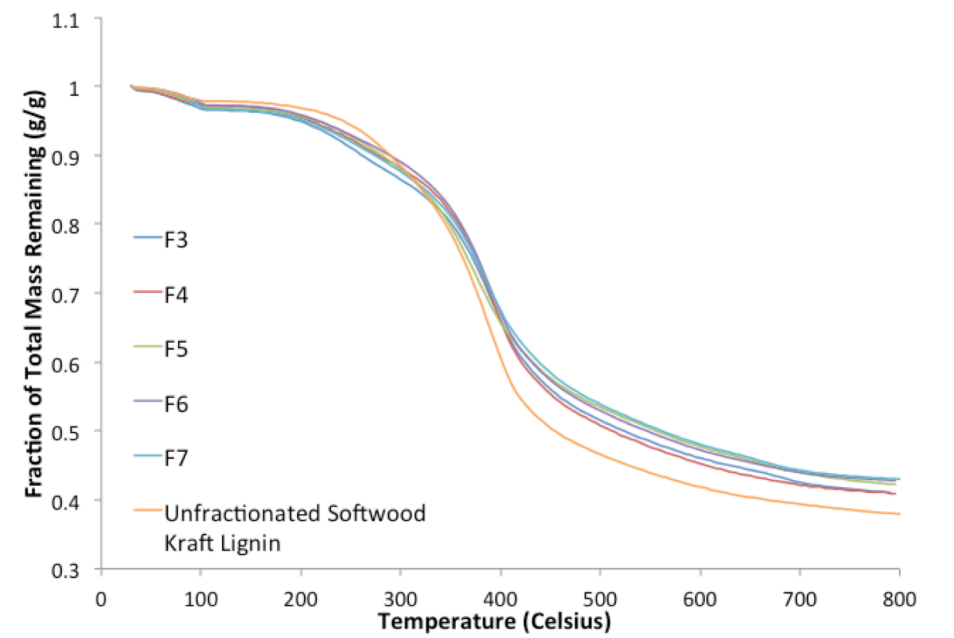

Figure 3.8. Lignin fraction mass loss by TGA.

The mass-loss curves (Figure 3.8) are normalized to include only the mass remaining after complete water volatilization from the samples has occurred at 105-115°C. These results show comparable mass volatilization profiles for all fractionated samples, with the mass fraction of the original sample remaining being 0.421–0.445 when the temperature reaches 800°C. The

derivative curves for the rate of volatilization (-dm/dT) are presented in Figure 3.9 and exhibit three distinct peaks, representing the loss of water (50-110°C), the loss of polysaccharides (200-300°C), and loss of the bulk lignin (300-500°C) (Garcia-Perez, Chaala, Yang, & Roy, 2001).

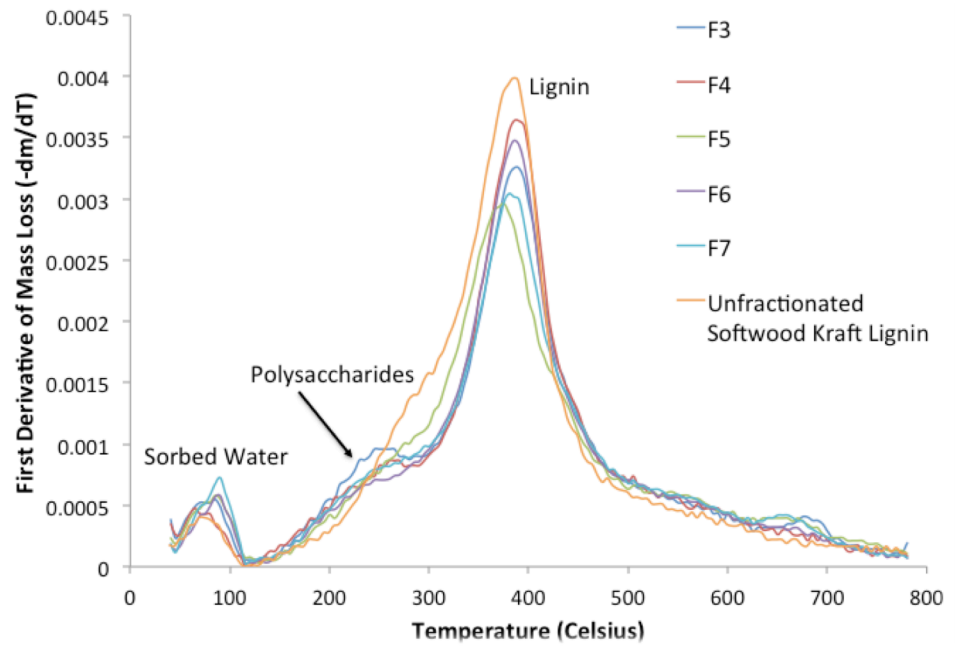

Figure 3.9. First derivatives of mass loss curves for lignin fractions.

Minor differences in these three peaks were observed among the lignin fractions (F3–F7). The water peak is associated with the sorbed water, and the thermal behavior of this water (*i.e.,* the heat of desorption of water in the heating range of 80-130°C) has been correlated to the phenolic hydroxyl and carboxyl content of the lignin (Vasile, Popescu, Stoleriu, & Gosselink, 2006). The peak associated with aliphatic hydroxyl groups, corresponding primarily to polysaccharides (Garcia-Perez et al., 2001; Mousavioun & Doherty, 2010), is most prominent for fractions F3 and F4 (which both show a maximum rather than an inflection point as in the other samples) and is in agreement with the composition results of Figure 3.2, which show the highest

polysaccharide content for these fractions. The mass-loss peak associated with the bulk of the lignin is relatively unchanged between lignin fractions with the exception of fraction F5, which exhibits a lower onset temperature. As would be expected, the derivative curve for the unfractionated lignin bounds all of the derivative curves for the lignin fractions (Figure 3.9).

3.3.5 Analytical pyrolysis

The distribution and relative abundance of aromatic monomers generated from the lignin fractions by analytical pyrolysis are presented in Figure 3.10. Aromatic compounds are grouped as dimethoxybenzene or dimethoxyphenol (S), guaiacol (G), or phenol (Ph) monomers containing one (C_1), two (C_2), or three (C_3) additional carbons, with abundances being defined as a percentage of the total summed peak areas. Identification of the aromatic compounds used in this analysis is provided in the Appendix on page 89, Table B.1.

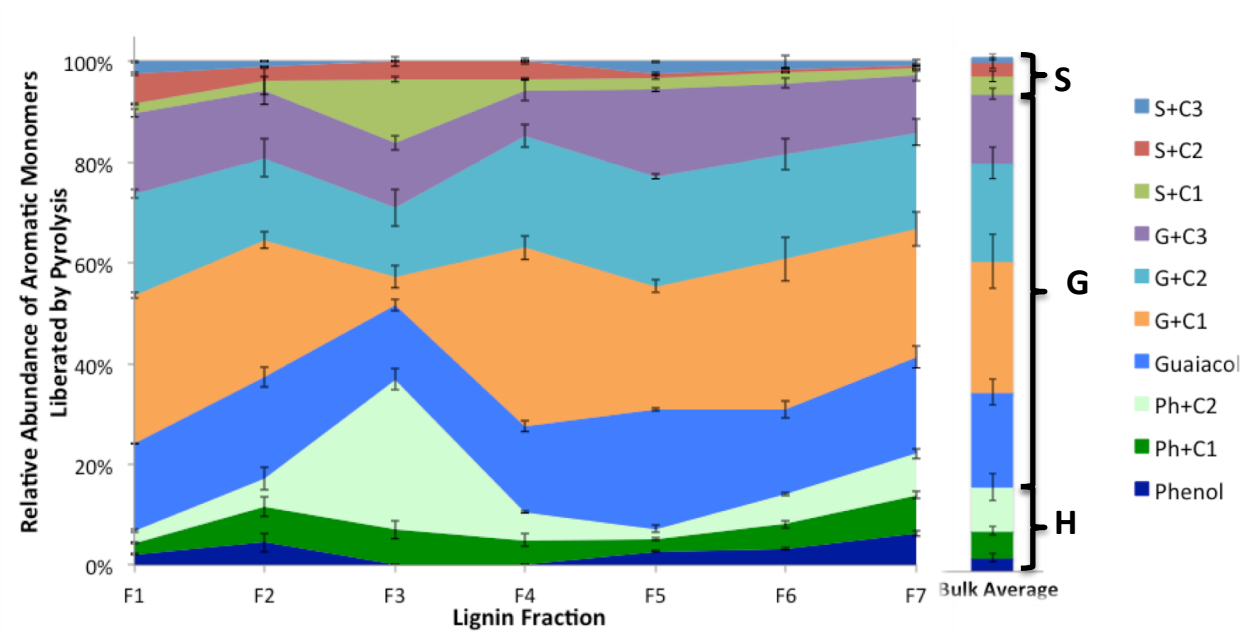

Figure 3.10. Relative normalized abundance of aromatic monomers released by pyrolysis of lignin.

As the calculated abundances do not consider differences in response factors for each compound, this approach is an approximation of the relative abundance of these sources of aromatic monomers. However, this is the method that is generally applied by researchers for S/G ratio estimation (Sykes et al., 2009). It should be noted that the dimethoxylated aromatics are not likely to originate from syringyl lignins, as most gymnosperms lack the enzymes required for sinapyl alcohol biosynthesis (Weng & Chapple, 2010). Instead, these dimethoxylated aromatics are most likely the result of chemical rearrangement during pyrolysis, or they may represent a single carbon remaining from a residual alkyl-aryl ether bond.

As shown in Figure 3.10, all fractions exhibit a comparable distribution of aromatic products, albeit with notable exceptions. Interestingly, while the dimethoxylated lignins comprise a small fraction of total aromatics (with a mass-averaged bulk composition of 6%), they encompass a wide range of abundances among the fractions (3.6-14.5%). In particular, these are most enriched in the fractions recovered at the more alkaline pH values and especially fraction F3. The unmethoxylated phenolics comprise 11% of the mass-averaged bulk composition but have a range of between 7 and 34% of the total aromatics across all fractions. Again, fraction F3 shows substantially more Ph+C_2 (primarily consisting of *p*-xylenol) relative to the other fractions. Considering that fraction F3 represents only 3.1% of the mass of the lignin, the overall contribution of this difference is relatively minor. Nevertheless, this result provides evidence that the lignins are more likely to form unmethoxylated phenolics upon pyrolysis (*e.g.*, non-guaiacyl lignins) that phase-separate out at higher pHs, and that the guaiacyl-only monomers preferentially separate out at the lower pH values.

3.3.6 Property correlations

Many of the quantifiable properties of the lignins recovered in the pH range where the majority of the mass is recovered (*i.e.,* fractions F3-F7) correlate to each other and to the pH at which the fractions were recovered. With the use of a correlation map (Figure 3.11), these linear dependencies are presented simultaneously for fractions F3-F7 and show exceptionally strong correlation, with $|R| > 0.95$ between a number of the lignin properties, as discussed below.

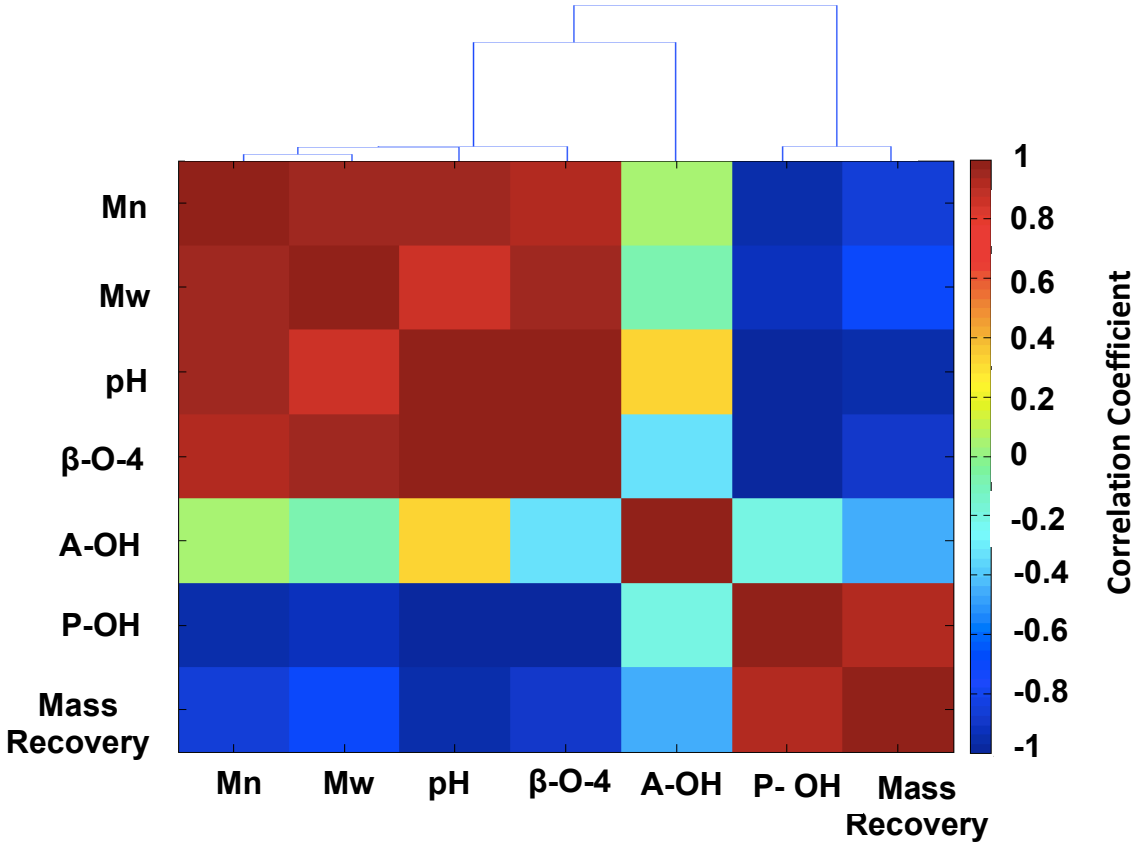

Figure 3.11. Correlation map for lignin properties of fractions F3-F7 demonstrating strong correlations between most properties; A-OH = Aliphatic OH, P-OH = Phenolic OH.

The first trend of importance observed is the relationship between the phenolic hydroxyl content and the cumulative mass recovery, which can serve as a proxy for the alkali solubility. A high alkali solubility means that the species in a given lignin fraction are highly soluble in the

caustic black liquor and tend to stay in solution, and not separate out as a separate lignin phase, even as the pH is lowered via CO_2 acidification. The phenolic hydroxyl content is known to contribute to improved alkali solubility (Froass, Ragauskas, & Jiang, 1998; Lapierre et al., 1989), which is one reason for lignin solubilization and removal from the cell wall during alkaline pulping (D. J. Dong & Fricke, 1995). For example, the range of phenolic hydroxyl content in the kraft lignins in the present work (from 0.47 to 0.69 mol per monomer, see Figure 3.4) is significantly higher than the phenolic hydroxyl content of relatively unmodified wood lignins (typically less than 0.1 mol/lignin monomer) (Chen, 1992). This positive relationship between alkali solubility and free phenolic content has been observed in the literature, for example, for the acidification of steam-exploded aspen lignin (H. L. Chum, Ratcliff, Schroeder, & Sopher, 1984) and for the CO_2-based precipitation of softwood kraft lignins (Nagy et al., 2010). For the latter case, the phenolic hydroxyl content (as well as carboxyl hydroxyl and aliphatic hydroxyl content) as determined by ^{31}P NMR of phosphitylated lignin correlates with alkali solubility, that is, with a tendency for the lignin fraction to remain in solution with decreasing pH. Dong et al. (D. J. Dong, Fricke, Moudgil, & Johnson, 1996) identified a strong pH-dependence in the zeta potential of kraft lignins, indicating that ionizable groups in lignin may contribute significantly to their aggregation or repulsion. Finally, we note that the acid dissociation constant of phenolic hydroxyl groups (pK_a = 10.5) is well within the range of our pH-based fractionation experiments (Guerra et al., 2007; Ragnar et al., 2000); thus, it represents an important property with respect to the phase partitioning of liquid lignin.

GPC-determined lignin molecular weights exhibited a strong negative correlation with the phenolic hydroxyl content and a strong positive correlation with the β-O-4 content (Figure

3.11). Both of these results are logical, considering that β-aryl ethers are the primary target of kraft delignification and that the scission of a β-aryl ether results in the generation of a "free" phenolic hydroxyl group, which confers alkali solubility to these lignin fragments and results in a decrease in the molecular weight. This relationship has been quantified in the literature, where the free phenolic content has been found to be strongly inversely correlated to the β-O-4 content in kraft delignified softwoods (Koda, Gaspar, Yu, & Argyropoulos, 2005) and to the molecular weight in diverse lignins modified by fungal laccases (van de Pas et al., 2011).

3.4 Conclusions

Liquid lignin fractions solvated in water were recovered from softwood kraft black liquor using a novel aqueous fractionation process involving sequential CO_2 acidification. The bulk of the material was found to phase-separate between a pH of 11.6 and 10.0. The fractions were characterized for a range of structural features. Based on the aromatic methoxyl content of these fractions, it was concluded that the majority of the material by mass in all recovered fractions is lignin. However, the fractions recovered at the highest pHs were enriched in aliphatic extractives and polysaccharides relative to the other lignin fractions. Additionally, these higher-pH lignin fractions were found by pyrolysis GC/MS to yield the highest fraction of non-guaiacyl lignins, with the dimethoxylated aromatics potentially corresponding to higher aryl ether content. The aliphatic hydroxyl content was not discernibly different between all of the various fractions, while DGA/DTA curves showed differences only in the polysaccharide mass-loss peaks.

However, these solvated liquid-lignin fractions exhibited several notable trends in their structural properties that can be related to their phase-partitioning behavior with respect to pH. Specifically, the phenolic hydroxyl content of a lignin fraction as determined by ^{1}H NMR was

found to exhibit a strong negative linear function with respect to the pH at which that fraction phase-separated. In other words, lignin fractions with a lower phenolic hydroxyl content were found to phase-separate from solution first, that is, at the highest pH values. The phenolic hydroxyl content also had a strong negative correlation to the estimated molecular weights of the lignin fractions as determined by GPC, but a strong positive correlation to the estimated β-O-4 content as determined by quantitative ^{13}C NMR. Thus, fractions with the highest molecular weights and β-O-4 content separated out to form a liquid-lignin phase at a higher pH. Taken together, this study provides encouraging evidence that our unique pH-based fractionation approach can be employed to generate kraft lignins that are enriched or depleted in various structural properties relative to the bulk lignin.

APPENDIX

APPENDIX

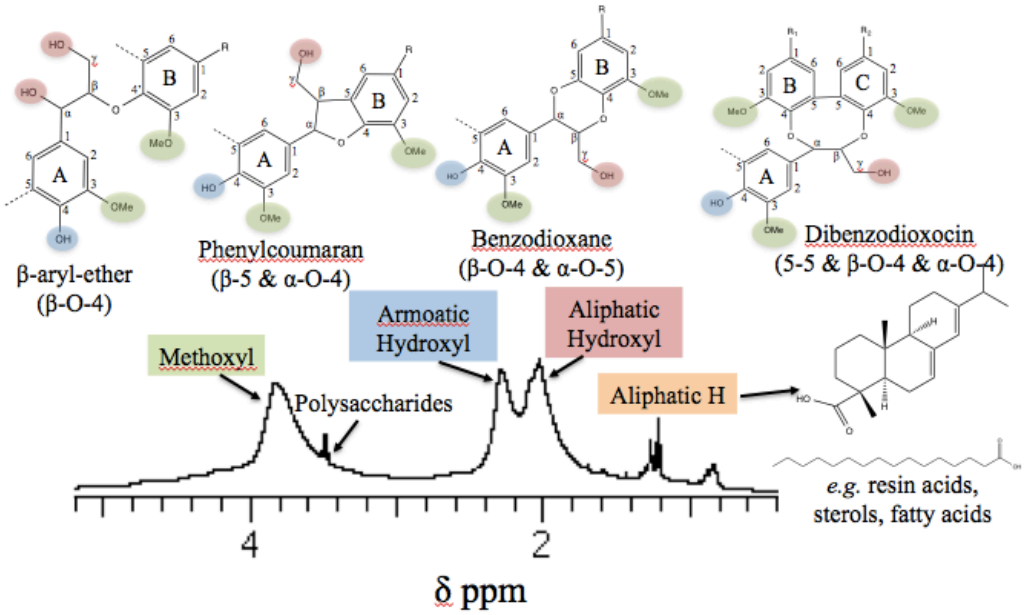

Figure B.1. Representative ^{1}H NMR spectra of acetylated lignin fractions showing identification of typical structures in lignin as well as aliphatic protons that might be found in softwood extractives.

