

INFLUENCE OF PRETREATMENT, LIGNIN EXTRACTION, AND CHEMICAL MODIFICATION
ON LIGNIN PROPERTIES AND THE PERFORMANCE OF LIGNIN-FORMALDEHYDE
RESINS AND LIGNIN-PLA COMPOSITE MATERIALS

by

Brian Keith Saulnier

A dissertation submitted in partial fulfillment
of the requirements for the degree

of

Doctor of Philosophy

in

Chemical Engineering

MONTANA STATE UNIVERSITY
Bozeman, Montana

December 2022

©COPYRIGHT

by

Brian Keith Saulnier

2022

All Rights Reserved

ACKNOWLEDGEMENTS

First and foremost I would like to thank my advisor Dr. David Hodge for all his support and mentorship during my time here at Montana State. He has been a great facilitator of my professional development, helping me to become a better scientist, academic, and writer.

I also wish to acknowledge all the intellectual support, moral support, and lab assistance all the members of my lab group have provided during the many hours of working in the lab. The Hodge lab team has changed a lot over my 4 years here with new members arriving and departing including Joe Phongpreecha, Sandip Singh, Anthony Savoy, Villő Enikő Bécsy-Jakab, Dilara Dulger, Dylan Cousins, Will Otto, Mingfei Li, and Alex Brunat. What has remained constant is the unwavering support they have provided.

Finally I would like to recognize those members of other labs that have been crucial contributors to the successful completion of my research work and the writing of this thesis. Mohsen Siahkamari and Dr. Mojgan Nejad at Michigan State University for their work with formulation and characterization of lignin-formaldehyde resins and adhesives. Seth Kane and Dr. Cecily Ryan for their instruction and mentorship of the proper fabrication and characterization of composite materials. Dan Samborsky for his assistance in gathering tensile data. And finally Rhys Trousdale and the Walker lab group for training and the kindly allowed use of their instruments. Completing this project required the assistance of many different people, it would have been impossible to complete alone.

TABLE OF CONTENTS

1. INTRODUCTION AND BACKGROUND	1
General Introduction	1
Feedstock Selection	4
Pretreatment and Lignin Extraction Methods and Conditions.....	6
Lignin Fractionation.....	11
Lignin Chemical Modification.....	15
References.....	18
2. IMPACT OF DILUTE ACID PRETREATMENT CONDITIONS ON <i>p</i> -COUMARATE REMOVAL IN DIVERSE MAIZE LINES	26
Contribution of Authors and Co-Authors	26
Manuscript Information	27
Abstract	28
Introduction.....	28
Methods.....	32
Biomass Feedstock.....	32
Pretreatment Conditions.....	32
Composition Analysis.....	32
Esterified <i>p</i> -Coumarate Content	33
Enzymatic Hydrolysis.....	33
Methyl Trans <i>p</i> -Coumarate Hydrolysis	34
Results and Discussion	35
Biomass and Pretreatment.....	35
Impact of Pretreatment on Cell Wall Composition and Enzymatic.....	38
Hydrolysis Yields	
Correlation of Data	42
Ester Hydrolysis Kinetics	44
Conclusions.....	47
Acknowledgements.....	47
Supplementary Data.....	48
References.....	48
3. EFFECT OF DILUTE ACID PRETREATMENT AND LIGNIN EXTRACTION CONDITIONS ON LIGNIN PROPERTIES AND SUITABILITY AS A PHENOL REPLACEMENT IN PHENOL-FORMALDEHYDE WOOD ADHESIVES	52
Contribution of Authors and Co-Authors	52
Manuscript Information	53
Abstract.....	54
Introduction.....	54

TABLE OF CONTENTS CONTINUED

Materials and Methods.....	59
Dilute Acid Pretreatment	59
Enzymatic Hydrolysis.....	60
Lignin Extraction and Recovery	61
Composition Analysis.....	61
<i>p</i> -Coumarate Content.....	62
Gel Permeation Chromatography	62
Scale-Up of Pretreatment, Hydrolysis, and Lignin Extraction	63
Preparation of Resins and Adhesives.....	64
Resin and Adhesive Property Characterization	65
Results and Discussion	67
Biomass Pretreatment and Enzymatic Hydrolysis.....	67
Lignin Extraction, Recovery, and Characterization.....	68
Scale-Up of Pretreatment, Hydrolysis, and Lignin Extraction and Recovery	76
PF Resin and Adhesive Synthesis, Characterization, and Testing.....	79
Conclusions.....	82
Acknowledgements.....	84
Supplementary Data.....	84
References.....	85
 4. FRACTIONAL PRECIPITATION OF CORN STOVER FORMIC ACID LIQUORS	 91
Contribution of Authors and Co-Authors	91
Manuscript Information	92
Abstract	93
Introduction.....	93
Materials and Methods.....	96
Formic Acid Pretreatment and Lignin Extraction.....	96
Lignin Fractional Precipitation	96
Gel Permeation Chromatography	97
Composition Analysis.....	97
Enzymatic Hydrolysis.....	98
<i>p</i> -Coumarate Content.....	98
Results and Discussion	99
Composition and Mass Yields	99
Lignin Properties.....	102
Glucose Hydrolysis Yields	104
Suggestions for Future Work.....	107
Conclusions.....	108
References.....	108

TABLE OF CONTENTS CONTINUED

5. INCORPORATION OF FUNCTIONALIZED CORN STOVER LIGNIN FRACTIONS INTO PLA COMPOSITE MATERIALS.....	114
Contribution of Authors and Co-Authors	114
Manuscript Information	115
Abstract	116
Introduction.....	116
Materials and Methods.....	121
Lignin Generation and Fractionation	121
Biopolymer Functionalization	122
Composition Analysis.....	123
Composite Synthesis and Characterization.....	123
Gel Permeation Chromatography (GPC).....	124
Results and Discussion	126
Biomass Fractionation and Biopolymer Recovery	126
NMR Characterization of Functionalized Biopolymers	131
Synthesis of Lignin-PLA Composites	136
Characterization of Composite Thermal Properties.....	141
Molar Mass Distribution of Biopolymers, Functionalized Biopolymers, And PLA-Lignin blends.....	147
Composite Mechanical Properties	150
Conclusions.....	152
Supplementary Data.....	153
References.....	153
6. CONCLUSIONS.....	165
REFERENCES CITED.....	168

LIST OF TABLES

Table	Page
1. Dilute Acid Pretreatment Conditions and the Calculated Values for the Combined Severity factor (CSF)	37
2. Composition of Biomass	48
3. Conditions Used for Dilute Acid Pretreatment Screening at the 100 mL Scale	60
4. Properties of Lignin-Formaldehyde Resins	79
5. Properties of Original and Modified Lignin-Formaldehyde Adhesives	80
6. Elemental Composition of Recovered Lignins from Scale Up	84
7. Original and Modified Adhesive Formulation Components	85

LIST OF FIGURES

Figure	Page
1. Basic Biorefinery Process	2
2. Strategies for the Upgrading of Lignin Properties for Value-Added Applications	4
3. Pretreatment Methods for Biomass Deconstruction	6
4. Chemical Processes for the Fractionation of Lignin	11
5. Potential Sites Within Lignins for Incorporation into Phenol-Formaldehyde Resins	34
6. Diversity of <i>p</i> CA and Lignin in Corn Stover Samples	36
7. Impact of Pretreatment Conditions on Cell Wall Xylan Content for Distinct Maize Genotypes, All Samples for as a Function of Time, and The Initial pH for the 3-hr Pretreatment Time	39
8. Impact of Pretreatment Conditions on Cell Wall <i>p</i> CA Content for Distinct Maize Genotypes, All Samples for as a Function of Time, and The Initial pH for the 3-hr Pretreatment Time	41
9. Impact of Pretreatment Conditions on Cell Wall Glucose Hydrolysis Yields for Distinct Maize Genotypes, All Samples for as a Function of Time, and the Initial pH for the 3-hr Pretreatment Time	41
10. Correlations between Glucose Hydrolysis Yields and Xylan Solubilization, Glucose Hydrolysis Yields and <i>p</i> CA Content, and Xylan Solubilization and <i>p</i> CA Content	42
11. Reaction Kinetics of the Dilute Acid-Catalyzed Hydrolysis of <i>trans-p</i> CA Methyl Ester, corn stover <i>p</i> CA-Lignin Ester, and Corn Stover Xylan	45
12. Impact of Pretreatment Conditions on Subsequent Enzymatic Hydrolysis Yields and Relationships between Hydrolysis Yields and Xylan Removal For 48-hr Hydrolysis Times at Two Enzyme Loadings	68
13. Impact of Pretreatment Conditions on Lignin Extraction and Recovery Yields from the Resulting Corn Stover Lignin Cake and Recovers Lignin Number average Molar Mass using either NaOH or Formic Acid Lignin	71

LIST OF FIGURES CONTINUED

Figure	Page
14. Chemical Properties of Diverse Lignins Recovered from Corn Stover Lignin Cakes Using NaOH or Formic Acid Extraction.....	74
15. Esterified <i>p</i> CA in Grass Lignins and the Relationship between Pretreatment And Extraction Conditions on <i>p</i> CA Solubilization and Pretreatment Temperature on <i>p</i> CA Hydrolysis Kinetics.....	75
16. Relationship Between <i>p</i> CA content and ³¹ P NMR Determined <i>p</i> -Hydroxyphenyl Monomer	76
17. Lap Shear Strength of Adhesives Isolated from Dilute Acid Pretreated Corn Stover Lignin Cake with Results from Our Previous Work	82
18. Process Diagram for Formic Acid Treatment of Corn Stover and the Recovery of Five Biopolymer Fractions.....	99
19. Formic Acid Fraction Composition and Recovered Mass Yields	100
20. <i>p</i> CA Content of Formic Acid Fractions and Formic Acid Fraction Molecular Weight	103
21. Glucose Hydrolysis Yields after 72 Hours of FAF1, Solid Residue after Formic Acid Pretreatment, and Dilute Acid Pretreated Corn Stover.....	105
22. Three Biopolymer Fractions are Recovered by Fractional pH Adjustment Of Alkali Liquors.....	125
23. Oxyalkylation of Lignin and Xylan with Propylene Carbonate and a DBU Catalyst.....	126
24. Possible Lignin-Lignin, Lignin-Xylan, and Lignin-PLA Crosslinking Reactions During Modification and Extrusion	128
25. Polymer Composition of Recovered Biopolymer Before and After Modification and Precipitated Biopolymer and Insoluble Cellulose Fraction Mass Yields	130
26. ¹ H NMR of Unmodified and Modified Biopolymer Fractions.....	133

LIST OF FIGURES CONTINUED

Figure	Page
27. ^{13}C NMR Spectra of Unmodified and Modified Biopolymer Fractions.....	134
28. Viscosity during Composite Extrusion and Percent Viscosity Drop During Extrusion.....	137
29. Confocal Images of Modified and Unmodified Biopolymer Composites	140
30. Results of DMA Experiments showing Storage Modulus for 5% and 20% Biopolymer Composites, and Loss Modulus for 5% and 20% Composites	142
31. Glass Transition Temperature, Melting Temperature, Cold Crystallization Temperature, and Degree of Crystallinity of 5% and 20% Composites.....	143
32. GPC Spectra of Modified and Unmodified Biopolymer Fractions, Composite Materials, and Acetylated Unmodified Biopolymers	148
33. Mechanical Properties: Tensile Strength and Modulus and Strain at Break For 20% Composite Materials	151
34. DSC Thermograms of 5% and 20% Composite Materials	153

ABSTRACT

Bio-ethanol can be produced from lignocellulosic biomass in a biorefinery as part of a three step process, a chemical or mechanical pretreatment, enzymatic hydrolysis of the cell wall, and fermentation of these sugars to ethanol. One of the byproducts of this process is lignin, a complex biopolymer composed of a heterogeneous aromatic structure. Lignin is often burned to provide energy for the biorefinery. Incorporating lignin into higher-value products is crucial to the viability of the biorefinery process and the full utilization of the renewable carbon contained in biomass. Challenges to the inclusion of lignin in value-added products include recalcitrance of the cell wall to deconstruction and lignin extraction, heterogeneity of the lignin chemical structure, polydisperse molecular weight distributions, and low reactivity. In this thesis we address these challenges by using feedstock selection, selection of pretreatment and lignin extraction process conditions, lignin fractional precipitation, and direct chemical modification of lignin. Chapter 1 provides an overall introduction and background of previous work. Chapter 2 uses a diverse panel of corn stover genotypes subjected to dilute acid pretreatment using a variety of process conditions. The response of the biomass to pretreatment was characterized with special attention given to glucose hydrolysis yields and *p*-coumarate (*p*CA) content. Chapter 3 uses a single corn stover source pretreated using a variety of dilute acid conditions followed by two different lignin extraction methods. The influence of pretreatment and lignin extraction conditions on lignin properties was characterized with focus on lignin *p*CA content. This study found that lignin-formaldehyde resins using lignin from optimized process conditions achieved lap shear strengths higher than conventional phenol-formaldehyde resins. Chapter 4 addresses lignin polydispersity and heterogeneity using the fractional precipitation of lignin from formic acid liquors to obtain differing molecular weight lignin fractions while allowing for successful enzymatic hydrolysis of cellulose. Chapter 5 uses fractional precipitation of corn stover alkali liquors along with modification using propylene carbonate to obtain a panel of multi-component biopolymer fractions for manufacture of biopolymer-PLA composite materials. These materials were fully characterized finding materials made with modified biopolymers exhibited better lignin dispersion, and improved thermal and mechanical properties.

CHAPTER ONE

INTRODUCTION AND BACKGROUND

General Introduction

Global fossil fuel usage and CO₂ emissions continue to rise due largely to increased electrical demand in developing countries [1], however, concerns of climate change are causing some countries to transition from a traditionally fossil fuel based economy to one based on renewable, sustainable, and low emission technologies. Many nations have entered pacts such as the 2015 Paris Agreement with pledges to limit global warming to less than 2 °C, reach net zero CO₂ emissions by 2050, and negative emissions by 2070 [1]. One proposed method to help reach these goals is through the adoption of a bioeconomy. A bioeconomy reduces emissions and reduces reliance on fossil fuels by the implementation of lignocellulosic biomass to make biofuels, biomaterials, and other bio based products such as platform chemicals and fertilizers [2]. Lignocellulosic biomass is an abundant and renewable resource with a varying composition based on feedstock source ranging from 25-55% cellulose, 10-40% hemicellulose, 5-25% lignin, and lesser amounts of pectin, proteins, and waxes [3]. In order for the most efficient utilization of this resource, all of these components must be used for the generation of bio-based products.

The most commonly suggested application for lignocellulosic biomass is within a biorefinery, which can make a variety of products from biomass, one such process uses the polysaccharide fractions of the biomass for the production of ethanol. This process generally starts with a chemical, mechanical, or biological pretreatment to improve access to the polysaccharides within the lignocellulosic cell wall. This pretreatment is then followed by enzymatic hydrolysis of cellulose into monomeric sugars, fermentation, and finally recovery of

ethanol for implementation in biofuels (**Fig. 1**). A downside of the biorefinery process is underutilization of the lignin fraction. Lignin is a structural polymer present in most plants that are composed of a complex aromatic structure that shows promise for use in fuels, materials, and platform chemicals. Despite many potential uses, both the pulp and paper industry and the biorefinery industry typically burn lignin as a fuel source [4]. Implementing lignin into value-added applications creates an opportunity for an increase in viability of the biorefinery process and generation of additional value to the bioeconomy.

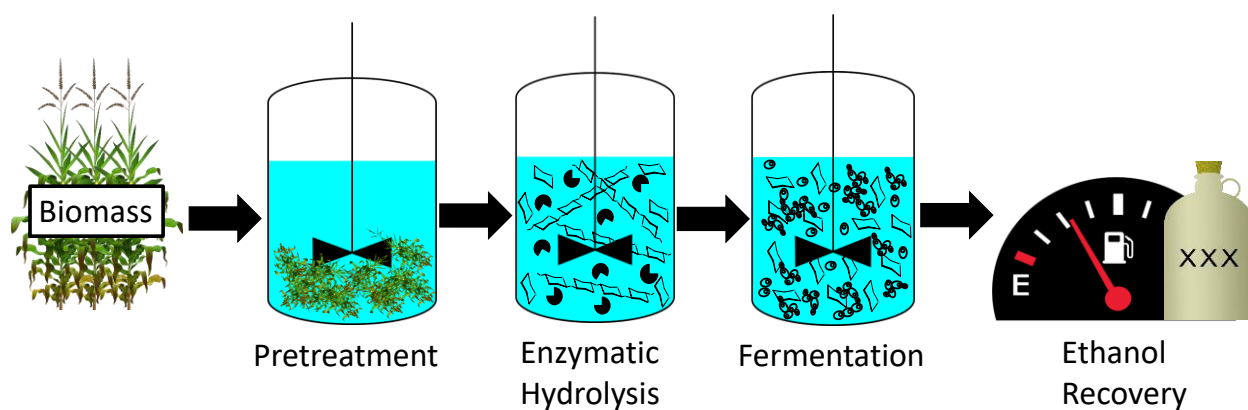


Figure 1: Basic Biorefinery Process

Despite the vast array of studies which have been conducted addressing challenges with lignin valorization, numerous difficulties with incorporating lignin into value-added products still exist. These challenges include recalcitrance of the biomass cell wall to deconstruction and lignin extraction, differences in lignin structure due to the feedstock and pretreatment method used, heterogeneous lignin chemical structure, and low lignin reactivity [4-6]. Four important methods for improving the properties of lignin and tailoring its chemical structure for a chosen application will be reviewed in this chapter. These methods include biomass feedstock selection,

selection of pretreatment and lignin extraction methods and processing conditions, lignin fractional precipitation, and direct chemical modification of lignin (**Fig. 2**). Feedstock selection influences the properties of recovered lignin through many means. The most drastic being differences in lignin chemical composition due to the type of biomass used (hardwood, softwood, graminaceous), however lignin structure can also be influenced by plant genetics, time of harvest, and plant anatomical fraction. The pretreatment process influences properties of recovered lignin based on the specific pretreatment method used as well as process conditions such as time, temperature, chemical loading, and milling size. Lignin fractionation splits bulk lignin into portions with differing characteristics exploiting properties such as molar mass (ultrafiltration) and solubility due to polarity and capacity for hydrogen bonding arising from different lignin functional groups (acidification, solvent systems). Lignin chemical modification can selectively tailor lignin properties by reaction with a wide variety of reagents. These reactions can modify lignin polarity, degree of crosslinking, reactivity, and surface properties. The application of these methods by selectively tailoring lignin structure for its utilization in phenol formaldehyde resins and PLA composite materials is the focus of the bulk of the work in this thesis. This section presents a general review of previous work exploring how lignin physical and chemical properties can be changed to influence the performance of value-added applications. Specific background for each of the studies in this thesis are presented at the beginning of their respective chapter.

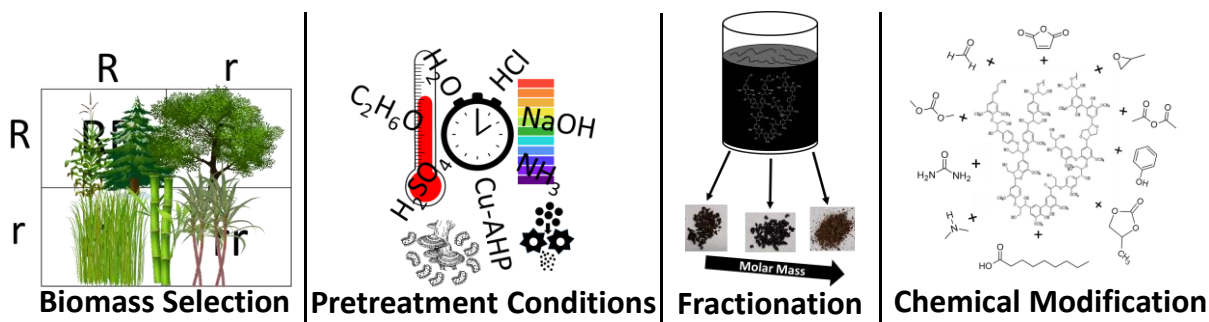


Figure 2: Strategies for the Upgrading of Lignin Properties for Value-Added Applications

Feedstock Selection

The main constituents of the lignin polymer backbone vary with the type of biomass they are derived from. Grass lignins contain disproportionate amounts of *p*-hydroxyphenyl (H) subunits and *p*-coumarate (*p*CA) end chains, in contrast softwoods contain mostly guaiacyl (G) subunits, and hardwoods primarily syringyl (S) subunits [7]. This leads to different linkages between subunits of different feedstocks, for example hardwood lignins contain a higher proportion of β -O-4 linkages than grass or softwood lignins [7]. These differences in the chemical structure of lignins from different backgrounds can be exploited by selecting feedstocks which contain lignins with a favorable structure for reactivity or compatibility with other chemical components of lignin based chemicals, resins, or materials. Previous studies investigated how different feedstocks respond to pretreatment, and how the resulting lignin structures affect their performance in a variety of value-added applications. Studies demonstrating how different feedstocks respond to pretreatment includes a study on steam explosion pretreatment using both softwoods and hardwoods, this study found differences in lignin molecular weight, functional groups, and β -O-4 content between the two feedstocks [8].

Similar results were found with organosolv pretreatment finding differences in lignin recovery yields and β -O-4 content between different wood feedstocks [9], differing lignin extraction yields and subunit ratios in miscanthus and typha grass [10], and differing lignin monomer composition and glass transition temperature in hardwood, softwood, and graminaceous feedstocks [11]. Work with formic acid extraction also found differences in thermal properties and recovery yields of lignins from a wide variety of feedstocks [12]. A study using a panel of *Arabidopsis* genotypes subjected to maleic acid treatment found differences in degree of biomass deconstruction due to differing lignin chemical structures [13].

Lignins from different feedstocks also affect the performance of lignin based chemicals, resins, and materials. The following studies demonstrate how variance in lignin subunits and intermolecular linkages due to different feedstock source result in unique mechanical and thermal properties of lignin-based products. A distinct difference in the mechanical and thermal properties of epoxy resins due to different monomer composition of two lignins derived from different wood feedstocks has been reported [14]. Phenol formaldehyde resins were also found to exhibit different properties based on lignin feedstock. This was attributed to different lignin molecular weight and subunit prevalence[15]. Studies on feedstock and properties of lignin based carbon fibers have found varying mechanical properties based on degree of lignin uniformity [16], and a relationship between mechanical properties, electroconductivity, and β -O-4 content of lignin from different feedstocks [7]. Differences in yields and composition of platform chemical pyrolysis produced from lignins of varying backgrounds has also been demonstrated [17]. The work presented in this thesis builds on these studies to further our knowledge of how different biomass sources respond differently to pretreatment and how the

chemical differences in the resulting biomass affect the performance of lignin based products.

Chapter 2 characterizes a diverse panel of corn stover genotypes subjected to dilute acid pretreatment with special focus given to their *p*CA content for enhanced reactivity in phenol formaldehyde resins

Pretreatment and Lignin Extraction Methods and Conditions

The primary function of biorefinery pretreatment is to improve access to cellulose by deconstruction of the cell wall, primarily targeting the removal of lignin and hemicellulose. The literature contains a wealth of different chemical, mechanical, and biological methods targeting deconstruction of the cell wall (**Fig. 3**). Recent work studying biomass pretreatment has moved away from only targeting cellulose accessibility for improvement of enzymatic saccharification to additionally targeting full utilization of the lignin fraction [18].

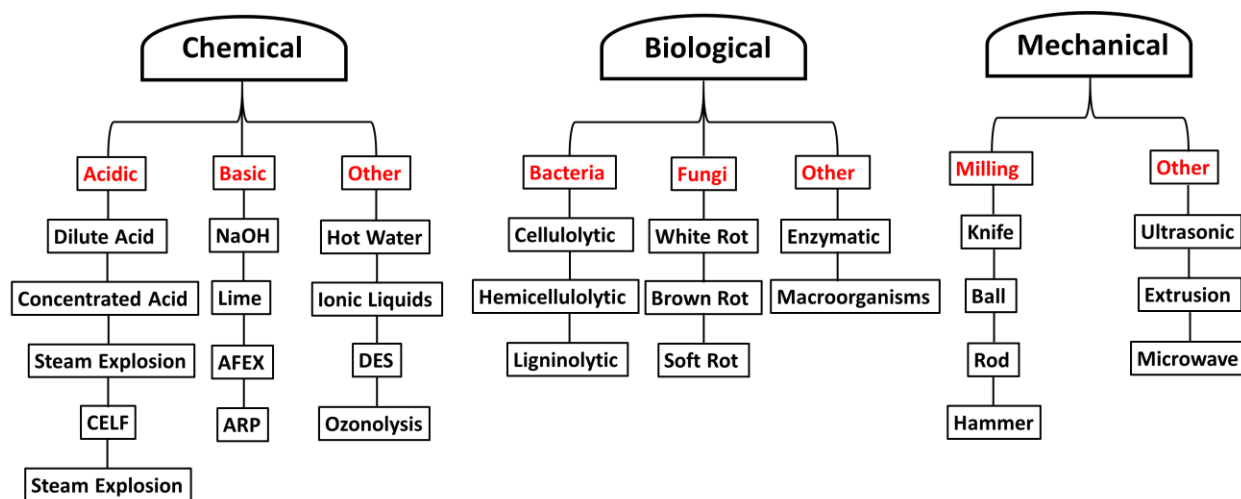


Figure 3: Pretreatment Methods for Biomass Deconstruction

Many studies have been conducted studying the effects of different pretreatment methods and different process conditions and the resulting lignin properties. The following review shows how the selection of different chemical pretreatment methods can influence properties of recovered lignins. One study used a wide variety of different pretreatments including dilute acid, alkaline, organosolv, and steam explosion pretreatments and found differences in the resulting molecular weight, glass transition temperature, and phenolic hydroxyl content of the recovered lignins [19]. Another study compared the results of steam explosion pretreatment to those of an acid hydrolysis pretreatment. Lignins from these pretreatments had different hydroxyl contents, molecular weights, and thermal stabilities [20]. This trend of differing lignin molecular weight and hydroxyl content is also represented with a study using organosolv and ammonia pretreatments [21]. A study using co-solvent-enhanced lignocellulosic fractionation (CELFF) pretreatment and Cu-catalyzed alkaline hydrogen peroxide pretreatment (Cu-AHP) found varied polysaccharide content, molar mass, β -aryl ether content, and aromatic monomer yield following thioacidolysis [22]. This is not an exhaustive review, other studies comparing lignin properties after different pretreatments include organosolv, aquasolve, liquid hot water, and soda pretreatments of rye straw [23], dilute acid, alkaline, ethanol, and liquid hot water pretreatment of sugarcane bagasse [24], dilute acid, alkali, sulfite, mechanical milling, and deep eutectic solvent pretreatment of softwoods and grasses [25], and dilute acid, autohydrolysis, organosolv, and 2-naphthol pretreatment of oil palm fronds [26].

In addition to obtaining lignins with specific properties using different chemical pretreatment methods, lignin properties can be tailored by careful selection of pretreatment process conditions such as pretreatment time, temperature, and chemical loading. This

phenomenon is demonstrated in the following studies. A study on the organosolv pulping of larch sawdust varies pretreatment temperature and sulfuric acid loading finding direct relationships between lignin β -ether bonds, phenolic hydroxyl content, aliphatic hydroxyl content, and molecular weight with pretreatment severity[27]. A similar study varying the time and temperature of ionic liquid pretreatment finds differences in lignin molecular weight, interunit linkages, and lignin degradation products based on pretreatment severity[28]. The trend of pretreatment severity impacting lignin molecular weight, cleavage of interunit bonds, and degradation products is reinforced in a study varying temperature of alkali pretreatment[29]. Other studies on the effect of pretreatment conditions on lignin structure have been conducted using a variety of pretreatments including liquid hot water[30], dilute acid[31], γ -valerolacetone (GVL)[32], steam explosion[33], sulfolane[34], deep eutectic solvents (DES)[35], and others.

Some studies go beyond characterizing lignin properties to explore relationships between pretreatment method, pretreatment conditions, and the performance of lignin value-added products, demonstrating how the selection of pretreatment method and conditions can be used to recover lignins with tailored chemical structures for specific products. A study with PF resins was conducted using both an acidic and basic organosolv pretreatment. This study found that lignin purity, molecular weight, and chemical structure affected the mechanical properties of the resins[36]. Another study using phenol-formaldehyde (PF) resins investigates the effects of pretreatment severity on lignin properties by employing organosolv pretreatments with different temperatures and times. They found that PF resin behavior was related to the degree of lignin structure condensation[37]. An additional study using organosolv pretreatment at two different temperatures presents a correlation between lignin hydroxyl content, monomer composition, and

thermal stability due to pretreatment severity, and the subsequent spinability and mechanical properties of carbon fibers[38]. A study using the CELF pretreatment method with different severities found relationships between lignin molecular weight, hydroxyl content, and the subsequent mechanical properties of lignin based polyurethanes[39].

Certain pretreatment methods will leave the recovered lignin fraction with high amounts of residual contaminants such as polysaccharides, inorganics, or extractives. An additional lignin extraction/purification step can be used to separate lignin from other polymers, remove impurities, and tailor the chemical structure of lignin for a target value-added application. Work using additional purification of lignin after pretreatment is more scarce than work with traditional pretreatment, however some studies exist which implement a lignin purification process and analyze the resulting changes in lignin properties with focus on their implementation into value-added products. For example, one study uses lignin from an ethanol pretreatment process and subjects it to two different purification processes; a cyclohexane-ethanol process and an alkaline extraction. The authors concluded that the two lignins differ in their suitability for PF resins due to changes in glass transition and molecular weight [40]. A study using organosolv pretreated poplar uses two different lignin purification processes, pyridine-acetic acid-water and dioxane-water. The purifications resulted in lignins with differing monomer content, molecular weight, β -O-4 content, and thermal stability[41]. Studies which examine the effects of different process conditions on extracted lignin properties also exist. A study using hardwood lignins purified with both alkaline and acid hydrolyses using different purification times, temperatures, and acid/base loadings found that the different extraction processes and conditions led to polysaccharide removal and changed lignin molecular weight[42].

The studies in this thesis provide additional insight into how a lignin's processing history including pretreatment method, pretreatment conditions, and lignin extraction/purification method changes the properties of recovered lignin, and how these properties affect properties of lignin-based products. Chapters 2 and 3 use dilute acid as a pretreatment method. Dilute acid pretreatment contributes to the deconstruction of the cell wall by removing xylan and redistributing lignin, disrupting the polymer network. Dilute acid is one of the preferred methods for the biorefinery process due to its low chemical costs and ability for high sugar hydrolysis yields. Dilute acid pretreatment severity can be described by its combined severity factor (CSF) given by the equation below. Where t corresponds to reaction time, T corresponds to reaction temperature, and pH is given as initial reaction pH.

$$\log_{10}(CSF) = \log_{10} \left[t e^{\frac{T-100}{14.75}} \right] - pH$$

The studies in this thesis use corn stover subjected to a variety of dilute acid pretreatment process conditions with varied severities changing time, temperature, and acid loading to characterize how biomass and lignin properties respond to diverse pretreatment conditions with a special focus on pCA and phenolic hydroxyl content for the optimization of lignin properties for incorporation in PF resins. Chapter 3 discusses the effect of additional lignin extraction/purification after pretreatment using both a formic acid and NaOH extraction method with characterization of purified lignin properties and properties of lignin-formaldehyde resins and adhesives.

Lignin Fractionation

Another technique that can be used to selectively modify lignin properties for a target application is lignin fractionation. These processes separate recovered lignin into multiple fractions of different molecular weights, often with different chemical properties, importantly an increase in homogeneity of the individual lignin fractions compared to the original bulk lignin. Chemical methods which may be used to achieve lignin molecular weight fractionation include fractional precipitation of lignin containing alkali liquors using acidification, and lignin fractionation using a single solvent or multiple solvent system (**Fig. 4**).

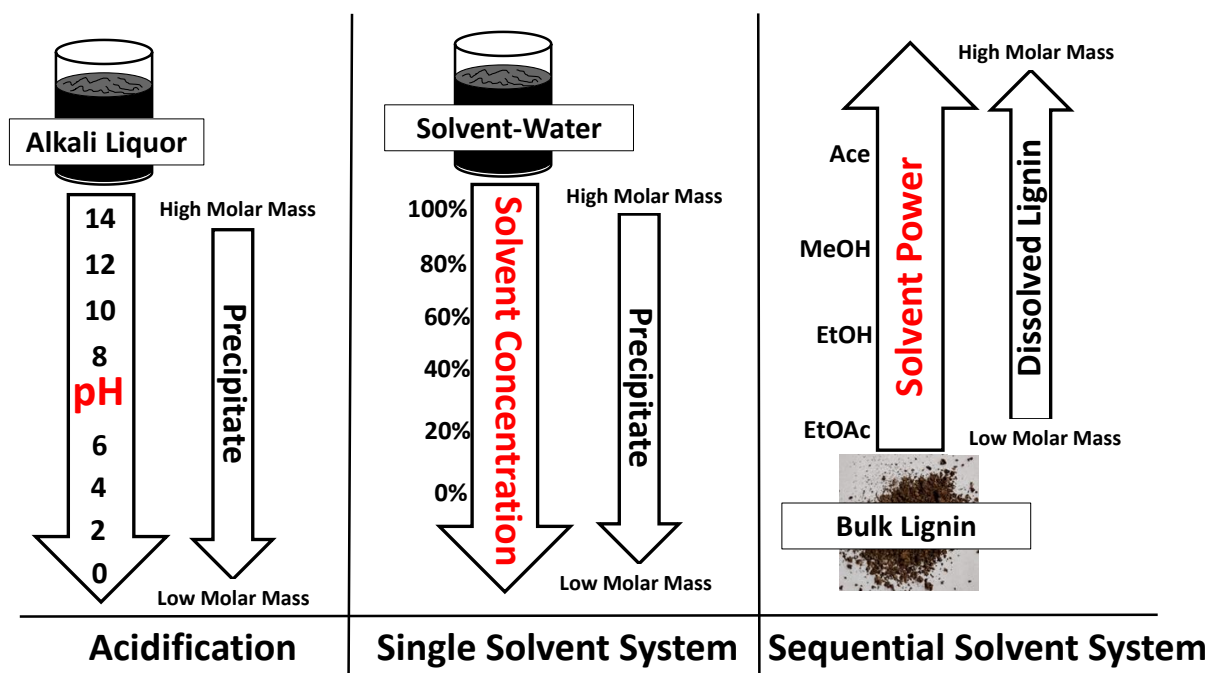


Figure 4: Chemical Processes for the Fractionation of Lignin

Fractional lignin precipitation by pH adjustment (acidification) is one of most prevalent fractionation methods reported in the literature. The ensuing literature demonstrates how

different reagents can be used for lignin fractionation by acidification, and how acidification can be used to recover monodisperse lignins with specific molecular weights and chemical components. One common mineral acid used is HCl. An example of its use is seen in a study using lignin containing alkaline liquor from corn stalk. In this study the authors used HCl to adjust the pH of the liquor to pH 5, pH 4, pH 3, and pH 2, obtaining three insoluble fractions. Recovered lignin fractions exhibited reduced molecular weight as recovery pH decreased with all fractions displaying much lower polydispersity than unfractionated lignin and a difference in particle size between fractions[43]. Other studies using hydrochloric acid have found changing elemental composition, ash content, and glass transition temperature of lignin fractions recovered from soft and hardwood kraft liquors[44], and differences in lignin subunit ratios, intermolecular linkages, and antioxidant capability in black liquor of a commercial lignin[45]. Another commonly used acid is sulfuric acid. A study with liquor from soda pulping of *Miscanthus* used H₂SO₄ to adjust liquor pH obtaining 9 lignin fractions between pH 12.64 and pH 0.72 finding increasing lignin yields with decreasing recovery pH. The authors also found increasing lignin purity as liquor pH decreased[46]. Using H₂SO₄ differing lignin hydroxyl content, polydispersity, and particle size in different fractions of alkali liquor of steam exploded sugarcane bagasse has been reported[47] along with a relationship between lignin recovery yields and silica content in lignin fractions from soda liquor of wheat straw[48]. Other reagents to adjust the pH of black liquor recovering fractionated lignins have been employed, including the use of H₃PO₄[49] and CO₂[50].

Solvent systems using either single or multiple solvents can also be used for the separation of lignin into molecular weight fractions, exploiting differences in lignin solubility

parameters. Lignin solubility in different solvents is a function of its polarity, strength of dispersion forces, and capacity for hydrogen bonding based on molar mass and functional groups such as phenolic hydroxyls. The subsequently presented literature demonstrates how changing solvent properties can be used to recover lignins with specific characteristics. The most common single solvent system is ethanol-water with its solvent polarity and hydrogen bonding capacity changing with each dilution by the addition of water. A study using Kraft lignin dissolved lignin in ethanol of differing concentrations from 0-100% recovering the insoluble portion. This study found that lignin molecular weight reaches a maximum at 80% ethanol and decreases with both increasing and decreasing ethanol concentration with another uptick in molecular weight at 50% ethanol. They also found lower polydispersity at high ethanol concentrations and more phenolic hydroxyls at low ethanol concentrations[51]. Another often employed single solvent system for lignin fractionation is acetone-water. One study using this system uses organosolv extracted switchgrass and pine lignins dissolved in 30-60% acetone. Lignin fractions were found to have lower molecular weight and polydispersity at lower acetone concentrations. Differences in functional group distribution were also found with lignins recovered at lower acetone concentrations containing more phenolic, and less aliphatic hydroxyl groups[52]. Many other single solvent system can be employed including acetic acid[53], methanol[54], THF[55], glycerol-ethanol[56], ionic liquids[57], and γ -valerolacetone[58].

Multiple solvents can also be used for the fractional separation of lignin, exploiting differences in solubility with a sequence of solvents of increasing solvent power. An example of how a solvent sequence for this procedure can be selected is shown in a study using industrial Kraft lignin. The authors in this study screen a wide panel of solvents for their ability to dissolve

lignin, with predictions of their success based on Hansen solubility parameter. The results of this study are then used to design a solvent sequence of increasing lignin dissolution ability. The final sequence was composed of ethyl acetate, ethanol, methanol, and acetone, and resulted in lignin fractions of increasing molecular weight and decreasing solubility with each subsequent solvent[59]. Another study applies this knowledge to design a solvent sequence composed of ether, ethyl acetate, methanol, acetone, and dioxane-water to fractionate organosolv bamboo lignin and goes a step further characterizing chemical structure of recovered lignin fractions. They found with each subsequent solvent extraction, recovered lignins were more polydisperse, with a higher molecular weight, lower sugar content, more β - β' bonds, and less phenolic hydroxyl groups[60]. Many other studies using different solvent sequences also exist[61-63].

The fractionation methods reviewed in this chapter show how these types of processes can be used to transform a low purity, heterogeneous, and low reactivity lignin into lignin fractions with increased homogeneity, purity, and a functional group configuration suited for specific lignin-based products. This thesis joins these previous studies using fractional precipitation methods to obtain lignins with tailored molecular weights, purities, and chemical structure. Chapter 4 uses a fractional precipitation process using sequential dilution of lignin containing formic acid liquors with characterization of the recovered lignin fractions and discussion of their suitability for PF resins and other value-added products. Chapter five uses acidification of black liquor from alkali treated corn stover to obtain a polysaccharide heavy fraction and two lignin fractions. These recovered biopolymers are then evaluated for use in PLA composite materials with thermal and mechanical characterization of extruded biopolymer-PLA composites.

Lignin Chemical Modification

The final strategy for the enhancement of lignin properties for value-added applications which is reviewed in this chapter is the direct chemical modification of lignin. Lignin's complex and heterogeneous structure, polar functional groups, and crosslinking between the lignin polymeric backbone obstructs the inclusion of lignin into many products. If a lignin's source and processing history result in unfeasible properties for the target application, chemical modification can be used to change the nature of the lignin structure. Many chemical modification processes are suggested and evaluated in the literature[64, 65]. Studies evaluating chemically modified lignin for inclusion into value-added products are numerous including Kraft lignin demethylation for lignin-polyethylenimine wood adhesives[66], esterification of Kraft lignin for the formation of lignin-polybutadiene thermoplastics[67], phenolation of sulfuric acid lignin for the manufacture of surfactants[68], amination of soda pulping lignin for PVC-lignin-wood flour composites[69], aminated lignin for polyurethane foams[70], amination of alkaline lignin for epoxy resins[71], nitrated alkaline lignin for polyurethanes[72], sulfonated Kraft lignin for use as a cement additive[73], lignin-PMMA grafting for electrospinning of nanofiber composites[74], and many others. This chapter will focus on reviewing studies on lignin chemical modification for the products employed in this thesis, namely lignin-formaldehyde wood adhesives and lignin-PLA composites.

Phenolation, demethylation, and methylation are the three most commonly studied modification procedures to increase lignin reactivity with formaldehyde. Phenolation reacts lignin with phenol under acidic conditions resulting in a reduction of molecular weight and the conversion of lignin aliphatic hydroxyl groups to phenolic hydroxyls[75]. This increase in

phenolic hydroxyl groups increases lignin reactivity with formaldehyde resulting in lower formaldehyde emissions[76], and improved mechanical properties[77] of wood products made with phenolated lignin-formaldehyde resin adhesives. Demethylation removes the methyl unit of the methoxy groups of S and G lignin subunits resulting in increased phenolic hydroxyl groups available for reaction with formaldehyde[78]. A study using iodocyclohexane to demethylate alkaline lignin from wheat straw found that an increase in available lignin hydroxyl groups in the modified lignin led to decreases in gelation time, decreased free formaldehyde, and increased bonding strength compared to resins made with unmodified lignin[78]. In a lignin methylation process lignin is reacted with formaldehyde prior to resin formation adding methylol groups to free positions on lignin aromatic rings and lignin side chains resulting in increased hydroxyl groups available for reaction with formaldehyde during resin formulation[79]. A study using methylolated organosolv lignin for the production of lignin-formaldehyde adhesive based particleboard found a decrease in gelation time and an increase in shear and bonding strengths of particleboards made with modified lignins[80]. Many other lignin modification methods have been employed prior to lignin-formaldehyde resin production including maleation[81], treatment in deep eutectic solvents[82], and glyoxalation[83].

Due to the low compatibility of lignin with PLA many studies use chemical modification of lignin prior to incorporation into lignin-PLA composite materials. One of the most commonly employed modification methods for PLA composites is acetylation. Acetylation replaces lignin hydroxyl groups with acetyl groups changing the polarity of the lignin molecule resulting in increased compatibility of modified lignin with PLA and decreased lignin hydrogen bonding leading to less agglomeration[84]. Previous work with acetylated Kraft and organosolv lignin has

found acetylation increases the thermal stability of the modified lignins and improves the mechanical properties of PLA composites at high levels of lignin incorporation when compared to materials made with non-acetylated lignin[85, 86]. This strategy of reducing lignin hydrogen bonding and changing its polarity by the modification of hydroxyl groups is used with esterification of lignin with many different chemicals. One study using soft and hardwood lignins esterified with fatty acids found an increase in PLA material ductility due to lignin modification[87]. Another study found an increase in ductility with pine lignin esterified with butyric, isobutyric, and crotonic anhydrides, forming PLA composites with higher elongation at break and tensile modulus when compared to composites made with unmodified lignin[88]. While the esterification of lignin with acetate and other esters is most common many other lignin modification methods have been developed to increase the interfacial interactions between lignin and PLA including alkylation[89], silanization[90, 91], and urea modification[92].

Chapter 5 of this thesis modifies lignin by the application of an oxyalkylation modification using propylene carbonate. This modification increases compatibility between lignin and PLA by converting lignin phenolic hydroxyl groups to aliphatic chains with terminal hydroxyls. This increases compatibility with PLA through multiple mechanisms. The oxyalkylation of lignin reduces its polarity, reduces hydrogen bonding between phenolic hydroxyls, and causes steric hindrance, reducing the ability of lignin to agglomerate with itself increasing interfacial interaction strength with PLA.

References

1. IEA *World Energy Outlook 2021*. 2021.
2. Pandey, A., R.D. Tyagi, and S. Varjani, *Biomass, biofuels, biochemicals: Circular bioeconomy—Current developments and future outlook*. 2021: Elsevier.
3. Sorek, N., T.H. Yeats, H. Szemenyei, H. Youngs, and C.R. Somerville, *The implications of lignocellulosic biomass chemical composition for the production of advanced biofuels*. Bioscience, 2014. **64**(3): p. 192-201.
4. Ragauskas, A.J., G.T. Beckham, M.J. Bidddy, R. Chandra, F. Chen, M.F. Davis, B.H. Davison, R.A. Dixon, P. Gilna, M. Keller, P. Langan, A.K. Naskar, J.N. Saddler, T.J. Tschaplinski, G.A. Tuskan, and C.E. Wyman, *Lignin valorization: improving lignin processing in the biorefinery*. Science, 2014. **344**(6185): p. 1246843.
5. Ullah, M., P. Liu, S. Xie, and S. Sun, *Recent Advancements and Challenges in Lignin Valorization: Green Routes towards Sustainable Bioproducts*. Molecules, 2022. **27**(18): p. 6055.
6. Sethupathy, S., G.M. Morales, L. Gao, H. Wang, B. Yang, J. Jiang, J. Sun, and D. Zhu, *Lignin valorization: status, challenges and opportunities*. Bioresource Technology, 2022: p. 126696.
7. Li, Q., C. Hu, M. Li, P. Truong, J. Li, H.-S. Lin, M.T. Naik, S. Xiang, B.E. Jackson, and W. Kuo, *Enhancing the multi-functional properties of renewable lignin carbon fibers via defining the structure–property relationship using different biomass feedstocks*. Green Chemistry, 2021. **23**(10): p. 3725-3739.
8. Li, J., G. Gellerstedt, and K. Toven, *Steam explosion lignins; their extraction, structure and potential as feedstock for biodiesel and chemicals*. Bioresource Technology, 2009. **100**(9): p. 2556-2561.
9. Zijlstra, D.S., C.A. Analbers, J. Korte, E. Wilbers, and P.J. Deuss, *Efficient Mild Organosolv Lignin Extraction in a Flow-Through Setup Yielding Lignin with High β -O-4 Content*. Polymers (Basel), 2019. **11**(12).
10. Timilsena, Y.P., I.G. Audu, S.K. Rakshit, and N. Brosse, *Impact of the lignin structure of three lignocellulosic feedstocks on their organosolv delignification. Effect of carbonium ion scavengers*. Biomass and Bioenergy, 2013. **52**: p. 151-158.
11. Sammons, R.J., D.P. Harper, N. Labbé, J.J. Bozell, T. Elder, and T.G. Rials, *Characterization of organosolv lignins using thermal and FT-IR spectroscopic analysis*. BioResources, 2013. **8**(2): p. 2752-2767.

12. Watkins, D., M. Nuruddin, M. Hosur, A. Tcherbi-Narteh, and S. Jeelani, *Extraction and characterization of lignin from different biomass resources*. Journal of Materials Research and Technology, 2015. **4**(1): p. 26-32.
13. Ciesielski, P.N., M.G. Resch, B. Hewetson, J.P. Killgore, A. Curtin, N. Anderson, A.N. Chiaramonti, D.C. Hurley, A. Sanders, M.E. Himmel, C. Chapple, N. Mosier, and B.S. Donohoe, *Engineering plant cell walls: tuning lignin monomer composition for deconstructable biofuel feedstocks or resilient biomaterials*. Green Chemistry, 2014. **16**(5): p. 2627-2635.
14. Gioia, C., M. Colonna, A. Tagami, L. Medina, O. Sevastyanova, L.A. Berglund, and M. Lawoko, *Lignin-Based Epoxy Resins: Unravelling the Relationship between Structure and Material Properties*. Biomacromolecules, 2020. **21**(5): p. 1920-1928.
15. Ghorbani, M., F. Liebner, H.W.G. van Herwijnen, L. Pfungen, M. Krahofer, E. Budjav, and J. Konnerth, *Lignin Phenol Formaldehyde Resoles: The Impact of Lignin Type on Adhesive Properties*. 2016, 2016. **11**(3): p. 15.
16. Li, Q., C. Hu, M. Li, P. Truong, M.T. Naik, D. Prabhu, L. Hoffmann, W.L. Rooney, and J.S. Yuan, *Discovering Biomass Structural Determinants Defining the Properties of Plant-Derived Renewable Carbon Fiber*. iScience, 2020. **23**(8): p. 101405.
17. Mendu, V., A.E. Harman-Ware, M. Crocker, J. Jae, J. Stork, S. Morton, A. Placido, G. Huber, and S. DeBolt, *Identification and thermochemical analysis of high-lignin feedstocks for biofuel and biochemical production*. Biotechnology for Biofuels, 2011. **4**(1): p. 43.
18. Yoo, C.G., X. Meng, Y. Pu, and A.J. Ragauskas, *The critical role of lignin in lignocellulosic biomass conversion and recent pretreatment strategies: A comprehensive review*. Bioresource Technology, 2020. **301**: p. 122784.
19. Glasser, W.G., C.A. Barnett, P.C. Muller, and K.V. Sarkanen, *The chemistry of several novel bioconversion lignins*. Journal of Agricultural and Food Chemistry, 1983. **31**(5): p. 921-930.
20. Lora, J.H. and W.G. Glasser, *Recent Industrial Applications of Lignin: A Sustainable Alternative to Nonrenewable Materials*. Journal of Polymers and the Environment, 2002. **10**(1): p. 39-48.
21. Yoo, C.G., M. Li, X. Meng, Y. Pu, and A.J. Ragauskas, *Effects of organosolv and ammonia pretreatments on lignin properties and its inhibition for enzymatic hydrolysis*. Green Chemistry, 2017. **19**(8): p. 2006-2016.
22. Bhalla, A., C.M. Cai, F. Xu, S.K. Singh, N. Bansal, T. Phongpreecha, T. Dutta, C.E. Foster, R. Kumar, B.A. Simmons, S. Singh, C.E. Wyman, E.L. Hegg, and D.B. Hodge, *Performance of three delignifying pretreatments on hardwoods: hydrolysis yields*,

- comprehensive mass balances, and lignin properties*. Biotechnology for Biofuels, 2019. **12**(1): p. 213.
23. Wörmeyer, K., T. Ingram, B. Saake, G. Brunner, and I. Smirnova, *Comparison of different pretreatment methods for lignocellulosic materials. Part II: Influence of pretreatment on the properties of rye straw lignin*. Bioresource Technology, 2011. **102**(5): p. 4157-4164.
 24. Xu, C., F. Liu, M.A. Alam, H. Chen, Y. Zhang, C. Liang, H. Xu, S. Huang, J. Xu, and Z. Wang, *Comparative study on the properties of lignin isolated from different pretreated sugarcane bagasse and its inhibitory effects on enzymatic hydrolysis*. International Journal of Biological Macromolecules, 2020. **146**: p. 132-140.
 25. Ma, R., X. Zhang, Y. Wang, and X. Zhang, *New Insights Toward Quantitative Relationships between Lignin Reactivity to Monomers and Their Structural Characteristics*. ChemSusChem, 2018. **11**(13): p. 2146-2155.
 26. Hussin, M.H., A.A. Rahim, M.N. Mohamad Ibrahim, M. Yemloul, D. Perrin, and N. Brosse, *Investigation on the structure and antioxidant properties of modified lignin obtained by different combinative processes of oil palm fronds (OPF) biomass*. Industrial Crops and Products, 2014. **52**: p. 544-551.
 27. Hochegger, M., B. Cottyn-Boitte, L. Cézard, S. Schober, and M. Mittelbach, *Influence of Ethanol Organosolv Pulping Conditions on Physicochemical Lignin Properties of European Larch*. International Journal of Chemical Engineering, 2019. **2019**: p. 1734507.
 28. Dutta, T., N.G. Isern, J. Sun, E. Wang, S. Hull, J.R. Cort, B.A. Simmons, and S. Singh, *Survey of Lignin-Structure Changes and Depolymerization during Ionic Liquid Pretreatment*. ACS Sustainable Chemistry & Engineering, 2017. **5**(11): p. 10116-10127.
 29. Xiao, L.-P., Y.-Y. Bai, Z.-J. Shi, Q. Lu, and R.-C. Sun, *Influence of alkaline hydrothermal pretreatment on shrub wood Tamarix ramosissima: Characteristics of degraded lignin*. Biomass and Bioenergy, 2014. **68**: p. 82-94.
 30. Ko, J.K., Y. Kim, E. Ximenes, and M.R. Ladisch, *Effect of liquid hot water pretreatment severity on properties of hardwood lignin and enzymatic hydrolysis of cellulose*. Biotechnol Bioeng, 2015. **112**(2): p. 252-62.
 31. Jensen, A., Y. Cabrera, C.-W. Hsieh, J. Nielsen, J. Ralph, and C. Felby, *2D NMR characterization of wheat straw residual lignin after dilute acid pretreatment with different severities*. Holzforschung, 2017. **71**(6): p. 461-469.
 32. Ahmed, M.A., J.H. Lee, A.A. Raja, and J.W. Choi, *Effects of Gamma-Valerolactone Assisted Fractionation of Ball-Milled Pine Wood on Lignin Extraction and Its Characterization as Well as Its Corresponding Cellulose Digestion*. Applied Sciences, 2020. **10**(5): p. 1599.

33. Montané, D., J. Salvadó, and X. Farriol, *Fractionation of wheat straw via steam-explosion pretreatment. Characteristics of the lignin obtained by alkali delignification of the steamed straw*. 1997.
34. Zhong, L., M. Xu, C. Wang, L. Shao, J. Mao, W. Jiang, X. Ji, G. Yang, J. Chen, and G. Lyu, *Pretreatment of willow using the alkaline-catalyzed sulfolane/water solution for high-purity and antioxidative lignin production*. International Journal of Biological Macromolecules, 2020. **159**: p. 287-294.
35. Chen, Z., X. Bai, H. Zhang, and C. Wan, *Insights into structural changes of lignin toward tailored properties during deep eutectic solvent pretreatment*. ACS Sustainable Chemistry & Engineering, 2020. **8**(26): p. 9783-9793.
36. Pang, B., S. Yang, W. Fang, T.-Q. Yuan, D.S. Argyropoulos, and R.-C. Sun, *Structure-property relationships for technical lignins for the production of lignin-phenol-formaldehyde resins*. Industrial crops and products, 2017. **108**: p. 316-326.
37. Dababi, I., O. Gimello, and E. Elaloui, *Organosolv lignin-based wood adhesive. Influence of the lignin extraction conditions on the adhesive performance*. Polymers (Basel) **8**. 2016.
38. Hosseinaei, O., D.P. Harper, J.J. Bozell, and T.G. Rials, *Role of Physicochemical Structure of Organosolv Hardwood and Herbaceous Lignins on Carbon Fiber Performance*. ACS Sustainable Chemistry & Engineering, 2016. **4**(10): p. 5785-5798.
39. Wang, Y.-Y., P. Sengupta, B. Scheidemann, Y. Pu, C.E. Wyman, C.M. Cai, and A.J. Ragauskas, *Effects of CELF pretreatment severity on lignin structure and the lignin-based polyurethane properties*. Frontiers in Energy Research, 2020. **8**: p. 149.
40. Park, Y., W. Doherty, and P.J. Halley, *Developing lignin-based resin coatings and composites*. Industrial crops and products, 2008. **27**(2): p. 163-167.
41. Gao, S., J. Zhao, X. Wang, Y. Guo, Y. Han, and J. Zhou, *Lignin structure and solvent effects on the selective removal of condensed units and enrichment of S-type lignin*. Polymers, 2018. **10**(9): p. 967.
42. Corderi, S., T. Renders, K. Servaes, K. Vanbroekhoven, T. De Roo, and K. Elst, *Strategies for the removal of polysaccharides from biorefinery lignins: process optimization and techno economic evaluation*. Molecules, 2021. **26**(11): p. 3324.
43. Wang, G. and H. Chen, *Fractionation of alkali-extracted lignin from steam-exploded stalk by gradient acid precipitation*. Separation and Purification Technology, 2013. **105**: p. 98-105.
44. Lourençon, T.V., F.A. Hansel, T.A. da Silva, L.P. Ramos, G.I.B. de Muniz, and W.L.E. Magalhães, *Hardwood and softwood kraft lignins fractionation by simple sequential acid precipitation*. Separation and Purification Technology, 2015. **154**: p. 82-88.