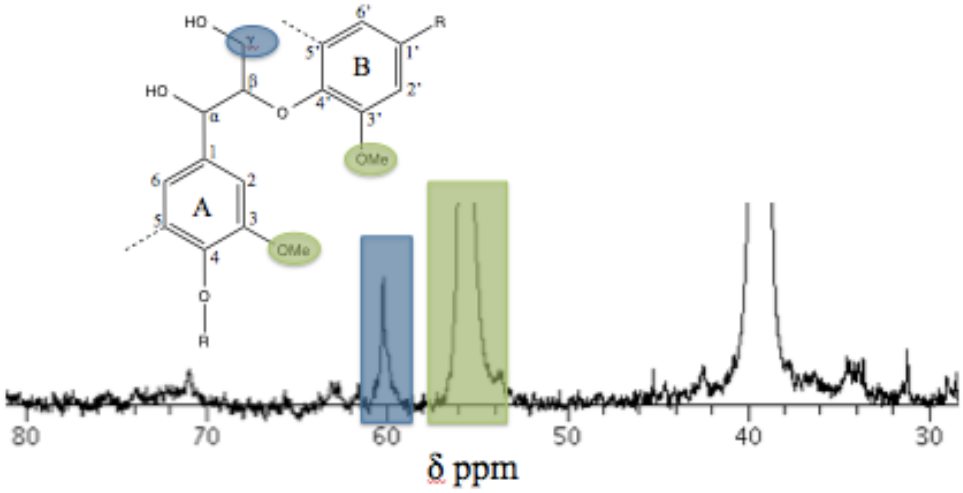

Figure B.2. Quantitative ^{13}C NMR spectra of a typical lignin fraction identifying the C_{γ} in a β-O-4 bond and a methoxyl group used to determine lignin β-O-4 content.

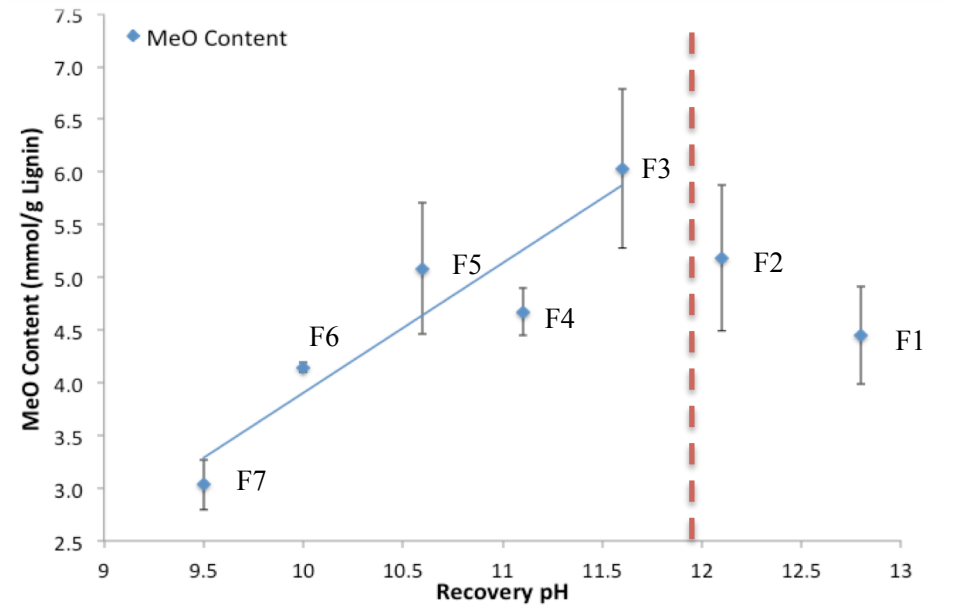

Figure B.3. Methoxyl content for the seven lignin fractions recovered.

Table B.1. Aromatic compound identification for pyro-GC/MS

Classification	Compound	Res. Time (Minutes)	Mass Peak (m/z)
S+C3	Methoxyeugenol	25.9	194
S+C2	4-Ethyl-2,6-Dimethoxyphenol	23.1	137
S+C2	3,4-Dimethoxystryene	20.9	164
S+C1	2,3-Dimethoxytoluene	17.6	152
S+C1	2,5-Dimethoxytoluene	18.4	137
G+C3	Dihydromethyleugenol	23.6	151
G+C3	Propiovanillione	24.7	151
G+C3	Guaicylacetone	24.2	137
G+C3	*p*-Propylguaiacol	20.1	137
G+C3	Isoeugenol	21.0	164
G+C2	Acetovanillone	23.5	151
G+C2	4-Vinylguaiacol	19.7	135
G+C2	4-Ethylguaiacol	18.9	137
G+C1	2-Methoxy-4-Methylphenol	17.3	138
G+C1	Vanillin	22.3	151
G+C1	2-Methoxy-6-Methylphenol	16.8	123
G+C1	3-Methoxy-5-Methylphenol	17.3	123
Guaiacol	Guaiacol	15.5	124
Ph+C2	3-Ethylphenol	18.1	107
Ph+C2	*p*-Xylenol	17.4	122
Ph+C1	*p*-Cresol	16.6	107
Ph+C1	*o*-Cresol	15.9	108
Phenol	Phenol	15.0	94

CHAPTER 4. HEMICELLULOSE DISSOLUTION AND THE INFLUENCE OF LIGNIN REMOVAL ON ENZYMATIC HYDROLYSIS FOR SODA PULPED HARDWOODS

4.1 Introduction

Lignocellulosic biomass feedstocks are targeted as one option to offset petroleum consumption and to offer a sustainable approach to the production of liquid transportation fuels and value-added chemicals. The complex structure within lignocellulosic biomass necessitates the use of a pretreatment process to break plant cell wall recalcitrance for conversion to fuels or chemicals (Himmel et al., 2007). A range of pretreatment variations have been researched to understand how the structural components within cell walls can be deconstructed under different conditions such as pH, temperature, and chemical loading (Mosier et al., 2005). Hemicellulose, an amorphous polysaccharide, is most significantly affected by pretreatments utilizing liquid hot water (LHW) (Y. Kim, Mosier, & Ladisch, 2009), dilute acid (Torget, Werdene, Himmel, & Grohmann, 1990), or high temperature alkaline pretreatments (Oka et al., 2013). Both LHW and dilute acid pretreatments dissolve hemicellulose into oligomeric fractions followed by hydrolysis to monomeric constituents (Marzialetti et al., 2008; VanWalsum et al., 1996). Conversely, a high temperature alkaline pretreatment, a process that is similar to chemical pulping, functions by removing hemicellulose through the peeling reaction and suspending the polysaccharide as a solute in the aqueous phase (Hamilton, 1962). The LHW and dilute acid pretreatments do not significantly affect the lignin structure aside from redistributing some of the aromatic polymer around the plant cell wall (Sannigrahi, Ragauskas, & Miller, 2008). Pretreatments that strongly affect lignin structures incorporate alkali, organic solvents, or oxidative chemicals for lignin removal (Chang, Nagwani, et al., 2001; Sarkanen et al., 1981; Venica, Chen, & Gratzl, 2008). Pretreatments of this type can either directly dissolve lignin fragments or remove the fragments

by breaking specific linkages between the lignin structures itself or the hemicellulose polysaccharide. Any of the disruptions made to the hemicellulose or lignin structure can result in the improvement for the conversion of cellulose to a biologically renewable liquid transportation fuel (Hendriks & Zeeman, 2009). Much of the impediment to commercializing the production of these types of fuels from cellulose lies in constructing capital-intensive pretreatment processes (Yang & Wyman, 2008). One way to overcome this issue is to look at industries that already have a well-developed infrastructure for the chemical processing of woody biomass feedstocks.

In North America, the pulp and paper industry is uniquely positioned to assist with the conversion of woody lignocellulose by already possessing a well-defined infrastructure of capital equipment. On a global scale kraft pulping is the chemical process of choice for obtaining cellulosic fibers for papermaking due to process economics that give sufficient recovery of cooking chemicals and excess power generation allowing kraft pulp mills to meet their own net power demands. During this process hemicellulose is solubilized by the peeling reaction and eventually degraded to isosaccharinic acids (Sjostrom, 1993). Lignin is primarily removed by the addition of Na_2S where the nucleophilic aromatic substitution occurs by sulfur at elevated temperatures. The obtained pulp is enriched in cellulosic fibers that are used in papermaking, while the dissolved non-cellulosic biopolymers are sent to a recovery boiler where they are burned for power generation. Current research and industrial implementation is now trending towards recovering these biopolymers by fractionating these components from the black liquor so they can be used as a source to generate renewable fuels or chemicals (Phillips et al., 2013). This idea of utilizing the non-cellulosic portion of the plant cell wall for the generation of fuels or chemicals is not new. In previous decades sulfite pulping was a more predominant form of chemical pulping. When an acidic sulfite cook was utilized the hemicellulose portion within the

woody biomass could be hydrolyzed to monomers in the spent liquor followed by fermentation to produce ethanol (Lawford & Rousseau, 1993). Alternatively, lignin can be recovered by techniques such as ultrafiltration (Jönsson et al., 2008) or pH adjustment by sparging CO_2 into the aqueous black liquor to precipitate lignin (Nagy et al., 2010; Velez & Thies, 2013), which has already investigated for commercialization (Lake & Blackburn, 2011; Tomani, 2010). Depending on the fractionation process utilized the lignin recovered can contain variations in it's composition and structure. As an example the CO_2 precipitation of lignin can contain variations in sugar content and functional groups that are correlated with the pH of fractionation that was achieved (Stoklosa et al., 2013). Although the current pulping industry heavily relies on sulfurous compounds in chemical pulping, a new direction should be initiated by utilizing soda pulping when it comes to producing renewable products from woody biomass. In this way sulfurous compounds can be removed from the processing of woody biomass that produces benefits both environmentally and for the recovery of lignin in a sulfur-free form that has greater product applications (Lora & Glasser, 2002).

Integrated Forest Biorefineries (IFBR) are envisioned to mostly utilize the non-cellulosic plant cell wall fractions for the production of fuels and chemicals by implementing an alkali pre-extraction (or pretreatment), while the cellulosic fraction can be sent to an actual pulping process to procure the pulp fiber needed for papermaking (Ragauskas et al., 2006; Van Heiningen, 2006). The economics associated with the transition to an IFBR must be favorable to an operational pulp mill for this to occur. A process simulation and economic analysis was performed on an IFBR working within three scenarios: traditional kraft pulping, hemicellulose pre-extraction prior to pulping for ethanol production, and hemicellulose pre-extraction prior to pulping coupled with the conversion of short cellulosic fibers to ethanol (Huang et al., 2009; Huang et

al., 2010). By converting both the extracted hemicellulose and short cellulosic fibers to ethanol a total of 10.04 MM gallons (0.038 MM m^3) of ethanol could be produced at $1.86 per gallon ($491 per m^3), which simultaneously increased pulping capacity by 22% but the lower solid contents in the black liquor would produce less steam than a traditional kraft mill (Huang et al., 2010). However, this lower production of steam could be offset from a 13% reduction in fuel usage by the lime kiln due to a lower loading of chemical inorganics (Huang et al., 2010). Furthermore, ethanol is not the only other fuel or value-added chemical that has the option of being produced in an IFBR. Hot water pre-extraction has been evaluated on both larch and birch prior to chemical pulping for the purpose of extracting hemicelluloses followed by the fermentation hemicellulosic sugars to lactic acid (Walton, Bischoff, van Heiningen, & van Walsum, 2010) or succinic acid (Helmerius et al., 2010), respectively, by different strains of bacteria. Although high titers of lactic acid or succinic acid were achieved, the hot water extraction had the additional effect of decreasing pulp yield due to unwanted hydrolysis of cellulosic microfibrils (Helmerius et al., 2010); but the pulp yields could be improved by adding polysulfide-anthraquinone (PSAQ) to produce a good papermaking pulp with similar yields (Horhammer et al., 2011). To ensure that an IFBR can be economical with traditional pulp mills the inclusion of black liquor gasification is envisioned to boost the attractiveness for this type of integrated processing. With black liquor gasification it is possible to recover the initial cooking chemicals in the form of green liquor, increase power generation by converting organics to synthesis gas that can be sent to a gas turbine or used in other conversion processes to produce liquid hydrocarbons, and to completely segregate environmentally hazardous sulfur compounds in a condensed form, especially at pressurized gasification at temperatures greater than 800°C (Backman, Frederick, & Hupa, 1993; Lindstrom, Jameel, Naithani, Kirkman, & Renard, 2007;

Salmenoja, 1993). The main cautionary note about black liquor gasification would be to consider proper operational parameters to decrease the amount of H_2S that can be formed during the process. Production of H_2S would require additional wet scrubbers that would also increase IFBR capital cost.

One understanding that is missing from IFBR evaluation is the effect that pulping processes have on obtaining a more readily digestible woody biomass substrate. The research discussed here focuses on the effect of soda pulping on three different hardwood species and the implications for the three major cell wall fractions as a substrate source for renewable fuel or chemical production. Soda pulping trials were conducted on three hardwood species in a laboratory scale digester at varying temperature and time parameters. During the heat-up phase and constant temperature cooking phase the solubilization of xylan and lignin was investigated to explore the potential for xylan recovery from the black liquor while attempting to balance the xylan retention against delignification for yields of monomeric sugars after enzymatic hydrolysis. Woody biomass delignification evaluated at different severity cooks was compared with initial hydrolysis rates to understand how lignin affects yield of sugars during the first hours of enzymatic hydrolysis. Also evaluated was the effect on hydrolysis yields due to different methods of comminution and different loadings of enzymes. Overall this work presents an option for deconstructing the entire plant cell wall contents from woody biomass in a pulping process to produce renewable fuels or chemicals.

4.2 Materials and Methods

4.2.1 Feedstock Composition

Three chipped hardwood species were analyzed in this study: *Acer saccharum* (sugar maple), *Betula pendula* (silver birch), and *Populus nigra X P. maximowiczii* cv. NM-6 (hybrid poplar). Samples were obtained from Curt Lindström of Smurfit-Kappa in Sweden (silver birch), Dr. Raymond Miller of Michigan State University (hybrid poplar), and Todd Smith of the Devereaux Saw Mill, Inc. in Pewamo, MI, USA (sugar maple). All samples had moisture contents around 5-8% and the composition for each feedstock was determined by NREL/TP 510-42618 (Sluiter et al., 2008) with slight modifications as discussed in Chapter 2, Section 2.2.1. Ash and extractive components were quantified according to NREL/TP 510-42622 (Sluiter, Hames, et al., 2005) and NREL/TP 510-42619 (Sluiter, Ruiz, et al., 2005), respectively.

4.2.2 Soda Pulping Trials

An M/K Systems, Inc. (Peabody, MA, USA) single vessel digester unit with a volume capacity of 10 L was used for soda pulping trials. A 4:1 (L:kg dry wood) liquid loading was selected with an 18% loading of NaOH (g NaOH per g dry wood); this corresponds to a NaOH concentration of 45 g/L. The wood chips were cooked to different H-factor levels. The H-factor is a single variable representation of the effect temperature and net cooking time have on delignifcation given by the equation in Figure 4.1 (Sjostrom, 1993).

$$H = \int_0^t \exp\left(43.2 - \frac{16{,}113}{T}\right) dt$$

Figure 4.1. H-factor relationship for chemical pulping

The temperature (T) is in Kelvin and time (t) in minutes. Table 4.1 outlines the feedstocks that were utilized for each cooking trial.

Table 4.1. Soda pulping trial conditions

Combined H-Factor (Heat Up Plus Cook)	Maximum Temperature (°C)	Temperature Ramp Rate (°C/min)	Total Cooking Time (Hours)	Feedstocks
114	150	0.75 to 135°C, 0.5 to 150°C	0.5	Poplar
525	150	0.75 to 135°C, 0.5 to 150°C	3	Poplar
1147	170	0.75 to 135°C, 0.5 to 170°C	1	Birch, Maple, Poplar

An additional LHW pretreatment was carried out on hybrid poplar in the same digester vessel with a 4:1 liquid loading at 160°C for 1.5 hours to compare the effectiveness of soda pulping to LHW pretreatment in terms of generating a digestible substrate by enzymatic hydrolysis.

For the most severe H-factor condition samples of the black liquor were collected during the heat up phase, and the constant temperature cook phase with the use of an inline condenser. Figure 4.2 shows a general temperature profile over the entire pulping trial marked with red lines indicating each sampling point during the heat up phase. At the constant temperature phase samples were collected at 5, 10, 20, 30, 45, and 60 minutes. After system cool down and pressure reduction the residual black liquor was collected in bulk. Wood chips were subjected to a thorough wash with deionized water by rinsing the chips in the vessel canister, followed by soaking the canister containing the wood chips in a 5-gallon container of water. The water was exchanged up to two times a day until the wash water was clear and the bulk wash liquid pH was neutral. The chips were then oven dried at 105°C to determine the yield.

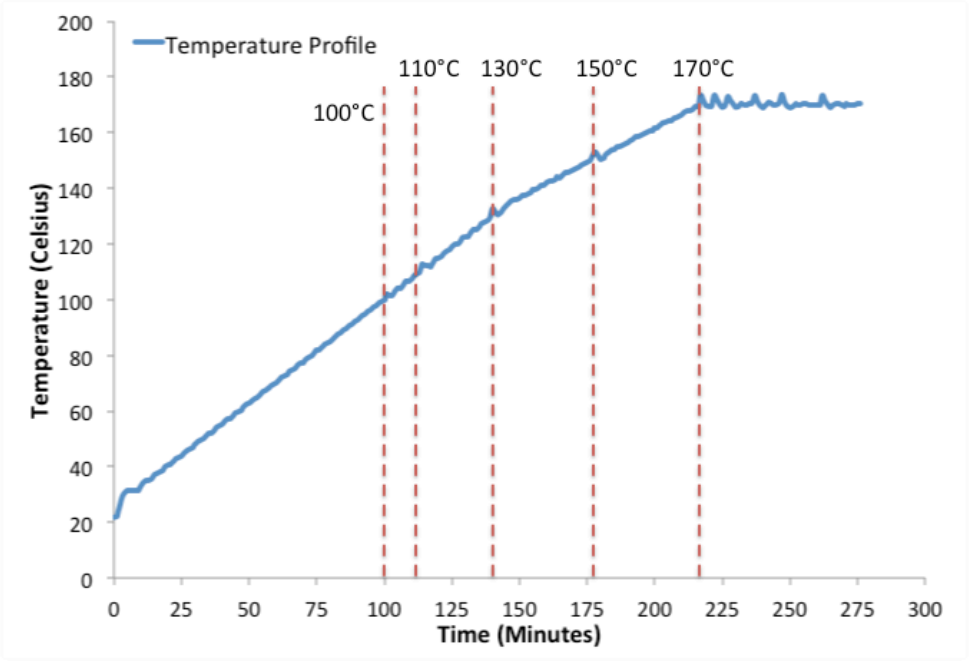

Figure 4.2. A general temperature profile for a H-factor cook at 1147.

4.2.3 Analysis of Black Liquor Fractions

Each black liquor fraction collected was analyzed for dissolved plant cell wall content to determine the kinetics of solubilization. Prior to this the pH of each fraction was measured by an Oakton Instruments (Vernon Hills, IL, USA) pH 510 bench top meter to determine any pH changes occurring during the process. Samples were then subjected to a 4% acid hydrolysis according the method NREL LAP TP-510-42623 (Sluiter et al., 2006). Around 4 mL of each black liquor sample was taken for analysis and diluted to a volume of 8 mL with water. The black liquor analysis follows the same procedure for alkali extracts outlined in Chapter 2, Section 2.2.2. The precipitated insoluble solids after hydrolysis were centrifuged and washed at 4,500 RPM for 5 minutes with deionized water in 15 mL conical tubes until the pH was neutral. The washed solids were transferred to aluminum trays that were previously weighed and placed

in an oven to dry at 105°C overnight. Gravimetric analysis of the solids followed to determine the total amount of precipitated material from the black liquor fractions. This material is considered to be the amount of Klason lignin removed at each sample point.

4.2.4 Particle Size Reduction for Enzymatic Hydrolysis

Washed and dried wood chips after soda pulping underwent particle size reduction in two steps. The first step combined the dried wood chips with deionized water at a solids consistency of 5% (w/w). This mixture of wood chips and water was added to a blender for disintegration. To produce a consistent disintegrated substrate between each feedstock, operation of the blender was standardized for the amount of pulses and total time used to disintegrate. For each feedstock around 20 pulses with the blender were sufficient to disintegrate the wood chips. This material was then blended at a constant power setting for 20 minutes to produce the final disintegrated feedstock sample. The disintegrated sample was filtered to remove free water, followed by drying at 50°C overnight. The second step consisted of using a Thomas Wiley® Mini Mill (Swedesboro, NJ, USA) and passing the feedstock through a 20 mesh screen. After milling the samples were collected in zip-top plastic bags for storage. A moisture content analysis was performed on all the feedstocks after milling with samples added to pre-weighed aluminum trays and dried in an oven for at least four hours at 105°C. Additionally, samples of birch, maple, and hybrid poplar (H-factor cooks of 1147 and 525) were left in a disintegrated particle size without knife milling to test if a preferential enzymatic breakdown occurred between larger and smaller particle size substrates.

4.2.5 Enzymatic Hydrolysis

Milled feedstock were added to 250 mL Erlenmeyer flasks at a 10% (w/v) solids loading in a citric acid buffer at a concentration of 50 mM and pH around 5.5. This pH was chosen based upon obtaining higher sugar yields from pretreated biomass (Lan et al., 2013; Z. J. Wang, Lan, & Zhu, 2013). To prevent the growth of unwanted microorganisms tetracycline was added to the slurry at a concentration of 40 µg/mL. Cellic® CTec2 and HTec2 from Novozymes (Bagsævard, Denmark) were the enzyme cocktails utilized in the experiments and added at a CTec to HTec ratio of 2:1. Protein concentrations were determined according to the Bradford Assay (Sigma-Aldrich). For all experiments the total enzyme loading was 20 mg enzyme per gram of glucan in the biomass sample, unless otherwise noted. A piece of Parafilm (Bemis Company, Neenah, WI, USA) was placed over the top of each flask to prevent evaporation losses. Hydrolysis was carried out in a shaking incubator at 50°C at 160 RPM. Hydrolysis was allowed to occur over a 5 day period. During sampling the flasks were removed from the incubator, briefly swirled, and then 400 µL of the hydrolysate was removed and placed in a 1.7 mL microcentrifuge tube, followed by centrifuging at 13,000 RPM for three minutes. After sampling a new piece of parafilm was placed on the top of the flasks before being returned to the incubator. After centrifugation the liquid sample was diluted ten times in water followed by syringe filtering through a 0.22 µm filter into HPLC vials. Samples were stored in a freezer until HPLC analysis. The conditions for HPLC were performed according to conditions given in Chapter 2, Section 2.2.1. Glucose and xylose concentrations were converted to their polymeric form, and yields for each were calculated as a ratio of the sugar released at the observed time point divided by the theoretical amount of glucan and xylan supplied based on the composition of the untreated or treated feedstock.

4.3 Results and Discussions

4.3.1 Biomass Composition and Yields After Pulping

The temperature and NaOH loading utilized in this study altered the original hardwood composition during soda pulping. Figure 4.3 presents the biomass composition after each soda pulping trial and the lone LHW pretreatment on hybrid poplar. The composition results are presented on a gram per dry gram original biomass basis. One of the first observations noticeable is how the cellulose (*i.e.* glucan) content is relatively unaffected at each condition analyzed.

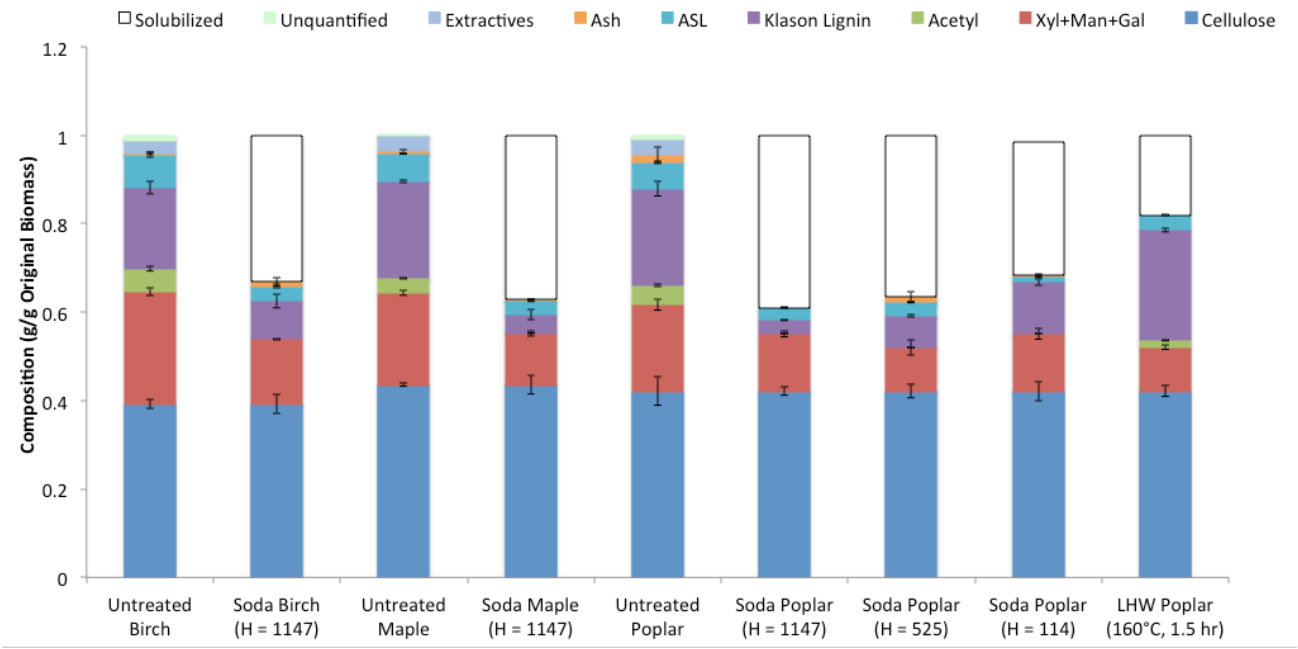

Figure 4.3. Composition of the three hardwood feedstocks before and after soda pulping, and after LHW pretreatment for hybrid poplar.

It should be expected that the amorphous portions of the cellulose chain can be removed by pretreatment (Sannigrahi et al., 2008), however, there is much less of this compared to the crystalline cellulose that it does not affect the overall cellulose composition. The HPLC method to quantify biomass composition, the same method utilized in Chapter 2, does not separate the individual sugar monomers of galactose, mannose, or xylose. For this reason the hemicellulose

content is referred to as “Xyl+Man+Gal”. An assumption can be made that the predominant hemicellulose component is xylan (Glasser et al., 2000) and this term will be used from this point forward.

In contrast to glucan, the soda pulping and LHW treatments altered the content of xylan and Klason lignin. The amount of xylan removed was similar for all soda pulping trials. Xylan content decreases after each treatment with the removed xylan dissolving into the black liquor or hydrolyzed by hot water. Greater xylan removal occurred during the LHW pretreatment on poplar, a potentially expected result since LHW pretreatments at high temperatures can readily hydrolyze the more susceptible hemicellulose fractions due to their lack of hydrogen bonding in the cell wall (Bobleter, 1994). For the soda pulping trials, acetyl side groups were completely saponified by alkali and only a small fraction of acetyl remained in the hybrid poplar after LHW. As Figure 4.3 shows Klason lignin was removed in large quantities from all soda pulping trials except for the lowest H-factor cook (114) and hardly any lignin removal for the LHW pretreatment. This removal of lignin should be expected as well since OH^- performs well in disrupting lignin bonds to dissolve substantial fractions in traditional pulping processes (Venica et al., 2008). In terms of the overall amount of material solubilized, the most severe pulping trial (H = 1147) produce similar amounts of material removed for all three feedstocks tested. The poplar feedstock tested at three different H-factor cooks had similar overall material dissolution at the 1147 and 525 cooks, but the low H-factor cook at 114 produced less material removed and more residual solids after cooking. The same trend for Klason lignin in the LHW pretreated poplar was present again for the overall amount of material removed from the LHW treated poplar due to the lack of chemical catalysts utilized.

When comparing different sets of alkali pulps cooked at different temperatures and times it is easiest to compare them according to the combined H-factor of the process. The combined variable H-factor was developed to describe the extent of delignifcation on a woody biomass feedstock during alkali chemical pulping (Sjostrom, 1993). In Figure 4.4 the content xyland and Klason lignin are plotted as a function of H-factor, along with the pulp yield. The trends presented are obvious; with increasing H-factor the pulp yield decreases, while the residual content of xylan and Klason lignin in the poplar decrease as well.

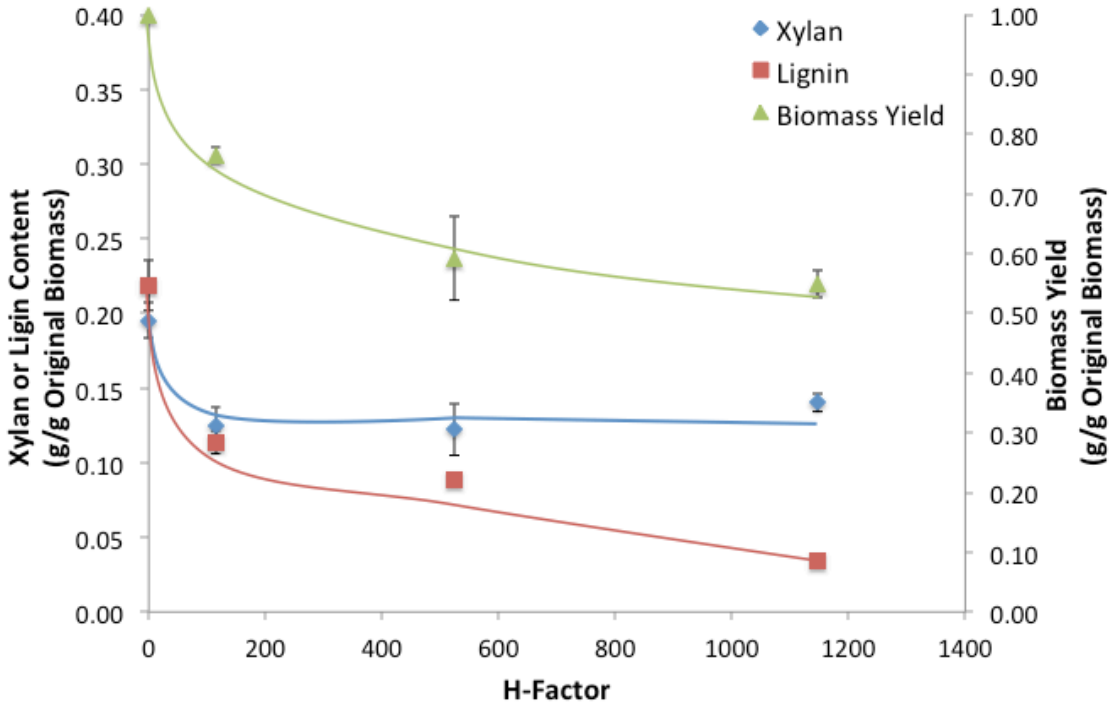

Figure 4.4. Pulp yield, and residual xylan and Klason lignin content as a function of H-factor.

The high temperatures and chemical loading utilized have an expectation that larger amounts of xylan can be removed as opposed to lower temperatures and lower NaOH loadings, which have a tendency to lower the pH during the release of organic acids that neutralize OH^- (Lehto & Alen, 2013). That should not be the case in this work due to the high loading of NaOH utilized.

Although the xylan content in the untreated poplar did decrease after soda pulping, the residual xylan remaining was consistent for the three separate pulping studies. Xylan extraction is known to be dependent upon both temperature and alkali loading (Testova et al., 2014). The data presented in Figure 4.4 show that xylan was removed during soda pulping, but the trials had an overall greater effect on Klason lignin removal. It must be noted that a careful balance must be obtained when trying to extract desirable plant cell wall components. In this case the greater amount of xylan remaining in the residual solids is encouraging because it allows the xylan to be recovered as a monomeric sugar during downstream enzymatic hydrolysis, or to serve as a fiber adhesive to increase the pulp strength properties (Danielsson & Lindstrom, 2005; Molin & Teder, 2002). The lower quantity of lignin remaining in the residual solids indicates the opportunity to recover a majority of the lignin from the aqueous black liquor, and, in terms of downstream pulp processing, lower chemical loadings can be achieved in a pulp bleaching process for the purpose of oxidizing the residual lignin.

4.3.2 Hemicellulose and Klason Lignin Solubilization During Pulping

The collected samples of black liquor were analyzed for the amount of dissolved xylan and Klason lignin in the aqueous phase. Figures 4.5 – 4.7 present the kinetics of solubilization for both the xylan and Klason lignin portions after the high severity soda pulping trial (H = 1147) for all three feedstocks. The amount of xylan and Klason lignin was determined after a 4% acid hydrolysis on the black liquor samples. The dashed black line on each graph indicates the time point when the cook reached a constant temperature of 170°C. A time of -116 minutes indicates when the reactor vessel reached a temperature of 100°C, which was 100 minutes into the heat up phase of the pulp trial. For the solubilization of xylan the trends show a gradual increase in the

amount of xylan solubilized from each hardwood species until 170°C. Upon reaching constant temperature the amount of xylan solubilized decreases for both birch and poplar after 5 minutes at 170°C, while the amount of xylan solubilized from maple increases 10 minutes into the cook phase followed by a gradual decrease.

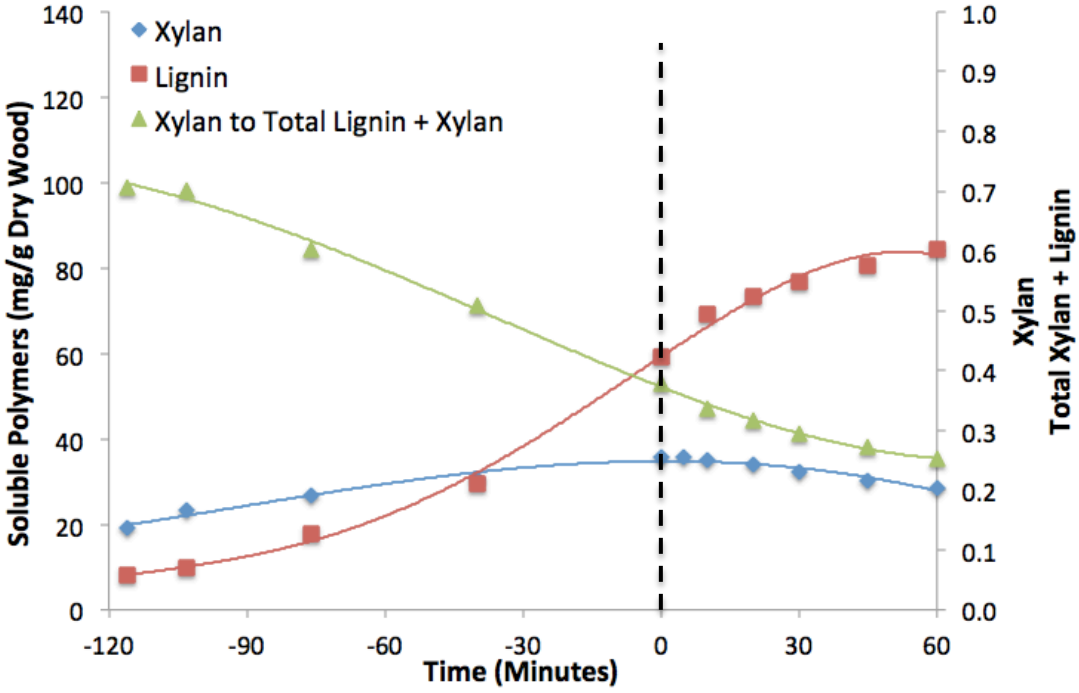

Figure 4.5. Solubilization of xylan and Klason lignin during birch soda pulping (H = 1147).

The decrease in xylan can be attributed to degradation during the cook phase by the xylan breaking down to form saccharinic acids (Giudici & Park, 1996; Kakola, Alen, Pakkanen, Matilainen, & Lahti, 2007; Ponder & Richards, 1997b). Xylan re-adsorption to cellulose could also be responsible for the retention of a substantial fraction of the xylan.

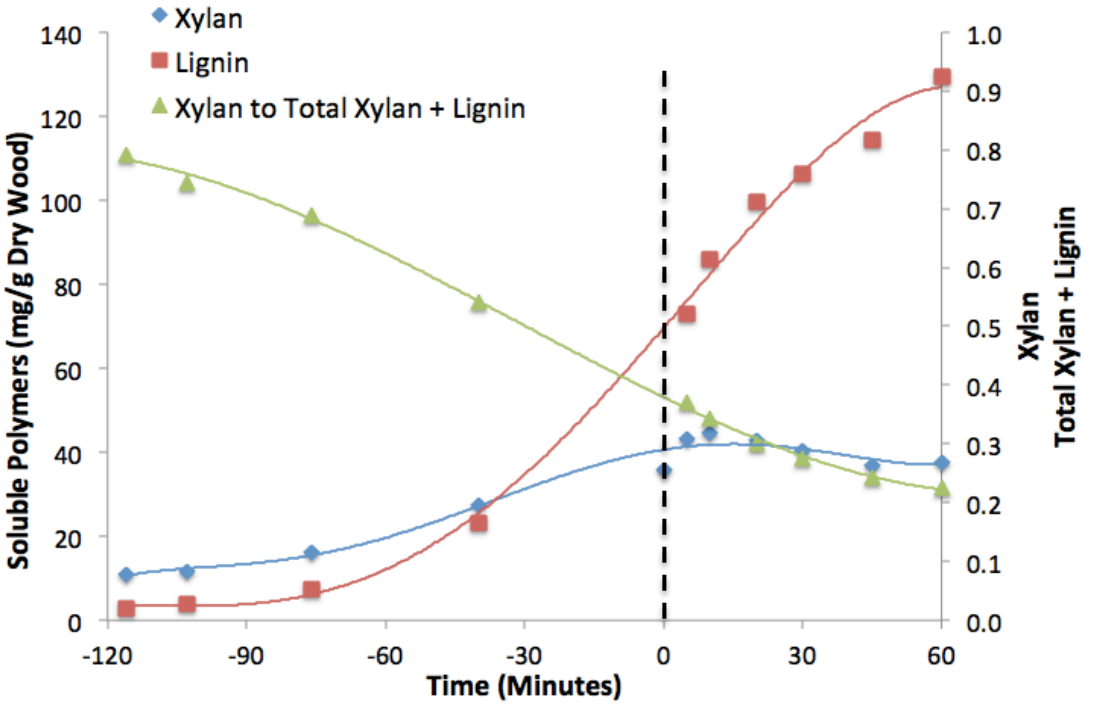

Figure 4.6. Solubilization of xylan and Klason lignin during maple soda pulping (H = 1147).