45. Du, B., Y. Bai, Z. Pan, J. Xu, Q. Wang, X. Wang, G. Lv, and J. Zhou, *pH fractionated lignin for the preparation of lignin-based magnetic nanoparticles for the removal of methylene blue dye*. Separation and Purification Technology, 2022. **295**: p. 121302.
46. García, A., A. Toledano, L. Serrano, I. Egüés, M. González, F. Marín, and J. Labidi, *Characterization of lignins obtained by selective precipitation*. Separation and Purification Technology, 2009. **68**(2): p. 193-198.
47. Brenelli, L.B., F. Mandelli, A.Z. Mercadante, G.J.d.M. Rocha, S.A. Rocco, A.F. Craievich, A.R. Gonçalves, D.d.C. Centeno, M. de Oliveira Neto, and F.M. Squina, *Acidification treatment of lignin from sugarcane bagasse results in fractions of reduced polydispersity and high free-radical scavenging capacity*. Industrial Crops and Products, 2016. **83**: p. 94-103.
48. Gilarranz, M.A., F. Rodriguez, M. Oliet, and J.A. Revenga, *Acid Precipitation and Purification of Wheat Straw Lignin*. Separation Science and Technology, 1998. **33**(9): p. 1359-1377.
49. Domínguez-Robles, J., E. Espinosa, D. Savy, A. Rosal, and A. Rodriguez, *Biorefinery Process Combining Specel® Process and Selective Lignin Precipitation using Mineral Acids*. 2016, 2016. **11**(3): p. 17.
50. Stoklosa, R.J., J. Velez, S. Kelkar, C.M. Saffron, M.C. Thies, and D.B. Hodge, *Correlating lignin structural features to phase partitioning behavior in a novel aqueous fractionation of softwood Kraft black liquor*. Green Chemistry, 2013.
51. Goldmann, W.M., J. Ahola, M. Mikola, and J. Tanskanen, *Solubility and fractionation of Indulin AT kraft lignin in ethanol-water media*. Separation and Purification Technology, 2019. **209**: p. 826-832.
52. Sadeghifar, H., T. Wells, R.K. Le, F. Sadeghifar, J.S. Yuan, and A. Jonas Ragauskas, *Fractionation of organosolv lignin using acetone: water and properties of the obtained fractions*. ACS Sustainable Chemistry & Engineering, 2017. **5**(1): p. 580-587.
53. Klett, A.S., P.V. Chappell, and M.C. Thies, *Recovering ultraclean lignins of controlled molecular weight from Kraft black-liquor lignins*. Chemical Communications, 2015. **51**(64): p. 12855-12858.
54. Zhang, H., Y. Bai, B. Yu, X. Liu, and F. Chen, *A practicable process for lignin color reduction: fractionation of lignin using methanol/water as a solvent*. Green Chemistry, 2017. **19**(21): p. 5152-5162.
55. Meng, X., A. Parikh, B. Seemala, R. Kumar, Y. Pu, C.E. Wyman, C.M. Cai, and A.J. Ragauskas, *Characterization of fractional cuts of co-solvent enhanced lignocellulosic fractionation lignin isolated by sequential precipitation*. Bioresource Technology, 2019. **272**: p. 202-208.

56. Liu, C., C. Si, G. Wang, H. Jia, and L. Ma, *A novel and efficient process for lignin fractionation in biomass-derived glycerol-ethanol solvent system*. Industrial Crops and Products, 2018. **111**: p. 201-211.
57. Chambon, C.L., V. Fitriyanti, P. Verdía, S.M. Yang, S. Hérou, M.-M. Titirici, A. Brandt-Talbot, P.S. Fennell, and J.P. Hallett, *Fractionation by Sequential Antisolvent Precipitation of Grass, Softwood, and Hardwood Lignins Isolated Using Low-Cost Ionic Liquids and Water*. ACS Sustainable Chemistry & Engineering, 2020. **8**(9): p. 3751-3761.
58. Xu, Y.-H., P. Zeng, M.-F. Li, J. Bian, and F. Peng, *γ -Valerolactone/water system for lignin fractionation to enhance antibacterial and antioxidant capacities*. Separation and Purification Technology, 2021. **279**: p. 119780.
59. Duval, A., F. Vilaplana, C. Crestini, and M. Lawoko, *Solvent screening for the fractionation of industrial kraft lignin*. Holzforschung, 2016. **70**(1): p. 11-20.
60. Li, M.-F., S.-N. Sun, F. Xu, and R.-C. Sun, *Sequential solvent fractionation of heterogeneous bamboo organosolv lignin for value-added application*. Separation and Purification Technology, 2012. **101**: p. 18-25.
61. Hemmingson, J.A., *Steam-Explosion Lignins: Fractionation, Composition, Structure and Extractives*. Journal of Wood Chemistry and Technology, 1987. **7**(4): p. 527-553.
62. Jiang, X., D. Savithri, X. Du, S. Pawar, H. Jameel, H.-m. Chang, and X. Zhou, *Fractionation and characterization of kraft lignin by sequential precipitation with various organic solvents*. ACS Sustainable Chemistry & Engineering, 2017. **5**(1): p. 835-842.
63. Arshanitsa, A., J. Ponomarenko, T. Dizhbite, A. Andersone, R.J.A. Gosselink, J.v.d. Putten, M. Lauberts, and G. Telysheva, *Fractionation of technical lignins as a tool for improvement of their antioxidant properties*. Journal of Analytical and Applied Pyrolysis, 2013. **103**: p. 78-85.
64. Laurichesse, S. and L. Avérous, *Chemical modification of lignins: Towards biobased polymers*. Progress in Polymer Science, 2014. **39**(7): p. 1266-1290.
65. Figueiredo, P., K. Lintinen, J.T. Hirvonen, M.A. Kostianen, and H.A. Santos, *Properties and chemical modifications of lignin: Towards lignin-based nanomaterials for biomedical applications*. Progress in Materials Science, 2018. **93**: p. 233-269.
66. Liu, Y. and K. Li, *Preparation and Characterization of Demethylated Lignin-Polyethylenimine Adhesives*. The Journal of Adhesion, 2006. **82**(6): p. 593-605.
67. Saito, T., R.H. Brown, M.A. Hunt, D.L. Pickel, J.M. Pickel, J.M. Messman, F.S. Baker, M. Keller, and A.K. Naskar, *Turning renewable resources into value-added polymer: development of lignin-based thermoplastic*. Green Chemistry, 2012. **14**(12): p. 3295-3303.

68. Matsushita, Y. and S. Yasuda, *Reactivity of a condensed-type lignin model compound in the Mannich reaction and preparation of cationic surfactant from sulfuric acid lignin*. Journal of Wood Science, 2003. **49**(2): p. 166-171.
69. Yue, X., F. Chen, and X. Zhou, *Improved interfacial bonding of PVC/wood-flour composites by lignin amine modification*. BioResources, 2011. **6**(2): p. 2022-2044.
70. Huo, S.-P., M.-C. Nie, Z.-W. Kong, G.-M. Wu, and J. Chen, *Crosslinking kinetics of the formation of lignin-aminated polyol-based polyurethane foam*. Journal of Applied Polymer Science, 2012. **125**(1): p. 152-157.
71. Pan, H., G. Sun, and T. Zhao, *Synthesis and characterization of aminated lignin*. International Journal of Biological Macromolecules, 2013. **59**: p. 221-226.
72. Huang, J. and L. Zhang, *Effects of NCO/OH molar ratio on structure and properties of graft-interpenetrating polymer networks from polyurethane and nitrolignin*. Polymer, 2002. **43**(8): p. 2287-2294.
73. He, W. and P. Fatehi, *Preparation of sulfomethylated softwood kraft lignin as a dispersant for cement admixture*. RSC Advances, 2015. **5**(58): p. 47031-47039.
74. Kai, D., S. Jiang, Z.W. Low, and X.J. Loh, *Engineering highly stretchable lignin-based electrospun nanofibers for potential biomedical applications*. Journal of Materials Chemistry B, 2015. **3**(30): p. 6194-6204.
75. Jiang, X., J. Liu, X. Du, Z. Hu, H.-m. Chang, and H. Jameel, *Phenolation to Improve Lignin Reactivity toward Thermosets Application*. ACS Sustainable Chemistry & Engineering, 2018. **6**(4): p. 5504-5512.
76. Younesi-Kordkheili, H., A. Pizzi, and G. Niyatzade, *Reduction of formaldehyde emission from particleboard by phenolated kraft lignin*. The Journal of Adhesion, 2016. **92**(6): p. 485-497.
77. Çetin, N.S. and N. Özmen, *Use of organosolv lignin in phenol-formaldehyde resins for particleboard production: II. Particleboard production and properties*. International Journal of Adhesion and Adhesives, 2002. **22**(6): p. 481-486.
78. Song, Y., Z. Wang, N. Yan, R. Zhang, and J. Li, *Demethylation of Wheat Straw Alkali Lignin for Application in Phenol Formaldehyde Adhesives*. Polymers, 2016. **8**(6): p. 209.
79. Campbell, A.G. and A.R. Walsh, *The present status and potential of kraft lignin-phenol-formaldehyde wood adhesives*. The Journal of Adhesion, 1985. **18**(4): p. 301-314.
80. Stuecker, A., J., J. Podschun, M. Heitmann, and R. Lehnen, *Organosolv Lignin in Phenol-Formaldehyde Resins-Effect of Molecular Weight and Lignin Methylation*. Towards Forest Products and Processes with Lower Environmental Impact, 2014: p. 251.

81. Younesi-Kordkheili, H., *Maleated lignin coreaction with phenol-formaldehyde resins for improved wood adhesives performance*. International Journal of Adhesion and Adhesives, 2022. **113**: p. 103080.
82. Lian, H., S. Hong, A. Carranza, J.D. Mota-Morales, and J.A. Pojman, *Processing of lignin in urea–zinc chloride deep-eutectic solvent and its use as a filler in a phenol-formaldehyde resin*. RSC Advances, 2015. **5**(36): p. 28778-28785.
83. Younesi-Kordkheili, H. and A. Pizzi, *A Comparison among Lignin Modification Methods on the Properties of Lignin–Phenol–Formaldehyde Resin as Wood Adhesive*. Polymers, 2021. **13**(20): p. 3502.
84. Kim, Y., J. Suhr, H.-W. Seo, H. Sun, S. Kim, I.-K. Park, S.-H. Kim, Y. Lee, K.-J. Kim, and J.-D. Nam, *All biomass and UV protective composite composed of compatibilized lignin and poly (lactic-acid)*. Scientific Reports, 2017. **7**(1): p. 1-11.
85. Gordobil, O., R. Delucis, I. Egüés, and J. Labidi, *Kraft lignin as filler in PLA to improve ductility and thermal properties*. Industrial Crops and Products, 2015. **72**: p. 46-53.
86. Gordobil, O., I. Egüés, R. Llano-Ponte, and J. Labidi, *Physicochemical properties of PLA lignin blends*. Polymer Degradation and Stability, 2014. **108**: p. 330-338.
87. Gordobil, O., I. Egüés, and J. Labidi, *Modification of Eucalyptus and Spruce organosolv lignins with fatty acids to use as filler in PLA*. Reactive and Functional Polymers, 2016. **104**: p. 45-52.
88. Vila, C., V. Santos, B. Saake, and J.C. Parajó, *Manufacture, characterization, and properties of poly-(lactic acid) and its blends with esterified pine lignin*. BioResources, 2016. **11**(2): p. 5322-5332.
89. Zhang, N., M. Zhao, G. Liu, J. Wang, Y. Chen, and Z. Zhang, *Alkylated lignin with graft copolymerization for enhancing toughness of PLA*. Journal of Materials Science, 2022. **57**(19): p. 8687-8700.
90. Zhu, J., L. Xue, W. Wei, C. Mu, M. Jiang, and Z. Zhou, *Modification of Lignin with Silane Coupling Agent to Improve the Interface of Poly(L-lactic) Acid/Lignin Composites*. 2015, 2015. **10**(3): p. 11.
91. Qian, S., K. Sheng, K. Yu, L. Xu, and C.A. Fontanillo Lopez, *Improved properties of PLA biocomposites toughened with bamboo cellulose nanowhiskers through silane modification*. Journal of Materials Science, 2018. **53**(15): p. 10920-10932.
92. Zhang, R., X. Xiao, Q. Tai, H. Huang, and Y. Hu, *Modification of lignin and its application as char agent in intumescent flame-retardant poly (lactic acid)*. Polymer Engineering & Science, 2012. **52**(12): p. 2620-2626.

CHAPTER 2

IMPACT OF DILUTE ACID PRETREATMENT CONDITIONS ON p-COUMARATE
REMOVAL IN DIVERSE MAIZE LINESContribution of Authors and Co-Authors

Author: Brian K. Saulnier

Contributions: Conducted corn stover pretreatment experiments. Characterized sugar content, Klason lignin content, ash content, and moisture content of raw and pretreated biomass. Conducted alkaline saponification for quantification of biomass pCA content. Employed enzymatic hydrolysis on raw and pretreated biomass for determination of sugar yields. Conducted model compound acid hydrolysis experiments. Wrote first draft of manuscript.

Co-Author: Thanaphong Phongpreecha

Contributions: Conducted corn stover pretreatment experiments. Aided in training of laboratory procedures. Provided editing and feedback for final manuscript draft.

Co-Author: Sandip K. Singh

Contributions: Provided guidance for laboratory procedures and aided in interpretation of data. Provided editing and feedback for final manuscript draft.

Co-Author: David B. Hodge

Contributions: Assisted in experimental design and selection of pretreatment conditions. Aided in interpretation of data. Constructed correlation plots. Provided assistance in the writing, editing, and finalization of subsequent manuscript drafts.

Manuscript Information

Brian K. Saulnier, Thanaphong Phongpreecha, Sandip K. Singh, David B. Hodge

Bioresource Technology

Status of Manuscript:

☐ Prepared for submission to a peer-reviewed journal

☐ Officially submitted to a peer-reviewed journal

☐ Accepted by a peer-reviewed journal

☒ Published in a peer-reviewed journal

Elsevier

Bioresource Technology 314 (2020) 123750

<https://doi.org/10.1016/j.biortech.2020.123750>

IMPACT OF DILUTE ACID PRETREATMENT CONDITIONS ON *p*-COUMARATE REMOVAL IN DIVERSE MAIZE LINES

Abstract

Prior work has identified that lignins recovered from dilute acid-pretreated corn stover exhibit superior performance in phenol–formaldehyde resins used in wood adhesive applications when compared to diverse process-modified lignins derived from other sources. This improved performance is hypothesized to be due to the higher content of unsubstituted phenolic groups specifically *p*-coumarate lignin esters. In this work, a diverse set of corn stover samples are employed that exhibit diversity in *p*-coumarate content and total lignin content to explore the relationship between dilute acid pretreatment conditions, *p*-coumarate ester hydrolysis, xylan solubilization, and the resulting glucose enzymatic hydrolysis yields. The goal of this study is to identify pretreatment conditions that preserve a significant fraction of the *p*-coumarate esters while simultaneously achieving high enzymatic hydrolysis yields. Kinetic parameters for *p*-coumarate ester hydrolysis were quantified and pretreatment-biomass combinations were identified that result in glucose hydrolysis yields of more than 90% while retaining nearly 50 mg *p*-coumarate/g lignin.

Introduction

Technologies for the creation of cellulosic biofuels from herbaceous feedstocks which implement a pretreatment and enzymatic hydrolysis have begun to be commercialized in the last few years [93]. However, there are still technological and economic challenges that limit widespread adoption of these biomass conversion routes. One approach to improving the process economics of the lignocellulose to biofuels processes is through diversification of the product portfolio obtained from these methods to include renewable, bio-based chemicals. This approach is widely recognized as an opportunity both to improve the economics of these processes and to buffer against market fluctuations. In particular, there is an opportunity for technologies that can be integrated with conversion processes which employ a biomass pretreatment/fractionation

strategy, and allow for the utilization of all or a fraction of the process-modified lignins for purposes other than as a solid fuel for process heat and power.

Lignin represents one of the few renewable sources of aromatic chemicals, and lignin valorization has been a subject of increasing recent research to enable the use of lignin as a feedstock for bio-based products [4]. Lignins may make up more than 20% of the mass entering a biorefinery, the extraction of additional value from these biorefinery-derived lignins has great potential to improve the economic competitiveness of these facilities. Commercialization of co-products from wood lignins derived from Kraft pulping has long proved challenging. However a lignin's origin (*i.e.*, hardwood, softwood, or grass) and processing history (*i.e.*, alkaline pulping, organosolv pulping, sulfite pulping, dilute acid pretreatment, *etc.*) has an enormous impact on the structure, properties, and suitability of the lignin for a particular application. Dilute acid pretreatment has been studied for many decades at both the pilot [94] and demonstration scale [95], although use of the water-insoluble lignin remaining after enzymatic hydrolysis following dilute acid pretreatment has been limited to use as fuel in a boiler.

Phenol formaldehyde (PF) resins are commonly used as adhesives in engineered wood products including plywood, laminated veneer lumber, and oriented strand board. These resins represent a market of more than \$3.5 billion and 34% of the total adhesive market [96]. Lignins derived from biorefining or chemical pulping processes can serve as a potential bio-based feedstock alternative to the petroleum-derived phenol used in the synthesis of these resins. However, common challenges to the utilization of lignins in this application include low reactivity towards formaldehyde, high molecular weight, and high polydispersity [97]. The low reactivity of lignin towards formaldehyde when compared to phenol is mainly due to the lack of

reactive sites resulting from the *para* position of aromatic rings being bound in intramolecular linkages, while the *ortho* positions are often occupied by either methoxyl groups or β -5 linkages [64]. Due to the low reactivity of lignin with formaldehyde, resins made with non-modified lignin have historically exhibited much longer gelation times than traditional PF resins, and were only able to achieve the production of resins with acceptable mechanical properties using partial phenol replacement with <60% of phenol replaced with lignin [98, 99]. To address these challenges, many studies have functionalized lignin using approaches that include methylation [100], phenolation [101], and demethylation [78] to increase lignin reactivity and to yield mechanical properties comparable to conventional PF resin adhesives. As motivation for this work, it was recently demonstrated that a purified subset of dilute acid-pretreated corn stover lignin derived from the enzymatic hydrolysis residue (*i.e.*, the “lignin cake”) from a demonstration-scale biorefinery could be used as a 100% phenol replacement in phenol formaldehyde (PF) adhesives for plywood manufacturing with performance comparable to a commercial phenol resorcinol formaldehyde adhesive [102]. This is notable in that these results could not be achieved using other process lignins including softwood and hardwood lignins derived from Kraft pulping, corn stover lignins derived from organosolv pulping, and wheat straw lignin derived from soda pulping [103]. It has been hypothesized that the high quantified content of unsubstituted phenolic groups in the corn stover lignins, most importantly *p*-coumarate (*p*CA), are responsible for this lignin’s high level of incorporation into the PF resin polymer matrix.

A significant number of promising bioenergy feedstocks are graminaceous monocots or grasses, including agricultural residues such as corn stover, wheat straw, rice straw, and sugar

cane bagasse. One distinguishing feature of the graminaceous monocot lignins is the considerable incorporation of the *p*-hydroxycinnamic acids including ferulate and *p*CA [104, 105]. It is known that *p*CA is acylated to the lignin polymer at the γ -carbon of the side chain region primarily in syringyl moieties and to a lesser degree on arabinosyl side chains of arabinoxylan [106]. The *p*CA content of grasses is known to exhibit substantial diversity with respect to genotype, anatomical fraction, and maturity [107, 108] with the content in some fractions in maize reaching more than 150 mg *p*CA / g Klason lignin [106] or ~13% of the total aromatic content of the biomass. The prior processing history of the biomass (*i.e.*, pretreatment) as well as any lignin recovery and purification steps will also impact the *p*CA content of the lignin as the ester bond is labile to acid- or base-catalyzed hydrolysis. Therefore, if the hypothesis that *p*CA content is the most important contributor to lignin suitability in PF resins is correct, then the feedstock genotype, together with growth conditions, harvest time, and lignin processing history are all of critical importance for lignin suitability as a raw material for these applications.

In this study we seek to further understand the fate of *p*CA in corn stover lignins during dilute acid pretreatment with the goal of identifying how both the feedstock and the processing conditions could be optimally coupled to extract the most value from the lignin co-product. Specifically, we investigate the impact of plant genotype in diverse maize lines of differing initial lignin and *p*CA contents and dilute acid pretreatment conditions (pretreatment time and initial acid loading) on the response of the biomass to pretreatment (composition and enzymatic hydrolysis yields) with the goal of identifying the balance between high hydrolysis yields and low *p*CA solubilization (*i.e.*, higher quality lignin for co-products). Finally, we performed the

acid-catalyzed hydrolysis of a model pCA ester under conditions comparable to dilute acid pretreatment and estimated kinetic parameters for ester hydrolysis.

Methods

Biomass Feedstock

A panel of inbred maize lines were used as a biomass source and represent a subset of a wider diversity panel grown in Arlington, Wisconsin as reported in our previous work [107]. These include lines B7, F431, NC368, B14A, NK807, and 5220 and were selected based on extremes in the contents of lignin and pCA. Complete compositional data for these materials are provided in **Supplemental Table S1**.

Pretreatment Conditions

Pretreatments were conducted at 10% solid loadings, (g corn stover/g liquid solution) in dilute H₂SO₄ solution using 75-mL round bottom ChemGlass pressure vessels immersed in a temperature-controlled silicone oil bath heated by a hot plate. Dilute acid solutions varied in pH between 2.36 and 3.66 and treatment time varied between 45 and 360 minutes at 180 °C with a stir rate of 260 rpm to investigate treatment conditions with ranging severity factors. Treated biomass was separated from reaction liquor via vacuum filtration, washed with 500 mL of water, and air dried for 4 days at room temperature. Biomass yields after pretreatment were determined gravimetrically.

Composition Analysis

Klason lignin and sugar content of biomass was determined using the NREL laboratory analytical procedure (LAP, TP-510-42618) using a Bio-Rad Aminex HPX-87H column with a

mobile phase of 5 mM H₂SO₄ at 0.6 mL/min with a 65 °C column temperature for a 25 minute duration for sugar separation, quantification was conducted using an RID detector [109].

Remaining moisture content of biomass was determined gravimetrically after drying the biomass for 4 hours at 105 °C. Ash content was determined gravimetrically after heating the biomass at 620 °C for 4 hours according to the NREL LAP (TP-510-42622) [109].

Esterified *p*-Coumarate Content

Alkali-hydrolyzable *p*-coumarate content was determined via alkaline saponification. Biomass was placed in 40-mL Teflon-lined stainless-steel synthesis reactors (Shenzen Langce Ltd., Huizhou, China) with 12.5 mL of 3 M NaOH at 2% solid loading (0.25 g biomass). These reactors were heated to 180 °C for 2 hours with a stir rate of 280 rpm. After cooling, alkaline liquor was adjusted to below pH 2 using 150 µL H₂SO₄ and centrifuged at 13,400 rpm (10,400g) for 10 minutes to remove the precipitate. Cinnamic acids were quantified using an Agilent Poroshell 120 C18 column using a gradient of two solvents, 1% acetic acid in water (solvent A), and 1% acetic acid in 50/50% v/v methanol and water (solvent B). A 32 minute gradient was used reducing from 100% solvent A to 30% solvent A and 70% solvent B over 2 minute increments with at a flow rate of 0.4 mL/min and a column temperature of 40 °C. Acids were quantified using a DAD detector at 280 nm.

Enzymatic Hydrolysis

Enzymatic hydrolysis was conducted using CTec2 (Novozymes A/S, Bagsværd, Denmark) at an enzyme loading of 15 mg protein/ g glucan with a 10% (wt/liquid vol) solid biomass loading. The hydrolysis was conducted in a 0.05 M citrate buffer along with a 0.04 mg/mL tetracycline solution to inhibit bacterial growth. Samples were placed in 15-mL

centrifuge tubes and subjected to orbital shaking in an incubator at 50 °C for 24 hours. Sugar yields were quantified using an Aminex HPX-87H column (Bio-Rad, Hercules, CA) with detection by refraction index detector (RID).

Methyl Trans *p*-Coumarate Hydrolysis

A solution of 0.8 mg/mL methyl trans *p*-coumarate was suspended in dilute sulfuric acid of pH 2.86 and pH 3.36 within 40-mL Teflon-lined stainless-steel synthesis reactors. These reactors were heated to 180 °C for times varying between 3 and 17 hours with a stir rate of 280 rpm in a silicon oil bath. Decrease in reactant in the resulting reaction mixture was analyzed via HPLC using an Agilent Poroshell 120 C18 column. A 90 minute gradient from 100% solvent A to 70% solvent B and 30% solvent A with 6 minute increments was used with a flow rate of 0.4 mL/min and a column temperature of 40 °C. Methyl trans *p*-coumarate was quantified using a DAD detector at 280 nm.

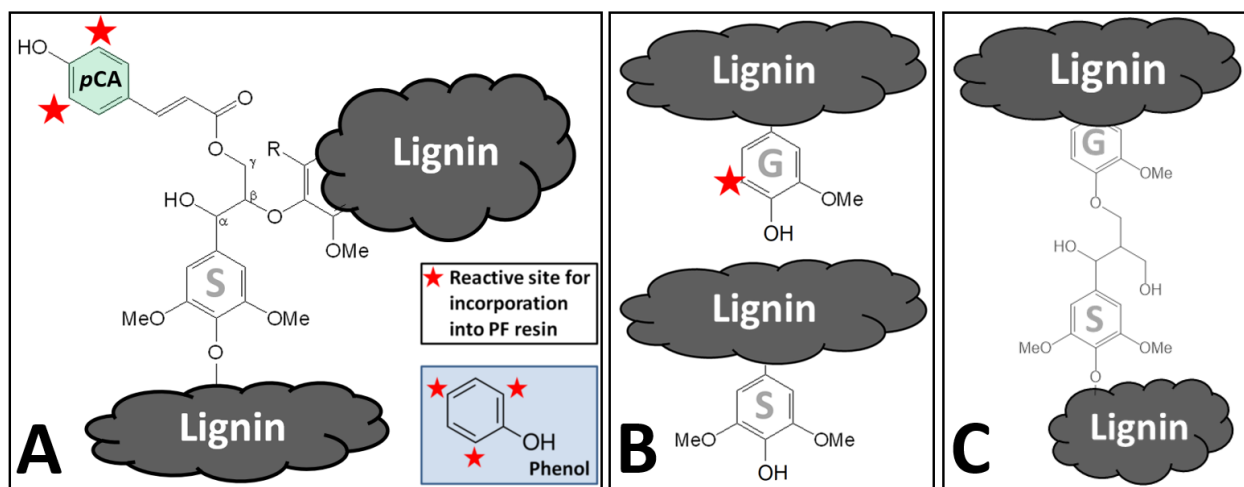


Fig. 1: Potential sites within lignins for incorporation into phenol-formaldehyde resins.

Results and Discussion

Biomass and Pretreatment

The content of *pCA* within lignins has been proposed to be a key feature impacting the utility of dilute acid-pretreated corn stover lignins in phenol-formaldehyde resin applications [103]. Critically, *pCA* should be substantially more reactive than other monomers within the lignin backbone as it has two *ortho* sites available to react with formaldehyde (**Fig. 1A**) relative to the three positions available in phenol (two *ortho* and one *para*). This can be contrasted with polymeric lignin, which for a chain ending in a free phenolic group will only have one or zero available *ortho* sites for guaiacyl or syringyl monomers, respectively (**Fig. 1B**); meanwhile lignin monomers within the interior of the lignin polymer will have zero sites that are *ortho* or *para* to a phenolic group (**Fig.1C**). Furthermore, ester-linked *pCA* is susceptible to acid- (and base-) catalyzed hydrolysis during pretreatments. As such, the impact of pretreatment conditions on the fate of *pCA* is an important, and yet largely unexplored topic for both understanding the mass flows within a biorefinery as well as the potential for increasing co-product value. If *pCA* content is important for lignin value in co-products applications, then the choice of conditions for pretreatments can be considered as a balance between **(1)** economic constraints, **(2)** conditions that optimize yields, and **(3)** conditions that preserve *pCA* content in the lignin.

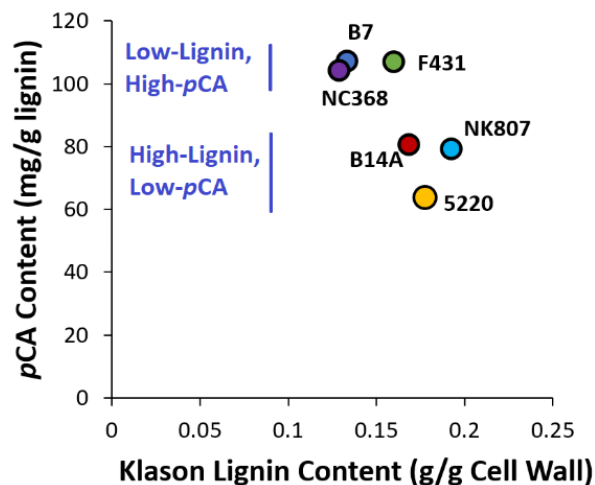


Fig. 2: Diversity of *pCA* and lignin in corn stover samples.

In addition to being impacted by processing, both Klason lignin content and *pCA* content are known to contribute to cell wall recalcitrance in corn stover. As an example, our prior work demonstrated with an expanded maize diversity panel that both initial *pCA* content and initial Klason lignin content exhibited a statistically significant inverse correlation to glucose hydrolysis yields ($p < 0.05$) in untreated biomass subjected to enzymatic hydrolysis only [110]. In this study, in order to develop a better understanding of how both lignin content and *pCA* content impact the response of the biomass to dilute acid pretreatment and enzymatic hydrolysis, and the response of the lignin-associated *pCA* to dilute acid pretreatment, we screened a diverse set of corn stover samples to a range of pretreatment severities. The six corn stover genotypes (**Fig. 2**) were selected based on their diversity in *pCA* content, which ranged from 63.8-107.2 mg *pCA*/g lignin, and Klason lignin content (12.9-19.3% by mass). The selected genotypes fall into two general categories of high-lignin, low-*pCA* (B14A, NK807, 5220) and low-lignin, high-*pCA* (B7, NC368, F431). The complete composition profiles of all six genotypes are available in **Supplemental Table S1**. Critically, as shown in **Figure 2**, *pCA* can

comprise more than 10% of the aromatic content of lignins in graminaceous monocots such as maize. For an integrated biorefinery processing produced corn stover, this represents an enormous reservoir of potentially recoverable, utilizable aromatics. For example in an integrated biorefinery processing 2000 tonnes/day of corn stover, the data in **Figure 2** represents between 23 and 34 tonnes/day of *p*CA passing through the biorefinery.

Table 1: Dilute acid pretreatment conditions and the calculated value for the combined severity factor (CSF)

Time (hr)	Initial pH	Temperature	CSF
0.75	3.36	180 °C	0.65
1.5	3.36	180 °C	0.95
3	3.36	180 °C	1.25
3	2.86	180 °C	1.75
6	3.66	180 °C	1.25
6	3.16	180 °C	1.75

The six maize genotypes were subjected to dilute acid pretreatment over a range of severities according to the conditions in **Table 1**. Additionally, the values for the combined severity factor (CSF) are presented using the equation for CSF (based on initial pH) and parameters developed by Chum *et al.* [111]. Conditions for pretreatment were selected based on the CSF range identified by Schell *et al.* [94] as achieving glucose hydrolysis yields >70% for dilute acid-pretreated corn stover. While the CSF values were within a comparable range, the pretreatment times were much longer (up to 6 hr rather than <10 min) and the initial pH values were much higher (*i.e.*, lower acid loadings were utilized). The reasons these milder range of conditions were chosen are so that **(1)** these milder acid loadings would allow the reaction to take place in reactors without use of expensive, corrosion-resistance alloys, substantially lowering capital costs, **(2)** improve the selectivity of xylan hydrolysis to xylose dehydration to furfural,

and **(3)** potentially decrease the hydrolysis of esterified *p*CA while simultaneously achieving pretreatment performance targets. It should be noted that not all biomass-pretreatment combinations were employed in this study.

Impact of Pretreatment on Cell Wall Composition and Enzymatic Hydrolysis Yields

The impact of pretreatment conditions on the solubilization of key cell wall components was assessed with a focus on xylan and *p*CA. Complete compositional profiles for all biomass-pretreatment combinations are provided in **Supplemental Table S1**. Xylans are a major structural component of the plant cell walls of angiosperms and are involved in non-covalent cross-links between cellulose microfibrils [110]. Hemicelluloses inhibit the ability of cellulase enzymes to access cellulose within the cell wall structure and reduce the effectiveness of enzyme hydrolysis and, consequently, the overall potential biofuel yield. Hemicellulose removal is typically a target of biomass deconstruction processes either during pretreatment by hydrolysis and/or solubilization or during enzymatic hydrolysis by xylanolytic enzymes. During dilute acid pretreatments, acid catalyzes the hydrolysis of the glycosidic bonds with xylans resulting in their removal from the cell wall in the form of its constituent oligomers and monomers, while lignin is melted and redistributed within cell wall [112, 113]. These two outcomes of dilute acid pretreatment allow the cellulase enzyme cocktail access to the cellulose, resulting in higher total glucose hydrolysis yields. The amount of xylan removed and solubilized during dilute acid pretreatments is commonly used to evaluate the effectiveness of different dilute acid pretreatment conditions and how we expect these conditions to influence the maximum obtainable hydrolysis yield from pretreated corn stover [94, 114].

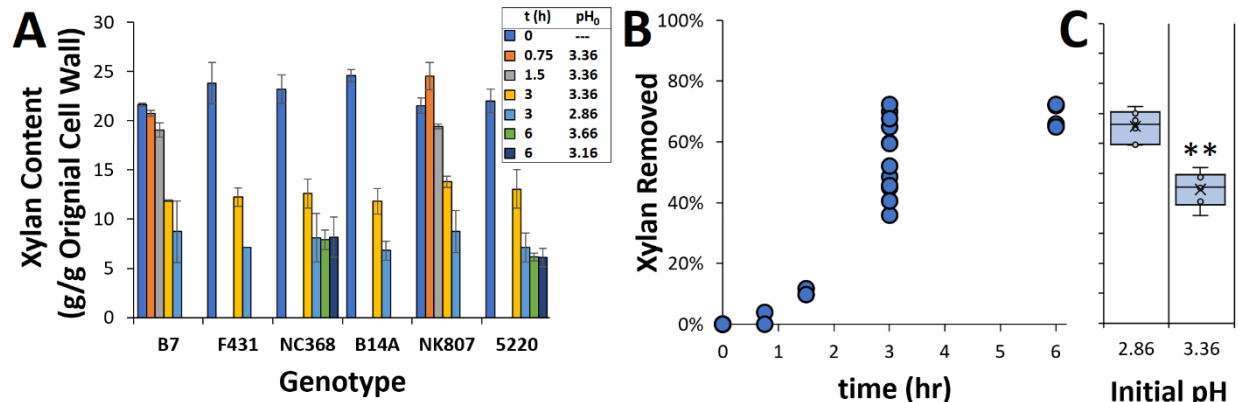


Figure 3: Impact of pretreatment conditions on cell wall xylan content for (A) distinct maize genotypes, (B) all samples for as a function of time, and (C) the initial pH for the 3-hr pretreatment time. ** represents $p < 0.001$.

The primary contributors to the effectiveness of a dilute acid pretreatment are temperature, time, and acid catalyst concentration. As all the experiments were performed at 180°C, the time and initial pH are the only two factors that will be contributing to differences in responses in this work. The results for changes to xylan content are presented in **Figure 3** and show several notable trends. Specifically, when all samples are pooled, the xylan solubilization can be shown to be a strong, significant function of time, with longer pretreatment times resulting in significantly higher levels of xylan solubilization (**Fig. 3B**). For the 3-hr pretreatment times, where the most data points were collected, even the minor differences in the initial pH have a significant ($p < 0.001$) impact on the xylan solubilization during pretreatment (**Fig. 3C**).

In prior work, *p*CA solubilization during acidic treatments has been studied primarily to understand its role as an inhibitor of either cellulases during enzymatic hydrolysis when present in hydrolysate liquors [115, 116] or intact in the cell wall [117, 118], as well as during microbial fermentation [119-121], or used as a standard analytical tool for the analysis of *p*CA-polysaccharide esters [106]. However, a detailed information on the kinetics or hydrolysis of

*p*CA-lignin esters during chemical pretreatments is scarce in the literature. As one example, it is known that hydrolysis using 0.1 M trifluoroacetic acid at 100°C for 1 hour is capable of hydrolyzing glycosidic bonds within arabinoxylan while preserving the ester bond of *p*CA acylated to arabinosyl subunits [106]. One prior study showed that *p*CA was the most abundant aromatic monomer in liquors derived from dilute acid pretreatment of corn stover and the quantified content was equivalent to 16.7 mg *p*CA/g original Klason lignin [119]. The pretreatment conditions in this study were 190°C, an estimated initial pH of 0.7, and a residence time of <10 minutes. Other work in sugarcane bagasse has shown that pretreatment under acidic conditions resulted in less *p*CA in the hydrolysate when compared to steam pretreatment and pretreatment in the presence of SO₂ due to the further degradation of *p*CA and other aromatic acids during pretreatment [122].

In the present work, dilute acid pretreatment is shown to result in extensive hydrolysis of *p*CA (**Fig. 4**). The results for *p*CA removal correspond with those for xylan removal and show that both time and initial pH are key parameters in its removal from the cell wall. Under the most severe pretreatment conditions (6-hr pretreatment time), a 6.5-fold reduction in *p*CA content was observed. As with the xylan solubilization, pretreatment time was found to correlate to reduction in *p*CA content (**Fig. 4B**). For every genotype subjected to 3-hr pretreatments, the initial pH had a statistically significant impact on the *p*CA removal (**Fig. 4C**).

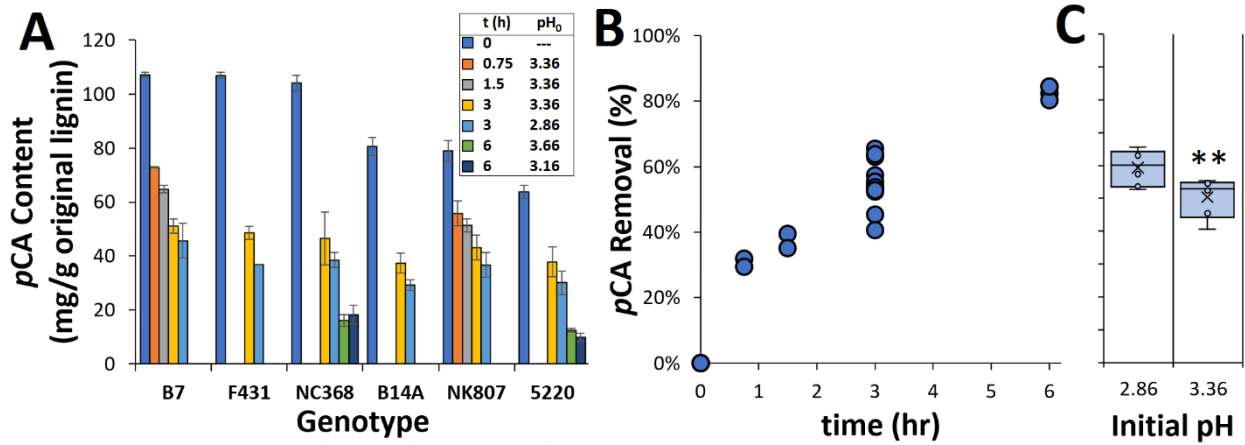


Figure 4: Impact of pretreatment conditions on cell wall pCA content for (A) distinct maize genotypes, (B) all samples for as a function of time, and (C) the initial pH for the 3-hr pretreatment time. ** represents $p < 0.001$.

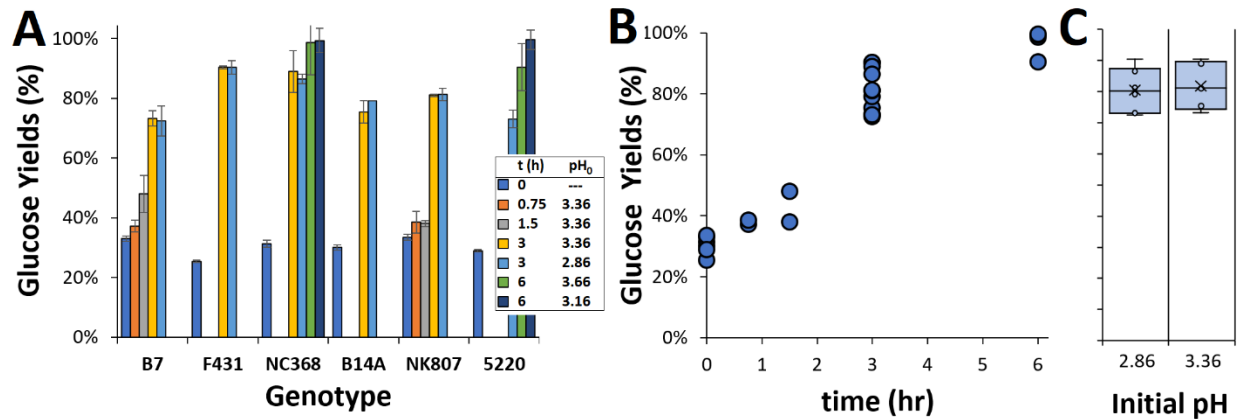


Figure 5: Impact of pretreatment conditions on cell wall glucose hydrolysis yields for (A) distinct maize genotypes, (B) all samples for as a function of time, and (C) the initial pH for the 3-hr pretreatment time.

Enzymatic hydrolysis of cellulose to glucose provides an assessment of the relative recalcitrance of the cell wall and, additionally, is a key step determining the economic feasibility of ethanol production from lignocellulosic biomass. Enzymatic hydrolysis is considered to be one of the largest contributions to the operating cost in a biorefinery with enzymes estimated to contribute up to \$1 per gallon of ethanol produced [123]. It can be observed that, with the

exception of the B7, the low-lignin biomass (F431, NC368) generally exhibited higher hydrolysis yields than the high-lignin biomass (B14A, NK807, 5220). As lignin is not removed during batch dilute acid pretreatments, but only melted and redistributed, this could be expected as redeposited lignin can prevent hydrolytic enzymes from accessing glycosidic bonds within cellulose. Comparable to the impacts of dilute acid pretreatment on xylan and *p*CA removal, longer dilute acid pretreatment times result in greater hydrolysis yields due to increases hemicellulose removal during pretreatment. However, unlike the other results following three-hour pretreatment whereby differences in the initial pH resulted in differences in xylan (**Fig. 3B**) and *p*CA solubilization (**Fig. 4B**), a significant difference in hydrolysis yields between differing pH pretreatments was not observed (**Fig. 5C**).

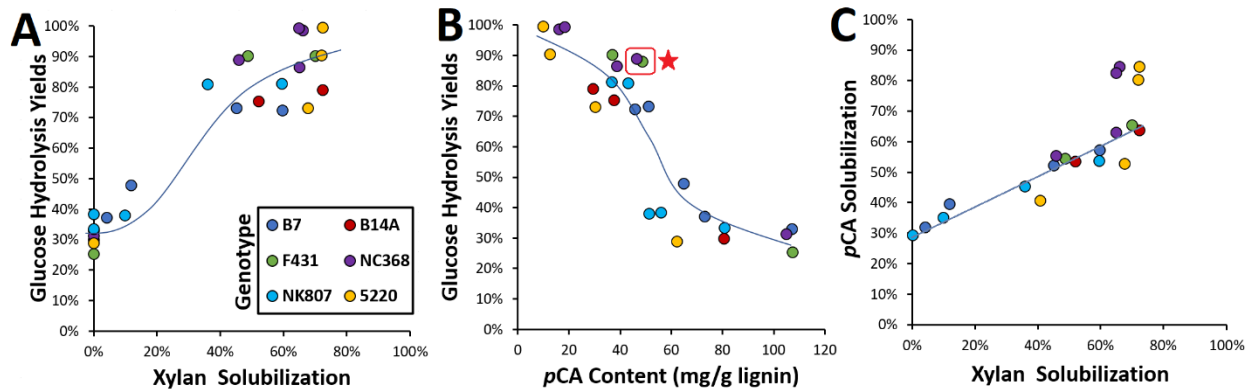


Figure 6: Correlations between (A) glucose hydrolysis yields and xylan solubilization, (B) glucose hydrolysis yields and *p*CA content, and (C) xylan solubilization and *p*CA content. Biomass-pretreatment combinations resulting in high hydrolysis yields and high *p*CA content are highlighted with a “★”.

Correlation of Data

Select trends within the data set are next plotted in order to highlight key relationships between pretreatment outcomes (**Fig. 6**). The first key observation is that xylan solubilization is

generally correlated to glucose hydrolysis yields across all pretreatment conditions and genotypes (**Fig. 6A**). Xylan removal (or alternatively xylose generation) has been used as a proxy for the extent of pretreatment and has been shown to exhibit correlations to the enzymatic hydrolysis yields during dilute acid pretreatments [124], with one study showing strong linear correlations between xylan removal and enzymatic hydrolysis yields in corn stover subjected to dilute acid pretreatment in both batch and flowthrough reactor configurations [114]. Other work in a continuous pilot-scale reactor showed that rather than exhibiting a continuous linear increase in hydrolysis yields with xylan removal that the glucose hydrolysis yields in dilute acid-pretreated corn stover reached a maximum at xylan solubilization levels of >60% [94]. Our data show that for each of the six genotypes, xylan solubilization above 50% did not result substantial improvements in glucose hydrolysis yields (**Fig. 6A**). As shown previously, the high-lignin biomass generally exhibited lower yields than the low-lignin biomass.

The glucose hydrolysis yields are plotted against the *pCA* content for all biomass-pretreatment combinations to highlight the balance between achieving high hydrolysis yields versus hydrolyzing *pCA* during the pretreatment (**Fig. 6B**). These results show a sigmoidal relationship between these two variables and it can be observed that at least two biomass-pretreatment combinations (highlighted in **Fig. 6B** with a “★”) are capable of achieving hydrolysis yields >90% while retaining more than 50 mg *pCA*/g original Klason lignin. It is notable that both genotypes (NC368 and F431) are from the low-lignin, high-*pCA* category suggesting that the two objectives of high sugar yields and high *pCA* retention can be achieved simultaneously by selection of appropriate feedstock-pretreatment combinations. The conditions

used for pretreatment (3 hr, $\text{pH}_0 = 3.36$, 180°C) at the higher initial pH was shown to result in less *p*CA removal (**Fig. 4C**) while not having a significant impact on hydrolysis yields (**Fig. 5C**).

The next within-dataset correlation exhibiting notable trends is the relationship between the *p*CA solubilization and the xylan solubilization (**Fig. 6C**). For this, a strong linear correlation can be observed for all biomass samples except for samples with *p*CA solubilization greater than ~65%. This indicates that under the conditions tested the rate of hydrolysis of an acid-catalyzed *p*CA-lignin ester linkage is of the same order of magnitude as the hydrolysis of a glycosidic bond within xylan. In the next section, these kinetics for *p*CA ester hydrolysis are explored in more detail.

Ester Hydrolysis Kinetics

In order to further understand the kinetics of *p*CA ester hydrolysis, a kinetic study was next performed using *trans-p*CA methyl ester as a model compound with the results compared to those from corn stover. The hydrolysis of phenolic esters has been studied since at least 1937 when Hammett developed a relationship relating the rate of reaction of substituted aromatic compounds to the structure of their unique substitution [125]. Kinetic models based on modified versions of the Hammett equation have been developed to explain the hydrolysis of various esters catalyzed by both dilute and concentrated acid [126, 127]. In addition to these models these kinetic principles have been used to explore the mechanism behind acid-catalyzed ester hydrolysis. Shi *et. al.* suggest that an ester bond is hydrolyzed by acid according to a two-step mechanism; an alkyl-oxygen protonation which creates an acylium ion followed by a trimolecular hydrolysis [44].

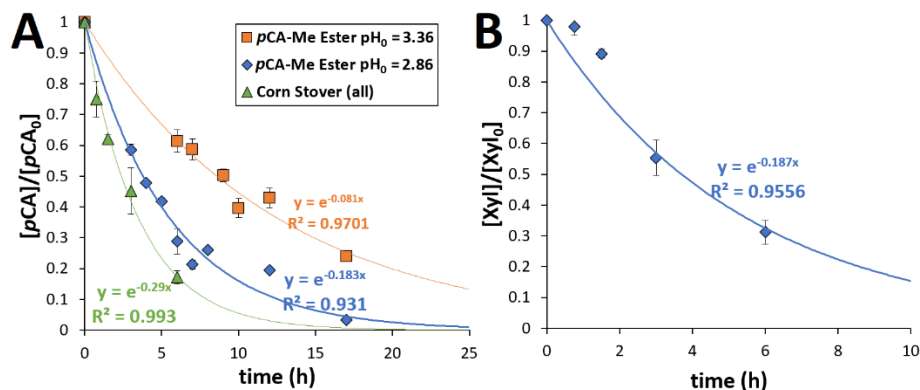


Fig. 7: Reaction kinetics of the dilute acid-catalyzed hydrolysis of **(A)** *trans*-pCA methyl ester and corn stover pCA-lignin ester and **(B)** corn stover xylan.

In the present work, the pCA methyl ester was hydrolyzed under conditions comparable to those used for our dilute acid pretreatments of corn stover at times ranging from 2 to 17 hours, initial pH values of 3.36 and 2.86, and a temperature of 180°C. The goal of this set of experiments was to gain insight into the kinetics of the acid-catalyzed hydrolysis of the pCA-lignin ester bond responsible for the removal of pCA during dilute acid pretreatment. Results are plotted as a dimensionless conversion such that all biomass samples with varying pCA contents could be plotted for comparison (**Fig. 7A**). Exponential functions were fit to the curves with the assumption that ester hydrolysis kinetics could be approximated by a first-order reaction with respect to pCA concentration with rate constants estimated to be 0.081 h⁻¹ (pH₀ = 3.36) and 0.183 h⁻¹ (pH₀ = 2.86). This is consistent with increasing acid catalyst concentration increasing the rate constant as a contributor to activation energy or alternatively as a concentration dependence in an elementary rate law. These results can be compared to the pCA hydrolysis kinetics pooled from all the biomass-pretreatment conditions screened in this work with pH₀ ranging from 2.86 to 3.66 (**Fig. 7A**). Assuming first-order kinetics for this reaction, the estimated

rate constant for xylan hydrolysis and removal from the corn stover is 0.29 h^{-1} . Although this is the same order of magnitude as the rate constant for model compound ester hydrolysis, it is still significantly higher. Potential reasons for this discrepancy could be either differing mechanisms for the hydrolysis of a *p*CA-lignin ester versus a *p*CA-methyl ester. A second possibility is that the presence of biomass during the reaction results in generation of additional acid through autohydrolysis of acetyl groups within the hemicellulose and further decreases the pH during the pretreatment. However, it has been shown that pH decreases in liquid hot water and dilute acid pretreatments do not generally proceed below pH 3.0 [128].

The results of the *p*CA ester hydrolysis kinetics can be compared to those of the xylan in corn stover (**Fig. 7B**). For this, data were selected from all samples and conditions that used a $\text{pH}_0 \geq 3.16$ as the lower pH condition resulted in substantially higher xylan hydrolysis (**Fig. 3C**). An exponential curve is fit to the data under the assumption that xylan solubilization can be approximated by first-order reaction kinetics yielding a rate constant of 0.187 h^{-1} which is of the same order of magnitude as the *p*CA hydrolysis (**Fig. 7A**). This finding of comparable rate constants for *p*CA and xylan hydrolysis is supported by our previous observation of a linear correlation between xylan and *p*CA removal during pretreatment (**Fig. 6C**). Xylan removal from the cell wall involves multiple step including hydrolysis of cellulose-associated insoluble xylan to yield water-soluble oligomeric xylan. Unless this xylan is further hydrolyzed to monomeric xylose, these xylooligomers may be able to absorb back onto the cellulose. More complex models for xylan hydrolysis within biomass have been developed that include two pools of xylan (“slow-reacting” and “fast-reacting”) that first hydrolyze to oligomers followed by hydrolysis of oligomers to monomers with each of these reactions exhibiting differing rate constants that are

elementary with respect to xylan and acid concentration [94]. Using the expressions developed by Schell et al. [94] for the hydrolysis of xylan during dilute acid pretreatment of corn stover, values for the rate constants for “slow-reacting” xylan (presumably the rate-limiting step) are calculated to be 0.48 h^{-1} for a pH of 2.86 and 0.152 h^{-1} for a pH 3.36, which bounds our estimated rate constant of 0.127 h^{-1} .

Conclusions

A panel of six diverse maize genotypes exhibiting a range of pCA and lignin contents was used to study the relationship between dilute acid pretreatment conditions and the biomass response to pretreatment with the goals understanding pCA ester hydrolysis kinetics during pretreatment. For this, a range of pretreatment conditions utilizing mild dilute acid (initial pH ≥ 2.86) were employed over times ranging from 45 min to 6 h at 180°C . Strong correlations between xylan solubilization and pCA solubilization were identified. Using a model pCA-ester, kinetic parameters for acid-catalyzed ester hydrolysis were estimated and were of the same order of magnitude as those for xylan hydrolysis under comparable conditions.

Acknowledgements

This work was supported by AFRI competitive grant no. 2018-67009-27900/1015055 from the USDA National Institute of Food and Agriculture.

Supplementary Data

Supplemental Table S1: Composition of Biomass

Time (hr)	Initial pH	Temp. (°C)	Combined Severity Factor	Maize Genotype	Glucan (%)	Xylan (%)	Acetate (%)	Klason Lignin (%)	p CA (mg/g original lignin)	Glucose Hydrolysis Yield (g/g)
Untreated Biomass				S220	31.9	22.0	3.4	17.8	63.8	0.289
				B14A	35.5	24.6	3.6	16.8	80.6	0.300
				B7	32.4	21.6	3.5	13.4	107.1	0.330
				F431	36.3	23.8	4.0	16.0	107.0	0.253
				NC368	31.7	23.2	4.0	12.9	104.1	0.313
				NK807	35.1	21.5	3.8	19.2	79.1	0.335
0.75	3.36	180	0.65	B7	40.1	25.7	4.3	9.6	72.9	0.372
				NK807	39.4	27.6	4.5	10.7	55.8	0.385
1.5	3.36	180	0.95	B7	40.7	23.9	3.8	9.1	64.8	0.479
				NK807	43.8	24.2	4.0	15.4	51.3	0.380
3	3.36	180	1.25	S220	48.4	19.8	2.4	18.2	37.8	
				B14A	54.4	18.1	2.3	17.6	37.4	0.754
				B7	48.7	17.9	2.7	20.0	51.1	0.732
				F431	56.3	18.9	2.8	11.4	48.6	0.903
				NC368	49.5	19.7	2.9	15.9	46.5	0.890
				NK807	49.4	19.4	2.7	18.9	43.2	0.810
3	2.86	180	1.75	S220	54.7	12.2	1.6	19.3	30.1	0.731
				B14A	57.1	11.0	1.7	20.4	29.2	0.791
				B7	50.2	13.5	2.3	23.5	45.7	0.724
				F431	54.9	10.8	1.0	18.3	36.9	0.903
				NC368	52.2	13.4	2.1	22.9	38.6	0.865
				NK807	52.4	13.0	1.9	24.3	36.6	0.812
6	3.66	180	1.25	S220	53.3	10.3	1.1	23.8	12.5	0.904
				NC368	53.1	13.2	1.7	23.6	16.1	0.986
6	3.16	180	1.75	S220	54.1	10.3	1.2	23.6	9.8	0.996
				NC368	52.7	13.6	1.6	23.4	18.2	0.993

ERROR (+/-)

Time (hr)	Initial pH	Temp. (°C)	Combined Severity Factor	Maize Genotype	Glucan (%)	Xylan (%)	Acetate (%)	Klason Lignin (%)	p CA (mg/g original lignin)	Glucose Hydrolysis Yield (g/g)
Untreated Biomass				S220	0.6	1.2	0.3	0.1	2.5	0.004
				B14A	0.2	0.6	0.1	0.7	3.3	0.008
				B7	2.2	0.1	0.1	0.3	0.9	0.009
				F431	0.8	2.1	0.4	0.3	1.1	0.004
				NC368	2.4	1.5	0.2	1.2	2.8	0.012
				NK807	0.7	0.8	0.1	0.5	3.8	0.010
0.75	3.36	180	0.75	B7	0.9	0.3	0.1	0.0	0.2	0.020
				NK807	1.4	1.4	0.1	1.0	4.6	0.036
1.5	3.36	180	1.5	B7	1.9	0.7	0.2	1.0	1.3	0.062
				NK807	1.4	0.2	0.0	4.2	2.4	0.010
3	3.36	180	1.25	S220	0.9	2.0	0.1	2.0	5.5	
				B14A	0.6	1.3	0.1	1.8	3.7	0.038
				B7	2.4	0.1	0.1	1.7	2.6	0.026
				F431	1.9	0.9	0.8	0.5	2.4	0.004
				NC368	1.5	1.5	0.2	4.1	9.7	0.069
				NK807	0.7	0.5	0.0	0.5	4.6	0.004
3	2.86	180	1.75	S220	1.2	1.5	0.3	3.2	4.4	0.030
				B14A	2.2	1.0	0.1	2.1	1.9	0.000
				B7	3.4	3.1	0.6	2.6	6.4	0.050
				F431					0.1	0.023
				NC368	5.8	2.5	0.4	0.0	2.8	0.016
				NK807	0.6	2.1	0.5	1.7	4.6	0.021
6	3.66	180	1.25	S220	1.5	0.4	0.1	0.5	0.6	0.079
				NC368	1.4	1.0	0.2	0.5	2.3	0.108
6	3.16	180	1.75	S220	2.4	0.9	0.2	1.0	1.5	0.033
				NC368	3.2	2.0	0.3	1.0	3.4	0.040

References

4. Ragauskas, A.J., G.T. Beckham, M.J. Bidy, R. Chandra, F. Chen, M.F. Davis, B.H. Davison, R.A. Dixon, P. Gilna, M. Keller, P. Langan, A.K. Naskar, J.N. Saddler, T.J. Tschaplinski, G.A. Tuskan, and C.E. Wyman, *Lignin valorization: improving lignin processing in the biorefinery*. Science, 2014. **344**(6185): p. 1246843.
44. Lourençon, T.V., F.A. Hansel, T.A. da Silva, L.P. Ramos, G.I.B. de Muniz, and W.L.E. Magalhães, *Hardwood and softwood kraft lignins fractionation by simple sequential acid precipitation*. Separation and Purification Technology, 2015. **154**: p. 82-88.
64. Laurichesse, S. and L. Avérous, *Chemical modification of lignins: Towards biobased polymers*. Progress in Polymer Science, 2014. **39**(7): p. 1266-1290.
78. Song, Y., Z. Wang, N. Yan, R. Zhang, and J. Li, *Demethylation of Wheat Straw Alkali Lignin for Application in Phenol Formaldehyde Adhesives*. Polymers, 2016. **8**(6): p. 209.

93. Warner, E., A. Schwab, and D. Bacovsky, *2016 Survey of Non-Starch Alcohol and Renewable Hydrocarbon Biofuels Producers*. 2017, NREL (National Renewable Energy Laboratory (NREL), Golden, CO (United States)).
94. Schell, D.J., J. Farmer, M. Newman, and J.D. McMillan, *Dilute-sulfuric acid pretreatment of corn stover in pilot-scale reactor*. *Applied Biochemistry and Biotechnology*, 2003. **105**(1): p. 69-85.
95. Carlson, D.C., *System for the Treatment of Biomass*. 2020: US.
96. Gomez-Bueso, J. and Haupt, *Wood Composite Adhesives*, in *Phenolic Resins: A Century of Progress*, L. Pilato, Editor. 2010, Springer-Verlag: Berlin.
97. Mansouri, N.-E. and J. Salvadó, *Structural characterization of technical lignins for the production of adhesives: Application to lignosulfonate, kraft, soda-anthraquinone, organosolv and ethanol process lignins*. *Industrial Crops and Products*, 2006. **24**: p. 8-16.
98. Vázquez, G., G. Antorrena, J. González, and J. Mayor, *Lignin-phenol-formaldehyde adhesives for exterior grade plywoods*. *Bioresource Technology*, 1995. **51**(2): p. 187-192.
99. Danielson, B. and R. Simonson, *Kraft lignin in phenol formaldehyde resin. Part 1. Partial replacement of phenol by kraft lignin in phenol formaldehyde adhesives for plywood*. *Journal of Adhesion Science and Technology*, 1998. **12**(9): p. 923-939.
100. Mansouri, N.E.E., A. Pizzi, and J. Salvado, *Lignin-based polycondensation resins for wood adhesives*. *Journal of Applied Polymer Science*, 2007. **103**(3): p. 1690-1699.
101. Jiang, X., J. Liu, X. Du, Z. Hu, H.-m. Chang, and H. Jameel, *Phenolation to Improve Lignin Reactivity toward Thermosets Application*. *ACS sustainable chemistry & engineering*., 2018. **6**(4): p. 5504-5512.
102. Kalami, S., M. Arefmanesh, E. Master, and M. Nejad, *Replacing 100% of phenol in phenolic adhesive formulations with lignin*. *Journal of Applied Polymer Science*, 2017. **134**(30): p. 45124.
103. Kalami, S., N. Chen, H. Borazjani, and M. Nejad, *Comparative analysis of different lignins as phenol replacement in phenolic adhesive formulations*. *Ind Crop Prod*, 2018. **125**: p. 520-528.
104. Carpita, N.C., *Structure and biogenesis of the cell walls of grasses*. *Annual Review of Plant Physiology and Plant Molecular Biology*, 1996. **47**: p. 445-476.
105. Hatfield, R.D., J. Ralph, and J.H. Grabber, *Cell wall cross-linking by ferulates and diferulates in grasses*. *Journal of the Science of Food and Agriculture*, 1999. **79**(3): p. 403-407.

106. Hatfield, R.D., J.M. Marita, and K. Frost, *Characterization of p-coumarate accumulation, p-coumaroyl transferase, and cell wall changes during the development of corn stems*. Journal of the Science of Food and Agriculture, 2008. **88**(14): p. 2529-2537.
107. Li, M., M. Heckwolf, J.D. Crowe, D.L. Williams, T.D. Magee, S.M. Kaeppler, N. de Leon, and D.B. Hodge, *Cell-wall properties contributing to improved deconstruction by alkaline pre-treatment and enzymatic hydrolysis in diverse maize (Zea mays L.) lines*. Journal of experimental botany, 2015. **66**(14): p. 4305-4315.
108. Zhang, Y., Q. Hou, W. Xu, M. Qin, Y. Fu, Z. Wang, S. Willför, and C. Xu, *Revealing the structure of bamboo lignin obtained by formic acid delignification at different pressure levels*. Industrial Crops and Products, 2017. **108**: p. 864-871.
109. Sluiter, A., B. Hames, R. Ruiz, C. Scarlata, J. Sluiter, and D. Templeton, *Determination of ash in biomass*. National Renewable Energy Laboratory, 2008 (NREL/TP-510-42622).
110. Ong, R., S. Chundawat, D. Hodge, S. Keskar, and B. Dale, *Linking Plant Biology and Pretreatment: Understanding the Structure and Organization of the Plant Cell Wall and Interactions with Cellulosic Biofuel Production*. 2014. p. 231-253.
111. Chum, H.L., D.K. Johnson, and S.K. Black, *Organosolv pretreatment for enzymic hydrolysis of poplars. 2. Catalyst effects and the combined severity parameter*. Industrial & engineering chemistry research, 1990. **29**(2): p. 156-162.
112. Donohoe, B.S., S.R. Decker, M.P. Tucker, M.E. Himmel, and T.B. Vinzant, *Visualizing lignin coalescence and migration through maize cell walls following thermochemical pretreatment*. Biotechnol Bioeng, 2008. **101**(5): p. 913-25.
113. Selig, M.J., S. Viamajala, S.R. Decker, M.P. Tucker, M.E. Himmel, and T.B. Vinzant, *Deposition of lignin droplets produced during dilute acid pretreatment of maize stems retards enzymatic hydrolysis of cellulose*. Biotechnol Prog, 2007. **23**(6): p. 1333-9.
114. Yang, B. and C.E. Wyman, *Effect of Xylan and Lignin Removal by Batch and Flowthrough Pretreatment on the Enzymatic digestibility of Corn Stover Cellulose*. Biotechnol Bioeng, 2004. **86**(1): p. 88-95.
115. Martin, S.A. and D.E. Akin, *Effect of phenolic monomers on the growth and beta-glucosidase activity of Bacteroides ruminicola and on the carboxymethylcellulase, beta-glucosidase, and xylanase activities of Bacteroides succinogenes*. Appl Environ Microbiol, 1988. **54**(12): p. 3019-22.
116. Theodorou, M.K., D.J. Gascoyne, D.E. Akin, and R.D. Hartley, *Effect of phenolic acids and phenolics from plant cell walls on rumenlike fermentation in consecutive batch culture*. Appl Environ Microbiol, 1987. **53**(5): p. 1046-50.

117. Hartley, R.D. and E.C. Jones, *Phenolic components and degradability of the cell walls of the brown midrib mutant, bm3, of Zea mays*. Journal of the Science of Food and Agriculture, 1978. **29**(9): p. 777-782.
118. Hartley, R.D., *p-Coumaric and ferulic acid components of cell walls of ryegrass and their relationships with lignin and digestibility*. Journal of the Science of Food and Agriculture, 1972. **23**(11): p. 1347-1354.
119. Chundawat, S.P.S., R. Vismeh, L.N. Sharma, J.F. Humpala, L. da Costa Sousa, C.K. Chambliss, A.D. Jones, V. Balan, and B.E. Dale, *Multifaceted characterization of cell wall decomposition products formed during ammonia fiber expansion (AFEX) and dilute acid based pretreatments*. Bioresource Technology, 2010. **101**(21): p. 8429-8438.
120. Du, B., L.N. Sharma, C. Becker, S.F. Chen, R.A. Mowery, G.P. van Walsum, and C.K. Chambliss, *Effect of varying feedstock-pretreatment chemistry combinations on the formation and accumulation of potentially inhibitory degradation products in biomass hydrolysates*. Biotechnol Bioeng, 2010. **107**(3): p. 430-40.
121. Mitchell, V.D., C.M. Taylor, and S. Bauer, *Comprehensive Analysis of Monomeric Phenolics in Dilute Acid Plant Hydrolysates*. BioEnergy Research, 2014. **7**(2): p. 654-669.
122. Martin, C., M. Galbe, N.O. Nilvebrant, and L.J. Jonsson, *Comparison of the fermentability of enzymatic hydrolyzates of sugarcane bagasse pretreated by steam explosion using different impregnating agents*. Appl Biochem Biotechnol, 2002. **98-100**: p. 699-716.
123. Kumar, D. and G.S. Murthy, *Stochastic molecular model of enzymatic hydrolysis of cellulose for ethanol production*. Biotechnology for Biofuels, 2013. **6**(1): p. 63.
124. Yao, L., H. Yang, C.G. Yoo, Y. Pu, X. Meng, W. Muchero, G.A. Tuskan, T. Tschaplinski, and A.J. Ragauskas, *Understanding the influences of different pretreatments on recalcitrance of Populus natural variants*. Bioresource Technology, 2018. **265**: p. 75-81.
125. Hammett, L.P., *The Effect of Structure upon the Reactions of Organic Compounds. Benzene Derivatives*. Journal of the American Chemical Society, 1937. **59**(1): p. 96-103.
126. Taft, R.W., *Linear Free Energy Relationships from Rates of Esterification and Hydrolysis of Aliphatic and Ortho-substituted Benzoate Esters*. Journal of the American Chemical Society, 1952. **74**(11): p. 2729-2732.
127. Yates, K., *Kinetics of ester hydrolysis in concentrated acid*. Accounts of Chemical Research, 1971. **4**(4): p. 136-144.
128. Kapu, N.S., Z. Yuan, X.F. Chang, R. Beatson, D.M. Martinez, and H.L. Trajano, *Insight into the evolution of the proton concentration during autohydrolysis and dilute-acid hydrolysis of hemicellulose*. Biotechnol Biofuel, 2016. **9**: p. 224.

CHAPTER 3

EFFECT OF DILUTE ACID PRETREATMENT AND LIGNIN EXTRACTION CONDITIONS
ON LIGNIN PROPERTIES AND SUITABILITY AS A PHENOL REPLACEMENT IN
PHENOL-FORMALDEHYDE WOOD ADHESIVES

Contribution of Authors and Co-Authors

Author: Brian K. Saulnier

Contributions: Designed experiments and selected pretreatment conditions. Conducted large and small scale corn stover pretreatment experiments. Subjected pretreated biomass to enzymatic hydrolysis at large and small scales for the production of lignin cake. Extracted lignin from lignin cake at small and large scales using NaOH and formic acid methods. Analyzed all produced materials for structural composition. Determined *p*CA content of biomass and lignin using alkaline saponification method. Wrote first draft of manuscript.

Co-Author: Mohsen Siahkamari

Contributions: Characterized lignin functional groups by ^{31}P NMR method. Analyzed lignins for elemental composition. Formulated and characterized lignin-formaldehyde resins and adhesives. Optimized adhesive component ratios. Evaluated adhesives for mechanical performance via lap shear strength tests. Wrote first draft of resin and adhesive property discussion.

Co-Author: Sandip K. Singh

Contributions: Determined molecular weight of extracted lignins using GPC method. Provided guidance on lab techniques and aided in data interpretation. Assisted with editing of final manuscript draft.

Co-Author: Mojgan Nejad

Contributions: Provided assistance in design of experiments. Aided in the interpretation of data. Gave feedback and editing assistance for final draft of manuscript.

Co-Author: David B. Hodge

Contributions: Helped with experimental design and selection of pretreatment and lignin extraction conditions. Aided in interpretation of data. Provided assistance in the writing, editing, and finalization of subsequent manuscript drafts.

Manuscript Information

Brian Saulnier, Mohsen Siahkamari, Sandip K. Singh, Mojgan Nejad, David B. Hodge

Journal of Agricultural and Food Chemistry

Status of Manuscript: [Put an x in one of the options below, delete this]

☐ Prepared for submission to a peer-reviewed journal

☒ Officially submitted to a peer-reviewed journal

☐ Accepted by a peer-reviewed journal

☐ Published in a peer-reviewed journal

American Chemical Society

Submitted October 19, 2022

EFFECT OF DILUTE ACID PRETREATMENT AND LIGNIN EXTRACTION CONDITIONS
OF LIGNIN PROPERTIES AND SUITABILITY AS A PHENOL REPLACEMENT IN
PHENOL-FORMALDEHYDE WOOD ADHESIVES

Abstract

Corn stover was subjected dilute acid pretreatment to assess the impact of pretreatment conditions on lignin extractability, properties, and utility as a phenol replacement in wood phenol-formaldehyde (PF) adhesives. It was identified that both formic acid and NaOH could extract and recover 60% to 70% of the lignin remaining after pretreatment and enzymatic hydrolysis under the mildest pretreatment conditions while simultaneously achieving reasonable enzymatic hydrolysis yields (>60%). The availability of reaction sites for incorporation of lignins into the PF polymer matrix (i.e., unsubstituted phenolic hydroxyl groups) was shown to be strongly impacted by the pretreatment time and the recovery. Finally, a 100% lignin-based wood adhesive formulated using formic acid-extracted lignin was demonstrated to have a dry sheer strength exceeding that of a conventional PF adhesive. These findings suggest that both pretreatment and lignin extraction conditions can be tailored to yield lignins with properties targeted for this co-product application.

Introduction

Lignocellulosic biomass represents a significant renewable resource that can serve as a potential feedstock for the production of fuels, chemicals, and materials. One approach to renewable fuels is through a chemical pretreatment of biomass followed by enzymatic hydrolysis of the polysaccharides to yield fermentable sugars that can be biologically or chemically upgraded to fuels and chemicals [129]. While the structural polysaccharide content of lignocellulose can be targeted for depolymerization, conversion, and high yields, the utilization of the lignin fraction is both necessary to improve overall process economics[130] and challenging for applications other than direct combustion due to lignin heterogeneity and its modification during processing. Three general approaches can be applied for integrating lignin

recovery and utilization into lignocellulose biorefining processes. These include utilizing a pretreatment that solubilizes a fraction of the lignin (*e.g.*, alkali or organosolv) that can be recovered and utilized,[130] utilizing a “lignin first” pretreatment that integrates pretreatment with lignin solubilization and depolymerization,[131] and the approach that will be applied in the present work: extracting and recovering lignin from the water-insoluble “lignin cake” that remains after pretreatment and enzymatic hydrolysis using a lignin-solubilizing solvent [132].

Phenol formaldehyde (PF) resins are commonly used as adhesives in engineered wood products including plywood, laminated veneer lumber, and oriented strand board. These resins represent a market of more than \$3.5 billion and 34% of the total adhesive market [96]. Lignins derived from biorefining or chemical pulping processes can serve as a potential bio-based alternative to the petroleum-derived phenol used in the synthesis of these resins. However, common challenges to the utilization of lignins in this application include low reactivity towards formaldehyde, high molecular weight, and high polydispersity [97]. Formaldehyde can react with phenol at sites *para* and *ortho* to the hydroxyl group [133]. Low reactivity of lignin with formaldehyde relative to phenol is mainly due to the lack of reactive sites resulting from the *para* position of aromatic rings being bound in intramolecular linkages, while the *ortho* positions are often occupied by either methoxyl groups or β -5 linkages [64]. Due to the low reactivity of lignin with formaldehyde, resins made with non-modified lignin have historically exhibited much longer gelation times than traditional PF resins and were only able to produce resins with acceptable mechanical properties using partial phenol replacement with <60% of the phenol replaced by lignin [98, 99]. To address these challenges, many studies have functionalized lignin using approaches that include methylation,[100] phenolation,[101] and demethylation[78] to

increase lignin reactivity and to yield mechanical properties comparable to conventional PF resin adhesives. However, these lignin functionalization steps add additional cost to the lignin-formaldehyde resin production process.

Formulation and production of PF resins with phenol fully replaced by plant-derived lignin without the need for further functionalization reactions can result in a product more attractive for industrial use. Recent work demonstrated that a lignin-formaldehyde wood adhesive with mechanical properties similar to commercial PF resins can be formulated with 100% replacement of phenol using a NaOH extracted lignin from dilute acid pretreated corn stover [102, 134]. Grass lignins are known to exhibit high phenolic hydroxyl contents namely in the form of *p*-coumarate (*p*CA) esters [135]. We suggest that the success of this study in fully replacing phenol with lignin in PF resins is due to the use of a grass lignin (corn stover) opposed to hardwood derived lignins used in the previously mentioned studies [98, 99]. It has been hypothesized that high *p*CA content is especially important for successful replacement of phenol with lignin in PF resin, and we have shown that dilute acid pretreatment of corn stover can result in high levels of *p*CA being retained by the biomass while also allowing for high glucose hydrolysis yields [132]. This previous work focused on understanding the relationship between *p*CA, lignin content, and dilute acid pretreatment conditions specifically understanding the susceptibility of the *p*CA ester bond in lignin to hydrolysis during dilute acid pretreatment. We found that *p*CA solubilization and xylan solubilization during pretreatment are directly correlated with each other and with glucose hydrolysis yields following pretreatment. We also demonstrated using a *p*CA-ester model compound that acid catalyzed ester hydrolysis proceeds according to kinetic parameters of the same order of magnitude as xylan hydrolysis [132]. In this

study, we build on these results by using a single corn stover biomass source to (1) understand the relationship between pretreatment severity and lignin extractability, (2) understand the susceptibility of the *p*CA bond to hydrolysis during extraction using two approaches to extract lignin from the lignin cake, (3) determine if *p*CA content is the primary contributor to the “H” lignin signal in ^{31}P NMR, (4) scale-up pretreatment and lignin extraction and demonstrate successful 100% replacement of phenol with corn stover lignin, and (5) characterize resin and adhesive properties and optimize resin formulation for corn stover lignin.

The reactivity of lignin towards formaldehyde and the resulting resin properties are dependent on the feedstock used,[136] the method of biomass pretreatment,[36] and the conditions used for lignin extraction/purification [137]. Relevant to the present study is how *p*CA content varies between different feedstock sources, genotypes, plant tissue, and plant development stage. Prior work in our laboratory has focused on understanding both the distribution and fate of *p*CA during the biorefining of graminaceous feedstocks [107, 138, 139]. Importantly, we’ve identified up to 5-fold differences in the abundance of *p*CA in lignin across diverse bioenergy-relevant grasses and even nearly 3-fold differences within a single plant [140]. If the hypothesis that *p*CA content is the most important contributor to lignin suitability in PF resins is correct, then the implications are that the feedstock genotype, together with growth conditions, harvest time, and lignin processing history are all of importance for lignin suitability as a raw material for these applications. Critically, this understanding presents the opportunity to optimize feedstock selection based on lignin suitability for co-products in addition to properties such as cell wall recalcitrance or agronomic suitability.

Besides the impacts of pretreatment on lignin, any additional purification, extraction, or recovery steps are important parts of a lignin's processing history which can affect the subsequent lignin properties. While not studied as extensively as pretreatment, some studies have been conducted investigating lignin properties after additional purification processes. Previous work demonstrated that when compared to kraft lignin, lignin extracted from sugarcane bagasse using an acetosolv process displayed lower molecular mass, higher thermal stability, higher phenolic and total hydroxyl contents, and more *p*-hydroxyphenyl units [141]. Other work compared lignin extraction by organic acids to extraction via organic alcohols and showed higher lignin recovery yields using mixtures of formic and acetic acid versus acetic acid alone and versus ethanol and methanol. They also find little difference in the molecular weights of lignins extracted using the different solvent systems, and find higher content of *p*-hydroxyphenyl units in lignin extracted with organic acid versus organic alcohol [142]. These results illustrate the importance of feedstock, pretreatment method, and lignin extraction conditions on the suitability of a certain lignin for incorporation into PF resins based on the lignin's properties. Higher *p*-hydroxyphenyl content within lignin allows for more open reactive sites in the *ortho* position of the aromatic ring and thus increased reactivity with formaldehyde allowing for more complete phenol replacement in PF resins [143, 144]. The molecular weight distribution of a specific lignin has also been shown to have an impact on lignin reactivity towards formaldehyde, with lower molecular weight lignins demonstrated to be more reactive towards formaldehyde [145, 146]. One study suggests that this effect of increasing lignin reactivity as molecular weight decreases is due to higher phenolic hydroxyl content resulting from the cleavage of β -O-4 bonds in low molecular weight lignin fractions [147]. Prior work linked lignin thermal stability to

degree of incorporation into PF resins and hypothesized that a high molecular weight lignin contains fewer end chains which will result in lower volume mobility, causing more energy for chain rotation, and thus higher T_g values [148]. It was suggested that lower T_g values for a certain lignin indicate more free volume, opening a higher possibility for cross linking formation in PF resins [148]. Other work suggested that lignins with a higher thermal decomposition temperature can be incorporated into PF resins to heighten their thermal stability allowing for their use in high temperature applications [149].

For the present study, we investigate how dilute acid pretreatment conditions for corn stover impact lignin properties, lignin solubility/extractability, *p*CA content of the lignins through each processing step, and the resulting incorporation of these lignins into the polymer matrix of phenol-formaldehyde resins. Specifically, corn stover is subjected to a range of dilute acid pretreatment conditions (*i.e.*, varying acid loading, temperature, and time) followed by enzymatic hydrolysis and lignin extraction/recovery utilizing either formic acid or NaOH. Based on the results of this screening select conditions are identified to scale up the lignin generation to yield sufficient material for testing in materials applications. These lignins are next used to synthesize PF resins and corresponding wood adhesives with 100% of the phenol replaced with lignin. Finally, the physical and mechanical properties of these resins and adhesives are characterized and linked to differences in the chemical properties of the source lignins.

Materials and Methods

Dilute Acid Pretreatment

A commercial hybrid corn stover was obtained from Idaho National Laboratory after harvest in 2014 in Story County IA. The corn stover was first milled to pass a 2-inch screen

using a Vermeer bale grinder BG-480 followed by milling with a Bliss Hammermill to pass a 1-inch screen, and finally milled to pass a 20-mesh using a Wiley mini-mill (Thomas Scientific). Pretreatments were conducted in a 300-mL stainless steel sample cylinder (Swagelok, 316L-HDF4-300) submerged in fluidized aluminum oxide heated to the desired temperatures (Techne SBS-4, Cole-Parmer). Pretreatments were performed at solid loadings of 20% (mass dry corn stover/liquid volume) with initial values for pH ranging from pH 1-3, times ranging from 1 to 6 hours, and temperatures ranging from 150 to 200 °C corresponding to combined severity factors (CSF) of 1.0 to 4.0 calculated using the equation developed by Chum et al [111]. Heat-up was considered in the pretreatment time, while samples were air-cooled to room temperature following the targeted pretreatment time. The conditions used for pretreatment are presented in

Table 1.

Table 1: Conditions used for dilute acid pretreatment screening at the 100 mL scale.

Time	Initial pH	Temperature	CSF
6.0 h	3.0	150 °C	1.0
1.8 h	2.0	150 °C	1.5
1.0 h	1.25	150 °C	2.0
2.0 h	1.0	200 °C	4.0

Enzymatic Hydrolysis

After pretreatment, corn stover was subjected to enzymatic hydrolysis using CTec2 enzyme cocktail (Novozymes A/S, Bagsvaerd, Denmark). Hydrolysis was conducted at 10% biomass loading (wt/vol) with two different enzyme loadings (15 and 30 mg protein/ g glucan). Samples were hydrolyzed at a constant pH of 5 using a 1 M citrate buffer within 15-mL centrifuge tubes for 72 hours at 50 °C. A tetracycline solution was added to impede bacterial

growth (0.045 mg/mL). Hydrolysis liquor samples were taken at 24, 48, and 72 hours. Glucose yields were quantified using HPLC (Agilent Technologies, 1260 Infinity II) equipped with an Aminex HPX-87H column (Bio-Rad, Hercules, CA) using detection by RID.

Lignin Extraction and Recovery

Lignin was extracted from hydrolysis residues using both NaOH and formic acid extraction methods. NaOH extraction was conducted in 40-mL stainless steel synthesis reactors (Shenzhen Langce Ltd., Huizhou, China) using solid loadings of 20% (wt/vol) hydrolysis residue to 1 M NaOH corresponding to a biomass to NaOH loading of 200 mg NaOH/g biomass. Reactors were submersed in a silicon oil bath and heated on a hot plate to 85 °C for 1 hour. Lignin was precipitated from the resulting alkaline liquor by adding concentrated H₂SO₄ dropwise until a pH <2 was achieved. Lignin was separated from solution via vacuum filtration (Whatman Grade 42 Ashless Filter Papers), washed with DI water until neutral pH was reached, and dried at room temperature. Formic acid extraction was conducted using the same reactor system as the NaOH extraction method. Solid loadings of 10% (wt/vol) hydrolysis residue to 88% (w/w) formic acid was used. Reactors were refluxed at 101 °C for 1 hour. Formic acid lignin solution was collected, and solid residue was washed with an additional 50 mL of formic acid. This formic acid was added to the previously separated formic acid solution and lignin was precipitated by the addition of 300 mL DI water. Lignin was removed from solution via centrifugation and washed with additional DI water.

Composition Analysis

Untreated corn stover, pretreated corn stover, hydrolysis residues, and extracted lignins were analyzed via NREL LAP TP-510-42618 to determine structural polysaccharide and Klason

lignin contents [150]. Sugars were quantified via HPLC with an RID detector using a Bio-Rad Aminex HPX-87H column, a mobile phase of 5 mM H₂SO₄, a flow rate of 0.6 mL/min, and a column temperature of 65 °C. These samples were further analyzed for moisture and ash content, drying at 105 °C for 4 hours and measuring mass loss due to moisture, and subsequently measuring remaining ash content gravimetrically after combusting the biomass at 620 °C for 4 hours according to a method modified from the NREL laboratory analytical procedure LAP TP-510-42622.

p-Coumarate Content

An alkaline saponification was used to determine the amount of *p*-coumarate contained within pretreated biomass and extracted lignin. Samples were placed in stainless steel synthesis reactors (Shenzen Langce Ltd., Huizhou, China) with a solid loading of 2% (wt/vol) biomass to 3M NaOH. Reactors were heated to 180 °C for 2 hours while stirred with a magnetic stir bar at 280 rpm. Following the reaction, the alkaline liquor was adjusted to pH <2 by the dropwise addition of concentrated H₂SO₄. The resulting precipitate was separated via centrifugation and solution was removed for quantification of *p*-coumarate by HPLC using an Agilent PoroShell 120 C18 column and DAD detection at 280 nm. A gradient of two solvents was used: 1% acetic acid in water (solvent A), and 1% acetic acid in 50/50% v/v methanol and water (solvent B). The gradient ran for 32 minutes reducing from 100% solvent A to 30% solvent A and 70% solvent B using 2-minute increments with a column temperature of 40 °C and a flow rate of 0.4 mL/min.

Gel Permeation Chromatography

Gel permeation chromatography (GPC) was used to determine the molecular weight of extracted lignins. Prior to GPC analysis lignin samples were acetylated; 10 mg of each lignin

sample was added to 2.5 mL pyridine and stirred with a magnetic stir bar for 20 minutes at room temperature to fully dissolve the lignin samples. Next, 0.25 mL of acetic anhydride was added and stirred at room temperature for 20 hours. Acetylated lignin was removed from excess solvent by using a rotary evaporator to remove solvent at 40 °C. Remaining solid residue was further dried using a vacuum for 10 minutes at room temperature. Dry and solvent free acetylated lignin was dissolved in 2 mL THF, filtered twice using a 0.2 µm PTFE syringe filter, and prepared for NMR analysis. GPC analysis was conducted using an HPLC equipped with a Waters Styragel HR 4 (7.8 x 300 mm, Milford, MA, USA) column using THF as a mobile phase at a flow rate of 0.5 mL/min and a column temperature of 40 °C. A spectrum was obtained using a DAD detector at 280 nm and molecular weight quantification was achieved using a calibration curve constructed from ReadyCal polystyrene standards (Fluka Analytical) in the 250-70,000 Da range.

Scale-Up of Pretreatment, Hydrolysis, and Lignin Extraction

Pretreatment scale-up was performed at the pretreatment conditions corresponding to a CSF of 2.0 from **Table 1**. Corn stover was hammer-milled to pass a 3-mm screen (Model 158, Pellet Masters, Chippewa Falls, WI). Milled corn stover and dilute sulfuric acid solution were mixed in 16-inch 3 NPT 316 threaded stainless-steel pipes, sealed with pipe fittings and pipe dope, and allowed to equilibrate overnight. Sealed pipes were submerged in a water bath inside a 20 L stainless steel digester (RegMed model AU/E-20, São Paulo, Brazil) and heated to 150 °C and held for 1 hour. After pretreatment, the biomass was washed with water in a home brewing mesh filter bag and dried at 50 °C.

Hydrolysis was performed to yield “lignin cake” or the lignin-rich hydrolysis residue following enzymatic hydrolysis and was conducted in a five-gallon polyethylene bucket using

10% (wt/vol) solid loadings and a 25 mg protein/g glucan enzyme loading comparable to our previous work [151]. The bucket was heated to 50 °C in an incubator for 72 hours, stirring periodically for the first 24 hours, with periodic pH adjustment to pH 5.0. Following hydrolysis, lignin cake was washed 4 times using 1 L DI water in a Beckman Coulter Avanti JXN-26 centrifuge and dried at 50 °C for 48 hours.

Formic acid lignin extraction was conducted using 10% (wt/vol) solids in 88% (w/w) formic acid in a 5-L glass round bottom flask (USALab, RBF5L1N) in a stainless steel oil bath heated by a hot plate with an aluminum foil heating mantle. The round bottom flask was equipped with a condenser and the formic acid solution was allowed to boil at reflux for 1 hour. Remaining solids were washed twice with ~40% w/w formic acid, and lignin was precipitated by the addition of 13 L of DI water. Lignin was separated from solution and washed with additional water via centrifugation (Avanti JXN-26, Beckman Coulter).

NaOH lignin extraction was conducted in a stainless-steel digester (RegMed model AU/E-20, São Paulo, Brazil) equipped with 4 intermediate 1.5 L reactor vessels (Lid T4T-20). Lignin cake was submerged in 1 M NaOH and heated to 85 °C for 1 hour. Black liquor was separated from remaining solids using a home brewing mesh filter bag and a cider press. Liquor was adjusted to pH <2 using H₂SO₄ to precipitate lignin. Lignin was recovered via centrifugation (Avanti JXN-26, Beckman Coulter), and washed with DI water.

Preparation of Resins and Adhesives

Phenol-free lignin-based resins were prepared according to our previous work [102]. Briefly, 25 g of recovered lignin was first dissolved in 85 mL of 1M NaOH and then transferred to a 250 mL three-necked round bottom flask equipped with a condenser. Formaldehyde was

gradually added to the flask until a 1:2 molar ratio of formaldehyde to lignin phenolic hydroxyl was achieved. Using a digital hot plate with constant stirring, the flask was heated from room temperature to 65 °C over 30 minutes followed by incubation at 65 °C for 30 minutes. Next, 40 mL of 1M aqueous NaOH was added. The resulting mixture was gradually increased to 90 °C and the temperature was held constant for 2 more hours. After cooling the resin using an ice bath, some of the resin was frozen to prevent additional polymerization, while the rest was utilized to characterize chemical and physical properties. The developed resins were used to prepare lignin-based adhesives utilizing the same process used for commercial phenol-formaldehyde glue mix formulation as described in our prior work [134]. Adhesives are composed on a weight basis of 66% thawed resin combined with 8% wheat flour, 16% distilled water, 8% plywood extender (Modal), and 2% NaOH pellets. The lignin-based adhesives were stirred for 5 minutes using a high-speed mixer at 800 rpm until homogeneous.

Resin and Adhesive Property Characterization

Properties of developed resins and adhesives were evaluated according to ASTM-recommended test methods. All properties were measured in triplicate except for solid content which was tested in five replicates. For each sample solid content was measured according to ASTM D4426-01. In labeled pans 1 g of each resin was heated to 125 °C for 105 minutes. After heating, samples were cooled in a desiccator, weighed, and percent solid content was calculated. The pH of resins and adhesives was measured using a Mettler Toledo S220 digital pH meter. Rheology of formulated resins was analyzed using a Discovery HR-1 hybrid rheometer (TA Instrument) equipped with a 40-mm parallel plate geometry and a 1000 μm gap at 23 °C. Rheometer tests were performed at constant shear rates of 1000 (s^{-1}) to determine the viscosity of

resins and adhesives. Gelation time was determined following the method reported by Pizzi and Mittal [152]. A glass test tube containing 1 g of each resin sample was submerged in a beaker of boiling water. The resin was then stirred with a glass rod until a string formed around the rod. The gelation time was recorded from the time the test tube was immersed in boiling water to the time that the string was formed. The free formaldehyde level of resins was determined using the hydroxylamine hydrochloride method according to European Standard DIN EN ISO 9397. The alkalinity of resin and adhesive samples was measured according to Lorenz and Christiansen's recommended procedure [153]. Using an estimated amount of NaOH in each sample, different amounts of resin or adhesive (2 to 10 g) were added to 100 mL distilled water. This solution was titrated to pH 3.5 using 0.1 N hydrochloric acid solution. The alkalinity is calculated based on the amount of resin used and the hydrochloric acid volume.

The adhesives were tested for strength of adhesion using a lap shear strength test according to ASTM D5868-01. For each measurement 0.12 g of adhesive was applied to one-fourth ($25.4 \times 25.4 \text{ mm}^2$) of the Douglas-fir veneer samples ($25.4 \text{ mm} \times 102 \text{ mm} \times 5.6 \text{ mm}$). Single-lap joint samples were prepared using a digital hot press (Carver, Inc., Model 3895.4PL1000). The automatic press instrument enables adhesives to cure between veneer samples by adjusting the temperature of the press plates and the required pressure. Utilizing the pressing conditions recommended by industry for commercial adhesives, the veneer samples were pressed for 4 min at 175°C under 1400 kPa. The adhesion strength (tensile strength at maximum load) of produced adhesives was measured using an Instron 5565 Universal testing equipment with a loading rate of 12.7 mm/min.

Results and Discussion

Biomass Pretreatment and Enzymatic Hydrolysis

For the first part of this study, corn stover was subjected to dilute sulfuric acid pretreatments performed over a range of times, temperatures, and initial pH values with the goal of generating lignins with substantial differences in properties. This set of materials was utilized to achieve two goals. First, the diverse lignins were used to develop a better understanding of how these differences in lignin properties impact the extractability/solubility during a lignin recovery process from a hydrolysis residue (“lignin cake”). Second, through the characterization of both lignin recovery yields, and lignin properties, combinations of conditions for pretreatment and lignin extraction can be identified that may yield lignins that are optimal for application as a phenol replacement in phenol-formaldehyde wood adhesives.

The four pretreatment conditions are outlined in **Table 1**. The three moderate conditions at 150 °C chosen in this study span a range of CSFs expected to result in high glucose hydrolysis yields[94, 132] as well as moderate modification of lignin properties and extractability in solvent [154]. A fourth significantly more severe condition at higher temperature (200 °C) and the lowest initial pH (1.0) corresponding to a CSF of 4.0 was chosen to assess the effects of high-severity pretreatments on the subsequent lignin properties as well as enzymatic hydrolysis yields. The pretreated biomass was first tested for its response to enzymatic hydrolysis and xylan solubilization. These results are presented in **Figure 1** and show several key trends. First, it can be observed that increasing time and enzyme loading during enzymatic hydrolysis both increase yields (**Fig. 1A**), as would be expected. However, increasing values of CSF are not necessarily correlated to increasing hydrolysis yields. One reason for this is that the mildest pretreatment in

terms of CSF (*i.e.*, CSF = 1.0) was also the longest pretreatment (6 hr) and gave the strongest response of the three pretreatments performed at 150 °C. Xylan, is distributed on the surface of the cellulose fibers as well as being present in the inter-fibrillar space[155], and has been shown to act as a barrier inhibiting the access of enzymes to cellulose [156]. Xylan removal is one of the key outcomes of dilute acid pretreatment and the correlation between xylan removal and enzymatic hydrolysis yields in pretreated corn stover is well-established [94, 132]. This study reinforces this finding showing a strong correlation between xylan removal and enzymatic hydrolysis yields following dilute acid pretreatment (**Fig. 1B**).

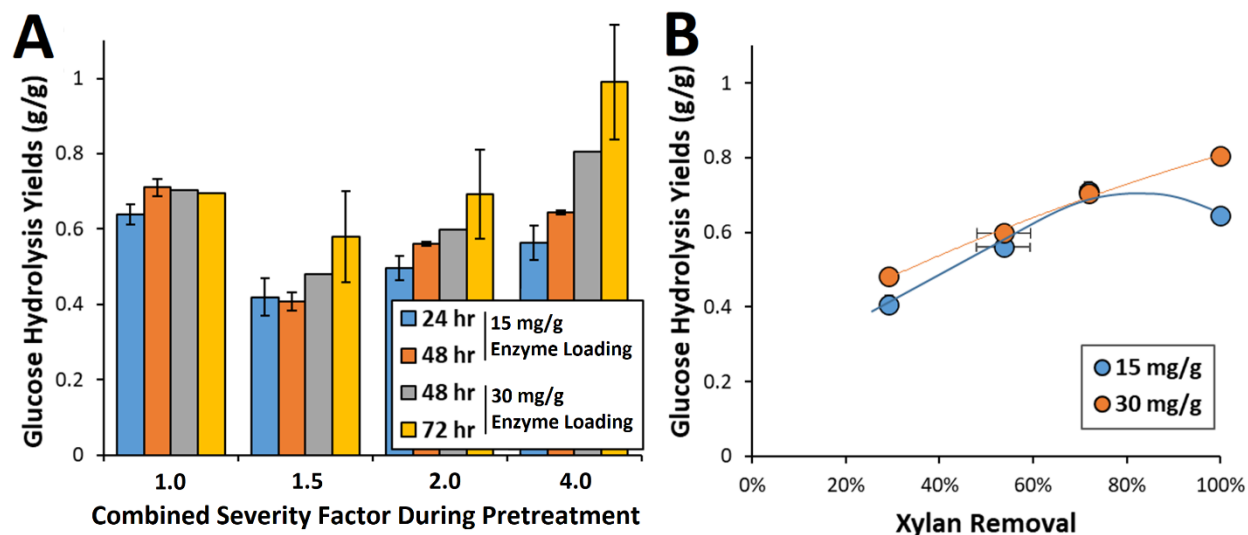


Figure 1: Impact of pretreatment conditions on (A) subsequent enzymatic hydrolysis yields and (B) relationship between hydrolysis yields and xylan removal for 48-hr hydrolysis times at two enzyme loadings.

Lignin Extraction, Recovery, and Characterization

Challenges to utilizing lignin within enzymatic hydrolysis residue (*i.e.*, “lignin cake”) for biobased materials and chemicals include (1) the high level of contamination with other “non-

lignin” components (unhydrolyzed polysaccharides, ash, cellulase protein) that necessitates purification, (2) the relatively poor solubility of the lignins in many solvents that would be necessary for both purification/separation of the lignins from the lignin cake, and for processing in many of the target applications, and (3) the substantial modification of the lignins during pretreatment that may limit its utility in many applications. To address these challenges, two approaches (NaOH and formic acid treatment) were next applied to extract, solubilize, and recover the lignin from the hydrolysis residue. The lignin recovery yields were assessed and select properties of the recovered lignins were characterized (molar mass, abundance of *p*CA and other functional groups, and contamination of the recovered lignins with polysaccharides and ash).

Lignin solubility is key for its utilization in many chemical and materials applications that involve chemical modifications such as cross-linking, functionalization, or depolymerization. Pretreatments of lignocellulose using NaOH are well-established technologies[157] and target lignin (as well as hemicellulose) removal primarily through two mechanisms. First, alkali contributes to the chemical cleavage of β -aryl ethers in lignin at elevated temperature as well as hydrolysis of ester bonds (*e.g.*, ferulate and *p*-coumarate) within and between lignin and xylan in grasses such as corn stover. Second, these lignin and xylan fragments are more soluble in aqueous alkali, which facilitates their removal from the plant cell wall. Consequently, NaOH treatments may be employed after enzymatic hydrolysis for lignin extraction and recovery for lignin utilization in co-products applications [158, 159]. One advantage of utilizing NaOH extraction and solubilization of lignin is that lignin-formaldehyde resins are ultimately formulated under alkaline conditions and potentially this approach could

remove a lignin precipitation/recovery step. As a potential disadvantage of NaOH lignin extraction, previous studies have shown that NaOH treatment of lignin results in reduction of phenolic hydroxyl content especially the reduction of *p*-hydroxyphenyl subunits,[160] presumably due to the alkali-catalyzed hydrolysis esters of *p*CA pendant groups on lignin [132]. Notably, our prior work utilized NaOH extraction to isolate lignin from polysaccharide-rich residues such as lignin cake [102]. A formic acid lignin extraction is also employed as it is hypothesized that this may result in a higher retention of *p*-hydroxyphenyl and *p*CA for incorporation into PF resins. Formic acid lignin purification and fractionation has become the subject of increasing research in recent years [161]. Formic acid extraction processes have been shown be successful in separating lignin from carbohydrates and ash components in multiple types of biomass while retaining high levels of phenolic hydroxyls and *p*CA within the lignin structure [108, 162]. These high-purity lignins with an absence of polysaccharides and inorganics while retaining reactive phenolics such as *p*-hydroxyphenyl and *p*CA exhibit promise for lignins with ideal properties for reaction with formaldehyde and creation of PF resins.

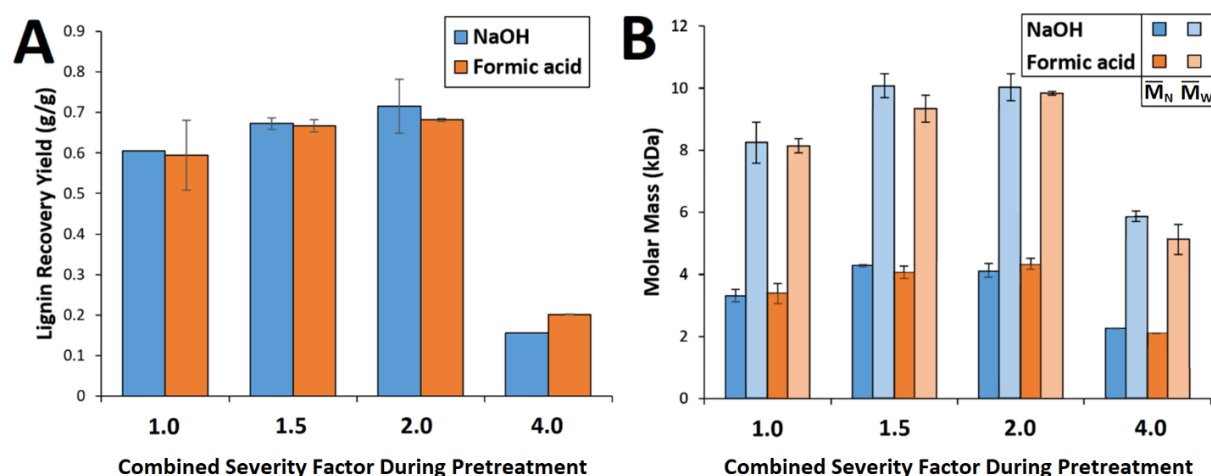


Figure 2: Impact of pretreatment conditions on (A) lignin extraction and recovery yields from the resulting corn stover lignin cake and (B) recovered lignin number average molar mass using either NaOH or formic acid lignin extraction.

The formic acid and NaOH lignin extractions used in this study were both conducted under relatively low temperature (101 °C and 85 °C, respectively) and atmospheric pressure. Extraction and recovery under these conditions resulted in high lignin recovery yields from the lignin cake in the 60-70% range for both formic acid and NaOH extractions under all pretreatment conditions except the most severe condition at CSF 4 (**Fig. 2A**). Importantly, these yields of 70% under relatively mild extraction conditions are significant relative to prior work. As one example, increasing CSF from 0.5 to 1.5 increased lignin recoveries from ~45% to 57% in dilute acid-pretreated and enzymatically hydrolyzed corn cob using ball-milling, additional acid hydrolysis, and extraction with acidic dioxane-water mixtures [154]. As another example, prior work demonstrated that despite significant lignin solubilization, direct alkaline pretreatment of corn stover under relatively comparable conditions to those used in the present work resulted in approximately 35% recovery of the lignin [163]. One possible implication of this is that prior dilute acid pretreatment act synergistically with subsequent alkaline solubilization to improve the

recoverability of the lignin. One notable exception to the high lignin recovery yields is the for the most severe pretreatment (CSF = 4.0) of corn stover, with yields of less than 20% (**Fig. 2A**). It can be hypothesized that under these severe conditions, the lignin becomes more highly condensed and “char-like”, both reducing its solubility in aqueous alkali and formic acid, and reducing its chemical utility. Acid hydrolysis residue following furfural production from corn cob is comparable to these high-severity pretreated materials, and it’s notable that low lignin yields (less than 30%) using alkali extraction have been identified from these materials as well [164]. Overall, these results suggest that some prior acid pretreatment may improve lignin recoverability relative to only alkaline pretreatment, and harsh acid pretreatments render the lignin less extractable. The results for recovered lignin molar mass (**Fig. 2B**) show that both number average ($\bar{M}_N$) and weight average ($\bar{M}_W$) molar masses increase as CSF increases from 1.0 to 2.0 ($p < 0.05$). At the most severe CSF (4.0), the values for recovered lignin molar mass drop by approximately 50% relative to those recovered from other pretreatment conditions. Coupled with the low lignin recovery yields from most severe CSF, it can be hypothesized that the bulk of the lignin becomes highly cross-linked and extraction solvent-insoluble such that only a small low-MW subset of the lignin is recoverable from this sample.

Lignins generated using both extraction methods over the entire pretreatment range were next subjected to characterization with respect to lignin purity/composition, *pCA* content, and phenolic hydroxyl content. This was performed with the goal of identifying coupled pretreatment and lignin recovery conditions that can (1) maximize lignin recovery yields and (2) generate lignins with properties suitable for subsequent use in PF resins. Specifically, the identified conditions will be used for subsequent scale-up for producing lignins of sufficient quantity for

production and property testing of PF resins and the resulting adhesives. The “ideal” lignin properties for this application include high phenolic hydroxyl content and/or *pCA* content, and low content of ash and polysaccharides. Results show that the recovered lignins all exhibited less than 2% by weight ash content with formic acid extracted lignin exhibiting ~10 to 20-fold higher ash than the NaOH lignin (**Fig. 3A**). This discrepancy disagrees with previous literature,[108] and may be due to different washing methods employed after the two lignin extractions. All lignins contained less than 5% polysaccharide content by weight except for the NaOH lignin from the CSF 1.5 condition (**Fig. 3B**). It is unknown why this lignin showed such high sugar content after lignin extraction.

The NaOH and formic acid-extracted and recovered lignins were next subjected to ^{31}P NMR characterization to determine the relative abundance of functional groups in the lignin (**Fig. 3B**) and, importantly, phenolic hydroxyl content, which is critical for lignin incorporation into PF resins. It should be noted that not all lignins generated in this study were analyzed due to poor solubility in the solvents used during derivatization and NMR characterization. In agreement with previous literature lignins extracted with formic acid showed reduced aliphatic hydroxyl content and increased phenolic hydroxyl content when compared to NaOH extracted lignins (**Fig. 3B**) [108, 162]. This supports our previous hypothesis that formic acid lignin will exhibit more favorable properties compared to NaOH lignin for implementation in PF resins. Prior literature on solvent extraction of dilute acid-pretreated corn cob found that increasing CSF from 0.5 to 1.5 increased the total phenolic hydroxyl content from 2.6 to 3.1 mmol/g,[154] which is the same magnitude as our results.

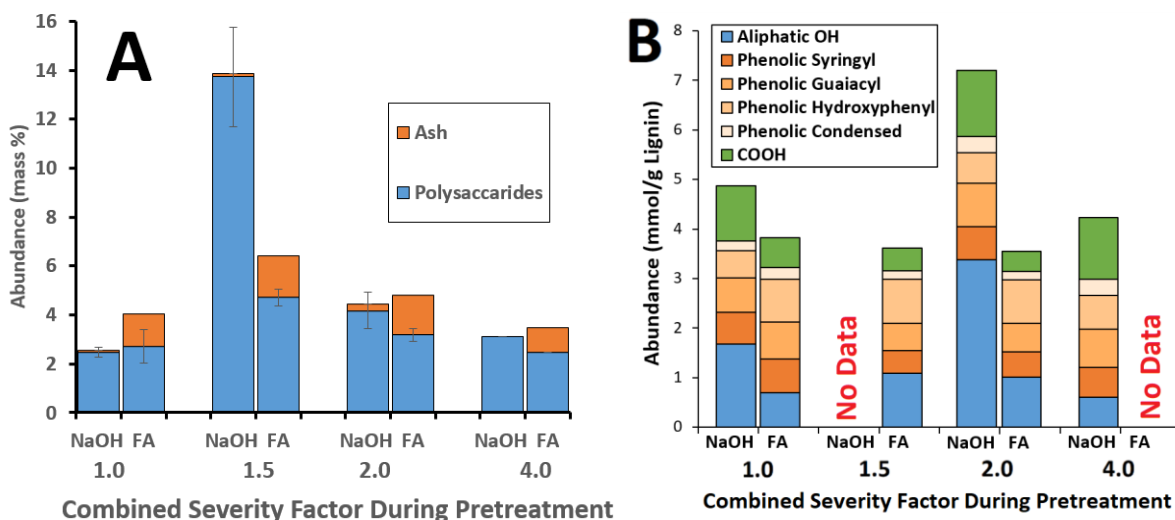


Figure 3: Chemical properties of diverse lignins recovered from corn stover lignin cakes using NaOH or formic acid (FA) extraction.

The presence of *p*CA ester groups on lignins derived from graminaceous feedstocks have been hypothesized to be a key feature of their improved incorporation into PF resins relative to wood lignins derived from other processes (**Fig. 4A**) [132, 134]. However, these labile ester linkages are highly susceptible to alkaline and acid-catalyzed hydrolysis during both pretreatment and lignin recovery stages. In the present work, *p*CA content in the extracted lignins was quantified using alkaline saponification (**Fig. 4B**). The results show several key trends. First, the dilute acid pretreatment can hydrolyze a significant amount of the *p*CA from the lignin (16%-96% removal, **Fig. 4B**). A second key trend is that the formic acid-extracted lignins have a significantly higher *p*CA content than the NaOH-extracted lignin ($p < 0.05$). This indicates that the combination of temperature, time, and NaOH concentration required to solubilize the lignin is also capable of hydrolyzing the majority of the *p*CA esters remaining in the lignin. However, the formic acid extraction can solubilize a significant fraction of the lignin (greater than 60%, **Fig. 2A**) while preserving approximately 50% of the *p*CA esters remaining on the lignins (**Fig.**

4B). A third and final trend is that, as identified in our prior work,[132] *p*CA hydrolysis during dilute acid pretreatment approximates first-order reaction kinetics with respect to *p*CA content and temperature and time rather than pH (or CSF) are strongest predictors of *p*CA hydrolysis (**Fig. 4C**). Fitting trendlines to the 150 °C and the 200 °C data it is possible to estimate rate constants as 0.148 hr⁻¹ and 1.632 hr⁻¹, respectively. However, developing correlations using only two data points for the 200 °C condition is not expected to provide an accurate estimate, but is useful for providing an order of magnitude assessment. Combining these results with prior work on corn stover *p*CA hydrolysis at 180 °C is able to yield order of magnitude estimates for activation energy (E_a) and pre-exponential constant (A) for this reaction system (**Fig. 4C**).