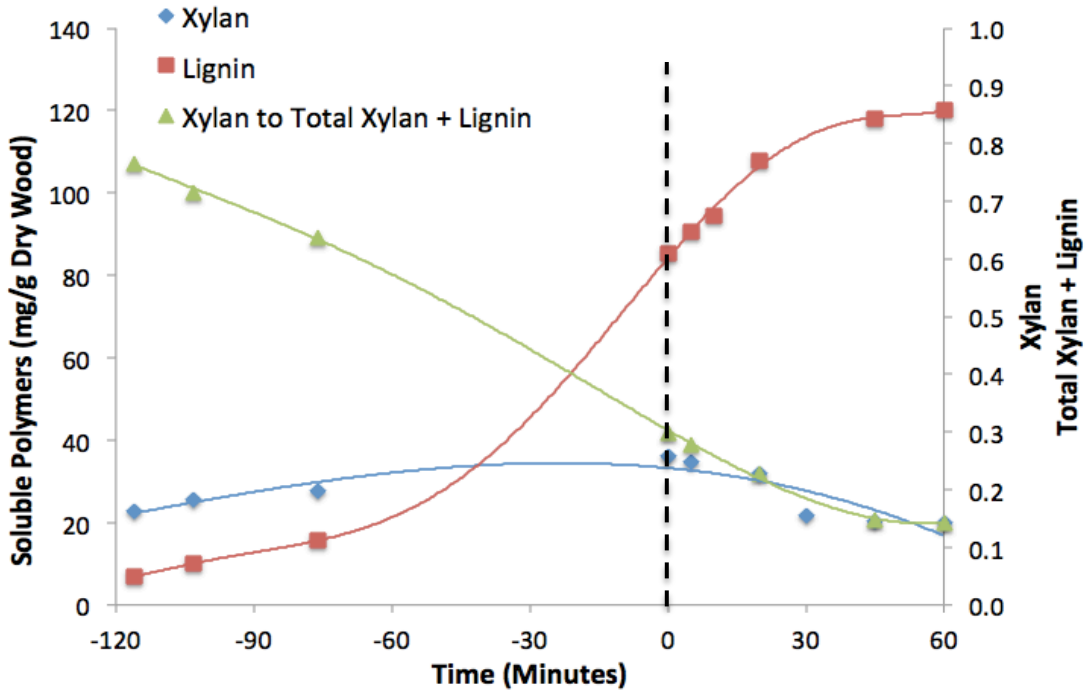

Figure 4.7. Solubilization of xylan and Klason lignin during poplar soda pulping (H = 1147).

Alkali solutions at higher temperatures also contribute more towards cleaving the negatively charged 4-O-MeGA substitutions on the xylan chain that will help to promote xylan aggregation in the aqueous solution, thus decreasing solubility and increasing xylan adsorption to cellulose (Linder et al., 2003). Furthermore, xylan present in the surfaces of hardwood kraft fibers have been shown to contain lower frequencies of uronic acid side groups and a 30% higher average molecular mass, thus lending more credence for xylan re-adsorption to kraft pulps during the course of a cook (Dahlman, Jacobs, & Sjoberg, 2003). A combination of degradation and adsorption would indeed indicate a loss in quantifiable xylan contained in the aqueous black liquor. Additionally, the amount of xylan that is lost from solution appears to be affected by species type. More xylan is lost from hybrid poplar as opposed to birch or maple. Xylan is the dominant hemicellulose polysaccharide in hardwoods, however, GMs can account for 2-5% of the dry wood weight (Sjostrom, 1993) and it has been shown that deacetylated mannans can strongly and irreversibly absorb to cellulosic surfaces (Hannuksela, Tenkanen, & Holmbom, 2002). Since GMs were not directly quantified by the HPLC method utilized in this study it is not known whether the three feedstocks assayed contained different amounts of GM that could influence the overall amount of hemicellulose lost from the black liquor during pulping.

Conversely, the dissolution of Klason lignin during the soda pulping trials tracks closely to the total mass loss and increases throughout the duration of the trial as evidenced in Figures 4.5 – 4.7. At the start of the constant temperature phase the amount of Klason lignin dissolved into the black liquor increases for all three hardwood feedstocks, albeit a lower amount of Klason lignin is removed from the birch when compared to maple and hybrid poplar. For a typical alkali pulping process three stages of delignification occur: initial, bulk, and residual (Sjostrom, 1993). The initial stage of delignification is controlled by diffusion, while bulk delignification is

controlled by chemical reactions and occurs at temperatures above 140°C until the residual phase where there is relatively little lignin remaining in the woody biomass (≈ 10%) (Sjostrom, 1993). The trends presented in Figures 4.5 – 4.7 show increases in delignification for all three feedstocks after 40 minutes, corresponding to a temperature of 130°C. When comparing the three H-factor cooks performed on hybrid poplar in Figure 4.4 the highest H-factor (H = 1147) could remove up to 84% of the Klason lignin (*i.e.* a composition yield around 0.031 g final per g original biomass), which would be expected with the alkali concentration and temperature utilized. The extent of delignification that can occur in the bulk stage is dependent upon the molar alkali concentration that is supplied during the initial stage of delignification (Olm & Tistad, 1979). With OH^- being a strong nucleophile the most notable reactions that occur during the bulk delignification stage include the cleavage of β-aryl ether bonds in phenolic and non-phenolic units, cleavage of α-aryl ether bonds in phenolic units, and condensation reactions (Gierer, 1980). Concomitantly, hemicellulose is removed and/or degraded during bulk stage delignification. In a study conducted on softwood, xylan removal has been shown to be linearly proportional to delignifcation while polysaccharide peeling was minor, but GM was dissolved rapidly and 45% degraded by the peeling reaction (Wigell, Brelid, & Theliander, 2007). However, the data presented in Figures 4.5 – 4.7 indicate that xylan selectivity to lignin removal is strongly a function of time.

4.3.3 Enzymatic Hydrolysis of Residual Soda Pulped Hardwoods

The insoluble hardwood feedstocks after alkali pulping were investigated by enzymatic hydrolysis to determine the recovery of monomeric sugars. Investigations into the recovery of soluble sugars from woody biomass after alkali pulping have not been carried out since chemical

pulps have strictly been reserved for the production of paper. However, pretreatments similar to chemical pulping, such as the Organosolv or SPORL process, have been evaluated as a way to recover and convert the polysaccharide portion of woody biomass to fuels or chemicals (Araque et al., 2008; Del Rio, Chandra, & Saddler, 2010; Lan et al., 2013; Pan et al., 2006; Puls & Saake, 2004; G. S. Wang et al., 2009; Z. J. Wang et al., 2013; Zhu et al., 2009). (Lan et al., 2013; G. S. Wang et al., 2009; Z. J. Wang et al., 2013; Zhu et al., 2009).

Each feedstock after pulping was subjected to enzymatic hydrolysis. The woody biomass went through a two-stage particle size reduction that was discussed in Section 4.2.4. Figure 4.8 shows the glucose hydrolysis yields for birch, maple, and poplar after the high H-factor cook, and Figure 4.9 shows the xylose hydrolysis yields for the same feedstocks. The yield of sugar was calculated based on the concentration of glucan or xylan released into solution at each time point divided by the amount of glucan or xylan supplied to the overall process (*i.e.* the initial pulping process). It is evident that the sugar yields after soda pulping increase significantly when compared to each untreated feedstock. High yields of glucose (> 80%) were achieved by the addition of 20 mg of enzyme per g glucan for each feedstock shown in Figure 4.8. Xylose yields were lower than 40% for each feedstock in Figure 4.9. The low yields account for xylan extraction during the pulping process, and xylan degradation from the peeling reaction. The increase in sugar yields for the treated feedstocks can be attributed to lignin removal since it is understood that the removal of lignin will increase the exposed cellulose surface area and lessen the loss of enzymes due to non-productive binding (Mansfield, Mooney, & Saddler, 1999). The yields of soluble sugars after a chemical pulping treatment have not been tested extensively. Other forms of alkaline pretreatment, such as AFEX™ or lime, have been utilized on woody biomass to test the efficacy of plant cell wall deconstruction.

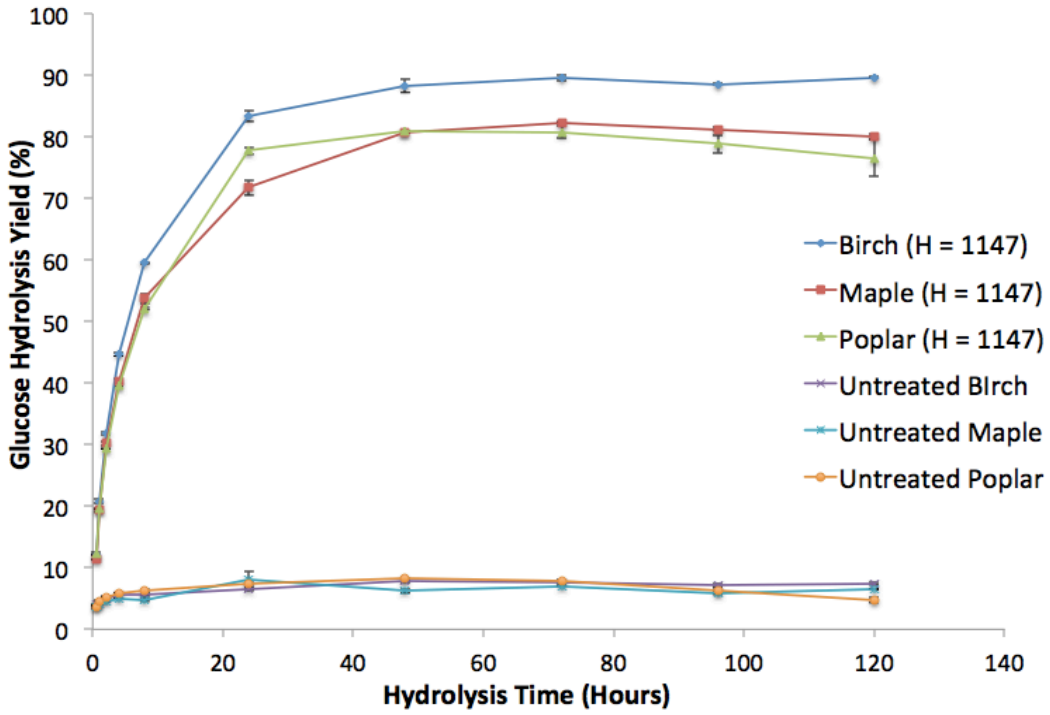

Figure 4.8. Glucose enzymatic hydrolysis yields for birch, maple, and poplar after the high H-factor trial; enzyme loading 20 mg per g of glucan.

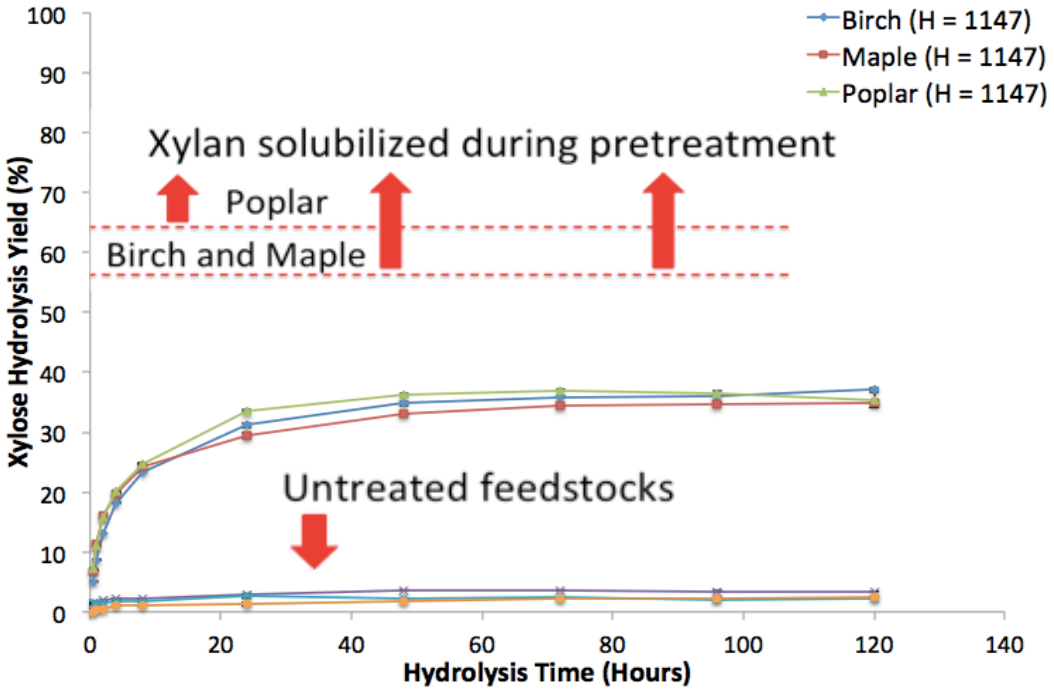

Figure 4.9. Xylose enzymatic hydrolysis yields for birch, maple, and poplar after the high H-factor trial; enzyme loading 20 mg per g of glucan.

Black locust treated by AFEX™ at 180°C and 1.0 g NH_3 per g dry wood, followed by milling, was shown to greatly improve biomass digestibility at high enzyme loadings by generating close to 80% yields of monomeric glucose and hemicellulose sugars after three days of hydrolysis (Garlock, Wong, Balan, & Dale, 2012). However, AFEX™ is a unique alkaline pretreatment since it does not function by changing the composition of the biomass material but instead opens up the pore structure of the cell wall and converts the cellulose chain into a more digestible allomorph (Chundawat, Bellesia, et al., 2011; Chundawat, Donohoe, et al., 2011). Lime pretreatments on poplar have indicated up to 77% and 74% monomeric sugar conversions for glucose and xylose, respectively, after three days of enzymatic hydrolysis, but lime pretreatments also increase the ash content of the biomass due to calcium incorporation (Chang, Nagwani, et al., 2001). More recently, Oka et al. (2013) determined that alkaline treatment at 150°C with 1 N and 5 N NaOH solutions on cedar and eucalyptus produced different monomeric conversion yields after 24 hour hydrolysis. The glucose conversion for cedar reached 10% after 1 N NaOH treatment and 80% after 5 N NaOH treatment, while eucalyptus could reach 40% conversion after 1 N NaOH treatment and 70% conversion after 5 N NaOH treatment (Oka et al., 2013). In this research the NaOH concentration was around 1.1 M and following 24 hours of hydrolysis a 70% or higher yield of glucose could be achieved for birch, maple, and poplar. The yields in Figure 4.8 are higher than what was achieved in the study by Oka et al. (2013) with 1 N NaOH at 150°C, but differences in yield can be attributed species difference (*i.e.* cedar is a softwood and should have a higher lignin content), type of enzymes used, and enzyme loading.

Yields for enzymatic hydrolysis were also compared based on different pulping or pretreatment conditions, substrate particle size, and enzyme loading. Figure 4.10 shows the glucose yields for hybrid poplar after the three H-factor trials. The yields of glucose follow the

exact same trend in Figure 4.8 up until 24 hour hydrolysis, where a slight increase in the glucose yield for the 1147 cook. The 525 H-factor cook utilized lower temperature (150°C) but longer pulping time (3 hours) that could affect the macrostructure of the woody biomass plant cell wall by redistributing lignin more evenly over the fibers to increase the cellulose surface area to enzyme exposure. The xylose hydrolysis yields in Figure 4.11 are also improved for the 525 H-factor cook indicating a better preferential breakdown of the polysaccharides to monomers by the enzymes. Figures 4.3 and 4.4 indicate that poplar had more lignin removed with the increase in H-factor severity. Previous work has related the level of delignification to enzymatic hydrolysis response in hardwoods and softwoods (Mansfield et al., 1999; Schwald, Brownell, & Saddler, 1988; Yu, Jameel, Chang, & Park, 2011) and grasses (Li et al., 2012; Ohgren, Bura, Saddler, & Zacchi, 2007; Williams & Hodge, 2014). These studies show that a larger quantity of lignin removal typically improve sugar yields during enzymatic hydrolysis. However, Figures 4.10 and 4.11 indicate that greater sugar yields are obtained from feedstocks with higher residual lignin content. While this data is slightly confounding it could be an expected result. Previously, delignification below 5%, along with xylan removal, resulted in overall decreased digestibility potentially due to aggregation of cellulose microfibrils (Ishizawa et al., 2009).

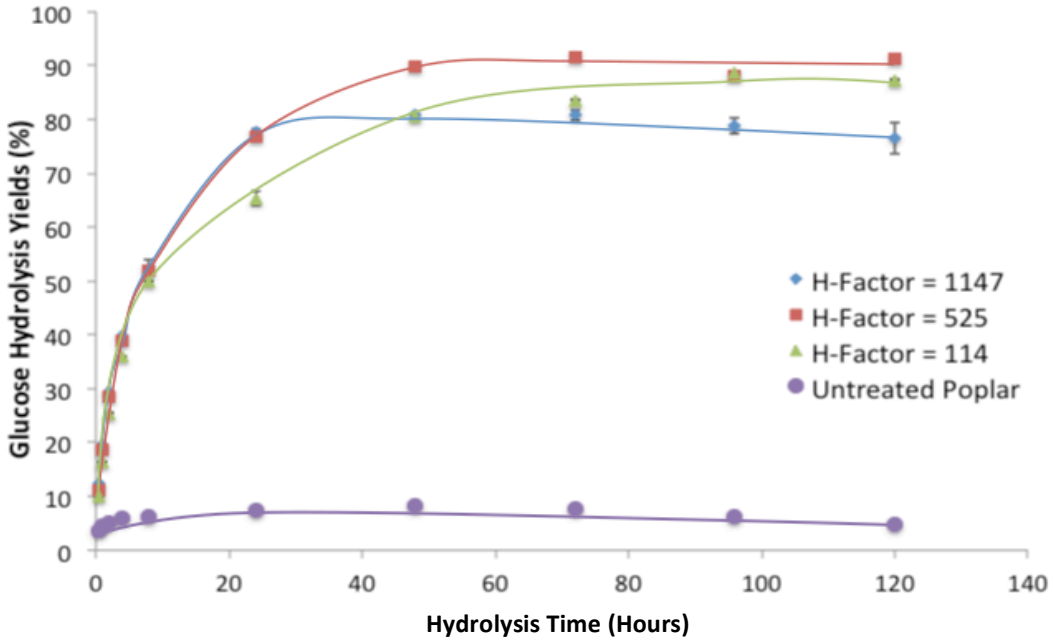

Figure 4.10. Glucose enzymatic hydrolysis yields for soda pulped poplar at an H-factor of 1147, 525, and 114; enzyme loading of 20 mg per g glucan.

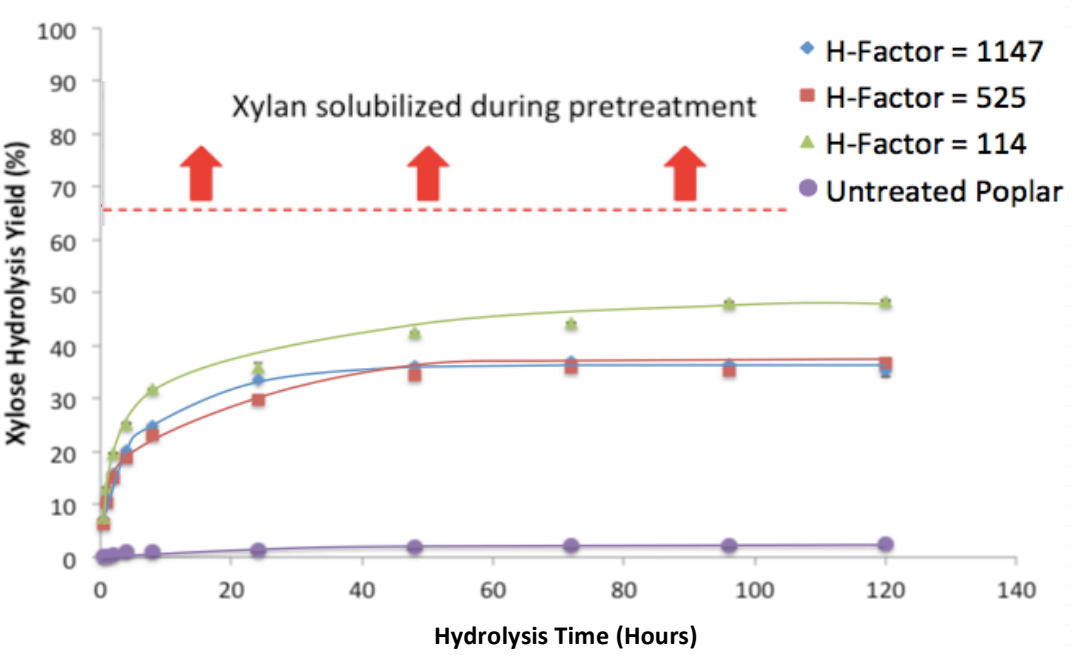

Figure 4.11. Xylose enzymatic hydrolysis yields for soda pulped poplar at an H-factor of 1147, 525, and 114; enzyme loading of 20 mg per g glucan.