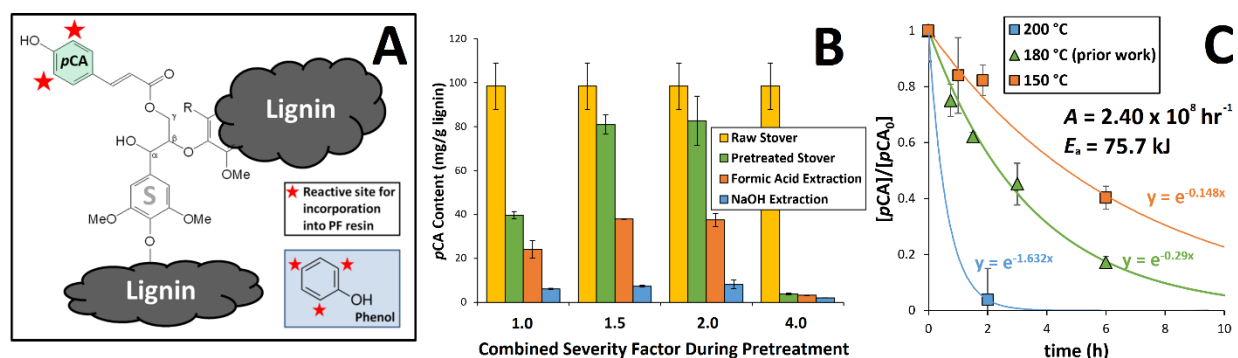


Figure 4: Esterified *p*CA in grass lignins (**A**) and the relationship between (**B**) pretreatment and extraction conditions on *p*CA solubilization and (**C**) pretreatment temperature on *p*CA hydrolysis kinetics.

The *p*-hydroxyphenyl aromatic hydroxyl content of a lignin is a key hypothesized component of the utility of these lignins as a phenol replacement in PF resins. However, from the literature it is not clear how much of this can be attributable to *p*CA esters within the lignin. For the current work, this relationship was explored for the samples for which data are available using *p*CA determined by base-catalyzed hydrolysis followed by HPLC quantification

(saponification), and the *p*-hydroxyphenyl contents determined by ^{31}P NMR. A comparison of these results shows a strong correlation between these two measured quantities (**Fig. 5**).

Specifically, this shows that the *p*CA may contribute to more than 40% of the *p*-hydroxyphenyl content of this lignin for the highest *p*CA-containing samples, while the *p*CA-poor samples still have a significant level (~55 mmol/g lignin) of *p*-hydroxyphenyl functional groups.

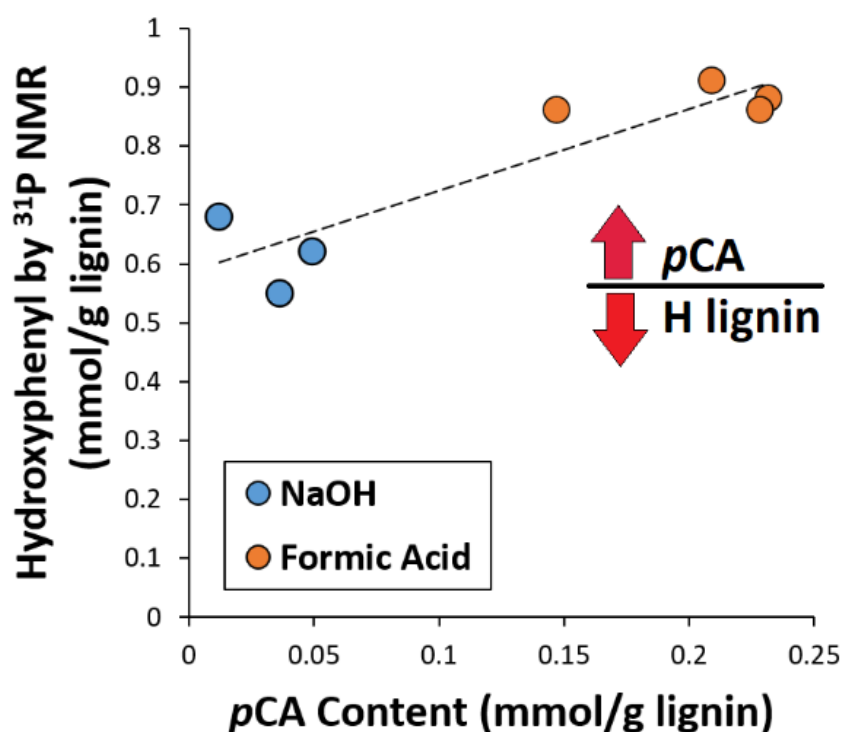


Figure 5: Relationship between *p*CA content and ^{31}P NMR-determined *p*-hydroxyphenyl monomer.

Scale Up of Pretreatment, Hydrolysis, and Lignin Extraction and Recovery

Scale up of lignin production was next performed to generate sufficient quantities of lignin for subsequent synthesis of lignin-based resins and testing of lignin-based adhesives.

Based on the conditions previously screened for biomass pretreatment and lignin

extraction/recovery, an “ideal” set of conditions can be identified to meet economic objectives including high yields for both lignocellulosic sugar-derived biofuels and lignin-based co-products, as well as lignin properties suitable for these co-products. Specifically, these criteria could be envisioned as comprising (1) high sugar hydrolysis yields (**Fig. 1**), (2) high lignin recovery yields (**Fig. 2**), and (3) high *p*-hydroxyphenyl or *p*CA contents in the recovered lignins (**Figs. 3B** and **4A**). For the first criterion (hydrolysis), pretreatments at CSF 1.0, 2.0, and 4.0 were capable of glucose hydrolysis yields greater than 60% (**Fig. 1**). For the second selection criterion (lignin recovery yields), both NaOH and formic acid extraction could give recovery yields greater than 60% at CSF 1.0, 1.5, and 2.0 (**Fig. 2**). Finally, for the third selection criteria (*p*-hydroxyphenyl or *p*CA content), it is clear (**Fig. 4B**) that only the formic acid-extracted lignin from CSF 1.0, 1.5, and 2.0 retain much of the original *p*CA (20-40% of the original) compared to the NaOH-extracted lignin (less than 8%). However, both NaOH- and formic acid-extracted lignins retain a large portion of the phenolic *p*-hydroxyphenyl content (**Fig. 5**) that may be hypothesized to be present in lignin structures other than *p*CA esters. The formic acid-extracted lignins from CSF 1.0, 1.5, and 2.0 all had comparable levels of *p*-hydroxyphenyl contents (0.86-0.88 mmol/g lignin, **Figs. 3B** and **5**). Based on these selection criteria, the CSF 2.0 pretreatment was chosen for scale-up of lignin generation for subsequent PF resin and adhesive synthesis, characterization, and testing. Both formic acid and NaOH extraction methods were used after pretreatment for comparative results. While the formic acid-extracted lignin is expected to represent the “ideal” lignin, the NaOH-extracted lignin was chosen to test the hypothesis that the adhesive mechanical performance will be compromised due to the lower *p*-hydroxyphenyl and *p*CA contents in this lignin relative to the formic acid-extracted lignin.

The scale-up of pretreatment, enzymatic hydrolysis, and extraction was completed and additional characterization was performed to validate that processes performance at large scale was comparable to lab scale, and that lignin properties were comparable. The glucose hydrolysis yields for the scaled-up experiments ($55.4\% \pm 1.5\%$) were within the expected range of the small-scale results (**Fig. 1A**). Additionally, analysis of recovered lignins for functional groups by ^{31}P NMR, molar mass by GPC, xylan content, and alkaline saponification for *p*CA contents all showed close correspondence (generally within 5%) between the large-scale and small-scale lignins (data not shown). One exception was for the ash content (**Supplemental Table S1**), where the formic acid-extracted lignin had a lower ash content at the large scale (0.46% vs. 1.61%) and the NaOH-extracted lignin had a higher ash content at the large scale (0.97% vs. 0.26%). Potential reasons for these discrepancies may be due to slight differences in the lignin washing procedure between scales. One final interesting finding from the lignin characterization results was relatively high N content of the recovered lignins, which were 1.13% for formic acid-extracted lignin and 1.53% for NaOH-extracted lignin (**Supplemental Table S2**). Assuming that the majority of this N is structural protein in the corn stover, this yields a significant amount of protein in each of these lignins: 5.6% protein for formic acid-extracted lignin and 7.6% protein for NaOH-extracted lignin assuming a nitrogen-to-protein conversion factor of 4.95 for corn stover [165]. Many non-lignin compounds (*e.g.*, cutin/suberin, fat/waxes, and protein) can contribute to the Klason lignin measurement[166] and it is likely that a significant fraction of these may propagate through the pretreatment, extraction, and recovery steps to end up enriched in the final recovered “lignin”.

Table 2: Properties of lignin-formaldehyde resins (standard deviations of replicate measurements given in parenthesis).

Lignin Source		pH	Solid Content (%)	Free Formaldehyde Content (%)	Viscosity (mPa·s)	Alkalinity (%)	Gelation Time (min)
Pretreatment	Extraction						
CSF 2.0	Formic Acid	10.7 (0.1)	19.3 (0.1)	0.37 (0.02)	8.3	2.4 (0.1)	18.3 (0.2)
CSF 2.0	NaOH	11.3 (0.1)	21.7 (0.1)	0.56 (0.04)	8.4	2.9 (0.1)	20.4 (0.3)

PF Resin and Adhesive Synthesis, Characterization, and Testing

Lignin-formaldehyde resins were next synthesized using the formic acid-extracted lignin and the NaOH-extracted lignin followed by characterization. These resins exhibited significant differences in many of the characterized properties (**Table 2**) due to differences in lignin chemical composition (including impurities such as ash, xylan, and protein), and structure (molar mass and functional groups). Due to the nature of the lignin extraction process, the resin formulated with the NaOH extracted lignin had a higher pH and alkalinity than the resin formulated with formic acid extracted lignin ($p < 0.05$). Because of its higher phenolic hydroxyl content (**Fig. 3A**) more formaldehyde was used for the formulation of resin made with the formic acid-extracted lignin, which resulted in this resin exhibiting a lower solid content than the resin utilizing the NaOH-extracted lignin (**Table 2**). Resin solid content and viscosity are important properties for the subsequent resin adhesive performance. If adhesives exhibit low viscosity and solid content during the curing stage most of the adhesive will penetrate between layers in the wood during pressing leading to severely impaired adhesive performance [167]. The formic acid-extracted lignin contained more reactive sites for reaction with formaldehyde than NaOH-extracted lignin primarily due to higher *pCA* content (**Fig. 4**). These additional reactive sites

made the formic acid lignin more reactive with formaldehyde leading to a lower free formaldehyde content in the resulting resin (**Table 2**). Free formaldehyde content in PF resin has been shown to be correlated to increased formaldehyde emissions during the manufacture of engineered wood products [168]. This release of formaldehyde both during manufacture and from the finished product can lead to negative health effects in both manufacturing workers and end product users [169]. These results show that feedstock selection and lignin processing history can be used to tailor the reactivity of lignin with formaldehyde in order to create resins with low levels of free formaldehyde. The formic acid-extracted lignin also exhibited a faster gelation time than NaOH-extracted lignin (**Table 2**). This is likely also due to the increased number of available reactive sites in the formic acid-extracted lignin leading to increased crosslinking in the lignin-formaldehyde matrix causing accelerated polymer gelation [170]. Gelation time is an important metric which can influence resin curing parameters such as press time [171]. This relationship between lignin reactivity towards formaldehyde and resin gelation time shows how lignin processing conditions can be used to create lignin-formaldehyde resins with optimized curing parameters.

Table 3: Properties of original and modified lignin-formaldehyde adhesives

Adhesive Formulation	Lignin Source	pH	Solid Content%	Viscosity (mPa·s)
Original Formulation	Formic Acid-Extracted	13.3 (0.1)	27.5 (0.1)	210
Modified Formulation		12.5 (0.1)	29.5 (0.3)	420
	NaOH-Extracted	13.12 (0.2)	30.3 (0.1)	626

After characterizing these original resins, adhesives were made according to both the formulation applied in our previous work[102, 134], and using a modified formulation (**Supplemental Table S2**) in an attempt to modify physical properties of the lignin-formaldehyde resins for improved adhesive performance, namely higher lap shear strengths. This modified formulation resulted in higher viscosity and solid content when compared to the original resins (**Table 3**). Finally, the performance of these adhesives on wood was quantified using a lap shear strength test and was compared with results from previously published lignin-formaldehyde (or a PF control) adhesives from our group (**Fig. 6**). These results include the adhesive performance using the “original formulation” for the resin for an industrial lignin cake derived from dilute acid pretreatment of corn stover using NaOH extraction as well as the conventional phenol-formaldehyde control [134]. Several key results can be observed. Namely, the wood adhesive shear strength from formic acid-extracted lignin made with the conventional adhesive formulation from the present work was statistically indistinguishable from prior work as well as the PF control. Notably, many other lignin sources were unable to achieve comparable performance to the control adhesive [134]. A final observation is that the lignin-formaldehyde resin formulated using the formic acid-extracted lignin and the modified adhesive preparation exhibited a lap shear strength significantly higher (based on a paired t-test at a 95% confidence interval) than the conventional PF resin as well as the NaOH-extracted lignin using the same optimized adhesive formulation (**Fig. 6**). Overall, these are significant in that this validates a strategy for optimizing lignin properties for this target application while simultaneously achieving high sugar yields and high lignin recovery yields.

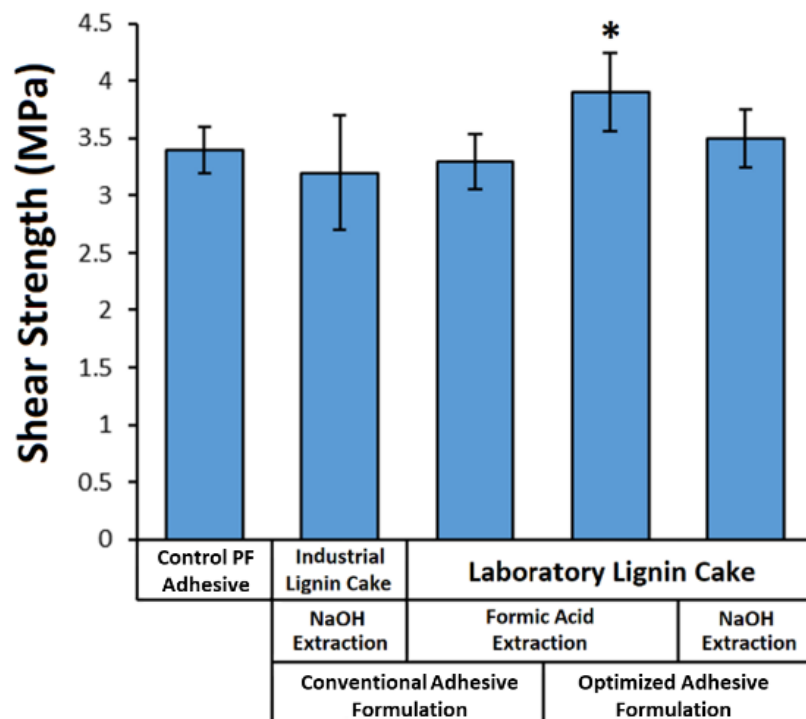


Figure 6: Lap shear strength of adhesives isolated from dilute acid-pretreated corn stover lignin cake with results from our prior work [134]. (Error bars represent standard deviations, * corresponds to a 95% confidence interval)

Conclusions

The type of feedstock used, pretreatment employed, and method of lignin extraction/purification all influence the properties of lignins extracted from a biorefinery process. Structural and chemical properties of a specific lignin such as phenolic hydroxyl content and molecular weight affect the ability of a lignin to be incorporated into PF resins for wood adhesives and the subsequent material properties of the formulated adhesives. Our previous studies have shown that lignin generated from NaOH extraction of dilute acid pretreated corn stover can be used to formulate PF resins with 100% of phenol replaced which exhibit material properties that are comparable to a conventional PF adhesive [134]. We have previously

hypothesized that the improved lignin incorporation in this study is largely due to high amounts of *p*-coumarate (*p*CA) present within grass lignins presenting additional phenolic hydroxyls for reaction with formaldehyde. In a previous study we have identified dilute acid pretreatment conditions that result in high hydrolysis yields while retaining high *p*CA levels within the biomass [132]. In this study, we expand on those finding by using corn stover to investigate a wide range of dilute acid pretreatment conditions and lignin extraction using NaOH and formic acid. A number of key findings can be highlighted. First, it was shown that relatively mild pretreatment conditions could result in lignin extraction yields of 60-70% using both lignin extraction approaches while simultaneously achieving glucose hydrolysis yields >60%. These yields are encouraging relative to prior literature. The harshest pretreatment condition resulted in low lignin extraction and recovery yields (<20%) as a likely consequence of lignin cross-linking during pretreatment. The main impurities in the recovered lignins were xylan (1.5-13%), ash (<2%), and potentially protein (up to 7.6% based on N content). The *p*CA content following pretreatment was demonstrated to be strong function of pretreatment time and first order kinetic parameters for the acid-catalyzed hydrolysis of *p*CA esters during pretreatment were estimated. Formic acid lignin extraction was shown to preserve significantly more *p*CA than the NaOH extraction. The “*p*-hydroxyphenyl” or “H” lignin content was shown to be positively correlated to *p*CA content, demonstrating that *p*CA can comprise a significant fraction of the “H” lignin in biomass. Finally, a combination of pretreatment and lignin extraction were scaled up to generate lignin-formaldehyde resins and wood adhesives and to test physical and material properties. It was shown that both formic acid and NaOH-extracted lignins could yield wood adhesives with dry sheer-strength properties comparable to a conventional industrial PF adhesive formulation

and that an optimized adhesive formulation could yield an adhesive with statistically higher sheer strength than the control. Overall, this work demonstrates that for a target application such as lignin-based wood adhesives, both pretreatment and lignin extraction conditions can be selected to yield lignins with optimized properties.

Acknowledgements

The authors would like to acknowledge Alex Brunat (Montana State University) for providing laboratory assistance for pretreatment and enzymatic hydrolysis experiments. This work was supported by AFRI competitive grant no. 2018-67009-27900/1015055 from the USDA National Institute of Food and Agriculture.

Supplementary Data

Supplemental Table S1: Elemental composition of recovered lignins from scale up.

Lignin	N (%)	S (%)	K (%)	Ca (%)	Na (%)	B (ppm)	Zn (ppm)	Mn (ppm)	Fe (ppm)	Cu (ppm)	Al (ppm)
Formic Acid Extracted	1.13	0.12	0.02	0.01	0.01	1	1	1	29	5	26
NaOH Extracted	1.53	0.37	0.01	0.01	0.38	1	3	1	222	16	49

Supplemental Table S2: Original and modified adhesive formulation components

Component	Amount (wt.%)				
	Formulated Lignin-Based Resin	NaOH	Water	Wheat flour	Alder Bark (Modal)
Original formulation	66	3	18	6.5	6.5
Modified formulation	66	2	16	8	8

References

36. Pang, B., S. Yang, W. Fang, T.-Q. Yuan, D.S. Argyropoulos, and R.-C. Sun, *Structure-property relationships for technical lignins for the production of lignin-phenol-formaldehyde resins*. Industrial crops and products, 2017. **108**: p. 316-326.
64. Laurichesse, S. and L. Avérous, *Chemical modification of lignins: Towards biobased polymers*. Progress in Polymer Science, 2014. **39**(7): p. 1266-1290.
78. Song, Y., Z. Wang, N. Yan, R. Zhang, and J. Li, *Demethylation of Wheat Straw Alkali Lignin for Application in Phenol Formaldehyde Adhesives*. Polymers, 2016. **8**(6): p. 209.
94. Schell, D.J., J. Farmer, M. Newman, and J.D. McMillan, *Dilute-sulfuric acid pretreatment of corn stover in pilot-scale reactor*. Applied Biochemistry and Biotechnology, 2003. **105**(1): p. 69-85.
96. Gomez-Bueso, J. and Haupt, *Wood Composite Adhesives*, in *Phenolic Resins: A Century of Progress*, L. Pilato, Editor. 2010, Springer-Verlag: Berlin.
97. Mansouri, N.-E. and J. Salvadó, *Structural characterization of technical lignins for the production of adhesives: Application to lignosulfonate, kraft, soda-anthraquinone, organosolv and ethanol process lignins*. Industrial Crops and Products, 2006. **24**: p. 8-16.
98. Vázquez, G., G. Antorrena, J. González, and J. Mayor, *Lignin-phenol-formaldehyde adhesives for exterior grade plywoods*. Bioresource Technology, 1995. **51**(2): p. 187-192.
99. Danielson, B. and R. Simonson, *Kraft lignin in phenol formaldehyde resin. Part 1. Partial replacement of phenol by kraft lignin in phenol formaldehyde adhesives for plywood*. Journal of Adhesion Science and Technology, 1998. **12**(9): p. 923-939.
100. Mansouri, N.E.E., A. Pizzi, and J. Salvado, *Lignin-based polycondensation resins for wood adhesives*. Journal of Applied Polymer Science, 2007. **103**(3): p. 1690-1699.

101. Jiang, X., J. Liu, X. Du, Z. Hu, H.-m. Chang, and H. Jameel, *Phenolation to Improve Lignin Reactivity toward Thermosets Application*. ACS sustainable chemistry & engineering., 2018. **6**(4): p. 5504-5512.
102. Kalami, S., M. Arefmanesh, E. Master, and M. Nejad, *Replacing 100% of phenol in phenolic adhesive formulations with lignin*. Journal of Applied Polymer Science, 2017. **134**(30): p. 45124.
107. Li, M., M. Heckwolf, J.D. Crowe, D.L. Williams, T.D. Magee, S.M. Kaeppler, N. de Leon, and D.B. Hodge, *Cell-wall properties contributing to improved deconstruction by alkaline pre-treatment and enzymatic hydrolysis in diverse maize (Zea mays L.) lines*. Journal of experimental botany, 2015. **66**(14): p. 4305-4315.
108. Zhang, Y., Q. Hou, W. Xu, M. Qin, Y. Fu, Z. Wang, S. Willför, and C. Xu, *Revealing the structure of bamboo lignin obtained by formic acid delignification at different pressure levels*. Industrial Crops and Products, 2017. **108**: p. 864-871.
111. Chum, H.L., D.K. Johnson, and S.K. Black, *Organosolv pretreatment for enzymic hydrolysis of poplars. 2. Catalyst effects and the combined severity parameter*. Industrial & engineering chemistry research, 1990. **29**(2): p. 156-162.
129. Ong, R.G., S.P. Chundawat, D.B. Hodge, S. Keskar, and B.E. Dale, *Linking plant biology and pretreatment: understanding the structure and organization of the plant cell wall and interactions with cellulosic biofuel production*, in *Plants and BioEnergy*. 2014, Springer New York. p. 231-253.
130. Yuan, Z., B.D. Bals, E.L. Hegg, and D.B. Hodge, *Technoeconomic evaluation of recent process improvements in production of sugar and high-value lignin co-products via two-stage Cu-catalyzed alkaline-oxidative pretreatment*. Biotechnology for Biofuels and Bioproducts, 2022. **15**(1): p. 45.
131. Phongpreecha, T., K.F. Christy, S.K. Singh, P. Hao, and D.B. Hodge, *Effect of catalyst and reaction conditions on aromatic monomer yields, product distribution, and sugar yields during lignin hydrogenolysis of silver birch wood*. Bioresource Technology, 2020. **316**: p. 123907.
132. Saulnier, B.K., T. Phongpreecha, S.K. Singh, and D.B. Hodge, *Impact of dilute acid pretreatment conditions on p-coumarate removal in diverse maize lines*. Bioresource Technology, 2020. **314**: p. 123750.
133. Siahkamari, M., S. Emmanuel, D.B. Hodge, and M. Nejad, *Lignin-Glyoxal: A Fully Biobased Formaldehyde-Free Wood Adhesive for Interior Engineered Wood Products*. ACS Sustainable Chemistry & Engineering, 2022. **10**(11): p. 3430-3441.

134. Kalami, S., N. Chen, H. Borazjani, and M. Nejad, *Comparative analysis of different lignins as phenol replacement in phenolic adhesive formulations*. Industrial Crops and Products, 2018. **125**: p. 520-528.
135. Hatfield, R.D., D.M. Rancour, and J.M. Marita, *Grass Cell Walls: A Story of Cross-Linking*. Front Plant Sci, 2017. **7**: p. 2056.
136. Ghorbani, M., F. Liebner, H.W. van Herwijnen, L. Pfungen, M. Krahofner, E. Budjav, and J. Konnerth, *Lignin phenol formaldehyde resoles: The impact of lignin type on adhesive properties*. BioResources, 2016. **11**(3): p. 6727-6741.
137. Dababi, I., O. Gimello, E. Elaloui, F. Quignard, and N. Brosse, *Organosolv lignin-based wood adhesive. Influence of the lignin extraction conditions on the adhesive performance*. Polymers, 2016. **8**(9): p. 340.
138. Li, M., D.L. Williams, M. Heckwolf, N. de Leon, S. Kaeppler, R.W. Sykes, and D. Hodge, *Prediction of Cell Wall Properties and Response to Deconstruction Using Alkaline Pretreatment in Diverse Maize Genotypes Using Py-MBMS and NIR*. BioEnergy Research, 2017. **10**(2): p. 329-343.
139. Austin, S., W.S. Kontur, A. Ulbrich, J.Z. Oshlag, W. Zhang, A. Higbee, Y. Zhang, J.J. Coon, D.B. Hodge, and T.J. Donohue, *Metabolism of Multiple Aromatic Compounds in Corn Stover Hydrolysate by Rhodopseudomonas palustris*. Environmental Science & Technology, 2015. **49**(14): p. 8914-8922.
140. Crowe, J.D., N. Feringa, S. Pattathil, B. Merritt, C. Foster, D. Dines, R.G. Ong, and D.B. Hodge, *Identification of developmental stage and anatomical fraction contributions to cell wall recalcitrance in switchgrass*. Biotechnology for Biofuels, 2017. **10**(1): p. 184.
141. Pinheiro, F.G.C., A.K.L. Soares, S.T. Santaella, L.M.A.e. Silva, K.M. Canuto, C.A. Cáceres, M.d.F. Rosa, J.P.d.A. Feitosa, and R.C. Leitão, *Optimization of the acetosolv extraction of lignin from sugarcane bagasse for phenolic resin production*. Industrial Crops and Products, 2017. **96**: p. 80-90.
142. Xu, F., J.-X. Sun, R. Sun, P. Fowler, and M.S. Baird, *Comparative study of organosolv lignins from wheat straw*. Industrial crops and products, 2006. **23**(2): p. 180-193.
143. Ramires, E.C., J.D. Megiatto, Jr., C. Gardrat, A. Castellan, and E. Frollini, *Valorization of an industrial organosolv-sugarcane bagasse lignin: Characterization and use as a matrix in biobased composites reinforced with sisal fibers*. Biotechnol Bioeng, 2010. **107**(4): p. 612-21.
144. Hoareau, W., F.B. Oliveira, S. Grelier, B. Siegmund, E. Frollini, and A. Castellan, *Fiberboards Based on Sugarcane Bagasse Lignin and Fibers*. Macromolecular Materials and Engineering, 2006. **291**(7): p. 829-839.

145. Mansouri, N.-E.E. and J. Salvadó, *Structural characterization of technical lignins for the production of adhesives: Application to lignosulfonate, kraft, soda-anthraquinone, organosolv and ethanol process lignins*. Industrial Crops and Products, 2006. **24**(1): p. 8-16.
146. Moubarik, A., N. Grimi, N. Boussetta, and A. Pizzi, *Isolation and characterization of lignin from Moroccan sugar cane bagasse: Production of lignin-phenol-formaldehyde wood adhesive*. Industrial Crops and Products, 2013. **45**: p. 296-302.
147. Solt, P., *Impact of molecular weight of kraft lignin on adhesive performance of lignin-based phenol-formaldehyde resins*. Forest products journal., 2018. **68**(4): p. 365.
148. Hussin, M.H., A.A. Rahim, M.N. Mohamad Ibrahim, and N. Brosse, *Physicochemical characterization of alkaline and ethanol organosolv lignins from oil palm (*Elaeis guineensis*) fronds as phenol substitutes for green material applications*. Industrial Crops and Products, 2013. **49**: p. 23-32.
149. El Mansouri, N.E., Q. Yuan, and F. Huang, *Characterization of alkaline lignins for use in phenol-formaldehyde and epoxy resins*. BioResources, 2011. **6**(3): p. 2647-2662.
150. Sluiter, A., B. Hames, R. Ruiz, C. Scarlata, J. Sluiter, D. Templeton, and D. Crocker, *Determination of structural carbohydrates and lignin in biomass*. Laboratory analytical procedure, 2008. **1617**(1): p. 1-16.
151. Banerjee, G., S. Car, T. Liu, D.L. Williams, S.L. Meza, J.D. Walton, and D.B. Hodge, *Scale-up and integration of alkaline hydrogen peroxide pretreatment, enzymatic hydrolysis, and ethanolic fermentation*. Biotechnol Bioeng, 2012. **109**(4): p. 922-31.
152. Pizzi, A. and K.L. Mittal, *Wood adhesives*. 2010: VSP.
153. Lorenz, L.F. and A.W. Christiansen, *Interactions of Phenolic Resin Alkalinity, Moisture Content, and Cure Behavior*. Industrial & Engineering Chemistry Research, 1995. **34**(12): p. 4520-4523.
154. Zhang, X., J. Zhu, L. Sun, Q. Yuan, G. Cheng, and D.S. Argyropoulos, *Extraction and characterization of lignin from corncob residue after acid-catalyzed steam explosion pretreatment*. Industrial Crops and Products, 2019. **133**: p. 241-249.
155. Crowe, J.D., P. Hao, S. Pattathil, H. Pan, S.-Y. Ding, D.B. Hodge, and J.K. Jensen, *Xylan Is Critical for Proper Bundling and Alignment of Cellulose Microfibrils in Plant Secondary Cell Walls*. Frontiers in Plant Science, 2021. **12**(2017).
156. Meng, X. and A.J. Ragauskas, *Recent advances in understanding the role of cellulose accessibility in enzymatic hydrolysis of lignocellulosic substrates*. Current Opinion in Biotechnology, 2014. **27**: p. 150-158.

157. Stoklosa, R.J. and D.B. Hodge, *Fractionation and Improved Enzymatic Deconstruction of Hardwoods with Alkaline Delignification*. BioEnergy Research, 2015. **8**(3): p. 1224-1234.
158. Zoia, L., A. Salanti, E.-L. Tolppa, D. Ballabio, and M. Orlandi, *Valorization of Side-Streams from a SSF Biorefinery Plant: Wheat Straw Lignin Purification Study*. 2017, 2017. **12**(1): p. 17.
159. Liu, Z.-H., N. Hao, Y.-Y. Wang, C. Dou, F. Lin, R. Shen, R. Bura, D.B. Hodge, B.E. Dale, A.J. Ragauskas, B. Yang, and J.S. Yuan, *Transforming biorefinery designs with 'Plug-In Processes of Lignin' to enable economic waste valorization*. Nature Communications, 2021. **12**(1): p. 3912.
160. Jung, W., D. Savithri, R. Sharma-Shivappa, and P. Kolar, *Changes in lignin chemistry of switchgrass due to delignification by sodium hydroxide pretreatment*. Energies, 2018. **11**(2): p. 376.
161. Yu, G., B. Li, C. Liu, Y. Zhang, H. Wang, and X. Mu, *Fractionation of the main components of corn stover by formic acid and enzymatic saccharification of solid residue*. Industrial Crops and Products, 2013. **50**: p. 750-757.
162. Villaverde, J.J., J. Li, M. Ek, P. Ligerio, and A. de Vega, *Native Lignin Structure of Miscanthus x giganteus and Its Changes during Acetic and Formic Acid Fractionation*. Journal of Agricultural and Food Chemistry, 2009. **57**(14): p. 6262-6270.
163. Karp, E.M., B.S. Donohoe, M.H. O'Brien, P.N. Ciesielski, A. Mittal, M.J. Bidy, and G.T. Beckham, *Alkaline Pretreatment of Corn Stover: Bench-Scale Fractionation and Stream Characterization*. ACS Sustainable Chemistry & Engineering, 2014. **2**(6): p. 1481-1491.
164. Li, R., X. Wang, Q. Lin, F. Yue, C. Liu, X. Wang, and J. Ren, *Structural Features of Lignin Fractionated From Industrial Furfural Residue Using Alkaline Cooking Technology and Its Antioxidant Performance*. Frontiers in Energy Research, 2020. **8**.
165. Chen, X., G. Zhao, Y. Zhang, L. Han, and W. Xiao, *Nitrogen-to-Protein Conversion Factors for Crop Residues and Animal Manure Common in China*. Journal of Agricultural and Food Chemistry, 2017. **65**(42): p. 9186-9190.
166. Bunzel, M., A. Schüßler, and G.r. Tchetssebu Saha, *Chemical characterization of Klason lignin preparations from plant-based foods*. Journal of agricultural and food chemistry, 2011. **59**(23): p. 12506-12513.
167. Haupt, R.A., *Phenolic resin-wood interaction*. Forest products journal, 1994. **44**(2): p. 69.
168. Carlson, F.E., E.K. Phillips, S.C. Tenhaeff, and W.D. Detlefsen, *A study of formaldehyde and other organic emissions from pressing of laboratory oriented strandboard*. Forest Products Journal, 1995. **45**(3): p. 71.

169. Dally, K.A., L.P. Hanrahan, M.A. Woodbury, and M.S. Kanarek, *Formaldehyde exposure in nonoccupational environments*. Arch Environ Health, 1981. **36**(6): p. 277-84.
170. Younesi-Kordkheili, H., *Ionic liquid modified lignin-phenol-glyoxal resin: a green alternative resin for production of particleboards*. The Journal of Adhesion, 2019. **95**(12): p. 1075-1087.
171. Shimkin, A.A., *Methods for the determination of the gel time of polymer resins and prepregs*. Russian Journal of General Chemistry, 2016. **86**(6): p. 1488-1493.

CHAPTER 4

FRACTIONAL PRECIPITATION OF CORN STOVER FORMIC ACID LIQUORS

Contribution of Authors and Co-Authors

Author: Brian K. Saulnier

Contributions: Conducted formic acid pretreatment of corn stover. Recovered, characterized, and conducted enzymatic hydrolysis on formic acid insoluble cellulose pulp. Employed fractionation process to separate formic acid liquors into four biopolymer fractions. Characterized sugar content, Klason lignin content, ash content, and moisture content of all recovered materials. Employed alkali saponification method to determine *p*CA content of recovered biopolymer fractions. Used GPC method to determine molar mass and polydispersity of recovered biopolymers. Wrote first draft of manuscript.

Co-Author: David B. Hodge

Contributions: Aided in interpretation of data. Provided assistance in the writing, editing, and finalization of subsequent manuscript drafts.

Manuscript Information

Brian Saulnier, David B. Hodge

Biomass Conversion and Biorefinery

Status of Manuscript: [Put an x in one of the options below, delete this]

☒ Prepared for submission to a peer-reviewed journal

☐ Officially submitted to a peer-reviewed journal

☐ Accepted by a peer-reviewed journal

☐ Published in a peer-reviewed journal

To be submitted to Biomass Conversion and Biorefinery

FRACTIONAL PRECIPITATION OF CORN STOVER FORMIC ACID LIQUORS

Abstract

To obtain maximum value from a biorefinery the process must obtain high cell wall polysaccharide hydrolysis yields while simultaneously implementing full utilization of the biomass lignin component. Primary barriers to lignin incorporation in value-added products include high polydispersity and heterogeneity of the lignin chemical structure. In this study we address these issues using the fractionation of lignin containing liquor following treatment of corn stover with 88% formic acid via step-wise dilution with water. This approach resulted in glucan and lignin losses <10%, achieved glucose hydrolysis yields of 39%, and recovered 3 lignin-rich fractions of differing molecular weights with high *p*-coumarate (*p*CA) retention. Future processes could be improved by the optimization of process conditions for reduced xylan degradation and improved biomass delignification allowing for increased glucose hydrolysis yields.

Introduction

Full utilization of lignocellulosic biomass, one of the most abundant natural resources, is necessary for the development of a bio-based and renewable economy. One use for lignocellulosic biomass is within a biorefinery for bioethanol production. A biorefinery process is composed of three steps. The first being a pretreatment of the biomass with the goal of improving access to the cell wall polysaccharides, namely cellulose and hemicellulose. Improved cell wall access is generally accomplished through the disruption of the higher order cell wall structure, hemicellulose removal, or lignin removal. Following pretreatment an enzymatic hydrolysis process is employed to convert cell wall polysaccharides into monomeric sugars. These sugars then undergo biological fermentation, and finally bioethanol is recovered.

The efficacy of the overall biorefinery process is dependent on the ability of the pretreatment to overcome the recalcitrance of the lignocellulosic cell wall, recover the majority of the cellulose contained within the biomass, and achieve high glucose hydrolysis yields. Many

pretreatment methods have been studied attempting to improve sugar yields from biomass including mechanical, chemical, and biological pretreatments. These different pretreatments all employ a unique strategy targeting different portions of the cell wall to improve enzymatic access. One of the most well studied pretreatment methods is dilute acid pretreatment. This pretreatment primarily targets hemicellulose removal while simultaneously chemically modifying cellulose, lignin, and redistributing lignin throughout the cell wall[172]. Another commonly used pretreatment, alkali pretreatment primarily targets the removal of lignin from the cell wall while also removing a portion of the hemicellulose and improving enzymatic access by changing the porosity, surface area, and crystallinity of the cellulose fraction[173]. In contrast to these two pretreatment methods formic acid pretreatment simultaneously targets the removal of lignin and hemicellulose from the cell wall while recovering the majority of the cellulose fraction[161]. These favorable characteristics have led to an increasing interest in formic acid processes in recent years. Formic acid pretreatment has been applied to a wide variety of feedstocks[174-177], with some studies reporting glucan recovery greater than 90%[178], lignin recovery greater than 70%[179], glucose hydrolysis yields exceeding 60%[161, 180], and ethanol production yields approaching 80% of theoretical[178].

The aforementioned studies focus mainly on the polysaccharide fractions of biomass while little attention is given to the recovery and utilization of the lignin fraction. Historically in biorefining and pulp and paper the lignin fraction recovered after pretreatment or a pulping process is burned as a fuel source[4]. To obtain maximum value from the biorefinery process the lignin fraction must be recovered and incorporated into value-added products. Recent work in the field has shifted from developing pretreatments for solely high sugar yields to additionally

prioritizing full utilization of the lignin fraction[18]. Many strategies for tailoring lignin properties for target applications have been developed including feedstock selection[8, 12, 132], selection of pretreatment method and process conditions[19, 22, 29, 132], lignin chemical modification[64, 73, 77, 79], and lignin fractionation.

Lignin fractionation processes separates lignin into fractions of differing molecular weights with different chemical structures and increased homogeneity compared to the unfractionated bulk lignin. The separation of these lignin fractions is achieved through the fractional pH adjustment of lignin containing alkaline liquors[43, 47, 50], successive ultrafiltration using membranes of different sizes[181, 182], consecutive lignin extraction in a multiple solvent system using solvents of increasing dissolving power[59, 60, 62], and fractionation by the sequential dilution of a single solvent system[51, 52, 57]. These chemical fractionation processes separate lignin into different fractions by exploiting their varied solubility in different solvents which can be described by their Hansen solubility parameter. The Hansen solubility parameter presents polymer solubility as a function of dispersion forces, polarity, and hydrogen bonding between the polymer and solvent. The selection of different organic solvents or their dilution with water results in changed solvent polarity and capacity for hydrogen bonding allowing for the selective dissolution of lignins with varied polarity and ability for hydrogen bonding based on their molecular weight and functional groups such as phenolic hydroxyls[59].

In this study we use a single step pretreatment and lignin extraction process using formic acid followed by the fractionation of the lignin and hemicellulose containing liquor using sequential dilution to obtain five different fractions. The recovered fractions were characterized and found to contain one cellulose heavy fraction, one hemicellulose heavy fraction, and three

primarily lignin fractions. Enzymatic hydrolysis yields of the cellulose fraction and overall process mass yields were evaluated. Lignin and hemicellulose heavy fractions were evaluated for molecular weight and chemical composition.

Materials and Methods

Formic Acid Pretreatment and Lignin Extraction

Formic acid lignin extraction was conducted using 10% (wt/vol) non dried corn stover in 88% (wt/wt) formic acid-water placed in a 500 mL glass round bottom flask submerged in an oil bath heated by a hot plate. The round bottom flask was equipped with a condenser and the formic acid solution was allowed to boil at reflux for 1 hour.

Lignin Fractional Precipitation

Insoluble material after formic acid treatment was separated from the bulk liquor by centrifugation (15,970 x g, 30 min, Avanti JXN-26, Beckman Coulter), washed with DI water three times, dried, and labeled FAF1. Reaction liquors containing 88% formic acid was diluted 3 times to 75%, 60%, and 50% formic acid. After each dilution insoluble material was separated by centrifugation, washed with DI water and labeled FAF2, FAF3, and FAF4, respectively. The remaining 50% formic acid liquor was separated from the remaining solids and recovered using a rotary evaporator. Recovered solids after evaporation were washed with DI water, separated by centrifugation, dried, and labeled FAF5. An overview of this process is given in a visual format in **Figure 1**.

Gel Permeation Chromatography

Gel permeation chromatography (GPC) was used to determine the molecular weight of recovered biopolymer fractions FAF2-FAF5. Prior to GPC analysis samples were acetylated; 10 mg of each sample was added to 2.5 mL pyridine and stirred with a magnetic stir bar for 20 minutes at room temperature until full dissolution. Next, 0.25 mL of acetic anhydride was added and stirred at room temperature for 20 hours. Acetylated samples were removed from excess solvent using a rotary evaporator at 40 °C. Remaining solid residue was further dried using a vacuum for 10 minutes at room temperature. Dry and solvent free acetylated samples were dissolved in 2 mL tetrahydrofuran (THF), filtered twice using a 0.2 µm PTFE syringe filter, and prepared for NMR analysis. GPC analysis was conducted using an HPLC equipped with a Waters Styragel HR 4 (7.8 x 300 mm, Milford, MA, USA) column using THF as a mobile phase at a flow rate of 0.5 mL/min and a column temperature of 40 °C. A spectra was obtained using a DAD detector at 280 nm and molecular weight quantification was achieved using a calibration curve constructed from Readycal polystyrene standards (Fluka Analytical) in the 250-70,000 Da range.

Composition Analysis

Raw corn stover and recovered fractions FAF1-FAF5 were analyzed via NREL LAP TP-510-42618 to determine structural polysaccharide and Klason lignin contents [150]. Sugars were quantified via HPLC with an RID detector using a Bio-Rad Aminex HPX-87H column, a mobile phase of 5 mM H₂SO₄, a flow rate of 0.6 mL/min, and a column temperature of 65 °C. Sample moisture content was determined using a moisture analyzer (OHAUS, MB 90).

Enzymatic Hydrolysis

To assess potential for sugar recovery from this process the cellulose heavy insoluble fraction after formic acid treatment (FAF1) was subjected to enzymatic hydrolysis using CTec2 enzyme cocktail (Novozymes A/S, Bagsvaerd, Denmark). Hydrolysis was conducted at 10% biomass loading (wt/vol) with an enzyme loading of 30 mg protein/ g glucan. Samples were hydrolyzed at a constant pH of 5 using a 1 M citrate buffer within 15-mL centrifuge tubes for 72 hours at 50 °C. A tetracycline solution was added to impede bacterial growth (0.045 mg/mL). Hydrolysis liquor samples were taken at the conclusion of the hydrolysis and glucose yields were quantified using HPLC (Agilent Technologies, 1260 Infinity II) equipped with an Aminex HPX-87H column (Bio-Rad, Hercules, CA) using detection by RID.

p-Coumarate Content

An alkaline saponification was used to determine the amount of *p*-coumarate contained within fractions FAF2-FAF5. Samples were placed in stainless steel synthesis reactors (Shenzen Langce Ltd., Huizhou, China) with a solid loading of 2% (wt/vol) biomass to 3M NaOH. Reactors were heated to 180 °C for 2 hours while stirred with a magnetic stir bar at 280 rpm. Following the reaction, the alkaline liquor was adjusted to pH <2 by the dropwise addition of concentrated H₂SO₄. The resulting precipitate was separated via centrifugation and solution was removed for quantification of *p*-coumarate by HPLC using an Agilent PoroShell 120 C18 column and DAD detection at 280 nm. A gradient of two solvents was used: 1% acetic acid in water (solvent A), and 1% acetic acid in 50/50% v/v methanol and water (solvent B). The gradient ran for 32 minutes reducing from 100% solvent A to 30% solvent A and 70% solvent B using 2-minute increments with a column temperature of 40 °C and a flow rate of 0.4 mL/min.

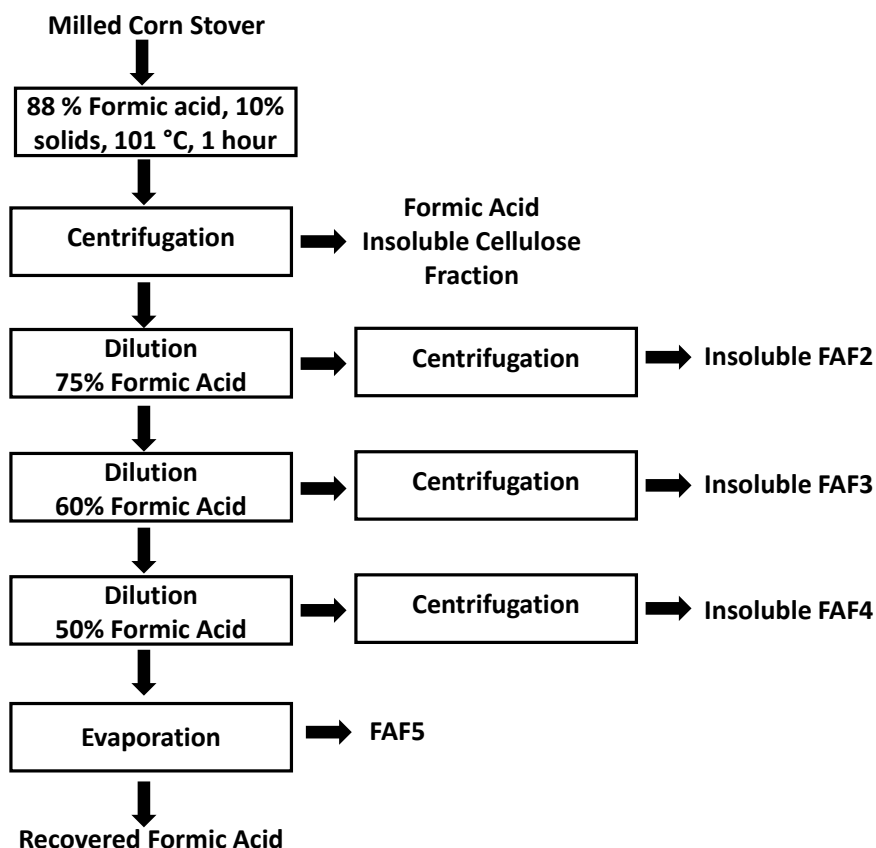


Figure 1: Process Diagram for Formic Acid Treatment of Corn Stover and the Recovery of Five Biopolymer Fractions

Results and Discussion

Composition and Mass Yields

Corn stover was subjected to formic acid pretreatment. Insoluble solids were labeled FAF1, the recovered liquor underwent stepwise dilution with DI water with insoluble precipitate at each step being recovered and labeled FAF2-FAF4, remaining liquor was evaporated and solids after evaporation were recovered and labeled FAF5 (**Fig. 1**). This fractional separation of formic acid liquor resulted in one formic acid insoluble cellulose heavy fraction (FAF1), three precipitated lignin heavy fractions (FAF2-FAF4), and one xylan/extractive heavy fraction

remaining after liquor evaporation (FAF5). The composition of each of these fractions can be seen in **Figure 2A**. All recovered lignin fractions were of high purity with >83% of their mass being attributed to Klason lignin. The formic acid insoluble fraction (FAF1) which was recovered for subsequent enzymatic hydrolysis contained 80% polysaccharides.

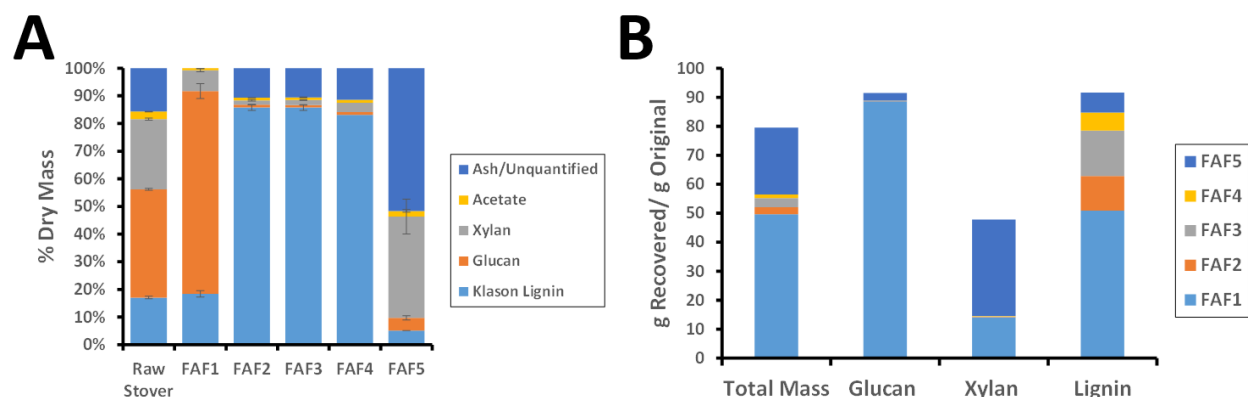


Figure 2: Formic Acid Fraction Composition (A) and Recovered Mass Yields (B)

Total recovery mass yields of each biopolymer component are given in **Figure 2B**. This approach recovered 80% of the total mass of the inflowing corn stover. Greater than 90% of the original glucan contained in the corn stover was recovered with the majority being present in the formic acid insoluble fraction in the form of cellulose (FAF1). High glucan recovery is crucial for achieving sugar yields necessary for the production of bioethanol. The high cellulose recovery yields in this study are comparable with those achieved by other pretreatment methods including dilute acid[183] and alkaline[163] pretreatments. Also important for the biorefinery process is the full recovery of the lignin fraction for lignin valorization. Formic acid treatment conditions used in this study were selected from our previous work which achieved high levels of delignification of lignin cake after the dilute acid pretreatment and enzymatic hydrolysis of

corn stover (Chapter 3). This study achieved high lignin recovery (>90%), however over half of this lignin remained in the formic acid insoluble (FAF1) fraction with an overall lignin solubilization of 50%. This is below the delignification yields of other studies using formic acid processes which used higher temperatures, higher formic acid concentrations, or longer reaction times[161, 184, 185]. The low lignin solubilization in this study led to inhibited hydrolysis yields (**Fig. 4**). Total xylan recovery yields were less than expected recovering 48% of the original xylan in the raw corn stover (**Fig. 2B**). The majority of this missing xylan is likely present in the unquantified portion of FAF5 in the form of degradation products and monosaccharides (**Fig. 2A**). The acid hydrolysis of biomass can lead to the depolymerization of polysaccharides to monosaccharides and further degradation to products such as furans and furfural [186, 187]. The hydrolysis of xylan into monomeric xylose followed by dehydration into furans and further degradation to furfural and other products occurs at a higher rate than the depolymerization of glucan and degradation of glucose under similar acidic conditions[188]. Xylan hydrolysis during formic acid treatment of biomass can be extensive with losses in xylan yields around 30% being reported in corn stover[189] and hardwood[190] dependent on processing conditions with more extensive hydrolysis occurring at higher reaction temperatures and times[191, 192]. It is reasonable to assume that xylan underwent significant depolymerization during our process and a large portion of the unquantified portion of FAF5 is composed of furfural and furans which remained soluble in the diluted formic acid liquor solution. The remaining portion of this unquantified fraction can be attributed to acid soluble lignin and extractives such as proteins, non-structural sugars, waxes, and inorganics.

Lignin Properties

Lignin and xylan heavy fractions FAF2-FAF5 were further analyzed for molecular weight, polydispersity index (PDI), and *p*-coumarate (*p*CA) content for evaluation of their suitability for incorporation into value-added products such as PF resins. The results of these analyses are summarized in **Figure 3**. Fraction FAF2-FAF5 *p*CA content is displayed in **Figure 3A** as mg *p*CA per g lignin in the biopolymer fraction. Our previous work has extensively examined the fate of *p*CA during pretreatment[132], and the effect of lignin *p*CA content on the properties of lignin-formaldehyde resins and wood adhesives (Chapter 3). We demonstrate that increasing *p*CA and H type lignin subunits lead to more favorable adhesive properties with adhesives formulated with lignin from the formic acid extraction of lignin cake from dilute acid pretreated corn stover exhibiting lap shear strength values significantly higher than commercial PF resin adhesives (Chapter 3). The lignin from this previous study contained 37.5 mg *p*CA per g lignin. All three lignin heavy fractions in this study (FAF2-FAF4) possessed lignin *p*CA content in the same range as this previous study (**Figure 3A**). This high *p*CA retention along with the high purity of these 3 fractions (**Figure 2A**) suggests suitability for use in lignin-formaldehyde resin adhesive applications. FAF5 contains a disproportionately high content of *p*CA with 123.2 mg/g lignin, higher even than raw corn stover. Our previous work has shown >80% *p*CA solubilization during pretreatment[132], and a 50% reduction during formic acid lignin extraction (Chapter 3). It is intuitive that the formic acid process used in this study will also solubilize a significant portion of *p*CA within the biomass. This solubilized *p*CA will remain in the reaction liquor after dilution being recovered in FAF5 after liquor evaporation.

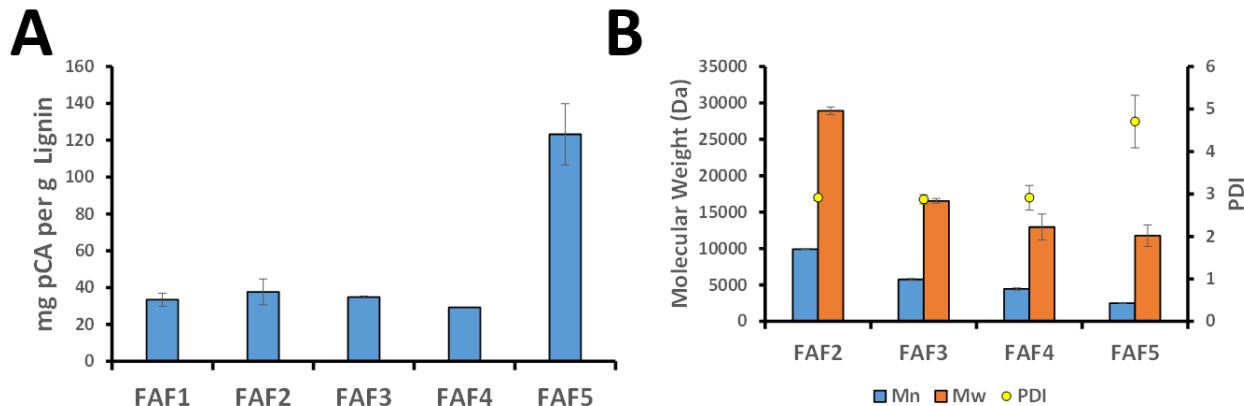


Figure 3: *p*CA Content of Formic Acid Fractions (**A**) and Formic Acid Fraction Molecular Weight (**B**)

Molecular weight values of FAF2-FAF5 quantified by GPC are seen in **Figure 3B**.

Lignin heavy fractions FAF2-FAF4 exhibited lower molecular weight with increasing dilution of the formic acid solution ($p < 0.05$) with unchanged PDI. FAF5 is of the lowest molecular weight with a significantly greater PDI. This reinforces that this fraction is composed of a wide range of materials including xylan, xylose, degradation products, acid insoluble lignin, and other extractives. Molecular weight polydispersity and the heterogeneity of the lignin chemical structure are two substantial barriers to the widespread utilization of lignin in value-added products[193]. Lignin fractionation strategies such as the one used in this study can be used to separate the bulk lignin into high and low molecular weight fractions with lower polydispersity and more uniform functional group distribution. High and low molecular weight lignin display different chemical properties and proportions of functional groups with low molecular lignin containing less aliphatic hydroxyl groups and more phenolic hydroxyls[194]. Many studies have shown molecular weight influence on the performance of lignin-based products[194, 195]. Due to its high reactivity and abundant phenolic hydroxyl groups low molecular weight lignin excels

in applications such as epoxy resins[196], PF resins (Chapter 3), polyethylene composites[197], and exhibit high performance as antioxidants[63, 198]. High molecular weight lignins have ideal properties for lignin based carbon fibers[195, 199] and polyurethanes[200]. The lignin heavy fractions obtained in this study (FAF2-FAF4) all well suited for a variety of value-added applications based on their *p*CA contents and varying molecular weights.

Glucose Hydrolysis Yields

Figure 4 shows glucose hydrolysis yields after 72 hours using formic acid insoluble cellulose fraction FAF1 from this study, average hydrolysis yields of solid residue from formic acid pretreatment in the literature, and hydrolysis yields of dilute acid pretreated corn stover from our previous work in Chapter 3 of this thesis. Hydrolyses of FAF1 obtained average glucose yields of 38.7%. The first study from the literature in **Figure 4** treats corn stover with the same concentration of formic acid used in this study (88%), however uses a lower temperature and longer pretreatment time. Hydrolysis of the insoluble fraction after this treatment resulted in yields between 32% and 63% based on enzyme loading[161]. The second study pretreats sugarcane bagasse under a variety of severities, varying reaction time, temperature, and formic acid concentration. Hydrolysis yields of the solid residue obtained after pretreatment with similar formic acid concentrations to those used in this study (80%-90%) obtained glucose yields from 30-54% after 72 hours dependent on pretreatment severity[185]. The final formic acid pretreatment study included in **Figure 4** used a flow through formic acid pretreatment of wheat straw with 72-90% formic acid at 120-130 °C. The resulting glucose yields from the hydrolysis of the remaining insoluble residue ranging from 15-73% based on enzyme loading, pretreatment temperature, and formic acid concentration[184].

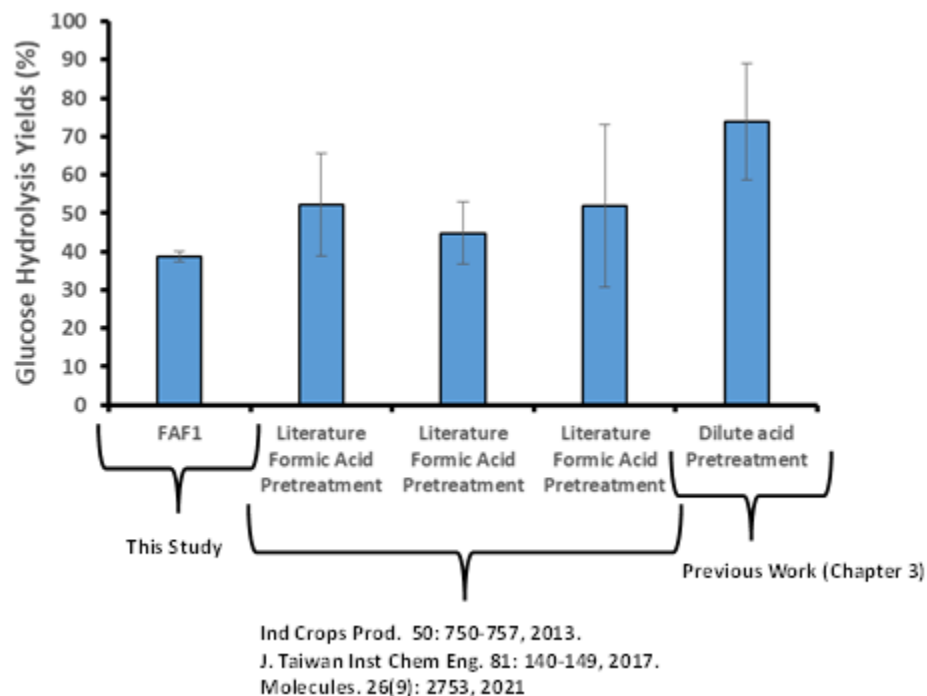


Figure 4: Glucose Hydrolysis Yields after 72 Hours of FAF1, Solid Residue after Formic Acid Pretreatment (Previous Studies), and Dilute Acid Pretreated Corn Stover (Chapter 3)

While the enzymatic hydrolysis yields obtained in this study are within the range of these other studies ($p > 0.05$), they are significantly lower than the best yields achieved ($p < 0.05$). The reasons for the lower yields in this study are likely due to multiple reasons. Our formic acid treatment only achieved delignification of the formic acid insoluble fraction (FAF1) of 49.1%. Using the same process conditions on lignin cake from corn stover after dilute acid pretreatment and hydrolysis we have previously obtained delignification $>70\%$ (Chapter 3). The aforementioned studies on formic acid pretreatment obtain delignification yields after formic acid treatment of 65.6%[161], 85%[185], and 72.4%[184] at the pretreatment conditions corresponding to the highest hydrolysis yields (**Fig. 4**), these delignification yields are significantly greater than those achieved in this study ($p < 0.05$). The presence of lignin in

biomass prior to hydrolysis has been shown previously to retard hydrolysis yields with greater lignin removal being correlated to higher glucose yields for a wide variety of pretreatments[22]. This phenomenon is a result of reduced steric hindrance and increased porosity of the cell wall following lignin removal[157, 201], nonspecific binding of cellulases to lignin[202], and the deposition of lignin droplets on the surface of biomass following pretreatment under acidic conditions[113]. The high amount of lignin retained in FAF1 is likely the leading cause of the lower hydrolysis yields achieved in this study compared to previous work. More work is required to optimize formic acid treatment conditions for more complete removal of lignin from raw biomass.

Another probable factor impeding hydrolysis yields in this study is the formylation of cellulose during pretreatment. Formylation is a reaction that occurs during formic acid pretreatment which replaces some of the cellulose hydroxyl groups with substituted formyl groups. The presence of these formyl groups has been shown to hinder the rate and extent of hydrolysis due to reduced ability of cellulases to recognize cellulose and the inhibition of cellulose-cellulase bonds[203]. The result of this inhibition is significant with one study showing an increase in formylation with increasing formic acid concentration and reaction severity, and demonstrated a 40% increase in hydrolysis yields at 72 hours after the implementation of an alkaline de-formylation process[185]. This stresses the importance of balancing the selection of formic acid treatment conditions for widespread solubilization of lignin while also limiting the degree of formylation.

Suggestions for Future Work

This study presents one scenario for the formic acid pretreatment of corn stover, hydrolysis of its cellulose component, and the fractionation of lignin containing formic acid liquors leading to the recovery of lignins with varied properties. A detailed mass balance on cell wall polymer recovery following pretreatment and fractionation was conducted, and lignin molecular weight and *pCA* content was characterized. Future work can build upon this process improving glucose hydrolysis yields, xylan recovery yields, and yields of the lignin rich fractions. Initial formic acid treatment conditions should be optimized to maximize lignin solubilization while minimizing the formylation of cellulose, degradation of xylose, and the solubilization of *pCA*. Due to the similar *pCA* content of the three lignin heavy fraction FAF2-FAF4 we suggest a two-step lignin precipitation/dilution, diluting formic acid to above its azeotrope concentration (77.5%) for recovery by simple distillation or to a lower percentage with the intention of reusing the dilute formic acid for further industrial processes. A xylan heavy fraction (FAF5) can be recovered as in this study after evaporation. In an ideal process the formic acid insoluble cellulose fraction will retain little lignin and remain primarily unmodified allowing for high hydrolysis yields for production of bioethanol. A high molecular weight and low molecular weight lignin fraction with high *pCA* content can be recovered and employed in PF resin and other value-added applications. If xylose degradation is successfully limited the fraction recovered after evaporation may be used for subsequent hydrolysis and fermentation. If extensive xylose degradation is still present this material may show utility for modification by propylene carbonate and inclusion in PLA composites (Chapter 5).

Conclusions

Fractionation of lignin containing liquor by step-wise dilution after formic acid treatment of corn stover was employed. Biomass was separated into five fractions: one formic acid insoluble cellulose heavy fraction (i.e., cellulose pulp), three lignin heavy fractions of different molecular weights, and one fraction composed primarily of xylan and extractives. This approach recovered 80% of the total incoming mass, 90% of the original glucan and lignin, and achieved hydrolysis yields of the cellulose heavy fraction of 39%. Lignin heavy fractions are well suited for PF resin applications due to their high retention of *p*CA. More work is needed on the optimization of process conditions to increase lignin solubilization while decreasing the formylation of cellulose, minimizing xylose degradation, and maintaining high *p*CA retention.

References

4. Ragauskas, A.J., G.T. Beckham, M.J. Bidy, R. Chandra, F. Chen, M.F. Davis, B.H. Davison, R.A. Dixon, P. Gilna, M. Keller, P. Langan, A.K. Naskar, J.N. Saddler, T.J. Tschaplinski, G.A. Tuskan, and C.E. Wyman, *Lignin valorization: improving lignin processing in the biorefinery*. Science, 2014. **344**(6185): p. 1246843.
8. Li, J., G. Gellerstedt, and K. Toven, *Steam explosion lignins; their extraction, structure and potential as feedstock for biodiesel and chemicals*. Bioresource Technology, 2009. **100**(9): p. 2556-2561.
12. Watkins, D., M. Nuruddin, M. Hosur, A. Tcherbi-Narteh, and S. Jeelani, *Extraction and characterization of lignin from different biomass resources*. Journal of Materials Research and Technology, 2015. **4**(1): p. 26-32.
18. Yoo, C.G., X. Meng, Y. Pu, and A.J. Ragauskas, *The critical role of lignin in lignocellulosic biomass conversion and recent pretreatment strategies: A comprehensive review*. Bioresource Technology, 2020. **301**: p. 122784.
19. Glasser, W.G., C.A. Barnett, P.C. Muller, and K.V. Sarkanen, *The chemistry of several novel bioconversion lignins*. Journal of Agricultural and Food Chemistry, 1983. **31**(5): p. 921-930.

22. Bhalla, A., C.M. Cai, F. Xu, S.K. Singh, N. Bansal, T. Phongpreecha, T. Dutta, C.E. Foster, R. Kumar, B.A. Simmons, S. Singh, C.E. Wyman, E.L. Hegg, and D.B. Hodge, *Performance of three delignifying pretreatments on hardwoods: hydrolysis yields, comprehensive mass balances, and lignin properties*. Biotechnology for Biofuels, 2019. **12**(1): p. 213.
29. Xiao, L.-P., Y.-Y. Bai, Z.-J. Shi, Q. Lu, and R.-C. Sun, *Influence of alkaline hydrothermal pretreatment on shrub wood Tamarix ramosissima: Characteristics of degraded lignin*. Biomass and Bioenergy, 2014. **68**: p. 82-94.
43. Wang, G. and H. Chen, *Fractionation of alkali-extracted lignin from steam-exploded stalk by gradient acid precipitation*. Separation and Purification Technology, 2013. **105**: p. 98-105.
47. Brenelli, L.B., F. Mandelli, A.Z. Mercadante, G.J.d.M. Rocha, S.A. Rocco, A.F. Craievich, A.R. Gonçalves, D.d.C. Centeno, M. de Oliveira Neto, and F.M. Squina, *Acidification treatment of lignin from sugarcane bagasse results in fractions of reduced polydispersity and high free-radical scavenging capacity*. Industrial Crops and Products, 2016. **83**: p. 94-103.
50. Stoklosa, R.J., J. Velez, S. Kelkar, C.M. Saffron, M.C. Thies, and D.B. Hodge, *Correlating lignin structural features to phase partitioning behavior in a novel aqueous fractionation of softwood Kraft black liquor*. Green Chemistry, 2013.
51. Goldmann, W.M., J. Ahola, M. Mikola, and J. Tanskanen, *Solubility and fractionation of Indulin AT kraft lignin in ethanol-water media*. Separation and Purification Technology, 2019. **209**: p. 826-832.
52. Sadeghifar, H., T. Wells, R.K. Le, F. Sadeghifar, J.S. Yuan, and A. Jonas Ragauskas, *Fractionation of organosolv lignin using acetone: water and properties of the obtained fractions*. ACS Sustainable Chemistry & Engineering, 2017. **5**(1): p. 580-587.
57. Chambon, C.L., V. Fitriyanti, P. Verdía, S.M. Yang, S. Hérou, M.-M. Titirici, A. Brandt-Talbot, P.S. Fennell, and J.P. Hallett, *Fractionation by Sequential Antisolvent Precipitation of Grass, Softwood, and Hardwood Lignins Isolated Using Low-Cost Ionic Liquids and Water*. ACS Sustainable Chemistry & Engineering, 2020. **8**(9): p. 3751-3761.
59. Duval, A., F. Vilaplana, C. Crestini, and M. Lawoko, *Solvent screening for the fractionation of industrial kraft lignin*. Holzforschung, 2016. **70**(1): p. 11-20.
60. Li, M.-F., S.-N. Sun, F. Xu, and R.-C. Sun, *Sequential solvent fractionation of heterogeneous bamboo organosolv lignin for value-added application*. Separation and Purification Technology, 2012. **101**: p. 18-25.

62. Jiang, X., D. Savithri, X. Du, S. Pawar, H. Jameel, H.-m. Chang, and X. Zhou, *Fractionation and characterization of kraft lignin by sequential precipitation with various organic solvents*. ACS Sustainable Chemistry & Engineering, 2017. **5**(1): p. 835-842.
63. Arshanitsa, A., J. Ponomarenko, T. Dizhbite, A. Andersone, R.J.A. Gosselink, J.v.d. Putten, M. Lauberts, and G. Telysheva, *Fractionation of technical lignins as a tool for improvement of their antioxidant properties*. Journal of Analytical and Applied Pyrolysis, 2013. **103**: p. 78-85.
64. Laurichesse, S. and L. Avérous, *Chemical modification of lignins: Towards biobased polymers*. Progress in Polymer Science, 2014. **39**(7): p. 1266-1290.
73. He, W. and P. Fatehi, *Preparation of sulfomethylated softwood kraft lignin as a dispersant for cement admixture*. RSC Advances, 2015. **5**(58): p. 47031-47039.
77. Çetin, N.S. and N. Özmen, *Use of organosolv lignin in phenol-formaldehyde resins for particleboard production: II. Particleboard production and properties*. International Journal of Adhesion and Adhesives, 2002. **22**(6): p. 481-486.
79. Campbell, A.G. and A.R. Walsh, *The present status and potential of kraft lignin-phenol-formaldehyde wood adhesives*. The Journal of Adhesion, 1985. **18**(4): p. 301-314.
113. Selig, M.J., S. Viamajala, S.R. Decker, M.P. Tucker, M.E. Himmel, and T.B. Vinzant, *Deposition of lignin droplets produced during dilute acid pretreatment of maize stems retards enzymatic hydrolysis of cellulose*. Biotechnol Prog, 2007. **23**(6): p. 1333-9.
132. Saulnier, B.K., T. Phongpreecha, S.K. Singh, and D.B. Hodge, *Impact of dilute acid pretreatment conditions on p-coumarate removal in diverse maize lines*. Bioresource Technology, 2020. **314**: p. 123750.
150. Sluiter, A., B. Hames, R. Ruiz, C. Scarlata, J. Sluiter, D. Templeton, and D. Crocker, *Determination of structural carbohydrates and lignin in biomass*. Laboratory analytical procedure, 2008. **1617**(1): p. 1-16.
157. Stoklosa, R.J. and D.B. Hodge, *Fractionation and Improved Enzymatic Deconstruction of Hardwoods with Alkaline Delignification*. BioEnergy Research, 2015. **8**(3): p. 1224-1234.
161. Yu, G., B. Li, C. Liu, Y. Zhang, H. Wang, and X. Mu, *Fractionation of the main components of corn stover by formic acid and enzymatic saccharification of solid residue*. Industrial Crops and Products, 2013. **50**: p. 750-757.
163. Karp, E.M., B.S. Donohoe, M.H. O'Brien, P.N. Ciesielski, A. Mittal, M.J. Bidy, and G.T. Beckham, *Alkaline Pretreatment of Corn Stover: Bench-Scale Fractionation and Stream Characterization*. ACS Sustainable Chemistry & Engineering, 2014. **2**(6): p. 1481-1491.

172. Pu, Y., F. Hu, F. Huang, B.H. Davison, and A.J. Ragauskas, *Assessing the molecular structure basis for biomass recalcitrance during dilute acid and hydrothermal pretreatments*. Biotechnology for biofuels, 2013. **6**(1): p. 1-13.
173. Kim, J.S., Y.Y. Lee, and T.H. Kim, *A review on alkaline pretreatment technology for bioconversion of lignocellulosic biomass*. Bioresource Technology, 2016. **199**: p. 42-48.
174. Marzioletti, T., S.J. Miller, C.W. Jones, and P.K. Agrawal, *Switchgrass pretreatment and hydrolysis using low concentrations of formic acid*. Journal of Chemical Technology & Biotechnology, 2011. **86**(5): p. 706-713.
175. Dong, L., X. Zhao, and D. Liu, *Kinetic modeling of atmospheric formic acid pretreatment of wheat straw with "potential degree of reaction" models*. RSC Advances, 2015. **5**(27): p. 20992-21000.
176. Cesaro, A., A. Conte, H. Carrère, E. Trably, F. Paillet, and V. Belgiorno, *Formic acid pretreatment for enhanced production of bioenergy and biochemicals from organic solid waste*. Biomass and bioenergy, 2020. **133**: p. 105455.
177. Sindhu, R., P. Binod, K. Satyanagalakshmi, K.U. Janu, K.V. Sajna, N. Kurien, R.K. Sukumaran, and A. Pandey, *Formic acid as a potential pretreatment agent for the conversion of sugarcane bagasse to bioethanol*. Appl Biochem Biotechnol, 2010. **162**(8): p. 2313-23.
178. Xu, J., M.H. Thomsen, and A.B. Thomsen, *Pretreatment on corn stover with low concentration of formic acid*. Journal of microbiology and biotechnology, 2009. **19**(8): p. 845-850.
179. Zhang, M., W. Qi, R. Liu, R. Su, S. Wu, and Z. He, *Fractionating lignocellulose by formic acid: characterization of major components*. Biomass and bioenergy, 2010. **34**(4): p. 525-532.
180. Dong, L., R. Wu, X. Zhao, and D. Liu, *Phenomenological modeling and evaluation of formic acid pretreatment of wheat straw with an extended combined severity factor for biomass fractionation and enzymatic saccharification to produce bioethanol*. Journal of the Taiwan Institute of Chemical Engineers, 2017. **81**: p. 140-149.
181. Humpert, D., M. Ebrahimi, and P. Czermak, *Membrane Technology for the Recovery of Lignin: A Review*. Membranes (Basel), 2016. **6**(3).
182. Weinwurm, F., A. Drljo, W. Waldmüller, B. Fiala, J. Niedermayer, and A. Friedl, *Lignin concentration and fractionation from ethanol organosolv liquors by ultra- and nanofiltration*. Journal of Cleaner Production, 2016. **136**: p. 62-71.

183. Zheng, Y., C. Lee, C. Yu, Y.-S. Cheng, R. Zhang, B.M. Jenkins, and J.S. VanderGheynst, *Dilute acid pretreatment and fermentation of sugar beet pulp to ethanol*. Applied Energy, 2013. **105**: p. 1-7.
184. Zhou, H., L. Tan, Y. Fu, H. Zhang, N. Liu, M. Qin, and Z. Wang, *Rapid Nondestructive Fractionation of Biomass (≤ 15 min) by using Flow-Through Recyclable Formic Acid toward Whole Valorization of Carbohydrate and Lignin*. ChemSusChem, 2019. **12**(6): p. 1213-1221.
185. Chang, X., J. Zhang, R. Wu, and X. Zhao, *Phenomenological modeling of formic acid fractionation of sugarcane bagasse by integration of operation parameters as an extended combined severity factor*. Molecules, 2021. **26**(9): p. 2753.
186. Lavarack, B.P., G.J. Griffin, and D. Rodman, *The acid hydrolysis of sugarcane bagasse hemicellulose to produce xylose, arabinose, glucose and other products*. Biomass and Bioenergy, 2002. **23**(5): p. 367-380.
187. Liu, X., M. Lu, N. Ai, F. Yu, and J. Ji, *Kinetic model analysis of dilute sulfuric acid-catalyzed hemicellulose hydrolysis in sweet sorghum bagasse for xylose production*. Industrial Crops and Products, 2012. **38**: p. 81-86.
188. Zhou, Z., D. Liu, and X. Zhao, *Conversion of lignocellulose to biofuels and chemicals via sugar platform: An updated review on chemistry and mechanisms of acid hydrolysis of lignocellulose*. Renewable and Sustainable Energy Reviews, 2021. **146**: p. 111169.
189. Lili, W., J. Yijun, L. Chunhu, L. Xiutao, M. Lingqian, W. Wei, and M. Xindong. *Microwave-assisted hydrolysis of corn cob for xylose production in formic acid*. in *2011 International Conference on Materials for Renewable Energy & Environment*. 2011.
190. Xu, J., Y. Fu, G. Tian, Q. Li, N. Liu, M. Qin, and Z. Wang, *Mild and efficient extraction of hardwood hemicellulose using recyclable formic acid/water binary solvent*. Bioresource Technology, 2018. **254**: p. 353-356.
191. Labauze, H. and B. Benjelloun-Mlayah, *Kinetics of xylan hydrolysis using an acetic and formic acid-based organosolv pretreatment*. Bioresource Technology Reports, 2021. **14**: p. 100690.
192. Lu, Y. and N.S. Mosier, *Biomimetic catalysis for hemicellulose hydrolysis in corn stover*. Biotechnol Prog, 2007. **23**(1): p. 116-23.
193. Sadeghifar, H. and A. Ragauskas, *Perspective on Technical Lignin Fractionation*. ACS Sustainable Chemistry & Engineering, 2020. **8**(22): p. 8086-8101.
194. Helander, M., H. Theliander, M. Lawoko, G. Henriksson, L. Zhang, and M.E. Lindström, *Fractionation of Technical Lignin: Molecular Mass and pH Effects*. 2013, 2013. **8**(2): p. 13.

195. Wells, T., M. Kosa, and A.J. Ragauskas, *Polymerization of Kraft lignin via ultrasonication for high-molecular-weight applications*. Ultrasonics Sonochemistry, 2013. **20**(6): p. 1463-1469.
196. Asada, C., S. Basnet, M. Otsuka, C. Sasaki, and Y. Nakamura, *Epoxy resin synthesis using low molecular weight lignin separated from various lignocellulosic materials*. International Journal of Biological Macromolecules, 2015. **74**: p. 413-419.
197. Huang, C., J. He, R. Narron, Y. Wang, and Q. Yong, *Characterization of Kraft Lignin Fractions Obtained by Sequential Ultrafiltration and Their Potential Application as a Biobased Component in Blends with Polyethylene*. ACS Sustainable Chemistry & Engineering, 2017. **5**(12): p. 11770-11779.
198. Xu, J., C. Li, L. Dai, C. Xu, Y. Zhong, F. Yu, and C. Si, *Biomass Fractionation and Lignin Fractionation towards Lignin Valorization*. ChemSusChem, 2020. **13**(17): p. 4284-4295.
199. Bai, J., S. Wang, Y. Li, Z. Wang, and J. Tang, *Effect of chemical structure and molecular weight on the properties of lignin-based ultrafine carbon fibers*. International Journal of Biological Macromolecules, 2021. **187**: p. 594-602.
200. Vanderlaan, M. and R. Thring, *Polyurethanes from Alcell® lignin fractions obtained by sequential solvent extraction*. Biomass and bioenergy, 1998. **14**(5-6): p. 525-531.
201. Crowe, J.D., R.A. Zarger, and D.B. Hodge, *Relating Nanoscale Accessibility within Plant Cell Walls to Improved Enzyme Hydrolysis Yields in Corn Stover Subjected to Diverse Pretreatments*. Journal of Agricultural and Food Chemistry, 2017. **65**(39): p. 8652-8662.
202. Palonen, H., F. Tjerneld, G. Zacchi, and M. Tenkanen, *Adsorption of Trichoderma reesei CBH I and EG II and their catalytic domains on steam pretreated softwood and isolated lignin*. J Biotechnol, 2004. **107**(1): p. 65-72.
203. Wu, R., X. Zhao, and D. Liu, *Structural Features of Formiline Pretreated Sugar Cane Bagasse and Their Impact on the Enzymatic Hydrolysis of Cellulose*. ACS Sustainable Chemistry & Engineering, 2016. **4**(3): p. 1255-1261.

CHAPTER 5

INCORPORATION OF FUNCTIONALIZED CORN STOVER LIGNIN FRACTIONS INTO
PLA COMPOSITE MATERIALSContribution of Authors and Co-Authors

Author: Brian K. Saulnier

Contributions: Conducted alkali pretreatments and fractionation of black liquor to obtain three biopolymer fractions. Characterized polysaccharide, lignin, ash, and moisture content of all recovered materials. Functionalized biopolymer fractions with propylene carbonate. Analyzed extent of biopolymer functionalization using ^1H and ^{13}C NMR. Prepared neat PLA, unmodified, and modified biopolymer composite materials in rectangular and dogbone geometries via extrusion and injection molding at two different biopolymer incorporation levels. Imaged biopolymer-PLA composites using confocal microscopy. Subjected all materials to dynamic mechanical analysis (DMA) experiments. Analyzed all materials using differential scanning calorimetry (DSC). Used GPC method to determine molar mass distributions of recovered biopolymers and materials. Conducted tensile tests on all materials. Wrote first draft of manuscript.

Co-Author: Seth Kane

Contribution: Aided in training of laboratory techniques. Assisted with data interpretation. Provided feedback on final draft of manuscript.

Co-Author: Cecily Ryan

Contributions: Aided in the design of experiments. Provided guidance for laboratory procedures and aided in interpretation of data. Provided editing and feedback for final manuscript draft.

Co-Author: David B. Hodge

Contributions: Assisted in experimental design. Aided in interpretation of data. Provided assistance in the writing, editing, and finalization of subsequent manuscript drafts.

Manuscript Information

Brian K. Saulnier, Seth Kane, Cecily Ryan, David B. Hodge

Biomacromolecules

Status of Manuscript: [Put an x in one of the options below, delete this]

☒ Prepared for submission to a peer-reviewed journal

☐ Officially submitted to a peer-reviewed journal

☐ Accepted by a peer-reviewed journal

☐ Published in a peer-reviewed journal

To be submitted to Biomacromolecules

INCORPORATION OF FUNCTIONALIZED CORN STOVER LIGNIN FRACTIONS INTO PLA COMPOSITE MATERIALS

Abstract

Poly lactide (PLA) is a common bio-derived plastic, however it suffers from some thermal and mechanical shortcomings such as brittleness, low thermal stability, and slow crystallization. One way to improve these properties is to make composite materials with biopolymers such as lignin or cellulose. Poor compatibility of biopolymer and PLA often leads to poor biopolymer dispersion and reduced material properties. In this study we use complex biopolymer fractions not previously used in PLA materials composed of lignin, xylan, and inorganics recovered from the fractional precipitation of corn stover alkali liquor. These biopolymer fractions are modified using the oxyalkylation of lignin and xylan hydroxyl groups by reaction with propylene carbonate. Modified biopolymer composite materials exhibited enhanced dispersion, interfacial interactions, and crosslinking during extrusion leading to thermal and mechanical properties improved over their unmodified counterparts.

Introduction

Lignin is one of the primary structural components of the cell walls of vascular plants (*i.e.*, lignocellulose) and represents an abundant and renewable source of aromatics with many promising value-added applications [204]. Utilization requires extraction and recovery of water-insoluble lignin from the cell wall where it is integrated within a matrix of other structural biopolymers (cellulose and hemicellulose) through a combination of physical entanglement and, for graminaceous feedstocks such as corn stover, covalent crosslinks with the hemicellulose xylan [105]. Thus, lignin utilization requires technologies that solubilize the lignin, often through integration with functionalization (*e.g.*, sulfonation) and/or depolymerization (*e.g.*, alkaline pulping) to yield lignins that often exhibit significantly different chemical and physical properties relative to the “native” lignin as it is found in the cell wall. For this reason, industrial utilization of lignin is envisioned as integrated with technologies for utilization of the

polysaccharide fraction such as chemical pulping or biorefining of lignocellulose. Besides the challenge of extracting and recovering lignin, additional hurdles to incorporating lignin into value-added products include the heterogeneity of its chemical structure and its polydisperse molecular weight distributions [205]. This heterogeneity arises from both the natural variability in the lignin structure, which varies between plant source[138] and anatomy[140]. Lignin heterogeneity is further aggravated by the many different biomass pretreatment and lignin extraction processes used and by the presence of other non-lignin contaminants that derive from the biomass such as hemicellulose, ash, or protein [206] or from the extraction or recovery process itself (*e.g.*, ash). The consequences of this structural heterogeneity are differences in its reactivity and solubility during processing and differences in the properties of its final products [207].

One approach for reducing the lignin heterogeneity is solvent fractionation [193, 208]. As lignin solubilization is already one of the requirements for lignin extraction and utilization, selective precipitation or solubilization is one approach to generate subsets of lignin that are enriched or depleted in target properties (*e.g.*, phenolic hydroxyl content, low molar mass, *etc.*). These approaches include sequential acidification of liquors containing alkali-solubilized lignins from pulping (*e.g.*, kraft or soda pulping) or alkaline pretreatments using weak (*e.g.*, CO₂) or strong acids (*e.g.*, H₂SO₄) to yield lignins of differing properties [209-211]. Fractionation via acidification is conducted using a variety of reagents including HCl[43], H₂SO₄[49], CO₂[50], and H₃PO₄[49], and has been employed using a variety of different feedstocks including hardwood[44], softwood[44], kraft liquor[50], corn stover[43], sugarcane bagasse[47], *Miscanthus* [46], and wheat straw[48]. Feedstock source influences the chemical structure and

properties of extracted lignins. Lignin from softwood and hardwood feedstocks are composed primarily of guaiacyl (G) and syringyl (S) subunits, while lignin from graminaceous feedstocks contain higher proportions of *p*-hydroxyphenyl (H) subunits and *p*-coumarate (*p*CA) end chains[7] leading to different intramolecular linkages between subunits[8, 9]. These chemical differences due to lignin source further influence the properties of lignin-based products with changes in mechanical[15, 16] and thermal[14] properties of lignin-based resins and materials based on feedstock source. Fractionation of lignin via acidification results in many differences in properties of recovered lignin fractions including variations in lignin yields[44, 46, 48, 194], ash content[44], glass transition temperature (T_g)[44], lignin acid solubility[194], sulfur content[194], particle size[43], polydispersity[43, 46, 47], phenolic hydroxyl content[43, 45, 47, 50], surface morphology[45], and antioxidant capability[212].

Organic solvent fractionation processes have also been used to obtain lignin fractions of reduced polydispersity such as fractionation of lignin using different solvent-water solutions of decreasing concentration including ethanol[51], acetic acid[53], acetone[213], THF[55], methanol [54], propyleneglycol monomethyl ether[214], ionic liquids[57], dihydrolevoglucosenone[215], and γ -valerolactone[58]. Lignin may also be fractionated using a series of solvents of increasing solvent power as related to their Hansen solubility parameters[59]. This process of sequential solvent fractionation has been widely implemented using a variety of different solvent sequences and feedstocks[60-63]. More monodisperse lignins can also be separated and recovered using membrane filtration [181, 182, 216]. By implementing the fractionation strategies reviewed above lignin properties can be tailored for better performance in a specific value-added applications[205]. Lignin has been applied to a wide

variety of different applications and products. These products include a fuel for combustion, low-value fillers for concrete and glue, carbon fiber, platform chemicals, plastic composites, polymeric foams, flavoring agents, fragrances, and others[4, 204]. In this study we focus on utilizing our extracted biopolymer fractions as an additive for thermoplastic composites, specifically lignin-poly lactide (PLA) composites.

PLA shows promise as a renewable biobased plastic with the ability for rapid degradation[217]. However, PLA suffers from some thermal and mechanical shortcomings such as brittleness, low thermal stability, and slow crystallization[218]. One way to address these challenges and improve the thermal and mechanical properties of PLA is to include a copolymer additive such as lignin. The utilization of lignin in PLA composites can help to improve their properties while simultaneously providing additional value for a biorefinery as a co-product. Prior work has employed lignins from a wide range of sources, including kraft[85, 219], alkali[86], organosolv[86, 220], lignosulfonate[221], and lignin nanoparticles[222]. This study differs from previous work using fractionated biopolymers recovered from corn stover alkali liquor instead of a single bulk lignin source. Due to this recovery process these biopolymers exhibit two key differences from previously used biopolymers. Lignin portions of the biopolymer fractions are expected to have more monodisperse molecular weight and a more homogeneous chemical structure from the use of fractional recovery as opposed to a single step process. This allows for the exploration of how lignin properties resulting from different recovery conditions influence the properties of lignin-PLA materials. The biopolymers used in this study also contain higher proportions of non-lignin materials including polysaccharides and inorganics providing insight into the efficacy of hemicellulose incorporation into the PLA

polymer matrix, and how lignin purity affects material properties. The alkali pretreatment conditions used in this study also differ from previous work using lower temperature and reaction time than many other studies[163, 223, 224]. Differing severities of alkali reaction influence degree of delignification, glucose hydrolysis yields, and lignin chemical structure.

Some studies show an increase in thermal stability of PLA materials upon the addition of lignin[85, 86, 220], while other studies show decreased thermal degradation temperatures[219, 221]. These discrepancies can be explained by differences arising from the source, extraction, and recovery approaches that yield lignins with differences in chemical composition, molar mass distributions, the presence of impurities, as well as differences in particle size, their distribution, and surface properties of the lignins. As a general trend, tensile strength of lignin-PLA composites decreases with increasing lignin content[85, 86, 219, 220, 225]. Other work identified changes in hydrolytic degradation[86, 226], impact strength[221], viscosity during extrusion[220], light transmittance[227], and crystallinity[228] of lignin-PLA materials.

Chemical functionalization of lignin has been employed as one approach to improve the properties of these composite materials by improving lignin's compatibility with PLA. Functionalization approaches include carboxylation[229], urea modification[92], acetylation[85], alkylation[89], esterification with other reagents[87, 230], and silanization[90]. In the present study we modify lignin in our biopolymer fractions using an oxyalkylation reaction with propylene carbonate. We hypothesize that several factors may contribute to improved compatibility of oxyalkylated lignin with PLA. First, the replacement of lignin phenolic hydroxyls with aliphatic hydroxyls decreases the polarity of lignin and the added aliphatic chains create steric hindrance decreasing the ability of lignin to form aggregates with itself, decreasing

aromatic π - π stacking and hydrogen bonding between phenolic hydroxyls. This allows the modified lignin to exhibit increased hydrogen bonding and van der Waals interactions with PLA[231]. These aliphatic hydroxyls may also increase the compatibility of lignin and PLA due to the higher selectivity of PLA for covalent bonding with aliphatic hydroxyl groups when compared to phenolic hydroxyl and carboxyl groups[89]. Propylene carbonate possesses advantages over many of the other reagents used for lignin modification including low cost, low toxicity, low volatility, and biodegradability[232]. Its use is especially attractive with the increasing push for environmentally friendly processes because propylene carbonate's usual manufacture from epoxides is a carbon negative process[233].

The work in this manuscript uses alkali pretreatment of corn stover followed by fractional precipitation of black liquor to obtain four biopolymer fractions. One NaOH insoluble cellulose pulp, two lignin rich fractions, and one xylan rich fraction. These biopolymers were subjected to an oxyalkylation modification with propylene carbonate and assessed for extent of modification via ^{13}C NMR. Modified and unmodified biopolymers were incorporated into extruded and injection molded PLA composites at incorporation levels of 5% and 20%. The rheological, thermal, and mechanical properties of the resulting composite materials were characterized.

Materials and Methods

Lignin Generation and Fractionation

A commercial hybrid corn stover used in the present work was obtained from Idaho National Laboratory after harvest in 2017 in Story County, Iowa. The corn stover was first milled to pass a 2-inch screen using a Vermeer bale grinder BG-480 followed by milling with a Bliss Hammermill to pass a 1-inch screen, and finally milled to pass a 20-mesh using a Wiley

mini-mill (Thomas Scientific). Milled corn stover was subjected to NaOH pretreatment in a stainless-steel tumbling pulp digester (RegMed model AU/E-20, São Paulo, Brazil) equipped with 4 one-liter capacity tubes. Stover was mixed with 1 M NaOH at a 20% solid loading (wt/vol) and heated to 85 °C for 1 hour. Remaining solids after pretreatment were separated with a home brewing mesh filter bag and washed with an excess of water. Pretreatment liquor was sequentially adjusted from an initial pH of 13.8 to pH 9, 5, and pH 1.5 at room temperature via the dropwise addition of 6 M H₂SO₄. Quantification of pH was conducted using a pH probe (FE150, Fischer). After each pH adjustment insoluble solids were separated via centrifugation, washed with dilute H₂SO₄, and vacuum dried at 50 °C for 48 hours to obtain three biopolymer fractions (**Fig. 1**).

Biopolymer Functionalization

For hydroxyalkylation, 15g dried biopolymer samples recovered from NaOH fractionation were placed in a 500 mL Parr reactor with 20 molar equivalents of propylene carbonate per mole of total lignin hydroxyl content, 0.1 molar equivalents of 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU) was added as a catalyst (Thermo Scientific 6674-22-2). Hydroxyl values were estimated based on values measured previously from similar lignins via ³¹P NMR. Reactions were conducted under nitrogen atmosphere for 3 hours at 170 °C. Following reaction, modified biopolymers were precipitated with 10 times weight equivalent of DI water and washed three times via centrifugation. To assess the degree of modification with propylene carbonate unmodified and modified biopolymer samples were subjected to ¹³C and ¹H NMR analysis. To prepare samples for NMR analysis 30 mg of each sample was dissolved in DMSO-d₆ with 3 mg chromium (III) acetylacetonate added as a relaxation agent and 3 mg dioxane added as

an internal standard. NMR experiments were conducted on a Bruker 600 MHz Avance III NMR Spectrometer. Experiments were conducted at 300 K with 64 scans for ^1H experiments and 20480 scans for ^{13}C experiments.

Composition Analysis

Polysaccharide and Klason lignin contents of all biomass and recovered biopolymer fractions as well as functionalized biopolymers were determined using a composition analysis procedure according to NREL LAP TP-510-42618[150]. Klason lignin was determined gravimetrically and sugar quantification was obtained via HPLC (Agilent 1260 series) equipped with an RID detector, a Bio-Rad Aminex HPX-87H column, a mobile phase of 5 mM H_2SO_4 , a flow rate of 0.6 mL/min, and a column temperature of 65°C. Sample moisture content was determined using a moisture analyzer (OHAUS, MB 90). Ash content was determined gravimetrically after sample combustion at 620 °C, a procedure modified from NREL LAP TP-510-42622[234].

Composite Synthesis and Characterization

Recovered biopolymers or functionalized biopolymers and PLA were mixed at 5% and 20% w/w biopolymer to PLA in a Thermo Fisher Scientific HAAKE Minilab II dual screw extruder at 168 °C for 5 minutes. Test bars for DMA and tensile analysis were formed using a Thermo Fisher Scientific Minijet Pro injection molder with injection parameters varying for each composition to avoid incomplete injections or air bubbles within test composites (± 5 °C).

PLA-biopolymer composites were subjected to three-point bending tests using a TA Instruments Q800 dynamic mechanical analyzer (DMA). Storage and loss modulus were

measured as a function of temperature with a temperature ramp from 30-140 °C with a constant frequency and amplitude.

To examine biopolymer particle size and dispersion 5% biopolymer-PLA composites were imaged using a Leica TCS SP5 Confocal Scanning Laser Microscope. Lignin fluorophores were excited using a 633 nm laser and images were taken randomly throughout the material at 3 magnifications using 10x dry, 20x dry, and 63x water submersed objectives.

To investigate crystallinity and thermal properties, 5% and 20% biopolymer-PLA composite materials were analyzed via differential scanning calorimetry. To a hermetically sealed aluminum pan 3-5 mg of each material was added. These samples were analyzed using a TA Instruments DSC 2500 with a 10 °C/minute heating gradient from -20 °C to 200 °C followed by cooling at 10 °C/minute back to -20 °C.

After extrusion composite materials were injection molded into type V dogbone geometries according to ASTM standard D638[235]. Tensile tests were conducted on an Instron 8562 load frame equipped with a 2620 series dynamic strain gauge extensometer at a rate of 1mm/minute until failure.

Gel-Permeation Chromatography (GPC)

Unmodified and modified biopolymer fractions and composites were prepared for analysis by dissolution in 3 mL THF and filtration using 0.2 µm PTFE syringe filters. Samples were analyzed for relative molecular weight using an HPLC with a Waters Styragel HR 4 (7.8 x 300 mm, Milford, MA, USA) column using THF as a mobile phase at a flow rate of 0.5 mL/min and a column temperature of 40 °C. Spectral data was collected using a DAD detector at 280 nm. Due to low solubility in THF unmodified biopolymer fractions were also analyzed after

acetylation. The acetylation procedure was conducted on 10 mg of each sample solubilized in 4 mL of pyridine by 30 minutes of stirring at room temperature. After full solubilization 0.25 mL of acetic anhydride was added and allowed to react for 20 hours at room temperature. Solvent was removed from acetylated biopolymer through the use of a rotary evaporator at 40 °C and dried under vacuum for 10 minutes.

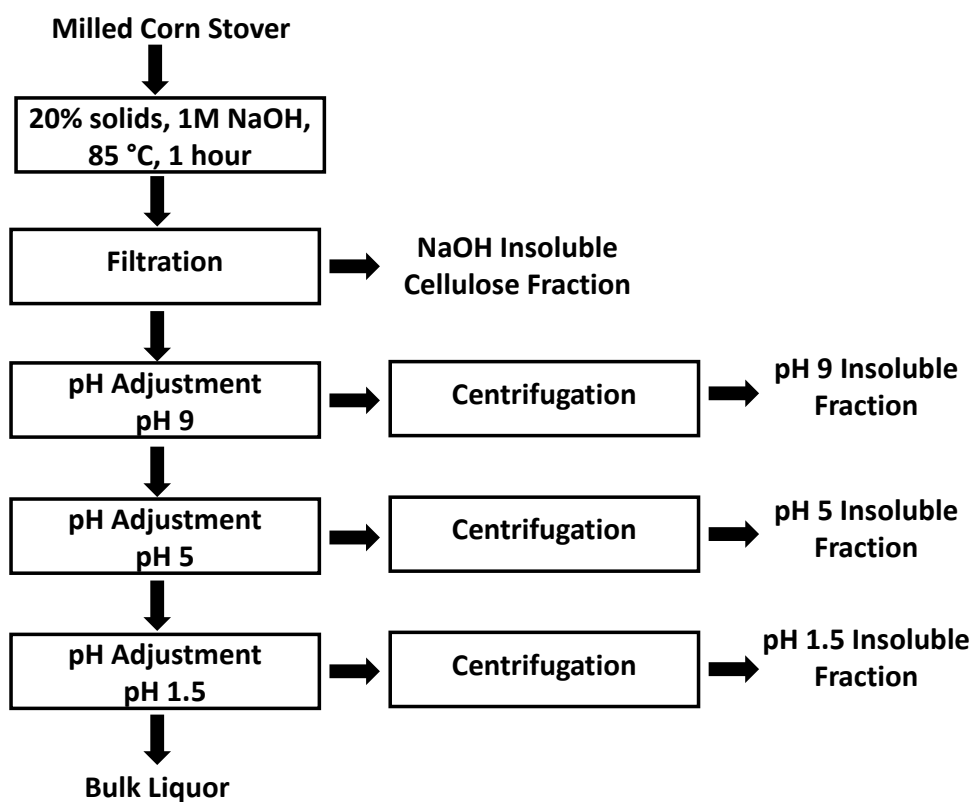
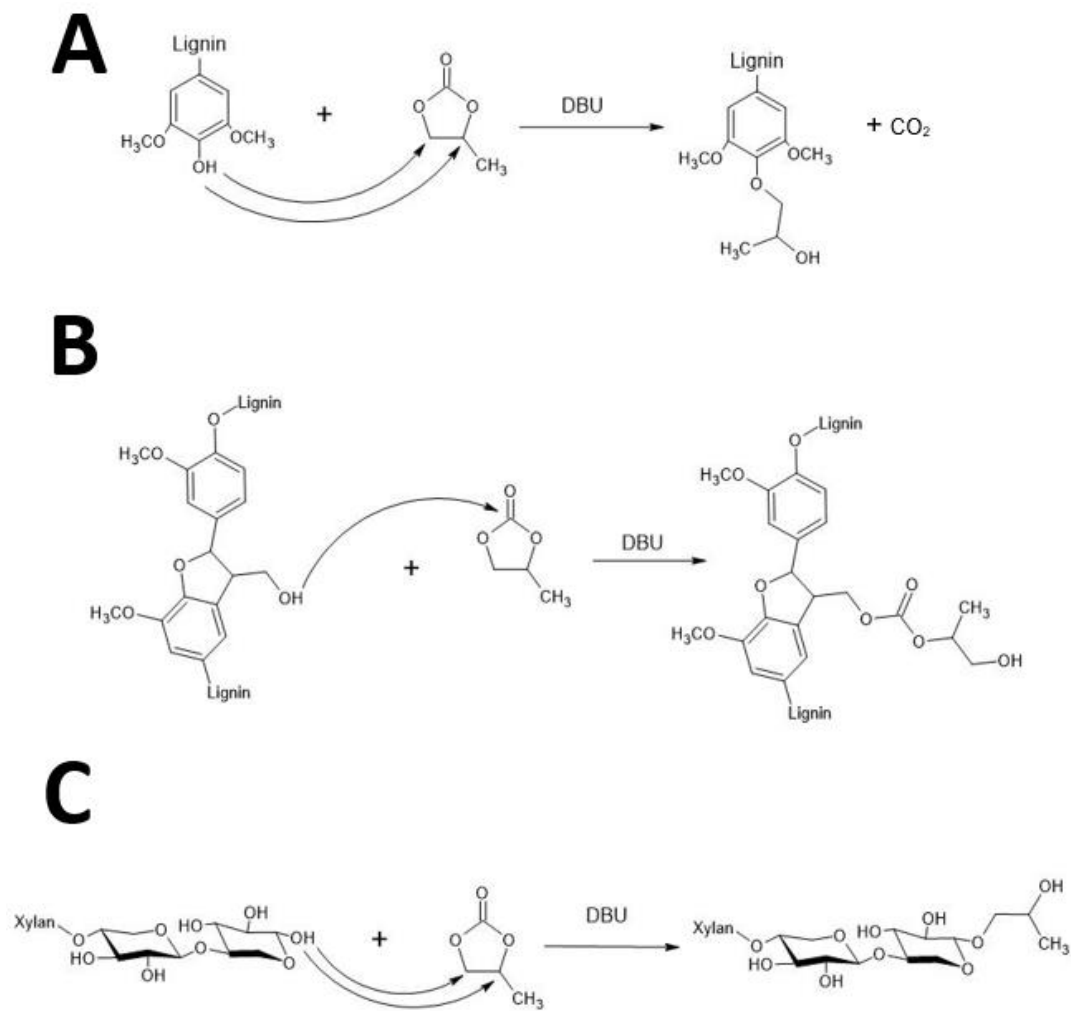


Figure 1: Three Biopolymer Fractions are recovered by Fractional pH Adjustment of Alkali Liquors

Results and Discussion

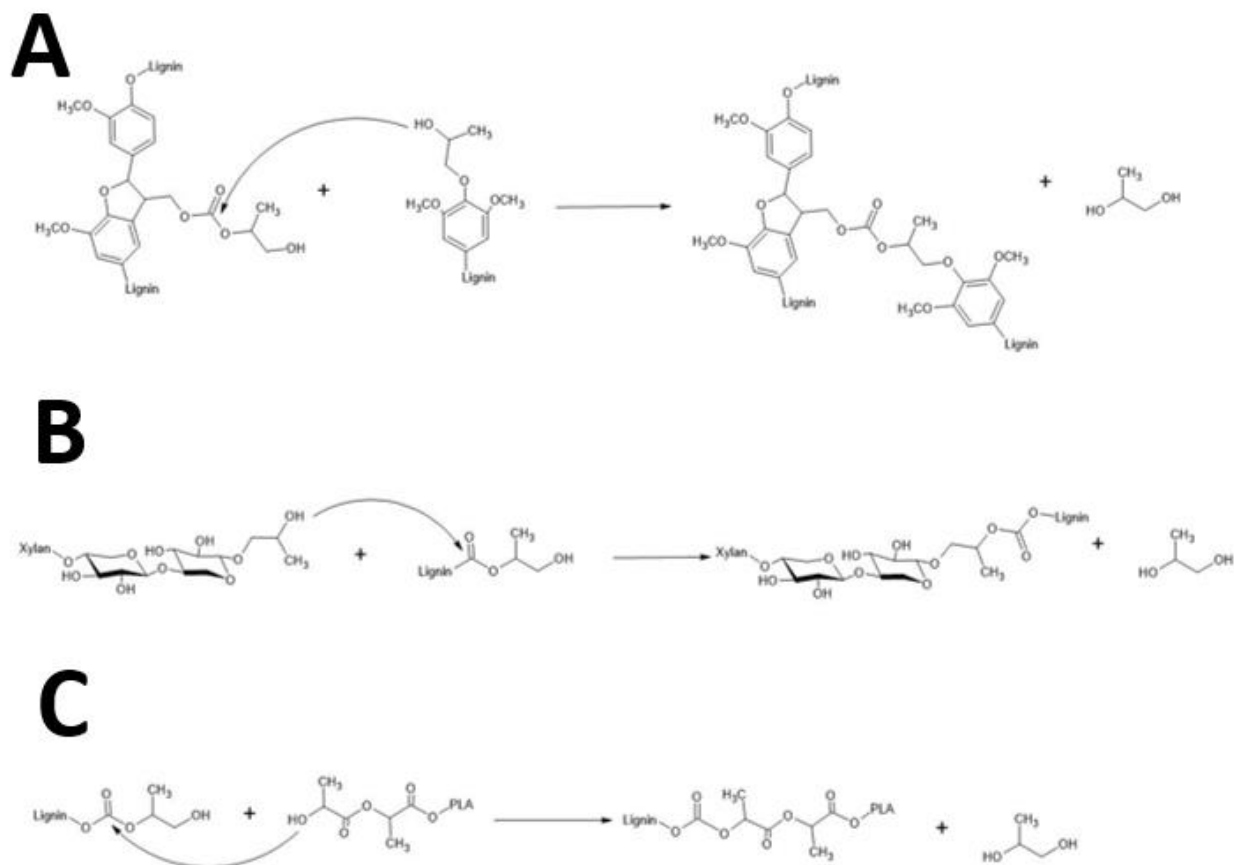
Biomass Fractionation and Biopolymer Recovery

Three biopolymer fractions were recovered from alkali liquor of pretreated corn stover using a three-step fractional precipitation with pH adjustment to recover insoluble precipitates at pH 9, pH 5, and pH 1.5 as outlined in **Figure 1**. These fractions will be referred to as pH 9, pH 5, and pH 1.5 biopolymer fractions throughout this manuscript.



Scheme 1: Oxyalkylation of Lignin (**A+B**) and Xylan (**C**) with Propylene Carbonate and a DBU Catalyst

These biopolymer fractions have significant impurities, including inorganics and hemicellulose due to the significant amount of xylan solubilized during pretreatment. Previous work has been conducted on the incorporation of polysaccharides into PLA materials. However, most of these studies focus on cellulose-PLA blends[236-238]. Extensive work has also been performed on modified cellulose-PLA composites with cellulose compatibilized by chemical modifications including acetylation[239], silanization[91], glycidyl methacrylate grafting[240], esterification[241], carboxylation[242], and others. Work in the literature on hemicellulose-PLA composites is much more limited with one study incorporating a galactoglucomannan based spruce hemicellulose in solvent casted PLA composites[243] and other studies investigating copolymers of xylan grafted with L-lactide[243-246]. To our knowledge there are no studies concerning the melt extrusion of xylan-PLA composite materials or studies using multicomponent xylan rich and lignin rich biopolymers as are used in this study. We hypothesize that the xylan contained in our biomass fractions will also undergo modification with propylene carbonate (**Scheme 1C**) and xylan will be incorporated into a combined PLA-lignin-xylan matrix through van der Waals interactions, hydrogen bonding, and ether bonding (**Scheme 2B**). Previous work has been conducted modifying xylan using propylene carbonate in DMSO[247], grafting propylene carbonate onto xylan in ionic liquids[248], and reacting xylan directly in propylene carbonate under similar conditions to those used in this study[249]. Studies also exist exploring modification of xylan using other cyclic carbonates such as propylene oxide[250, 251]. To our knowledge no studies have been conducted applying oxyalkylated polysaccharides to PLA composite materials.