For comparison, LHW pretreated poplar also underwent enzymatic hydrolysis to assess hydrolysis yield differences when compared to soda pulping. Figure 4.12 presents the glucose hydrolysis yields for LHW pretreated poplar at two different enzyme loadings.

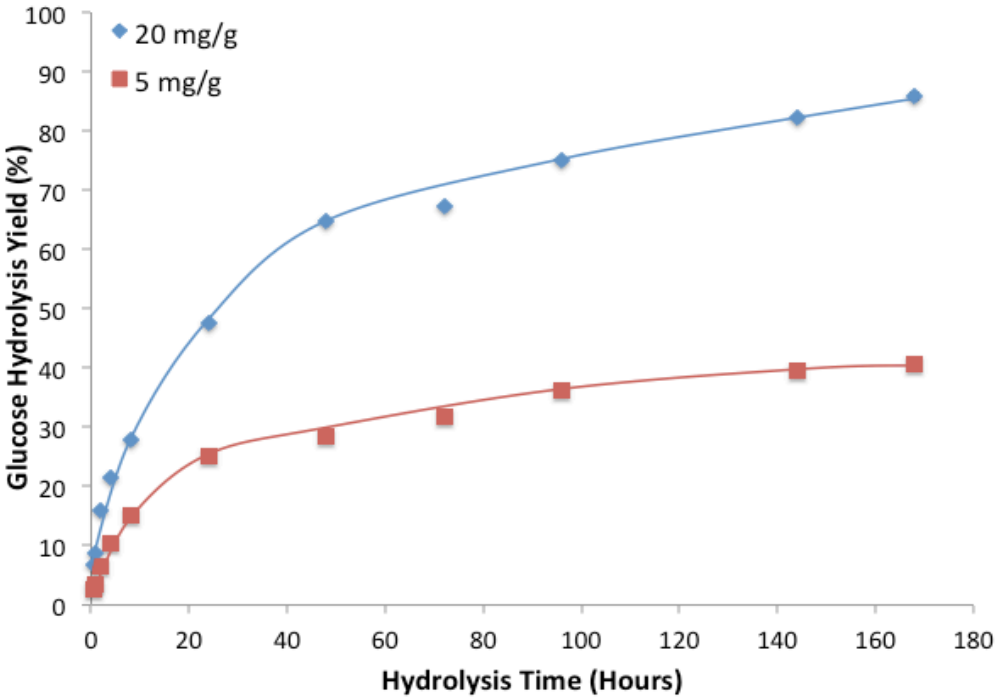

Figure 4.12. Glucose enzymatic hydrolysis yields for LHW poplar; enzyme loadings of 20 and 5 mg per g glucan.

The LHW pretreatment on poplar starts to level off around a glucan yield of 70% after 4 day hydrolysis. When compared to the soda pulped poplar the sugar release for LHW treated poplar is slower based on Figures 4.8 and 4.10 showing a consistent yield of 80% after 1 day hydrolysis. Xylose yields for LHW pretreated poplar are given in Figure 4.13. The xylose yields steadily increase for the LHW pretreated poplar over the course of hydrolysis to reach a final conversion around 30%, but a 40% yield of xylose can be achieved after 24 hour hydrolysis for the soda pulped poplar as indicated in Figures 4.9 and 4.11. Xylose yields were corrected to account for

losses by solubilization during pretreatment, and possible degradation by dehydration reactions; both losses are common for LHW pretreatments (Garrote, Dominguez, & Parajo, 1999).

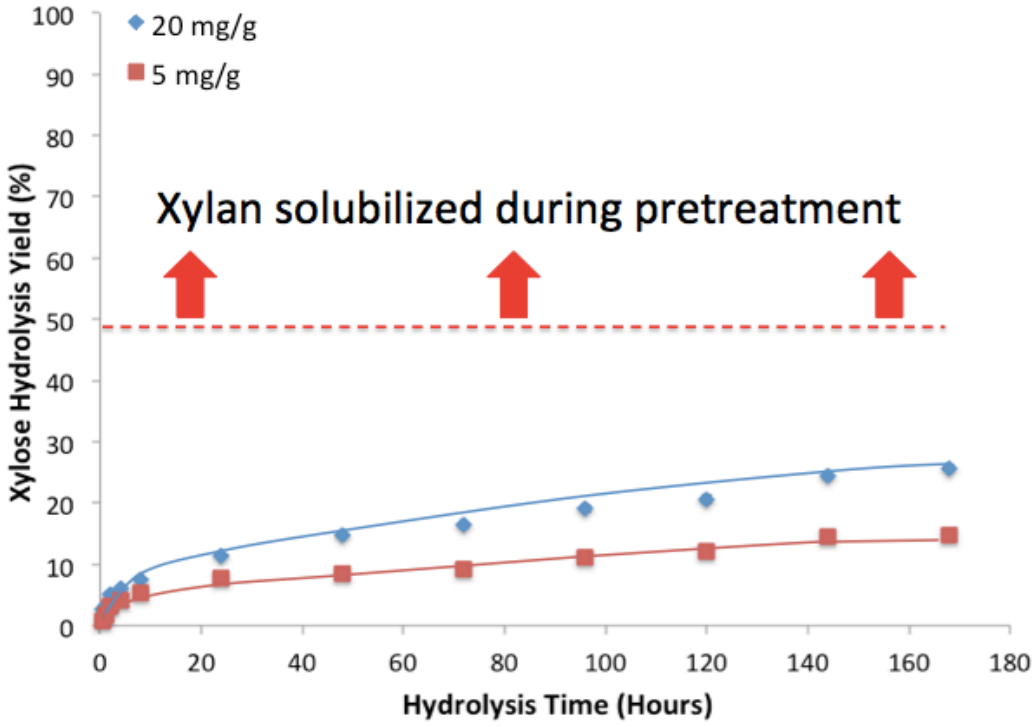

Figure 4.13. Xylose enzymatic hydrolysis yields for LHW poplar; enzyme loadings of 20 and 5 mg per g glucan.

The mechanism of LHW pretreatments is the dissolution and hydrolysis of hemicellulose, while the lignin fraction is only altered structurally and not removed in large quantities (Y. Kim et al., 2009). The composition chart in Figure 4.3 clearly shows a higher lignin content for LHW pretreated poplar as opposed to soda pulped poplar at the high H-factor cook. This higher lignin content probably influences the lower hydrolysis yields obtained for the LHW preatreated poplar.

Hydrolysis yields were also evaluated by varying the loading of enzymes for hydrolysis or the substrate particle size. Figure 4.14 and 4.15 presents the hydrolysis yields for glucose and xylose from soda pulped birch at varying enzyme loadings.

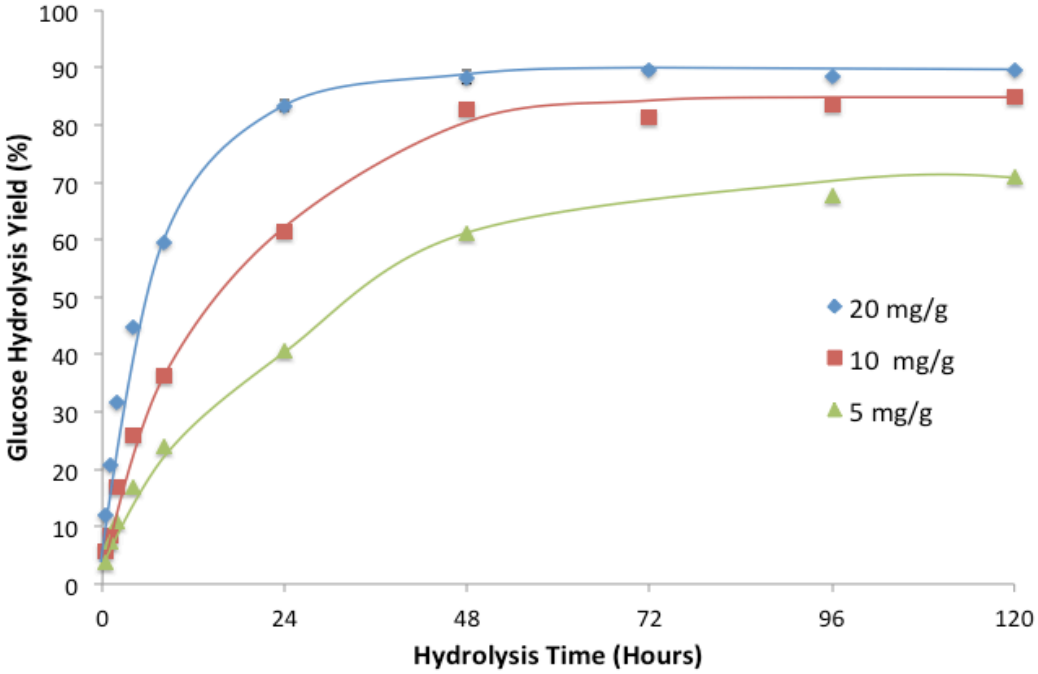

Figure 4.14. Glucose enzymatic hydrolysis for soda pulped birch (H = 1147) at enzyme loadings of 20, 10, and 5 mg per g glucan.

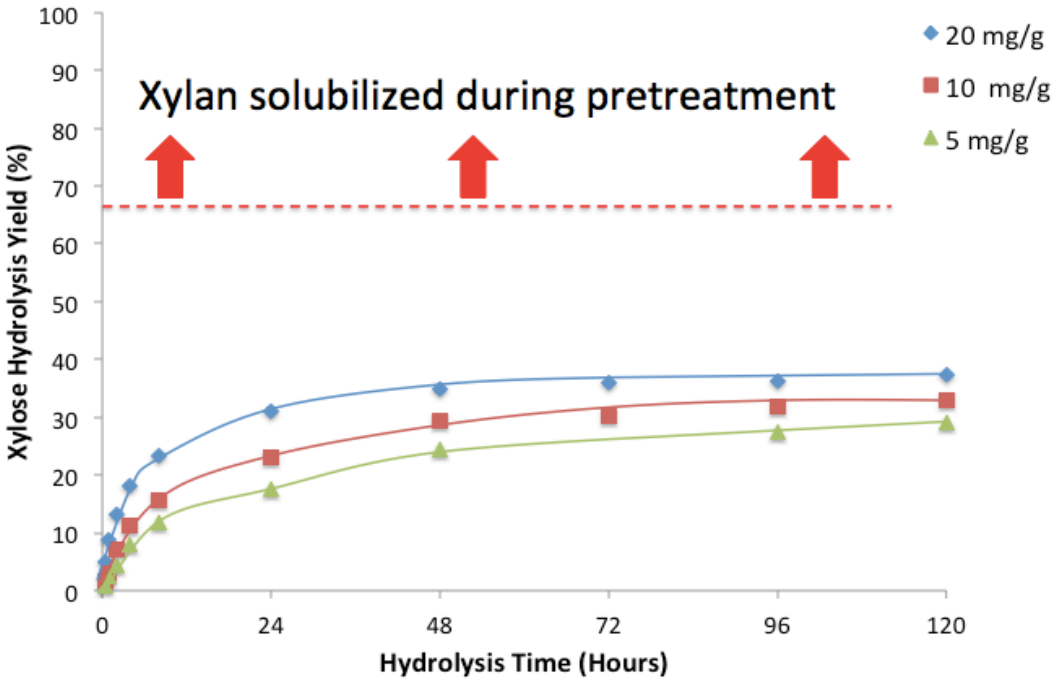

Figure 4.15. Xylose enzymatic hydrolysis for soda pulped birch (H = 1147) at enzyme loadings of 20, 10, and 5 mg per g glucan.

The most obvious trend in Figures 4.14 and 4.15 is that hydrolysis yields decrease when lower enzyme additions are utilized. Excluding pretreatment costs the high cost of enzyme production for biofuels produced by the biochemical platform is one of the limiting steps that challenge commercialization (Klein-Marcuschamer, Oleskowicz-Popiel, Simmons, & Blanch, 2012). By generating high sugar yields at lower enzyme addition it is possible for the production of biofuels from lignocellulosic biomass to be competitive. The lower enzyme dosages used here show that a 10 mg enzyme addition per g glucan can achieve very similar glucan and xylan yields when compared to 20 mg enzyme addition. However, the 5 mg enzyme addition only produces a glucan yield around 70% and a xylan yield below 30%, thus wasting sugars that can be recovered at the higher enzyme loading conditions.

Additionally, since a two-stage post-treatment milling was performed on the biomass to decrease the wood chip particle size, an extra round of enzymatic hydrolysis studies was carried out on the wood chips that were only "disintegrated" in a laboratory blender to determine hydrolysis yields. The disintegration step gave the wood chips a more exposed surface and unlocked a looser fiber structure. Figure 4.16 and 4.17 plot the glucose and xylose hydrolysis yields obtained for disintegrated wood chips versus the yields from knife-milling. The yields show each time point for birch and poplar after the high severity trial (H = 1147), and poplar after the mid-severity trial (H = 525). Initially the yields in Figures 4.16 and 4.17 do not appear to vary greatly, which would be an unexpected result since a larger particle size have produced lower hydrolysis yields due to a lower availability of cellulose surface area, or a diffusion limited step for the enzymes to access the polysaccharides in the substrate (Jorgensen, Kristensen, & Felby, 2007).

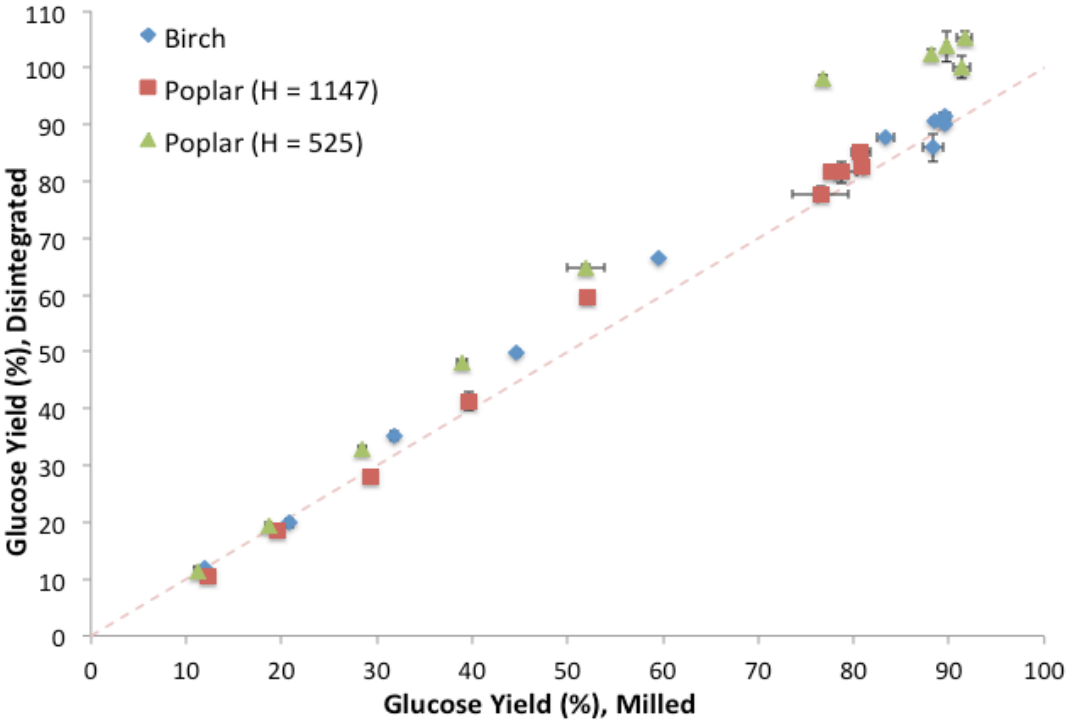

Figure 4.16. Disintegration hydrolysis yields versus knife-milled hydrolysis yields for glucose.

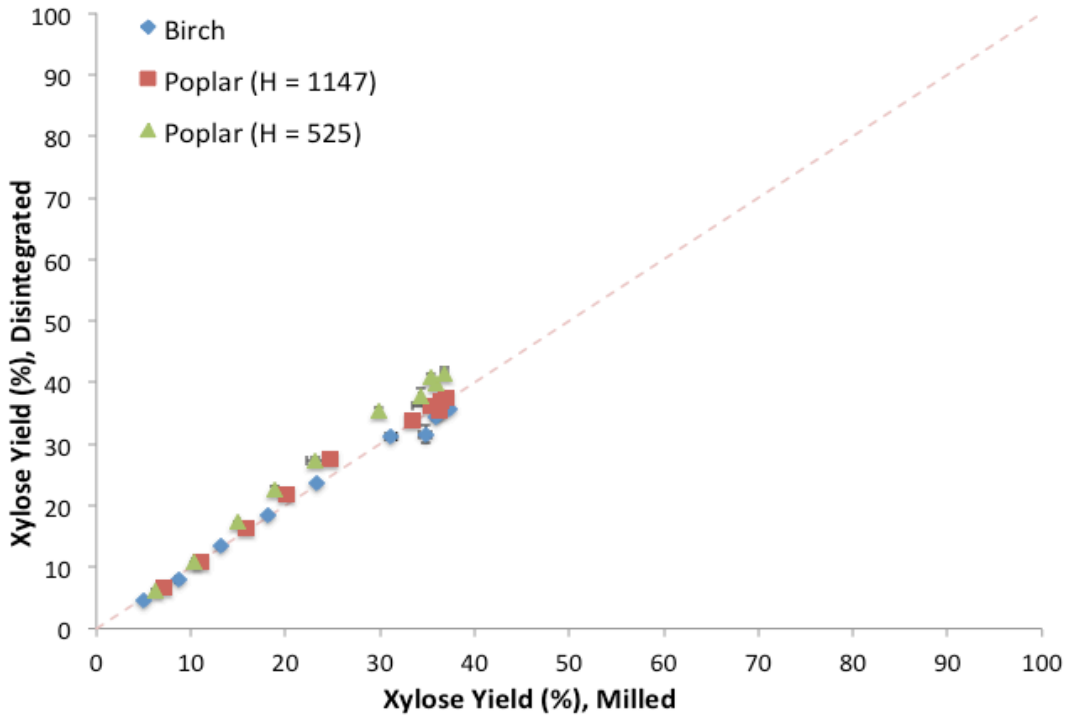

Figure 4.17. Disintegration hydrolysis yields versus knife-milled hydrolysis yields for xylose.

A paired T-test was used, at a significance level of 0.05, to statistically compare the glucose and xylose hydrolysis yields obtained for each particle size reduction method. The glucose hydrolysis yields for soda birch indicated statistically significant differences with a P-value 0f 0.0466, but the xylose hydrolysis yields produced no statistical differences with a P-value of 0.0619. Both P-values are came very close to the cutoff criteria so the effect of particle size on hydrolysis yields cannot be adequately considered to be important. For the high severity poplar the statistics indicate that no statistical differences exist for the glucose or xylose yields, both of which produce P-values greater than 0.05. Alternatively, the mid-severity poplar trial produced statistical differences for the glucose and xylose hydrolysis yields with P-values less than 0.002. Overall, differences in particle size did not make significant contributions to the overall hydrolysis yields. It should be understood that with additional biomass milling a lower quantity of enzymes can achieve high sugar yields (Garlock et al., 2012). Similar yields of soluble sugars after enzymatic hydrolysis with lower enzyme loadings and larger particle size shown in Figures 4.14 and 4.15 are encouraging for the aspect of producing a digestible feedstock after a soda pulping process.

4.3.4 Initial Hydrolysis Rates

The yields of soluble sugars after enzymatic hydrolysis were evaluated further to understand an initial rate of hydrolysis and the affect of lignin on the initial rate. The initial rates of hydrolysis were determined based upon a linear regression of the sugar released over the sample times of 0.5, 1, and 2 hours. Figures 4.18 and 4.19 plot the initial hydrolysis rates for glucose and xylose, respectively, against the residual lignin content for each treated and untreated biomass sample.