Scheme 2: Possible Lignin-Lignin (**A**), Lignin-Xylan (**B**), and Lignin-PLA (**C**) Crosslinking Reactions during Modification and Extrusion

As seen in **Figure 2A** the pH 9 fraction was composed primarily of xylan with around 30% Klason lignin and a high amount of inorganics with 24% ash. The pH 5 and pH 1.5 fractions were composed primarily of lignin with smaller amounts of xylan and inorganics, however these fractions contained a higher proportion of acid soluble materials. **Figure 2B** shows the mass yields of the recovered fractions in terms of total mass recovered, recovered lignin, recovered xylan, and recovered glucan. The alkali pretreatment and fractionation process resulted in 70% recovery of the original mass of the corn stover with 50% of the original mass

being recovered in the cellulose dominant fraction and the remaining 20% belonging to the 3 biopolymer fractions. These yields are similar to yields obtained by other studies using alkali pretreatment of corn stover under similar conditions[163, 252, 253]. The remaining 30% likely remained soluble in solution below pH 1.5, or resolubilized and was lost during the washing of the biopolymer fractions. After alkali pretreatment 89% of the original glucan and 48% of the original xylan contained in corn stover remained in the alkali insoluble fraction, these results agree with previous studies[163]. High glucan recovery in the insoluble fraction after alkali treatment as we have achieved in this study is important for both paper production and the production of ethanol from lignocellulosic biomass. Around 70% of the xylan in corn stover was recovered in the combined solid fractions, likely in polymeric form as lignin-xylan or glucan-xylan complexes. The majority of the remaining 30% of xylan unaccounted for is likely present in the remaining liquor after adjustment to pH 1.5 as either polymeric xylan, oligomers, monomeric xylose, or degradation products. These sugars could likely be recovered and used for subsequent fermentation or other value-added applications. A total yield of 57.5% of the original corn stover lignin was recovered in the isolated solid fractions with 47% contained in the three biopolymer fractions. The low level of lignin contained in the alkali insoluble cellulose-rich fraction is typical for alkali pretreatment[163]. Unaccounted for lignin is a result of a few different mechanisms. A study with alkali pretreatment of switchgrass shows that high molecular weight lignin which solubilizes during pretreatment redeposits on the surface of the insoluble fraction and is removed after subsequent washing resulting in up to a 20% reduction in original lignin recovery[223]. However this study used temperatures >100 °C which likely results in

greater lignin solubilization during pretreatment compared to the conditions used in this study (85 °C). Some unrecovered lignin also remained soluble in the liquor after pH adjustment.

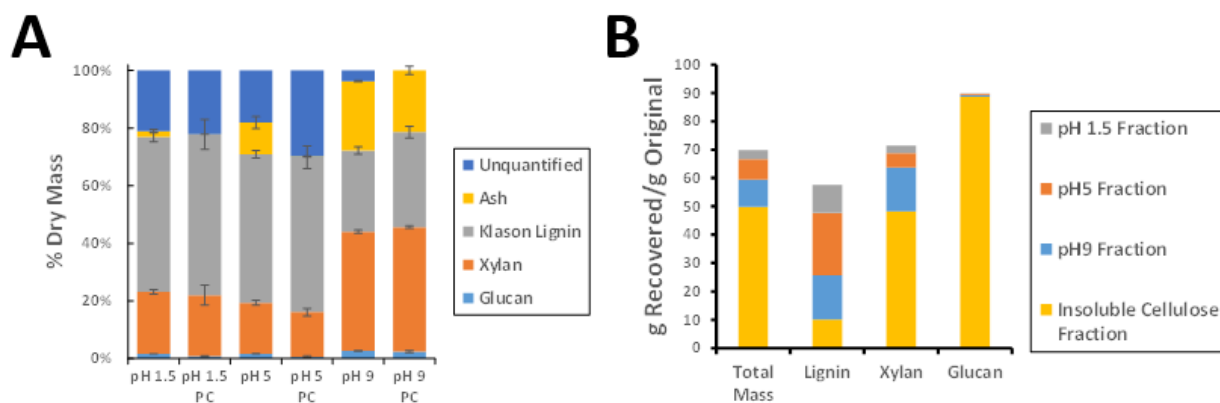


Figure 2: Polymer Composition of Recovered Biopolymers before and after Modification (A), and Precipitated Biopolymer and Insoluble Cellulose Fraction Mass Yields (B)

Extracted biopolymer fractions were modified via an oxyalkylation reaction with propylene carbonate (**Scheme 1**) according to previously optimized conditions in the literature[254]. These modified fractions will be referred to throughout the manuscript as pH 9 PC, pH 5 PC, and pH 1.5 PC biopolymer fractions. After reaction, modified fractions were again analyzed for composition (**Fig. 2A**). After functionalization no significant changes in polysaccharide or lignin content were observed. Modification of each fraction resulted in a reduction of ash, with the most significant change being observed in the pH 5 fraction. Unmodified and modified fractions were further analyzed via ^1H and ^{13}C NMR to verify the successful functionalization of lignin.

NMR Characterization of Functionalized Biopolymers

To assess whether biopolymer modification was successful both modified and unmodified biopolymers were subjected to ^1H and ^{13}C NMR analysis. Results for ^1H experiments are shown in **Figure 3**. Peak assignments for ^1H spectra were made according to previous literature [232, 255, 256]. As seen in **Figure 3** peaks between 0.6 and 1.5 ppm correspond to methyl groups on lignin aliphatic chains. The spectra of all biopolymers modified with propylene carbonate show an increase in peak area in this region indicating that the oxyalkylation modification reaction proceeded according to **Scheme 1** adding aliphatic side chains with attached methyl groups to the lignin and xylan backbones, this is in agreement with previous literature [232, 256]. Peaks at 2.5 and 3.6 ppm correspond to $\text{DMSO-}d_6$ solvent and dioxane internal standard, respectively. Modified biopolymers show a large peak centered at 3.35 ppm. This peak can be attributed to a combination of signals from methine, methylene, and aliphatic hydroxyl groups of the new side chains on both lignin and xylan components of the modified biopolymers (**Scheme 1**). The appearance of this peak is obvious after modification of the lignin heavy pH 1.5 and pH 5 fractions. On the other hand, this peak is prominent in both the unmodified and modified forms of the highest pH isolated fraction (pH 9). This can be attributed to two factors, firstly the higher molecular weight lignin contained in these fractions (isolated at higher pH) likely contains more aliphatic hydroxyls when compared to the lower molecular weight fractions. This trend has been shown in multiple previous studies on lignin fractional precipitation [44, 257-259]. Secondly, a large portion of this peak can be attributed to hydroxyls on xylan. Peaks at 4.3, 5, and 5.1 ppm can be assigned to protons in $\beta\text{-O-4}$ linkages, and may also have contributions from DMSO soluble hemicelluloses[260]. Biopolymer fractions recovered at pH 1.5 and pH 5 showed a reduction in these peaks after modification with

propylene carbonate. This may signify the cleavage of lignin β -O-4 linkages during the modification reaction due to the long reaction times and high temperatures. This reduction may also be due to the reduced DMSO solubility of modified biopolymers due to crosslinking. Propylene carbonate modified pH9 biopolymer fraction showed an increase in these peaks over its unmodified counterpart, the reason for this is unknown, it may be a result of new bonds formed between crosslinked xylan-xylan and xylan lignin complexes (**Scheme 2B**). Peaks above 6 ppm can be attributed to aromatic ring protons and phenolic hydroxyls. A reduction in these peaks in the pH 1.5 and pH 5 modified fractions supports the reduction of phenolic hydroxyls as a result of reaction with propylene carbonate. Modified and unmodified pH 9 fractions show less peaks in this region, this can be explained by their lower lignin content, lower DMSO solubility, and the increased aliphatic character of their lignins compared to the other fractions.

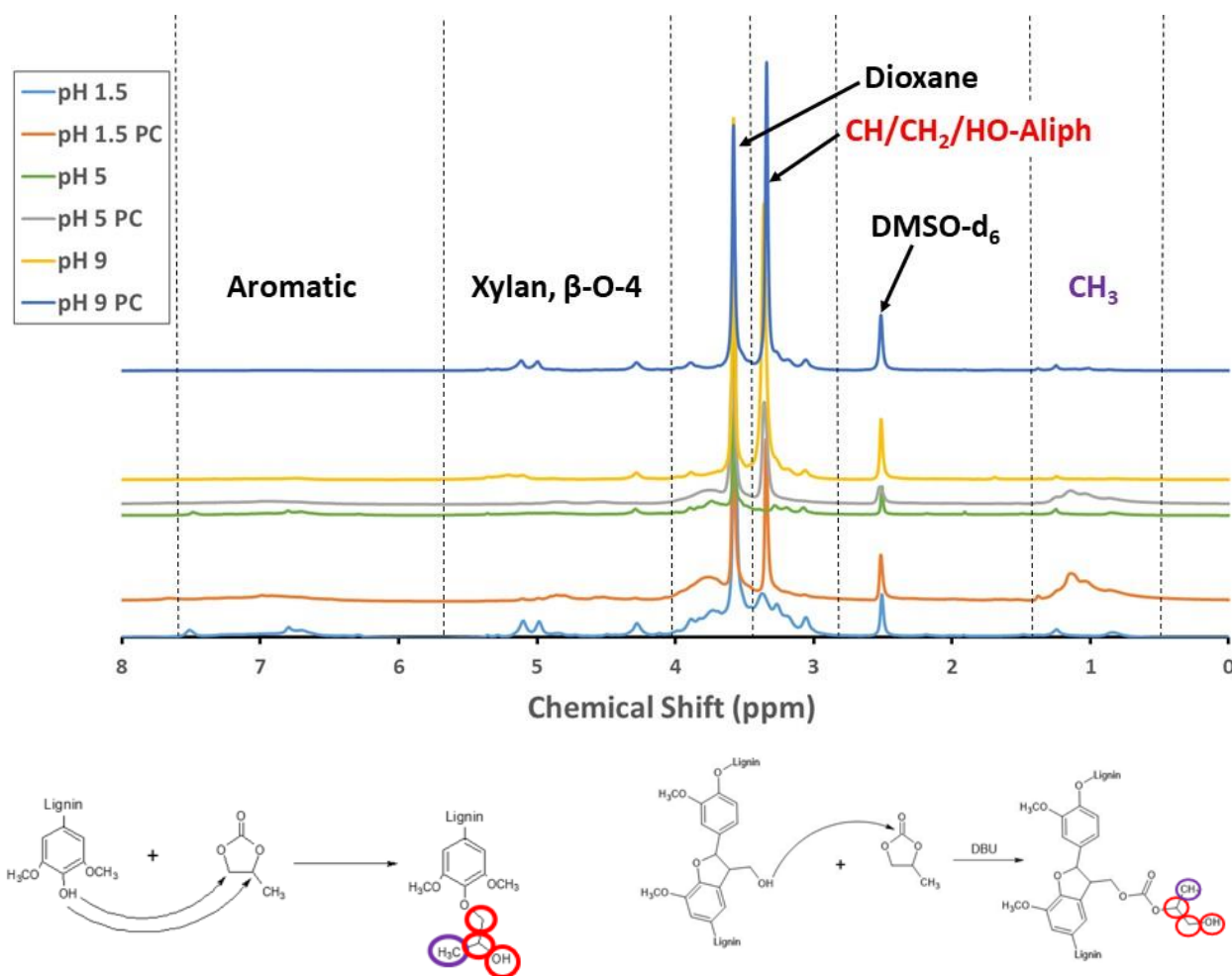


Figure 3: ^1H NMR Spectra of Unmodified and Modified Biopolymer Fractions

Figure 4 shows ^{13}C NMR data for unmodified and modified biopolymers. Peak assignments for the aromatic region of ^{13}C NMR were made according to previous literature[261]. In the aromatic region of unmodified biopolymer fractions, peaks signifying the presence of *p*-coumarate (*p*CA) (116, 130, and 144 ppm), and ferulic acid (FA) (111, 115, and 124 ppm) can be observed with especially strong *p*CA peaks in the pH 1.5 fraction. Small syringyl (104, 107 ppm) and syringyl- β -O-4 bond (86 ppm) can be resolved. A small peak signifying C5 of the guaiacyl unit can be seen at 115 ppm, all other guaiacyl peaks are very faint

or unable to be resolved. This agrees with previous literature and our previous work which shows the predominance of syringyl and *p*CA subunits over guaiacyl in corn stover lignins [132, 261].

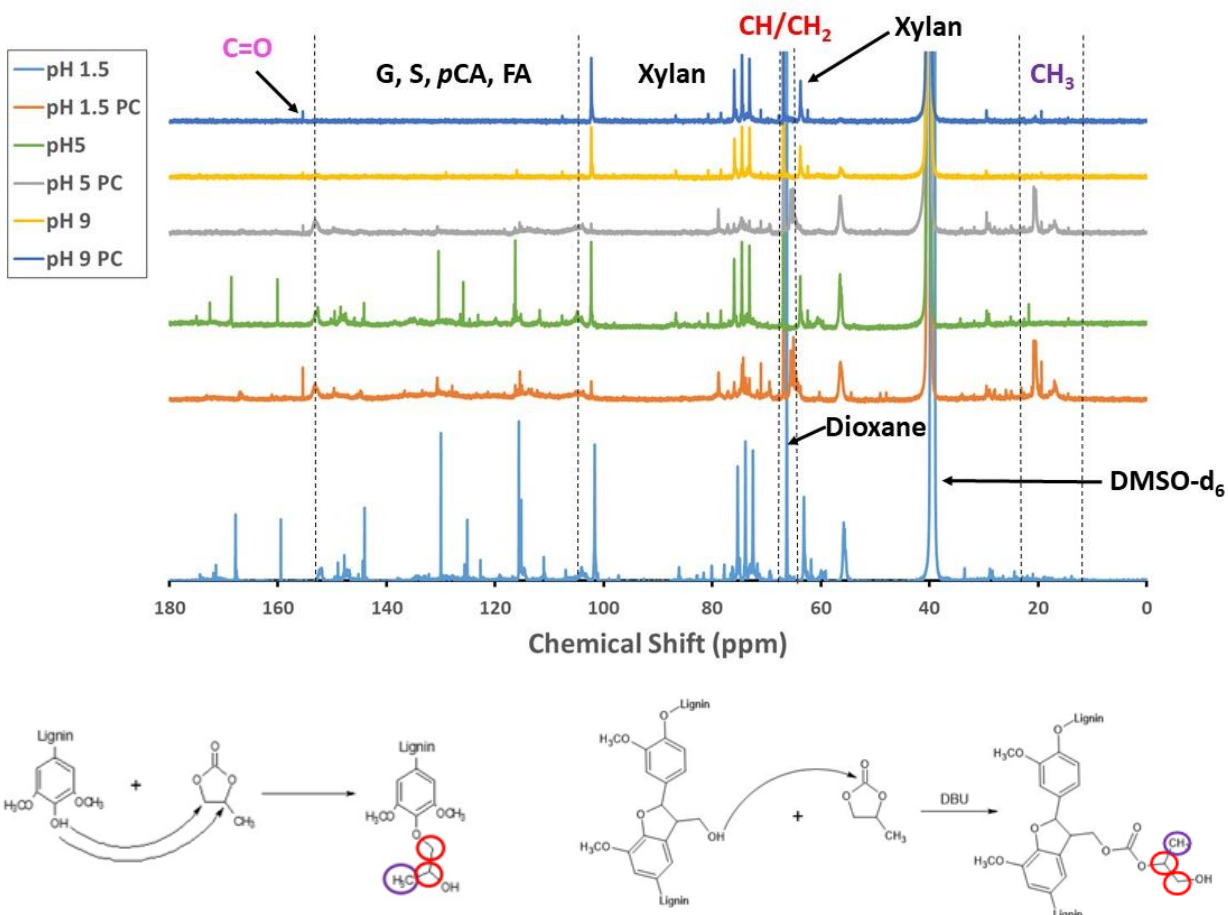


Figure 4: ^{13}C NMR Spectra of Unmodified and Modified Biopolymer Fractions

Each ^{13}C NMR spectra displays a peak at 102 ppm corresponding to xylan[262], as well as peaks at 64 ppm and 72-76 ppm region corresponding to xylan and xylan oligimers[262, 263]. Specifically, the peak at 102 ppm corresponds to C1 of xylose, the 72-76 ppm region corresponds to C2-C4, and the peak at 64 ppm belongs to C5. Biopolymer fractions precipitated at pH 1.5 and 5 show stronger xylan peaks than the pH9 fraction despite containing a lower

proportion of xylan. This discrepancy can be explained by differing solubilities and crystallinities of hemicellulose in the different fractions. Lower pH biopolymer fractions precipitated at pH 1.5 and pH 5 show a drastic reduction in signal for both the aromatic lignin carbons and xylan carbons. This is likely due to the reduced solubility of the modified lignins in DMSO. We hypothesize that this reduction in solubility is due to the reaction of propylene carbonate with both xylan and lignin hydroxyls creating aliphatic side chains with terminal hydroxyls which can then react with each other to cause cross linking ester bonds between xylan and lignin (**Scheme 2B**). These results agree with those from proton NMR (**Fig. 3**). This finding may also be supported by the appearance of new peaks at 74.2, 71, and 69 ppm in modified fractions which may correspond to bonds between xylan and lignin. This hypothesis is also reinforced by the GPC data showing a shift to higher molecular weight in pH 1.5 and pH 5 biopolymer fractions after modification further signifying the presence of crosslinking reactions during modification (**Fig. 9**).

The presence of new aliphatic carbons can be seen in the ^{13}C NMR spectra. New peaks arise in the 16-22 ppm region in the spectra of propylene carbonate modified biopolymers which can be attributed to methyl groups on lignin and xylan aliphatic side chains [232, 264]. These new aliphatic chains and lignin-lignin and lignin-xylan ester linkages can also be seen represented by the appearance of peaks from 64-66 ppm which correspond to methine and methylene carbons [232, 265]. These results agree with the results from proton NMR (**Fig. 3**). A methoxyl peak is evident in all spectra at 56 ppm. A new peak at 155 ppm appears corresponding to the new ketone carbons in the aliphatic side chains of modified lignins and xylans.

Synthesis of Lignin-PLA Composites

After characterization, modified and unmodified biopolymers were mixed with PLA in proportions of 5% and 20% w/w, melt extruded, and injection molded into rectangular and dogbone geometries. Over the course of each 5-minute extrusion, screw torque values were collected and subsequently converted to viscosity. These viscosity values over the course of the 5-minute extrusion for materials with 20% biopolymer incorporation are shown below in **Figure 5A**. Neat PLA was the most viscous material during extrusion and underwent the least significant reduction in viscosity over the course of the extrusion (**Fig. 5B**). Modified biopolymer fractions exhibited higher viscosities during extrusion than their unmodified counterparts with pH9 PC fraction undergoing a higher increase in viscosity after modification when compared to the fractions recovered at pH5 and pH1.5 (**Fig. 5A**). Modified materials also experienced a significantly lower decrease in viscosity over the course of the extrusion when compared to unmodified lignin materials (**Fig. 5B**).

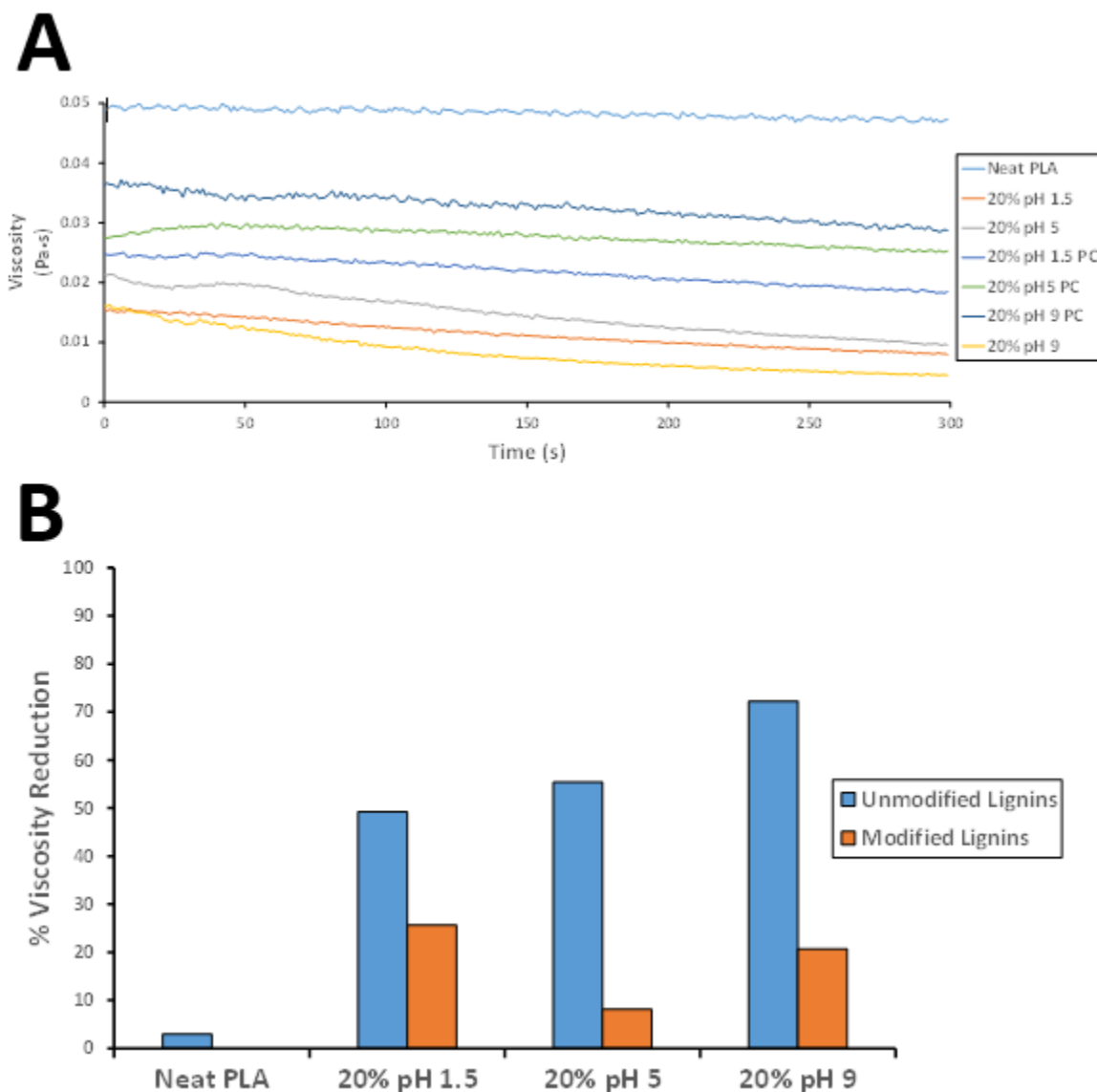


Figure 5: Viscosity during Composite Extrusion (**A**) and % Viscosity Drop during Extrusion (**B**)

Extrusion of PLA composites with our multi-component (lignin, xylan, ash, extractives) biopolymer fractions is a complex system and changes in rheology over the course of the extrusion likely arises from multifaceted sources. It is well established that PLA can undergo degradation during extrusion due to hydrolysis reactions, oxidative degradation, cis-elimination, and trans-esterification especially under high temperatures or in the presence of water[266]. This

degradation results in reduced viscosity of the material due to reduced molecular weight[266]. These depolymerization reactions are almost certainly the cause of the slight reduction in viscosity over the course of the extrusion of neat PLA (**Fig. 5**). Previous studies on the rheology of lignin-PLA composites show a decrease in viscosity with increasing lignin addition which they hypothesize is due to lignin plasticizer effects or the disruption of hydrogen bonding in the PLA network[220, 267]. These results agree with the lower viscosity we observed in all biopolymer composites (**Fig. 5A**). We hypothesize that the higher viscosity and reduced viscosity drop of modified biopolymer materials when compared to their unmodified counterparts is a result of stronger interfacial interactions[231] and increased chemical bonding[89] between modified biopolymers and PLA due to their added aliphatic hydroxyl groups (**Scheme 2**), leading to an increased molecular weight material and less degradation over the course of the extrusion. The presence of these additional crosslinking reactions during extrusion is supported by the increased molecular weight of modified biopolymer-PLA materials (**Fig. 9B**). The presence of a long shoulder at lower elution times on the GPC peaks of pH 1.5 PC and pH 5 PC which are not present in their lignin spectra indicates crosslinks between biopolymer constituent xylan, lignin, and PLA under the high temperature melt extrusion conditions. Reaction between lignins and PLA during extrusion has previously been demonstrated[221]. We hypothesize that the higher increase in viscosity after the modification of the pH 9 fraction when compared to the other fractions is due to its high hemicellulose content and the formation of lignin-xylan-PLA crosslinks during extrusion. Modified xylan may also have better affinity for PLA than modified lignin leading to a higher extent of reaction. This hypothesis is difficult to support with GPC due to the insolubility of xylan in THF.

Interfacial interactions and chemical bonding between lignin, xylan, and PLA are not the only factors that affect the changes in rheology between materials made with biopolymers. Our biopolymer fractions also showed a decrease in inorganic content after modification. A previous study investigating the effect of differing amounts of lignin inorganic impurities on lignin-PLA extruded materials found that with increasing ash content the thermal stability of lignin-PLA composites increases [220], which they attributed to inorganic catalysis leading to increased lignin-PLA crosslinking reactions. From our previous discussion we would expect this increase in thermal stability to result in increased viscosity, however the authors found the opposite with decreased viscosity as inorganic impurities increased[220]. This discrepancy highlights the complexity of examining the rheology of composite materials with multiple possible contributing factors. Ash removal during biopolymer modification may play a role in the viscosity increase in modified biopolymer materials, however the large increase in viscosity of the pH 9 PC fraction resulting in the greatest viscosity of all the modified materials (**Fig. 5A**) despite its high ash content (**Fig. 2A**) suggests that this is not the limiting factor influencing viscosity in our composite materials.

Another potential cause of the viscosity change seen in modified biopolymer materials is changes in particle size. As seen in the confocal images (**Fig. 6**), lignin particles in pH 5 and pH 1.5 fractions underwent a drastic size reduction and exhibited improved dispersity throughout the material following modification. A previous study using biocarbon has found that viscosity increases with decreasing filler particle size in PLA composites[268]. This disagrees with our findings of viscosity increase in pH 5 PC and pH 1.5 PC materials despite their reduced particle size. However, particle size changes still likely plays a role in the rheology of the composite

materials and may be a contributing factor to the heightened viscosity increase of the pH 9 PC fraction compared to the other fractions as the pH 9 PC modified fraction does not appear to undergo the same drastic particle size reduction (**Fig. 6**).

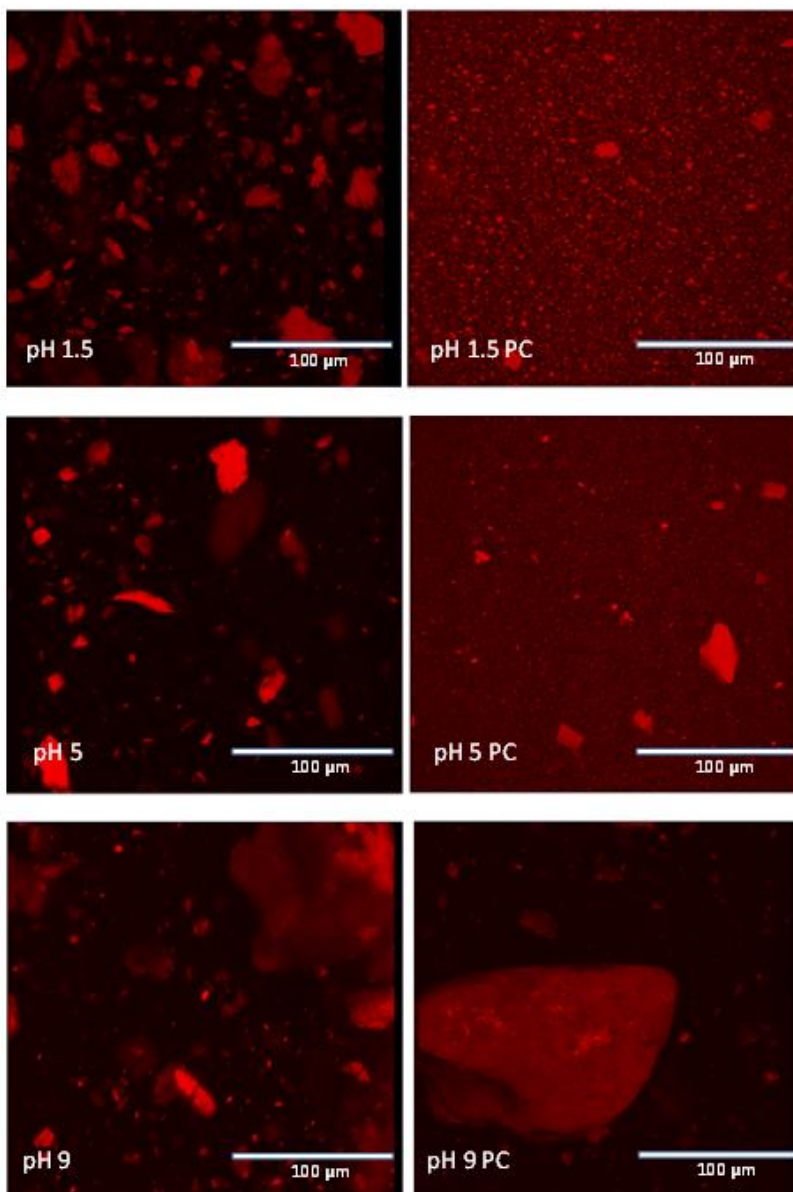


Figure 6: Confocal Images of Modified and Unmodified Biopolymer Composites

Characterization of Composite Thermal Properties

After composite manufacture, thermal and mechanical properties of extruded materials were analyzed to evaluate how each biopolymer fraction affected the feasibility of PLA materials for real-world use. Composite flexural properties were evaluated by dynamic mechanical analysis using a constant deformation amplitude and frequency over a temperature gradient. Storage modulus and loss modulus results are shown in **Figure 7**, the curves are averages of multiple replicate measurements. Storage modulus gives a good estimation of material stiffness and load bearing ability as it relates to temperature[269, 270]. In this study, we will split our discussion of the storage modulus of our composite materials into three temperature ranges: below the glass transition region (T_g), the transition region, and above T_g . As seen in **Figure 7A** 5% biopolymer-PLA composite materials exhibit relatively similar storage modulus values as temperature approaches T_g with 5% pH 5 PC and 5% pH 1.5 materials showing an increased storage modulus over neat PLA in the glass transition region. In the DMA plots of 20% biopolymer composites (**Fig. 7C**) more evident differences arrive. All but one of the composite materials have a higher storage modulus approaching T_g with 20% pH 1.5 and 20% pH 9 PC showing the highest storage modulus directly before T_g .

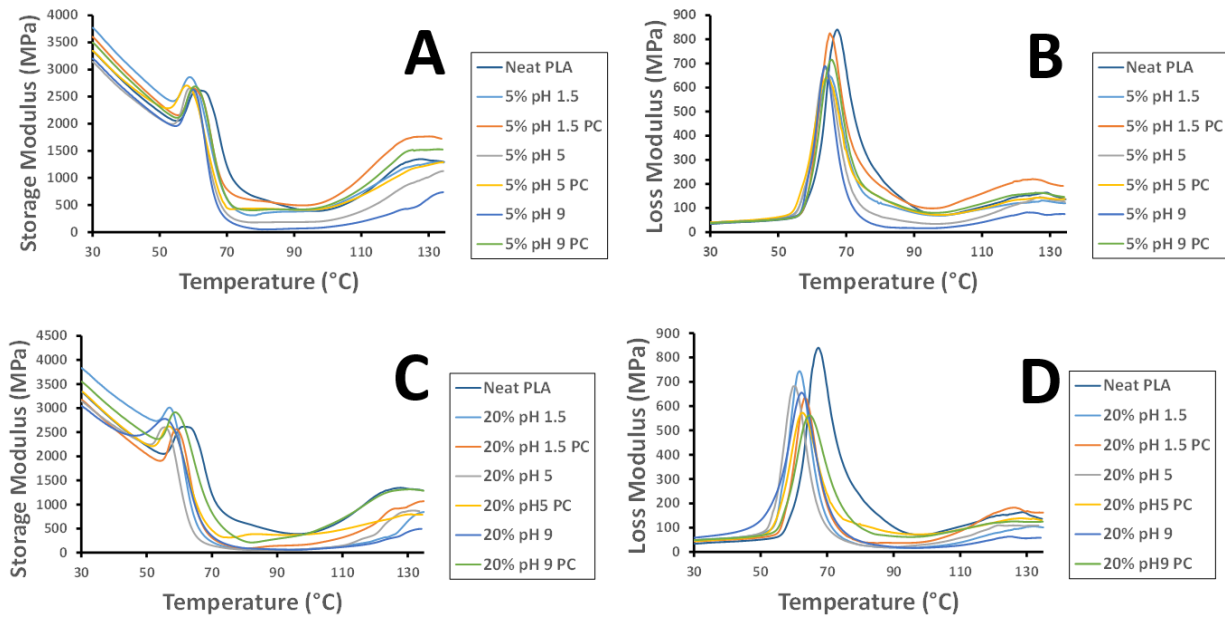


Figure 7: Results of DMA Experiments showing Storage Modulus for 5% (A) and 20% (C) Biopolymer Composites, and Loss Modulus for 5% (B) and 20% (D) Composites

As the glass transition occurs a sharp decrease in storage modulus can be seen in PLA and composite materials, this sharp drop can be attributed to increased polymer chain movement as the materials transition from a glassy to rubbery state[270]. In this transition region all composite materials at both 5% and 20% show significantly lower storage modulus than neat PLA (**Fig. 7A, 7C**). A previous study suggests that the reinforcement of PLA composites before the glass transition, but not in the transition region indicate that the increased storage modulus below T_g is caused by hydrogen bonding between PLA carboxyl groups and filler hydroxyls which are much more prevalent in the glassy state as compared to in the rubbery/transition state[271]. **Figure 7** shows a difference in the properties of different biopolymer-PLA composite materials over 80 °C. All modified biopolymer materials at 5% and 20% exhibit a higher storage modulus than their unmodified counterparts in the range of 80-130 °C, with a clear increase

between 100 and 125 °C. This increase in storage modulus can be attributed to the cold crystallization of PLA[270]. In the 5% biopolymer composites pH 1.5 PC and pH 9 PC materials surpass the storage modulus of neat PLA above 100 °C. These results correspond to these two materials having a high degree of crystallization in the DSC experiments (**Fig. 8D**).

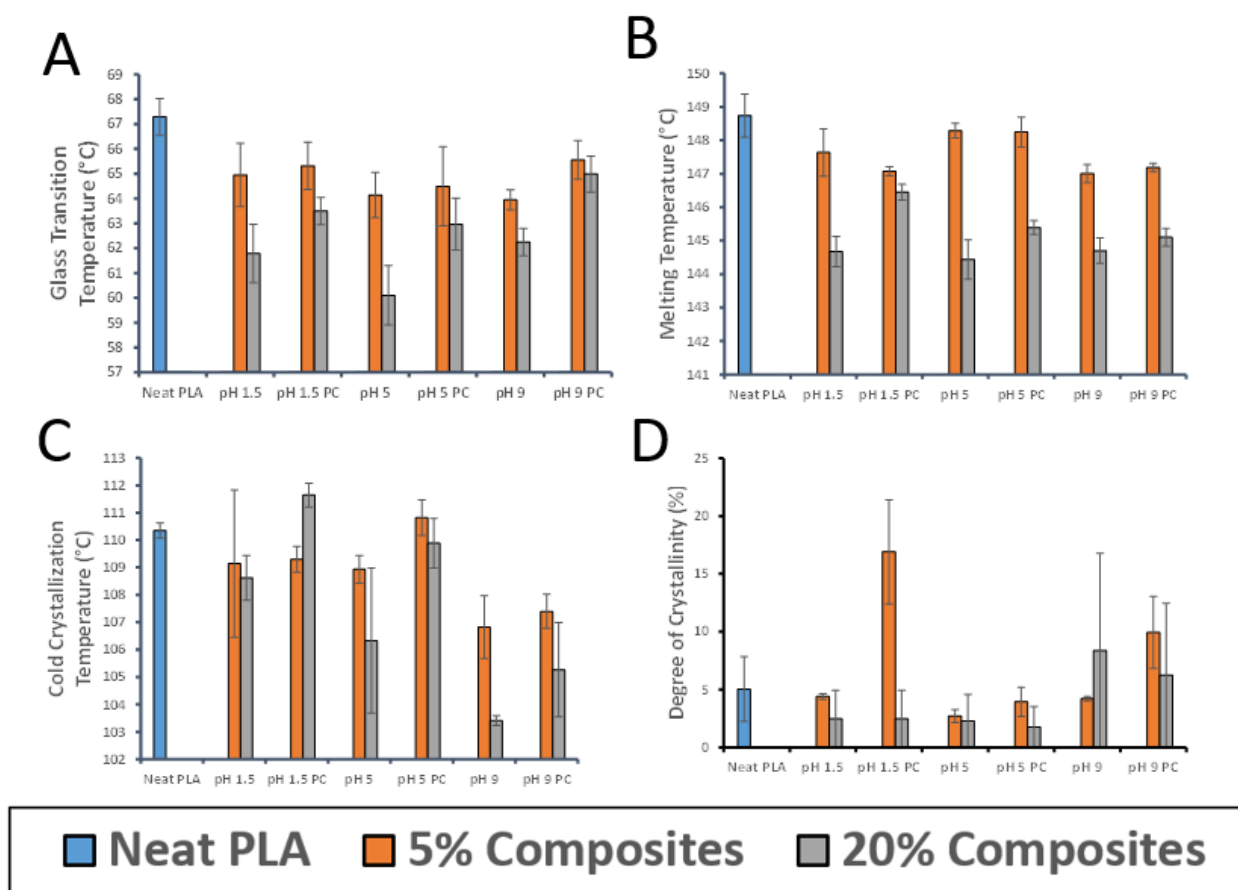


Figure 8: Glass Transition Temperature (A), Melting Temperature (B), Cold Crystallization Temperature (C), and Degree of Crystallinity (D) of 5% and 20% Composites

Material stiffness, or storage modulus, have been previously shown to increase with increasing degree of crystallinity[272]. This is an interesting finding suggesting that biopolymer properties can be tailored by chemical modification to improve the crystallization degree of PLA

materials and thus influence their mechanical properties. These composite materials show slightly different behavior at 20% lignin implementation with the 20% pH 9 PC material exhibiting a storage modulus almost identical to neat PLA above 100 °C, this result agrees with the DSC results showing that the pH9 PC material retains a high degree of crystallization at 20% (**Fig. 8D**). While the 20% pH 1.5 PC composite has a storage modulus significantly lower than neat PLA above 100 °C, it still has the highest storage modulus of all other composite materials above 120 °C (**Fig. 7C**). The reason for the higher magnitude decrease of crystallization enhancing properties of the pH 1.5 PC material at higher incorporation levels when compared to the pH 9 PC material is unknown. An interesting note in the crystallinity characterization from DSC (**Fig. 8D**) is the higher crystallinity error values seen in 20% biopolymer composites. The high variance in crystallinity measurements taken from the same samples suggests a complex crystallization process with different crystallinities throughout the polymer matrix. This can be explained by varied crosslinking reactions, different interfacial interactions leading to agglomeration, and the amount of nucleation sites provided by the mixed components of the biopolymer fractions leading to localized changes in the materials ability to crystallize. While the higher storage modulus can be directly explained by higher crystallinity in 5% pH 1.5 PC, 5% pH 9 PC, and 20% pH 9 PC materials, the increased storage modulus of all other modified materials over their unmodified counterparts despite similarly low degrees of crystallinity is harder to explain. Semi-crystalline polymers such as PLA can be described as a mixed two-phase system with ordered crystal lamella and an amorphous phase with an interphase region between them[273]. We suggest that the modulus increase of modified materials over unmodified in the cold crystallization region is due to stronger interfacial interactions between PLA crystals and

the biopolymer fillers due to increased dispersion forces, chemical bonding, and better particle dispersion.

Thermal transition temperatures taken from DMA and DSC results provide insight into changes in composite material properties resulting from different biopolymers. Transition temperature values are plotted in **Figure 8**, DSC thermograms are presented in **Supplementary Figure S1**. Glass transition temperature (T_g) was taken as the temperature at which the loss modulus reaches its maximum value. Of importance to note is the difference in T_g between modified and unmodified materials. All lignin-PLA composites had a glass transition temperature lower than neat PLA ($p < 0.05$) (**Fig. 8A**). However, in 20% composites all modified lignin materials possessed a significantly higher T_g than their unmodified counterparts ($p < 0.05$) (figure **8A**). This increase in the modified biopolymer materials is likely due to increase interfacial interactions and bonding between modified biopolymers and PLA resulting in reduced chain mobility, necessitating more energy or a higher temperature to exit the glassy state[274]. These results are further supported by the NMR and GPC result for modified biopolymers and composites (**Fig. 3, 4, and 9**). A previous study which incorporates propylene carbonate modified commercial Kraft lignin in PLA materials at inclusion levels of 1%-40% reports a conflicting trend. In their study modified lignin materials exhibited significantly lower T_g values compared to unmodified Kraft lignin materials in 20% and 40% lignin materials. They attribute this behavior to reduced hydrogen bonding ability of modified Kraft lignin due to reduction in phenolic hydroxyl content[275]. The material made with 20% pH 9 PC in this study had a significantly higher T_g than the modified fractions pH 1.5 PC and pH 5 PC ($p < 0.05$) (**Fig. 8A**). This is correlated with the viscosity and storage modulus results and can again be explained by

increased interfacial interactions and xylan-lignin-PLA crosslinking present in the xylan heavy biopolymer materials. Further insight into a materials thermal behavior can be gained by examining its cold crystallization and melting temperatures (**Figs. 8B** and **8C**). Most significant changes in these transition temperatures were seen in composites with 20% biopolymer incorporation. All 20% biopolymer composites exhibited lower melting temperature than neat PLA and their 5% biopolymer equivalents ($p < 0.05$). Trends in cold crystallization temperature are less clear. Non modified and modified composites with 20% pH 1.5 and pH 9 biopolymers had a lower crystallization temperature than neat PLA ($p < 0.05$) with 20% pH 1.5 PC having a higher crystallization temperature than 20% pH 1.5 materials ($p < 0.05$). The lessening of thermal transition temperatures when compared to neat PLA indicates that biopolymer incorporation into PLA composite materials lessens its thermal stability and may lead to struggles in high temperature applications. The lessening of T_g reduction in modified materials indicates promise for this propylene carbonate modification process for improving the thermal stability of biopolymer-PLA composites. The aforementioned study using Kraft lignin again disagrees with the trends we see in these thermal transition temperatures. They do not observe melt temperatures lower than neat PLA in modified or unmodified lignin composites even at high lignin incorporation levels ($>20\%$). They also report an increase in cold crystallization temperature over neat PLA in materials with 20% modified and unmodified Kraft lignin, with unmodified lignin materials having higher crystallization temps than unmodified[275]. The disagreement in thermal transition trends between our work and this previous study are likely due to chemical differences between the lignins used and the presence of polysaccharides in our biopolymer fractions.

All but two of the biopolymer-PLA composites had a lower magnitude of loss modulus than neat PLA ($p < 0.05$) (**Figs. 7B and 7D**). The loss modulus of a material presents its ability to dissipate energy as heat and is directly related to viscosity in the transition region [276-278]. This decrease in loss modulus from neat PLA in the DMA results of lignin materials agrees with our direct viscosity measurements during extrusion (**Fig. 5A**), although these viscosity measurements were at temperatures much higher than the transition region. However, at the level of 20% biopolymer incorporation in the composites non-modified biopolymer materials showed higher loss modulus values than their modified counterparts ($p < 0.05$), this disagrees with the results from extrusion viscosity. A study using natural fiber-reinforced PLA materials with explains that a material's loss modulus is related to the amount of energy it creates in the form of heat due to intermolecular friction during an applied load[278]. This can explain the differences in loss modulus between our modified and unmodified biopolymer materials. Modified biopolymer materials exhibit greater crosslinking, inhibiting the ability of polymer chains to move, thus causing reduced intermolecular friction. The larger, rougher, and less spherical particles in unmodified biopolymer materials as seen in the confocal imagery (**Fig. 6**) may also contribute to their higher loss modulus values due to increased friction between the rough particle surfaces and PLA.

Molar Mass Distribution of Biopolymers, Functionalized Biopolymers, and PLA-Lignin blends

Biopolymer fractions were next analyzed for molar mass distributions via gel permeation chromatography (GPC) (**Fig. 9**). Additionally, acetylated version of unmodified fractions were also analyzed due to low solubility in THF (**Fig. 9C**). These acetylated biopolymers exhibit bimodal peaks with a higher molecular weight peak attributable to lignin and a lower molecular

weight peak that may be attributable to xylan. In spectra of unmodified and modified biopolymer fractions and composites directly solubilized in THF, lignin peaks are present while xylan peaks are not due to low solubility in THF. For this reason, we can only comment on relative molecular weights and crosslinking of the lignin portions of modified biopolymer fractions and biopolymer-PLA materials. The pH 9 fraction is composed primarily of xylan (**Fig. 8C**) with a portion of its lignin component being higher molecular weight than the lower pH fractions. Biopolymer fractions recovered at pH 1.5 and pH 5 are shown to contain both lignin and xylan components with the pH 1.5 fraction lignin component being less polydisperse and lower molecular weight than the pH 5 fraction. These results agree with those of previous studies showing the precipitation of lower molecular weight lignins at lower pH [43, 44, 50].

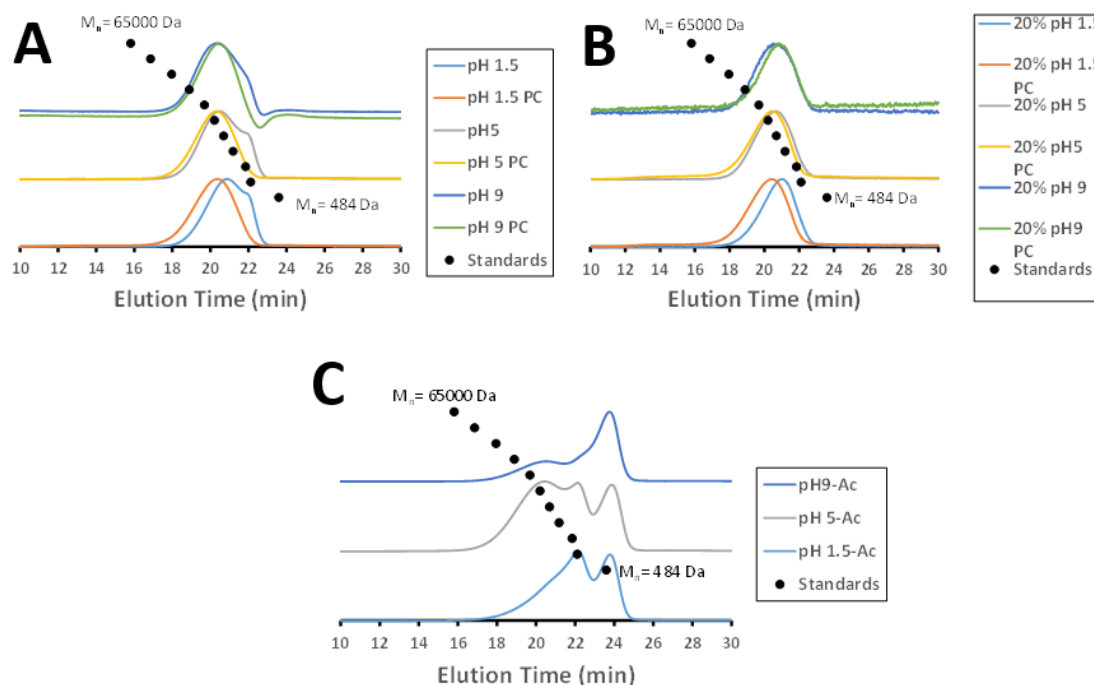


Figure 9: GPC Spectra of Modified and Unmodified Biopolymer Fractions (A), Composite Materials (B), and Acetylated Unmodified Biopolymers (C)

Figure 8A shows molar mass distributions of THF-soluble portions of both modified and unmodified biopolymer fractions. After modification the lignin portions of pH 1.5 and 5 fractions appear to undergo a reduction in polydispersity and shift to higher molecular weight. This suggests that in addition to the modification of hydroxyl groups with propylene carbonate (**Scheme 1**) these biopolymer fractions likely experienced lignin-lignin or lignin-xylan crosslinking reactions during the modification procedure as previously suggested by NMR characterization (**Scheme 2, Fig. 3 and 4**). The GPC spectra of the THF soluble portion of the pH 9 and pH 9 PC fractions show a less obvious molecular weight shift. This is likely due to the low signal intensity of these fractions during GPC analysis due to their low lignin content or may indicate the low solubility of the lignin portion of the pH 9 fraction during the functionalization reaction suggesting that most of the property changes of pH 9 PC modified materials are due to the modification of their xylan component.

Figure 8B shows GPC spectra of THF soluble portions of 20% biopolymer-PLA composites with both modified and unmodified biopolymer fractions. In addition to the shifts in molecular weight seen with the pH 1.5 PC and pH 5 PC biopolymer fractions, pH 1.5 PC and pH 5 PC modified composite GPC spectra contain a long tail at lower elution times indicating higher molecular weight material. This suggests that these modified materials undergo additional crosslinking during extrusion forming chemical bonds between lignin and PLA and forming lignin-xylan-PLA complexes (**Scheme 2**). This enhanced crosslinking behavior of modified biopolymer fractions during extrusion is one mechanism behind their improved thermal and mechanical properties. Large differences were not observed in the pH9 and pH9 PC composite materials. This is likely due to the previously mentioned low solubility of the xylan portion of

these fractions in THF which reacted with propylene carbonate and is the main contributor to any crosslinking of these materials during extrusion.

Composite Mechanical Properties

Materials manufactured with 20% incorporation of biopolymers were evaluated for mechanical properties via tensile testing. The results of these tests in the form of tensile strength, tensile modulus, and strain at break are presented in **Figure 10**. Unfortunately, tensile tests were unable to be conducted with the unmodified 20% pH 9 material due to the brittle nature of this material resulting in fracturing of the dogbones when clamped for tensile testing. All composite materials displayed a reduced tensile strength with respect to neat PLA ($p < 0.05$) (**Fig. 10A**). A reduction in tensile strength of PLA with increasing incorporation of lignin is widely reported in the literature [219, 220, 279-281]. This phenomenon is generally explained by the incompatibility of lignin and PLA leading to poor interfacial adhesion and poor lignin dispersity. The pH 9 PC material has the highest tensile strength of all the composites with a significantly higher ($p < 0.05$) tensile strength than the pH 1.5 PC and pH 5 PC material (**Fig. 10A**). The pH 5 PC material also exhibits a higher tensile strength ($p < 0.05$) than its unmodified counterpart. These trends in tensile strength mirror those of viscosity during extrusion and material T_g . This suggests that tensile strength is also related to the strength of interfacial interactions between the biopolymer fractions and PLA and the degree of crosslinking which occurs during biopolymer modification and during extrusion.

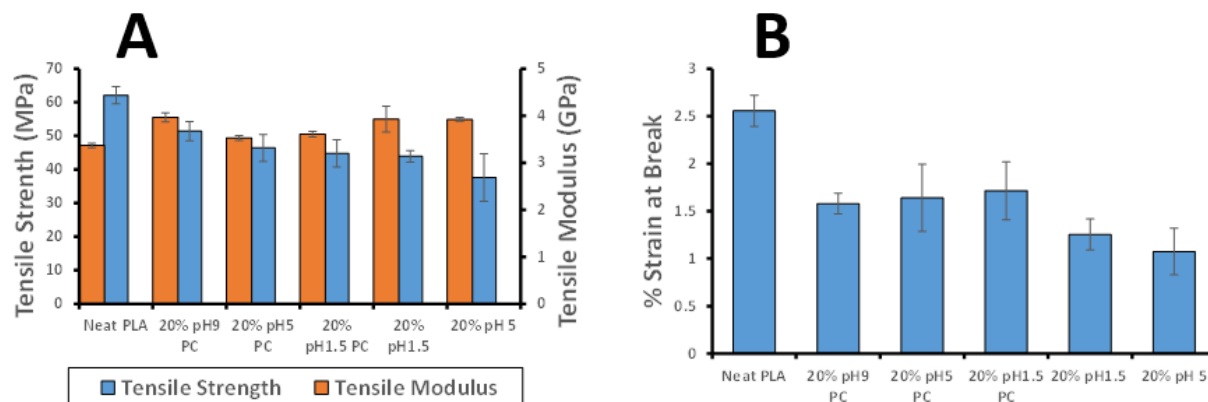


Figure 10: Mechanical Properties: Tensile Strength and Modulus (**A**), and Strain at Break (**B**) for 20% Composite Materials

Biopolymer composites all exhibited a significantly higher tensile modulus than neat PLA ($p < 0.05$) (**Fig. 10A**). Tensile modulus is a measure of the stiffness of a material. These results indicating an increase in stiffness upon the addition of biopolymer to PLA composites agrees with our results for the increase in storage modulus below T_g and the results of other studies with lignin-PLA[85, 220] and cellulose-PLA[237, 238, 282] composite materials. The pH 9 PC material exhibited a higher modulus than pH 1.5 PC and pH 5 PC materials ($p < 0.05$) again reinforcing that this xylan-heavy fraction has increased interfacial interactions with PLA due to functionalization of xylan and underwent greater xylan-lignin-PLA crosslinking during extrusion when compared to the other modified fractions. Interestingly, the unmodified pH5 fraction material possessed a higher modulus than its modified counterpart ($p < 0.05$). This result differs from the storage modulus results from DMA. The reason for this discrepancy is unknown.

Ductility of a material can be presented by its percent strain or elongation at the breaking point of a tensile test. These results for neat PLA and all composite materials are given in **Figure 10B**. Neat PLA was more ductile than all the composite materials ($p < 0.05$). However, all modified

biopolymer materials had a larger percent strain at break than the unmodified biopolymer materials ($p < 0.05$). This behavior can again be explained by the improved interfacial compatibility of the modified biopolymer fractions[283].

The mechanical properties of our biopolymer-PLA materials differ from those reported in the Kraft lignin paper. Materials made with Kraft lignin did not exhibit increased tensile modulus, and a reduction in tensile strength of modified Kraft lignin materials was seen in 20% materials. At 20% lignin incorporation a reduction in elongation at break of modified Kraft lignin materials was also reported[275]. These differences in the mechanical properties of materials made with our biopolymer fractions and those made with an industrial Kraft lignin illustrates the influence that the presence of polysaccharides and different lignin chemistries can have on the compatibility of PLA with biomass biopolymers.

Conclusions

Three biopolymer fractions were recovered from the pH adjustment and fractional precipitation of alkali liquor from corn stover. These biopolymer fractions containing primarily lignin, xylan, and inorganics were modified by reaction with propylene carbonate. Modified fractions were characterized by ^1H NMR, ^{13}C NMR, and GPC to verify the modification of lignin and xylan hydroxyl groups. PLA composites with the incorporation of 5% and 20% recovered biopolymer were manufactured via melt extrusion and injection molding. Modification of biopolymer fractions was found to increase material stiffness, T_g , tensile strength, percent strain at break, and dispersion of biopolymer throughout the composite. Materials made with the xylan heavy fraction recovered at the highest pH (pH9) exhibited the highest tensile strength, storage modulus, and T_g . Improvement of material properties after biopolymer modification was

hypothesized to be a result of increased interfacial interactions in the form of chemical bonding and van der Waals interactions with PLA and crosslinking during extrusion. The xylan-heavy fraction was hypothesized to have the largest improvement in properties after modification due to high affinity of propylene carbonate reaction with xylan and the formation of xylan-lignin-PLA crosslinked complexes during extrusion.

Supplementary Data

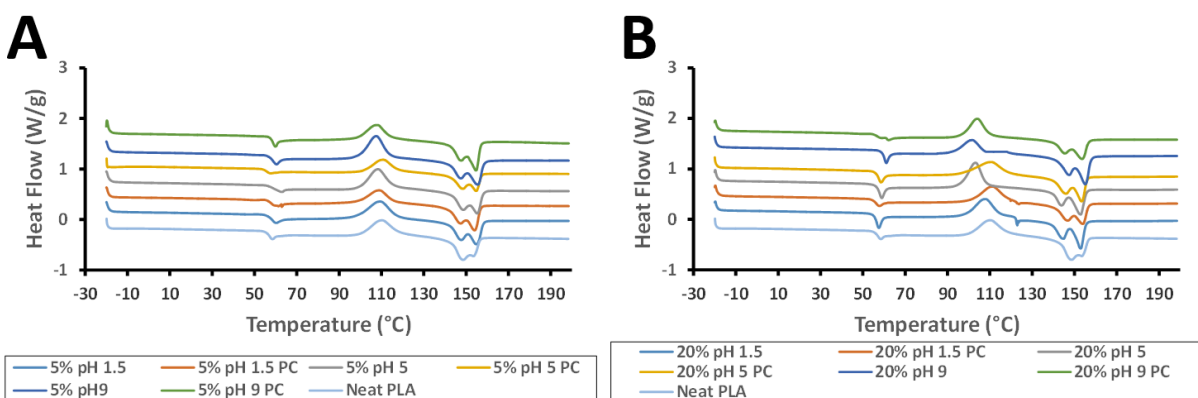


Figure S1: DSC Thermograms of 5% (A) and 20% (B) Composite Materials

References

4. Ragauskas, A.J., G.T. Beckham, M.J. Bidy, R. Chandra, F. Chen, M.F. Davis, B.H. Davison, R.A. Dixon, P. Gilna, M. Keller, P. Langan, A.K. Naskar, J.N. Saddler, T.J. Tschaplinski, G.A. Tuskan, and C.E. Wyman, *Lignin valorization: improving lignin processing in the biorefinery*. Science, 2014. **344**(6185): p. 1246843.
7. Li, Q., C. Hu, M. Li, P. Truong, J. Li, H.-S. Lin, M.T. Naik, S. Xiang, B.E. Jackson, and W. Kuo, *Enhancing the multi-functional properties of renewable lignin carbon fibers via defining the structure–property relationship using different biomass feedstocks*. Green Chemistry, 2021. **23**(10): p. 3725-3739.

8. Li, J., G. Gellerstedt, and K. Toven, *Steam explosion lignins; their extraction, structure and potential as feedstock for biodiesel and chemicals*. Bioresource Technology, 2009. **100**(9): p. 2556-2561.
9. Zijlstra, D.S., C.A. Analbers, J. Korte, E. Wilbers, and P.J. Deuss, *Efficient Mild Organosolv Lignin Extraction in a Flow-Through Setup Yielding Lignin with High β -O-4 Content*. Polymers (Basel), 2019. **11**(12).
14. Gioia, C., M. Colonna, A. Tagami, L. Medina, O. Sevastyanova, L.A. Berglund, and M. Lawoko, *Lignin-Based Epoxy Resins: Unravelling the Relationship between Structure and Material Properties*. Biomacromolecules, 2020. **21**(5): p. 1920-1928.
15. Ghorbani, M., F. Liebner, H.W.G. van Herwijnen, L. Pfungen, M. Krahofer, E. Budjav, and J. Konnerth, *Lignin Phenol Formaldehyde Resoles: The Impact of Lignin Type on Adhesive Properties*. 2016, 2016. **11**(3): p. 15.
16. Li, Q., C. Hu, M. Li, P. Truong, M.T. Naik, D. Prabhu, L. Hoffmann, W.L. Rooney, and J.S. Yuan, *Discovering Biomass Structural Determinants Defining the Properties of Plant-Derived Renewable Carbon Fiber*. iScience, 2020. **23**(8): p. 101405.
43. Wang, G. and H. Chen, *Fractionation of alkali-extracted lignin from steam-exploded stalk by gradient acid precipitation*. Separation and Purification Technology, 2013. **105**: p. 98-105.
44. Lourençon, T.V., F.A. Hansel, T.A. da Silva, L.P. Ramos, G.I.B. de Muniz, and W.L.E. Magalhães, *Hardwood and softwood kraft lignins fractionation by simple sequential acid precipitation*. Separation and Purification Technology, 2015. **154**: p. 82-88.
45. Du, B., Y. Bai, Z. Pan, J. Xu, Q. Wang, X. Wang, G. Lv, and J. Zhou, *pH fractionated lignin for the preparation of lignin-based magnetic nanoparticles for the removal of methylene blue dye*. Separation and Purification Technology, 2022. **295**: p. 121302.
46. García, A., A. Toledano, L. Serrano, I. Egüés, M. González, F. Marín, and J. Labidi, *Characterization of lignins obtained by selective precipitation*. Separation and Purification Technology, 2009. **68**(2): p. 193-198.
47. Brenelli, L.B., F. Mandelli, A.Z. Mercadante, G.J.d.M. Rocha, S.A. Rocco, A.F. Craievich, A.R. Gonçalves, D.d.C. Centeno, M. de Oliveira Neto, and F.M. Squina, *Acidification treatment of lignin from sugarcane bagasse results in fractions of reduced polydispersity and high free-radical scavenging capacity*. Industrial Crops and Products, 2016. **83**: p. 94-103.
48. Gilarranz, M.A., F. Rodriguez, M. Oliet, and J.A. Revenga, *Acid Precipitation and Purification of Wheat Straw Lignin*. Separation Science and Technology, 1998. **33**(9): p. 1359-1377.

49. Domínguez-Robles, J., E. Espinosa, D. Savy, A. Rosal, and A. Rodriguez, *Biorefinery Process Combining Specel® Process and Selective Lignin Precipitation using Mineral Acids*. 2016, 2016. **11**(3): p. 17.
50. Stoklosa, R.J., J. Velez, S. Kelkar, C.M. Saffron, M.C. Thies, and D.B. Hodge, *Correlating lignin structural features to phase partitioning behavior in a novel aqueous fractionation of softwood Kraft black liquor*. Green Chemistry, 2013.
51. Goldmann, W.M., J. Ahola, M. Mikola, and J. Tanskanen, *Solubility and fractionation of Indulin AT kraft lignin in ethanol-water media*. Separation and Purification Technology, 2019. **209**: p. 826-832.
53. Klett, A.S., P.V. Chappell, and M.C. Thies, *Recovering ultraclean lignins of controlled molecular weight from Kraft black-liquor lignins*. Chemical Communications, 2015. **51**(64): p. 12855-12858.
54. Zhang, H., Y. Bai, B. Yu, X. Liu, and F. Chen, *A practicable process for lignin color reduction: fractionation of lignin using methanol/water as a solvent*. Green Chemistry, 2017. **19**(21): p. 5152-5162.
55. Meng, X., A. Parikh, B. Seemala, R. Kumar, Y. Pu, C.E. Wyman, C.M. Cai, and A.J. Ragauskas, *Characterization of fractional cuts of co-solvent enhanced lignocellulosic fractionation lignin isolated by sequential precipitation*. Bioresource Technology, 2019. **272**: p. 202-208.
57. Chambon, C.L., V. Fitriyanti, P. Verdía, S.M. Yang, S. Hérou, M.-M. Titirici, A. Brandt-Talbot, P.S. Fennell, and J.P. Hallett, *Fractionation by Sequential Antisolvent Precipitation of Grass, Softwood, and Hardwood Lignins Isolated Using Low-Cost Ionic Liquids and Water*. ACS Sustainable Chemistry & Engineering, 2020. **8**(9): p. 3751-3761.
58. Xu, Y.-H., P. Zeng, M.-F. Li, J. Bian, and F. Peng, *γ -Valerolactone/water system for lignin fractionation to enhance antibacterial and antioxidant capacities*. Separation and Purification Technology, 2021. **279**: p. 119780.
59. Duval, A., F. Vilaplana, C. Crestini, and M. Lawoko, *Solvent screening for the fractionation of industrial kraft lignin*. Holzforschung, 2016. **70**(1): p. 11-20.
60. Li, M.-F., S.-N. Sun, F. Xu, and R.-C. Sun, *Sequential solvent fractionation of heterogeneous bamboo organosolv lignin for value-added application*. Separation and Purification Technology, 2012. **101**: p. 18-25.
61. Hemmingson, J.A., *Steam-Explosion Lignins: Fractionation, Composition, Structure and Extractives*. Journal of Wood Chemistry and Technology, 1987. **7**(4): p. 527-553.

62. Jiang, X., D. Savithri, X. Du, S. Pawar, H. Jameel, H.-m. Chang, and X. Zhou, *Fractionation and characterization of kraft lignin by sequential precipitation with various organic solvents*. ACS Sustainable Chemistry & Engineering, 2017. **5**(1): p. 835-842.
63. Arshanitsa, A., J. Ponomarenko, T. Dizhbite, A. Andersone, R.J.A. Gosselink, J.v.d. Putten, M. Lauberts, and G. Telysheva, *Fractionation of technical lignins as a tool for improvement of their antioxidant properties*. Journal of Analytical and Applied Pyrolysis, 2013. **103**: p. 78-85.
85. Gordobil, O., R. Delucis, I. Egüés, and J. Labidi, *Kraft lignin as filler in PLA to improve ductility and thermal properties*. Industrial Crops and Products, 2015. **72**: p. 46-53.
86. Gordobil, O., I. Egüés, R. Llano-Ponte, and J. Labidi, *Physicochemical properties of PLA lignin blends*. Polymer Degradation and Stability, 2014. **108**: p. 330-338.
87. Gordobil, O., I. Egüés, and J. Labidi, *Modification of Eucalyptus and Spruce organosolv lignins with fatty acids to use as filler in PLA*. Reactive and Functional Polymers, 2016. **104**: p. 45-52.
89. Zhang, N., M. Zhao, G. Liu, J. Wang, Y. Chen, and Z. Zhang, *Alkylated lignin with graft copolymerization for enhancing toughness of PLA*. Journal of Materials Science, 2022. **57**(19): p. 8687-8700.
90. Zhu, J., L. Xue, W. Wei, C. Mu, M. Jiang, and Z. Zhou, *Modification of Lignin with Silane Coupling Agent to Improve the Interface of Poly(L-lactic) Acid/Lignin Composites*. 2015, 2015. **10**(3): p. 11.
91. Qian, S., K. Sheng, K. Yu, L. Xu, and C.A. Fontanillo Lopez, *Improved properties of PLA biocomposites toughened with bamboo cellulose nanowhiskers through silane modification*. Journal of Materials Science, 2018. **53**(15): p. 10920-10932.
92. Zhang, R., X. Xiao, Q. Tai, H. Huang, and Y. Hu, *Modification of lignin and its application as char agent in intumescent flame-retardant poly (lactic acid)*. Polymer Engineering & Science, 2012. **52**(12): p. 2620-2626.
105. Hatfield, R.D., J. Ralph, and J.H. Grabber, *Cell wall cross-linking by ferulates and diferulates in grasses*. Journal of the Science of Food and Agriculture, 1999. **79**(3): p. 403-407.
132. Saulnier, B.K., T. Phongpreecha, S.K. Singh, and D.B. Hodge, *Impact of dilute acid pretreatment conditions on p-coumarate removal in diverse maize lines*. Bioresource Technology, 2020. **314**: p. 123750.
138. Li, M., D.L. Williams, M. Heckwolf, N. de Leon, S. Kaeppler, R.W. Sykes, and D. Hodge, *Prediction of Cell Wall Properties and Response to Deconstruction Using Alkaline*

- Pretreatment in Diverse Maize Genotypes Using Py-MBMS and NIR*. BioEnergy Research, 2017. **10**(2): p. 329-343.
140. Crowe, J.D., N. Feringa, S. Pattathil, B. Merritt, C. Foster, D. Dines, R.G. Ong, and D.B. Hodge, *Identification of developmental stage and anatomical fraction contributions to cell wall recalcitrance in switchgrass*. Biotechnology for Biofuels, 2017. **10**(1): p. 184.
 150. Sluiter, A., B. Hames, R. Ruiz, C. Scarlata, J. Sluiter, D. Templeton, and D. Crocker, *Determination of structural carbohydrates and lignin in biomass*. Laboratory analytical procedure, 2008. **1617**(1): p. 1-16.
 163. Karp, E.M., B.S. Donohoe, M.H. O'Brien, P.N. Ciesielski, A. Mittal, M.J. Bidy, and G.T. Beckham, *Alkaline Pretreatment of Corn Stover: Bench-Scale Fractionation and Stream Characterization*. ACS Sustainable Chemistry & Engineering, 2014. **2**(6): p. 1481-1491.
 181. Humpert, D., M. Ebrahimi, and P. Czermak, *Membrane Technology for the Recovery of Lignin: A Review*. Membranes (Basel), 2016. **6**(3).
 182. Weinwurm, F., A. Drljo, W. Waldmüller, B. Fiala, J. Niedermayer, and A. Friedl, *Lignin concentration and fractionation from ethanol organosolv liquors by ultra- and nanofiltration*. Journal of Cleaner Production, 2016. **136**: p. 62-71.
 193. Sadeghifar, H. and A. Ragauskas, *Perspective on Technical Lignin Fractionation*. ACS Sustainable Chemistry & Engineering, 2020. **8**(22): p. 8086-8101.
 194. Helander, M., H. Theliander, M. Lawoko, G. Henriksson, L. Zhang, and M.E. Lindström, *Fractionation of Technical Lignin: Molecular Mass and pH Effects*. 2013, 2013. **8**(2): p. 13.
 204. Becker, J. and C. Wittmann, *A field of dreams: Lignin valorization into chemicals, materials, fuels, and health-care products*. Biotechnology Advances, 2019. **37**(6): p. 107360.
 205. Pang, T., G. Wang, H. Sun, W. Sui, and C. Si, *Lignin fractionation: Effective strategy to reduce molecule weight dependent heterogeneity for upgraded lignin valorization*. Industrial Crops and Products, 2021. **165**: p. 113442.
 206. Wang, G., X. Liu, B. Yang, C. Si, A.M. Parvez, J. Jang, and Y. Ni, *Using Green γ -Valerolactone/Water Solvent To Decrease Lignin Heterogeneity by Gradient Precipitation*. ACS Sustainable Chemistry & Engineering, 2019. **7**(11): p. 10112-10120.
 207. Yoo, C.G. and A.J. Ragauskas, *Opportunities and challenges of lignin utilization*. Lignin Utilization Strategies: From Processing to Applications, 2021: p. 1-12.

208. Alinejad, M., C. Henry, S. Nikafshar, A. Gondaliya, S. Bagheri, N. Chen, S.K. Singh, D.B. Hodge, and M. Nejad *Lignin-Based Polyurethanes: Opportunities for Bio-Based Foams, Elastomers, Coatings and Adhesives*. Polymers, 2019. **11**.
209. Merewether, J., *Lignin XIV. The Precipitation of Lignin from Kraft Black Liquor*. 1961.
210. Tomani, P., *The lignoboost process*. Cellulose Chemistry and Technology, 2010. **44**.
211. Hubbe, M.A., R. Alén, M. Paleologou, M. Kannangara, and J. Kihlman, *Lignin recovery from spent alkaline pulping liquors using acidification, membrane separation, and related processing steps: A review*. BioResources, 2019. **14**(1): p. 2300-2351.
212. Ma, P., Y. Gao, and H. Zhai, *Fractionated Wheat Straw Lignin and Its Application as Antioxidant*. 2013, 2013. **8**(4): p. 15.
213. Boeriu, C.G., F.I. Fițigău, R.J.A. Gosselink, A.E. Frissen, J. Stoutjesdijk, and F. Peter, *Fractionation of five technical lignins by selective extraction in green solvents and characterisation of isolated fractions*. Industrial Crops and Products, 2014. **62**: p. 481-490.
214. Jääskeläinen, A.S., T. Liitiä, A. Mikkelsen, and T. Tamminen, *Aqueous organic solvent fractionation as means to improve lignin homogeneity and purity*. Industrial Crops and Products, 2017. **103**: p. 51-58.
215. Duval, A. and L. Avérous, *Dihydrolevoglucosenone (Cyrene™) as a versatile biobased solvent for lignin fractionation, processing, and chemistry*. Green Chemistry, 2022. **24**(1): p. 338-349.
216. Allegretti, C., S. Fontanay, Y. Krauke, M. Luebbert, A. Strini, J. Troquet, S. Turri, G. Griffini, and P. D'Arrigo, *Fractionation of Soda Pulp Lignin in Aqueous Solvent through Membrane-Assisted Ultrafiltration*. ACS Sustainable Chemistry & Engineering, 2018. **6**(7): p. 9056-9064.
217. Elsayy, M.A., K.-H. Kim, J.-W. Park, and A. Deep, *Hydrolytic degradation of polylactic acid (PLA) and its composites*. Renewable and Sustainable Energy Reviews, 2017. **79**: p. 1346-1352.
218. Zhai, S., Q. Liu, Y. Zhao, H. Sun, B. Yang, and Y. Weng, *A review: Research progress in modification of poly (lactic acid) by lignin and cellulose*. Polymers, 2021. **13**(5): p. 776.
219. Anwer, M.A.S., H.E. Naguib, A. Celzard, and V. Fierro, *Comparison of the thermal, dynamic mechanical and morphological properties of PLA-Lignin & PLA-Tannin particulate green composites*. Composites Part B: Engineering, 2015. **82**: p. 92-99.
220. Gao, Y., W. Qu, Y. Liu, H. Hu, E. Cochran, and X. Bai, *Agricultural residue-derived lignin as the filler of polylactic acid composites and the effect of lignin purity on the composite performance*. Journal of Applied Polymer Science, 2019. **136**(35): p. 47915.

221. Mimini, V., E. Sykacek, S.N.A. Syed Hashim, J. Holzweber, H. Hettegger, K. Fackler, A. Potthast, N. Mundigler, and T. Rosenau, *Compatibility of kraft lignin, organosolv lignin and lignosulfonate with PLA in 3D printing*. Journal of Wood Chemistry and Technology, 2019. **39**(1): p. 14-30.
222. Cavallo, E., X. He, F. Luzi, F. Dominici, P. Cerrutti, C. Bernal, M.L. Foresti, L. Torre, and D. Puglia, *UV Protective, antioxidant, antibacterial and compostable polylactic acid composites containing pristine and chemically modified lignin nanoparticles*. molecules, 2020. **26**(1): p. 126.
223. Karp, E.M., M.G. Resch, B.S. Donohoe, P.N. Ciesielski, M.H. O'Brien, J.E. Nill, A. Mittal, M.J. Biddu, and G.T. Beckham, *Alkaline pretreatment of switchgrass*. ACS Sustainable Chemistry & Engineering, 2015. **3**(7): p. 1479-1491.
224. Kuhn, E.M., M.H. O'Brien, P.N. Ciesielski, and D.J. Schell, *Pilot-scale batch alkaline pretreatment of corn stover*. ACS Sustainable Chemistry & Engineering, 2016. **4**(3): p. 944-956.
225. Triwulandari, E., M. Ghazali, D. Sondari, M. Septiyanti, Y. Sampora, Y. Meliana, S. Fahmiati, W.K. Restu, and A. Haryono, *Effect of lignin on mechanical, biodegradability, morphology, and thermal properties of polypropylene/polylactic acid/lignin biocomposite*. Plastics, Rubber and Composites, 2019. **48**(2): p. 82-92.
226. da Silva, T.F., F. Menezes, L.S. Montagna, A.P. Lemes, and F.R. Passador, *Effect of lignin as accelerator of the biodegradation process of poly (lactic acid)/lignin composites*. Materials Science and Engineering: B, 2019. **251**: p. 114441.
227. Park, S.Y., J.-Y. Kim, H.J. Youn, and J.W. Choi, *Utilization of lignin fractions in UV resistant lignin-PLA biocomposites via lignin-lactide grafting*. International journal of biological macromolecules, 2019. **138**: p. 1029-1034.
228. Kumar Singla, R., S.N. Maiti, and A.K. Ghosh, *Crystallization, Morphological, and Mechanical Response of Poly(Lactic Acid)/Lignin-Based Biodegradable Composites*. Polymer-Plastics Technology and Engineering, 2016. **55**(5): p. 475-485.
229. Hong, S.-H., J.H. Park, O.Y. Kim, and S.-H. Hwang, *Preparation of chemically modified lignin-reinforced PLA biocomposites and their 3D printing performance*. Polymers, 2021. **13**(4): p. 667.
230. Anugwom, I., V. Lahtela, M. Kallioinen, and T. Kärki, *Lignin as a functional additive in a biocomposite: Influence on mechanical properties of polylactic acid composites*. Industrial Crops and Products, 2019. **140**: p. 111704.
231. Parit, M. and Z. Jiang, *Towards lignin derived thermoplastic polymers*. International Journal of Biological Macromolecules, 2020. **165**: p. 3180-3197.

232. Kühnel, I., J. Podschun, B. Saake, and R. Lehnen, *Synthesis of lignin polyols via oxyalkylation with propylene carbonate*. *Holzforschung*, 2015. **69**(5): p. 531-538.
233. Buysch, H.-J., *Carbonic Esters*, in *Ullmann's Encyclopedia of Industrial Chemistry*.
234. Sluiter, A., B. Hames, R. Ruiz, C. Scarlata, J. Sluiter, and D. Templeton, *Determination of ash in biomass (NREL/TP-510-42622)*. National Renewable Energy Laboratory, Golden, 2005. **19**.
235. International, A., *ASTM D638-14, Standard Test Method for Tensile Properties of Plastics*. 2015: ASTM International.
236. Vila, C., A. Campos, C. Cristovao, A. Cunha, V. Santos, and J. Parajó, *Sustainable biocomposites based on autohydrolysis of lignocellulosic substrates*. *Composites Science and Technology*, 2008. **68**(3-4): p. 944-952.
237. Haafiz, M.K.M., A. Hassan, Z. Zakaria, I.M. Inuwa, M.S. Islam, and M. Jawaaid, *Properties of polylactic acid composites reinforced with oil palm biomass microcrystalline cellulose*. *Carbohydrate Polymers*, 2013. **98**(1): p. 139-145.
238. Jonoobi, M., J. Harun, A.P. Mathew, and K. Oksman, *Mechanical properties of cellulose nanofiber (CNF) reinforced polylactic acid (PLA) prepared by twin screw extrusion*. *Composites Science and Technology*, 2010. **70**(12): p. 1742-1747.
239. Tian, S., H. Xie, H. Zhang, and S. Fu, *Efficient separation of acetylated cellulose from eucalyptus and its enhancement on the mechanical strength of polylactic acid*. *International Journal of Biological Macromolecules*, 2021. **191**: p. 100-107.
240. Pracella, M., M.M.-U. Haque, and D. Puglia, *Morphology and properties tuning of PLA/cellulose nanocrystals bio-nanocomposites by means of reactive functionalization and blending with PVAc*. *Polymer*, 2014. **55**(16): p. 3720-3728.
241. Spinella, S., G. Lo Re, B. Liu, J. Dorgan, Y. Habibi, P. Leclère, J.-M. Raquez, P. Dubois, and R.A. Gross, *Polylactide/cellulose nanocrystal nanocomposites: Efficient routes for nanofiber modification and effects of nanofiber chemistry on PLA reinforcement*. *Polymer*, 2015. **65**: p. 9-17.
242. Cui, X., A. Ozaki, T.-A. Asoh, and H. Uyama, *Cellulose modified by citric acid reinforced Poly(lactic acid) resin as fillers*. *Polymer Degradation and Stability*, 2020. **175**: p. 109118.
243. Xu, W., A. Pranovich, P. Uppstu, X. Wang, D. Kronlund, J. Hemming, H. Öblom, N. Moritz, M. Preis, and N. Sandler, *Novel biorenewable composite of wood polysaccharide and polylactic acid for three dimensional printing*. *Carbohydrate polymers*, 2018. **187**: p. 51-58.

244. Zhang, X., H. Wang, C. Liu, A. Zhang, and J. Ren, *Synthesis of thermoplastic xylan-lactide copolymer with amidine-mediated organocatalyst in ionic liquid*. Scientific reports, 2017. 7(1): p. 1-10.
245. Kerrouche, D., N. Rouba, and V. Chaleix, *Chemical Modification of Xylan*, in *Biotechnological Applications of Biomass*. 2020, IntechOpen.
246. Persson, J., O. Dahlman, and A.-C. Albertsson, *Birch xylan grafted with PLA branches of predictable length*. Bioresources, 2012. 7(3): p. 3640-3655.
247. Akil, Y., D. Lorenz, R. Lehnen, and B. Saake, *Safe and non-toxic hydroxyalkylation of xylan using propylene carbonate*. European Polymer Journal, 2016. 77: p. 88-97.
248. Zhang, X., M. Chen, C. Liu, A. Zhang, and R. Sun, *Ring-opening graft polymerization of propylene carbonate onto xylan in an ionic liquid*. Molecules, 2015. 20(4): p. 6033-47.
249. Akil, Y., R. Castellani, R. Lehnen, T. Budtova, and B. Saake, *Hydroxyalkylation of xylan using propylene carbonate: comparison of products from homo- and heterogeneous synthesis by HRMAS NMR and rheology*. Cellulose, 2018. 25(1): p. 217-231.
250. Jain, R.K., M. Sjöstedt, and W.G. Glasser, *Thermoplastic Xylan Derivatives with Propylene Oxide*. Cellulose, 2000. 7(4): p. 319-336.
251. Laine, C., A. Harlin, J. Hartman, S. Hyvärinen, K. Kammiovirta, B. Krogerus, H. Pajari, H. Rautkoski, H. Setälä, J. Sievänen, J. Uotila, and M. Vähä-Nissi, *Hydroxyalkylated xylans – Their synthesis and application in coatings for packaging and paper*. Industrial Crops and Products, 2013. 44: p. 692-704.
252. Li, Q., Y. Gao, H. Wang, B. Li, C. Liu, G. Yu, and X. Mu, *Comparison of different alkali-based pretreatments of corn stover for improving enzymatic saccharification*. Bioresource Technology, 2012. 125: p. 193-199.
253. Yu, H., M. Zhang, J. Ouyang, and Y. Shen, *Comparative study on four chemical pretreatment methods for an efficient saccharification of corn stover*. Energy & Fuels, 2014. 28(7): p. 4282-4287.
254. Kühnel, I., B. Saake, and R. Lehnen, *Oxyalkylation of lignin with propylene carbonate: Influence of reaction parameters on the ensuing bio-based polyols*. Industrial Crops and Products, 2017. 101: p. 75-83.
255. Marchessault, R.H., S. Coulombe, H. Morikawa, and D. Robert, *Characterization of aspen exploded wood lignin*. Canadian Journal of Chemistry, 1982. 60(18): p. 2372-2382.
256. Kühnel, I., B. Saake, and R. Lehnen, *Comparison of different cyclic organic carbonates in the oxyalkylation of various types of lignin*. Reactive and Functional Polymers, 2017. 120: p. 83-91.

257. Cui, C., R. Sun, and D.S. Argyropoulos, *Fractional Precipitation of Softwood Kraft Lignin: Isolation of Narrow Fractions Common to a Variety of Lignins*. ACS Sustainable Chemistry & Engineering, 2014. **2**(4): p. 959-968.
258. Li, X.-h. and S.-b. Wu, *Chemical Structure and Pyrolysis Characteristics of the Soda-Alkali Lignin Fractions*. 2014, 2014. **9**(4): p. 13.
259. Zhang, L., W. Peng, F. Wang, H. Bao, P. Zhan, J. Chen, and Z. Tong, *Fractionation and quantitative structural analysis of lignin from a lignocellulosic biorefinery process by gradient acid precipitation*. Fuel, 2022. **309**: p. 122153.
260. Kumar, S., *Corn Cob Xylan-based Nanoparticles: Ester Prodrug of 5-Aminosalicylic Acid for Possible Targeted Delivery of Drug*. Journal of Pharmaceutical Sciences & Research, 2012. **4**: p. 195.
261. Kim, H., J. Ralph, and T. Akiyama, *Solution-state 2D NMR of Ball-milled Plant Cell Wall Gels in DMSO-d₆*. BioEnergy Research, 2008. **1**(1): p. 56-66.
262. Kim, H. and J. Ralph, *Solution-state 2D NMR of ball-milled plant cell wall gels in DMSO-d₆/pyridine-d₅*. Organic & Biomolecular Chemistry, 2010. **8**(3): p. 576-591.
263. Wende, G. and S.C. Fry, *2-O- β -D-xylopyranosyl-(5-O-feruloyl)-L-arabinose, a widespread component of grass cell walls*. Phytochemistry, 1997. **44**(6): p. 1019-1030.
264. Kühnel, I.S., B.; Lehnen, R. Oxyalkylation of lignin with propylene carbonate: Influence of reaction parameters on the ensuing bio-based polyols. Ind. Crops Prod. 2017, 101, 75–83.
265. Ralph, S., L. Landucci, and J. Ralph, *NMR Database of Lignin and Cell Wall Model Compounds*. 2004.
266. Al-Itry, R., K. Lamnawar, and A. Maazouz, *Improvement of thermal stability, rheological and mechanical properties of PLA, PBAT and their blends by reactive extrusion with functionalized epoxy*. Polymer Degradation and Stability, 2012. **97**(10): p. 1898-1914.
267. Kim, D.H., H.J. Kang, and Y.S. Song, *Rheological and thermal characteristics of three-phase eco-composites*. Carbohydr Polym, 2013. **92**(2): p. 1006-11.
268. Nagarajan, V., A.K. Mohanty, and M. Misra, *Biocomposites with Size-Fractionated Biocarbon: Influence of the Microstructure on Macroscopic Properties*. ACS Omega, 2016. **1**(4): p. 636-647.
269. Gupta, M. and R. Singh, *Flexural and dynamic mechanical analysis (DMA) of polylactic acid (PLA) coated sisal fibre reinforced polyester composite*. Materials Today: Proceedings, 2018. **5**(2): p. 6109-6114.

270. Cristea, M., D. Ionita, and M.M. Iftime, *Dynamic mechanical analysis investigations of PLA-based renewable materials: How are they useful?* Materials, 2020. **13**(22): p. 5302.
271. Lee, J.-S., G.H. Hwang, Y.S. Kwon, and Y.G. Jeong, *Impacts of cellulose nanofibril and physical aging on the enthalpy relaxation behavior and dynamic mechanical thermal properties of Poly(lactic acid) composite films.* Polymer, 2020. **202**: p. 122677.
272. Mathew, A.P., K. Oksman, and M. Sain, *The effect of morphology and chemical characteristics of cellulose reinforcements on the crystallinity of polylactic acid.* Journal of Applied Polymer Science, 2006. **101**(1): p. 300-310.
273. Sohn, S., *Crystallization behavior of bisphenol-A polycarbonate: Effects of crystallization time, temperature, and molar mass.* 2000, Virginia Polytechnic Institute and State University.
274. Yu, F., T. Liu, X. Zhao, X. Yu, A. Lu, and J. Wang, *Effects of talc on the mechanical and thermal properties of polylactide.* Journal of Applied Polymer Science, 2012. **125**(S2): p. E99-E109.
275. Esakkimuthu, E.S., D. DeVallance, I. Pylypchuk, A. Moreno, and M.H. Sipponen, *Multifunctional lignin-poly (lactic acid) biocomposites for packaging applications.* Frontiers in Bioengineering and Biotechnology, 2022. **10**.
276. Yamoum, C., J. Maia, and R. Magaraphan, *Rheological and thermal behavior of PLA modified by chemical crosslinking in the presence of ethoxylated bisphenol A dimethacrylates.* Polymers for Advanced Technologies, 2017. **28**(1): p. 102-112.
277. Semba, T., K. Kitagawa, U.S. Ishiaku, and H. Hamada, *The effect of crosslinking on the mechanical properties of polylactic acid/polycaprolactone blends.* Journal of Applied Polymer Science, 2006. **101**(3): p. 1816-1825.
278. Manral, A., F. Ahmad, and V. Chaudhary, *Static and dynamic mechanical properties of PLA bio-composite with hybrid reinforcement of flax and jute.* Materials Today: Proceedings, 2020. **25**: p. 577-580.
279. Wang, N., C. Zhang, W. Zhu, and Y. Weng, *Improving Interfacial Adhesion of PLA/Lignin Composites by One-Step Solvent-Free Modification Method.* Journal of Renewable Materials, 2020. **8**(9): p. 1139--1149.
280. Guo J, C.X., Wang J, He Y, Xie H, Zheng Q (2020). The influence of compatibility on the structure and properties of PLA/Lignin biocomposites by chemical modification. Polymers. 12(1), 56.
281. Park, C.W., W.J. Youe, S.J. Kim, S.Y. Han, J.S. Park, E.A. Lee, G.J. Kwon, Y.S. Kim, N.H. Kim, and S.H. Lee, *Effect of Lignin Plasticization on Physico-Mechanical Properties of Lignin/Poly(Lactic Acid) Composites.* Polymers (Basel), 2019. **11**(12).

282. Bledzki, A.K., A. Jaszkievicz, and D. Scherzer, *Mechanical properties of PLA composites with man-made cellulose and abaca fibres*. Composites Part A: Applied science and manufacturing, 2009. **40**(4): p. 404-412.
283. Li, X., H. Yu, J. Ding, L. Shen, and X. Zhao, *Synergistically enhanced toughness and water vapour barrier performance of polylactic acid by graphene oxide/acetylated lignin composite*. Materials Letters, 2022. **313**: p. 131829.

CHAPTER SIX

CONCLUSIONS

Full utilization of the lignin fraction from biorefining is key to the advancement of the field. The work in this thesis contributes to this goal by using feedstock selection, selection of pretreatment and lignin extraction conditions, lignin fractionation, and direct chemical modification of lignin to overcome common barriers to lignin utilization in value-added products.

Chapter two uses a diverse panel of six corn stover genotypes subjected to dilute acid pretreatments of different severities with varied pretreatment time, pretreatment temperature, and acid loading. Solubilization of *p*CA during pretreatment was correlated with xylan solubilization with pretreatment time being the crucial factor for *p*CA and xylan removal. The removal of *p*CA was also correlated to glucose hydrolysis yields after pretreatment. Using low lignin, high *p*CA containing corn stover genotypes under optimal dilute acid pretreatment conditions this correlation demonstrates how biomass-pretreatment combinations can be selected to achieve >90% hydrolysis yields while retaining >50mg *p*CA/g lignin in pretreated biomass. Kinetic parameters for *p*CA-ester hydrolysis were estimated using acid hydrolysis of a model compound.

Chapter three uses a single corn stover source subjected to a variety of pretreatment conditions followed by enzymatic hydrolysis and two lignin extraction methods using NaOH and formic acid. Xylan and *p*CA removal were again found to be correlated to dilute acid pretreatment time with ideal pretreatment and lignin extraction conditions achieving lignin extraction yields of 60-70% while simultaneously achieving glucose hydrolysis yields >60%. Lignins extracted using the formic acid method retained high levels of *p*-hydroxyphenyl and

*p*CA subunits with *p*CA accounting for >40% of the *p*-hydroxyphenyl ³¹P NMR signal. These excess reactive functional groups caused formic acid extracted lignins to exhibit superior lignin-formaldehyde resin and adhesive properties when compared to NaOH extracted lignins with adhesives formulated with 100% phenol replaced with formic acid lignin exhibiting lap shear strengths significantly greater than conventional PF resin adhesives.

Chapter four employs a lignin fractionation process using the sequential dilution of lignin containing liquors after the formic acid treatment of corn stover. This process recovered a cellulose heavy formic acid insoluble fraction with >90% original biomass glucan recovered. The enzymatic hydrolysis of this fraction achieved sugar yields of 39%, these yields are lower than previous studies using formic acid due to low lignin removal and high formylation of cellulose as a result of the formic acid treatment conditions used in this study. Three lignin heavy fractions of differing molecular weights were recovered, all preserving high lignin *p*CA content. A xylan and extractives heavy fraction was recovered with significant xylose degradation observed. More work is needed on the optimization of formic acid fractionation process conditions to increase lignin solubilization, decreasing the formylation of cellulose, minimize xylose degradation, and maintain high *p*CA retention.

Chapter five separates black liquor of alkali pretreated corn stover into four biopolymer fractions, an alkali insoluble cellulose fraction, a xylan heavy fraction, and two lignin heavy fractions. This fractionation process recovered >90% of the original biomass glucan, >70% original xylan, and >50% original lignin. Lignin and xylan heavy biopolymer fractions were modified via an oxyalkylation reaction with propylene carbonate and modified and unmodified biopolymers were included in extruded and injection molded PLA materials at two different

incorporation levels (5% and 20%). Modification of biopolymer fractions was found to increase material stiffness, T_g , tensile strength, % strain at break, and dispersion of biopolymer throughout the composite. Materials made with the xylan heavy fraction recovered at the highest pH (pH9) exhibited the best thermal and mechanical properties.

REFERENCES CITED

1. IEA *World Energy Outlook 2021*. 2021.
2. Pandey, A., R.D. Tyagi, and S. Varjani, *Biomass, biofuels, biochemicals: Circular bioeconomy—Current developments and future outlook*. 2021: Elsevier.
3. Sorek, N., T.H. Yeats, H. Szemenyei, H. Youngs, and C.R. Somerville, *The implications of lignocellulosic biomass chemical composition for the production of advanced biofuels*. Bioscience, 2014. **64**(3): p. 192-201.
4. Ragauskas, A.J., G.T. Beckham, M.J. Bidddy, R. Chandra, F. Chen, M.F. Davis, B.H. Davison, R.A. Dixon, P. Gilna, M. Keller, P. Langan, A.K. Naskar, J.N. Saddler, T.J. Tschaplinski, G.A. Tuskan, and C.E. Wyman, *Lignin valorization: improving lignin processing in the biorefinery*. Science, 2014. **344**(6185): p. 1246843.
5. Ullah, M., P. Liu, S. Xie, and S. Sun, *Recent Advancements and Challenges in Lignin Valorization: Green Routes towards Sustainable Bioproducts*. Molecules, 2022. **27**(18): p. 6055.
6. Sethupathy, S., G.M. Morales, L. Gao, H. Wang, B. Yang, J. Jiang, J. Sun, and D. Zhu, *Lignin valorization: status, challenges and opportunities*. Bioresource Technology, 2022: p. 126696.
7. Li, Q., C. Hu, M. Li, P. Truong, J. Li, H.-S. Lin, M.T. Naik, S. Xiang, B.E. Jackson, and W. Kuo, *Enhancing the multi-functional properties of renewable lignin carbon fibers via defining the structure–property relationship using different biomass feedstocks*. Green Chemistry, 2021. **23**(10): p. 3725-3739.
8. Li, J., G. Gellerstedt, and K. Toven, *Steam explosion lignins; their extraction, structure and potential as feedstock for biodiesel and chemicals*. Bioresource Technology, 2009. **100**(9): p. 2556-2561.
9. Zijlstra, D.S., C.A. Analbers, J. Korte, E. Wilbers, and P.J. Deuss, *Efficient Mild Organosolv Lignin Extraction in a Flow-Through Setup Yielding Lignin with High β -O-4 Content*. Polymers (Basel), 2019. **11**(12).
10. Timilsena, Y.P., I.G. Audu, S.K. Rakshit, and N. Brosse, *Impact of the lignin structure of three lignocellulosic feedstocks on their organosolv delignification. Effect of carbonium ion scavengers*. Biomass and Bioenergy, 2013. **52**: p. 151-158.
11. Sammons, R.J., D.P. Harper, N. Labbé, J.J. Bozell, T. Elder, and T.G. Rials, *Characterization of organosolv lignins using thermal and FT-IR spectroscopic analysis*. BioResources, 2013. **8**(2): p. 2752-2767.

12. Watkins, D., M. Nuruddin, M. Hosur, A. Tcherbi-Narteh, and S. Jeelani, *Extraction and characterization of lignin from different biomass resources*. Journal of Materials Research and Technology, 2015. **4**(1): p. 26-32.
13. Ciesielski, P.N., M.G. Resch, B. Hewetson, J.P. Killgore, A. Curtin, N. Anderson, A.N. Chiaramonti, D.C. Hurley, A. Sanders, M.E. Himmel, C. Chapple, N. Mosier, and B.S. Donohoe, *Engineering plant cell walls: tuning lignin monomer composition for deconstructable biofuel feedstocks or resilient biomaterials*. Green Chemistry, 2014. **16**(5): p. 2627-2635.
14. Gioia, C., M. Colonna, A. Tagami, L. Medina, O. Sevastyanova, L.A. Berglund, and M. Lawoko, *Lignin-Based Epoxy Resins: Unravelling the Relationship between Structure and Material Properties*. Biomacromolecules, 2020. **21**(5): p. 1920-1928.
15. Ghorbani, M., F. Liebner, H.W.G. van Herwijnen, L. Pfungen, M. Krahofer, E. Budjav, and J. Konnerth, *Lignin Phenol Formaldehyde Resoles: The Impact of Lignin Type on Adhesive Properties*. 2016, 2016. **11**(3): p. 15.
16. Li, Q., C. Hu, M. Li, P. Truong, M.T. Naik, D. Prabhu, L. Hoffmann, W.L. Rooney, and J.S. Yuan, *Discovering Biomass Structural Determinants Defining the Properties of Plant-Derived Renewable Carbon Fiber*. iScience, 2020. **23**(8): p. 101405.
17. Mendu, V., A.E. Harman-Ware, M. Crocker, J. Jae, J. Stork, S. Morton, A. Placido, G. Huber, and S. DeBolt, *Identification and thermochemical analysis of high-lignin feedstocks for biofuel and biochemical production*. Biotechnology for Biofuels, 2011. **4**(1): p. 43.
18. Yoo, C.G., X. Meng, Y. Pu, and A.J. Ragauskas, *The critical role of lignin in lignocellulosic biomass conversion and recent pretreatment strategies: A comprehensive review*. Bioresource Technology, 2020. **301**: p. 122784.
19. Glasser, W.G., C.A. Barnett, P.C. Muller, and K.V. Sarkanen, *The chemistry of several novel bioconversion lignins*. Journal of Agricultural and Food Chemistry, 1983. **31**(5): p. 921-930.
20. Lora, J.H. and W.G. Glasser, *Recent Industrial Applications of Lignin: A Sustainable Alternative to Nonrenewable Materials*. Journal of Polymers and the Environment, 2002. **10**(1): p. 39-48.
21. Yoo, C.G., M. Li, X. Meng, Y. Pu, and A.J. Ragauskas, *Effects of organosolv and ammonia pretreatments on lignin properties and its inhibition for enzymatic hydrolysis*. Green Chemistry, 2017. **19**(8): p. 2006-2016.
22. Bhalla, A., C.M. Cai, F. Xu, S.K. Singh, N. Bansal, T. Phongpreecha, T. Dutta, C.E. Foster, R. Kumar, B.A. Simmons, S. Singh, C.E. Wyman, E.L. Hegg, and D.B. Hodge, *Performance of three delignifying pretreatments on hardwoods: hydrolysis yields*,

- comprehensive mass balances, and lignin properties*. Biotechnology for Biofuels, 2019. **12**(1): p. 213.
23. Wörmeyer, K., T. Ingram, B. Saake, G. Brunner, and I. Smirnova, *Comparison of different pretreatment methods for lignocellulosic materials. Part II: Influence of pretreatment on the properties of rye straw lignin*. Bioresource Technology, 2011. **102**(5): p. 4157-4164.
 24. Xu, C., F. Liu, M.A. Alam, H. Chen, Y. Zhang, C. Liang, H. Xu, S. Huang, J. Xu, and Z. Wang, *Comparative study on the properties of lignin isolated from different pretreated sugarcane bagasse and its inhibitory effects on enzymatic hydrolysis*. International Journal of Biological Macromolecules, 2020. **146**: p. 132-140.
 25. Ma, R., X. Zhang, Y. Wang, and X. Zhang, *New Insights Toward Quantitative Relationships between Lignin Reactivity to Monomers and Their Structural Characteristics*. ChemSusChem, 2018. **11**(13): p. 2146-2155.
 26. Hussin, M.H., A.A. Rahim, M.N. Mohamad Ibrahim, M. Yemloul, D. Perrin, and N. Brosse, *Investigation on the structure and antioxidant properties of modified lignin obtained by different combinative processes of oil palm fronds (OPF) biomass*. Industrial Crops and Products, 2014. **52**: p. 544-551.
 27. Hochegger, M., B. Cottyn-Boitte, L. Cézard, S. Schober, and M. Mittelbach, *Influence of Ethanol Organosolv Pulping Conditions on Physicochemical Lignin Properties of European Larch*. International Journal of Chemical Engineering, 2019. **2019**: p. 1734507.
 28. Dutta, T., N.G. Isern, J. Sun, E. Wang, S. Hull, J.R. Cort, B.A. Simmons, and S. Singh, *Survey of Lignin-Structure Changes and Depolymerization during Ionic Liquid Pretreatment*. ACS Sustainable Chemistry & Engineering, 2017. **5**(11): p. 10116-10127.
 29. Xiao, L.-P., Y.-Y. Bai, Z.-J. Shi, Q. Lu, and R.-C. Sun, *Influence of alkaline hydrothermal pretreatment on shrub wood Tamarix ramosissima: Characteristics of degraded lignin*. Biomass and Bioenergy, 2014. **68**: p. 82-94.
 30. Ko, J.K., Y. Kim, E. Ximenes, and M.R. Ladisch, *Effect of liquid hot water pretreatment severity on properties of hardwood lignin and enzymatic hydrolysis of cellulose*. Biotechnol Bioeng, 2015. **112**(2): p. 252-62.
 31. Jensen, A., Y. Cabrera, C.-W. Hsieh, J. Nielsen, J. Ralph, and C. Felby, *2D NMR characterization of wheat straw residual lignin after dilute acid pretreatment with different severities*. Holzforschung, 2017. **71**(6): p. 461-469.
 32. Ahmed, M.A., J.H. Lee, A.A. Raja, and J.W. Choi, *Effects of Gamma-Valerolactone Assisted Fractionation of Ball-Milled Pine Wood on Lignin Extraction and Its Characterization as Well as Its Corresponding Cellulose Digestion*. Applied Sciences, 2020. **10**(5): p. 1599.

33. Montané, D., J. Salvadó, and X. Farriol, *Fractionation of wheat straw via steam-explosion pretreatment. Characteristics of the lignin obtained by alkali delignification of the steamed straw*. 1997.
34. Zhong, L., M. Xu, C. Wang, L. Shao, J. Mao, W. Jiang, X. Ji, G. Yang, J. Chen, and G. Lyu, *Pretreatment of willow using the alkaline-catalyzed sulfolane/water solution for high-purity and antioxidative lignin production*. International Journal of Biological Macromolecules, 2020. **159**: p. 287-294.
35. Chen, Z., X. Bai, H. Zhang, and C. Wan, *Insights into structural changes of lignin toward tailored properties during deep eutectic solvent pretreatment*. ACS Sustainable Chemistry & Engineering, 2020. **8**(26): p. 9783-9793.
36. Pang, B., S. Yang, W. Fang, T.-Q. Yuan, D.S. Argyropoulos, and R.-C. Sun, *Structure-property relationships for technical lignins for the production of lignin-phenol-formaldehyde resins*. Industrial crops and products, 2017. **108**: p. 316-326.
37. Dababi, I., O. Gimello, and E. Elaloui, *Organosolv lignin-based wood adhesive. Influence of the lignin extraction conditions on the adhesive performance*. Polymers (Basel) **8**. 2016.
38. Hosseinaei, O., D.P. Harper, J.J. Bozell, and T.G. Rials, *Role of Physicochemical Structure of Organosolv Hardwood and Herbaceous Lignins on Carbon Fiber Performance*. ACS Sustainable Chemistry & Engineering, 2016. **4**(10): p. 5785-5798.
39. Wang, Y.-Y., P. Sengupta, B. Scheidemann, Y. Pu, C.E. Wyman, C.M. Cai, and A.J. Ragauskas, *Effects of CELF pretreatment severity on lignin structure and the lignin-based polyurethane properties*. Frontiers in Energy Research, 2020. **8**: p. 149.
40. Park, Y., W. Doherty, and P.J. Halley, *Developing lignin-based resin coatings and composites*. Industrial crops and products, 2008. **27**(2): p. 163-167.
41. Gao, S., J. Zhao, X. Wang, Y. Guo, Y. Han, and J. Zhou, *Lignin structure and solvent effects on the selective removal of condensed units and enrichment of S-type lignin*. Polymers, 2018. **10**(9): p. 967.
42. Corderi, S., T. Renders, K. Servaes, K. Vanbroekhoven, T. De Roo, and K. Elst, *Strategies for the removal of polysaccharides from biorefinery lignins: process optimization and techno economic evaluation*. Molecules, 2021. **26**(11): p. 3324.
43. Wang, G. and H. Chen, *Fractionation of alkali-extracted lignin from steam-exploded stalk by gradient acid precipitation*. Separation and Purification Technology, 2013. **105**: p. 98-105.
44. Lourençon, T.V., F.A. Hansel, T.A. da Silva, L.P. Ramos, G.I.B. de Muniz, and W.L.E. Magalhães, *Hardwood and softwood kraft lignins fractionation by simple sequential acid precipitation*. Separation and Purification Technology, 2015. **154**: p. 82-88.

45. Du, B., Y. Bai, Z. Pan, J. Xu, Q. Wang, X. Wang, G. Lv, and J. Zhou, *pH fractionated lignin for the preparation of lignin-based magnetic nanoparticles for the removal of methylene blue dye*. Separation and Purification Technology, 2022. **295**: p. 121302.
46. García, A., A. Toledano, L. Serrano, I. Egüés, M. González, F. Marín, and J. Labidi, *Characterization of lignins obtained by selective precipitation*. Separation and Purification Technology, 2009. **68**(2): p. 193-198.
47. Brenelli, L.B., F. Mandelli, A.Z. Mercadante, G.J.d.M. Rocha, S.A. Rocco, A.F. Craievich, A.R. Gonçalves, D.d.C. Centeno, M. de Oliveira Neto, and F.M. Squina, *Acidification treatment of lignin from sugarcane bagasse results in fractions of reduced polydispersity and high free-radical scavenging capacity*. Industrial Crops and Products, 2016. **83**: p. 94-103.
48. Gilarranz, M.A., F. Rodriguez, M. Oliet, and J.A. Revenga, *Acid Precipitation and Purification of Wheat Straw Lignin*. Separation Science and Technology, 1998. **33**(9): p. 1359-1377.
49. Domínguez-Robles, J., E. Espinosa, D. Savy, A. Rosal, and A. Rodriguez, *Biorefinery Process Combining Specel® Process and Selective Lignin Precipitation using Mineral Acids*. 2016, 2016. **11**(3): p. 17.
50. Stoklosa, R.J., J. Velez, S. Kelkar, C.M. Saffron, M.C. Thies, and D.B. Hodge, *Correlating lignin structural features to phase partitioning behavior in a novel aqueous fractionation of softwood Kraft black liquor*. Green Chemistry, 2013.
51. Goldmann, W.M., J. Ahola, M. Mikola, and J. Tanskanen, *Solubility and fractionation of Indulin AT kraft lignin in ethanol-water media*. Separation and Purification Technology, 2019. **209**: p. 826-832.
52. Sadeghifar, H., T. Wells, R.K. Le, F. Sadeghifar, J.S. Yuan, and A. Jonas Ragauskas, *Fractionation of organosolv lignin using acetone: water and properties of the obtained fractions*. ACS Sustainable Chemistry & Engineering, 2017. **5**(1): p. 580-587.
53. Klett, A.S., P.V. Chappell, and M.C. Thies, *Recovering ultraclean lignins of controlled molecular weight from Kraft black-liquor lignins*. Chemical Communications, 2015. **51**(64): p. 12855-12858.
54. Zhang, H., Y. Bai, B. Yu, X. Liu, and F. Chen, *A practicable process for lignin color reduction: fractionation of lignin using methanol/water as a solvent*. Green Chemistry, 2017. **19**(21): p. 5152-5162.
55. Meng, X., A. Parikh, B. Seemala, R. Kumar, Y. Pu, C.E. Wyman, C.M. Cai, and A.J. Ragauskas, *Characterization of fractional cuts of co-solvent enhanced lignocellulosic fractionation lignin isolated by sequential precipitation*. Bioresource Technology, 2019. **272**: p. 202-208.

56. Liu, C., C. Si, G. Wang, H. Jia, and L. Ma, *A novel and efficient process for lignin fractionation in biomass-derived glycerol-ethanol solvent system*. Industrial Crops and Products, 2018. **111**: p. 201-211.
57. Chambon, C.L., V. Fitriyanti, P. Verdía, S.M. Yang, S. Hérou, M.-M. Titirici, A. Brandt-Talbot, P.S. Fennell, and J.P. Hallett, *Fractionation by Sequential Antisolvent Precipitation of Grass, Softwood, and Hardwood Lignins Isolated Using Low-Cost Ionic Liquids and Water*. ACS Sustainable Chemistry & Engineering, 2020. **8**(9): p. 3751-3761.
58. Xu, Y.-H., P. Zeng, M.-F. Li, J. Bian, and F. Peng, *γ -Valerolactone/water system for lignin fractionation to enhance antibacterial and antioxidant capacities*. Separation and Purification Technology, 2021. **279**: p. 119780.
59. Duval, A., F. Vilaplana, C. Crestini, and M. Lawoko, *Solvent screening for the fractionation of industrial kraft lignin*. Holzforschung, 2016. **70**(1): p. 11-20.
60. Li, M.-F., S.-N. Sun, F. Xu, and R.-C. Sun, *Sequential solvent fractionation of heterogeneous bamboo organosolv lignin for value-added application*. Separation and Purification Technology, 2012. **101**: p. 18-25.
61. Hemmingson, J.A., *Steam-Explosion Lignins: Fractionation, Composition, Structure and Extractives*. Journal of Wood Chemistry and Technology, 1987. **7**(4): p. 527-553.
62. Jiang, X., D. Savithri, X. Du, S. Pawar, H. Jameel, H.-m. Chang, and X. Zhou, *Fractionation and characterization of kraft lignin by sequential precipitation with various organic solvents*. ACS Sustainable Chemistry & Engineering, 2017. **5**(1): p. 835-842.
63. Arshanitsa, A., J. Ponomarenko, T. Dizhbite, A. Andersone, R.J.A. Gosselink, J.v.d. Putten, M. Lauberts, and G. Telysheva, *Fractionation of technical lignins as a tool for improvement of their antioxidant properties*. Journal of Analytical and Applied Pyrolysis, 2013. **103**: p. 78-85.
64. Laurichesse, S. and L. Avérous, *Chemical modification of lignins: Towards biobased polymers*. Progress in Polymer Science, 2014. **39**(7): p. 1266-1290.
65. Figueiredo, P., K. Lintinen, J.T. Hirvonen, M.A. Kostianen, and H.A. Santos, *Properties and chemical modifications of lignin: Towards lignin-based nanomaterials for biomedical applications*. Progress in Materials Science, 2018. **93**: p. 233-269.
66. Liu, Y. and K. Li, *Preparation and Characterization of Demethylated Lignin-Polyethylenimine Adhesives*. The Journal of Adhesion, 2006. **82**(6): p. 593-605.
67. Saito, T., R.H. Brown, M.A. Hunt, D.L. Pickel, J.M. Pickel, J.M. Messman, F.S. Baker, M. Keller, and A.K. Naskar, *Turning renewable resources into value-added polymer: development of lignin-based thermoplastic*. Green Chemistry, 2012. **14**(12): p. 3295-3303.