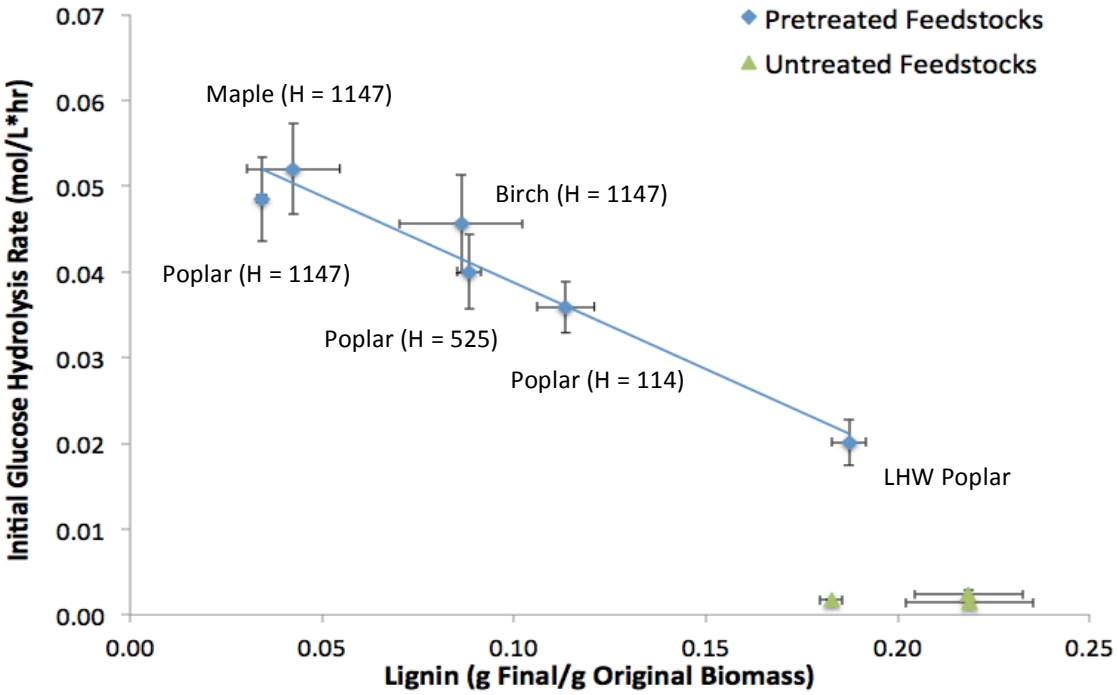

Figure 4.18. Initial glucose hydrolysis rates versus residual lignin content.

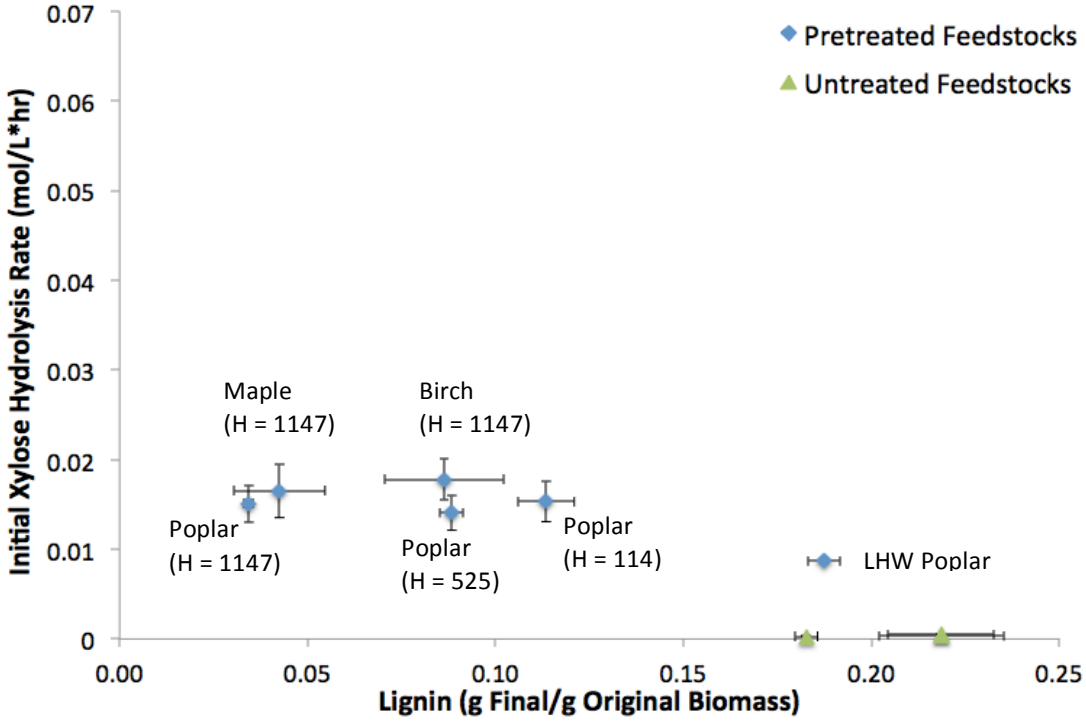

Figure 4.19. Initial xylose hydrolysis rates versus residual lignin content.

The initial rates for glucose and xylose release from untreated feedstocks are extremely low, an expected result since less than 10% yields of soluble sugars could be recovered from untreated feedstocks. When evaluating the initial rates of hydrolysis for birch, maple, and poplar at the 1147 H-factor trial, very little difference exists between both the release of glucose and xylose. The hybrid poplar treated at three different H-factors shows a steady decrease in the rate of glucose release, but the rate of xylose release does not appear to change much at all. The difference in the initial glucose rates for the hybrid poplar substrates is another indication of the role that lignin plays during enzymatic hydrolysis. Lower H-factor pulps show to contain more lignin in the residual substrate, as Figures 4.3 and 4.4 indicate, and although high sugar yields are still obtained with continued hydrolysis, the greater amount of lignin in the residual substrate could play a role in slowing down the action of the enzymes during the first couple of hours of hydrolysis. This can be supported from the initial rates achieved on LHW poplar, which are lower than the soda pulped poplar. Since LHW poplar contained the highest lignin content this is an extra indication the role lignin has in slowing down the rates of sugar release during the first house of enzymatic hydrolysis.

Furthermore, Figures 4.18 and 4.19 indicate a correlation between residual lignin content and the initial hydrolysis rates. Figure 4.19 clearly indicates no change in the rate of xylose release at short hydrolysis times, while Figure 4.18 shows a steady increase in the glucose release rate with decreasing lignin content (*i.e.* higher H-factor). More importantly, Figure 4.18 shows that even the initial glucose hydrolysis rate for LHW poplar falls in line with this trend. It is also interesting to see that the initial xylose hydrolysis rate in Figure 4.19 is independent of lignin content. A 95% confidence interval test was performed to see if the initial hydrolysis rates could be correlated to residual lignin content. The negative slope in Figure 4.18 for glucose did

not cross a value of zero for the confidence interval test; thus indicating that a correlation exists between the initial glucose hydrolysis rates and residual lignin content. The confidence interval test on the initial xylose hydrolysis rates confirmed that no correlation could be made to the residual lignin content. Overall, for similarly milled, pretreated materials, lignin content correlates strongly to the initial glucose hydrolysis yields.

4.4 Conclusions

Soda pulping on three different hardwood species was analyzed to determine hemicellulose and lignin dissolution during the pulping trial, and the processes ability to produce a digestible substrate for enzymes to recover monomeric sugars. Composition analysis after soda pulping indicated high removal amounts for lignin, but the xylan portion of the woody biomass could be recovered up to 70% of the initial material. For hybrid poplar three different H-factor pulping trials were evaluated and indicated decreasing yields of lignin with increasing H-factor, *i.e.* severity. At the highest H-factor setting around 10% of the lignin remained in the residual wood chips, while all three H-factor trials could retain around 60-70% of the initial xylan. During the course of soda pulping, the dissolution of xylan increased up to a point followed by slight decreases in the amount of xylan dissolved in the aqueous phase. Klason lignin dissolution continually increased throughout the trial.

The enzymatic hydrolysis of the residual wood chips indicated how soda pulping could produce a readily digestible substrate. The highest H-factor cooks (H = 1147) produced very similar glucose and xylose yields over a 5-day hydrolysis interval with final yields around 80% for glucose and 40% for xylose (after accounting for losses). When comparing different H-factor cooks on poplar (H = 1147 versus H = 525), the initial yields of sugars up to 24 hour hydrolysis

were essentially the same, but a slight increase in the final yields of glucose and xylose, around 90% and 50%, respectively, occurred for the poplar cooked at an H-factor of 525. Also by decreasing the enzyme loading to 10 mg of enzyme per g of glucan, similar yields of glucose and xylose were achieved. This is noteworthy since substantial delignification and substantial particle size reduction may not be necessary. Additionally, a larger substrate particle size did not appear to hinder the yields of monomeric sugars when compared to knife-milled substrates. Furthermore, the effect that lignin has on hydrolysis was indicated from the calculation of initial hydrolysis rates. Lower residual lignin content indicated a higher initial glucose hydrolysis rate could be achieved. Conversely, the initial xylose hydrolysis rates did not appear to be affected by the residual lignin content. This study shows how soda pulping can be utilized effectively to produce a residual black liquor stream that has high contents of hemicellulose and lignin for downstream recovery in a biorefinery setting, and an altered woody biomass feedstock for downstream enzymatic hydrolysis to recover monomeric sugars for conversion to liquid fuels or value-added chemicals.

REFERENCES

REFERENCES

Al-Dajani, W.W., & Tschirner, U.W. (2008). Pre-extraction of hemicelluloses and subsequent kraft pulping Part I: alkaline extraction. *Tappi Journal, 7*(6), 3-8.

Alekhina, M., Mikkonen, K.S., Alén, R., Tenkanen, M., & Sixta, H. (2013). Carboxymethylation of alkali extracted xylan for preparation of bio-based packaging films. *Carbohydrate Polymers*.

Andersson, A., Persson, T., Zacchi, G., Stalbrand, H., & Jonsson, A. S. (2007). Comparison of diafiltration and size-exclusion chromatography to recover hemicelluloses from process water from thermomechanical pulping of spruce. *Applied Biochemistry and Biotechnology, 137*, 971-983.

Andersson, C., Hodge, D., Berglund, K. A., & Rova, U. (2007). Effect of different carbon sources on the production of succinic acid using metabolically engineered Escherichia coli. *Biotechnology Progress, 23*(2), 381-388.

Araque, E., Parra, C., Freer, J., Contreras, D., Rodriguez, J., Mendonca, R., & Baeza, J. (2008). *Evaluation of organosolv pretreatment for the conversion of Pinus radiata D. Don to ethanol*.

Backman, R., Frederick, W. J., & Hupa, M. (1993). Basic studies on black-liquor pyrolysis and char gasification. *Bioresource Technology, 46*(1-2), 153-158.

Banerjee, G., Car, S., Scott-Craig, J. S., Hodge, D. B., & Walton, J. D. (2011). Alkaline peroxide pretreatment of corn stover: effects of biomass, peroxide, and enzyme loading and composition on yields of glucose and xylose. *Biotechnology for Biofuels, 4*, 15.

Barford, R. A., Magidman, P., Phillips, J. G., & Fishman, M. L. (1986). Estimation of degree of methylation of pectin by pyrolysis-gas chromatography *Analytical Chemistry, 58*(12), 2576-2578.

Baumberger, S., Abaecherli, A., Fasching, M., Gellerstedt, G., Gosselink, R., Hortling, B., . . . de Jong, E. (2007). Molar mass determination of lignins by size-exclusion chromatography: towards standardisation of the method. *Holzforschung, 61*(4), 459-468.

Bian, J., Peng, F., Peng, P., Xu, F., & Sun, R. C. (2010). Isolation and fractionation of hemicelluloses by graded ethanol precipitation from Caragana korshinskii. *Carbohydrate Research, 345*(6), 802-809.

Bobleter, O. (1994). Hydrothermal degradation of polymers derived from plants. *Progress in Polymer Science, 19*(5), 797-841.

Borrega, M., Nieminen, K., & Sixta, H. (2011). Degradation kinetics of the main carbohydrates in birch wood during hot water extraction in a batch reactor at elevated temperatures. *Bioresource Technology, 102*(22), 10724-10732.

Borrega, M., Tolonen, L.K., Bardot, F., Testova, L., & Sixta, H. (2012). Potential of hot water extraction of birch wood to produce high-purity dissolving pulp after alkaline pulping. *Bioresource Technology*.

Calvo-Flores, F. G., & Dobado, J. A. (2010). Lignin as Renewable Raw Material. *Chemsuschem, 3*(11), 1227-1235.

Carpita, N. C. (1983). Hemicellulosic polymers of cell-walls of *Zea* coleoptiles *Plant Physiology, 72*(2), 515-521.

Carpita, N. C. (1996). Structure and biogenesis of the cell walls of grasses. *Annual Review of Plant Physiology and Plant Molecular Biology, 47*, 445-476.

Carpita, N. C., & Whittern, D. (1986). A highly substituted glucuronoarabinoxylan from developing maize coleoptiles *Carbohydrate Research, 146*(1), 129-140.

Cathala, B., Saake, B., Faix, O., & Monties, B. (2003). Association behaviour of lignins and lignin model compounds studied by multidetector size-exclusion chromatography. *Journal of Chromatography A, 1020*(2), 229-239.

Chang, V. S., Kaar, W. E., Burr, B., & Holtzapple, M. T. (2001). Simultaneous saccharification and fermentation of lime-treated biomass. *Biotechnology Letters, 23*(16), 1327-1333.

Chang, V. S., Nagwani, M., Kim, C. H., & Holtzapple, M. T. (2001). Oxidative lime pretreatment of high-lignin biomass - Poplar wood and newspaper. *Applied Biochemistry and Biotechnology, 94*(1), 1-28.

Chen, CL. (1992). *Methods in Lignin Chemistry*. Heidelberg: Springer.

Cheng, H., Zhan, H., Fu, S., & Lucia, L. A. (2010). Alkali extraction of hemicellulose from depithed corn stover and effects on soda-aq-pulping. *Bioresources, 6*(1), 196-206.

Chum, H., Diebold, J., Scahill, J., Johnson, D., Black, S., Schroeder, H., & Kreibich, R. E. (1989). Biomass pyrolysis oil feedstocks for phenolic adhesives. *Acs Symposium Series, 385*, 135-151.

Chum, H. L., Johnson, D. K., Tucker, M. P., & Himmel, M. E. (1987). Some aspects of lignin characterization by high-performance size exclusion chromatography using styrene divinylbenzene copolymer gels. *Holzforschung, 41*(2), 97-108.

Chum, H. L., Ratcliff, M., Schroeder, H. A., & Sopher, D. W. (1984). Electrochemistry of biomass-derived materials. 1. Characterization, fractionation, and reductive electrolysis

of ethanol-extracted explosively-depressurized aspen lignin. *Journal of Wood Chemistry and Technology, 4*(4), 505-532.

Chundawat, S. P. S., Bellesia, G., Uppugundla, N., Sousa, L. D., Gao, D. H., Cheh, A. M., . . . Dale, B. E. (2011). Restructuring the Crystalline Cellulose Hydrogen Bond Network Enhances Its Depolymerization Rate. *Journal of the American Chemical Society, 133*(29), 11163-11174.

Chundawat, S. P. S., Donohoe, B. S., Sousa, L. D., Elder, T., Agarwal, U. P., Lu, F. C., . . . Dale, B. E. (2011). Multi-scale visualization and characterization of lignocellulosic plant cell wall deconstruction during thermochemical pretreatment. *Energy & Environmental Science, 4*(3), 973-984.

Compere, A.L., & Griffith, W.L. (2009). Preparation and analysis of biomass lignins. In J. R. Mielenz (Ed.), *Biofuels: Methods and Protocols* (Vol. 581, pp. 185-212).

Compere, A.L., Griffith, W.L., Leitten, C.F., & Pickel, J.M. (2005). Evaluation of lignin from alkaline-pulped hardwood black liquor: ORNL.

Contreras, S., Gaspar, A.R., Guerra, A., Lucia, L.A., & Argyropoulos, D.S. (2008). Propensity of lignin to associate: light scattering photometry study with native lignins. *Biomacromolecules, 9*(12), 3362-3369.

Crestini, C., Melone, F., Sette, M., & Saladino, R. (2011). Milled wood lignin: a linear oligomer. *Biomacromolecules, 12*(11), 3928-3935.

Dahlman, O., Jacobs, A., & Sjoberg, J. (2003). Molecular properties of hemicelluloses located in the surface and inner layers of hardwood and softwood pulps. *Cellulose, 10*(4), 325-334.

Dai, Q. Z., Jameel, H., & Chang, H. M. (2006). Precipitation of extractives onto kraft pulps during black liquor recycling in extended delignification process. *Journal of Wood Chemistry and Technology, 26*(1), 35-51.

Dale, B. (2008). Biofuels: Thinking clearly about the issues. *Journal of Agricultural and Food Chemistry, 56*(11), 3885-3891.

Dale, B. E., & Ong, R. G. (2012). Energy, wealth, and human development: Why and how biomass pretreatment research must improve. *Biotechnology Progress, 28*(4), 893-898.

Danielsson, S., & Lindstrom, M. E. (2005). Influence of birch xylan adsorption during kraft cooking on softwood pulp strength. *Nordic Pulp & Paper Research Journal, 20*(4), 436-441.

David, K., & Ragauskas, A. J. (2010). Switchgrass as an energy crop for biofuel production: A review of its ligno-cellulosic chemical properties. *Energy & Environmental Science, 3*(9), 1182-1190.

Del Rio, L. F., Chandra, R. P., & Saddler, J. N. (2010). The Effect of Varying Organosolv Pretreatment Chemicals on the Physicochemical Properties and Cellulolytic Hydrolysis of Mountain Pine Beetle-Killed Lodgepole Pine. *Applied Biochemistry and Biotechnology, 161*(1-8), 1-21.

Dong, D. (1993). *Characterization of Kraft Lignin and Investigation of Pulping Effects on Pulp Yield, Lignin Molecular Mass and Lignin Content of Black Liquor with a Central Composite Pulping Design.* University of Florida, Ann Arbor, MI. (9505752)

Dong, D. J., & Fricke, A. L. (1995). Effects of multiple pulping variables on the molecular-weight and molecular-weight distribution of kraft lignin. *Journal of Wood Chemistry and Technology, 15*(3), 369-393.

Dong, D. J., Fricke, A. L., Moudgil, B. M., & Johnson, H. (1996). Electrokinetic study of kraft lignin. *Tappi Journal, 79*(7), 191-197.

Ebringerova, A. (2006). Structural diversity and application potential of hemicelluloses. *Macromolecular Symposia, 232*, 1-12.

Ebringerova, A., Hromadkova, Z., Burchard, W., Dolega, R., & Vorwerg, W. (1994). Solution properties of water-insoluble rye-bran arabinoxylan *Carbohydrate Polymers, 24*(3), 161-169.

Elder, T.J., & Soltes, E.J. (1981). Pyrolysis of Lignocellulosic Materials. Phenolic Constituents of A Wood Pyrolytic Oil. *Wood and Fiber Science, 12*(4), 217-226.

EPA, United States. (2013). Questions and Answers on Changes to the Renewable Fuel Standard Program (RFS2). Retrieved March 17, 2014, 2014, from http://www.epa.gov/otaq/fuels/renewablefuels/compliancehelp/rfs2-aq.htm

Francis, R. C., Shin, S. J., Omori, S., Amidon, T. E., & Blain, T. J. (2006). Soda pulping of hardwoods catalyzed by anthraquinone and methyl substituted anthraquinones. *Journal of Wood Chemistry and Technology, 26*(2), 141-152.

Franco, H., Ferraz, A., Milagres, A. M. F., Carvalho, W., Freer, J., Baeza, J., & Mendonca, R. T. (2012). Alkaline sulfite/anthraquinone pretreatment followed by disk refining of Pinus radiata and Pinus caribaea wood chips for biochemical ethanol production. *Journal of Chemical Technology and Biotechnology, 87*(5), 651-657.

Froass, P. M., Ragauskas, A. J., & Jiang, J. (1996). Chemical structure of residual lignin from kraft pulp. *Journal of Wood Chemistry and Technology, 16*(4), 347-365.