68. Matsushita, Y. and S. Yasuda, *Reactivity of a condensed-type lignin model compound in the Mannich reaction and preparation of cationic surfactant from sulfuric acid lignin*. Journal of Wood Science, 2003. **49**(2): p. 166-171.
69. Yue, X., F. Chen, and X. Zhou, *Improved interfacial bonding of PVC/wood-flour composites by lignin amine modification*. BioResources, 2011. **6**(2): p. 2022-2044.
70. Huo, S.-P., M.-C. Nie, Z.-W. Kong, G.-M. Wu, and J. Chen, *Crosslinking kinetics of the formation of lignin-aminated polyol-based polyurethane foam*. Journal of Applied Polymer Science, 2012. **125**(1): p. 152-157.
71. Pan, H., G. Sun, and T. Zhao, *Synthesis and characterization of aminated lignin*. International Journal of Biological Macromolecules, 2013. **59**: p. 221-226.
72. Huang, J. and L. Zhang, *Effects of NCO/OH molar ratio on structure and properties of graft-interpenetrating polymer networks from polyurethane and nitrolignin*. Polymer, 2002. **43**(8): p. 2287-2294.
73. He, W. and P. Fatehi, *Preparation of sulfomethylated softwood kraft lignin as a dispersant for cement admixture*. RSC Advances, 2015. **5**(58): p. 47031-47039.
74. Kai, D., S. Jiang, Z.W. Low, and X.J. Loh, *Engineering highly stretchable lignin-based electrospun nanofibers for potential biomedical applications*. Journal of Materials Chemistry B, 2015. **3**(30): p. 6194-6204.
75. Jiang, X., J. Liu, X. Du, Z. Hu, H.-m. Chang, and H. Jameel, *Phenolation to Improve Lignin Reactivity toward Thermosets Application*. ACS Sustainable Chemistry & Engineering, 2018. **6**(4): p. 5504-5512.
76. Younesi-Kordkheili, H., A. Pizzi, and G. Niyatzade, *Reduction of formaldehyde emission from particleboard by phenolated kraft lignin*. The Journal of Adhesion, 2016. **92**(6): p. 485-497.
77. Çetin, N.S. and N. Özmen, *Use of organosolv lignin in phenol-formaldehyde resins for particleboard production: II. Particleboard production and properties*. International Journal of Adhesion and Adhesives, 2002. **22**(6): p. 481-486.
78. Song, Y., Z. Wang, N. Yan, R. Zhang, and J. Li, *Demethylation of Wheat Straw Alkali Lignin for Application in Phenol Formaldehyde Adhesives*. Polymers, 2016. **8**(6): p. 209.
79. Campbell, A.G. and A.R. Walsh, *The present status and potential of kraft lignin-phenol-formaldehyde wood adhesives*. The Journal of Adhesion, 1985. **18**(4): p. 301-314.
80. Stueker, A., J. Podschun, M. Heitman, and R. Lehnen, *Organosolv Lignin in Phenol-Formaldehyde Resins-Effect of Molecular Weight and Lignin Methylation*. Towards Forest Products and Processes with Lower Environmental Impact, 2014: p. 251.

81. Younesi-Kordkheili, H., *Maleated lignin coreaction with phenol-formaldehyde resins for improved wood adhesives performance*. International Journal of Adhesion and Adhesives, 2022. **113**: p. 103080.
82. Lian, H., S. Hong, A. Carranza, J.D. Mota-Morales, and J.A. Pojman, *Processing of lignin in urea–zinc chloride deep-eutectic solvent and its use as a filler in a phenol-formaldehyde resin*. RSC Advances, 2015. **5**(36): p. 28778-28785.
83. Younesi-Kordkheili, H. and A. Pizzi, *A Comparison among Lignin Modification Methods on the Properties of Lignin–Phenol–Formaldehyde Resin as Wood Adhesive*. Polymers, 2021. **13**(20): p. 3502.
84. Kim, Y., J. Suhr, H.-W. Seo, H. Sun, S. Kim, I.-K. Park, S.-H. Kim, Y. Lee, K.-J. Kim, and J.-D. Nam, *All biomass and UV protective composite composed of compatibilized lignin and poly (lactic-acid)*. Scientific Reports, 2017. **7**(1): p. 1-11.
85. Gordobil, O., R. Delucis, I. Egüés, and J. Labidi, *Kraft lignin as filler in PLA to improve ductility and thermal properties*. Industrial Crops and Products, 2015. **72**: p. 46-53.
86. Gordobil, O., I. Egüés, R. Llano-Ponte, and J. Labidi, *Physicochemical properties of PLA lignin blends*. Polymer Degradation and Stability, 2014. **108**: p. 330-338.
87. Gordobil, O., I. Egüés, and J. Labidi, *Modification of Eucalyptus and Spruce organosolv lignins with fatty acids to use as filler in PLA*. Reactive and Functional Polymers, 2016. **104**: p. 45-52.
88. Vila, C., V. Santos, B. Saake, and J.C. Parajó, *Manufacture, characterization, and properties of poly-(lactic acid) and its blends with esterified pine lignin*. BioResources, 2016. **11**(2): p. 5322-5332.
89. Zhang, N., M. Zhao, G. Liu, J. Wang, Y. Chen, and Z. Zhang, *Alkylated lignin with graft copolymerization for enhancing toughness of PLA*. Journal of Materials Science, 2022. **57**(19): p. 8687-8700.
90. Zhu, J., L. Xue, W. Wei, C. Mu, M. Jiang, and Z. Zhou, *Modification of Lignin with Silane Coupling Agent to Improve the Interface of Poly(L-lactic) Acid/Lignin Composites*. 2015, 2015. **10**(3): p. 11.
91. Qian, S., K. Sheng, K. Yu, L. Xu, and C.A. Fontanillo Lopez, *Improved properties of PLA biocomposites toughened with bamboo cellulose nanowhiskers through silane modification*. Journal of Materials Science, 2018. **53**(15): p. 10920-10932.
92. Zhang, R., X. Xiao, Q. Tai, H. Huang, and Y. Hu, *Modification of lignin and its application as char agent in intumescent flame-retardant poly (lactic acid)*. Polymer Engineering & Science, 2012. **52**(12): p. 2620-2626.

93. Warner, E., A. Schwab, and D. Bacovsky, *2016 Survey of Non-Starch Alcohol and Renewable Hydrocarbon Biofuels Producers*. 2017, NREL (National Renewable Energy Laboratory (NREL), Golden, CO (United States)).
94. Schell, D.J., J. Farmer, M. Newman, and J.D. McMillan, *Dilute-sulfuric acid pretreatment of corn stover in pilot-scale reactor*. *Applied Biochemistry and Biotechnology*, 2003. **105**(1): p. 69-85.
95. Carlson, D.C., *System for the Treatment of Biomass*. 2020: US.
96. Gomez-Bueso, J. and Haupt, *Wood Composite Adhesives*, in *Phenolic Resins: A Century of Progress*, L. Pilato, Editor. 2010, Springer-Verlag: Berlin.
97. Mansouri, N.-E. and J. Salvadó, *Structural characterization of technical lignins for the production of adhesives: Application to lignosulfonate, kraft, soda-anthraquinone, organosolv and ethanol process lignins*. *Industrial Crops and Products* 2006. **24**: p. 8-16.
98. Vázquez, G., G. Antorrena, J. González, and J. Mayor, *Lignin-phenol-formaldehyde adhesives for exterior grade plywoods*. *Bioresource Technology*, 1995. **51**(2): p. 187-192.
99. Danielson, B. and R. Simonson, *Kraft lignin in phenol formaldehyde resin. Part 1. Partial replacement of phenol by kraft lignin in phenol formaldehyde adhesives for plywood*. *Journal of Adhesion Science and Technology*, 1998. **12**(9): p. 923-939.
100. Mansouri, N.E.E., A. Pizzi, and J. Salvado, *Lignin-based polycondensation resins for wood adhesives*. *Journal of Applied Polymer Science*, 2007. **103**(3): p. 1690-1699.
101. Jiang, X., J. Liu, X. Du, Z. Hu, H.-m. Chang, and H. Jameel, *Phenolation to Improve Lignin Reactivity toward Thermosets Application*. *ACS sustainable chemistry & engineering*., 2018. **6**(4): p. 5504-5512.
102. Kalami, S., M. Arefmanesh, E. Master, and M. Nejad, *Replacing 100% of phenol in phenolic adhesive formulations with lignin*. *Journal of Applied Polymer Science*, 2017. **134**(30): p. 45124.
103. Kalami, S., N. Chen, H. Borazjani, and M. Nejad, *Comparative analysis of different lignins as phenol replacement in phenolic adhesive formulations*. *Ind Crop Prod*, 2018. **125**: p. 520-528.
104. Carpita, N.C., *Structure and biogenesis of the cell walls of grasses*. *Annual Review of Plant Physiology and Plant Molecular Biology*, 1996. **47**: p. 445-476.
105. Hatfield, R.D., J. Ralph, and J.H. Grabber, *Cell wall cross-linking by ferulates and diferulates in grasses*. *Journal of the Science of Food and Agriculture*, 1999. **79**(3): p. 403-407.

106. Hatfield, R.D., J.M. Marita, and K. Frost, *Characterization of p-coumarate accumulation, p-coumaroyl transferase, and cell wall changes during the development of corn stems*. Journal of the Science of Food and Agriculture, 2008. **88**(14): p. 2529-2537.
107. Li, M., M. Heckwolf, J.D. Crowe, D.L. Williams, T.D. Magee, S.M. Kaeppler, N. de Leon, and D.B. Hodge, *Cell-wall properties contributing to improved deconstruction by alkaline pre-treatment and enzymatic hydrolysis in diverse maize (Zea mays L.) lines*. Journal of experimental botany, 2015. **66**(14): p. 4305-4315.
108. Zhang, Y., Q. Hou, W. Xu, M. Qin, Y. Fu, Z. Wang, S. Willför, and C. Xu, *Revealing the structure of bamboo lignin obtained by formic acid delignification at different pressure levels*. Industrial Crops and Products, 2017. **108**: p. 864-871.
109. Sluiter, A., B. Hames, R. Ruiz, C. Scarlata, J. Sluiter, and D. Templeton, *Determination of ash in biomass*. National Renewable Energy Laboratory, 2008(NREL/TP-510-42622).
110. Ong, R., S. Chundawat, D. Hodge, S. Keskar, and B. Dale, *Linking Plant Biology and Pretreatment: Understanding the Structure and Organization of the Plant Cell Wall and Interactions with Cellulosic Biofuel Production*. 2014. p. 231-253.
111. Chum, H.L., D.K. Johnson, and S.K. Black, *Organosolv pretreatment for enzymic hydrolysis of poplars. 2. Catalyst effects and the combined severity parameter*. Industrial & engineering chemistry research, 1990. **29**(2): p. 156-162.
112. Donohoe, B.S., S.R. Decker, M.P. Tucker, M.E. Himmel, and T.B. Vinzant, *Visualizing lignin coalescence and migration through maize cell walls following thermochemical pretreatment*. Biotechnol Bioeng, 2008. **101**(5): p. 913-25.
113. Selig, M.J., S. Viamajala, S.R. Decker, M.P. Tucker, M.E. Himmel, and T.B. Vinzant, *Deposition of lignin droplets produced during dilute acid pretreatment of maize stems retards enzymatic hydrolysis of cellulose*. Biotechnol Prog, 2007. **23**(6): p. 1333-9.
114. Yang, B. and C.E. Wyman, *Effect of Xylan and Lignin Removal by Batch and Flowthrough Pretreatment on the Enzymatic digestibility of Corn Stover Cellulose*. Biotechnol Bioeng, 2004. **86**(1): p. 88-95.
115. Martin, S.A. and D.E. Akin, *Effect of phenolic monomers on the growth and beta-glucosidase activity of Bacteroides ruminicola and on the carboxymethylcellulase, beta-glucosidase, and xylanase activities of Bacteroides succinogenes*. Appl Environ Microbiol, 1988. **54**(12): p. 3019-22.
116. Theodorou, M.K., D.J. Gascoyne, D.E. Akin, and R.D. Hartley, *Effect of phenolic acids and phenolics from plant cell walls on rumenlike fermentation in consecutive batch culture*. Appl Environ Microbiol, 1987. **53**(5): p. 1046-50.

117. Hartley, R.D. and E.C. Jones, *Phenolic components and degradability of the cell walls of the brown midrib mutant, bm3, of Zea mays*. Journal of the Science of Food and Agriculture, 1978. **29**(9): p. 777-782.
118. Hartley, R.D., *p-Coumaric and ferulic acid components of cell walls of ryegrass and their relationships with lignin and digestibility*. Journal of the Science of Food and Agriculture, 1972. **23**(11): p. 1347-1354.
119. Chundawat, S.P.S., R. Vismeh, L.N. Sharma, J.F. Humpala, L. da Costa Sousa, C.K. Chambliss, A.D. Jones, V. Balan, and B.E. Dale, *Multifaceted characterization of cell wall decomposition products formed during ammonia fiber expansion (AFEX) and dilute acid based pretreatments*. Bioresource Technology, 2010. **101**(21): p. 8429-8438.
120. Du, B., L.N. Sharma, C. Becker, S.F. Chen, R.A. Mowery, G.P. van Walsum, and C.K. Chambliss, *Effect of varying feedstock-pretreatment chemistry combinations on the formation and accumulation of potentially inhibitory degradation products in biomass hydrolysates*. Biotechnol Bioeng, 2010. **107**(3): p. 430-40.
121. Mitchell, V.D., C.M. Taylor, and S. Bauer, *Comprehensive Analysis of Monomeric Phenolics in Dilute Acid Plant Hydrolysates*. BioEnergy Research, 2014. **7**(2): p. 654-669.
122. Martin, C., M. Galbe, N.O. Nilvebrant, and L.J. Jonsson, *Comparison of the fermentability of enzymatic hydrolyzates of sugarcane bagasse pretreated by steam explosion using different impregnating agents*. Appl Biochem Biotechnol, 2002. **98-100**: p. 699-716.
123. Kumar, D. and G.S. Murthy, *Stochastic molecular model of enzymatic hydrolysis of cellulose for ethanol production*. Biotechnology for Biofuels, 2013. **6**(1): p. 63.
124. Yao, L., H. Yang, C.G. Yoo, Y. Pu, X. Meng, W. Muchero, G.A. Tuskan, T. Tschaplinski, and A.J. Ragauskas, *Understanding the influences of different pretreatments on recalcitrance of Populus natural variants*. Bioresource Technology, 2018. **265**: p. 75-81.
125. Hammett, L.P., *The Effect of Structure upon the Reactions of Organic Compounds. Benzene Derivatives*. Journal of the American Chemical Society, 1937. **59**(1): p. 96-103.
126. Taft, R.W., *Linear Free Energy Relationships from Rates of Esterification and Hydrolysis of Aliphatic and Ortho-substituted Benzoate Esters*. Journal of the American Chemical Society, 1952. **74**(11): p. 2729-2732.
127. Yates, K., *Kinetics of ester hydrolysis in concentrated acid*. Accounts of Chemical Research, 1971. **4**(4): p. 136-144.
128. Kapu, N.S., Z. Yuan, X.F. Chang, R. Beatson, D.M. Martinez, and H.L. Trajano, *Insight into the evolution of the proton concentration during autohydrolysis and dilute-acid hydrolysis of hemicellulose*. Biotechnol Biofuel, 2016. **9**: p. 224.

129. Ong, R.G., S.P. Chundawat, D.B. Hodge, S. Keskar, and B.E. Dale, *Linking plant biology and pretreatment: understanding the structure and organization of the plant cell wall and interactions with cellulosic biofuel production*, in *Plants and BioEnergy*. 2014, Springer New York. p. 231-253.
130. Yuan, Z., B.D. Bals, E.L. Hegg, and D.B. Hodge, *Technoeconomic evaluation of recent process improvements in production of sugar and high-value lignin co-products via two-stage Cu-catalyzed alkaline-oxidative pretreatment*. *Biotechnology for Biofuels and Bioproducts*, 2022. **15**(1): p. 45.
131. Phongpreecha, T., K.F. Christy, S.K. Singh, P. Hao, and D.B. Hodge, *Effect of catalyst and reaction conditions on aromatic monomer yields, product distribution, and sugar yields during lignin hydrogenolysis of silver birch wood*. *Bioresource Technology*, 2020. **316**: p. 123907.
132. Saulnier, B.K., T. Phongpreecha, S.K. Singh, and D.B. Hodge, *Impact of dilute acid pretreatment conditions on p-coumarate removal in diverse maize lines*. *Bioresource Technology*, 2020. **314**: p. 123750.
133. Siahkamari, M., S. Emmanuel, D.B. Hodge, and M. Nejad, *Lignin-Glyoxal: A Fully Biobased Formaldehyde-Free Wood Adhesive for Interior Engineered Wood Products*. *ACS Sustainable Chemistry & Engineering*, 2022. **10**(11): p. 3430-3441.
134. Kalami, S., N. Chen, H. Borazjani, and M. Nejad, *Comparative analysis of different lignins as phenol replacement in phenolic adhesive formulations*. *Industrial Crops and Products*, 2018. **125**: p. 520-528.
135. Hatfield, R.D., D.M. Rancour, and J.M. Marita, *Grass Cell Walls: A Story of Cross-Linking*. *Front Plant Sci*, 2017. **7**: p. 2056.
136. Ghorbani, M., F. Liebner, H.W. van Herwijnen, L. Pfungen, M. Krahofer, E. Budjav, and J. Konnerth, *Lignin phenol formaldehyde resoles: The impact of lignin type on adhesive properties*. *BioResources*, 2016. **11**(3): p. 6727-6741.
137. Dababi, I., O. Gimello, E. Elaloui, F. Quignard, and N. Brosse, *Organosolv lignin-based wood adhesive. Influence of the lignin extraction conditions on the adhesive performance*. *Polymers*, 2016. **8**(9): p. 340.
138. Li, M., D.L. Williams, M. Heckwolf, N. de Leon, S. Kaeppler, R.W. Sykes, and D. Hodge, *Prediction of Cell Wall Properties and Response to Deconstruction Using Alkaline Pretreatment in Diverse Maize Genotypes Using Py-MBMS and NIR*. *BioEnergy Research*, 2017. **10**(2): p. 329-343.
139. Austin, S., W.S. Kontur, A. Ulbrich, J.Z. Oshlag, W. Zhang, A. Higbee, Y. Zhang, J.J. Coon, D.B. Hodge, and T.J. Donohue, *Metabolism of Multiple Aromatic Compounds in*

- Corn Stover Hydrolysate by Rhodopseudomonas palustris*. Environmental Science & Technology, 2015. **49**(14): p. 8914-8922.
140. Crowe, J.D., N. Feringa, S. Pattathil, B. Merritt, C. Foster, D. Dines, R.G. Ong, and D.B. Hodge, *Identification of developmental stage and anatomical fraction contributions to cell wall recalcitrance in switchgrass*. Biotechnology for Biofuels, 2017. **10**(1): p. 184.
 141. Pinheiro, F.G.C., A.K.L. Soares, S.T. Santaella, L.M.A.e. Silva, K.M. Canuto, C.A. Cáceres, M.d.F. Rosa, J.P.d.A. Feitosa, and R.C. Leitão, *Optimization of the acetosolv extraction of lignin from sugarcane bagasse for phenolic resin production*. Industrial Crops and Products, 2017. **96**: p. 80-90.
 142. Xu, F., J.-X. Sun, R. Sun, P. Fowler, and M.S. Baird, *Comparative study of organosolv lignins from wheat straw*. Industrial crops and products, 2006. **23**(2): p. 180-193.
 143. Ramires, E.C., J.D. Megiatto, Jr., C. Gardrat, A. Castellan, and E. Frollini, *Valorization of an industrial organosolv-sugarcane bagasse lignin: Characterization and use as a matrix in biobased composites reinforced with sisal fibers*. Biotechnol Bioeng, 2010. **107**(4): p. 612-21.
 144. Hoareau, W., F.B. Oliveira, S. Grelier, B. Siegmund, E. Frollini, and A. Castellan, *Fiberboards Based on Sugarcane Bagasse Lignin and Fibers*. Macromolecular Materials and Engineering, 2006. **291**(7): p. 829-839.
 145. Mansouri, N.-E.E. and J. Salvadó, *Structural characterization of technical lignins for the production of adhesives: Application to lignosulfonate, kraft, soda-anthraquinone, organosolv and ethanol process lignins*. Industrial Crops and Products, 2006. **24**(1): p. 8-16.
 146. Moubarik, A., N. Grimi, N. Boussetta, and A. Pizzi, *Isolation and characterization of lignin from Moroccan sugar cane bagasse: Production of lignin-phenol-formaldehyde wood adhesive*. Industrial Crops and Products, 2013. **45**: p. 296-302.
 147. Solt, P., *Impact of molecular weight of kraft lignin on adhesive performance of lignin-based phenol-formaldehyde resins*. Forest products journal., 2018. **68**(4): p. 365.
 148. Hussin, M.H., A.A. Rahim, M.N. Mohamad Ibrahim, and N. Brosse, *Physicochemical characterization of alkaline and ethanol organosolv lignins from oil palm (Elaeis guineensis) fronds as phenol substitutes for green material applications*. Industrial Crops and Products, 2013. **49**: p. 23-32.
 149. El Mansouri, N.E., Q. Yuan, and F. Huang, *Characterization of alkaline lignins for use in phenol-formaldehyde and epoxy resins*. BioResources, 2011. **6**(3): p. 2647-2662.

150. Sluiter, A., B. Hames, R. Ruiz, C. Scarlata, J. Sluiter, D. Templeton, and D. Crocker, *Determination of structural carbohydrates and lignin in biomass*. Laboratory analytical procedure, 2008. **1617**(1): p. 1-16.
151. Banerjee, G., S. Car, T. Liu, D.L. Williams, S.L. Meza, J.D. Walton, and D.B. Hodge, *Scale-up and integration of alkaline hydrogen peroxide pretreatment, enzymatic hydrolysis, and ethanolic fermentation*. Biotechnol Bioeng, 2012. **109**(4): p. 922-31.
152. Pizzi, A. and K.L. Mittal, *Wood adhesives*. 2010: VSP.
153. Lorenz, L.F. and A.W. Christiansen, *Interactions of Phenolic Resin Alkalinity, Moisture Content, and Cure Behavior*. Industrial & Engineering Chemistry Research, 1995. **34**(12): p. 4520-4523.
154. Zhang, X., J. Zhu, L. Sun, Q. Yuan, G. Cheng, and D.S. Argyropoulos, *Extraction and characterization of lignin from corncob residue after acid-catalyzed steam explosion pretreatment*. Industrial Crops and Products, 2019. **133**: p. 241-249.
155. Crowe, J.D., P. Hao, S. Pattathil, H. Pan, S.-Y. Ding, D.B. Hodge, and J.K. Jensen, *Xylan Is Critical for Proper Bundling and Alignment of Cellulose Microfibrils in Plant Secondary Cell Walls*. Frontiers in Plant Science, 2021. **12**(2017).
156. Meng, X. and A.J. Ragauskas, *Recent advances in understanding the role of cellulose accessibility in enzymatic hydrolysis of lignocellulosic substrates*. Current Opinion in Biotechnology, 2014. **27**: p. 150-158.
157. Stoklosa, R.J. and D.B. Hodge, *Fractionation and Improved Enzymatic Deconstruction of Hardwoods with Alkaline Delignification*. BioEnergy Research, 2015. **8**(3): p. 1224-1234.
158. Zoia, L., A. Salanti, E.-L. Tolppa, D. Ballabio, and M. Orlandi, *Valorization of Side-Streams from a SSF Biorefinery Plant: Wheat Straw Lignin Purification Study*. 2017, 2017. **12**(1): p. 17.
159. Liu, Z.-H., N. Hao, Y.-Y. Wang, C. Dou, F. Lin, R. Shen, R. Bura, D.B. Hodge, B.E. Dale, A.J. Ragauskas, B. Yang, and J.S. Yuan, *Transforming biorefinery designs with 'Plug-In Processes of Lignin' to enable economic waste valorization*. Nature Communications, 2021. **12**(1): p. 3912.
160. Jung, W., D. Savithri, R. Sharma-Shivappa, and P. Kolar, *Changes in lignin chemistry of switchgrass due to delignification by sodium hydroxide pretreatment*. Energies, 2018. **11**(2): p. 376.
161. Yu, G., B. Li, C. Liu, Y. Zhang, H. Wang, and X. Mu, *Fractionation of the main components of corn stover by formic acid and enzymatic saccharification of solid residue*. Industrial Crops and Products, 2013. **50**: p. 750-757.

162. Villaverde, J.J., J. Li, M. Ek, P. Ligeró, and A. de Vega, *Native Lignin Structure of Miscanthus x giganteus and Its Changes during Acetic and Formic Acid Fractionation*. Journal of Agricultural and Food Chemistry, 2009. **57**(14): p. 6262-6270.
163. Karp, E.M., B.S. Donohoe, M.H. O'Brien, P.N. Ciesielski, A. Mittal, M.J. Bidy, and G.T. Beckham, *Alkaline Pretreatment of Corn Stover: Bench-Scale Fractionation and Stream Characterization*. ACS Sustainable Chemistry & Engineering, 2014. **2**(6): p. 1481-1491.
164. Li, R., X. Wang, Q. Lin, F. Yue, C. Liu, X. Wang, and J. Ren, *Structural Features of Lignin Fractionated From Industrial Furfural Residue Using Alkaline Cooking Technology and Its Antioxidant Performance*. Frontiers in Energy Research, 2020. **8**.
165. Chen, X., G. Zhao, Y. Zhang, L. Han, and W. Xiao, *Nitrogen-to-Protein Conversion Factors for Crop Residues and Animal Manure Common in China*. Journal of Agricultural and Food Chemistry, 2017. **65**(42): p. 9186-9190.
166. Bunzel, M., A. Schüßler, and G.r. Tchetssebu Saha, *Chemical characterization of Klason lignin preparations from plant-based foods*. Journal of agricultural and food chemistry, 2011. **59**(23): p. 12506-12513.
167. Haupt, R.A., *Phenolic resin-wood interaction*. Forest products journal, 1994. **44**(2): p. 69.
168. Carlson, F.E., E.K. Phillips, S.C. Tenhaeff, and W.D. Detlefsen, *A study of formaldehyde and other organic emissions from pressing of laboratory oriented strandboard*. Forest Products Journal, 1995. **45**(3): p. 71.
169. Dally, K.A., L.P. Hanrahan, M.A. Woodbury, and M.S. Kanarek, *Formaldehyde exposure in nonoccupational environments*. Arch Environ Health, 1981. **36**(6): p. 277-84.
170. Younesi-Kordkheili, H., *Ionic liquid modified lignin-phenol-glyoxal resin: a green alternative resin for production of particleboards*. The Journal of Adhesion, 2019. **95**(12): p. 1075-1087.
171. Shimkin, A.A., *Methods for the determination of the gel time of polymer resins and prepreps*. Russian Journal of General Chemistry, 2016. **86**(6): p. 1488-1493.
172. Pu, Y., F. Hu, F. Huang, B.H. Davison, and A.J. Ragauskas, *Assessing the molecular structure basis for biomass recalcitrance during dilute acid and hydrothermal pretreatments*. Biotechnology for biofuels, 2013. **6**(1): p. 1-13.
173. Kim, J.S., Y.Y. Lee, and T.H. Kim, *A review on alkaline pretreatment technology for bioconversion of lignocellulosic biomass*. Bioresource Technology, 2016. **199**: p. 42-48.
174. Marziahetti, T., S.J. Miller, C.W. Jones, and P.K. Agrawal, *Switchgrass pretreatment and hydrolysis using low concentrations of formic acid*. Journal of Chemical Technology & Biotechnology, 2011. **86**(5): p. 706-713.

175. Dong, L., X. Zhao, and D. Liu, *Kinetic modeling of atmospheric formic acid pretreatment of wheat straw with "potential degree of reaction" models*. RSC Advances, 2015. **5**(27): p. 20992-21000.
176. Cesaro, A., A. Conte, H. Carrère, E. Trably, F. Paillet, and V. Belgiorno, *Formic acid pretreatment for enhanced production of bioenergy and biochemicals from organic solid waste*. Biomass and bioenergy, 2020. **133**: p. 105455.
177. Sindhu, R., P. Binod, K. Satyanagalakshmi, K.U. Janu, K.V. Sajna, N. Kurien, R.K. Sukumaran, and A. Pandey, *Formic acid as a potential pretreatment agent for the conversion of sugarcane bagasse to bioethanol*. Appl Biochem Biotechnol, 2010. **162**(8): p. 2313-23.
178. Xu, J., M.H. Thomsen, and A.B. Thomsen, *Pretreatment on corn stover with low concentration of formic acid*. Journal of microbiology and biotechnology, 2009. **19**(8): p. 845-850.
179. Zhang, M., W. Qi, R. Liu, R. Su, S. Wu, and Z. He, *Fractionating lignocellulose by formic acid: characterization of major components*. Biomass and bioenergy, 2010. **34**(4): p. 525-532.
180. Dong, L., R. Wu, X. Zhao, and D. Liu, *Phenomenological modeling and evaluation of formic acid pretreatment of wheat straw with an extended combined severity factor for biomass fractionation and enzymatic saccharification to produce bioethanol*. Journal of the Taiwan Institute of Chemical Engineers, 2017. **81**: p. 140-149.
181. Humpert, D., M. Ebrahimi, and P. Czermak, *Membrane Technology for the Recovery of Lignin: A Review*. Membranes (Basel), 2016. **6**(3).
182. Weinwurm, F., A. Drljo, W. Waldmüller, B. Fiala, J. Niedermayer, and A. Friedl, *Lignin concentration and fractionation from ethanol organosolv liquors by ultra- and nanofiltration*. Journal of Cleaner Production, 2016. **136**: p. 62-71.
183. Zheng, Y., C. Lee, C. Yu, Y.-S. Cheng, R. Zhang, B.M. Jenkins, and J.S. VanderGheynst, *Dilute acid pretreatment and fermentation of sugar beet pulp to ethanol*. Applied Energy, 2013. **105**: p. 1-7.
184. Zhou, H., L. Tan, Y. Fu, H. Zhang, N. Liu, M. Qin, and Z. Wang, *Rapid Nondestructive Fractionation of Biomass (≤ 15 min) by using Flow-Through Recyclable Formic Acid toward Whole Valorization of Carbohydrate and Lignin*. ChemSusChem, 2019. **12**(6): p. 1213-1221.
185. Chang, X., J. Zhang, R. Wu, and X. Zhao, *Phenomenological modeling of formic acid fractionation of sugarcane bagasse by integration of operation parameters as an extended combined severity factor*. Molecules, 2021. **26**(9): p. 2753.

186. Lavarack, B.P., G.J. Griffin, and D. Rodman, *The acid hydrolysis of sugarcane bagasse hemicellulose to produce xylose, arabinose, glucose and other products*. Biomass and Bioenergy, 2002. **23**(5): p. 367-380.
187. Liu, X., M. Lu, N. Ai, F. Yu, and J. Ji, *Kinetic model analysis of dilute sulfuric acid-catalyzed hemicellulose hydrolysis in sweet sorghum bagasse for xylose production*. Industrial Crops and Products, 2012. **38**: p. 81-86.
188. Zhou, Z., D. Liu, and X. Zhao, *Conversion of lignocellulose to biofuels and chemicals via sugar platform: An updated review on chemistry and mechanisms of acid hydrolysis of lignocellulose*. Renewable and Sustainable Energy Reviews, 2021. **146**: p. 111169.
189. Lili, W., J. Yijun, L. Chunhu, L. Xiutao, M. Lingqian, W. Wei, and M. Xindong, *Microwave-assisted hydrolysis of corn cob for xylose production in formic acid*. in *2011 International Conference on Materials for Renewable Energy & Environment*. 2011.
190. Xu, J., Y. Fu, G. Tian, Q. Li, N. Liu, M. Qin, and Z. Wang, *Mild and efficient extraction of hardwood hemicellulose using recyclable formic acid/water binary solvent*. Bioresource Technology, 2018. **254**: p. 353-356.
191. Labauze, H. and B. Benjelloun-Mlayah, *Kinetics of xylan hydrolysis using an acetic and formic acid-based organosolv pretreatment*. Bioresource Technology Reports, 2021. **14**: p. 100690.
192. Lu, Y. and N.S. Mosier, *Biomimetic catalysis for hemicellulose hydrolysis in corn stover*. Biotechnol Prog, 2007. **23**(1): p. 116-23.
193. Sadeghifar, H. and A. Ragauskas, *Perspective on Technical Lignin Fractionation*. ACS Sustainable Chemistry & Engineering, 2020. **8**(22): p. 8086-8101.
194. Helander, M., H. Theliander, M. Lawoko, G. Henriksson, L. Zhang, and M.E. Lindström, *Fractionation of Technical Lignin: Molecular Mass and pH Effects*. 2013, 2013. **8**(2): p. 13.
195. Wells, T., M. Kosa, and A.J. Ragauskas, *Polymerization of Kraft lignin via ultrasonication for high-molecular-weight applications*. Ultrasonics Sonochemistry, 2013. **20**(6): p. 1463-1469.
196. Asada, C., S. Basnet, M. Otsuka, C. Sasaki, and Y. Nakamura, *Epoxy resin synthesis using low molecular weight lignin separated from various lignocellulosic materials*. International Journal of Biological Macromolecules, 2015. **74**: p. 413-419.
197. Huang, C., J. He, R. Narron, Y. Wang, and Q. Yong, *Characterization of Kraft Lignin Fractions Obtained by Sequential Ultrafiltration and Their Potential Application as a Biobased Component in Blends with Polyethylene*. ACS Sustainable Chemistry & Engineering, 2017. **5**(12): p. 11770-11779.

198. Xu, J., C. Li, L. Dai, C. Xu, Y. Zhong, F. Yu, and C. Si, *Biomass Fractionation and Lignin Fractionation towards Lignin Valorization*. ChemSusChem, 2020. **13**(17): p. 4284-4295.
199. Bai, J., S. Wang, Y. Li, Z. Wang, and J. Tang, *Effect of chemical structure and molecular weight on the properties of lignin-based ultrafine carbon fibers*. International Journal of Biological Macromolecules, 2021. **187**: p. 594-602.
200. Vanderlaan, M. and R. Thring, *Polyurethanes from Alcell® lignin fractions obtained by sequential solvent extraction*. Biomass and bioenergy, 1998. **14**(5-6): p. 525-531.
201. Crowe, J.D., R.A. Zarger, and D.B. Hodge, *Relating Nanoscale Accessibility within Plant Cell Walls to Improved Enzyme Hydrolysis Yields in Corn Stover Subjected to Diverse Pretreatments*. Journal of Agricultural and Food Chemistry, 2017. **65**(39): p. 8652-8662.
202. Palonen, H., F. Tjerneld, G. Zacchi, and M. Tenkanen, *Adsorption of Trichoderma reesei CBH I and EG II and their catalytic domains on steam pretreated softwood and isolated lignin*. J Biotechnol, 2004. **107**(1): p. 65-72.
203. Wu, R., X. Zhao, and D. Liu, *Structural Features of Formiline Pretreated Sugar Cane Bagasse and Their Impact on the Enzymatic Hydrolysis of Cellulose*. ACS Sustainable Chemistry & Engineering, 2016. **4**(3): p. 1255-1261.
204. Becker, J. and C. Wittmann, *A field of dreams: Lignin valorization into chemicals, materials, fuels, and health-care products*. Biotechnology Advances, 2019. **37**(6): p. 107360.
205. Pang, T., G. Wang, H. Sun, W. Sui, and C. Si, *Lignin fractionation: Effective strategy to reduce molecule weight dependent heterogeneity for upgraded lignin valorization*. Industrial Crops and Products, 2021. **165**: p. 113442.
206. Wang, G., X. Liu, B. Yang, C. Si, A.M. Parvez, J. Jang, and Y. Ni, *Using Green γ -Valerolactone/Water Solvent To Decrease Lignin Heterogeneity by Gradient Precipitation*. ACS Sustainable Chemistry & Engineering, 2019. **7**(11): p. 10112-10120.
207. Yoo, C.G. and A.J. Ragauskas, *Opportunities and challenges of lignin utilization*. Lignin Utilization Strategies: From Processing to Applications, 2021: p. 1-12.
208. Alinejad, M., C. Henry, S. Nikafshar, A. Gondaliya, S. Bagheri, N. Chen, S.K. Singh, D.B. Hodge, and M. Nejad *Lignin-Based Polyurethanes: Opportunities for Bio-Based Foams, Elastomers, Coatings and Adhesives*. Polymers, 2019. **11**, DOI: 10.3390/polym11071202.
209. Merewether, J., *Lignin XIV. The Precipitation of Lignin from Kraft Black Liquor*. 1961.
210. Tomani, P., *The lignoboost process*. Cellulose Chemistry and Technology, 2010. **44**.

211. Hubbe, M.A., R. Alén, M. Paleologou, M. Kannangara, and J. Kihlman, *Lignin recovery from spent alkaline pulping liquors using acidification, membrane separation, and related processing steps: A review*. BioResources, 2019. **14**(1): p. 2300-2351.
212. Ma, P., Y. Gao, and H. Zhai, *Fractionated Wheat Straw Lignin and Its Application as Antioxidant*. 2013, 2013. **8**(4): p. 15.
213. Boeriu, C.G., F.I. Fițigău, R.J.A. Gosselink, A.E. Frissen, J. Stoutjesdijk, and F. Peter, *Fractionation of five technical lignins by selective extraction in green solvents and characterisation of isolated fractions*. Industrial Crops and Products, 2014. **62**: p. 481-490.
214. Jääskeläinen, A.S., T. Liitiä, A. Mikkelsen, and T. Tamminen, *Aqueous organic solvent fractionation as means to improve lignin homogeneity and purity*. Industrial Crops and Products, 2017. **103**: p. 51-58.
215. Duval, A. and L. Avérous, *Dihydrolevoglucosenone (Cyrene™) as a versatile biobased solvent for lignin fractionation, processing, and chemistry*. Green Chemistry, 2022. **24**(1): p. 338-349.
216. Allegretti, C., S. Fontanay, Y. Krauke, M. Luebbert, A. Strini, J. Troquet, S. Turri, G. Griffini, and P. D'Arrigo, *Fractionation of Soda Pulp Lignin in Aqueous Solvent through Membrane-Assisted Ultrafiltration*. ACS Sustainable Chemistry & Engineering, 2018. **6**(7): p. 9056-9064.
217. Elsaywy, M.A., K.-H. Kim, J.-W. Park, and A. Deep, *Hydrolytic degradation of polylactic acid (PLA) and its composites*. Renewable and Sustainable Energy Reviews, 2017. **79**: p. 1346-1352.
218. Zhai, S., Q. Liu, Y. Zhao, H. Sun, B. Yang, and Y. Weng, *A review: Research progress in modification of poly (lactic acid) by lignin and cellulose*. Polymers, 2021. **13**(5): p. 776.
219. Anwer, M.A.S., H.E. Naguib, A. Celzard, and V. Fierro, *Comparison of the thermal, dynamic mechanical and morphological properties of PLA-Lignin & PLA-Tannin particulate green composites*. Composites Part B: Engineering, 2015. **82**: p. 92-99.
220. Gao, Y., W. Qu, Y. Liu, H. Hu, E. Cochran, and X. Bai, *Agricultural residue-derived lignin as the filler of polylactic acid composites and the effect of lignin purity on the composite performance*. Journal of Applied Polymer Science, 2019. **136**(35): p. 47915.
221. Mimini, V., E. Sykacek, S.N.A. Syed Hashim, J. Holzweber, H. Hettegger, K. Fackler, A. Potthast, N. Mundigler, and T. Rosenau, *Compatibility of kraft lignin, organosolv lignin and lignosulfonate with PLA in 3D printing*. Journal of Wood Chemistry and Technology, 2019. **39**(1): p. 14-30.
222. Cavallo, E., X. He, F. Luzi, F. Dominici, P. Cerrutti, C. Bernal, M.L. Foresti, L. Torre, and D. Puglia, *UV Protective, antioxidant, antibacterial and compostable polylactic acid*

- composites containing pristine and chemically modified lignin nanoparticles*. *molecules*, 2020. **26**(1): p. 126.
223. Karp, E.M., M.G. Resch, B.S. Donohoe, P.N. Ciesielski, M.H. O'Brien, J.E. Nill, A. Mittal, M.J. Bidy, and G.T. Beckham, *Alkaline pretreatment of switchgrass*. *ACS Sustainable Chemistry & Engineering*, 2015. **3**(7): p. 1479-1491.
 224. Kuhn, E.M., M.H. O'Brien, P.N. Ciesielski, and D.J. Schell, *Pilot-scale batch alkaline pretreatment of corn stover*. *ACS Sustainable Chemistry & Engineering*, 2016. **4**(3): p. 944-956.
 225. Triwulandari, E., M. Ghozali, D. Sondari, M. Septiyanti, Y. Sampora, Y. Meliana, S. Fahmiati, W.K. Restu, and A. Haryono, *Effect of lignin on mechanical, biodegradability, morphology, and thermal properties of polypropylene/polylactic acid/lignin biocomposite*. *Plastics, Rubber and Composites*, 2019. **48**(2): p. 82-92.
 226. da Silva, T.F., F. Menezes, L.S. Montagna, A.P. Lemes, and F.R. Passador, *Effect of lignin as accelerator of the biodegradation process of poly (lactic acid)/lignin composites*. *Materials Science and Engineering: B*, 2019. **251**: p. 114441.
 227. Park, S.Y., J.-Y. Kim, H.J. Youn, and J.W. Choi, *Utilization of lignin fractions in UV resistant lignin-PLA biocomposites via lignin-lactide grafting*. *International journal of biological macromolecules*, 2019. **138**: p. 1029-1034.
 228. Kumar Singla, R., S.N. Maiti, and A.K. Ghosh, *Crystallization, Morphological, and Mechanical Response of Poly(Lactic Acid)/Lignin-Based Biodegradable Composites*. *Polymer-Plastics Technology and Engineering*, 2016. **55**(5): p. 475-485.
 229. Hong, S.-H., J.H. Park, O.Y. Kim, and S.-H. Hwang, *Preparation of chemically modified lignin-reinforced PLA biocomposites and their 3D printing performance*. *Polymers*, 2021. **13**(4): p. 667.
 230. Anugwom, I., V. Lahtela, M. Kallioinen, and T. Kärki, *Lignin as a functional additive in a biocomposite: Influence on mechanical properties of polylactic acid composites*. *Industrial Crops and Products*, 2019. **140**: p. 111704.
 231. Parit, M. and Z. Jiang, *Towards lignin derived thermoplastic polymers*. *International Journal of Biological Macromolecules*, 2020. **165**: p. 3180-3197.
 232. Kühnel, I., J. Podschun, B. Saake, and R. Lehnen, *Synthesis of lignin polyols via oxyalkylation with propylene carbonate*. *Holzforschung*, 2015. **69**(5): p. 531-538.
 233. Buysch, H.-J., *Carbonic Esters*, in *Ullmann's Encyclopedia of Industrial Chemistry*.

234. Sluiter, A., B. Hames, R. Ruiz, C. Scarlata, J. Sluiter, and D. Templeton, *Determination of ash in biomass (NREL/TP-510-42622)*. National Renewable Energy Laboratory, Golden, 2005. **19**.
235. International, A., *ASTM D638-14, Standard Test Method for Tensile Properties of Plastics*. 2015: ASTM International.
236. Vila, C., A. Campos, C. Cristovao, A. Cunha, V. Santos, and J. Parajó, *Sustainable biocomposites based on autohydrolysis of lignocellulosic substrates*. Composites Science and Technology, 2008. **68**(3-4): p. 944-952.
237. Haafiz, M.K.M., A. Hassan, Z. Zakaria, I.M. Inuwa, M.S. Islam, and M. Jawaaid, *Properties of polylactic acid composites reinforced with oil palm biomass microcrystalline cellulose*. Carbohydrate Polymers, 2013. **98**(1): p. 139-145.
238. Jonoobi, M., J. Harun, A.P. Mathew, and K. Oksman, *Mechanical properties of cellulose nanofiber (CNF) reinforced polylactic acid (PLA) prepared by twin screw extrusion*. Composites Science and Technology, 2010. **70**(12): p. 1742-1747.
239. Tian, S., H. Xie, H. Zhang, and S. Fu, *Efficient separation of acetylated cellulose from eucalyptus and its enhancement on the mechanical strength of polylactic acid*. International Journal of Biological Macromolecules, 2021. **191**: p. 100-107.
240. Pracella, M., M.M.-U. Haque, and D. Puglia, *Morphology and properties tuning of PLA/cellulose nanocrystals bio-nanocomposites by means of reactive functionalization and blending with PVAc*. Polymer, 2014. **55**(16): p. 3720-3728.
241. Spinella, S., G. Lo Re, B. Liu, J. Dorgan, Y. Habibi, P. Leclère, J.-M. Raquez, P. Dubois, and R.A. Gross, *Poly lactide/cellulose nanocrystal nanocomposites: Efficient routes for nanofiber modification and effects of nanofiber chemistry on PLA reinforcement*. Polymer, 2015. **65**: p. 9-17.
242. Cui, X., A. Ozaki, T.-A. Asoh, and H. Uyama, *Cellulose modified by citric acid reinforced Poly(lactic acid) resin as fillers*. Polymer Degradation and Stability, 2020. **175**: p. 109118.
243. Xu, W., A. Pranovich, P. Uppstu, X. Wang, D. Kronlund, J. Hemming, H. Öblom, N. Moritz, M. Preis, and N. Sandler, *Novel biorenewable composite of wood polysaccharide and polylactic acid for three dimensional printing*. Carbohydrate polymers, 2018. **187**: p. 51-58.
244. Zhang, X., H. Wang, C. Liu, A. Zhang, and J. Ren, *Synthesis of thermoplastic xylan-lactide copolymer with amidine-mediated organocatalyst in ionic liquid*. Scientific reports, 2017. **7**(1): p. 1-10.
245. Kerrouche, D., N. Rouba, and V. Chaleix, *Chemical Modification of Xylan*, in *Biotechnological Applications of Biomass*. 2020, IntechOpen.

246. Persson, J., O. Dahlman, and A.-C. Albertsson, *Birch xylan grafted with PLA branches of predictable length*. *Bioresources*, 2012. **7**(3): p. 3640-3655.
247. Akil, Y., D. Lorenz, R. Lehnen, and B. Saake, *Safe and non-toxic hydroxyalkylation of xylan using propylene carbonate*. *European Polymer Journal*, 2016. **77**: p. 88-97.
248. Zhang, X., M. Chen, C. Liu, A. Zhang, and R. Sun, *Ring-opening graft polymerization of propylene carbonate onto xylan in an ionic liquid*. *Molecules*, 2015. **20**(4): p. 6033-47.
249. Akil, Y., R. Castellani, R. Lehnen, T. Budtova, and B. Saake, *Hydroxyalkylation of xylan using propylene carbonate: comparison of products from homo- and heterogeneous synthesis by HRMAS NMR and rheology*. *Cellulose*, 2018. **25**(1): p. 217-231.
250. Jain, R.K., M. Sjöstedt, and W.G. Glasser, *Thermoplastic Xylan Derivatives with Propylene Oxide*. *Cellulose*, 2000. **7**(4): p. 319-336.
251. Laine, C., A. Harlin, J. Hartman, S. Hyvärinen, K. Kammiovirta, B. Krogerus, H. Pajari, H. Rautkoski, H. Setälä, J. Sievänen, J. Uotila, and M. Vähä-Nissi, *Hydroxyalkylated xylans – Their synthesis and application in coatings for packaging and paper*. *Industrial Crops and Products*, 2013. **44**: p. 692-704.
252. Li, Q., Y. Gao, H. Wang, B. Li, C. Liu, G. Yu, and X. Mu, *Comparison of different alkali-based pretreatments of corn stover for improving enzymatic saccharification*. *Bioresource Technology*, 2012. **125**: p. 193-199.
253. Yu, H., M. Zhang, J. Ouyang, and Y. Shen, *Comparative study on four chemical pretreatment methods for an efficient saccharification of corn stover*. *Energy & Fuels*, 2014. **28**(7): p. 4282-4287.
254. Kühnel, I., B. Saake, and R. Lehnen, *Oxyalkylation of lignin with propylene carbonate: Influence of reaction parameters on the ensuing bio-based polyols*. *Industrial Crops and Products*, 2017. **101**: p. 75-83.
255. Marchessault, R.H., S. Coulombe, H. Morikawa, and D. Robert, *Characterization of aspen exploded wood lignin*. *Canadian Journal of Chemistry*, 1982. **60**(18): p. 2372-2382.
256. Kühnel, I., B. Saake, and R. Lehnen, *Comparison of different cyclic organic carbonates in the oxyalkylation of various types of lignin*. *Reactive and Functional Polymers*, 2017. **120**: p. 83-91.
257. Cui, C., R. Sun, and D.S. Argyropoulos, *Fractional Precipitation of Softwood Kraft Lignin: Isolation of Narrow Fractions Common to a Variety of Lignins*. *ACS Sustainable Chemistry & Engineering*, 2014. **2**(4): p. 959-968.
258. Li, X.-h. and S.-b. Wu, *Chemical Structure and Pyrolysis Characteristics of the Soda-Alkali Lignin Fractions*. 2014, 2014. **9**(4): p. 13.

259. Zhang, L., W. Peng, F. Wang, H. Bao, P. Zhan, J. Chen, and Z. Tong, *Fractionation and quantitative structural analysis of lignin from a lignocellulosic biorefinery process by gradient acid precipitation*. Fuel, 2022. **309**: p. 122153.
260. Kumar, S., *Corn Cob Xylan-based Nanoparticles: Ester Prodrug of 5-Aminosalicylic Acid for Possible Targeted Delivery of Drug*. Journal of Pharmaceutical Sciences & Research, 2012. **4**: p. 195.
261. Kim, H., J. Ralph, and T. Akiyama, *Solution-state 2D NMR of Ball-milled Plant Cell Wall Gels in DMSO-d₆*. BioEnergy Research, 2008. **1**(1): p. 56-66.
262. Kim, H. and J. Ralph, *Solution-state 2D NMR of ball-milled plant cell wall gels in DMSO-d₆/pyridine-d₅*. Organic & Biomolecular Chemistry, 2010. **8**(3): p. 576-591.
263. Wende, G. and S.C. Fry, *2-O-β-d-xylopyranosyl-(5-O-feruloyl)-l-arabinose, a widespread component of grass cell walls*. Phytochemistry, 1997. **44**(6): p. 1019-1030.
264. Kühnel, I.S., B.; Lehnen, R. Oxyalkylation of lignin with propylene carbonate: Influence of reaction parameters on the ensuing bio-based polyols. Ind. Crops Prod. 2017, 101, 75–83.
265. Ralph, S., L. Landucci, and J. Ralph, *NMR Database of Lignin and Cell Wall Model Compounds*. 2004.
266. Al-Itry, R., K. Lamnawar, and A. Maazouz, *Improvement of thermal stability, rheological and mechanical properties of PLA, PBAT and their blends by reactive extrusion with functionalized epoxy*. Polymer Degradation and Stability, 2012. **97**(10): p. 1898-1914.
267. Kim, D.H., H.J. Kang, and Y.S. Song, *Rheological and thermal characteristics of three-phase eco-composites*. Carbohydr Polym, 2013. **92**(2): p. 1006-11.
268. Nagarajan, V., A.K. Mohanty, and M. Misra, *Biocomposites with Size-Fractionated Biocarbon: Influence of the Microstructure on Macroscopic Properties*. ACS Omega, 2016. **1**(4): p. 636-647.
269. Gupta, M. and R. Singh, *Flexural and dynamic mechanical analysis (DMA) of polylactic acid (PLA) coated sisal fibre reinforced polyester composite*. Materials Today: Proceedings, 2018. **5**(2): p. 6109-6114.
270. Cristea, M., D. Ionita, and M.M. Iftime, *Dynamic mechanical analysis investigations of PLA-based renewable materials: How are they useful?* Materials, 2020. **13**(22): p. 5302.
271. Lee, J.-S., G.H. Hwang, Y.S. Kwon, and Y.G. Jeong, *Impacts of cellulose nanofibril and physical aging on the enthalpy relaxation behavior and dynamic mechanical thermal properties of Poly(lactic acid) composite films*. Polymer, 2020. **202**: p. 122677.

272. Mathew, A.P., K. Oksman, and M. Sain, *The effect of morphology and chemical characteristics of cellulose reinforcements on the crystallinity of polylactic acid*. Journal of Applied Polymer Science, 2006. **101**(1): p. 300-310.
273. Sohn, S., *Crystallization behavior of bisphenol-A polycarbonate: Effects of crystallization time, temperature, and molar mass*. 2000, Virginia Polytechnic Institute and State University.
274. Yu, F., T. Liu, X. Zhao, X. Yu, A. Lu, and J. Wang, *Effects of talc on the mechanical and thermal properties of polylactide*. Journal of Applied Polymer Science, 2012. **125**(S2): p. E99-E109.
275. Esakkimuthu, E.S., D. DeVallance, I. Pylypchuk, A. Moreno, and M.H. Sipponen, *Multifunctional lignin-poly (lactic acid) biocomposites for packaging applications*. Frontiers in Bioengineering and Biotechnology, 2022. **10**.
276. Yamoum, C., J. Maia, and R. Magaraphan, *Rheological and thermal behavior of PLA modified by chemical crosslinking in the presence of ethoxylated bisphenol A dimethacrylates*. Polymers for Advanced Technologies, 2017. **28**(1): p. 102-112.
277. Semba, T., K. Kitagawa, U.S. Ishiaku, and H. Hamada, *The effect of crosslinking on the mechanical properties of polylactic acid/polycaprolactone blends*. Journal of Applied Polymer Science, 2006. **101**(3): p. 1816-1825.
278. Manral, A., F. Ahmad, and V. Chaudhary, *Static and dynamic mechanical properties of PLA bio-composite with hybrid reinforcement of flax and jute*. Materials Today: Proceedings, 2020. **25**: p. 577-580.
279. Wang, N., C. Zhang, W. Zhu, and Y. Weng, *Improving Interfacial Adhesion of PLA/Lignin Composites by One-Step Solvent-Free Modification Method*. Journal of Renewable Materials, 2020. **8**(9): p. 1139--1149.
280. Guo J, C.X., Wang J, He Y, Xie H, Zheng Q (2020). The influence of compatibility on the structure and properties of PLA/Lignin biocomposites by chemical modification. Polymers. 12(1), 56.
281. Park, C.W., W.J. Youe, S.J. Kim, S.Y. Han, J.S. Park, E.A. Lee, G.J. Kwon, Y.S. Kim, N.H. Kim, and S.H. Lee, *Effect of Lignin Plasticization on Physico-Mechanical Properties of Lignin/Poly(Lactic Acid) Composites*. Polymers (Basel), 2019. **11**(12).
282. Bledzki, A.K., A. Jaszkievicz, and D. Scherzer, *Mechanical properties of PLA composites with man-made cellulose and abaca fibres*. Composites Part A: Applied science and manufacturing, 2009. **40**(4): p. 404-412.

283. Li, X., H. Yu, J. Ding, L. Shen, and X. Zhao, *Synergistically enhanced toughness and water vapour barrier performance of polylactic acid by graphene oxide/acetylated lignin composite*. Materials Letters, 2022. **313**: p. 131829.