Froass, P. M., Ragauskas, A. J., & Jiang, J. E. (1998). NMR studies Part 3: Analysis of lignins from modern kraft pulping technologies. *Holzforschung, 52*(4), 385-390.

Garcia-Perez, M., Chaala, A., Yang, J., & Roy, C. (2001). Co-pyrolysis of sugarcane bagasse with petroleum residue. Part I: thermogravimetric analysis. *Fuel, 80*(9), 1245-1258.

Gargulak, J. D., & Lebo, S. E. (2000). Commercial use of lignin-based materials. In W. G. Glasser, R. A. Northey & T. P. Schultz (Eds.), *Lignin : Historical, Biological, and Materials Perspectives* (Vol. 742, pp. 304-320). Washington: Amer Chemical Soc.

Garlock, R. J., Wong, Y. S., Balan, V., & Dale, B. E. (2012). AFEX Pretreatment and Enzymatic Conversion of Black Locust (Robinia pseudoacacia L.) to Soluble Sugars. *Bioenergy Research, 5*(2), 306-318.

Garrote, G., Dominguez, H., & Parajo, J. C. (1999). Mild autohydrolysis: an environmentally friendly technology for xylooligosaccharide production from wood. *Journal of Chemical Technology and Biotechnology, 74*(11), 1101-1109.

Gidh, A. V., Decker, S. R., Vinzant, T. B., Himmel, M. E., & Williford, C. (2006). Determination of lignin by size exclusion chromatography using multi angle laser light scattering. *Journal of Chromatography A, 1114*(1), 102-110.

Gierer, J. (1980). Chemical aspects of kraft pulping *Wood Science and Technology, 14*(4), 241-266.

Girio, F. M., Fonseca, C., Carvalheiro, F., Duarte, L. C., Marques, S., & Bogel-Lukasik, R. (2010). Hemicelluloses for fuel ethanol: A review. *Bioresource Technology, 101*(13), 4775-4800.

Giudici, R., & Park, S. W. (1996). Kinetic model for kraft pulping of hardwood. *Industrial & Engineering Chemistry Research, 35*(3), 856-863.

Glasser, W. G., Dave, V., & Frazier, C. E. (1993). Molecular-weight distribution of (semi)commercial lignin derivatives. *Journal of Wood Chemistry and Technology, 13*(4), 545-559.

Glasser, W. G., Kaar, W. E., Jain, R. K., & Sealey, J. E. (2000). Isolation options for non-cellulosic heteropolysaccharides (HetPS). *Cellulose, 7*(3), 299-317.

Goring, D. A. I., & Timell, T. E. (1960). Molecular properties of 6 4-O-methylglucuronoxylans *Journal of Physical Chemistry, 64*(10), 1426-1430.

Gosselink, R.J.A., Abächerli, A., Semke, H., Malherbe, R., Käuper, P., Nadif, A., & Van Dam, J.E.G. (2004). Analytical protocols for characterisation of sulphur-free lignin. *Industrial Crops and Products, 19*(3), 271-281.

Griebl, A., Lange, T., Weber, H., Milacher, W., & Sixta, H. (2006). *Xylo-oligosaccharide (XOS) formation through hydrothermolysis of xylan derived from viscose process*.

Guerra, A., Gaspar, A.R., Contreras, S., Lucia, L.A., Crestini, C., & Argyropoulos, D.S. (2007). On the propensity of lignin to associate: a size exclusion chromatography study with lignin derivatives isolated from different plant species. *Phytochemistry, 68*(20), 2570-2583.

Hakala, T. K., Liitia, T., & Suurnakki, A. (2013). Enzyme-aided alkaline extraction of oligosaccharides and polymeric xylan from hardwood kraft pulp. *Carbohydrate Polymers, 93*(1), 102-108.

Hamilton, J.K. (1962). The behaviour of wood carbohydrates in technical pulping processes. *Pure and Applied Chemistry, 5*(1), 197-218.

Hampton, H. A., Haworth, W. N., & Hirst, E. L. (1929). Polysaccharides Part IV The constitution of xylan. *Journal of the Chemical Society*, 1739-1753.

Hannuksela, T., Tenkanen, M., & Holmbom, B. (2002). Sorption of dissolved galactoglucomannans and galactomannans to bleached kraft pulp. *Cellulose, 9*(3-4), 251-261.

Hansen, N. M. L., & Plackett, D. (2008). Sustainable films and coatings from hemicelluloses: A review. *Biomacromolecules, 9*(6), 1493-1505.

Hartley, R. D. (1983). Degradation of cell-walls of forages by sequential treatment with sodium-hydroxide and a commercial cellulase preparation *Journal of the Science of Food and Agriculture, 34*(1), 29-36.

Harton, S.E., Pingali, S.V., Nunnery, G.A., Baker, D.A., Walker, S.H., Muddiman, D.C., . . . Langan, P. (2012). Evidence for complex molecular architectures for solvent-extracted lignins. *Acs Macro Letters, 1*(5), 568-573.

Helmerius, J., von Walter, J. V., Rova, U., Berglund, K. A., & Hodge, D. B. (2010). Impact of hemicellulose pre-extraction for bioconversion on birch Kraft pulp properties. *Bioresource Technology, 101*(15), 5996-6005.

Hendriks, A., & Zeeman, G. (2009). Pretreatments to enhance the digestibility of lignocellulosic biomass. *Bioresource Technology, 100*(1), 10-18.

Himmel, M. E., Ding, S. Y., Johnson, D. K., Adney, W. S., Nimlos, M. R., Brady, J. W., & Foust, T. D. (2007). Biomass recalcitrance: Engineering plants and enzymes for biofuels production. *Science, 315*(5813), 804-807.

Hodge, D. B., Andersson, C., Berglund, K. A., & Rova, U. (2009). Detoxification requirements for bioconversion of softwood dilute acid hydrolyzates to succinic acid. *Enzyme and Microbial Technology, 44*(5), 309-316.

Holladay, J.E., Bozell, J.J., White, J.F., & Johnson, D. (2007). Top value-added chemicals from biomass. Volume II - Results of Screening for Potential Candidates from Biorefinery Lignin. In PNNL-16983 (Ed.).

Horhammer, H., Walton, S., & van Heiningen, A. (2011). A larch based biorefinery: pre-extraction and extract fermentation to lactic acid. *Holzforschung, 65*(4), 491-496.

Huang, H. J., Lin, W. L., Ramaswamy, S., & Tschirner, U. (2009). Process Modeling of Comprehensive Integrated Forest Biorefinery-An Integrated Approach. *Applied Biochemistry and Biotechnology, 154*(1-3), 205-216.

Huang, H. J., Ramaswamy, S., Al-Dajani, W. W., & Tschirner, U. (2010). Process modeling and analysis of pulp mill-based integrated biorefinery with hemicellulose pre-extraction for ethanol production: a comparative study. *Bioresource Technology, 101*(2), 624-631.

Ibn Yaich, A., Edlund, U., & Albertsson, A. C. (2012). Wood Hydrolysate Barriers: Performance Controlled via Selective Recovery. *Biomacromolecules, 13*(2), 466-473.

Iiyama, K., Lam, T. B. T., & Stone, B. A. (1990). Phenolic-acid bridges between polysaccharides and lignin in wheat internodes *Phytochemistry, 29*(3), 733-737.

International Energy Statistics - Petroleum Production. (2013).

Ishizawa, C. I., Jeoh, T., Adney, W. S., Himmel, M. E., Johnson, D. K., & Davis, M. F. (2009). Can delignification decrease cellulose digestibility in acid pretreated corn stover? *Cellulose, 16*(4), 677-686.

Izydorczyk, M. S., & MacGregor, A. W. (2000). Evidence of intermolecular interactions of beta-glucans and arabinoxylans. *Carbohydrate Polymers, 41*(4), 417-420.

Jackson, L. S., Heitmann, J. A., & Joyce, T. W. (1998). Production of dissolving pulp from recovered paper using enzymes. *Tappi Journal, 81*(3), 171-178.

Jacobs, A., & Dahlman, O. (2001). Characterization of the molar masses of hemicelluloses from wood and pulps employing size exclusion chromatography and matrix-assisted laser desorption ionization time-of-flight mass spectrometry. *Biomacromolecules, 2*(3), 894-905.

Janzon, R., Saake, B., & Puls, J. (2008). Upgrading of paper-grade pulps to dissolving pulps by nitren extraction: properties of nitren extracted xylans in comparison to NaOH and KOH extracted xylans. *Cellulose, 15*(1), 161-175.

Johansson, M. H., & Samuelson, O. (1977). Reducing end groups in birch xylan and their alkaline-degradation *Wood Science and Technology, 11*(4), 251-263.

Jönsson, A.S., Nordin, A.K., & Wallberg, O. (2008). Concentration and purification of lignin in hardwood kraft pulping liquor by ultrafiltration and nanofiltration. *Chemical Engineering Research and Design, 86*(11), 1271-1280.

Jorgensen, H., Kristensen, J. B., & Felby, C. (2007). Enzymatic conversion of lignocellulose into fermentable sugars: challenges and opportunities. *Biofuels Bioproducts & Biorefining-Biofpr, 1*(2), 119-134.

Kakola, J., Alen, R., Pakkanen, H., Matilainen, R., & Lahti, K. (2007). Quantitative determination of the main aliphatic carboxylic acids in wood kraft black liquors by high-performance liquid chromatography-mass spectrometry. *Journal of Chromatography A, 1139*(2), 263-270.

Karlsson, P., Roubroeks, J. P., Glasser, W. G., & Gatenholm, P. (2006). Optimization of the process conditions for the extraction of heteropolysaccharides from birch (Betula pendula). In J. J. Bozell & M. K. Patel (Eds.), *Feedstocks for the Future: Renewables for the Production of Chemicals and Materials* (Vol. 921, pp. 321-333). Washington: Amer Chemical Soc.

Kenealy, W.R., Houtman, C.J., Laplaza, J., Jeffries, T.W., & Horn, E.G. (2007). Pretreatments for converting wood into paper and chemicals. *Materials, Chemicals, and Energy From Forest Biomass (Argyropoulos, DS, ed.). Washington, DC: American Chemical Society*, 392-408.

Keyoumu, A., Sjodahl, R., Henriksson, G., Ek, M., Gellerstedt, G., & Lindstrom, M. E. (2004). Continuous nano- and ultra-filtration of kraft pulping black liquor with ceramic filters - A method for lowering the load on the recovery boiler while generating valuable side-products. *Industrial Crops and Products, 20*(2), 143-150.

Khiari, R., Mhenni, M. F., Belgacem, M. N., & Mauret, E. (2010). Chemical composition and pulping of date palm rachis and Posidonia oceanica - A comparison with other wood and non-wood fibre sources. *Bioresource Technology, 101*(2), 775-780.

Kim, T. H., Kim, J. S., Sunwoo, C., & Lee, Y. Y. (2003). Pretreatment of corn stover by aqueous ammonia. *Bioresource Technology, 90*(1), 39-47.

Kim, Y., Mosier, N. S., & Ladisch, M. R. (2009). Enzymatic Digestion of Liquid Hot Water Pretreated Hybrid Poplar. *Biotechnology Progress, 25*(2), 340-348.

Klein-Marcuschamer, D., Oleskowicz-Popiel, P., Simmons, B. A., & Blanch, H. W. (2012). The challenge of enzyme cost in the production of lignocellulosic biofuels. *Biotechnology and Bioengineering, 109*(4), 1083-1087.

Klinke, H. B., Ahring, B. K., Schmidt, A. S., & Thomsen, A. B. (2002). Characterization of degradation products from alkaline wet oxidation of wheat straw. *Bioresource Technology, 82*(1), 15-26.

Koda, K., Gaspar, A. R., Yu, L., & Argyropoulos, D. S. (2005). Molecular weight-functional group relations in softwood residual kraft lignins. *Holzforschung, 59*(6), 612-619.

Kohnke, T., Ostlund, A., & Brelid, H. (2011). Adsorption of Arabinoxylan on Cellulosic Surfaces: Influence of Degree of Substitution and Substitution Pattern on Adsorption Characteristics. *Biomacromolecules, 12*(7), 2633-2641.

Kongruang, S., Han, M. J., Breton, C. I. G., & Penner, M. H. (2004). Quantitative analysis of cellulose-reducing ends. *Applied Biochemistry and Biotechnology, 113*, 213-231.

Konn, J., Pranovich, A., & Holmbom, B. (2006). Dissolution of fibre material in alkaline pre-treatment and refining of spruce CTMP. *Holzforschung, 60*(1), 32-39.

Koshijima, T., Timell, T. E., & Zinbo, M. (1965). Number-average molecular weight of native hardwood xylans *Journal of Polymer Science Part C-Polymer Symposium*(11PC).

Krawczyk, H, Oinonen, P, & Jönsson, A.S. (2013). Combined membrane filtration and enzymatic treatment for recovery of high molecular mass hemicelluloses from chemithermomechanical pulp process water. *Chemical Engineering Journal, 225*, 292-299.

Lake, M.A., & Blackburn, J.C. (2011). United States of America Patent No. US20110294991 A1.

Lan, T. Q., Gleisner, R., Zhu, J. Y., Dien, B. S., & Hector, R. E. (2013). High titer ethanol production from SPORL-pretreated lodgepole pine by simultaneous enzymatic saccharification and combined fermentation. *Bioresource Technology, 127*, 291-297.

Lapierre, C., Jouin, D., & Monties, B. (1989). On the molecular-origin of the alkali solubility of gramineae lignins *Phytochemistry, 28*(5), 1401-1403.

Lau, M. W., Gunawan, C., & Dale, B. E. (2009). The impacts of pretreatment on the fermentability of pretreated lignocellulosic biomass: a comparative evaluation between ammonia fiber expansion and dilute acid pretreatment. *Biotechnology for Biofuels, 2*, 11.

Lawford, H. G., & Rousseau, J. D. (1993). Production of ethanol from pulp-mill hardwood and softwood spent sulfite liquors by genetically-engineered *Escherichia coli*. *Applied Biochemistry and Biotechnology, 39*, 667-685.

LeBel, R. G., Goring, D. A. I., & Timell, T. E. (1963). Solution Properties of Birch Xylan. I. Measurement of Molecular Weight. *Journal of Polymer Science: Part C, 2*(1), 19.

Lehto, J., & Alen, R. (2013). Alkaline Pre-treatment of Hardwood Chips Prior to Delignification. *Journal of Wood Chemistry and Technology, 33*(2), 77-91.

Li, M. Y., Foster, C., Kelkar, S., Pu, Y. Q., Holmes, D., Ragauskas, A., . . . Hodge, D. B. (2012). Structural characterization of alkaline hydrogen peroxide pretreated grasses exhibiting

diverse lignin phenotypes. *Biotechnology for Biofuels, 5*(38), (6 June 2012)-(2016 June 2012).

Linder, A., Bergman, R., Bodin, A., & Gatenholm, P. (2003). Mechanism of assembly of xylan onto cellulose surfaces. *Langmuir, 19*(12), 5072-5077.

Lindstrom, M., Jameel, H., Naithani, V., Kirkman, A., & Renard, J. (2007). Integrating Black Liquor Gasification and Pulping: A Review of Current Technology. In D. S. Argyropoulos (Ed.), *Materials, Chemicals, and Energy from Forest Biomass* (Vol. 954, pp. 434-462). Washington: Amer Chemical Soc.

Liu, W., Hou, Q. X., Mao, C. B., Yuan, Z. R., & Li, K. C. (2012). Effect of Hemicellulose Pre-extraction on the Properties and Bleachability of Aspen (Populus tremuloides) Chemithermomechanical Pulp. *Industrial & Engineering Chemistry Research, 51*(34), 11122-11127.

Liu, W., Yuan, Z. R., Mao, C. B., Hou, Q. X., & Li, K. C. (2012). Extracting hemicelluloses prior to aspen chemi-thermomechanical pulping: Effects of pre-extraction on pulp properties. *Carbohydrate Polymers, 87*(1), 322-327.

Liu, X., Fatehi, P., & Ni, Y. (2012). Removal of inhibitors from pre-hydrolysis liquor of kraft-based dissolving pulp production process using adsorption and flocculation processes. *Bioresource Technology, 116*, 492-496.

Lora, J. H., & Glasser, W. G. (2002). Recent industrial applications of lignin: A sustainable alternative to nonrenewable materials. *Journal of Polymers and the Environment, 10*(1-2), 39-48.

Loutfi, H, Blackwell, B, & Uloth, V. (1991). Lignin recovery from kraft black liquor: preliminary process design. *Tappi Journal, 74*(1), 203-210.

Madakadze, I. C., Radiotis, T., Li, J., Goel, K., & Smith, D. L. (1999). Kraft pulping characteristics and pulp properties of warm season grasses. *Bioresource Technology, 69*(1), 75-85.

Malawer, E.G., & Senak, L. (2004). Introduction to Size Exclusion Chromatography. In C. Wu (Ed.), *Handbook of Size Exclusion Chromatography and Related Techniques* (Second ed., Vol. 91). New York, NY, USA: Marcel Dekker, Inc.

Mamman, A. S., Lee, J. M., Kim, Y. C., Hwang, I. T., Park, N. J., Hwang, Y. K., . . . Hwang, J. S. (2008). Furfural: Hemicellulose/xylosederived biochemical. *Biofuels Bioproducts & Biorefining-Biofpr, 2*(5), 438-454.

Mansfield, S. D., Mooney, C., & Saddler, J. N. (1999). Substrate and enzyme characteristics that limit cellulose hydrolysis. *Biotechnology Progress, 15*(5), 804-816.

Mänttäri, M., Van der Bruggen, B., & Nyström, M. (2013). Nanofiltration. In S. Ramaswamy, H.-J. Huang & B. V. Ramarao (Eds.), *Separation and Purification Technologies in Biorefineries* (pp. 233-258).

Marzialetti, T., Olarte, M. B. V., Sievers, C., Hoskins, T. J. C., Agrawal, P. K., & Jones, C. W. (2008). Dilute acid hydrolysis of Loblolly pine: A comprehensive approach. *Industrial & Engineering Chemistry Research, 47*(19), 7131-7140.

Mayfield, C. A., Foster, C. D., Smith, C. T., Gan, J. B., & Fox, S. (2007). Opportunities, barriers, and strategies for forest bioenergy and bio-based product development in the Southern United States. *Biomass & Bioenergy, 31*(9), 631-637.

Mendes, C.V.T., Rocha, J.M.S., Sousa, G.D.A., & Carvalho, M.G.V.S. (2011). Extraction of hemicelluloses prior to kraft cooking: A step for an integrated biorefinery in the pulp mill. *O PAPEL, 72*(9), 79-83.

Miao, Z., Grift, T. E., Hansen, A. C., & Ting, K. C. (2011). Energy requirement for comminution of biomass in relation to particle physical properties. *Industrial Crops and Products, 33*(2), 504-513.

Molin, U., & Teder, A. (2002). Importance of cellulose/hemicellulose-ratio for pulp strength. *Nordic Pulp & Paper Research Journal, 17*(1), 14.

Monteil-Rivera, F., Phuong, M., Ye, M. W., Halasz, A., & Hawari, J. (2013). Isolation and characterization of herbaceous lignins for applications in biomaterials. *Industrial Crops and Products, 41*, 356-364.

Morreel, K., Kim, H., Lu, F. C., Dima, O., Akiyama, T., Vanholme, R., . . . Boerjan, W. (2010). Mass Spectrometry-Based Fragmentation as an Identification Tool in Lignomics. *Analytical Chemistry, 82*(19), 8095-8105.

Mosier, N., Wyman, C., Dale, B., Elander, R., Lee, Y. Y., Holtzapple, M., & Ladisch, M. (2005). Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresource Technology, 96*(6), 673-686.

Mousavioun, P., & Doherty, W. O. S. (2010). Chemical and thermal properties of fractionated bagasse soda lignin. *Industrial Crops and Products, 31*(1), 52-58.

N'diaye, S., Rigal, L., Larocque, P., & Vidal, P. F. (1996). Extraction of hemicelluloses from poplar, Populus tremuloides, using an extruder-type twin-screw reactor: A feasibility study. *Bioresource Technology, 57*(1), 61-67.

Nagy, M., Kosa, M., Theliander, H., & Ragauskas, A.J. (2010). Characterization of CO2 precipitated Kraft lignin to promote its utilization. *Green Chemistry, 12*(1), 31-34.

Naran, R., Black, S., Decker, S. R., & Azadi, P. (2009). Extraction and characterization of native heteroxylans from delignified corn stover and aspen. *Cellulose, 16*(4), 661-675.

Nowakowski, D. J., Bridgwater, A. V., Elliott, D. C., Meier, D., & de Wild, P. (2010). Lignin fast pyrolysis: Results from an international collaboration. *Journal of Analytical and Applied Pyrolysis, 88*(1), 53-72.

Ohgren, K., Bura, R., Saddler, J., & Zacchi, G. (2007). Effect of hemicellulose and lignin removal on enzymatic hydrolysis of steam pretreated corn stover. *Bioresource Technology, 98*(13), 2503-2510.

Ohman, F. (2006). *Precipitation and separation of lignin from kraft black liquor.* (Doctoral), Chalmers University of Technology.

Oka, D., Kobayashi, K., Isobe, N., Ogawa, Y., Yokoyama, T., Kimura, S., . . . Wada, M. (2013). Enzymatic hydrolysis of wood with alkaline treatment. *Journal of Wood Science, 59*(6), 484-488.

Olm, L., & Tistad, G. (1979). Kinetics of the initial-stage of kraft pulping *Svensk Papperstidning-Nordisk Cellulosa, 82*(15), 458-464.

Olsson, L., & HahnHagerdal, B. (1996). Fermentation of lignocellulosic hydrolysates for ethanol production. *Enzyme and Microbial Technology, 18*(5), 312-331.

Pan, X. J., Gilkes, N., Kadla, J., Pye, K., Saka, S., Gregg, D., . . . Saddler, J. (2006). Bioconversion of hybrid poplar to ethanol and co-products using an organosolv fractionation process: Optimization of process yields. *Biotechnology and Bioengineering, 94*(5), 851-861.

Pauly, M., & Keegstra, K. (2008). Cell‐wall carbohydrates and their modification as a resource for biofuels. *The Plant Journal, 54*(4), 559-568.

Peng, F., Ren, J. L., Xu, F., Bian, J., Peng, P., & Sun, R. C. (2010). Fractionation of Alkali-Solubilized Hemicelluloses from Delignified Populus gansuensis: Structure and Properties. *Journal of Agricultural and Food Chemistry, 58*(9), 5743-5750.

Perlack, R. D., & Stokes, B. J. (2011). *U.S. Billion-Ton Update: Biomass Supply for a Bioenergy and Bioproducts Industry*. Oak Ridge, TN: Oak Ridge National Laboratory.

Persson, T., Krawczyk, H., Nordin, A. K., & Jonsson, A. S. (2010). Fractionation of process water in thermomechanical pulp mills. *Bioresource Technology, 101*(11), 3884-3892.

Persson, T., Nordin, A.K., Zacchi, G., & Jönsson, A.S. (2007). Economic evaluation of isolation of hemicelluloses from process streams from thermomechanical pulping of spruce *Applied Biochemistry and Biotecnology* (pp. 741-752): Springer.

Phillips, R. B., Jameel, H., & Chang, H. M. (2013). Integration of pulp and paper technology with bioethanol production. *Biotechnology for Biofuels, 6*.

Pinto, P. C., Evtuguin, D. V., Neto, C. P., Silvestre, A. J. D., & Amado, F. M. L. (2002). Behavior of Eucalyptus globulus lignin during kraft pulping. II. Analysis by NMR, ESI/MS, and GPC. *Journal of Wood Chemistry and Technology, 22*(2-3), 109-125.

Pitifer, L. A., McLellan, M. R., & Vanburen, J. P. (1994). Analysis of pectin content and degree of polymerization in orange juice *Food Chemistry, 50*(1), 29-32.

Ponder, G. R., & Richards, G. N. (1997a). Arabinogalactan from Western larch .1. Effect of uronic acid groups on size exclusion chromatography. *Journal of Carbohydrate Chemistry, 16*(2), 181-193.

Ponder, G. R., & Richards, G. N. (1997b). Arabinogalactan from Western larch, Part III: alkaline degradation revisited, with novel conclusions on molecular structure. *Carbohydrate Polymers, 34*(4), 251-261.

Potthast, A., Rosenau, T., & Kosma, P. (2006). Analysis of oxidized functionalities in cellulose. *Polysaccharides Ii, 205*, 1-48.

Pulp and Paper Capacities Survery 2011-2016. (2012). Rome, Italy.

Puls, J., & Saake, B. (2004). Industrially isolated hemicelluloses. In P. Gatenholm & M. Tenhanen (Eds.), *Hemicelluloses: Science and Technology* (Vol. 864, pp. 24-37).

Puls, J., Schroder, N., Stein, A., Janzon, R., & Saake, B. (2006). Xylans from oat spelts and birch kraft pulp. *Macromolecular Symposia, 232*, 85-92.

Ragauskas, A. J., Nagy, M., Kim, D.H., Eckert, C. A., Hallett, J. P., & Liotta, C. L. (2006). From wood to fuels: integrating biofuels and pulp production. *Industrial biotechnology, 2*(1), 55-65.

Ragnar, M., Lindgren, C.T., & Nilvebrant, N.O. (2000). pKa-values of guaiacyl and syringyl phenols related to lignin. *Journal of Wood Chemistry and Technology, 20*(3), 277-305.

Ralph, S.A., Ralph, J., & Landucci, L. (2004). NMR Database of Lignin and Cell Wall Model Compounds. Madison, WI: US Forest Products Laboratory.

Robert, D. R., Bardet, M., Gellerstedt, G., & Lindfors, E. L. (1984). Structural-changes in lignin during kraft cooking. 3. On the structure of dissolved lignins. *Journal of Wood Chemistry and Technology, 4*(3), 239-263.

Rosell, K. G., & Svensson, S. (1975). Studies of distribution of 4-O-methyl-D-glucuronic acid residues in birch xylan. *Carbohydrate Research, 42*(2), 297-304.

Saadatmand, S., Edlund, U., Albertsson, A. C., Danielsson, S., & Dahlman, O. (2012). Prehydrolysis in Softwood Pulping Produces a Valuable Biorefinery Fraction for Material Utilization. *Environmental Science & Technology, 46*(15), 8389-8396.

Saake, B., Kruse, T., & Puls, J. (2001). Investigation on molar mass, solubility and enzymatic fragmentation of xylans by multi-detected SEC chromatography. *Bioresource Technology, 80*(3), 195-204.

Salmenoja, K. (1993). Black-liquor gasification - theoretical and experimental studies. *Bioresource Technology, 46*(1-2), 167-171.

Sannigrahi, P., Ragauskas, A. J., & Miller, S. J. (2008). Effects of Two-Stage Dilute Acid Pretreatment on the Structure and Composition of Lignin and Cellulose in Loblolly Pine. *Bioenergy Research, 1*(3-4), 205-214.

Sarkanen, S., Teller, D.C., Hall, J., & McCarthy, J.L. (1981). Lignin. 18. Associative effects among organosolv lignin components. *Macromolecules, 14*(2), 426-434.

Saulnier, L., Vigouroux, J., & Thibault, J. F. (1995). Isolation and partial characterization of feruloylated oligosaccharides from maize bran. *Carbohydrate Research, 272*(2), 241-253.

Schwald, W., Brownell, H. H., & Saddler, J. N. (1988). Enzymatic-hydrolysis of steam treated aspen wood - Influence of partial hemicellulose and lignin removal prior to pretreatment *Journal of Wood Chemistry and Technology, 8*(4), 543-560.

Shuai, L., Yang, Q., Zhu, J. Y., Lu, F. C., Weimer, P. J., Ralph, J., & Pan, X. J. (2010). Comparative study of SPORL and dilute-acid pretreatments of spruce for cellulosic ethanol production. *Bioresource Technology, 101*(9), 3106-3114.

Sills, D. L., & Gossett, J. M. (2011). Assessment of commercial hemicellulases for saccharification of alkaline pretreated perennial biomass. *Bioresource Technology, 102*(2), 1389-1398.

Simpson, W., & TenWolde, A. (2007). Physical properties and moisture relations of wood. In U. S. D. o. Agriculture (Ed.), *The Encyclopedia of Wood*. New York: Skyhorse Publishing, Inc.

Sjostrom, E. (1993). *Wood Chemistry: Fundamentals and Applications*. San Diego, CA: Academic Press.

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., & Templeton, D. (2005). Determination of Ash in Biomass *NREL Laboratoy Analytical Procedures*. Golden, Colorado, United States: National Renewable Energy Laboratory.

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., & Templeton, D. (2006). Determination of Sugars, Byproducts, and Degradation Prodcuts in Liquid Fraction Process Samples *NREL Laboratory Analytical Procedures*. Golden, Colorado, United States: National Renewable Energy Laboratory.

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., & Crocker, D. (2008). Determination of Structural Carbohydrates and Lignin in Biomass *NREL Laboratory Analytical Procedure*. Golden, Colorado, United States: National Renewable Energy Laboratory.

Sluiter, A., Ruiz, R., Scarlata, C., Sluiter, J., & Templeton, D. (2005). Determination of Extractives in Biomass *NREL Laboratoy Analytical Procedures*. Golden, Colorado, United States: National Renewable Energy Laboratory.

Song, H., & Lee, S.Y. (2006). Production of succinic acid by bacterial fermentation. *Enzyme and Microbial Technology, 39*(3), 352-361.

Stewart, D. (2008). Lignin as a base material for materials applications: Chemistry, application and economics. *Industrial Crops and Products, 27*(2), 202-207.

Stoklosa, R.J., & Hodge, D.B. (2012). Extraction, Recovery, and Characterization of Hardwood and Grass Hemicelluloses for Integration into Biorefining Processes. *Industrial & Engineering Chemistry Research, 51*(34), 11045-11053.

Stoklosa, R.J., Velez, J., Kelkar, S., Saffron, C.M., Thies, M.C., & Hodge, D.B. (2013). Correlating lignin structural features to phase partitioning behavior in a novel aqueous fractionation of softwood Kraft black liquor. *Green Chemistry, 15*(10), 2904-2912.

Sturgeon, R. J. (1973). Determination of degree of polymerization of xylans *Carbohydrate Research, 30*(1), 175-178.

Suhas, Carrott, PJM, & Riberio Carrott, MML. (2007). Lignin - from natural adsorbent to activated carbon: A review. *Bioresource Technology, 98*(12), 2301-2312.

Sun, R. C. (2009). Detoxification and separation of lignocellulosic biomass prior to fermentation for bioethanol production by removal of lignin and hemicelluloses. *Bioresources, 4*(2), 452-455.

Sykes, R., Yung, M., Novaes, E., Kirst, M., Peter, G., & Davis, M. (2009). High-Throughput Screening of Plant Cell-Wall Composition Using Pyrolysis Molecular Beam Mass Spectroscopy. In J. R. Mielenz (Ed.), *Biofuels: Methods and Protocols* (Vol. 581, pp. 169-183).

Teleman, A., Tenkanen, M., Jacobs, A., & Dahlman, O. (2002). Characterization of O-acetyl-(4-O-methylglucurono)xylan isolated from birch and beech. *Carbohydrate Research, 337*(4), 373-377.

Testova, L., Roselli, A., Costabel, L., Kovasin, K., Tenkanen, M., & Sixta, H. (2014). Combined Production of Polymeric Birch Xylan and Paper Pulp by Alkaline Pre-extraction Followed by Alkaline Cooking. *Industrial & Engineering Chemistry Research, 53*(19), 8302-8310.

Timell, T. E. (1960). Isolation and properties of an O-acetyl-4-O-methylglucurono-xyloglycan from the wood of white birch (*Betula papyrifera)*. *Journal of the American Chemical Society, 82*(19), 5211-5215.

Tomani, P. (2010). The lignoboost process. *Cellulose Chemistry & Technology, 44*(1), 53.

Torget, R., Werdene, P., Himmel, M., & Grohmann, K. (1990). Dilute acid pretreatment of short rotation woody and herbaceous crops *Applied Biochemistry and Biotechnology, 24-5*, 115-126.

Tunc, M.S., & van Heiningen, A.R.P. (2008). Hydrothermal dissolution of mixed southern hardwoods. *Holzforschung, 62*(5), 539-545.

van de Pas, D., Hickson, A., Donaldson, L., Lloyd-Jones, G., Tamminen, T., Fernyhough, A., & Mattinen, M.L. (2011). Characterization of fractionated lignins polymerized by fungal laccases. *Bioresources, 6*(2), 1105-1121.

Van Heiningen, A. (2006). Converting a kraft pulp mill into an integrated forest biorefinery. *Pulp & Paper-Canada, 107*(6), 38-43.

VanWalsum, G. P., Allen, S. G., Spencer, M. J., Laser, M. S., Antal, M. J., & Lynd, L. R. (1996). Conversion of lignocellulosics pretreated with liquid hot water to ethanol. *Applied Biochemistry and Biotechnology, 57-8*, 157-170.

Vasile, C., Popescu, M.C, Stoleriu, A., & Gosselink, R.J.A. (2006). New Tends in Natural and Synthetic Polymer Science. In C. Vasile & G. Zaikov (Eds.), (pp. 135-163). New York: Nova Science Publishers, Inc.

Velez, J., & Thies, M.C. (2013). Solvated Liquid-Lignin Fractions from a Kraft Black Liquor. *Bioresource Technology*.

Vena, P.F., Brienzo, M., del Prado García-Aparicio, M., Görgens, J.F., & Rypstra, T. Hemicelluloses extraction from giant bamboo (Bambusa balcooa Roxburgh) prior to kraft or soda-AQ pulping and its effect on pulp physical properties. *Holzforschung*, 1-8.

Venica, A. D., Chen, C. L., & Gratzl, J. S. (2008). Soda-AQ delignification of poplar wood. Part 1: Reaction mechanism and pulp properties. *Holzforschung, 62*(6), 627-636.

Villaverde, J. J., Li, J. B., Ek, M., Ligero, P., & de Vega, A. (2009). Native Lignin Structure of Miscanthus x giganteus and Its Changes during Acetic and Formic Acid Fractionation. *Journal of Agricultural and Food Chemistry, 57*(14), 6262-6270.

Vishtal, A.G., & Kraslawski, A. (2011). Challenges in industrial applications of technical lignins. *Bioresources, 6*(3), 3547-3568.

Vlasenko, E.Y., Ryan, A.I., Shoemaker, C.F., & Shoemaker, S.P. (1998). The use of capillary viscometry, reducing end-group analysis, and size exclusion chromatography combined with multi-angle laser light scattering to characterize endo-1,4-beta-D-glucanases on carboxymethylcellulose : A comparative evaluation of the three methods. *Enzyme and Microbial Technology, 23*(6), 350-359.

Walton, S.L., Bischoff, K.M., van Heiningen, A.R.P., & van Walsum, G.P. (2010). Production of lactic acid from hemicellulose extracts by Bacillus coagulans MXL-9. *Journal of Industrial Microbiology & Biotechnology, 37*(8), 823-830.

Wang, G.S., Pan, X.J., Zhu, J.Y., Gleisner, R., & Rockwood, D. (2009). Sulfite pretreatment to overcome recalcitrance of lignocellulose (SPORL) for robust enzymatic saccharification of hardwoods. *Biotechnology Progress, 25*(4), 1086-1093.

Wang, Z.J., Lan, T.Q., & Zhu, J.Y. (2013). Lignosulfonate and elevated pH can enhance enzymatic saccharification of lignocelluloses. *Biotechnology for Biofuels, 6*.

Wei, Z.J., Yang, Y., Yang, R., & Wang, C.Y. (2012). Alkaline lignin extracted from furfural residues for pH-responsive Pickering emulsions and their recyclable polymerization. *Green Chemistry, 14*(11), 3230-3236.

Weng, J.K., & Chapple, C. (2010). The origin and evolution of lignin biosynthesis. *New Phytologist, 187*(2), 273-285.

Werpy, T., Frye, J., & Holladay, J. (2006). Succinic acid–a model building block for chemical production from renewable resources. *Biorefineries-Industrial Processes and Products: Status Quo and Future Directions*, 367-379.

Whistler, R.L., & Richards, E L. (1970). Hemicelluloses. In W. Pigman & D. Horton (Eds.), *The Carbohydrates - Chemistry and Biochemistry* (Second Edition ed., Vol. 2A, pp. 447-469). New York: Academic Press.

White, E.M. (2010). *Woody Biomass for Bioenergy and Biofuels in the United States - A Briefing Paper*. Portland, OR.

Wigell, A., Brelid, H., & Theliander, H. (2007). Degradation/dissolution of softwood hemicellulose during alkaline cooking at different temperatures and alkali concentrations. *Nordic Pulp & Paper Research Journal, 22*(4), 488-494.

Wilkie, K.C.B., & Woo, S.L. (1977). Hemicelluloses of bamboos *Arundinaria japonica* and *Arundinaria anceps*. 2. Heteroxylan and hemicellulosic materials from bamboo leaves, and a reconsideration of general nature of commonly occurring xylans and other hemicelluloses. *Carbohydrate Research, 57*(AUG), 145-162.

Williams, D. L., & Hodge, D. B. (2014). Impacts of delignification and hot water pretreatment on the water induced cell wall swelling behavior of grasses and its relation to cellulolytic enzyme hydrolysis and binding. *Cellulose, 21*(1), 221-235.

Williamson, M. (2013, April, 2013). Tembec: A Biorefiner Ahead of Its Time. *IPW Magazine,* 24-27.

Wyman, C.E., Dale, B.E., Elander, R.T., Holtzapple, M., Ladisch, M.R., & Lee, Y.Y. (2005). Coordinated development of leading biomass pretreatment technologies. *Bioresource Technology, 96*(18), 1959-1966.

Xu, J.L., Cheng, J.J., Sharma-Shivappa, R.R., & Burns, J.C. (2010). Sodium hydroxide pretreatment of switchgrass for ethanol production. *Energy & Fuels, 24*, 2113-2119.

Yang, B., & Wyman, C.E. (2008). Pretreatment: the key to unlocking low-cost cellulosic ethanol. *Biofuels Bioproducts & Biorefining-Biofpr, 2*(1), 26-40.

Yelle, D.J., Ralph, J., & Frihart, C.R. (2011). Delineating pMDI model reactions with loblolly pine via solution-state NMR spectroscopy. Part 1. Catalyzed reactions with wood models and wood polymers. *Holzforschung, 65*(2), 131-143.

Yoon, S.H., Tunc, M.S., & Van Heiningen, A. (2011). Near-neutral pre-extraction of hemicelluloses and subsequent kraft pulping of southern mixed hardwoods. *Tappi Journal, 10*(1), 7-15.

Yoon, S.H., van Heiningen, A., & Krishnagopalan, G.A. (2008). *Kraft Pulping Integrated with mild alkaline pre-extraction of southern mixed hardwoods*. Paper presented at the TAPPI Pulping and Enivonmental Coference, Portland, OR.

Yu, Z. Y., Jameel, H., Chang, H. M., & Park, S. (2011). The effect of delignification of forest biomass on enzymatic hydrolysis. *Bioresource Technology, 102*(19), 9083-9089.

Zeikus, J.G., Jain, M.K., & Elankovan, P. (1999). Biotechnology of succinic acid production and markets for derived industrial products. *Applied Microbiology and Biotechnology, 51*(5), 545-552.

Zhang, Y.H.P., & Lynd, L.R. (2005). Determination of the number-average degree of polymerization of cellodextrins and cellulose with application to enzymatic hydrolysis. *Biomacromolecules, 6*(3), 1510-1515.

Zhu, J.Y., Pan, X.J., Wang, G.S., & Gleisner, R. (2009). Sulfite pretreatment (SPORL) for robust enzymatic saccharification of spruce and red pine. *Bioresource Technology, 100*(8), 2411-2418.

Zhu, J.Y., Zhu, W.Y., Obryan, P., Dien, B.S., Tian, S., Gleisner, R., & Pan, X.J. (2010). Ethanol production from SPORL-pretreated lodgepole pine: preliminary evaluation of mass

balance and process energy efficiency. *Applied Microbiology and Biotechnology, 86*(5), 1355-1365.