

TOWARDS LIGNIN VALORIZATION FROM
DIVERSE BIOMASS FEEDSTOCKS

by

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DEDICATION

To my husband Bence, for your unwavering love and encouragement through every challenge. To my mother Vali, for your boundless care, sacrifices, and belief in me; your strength inspires me every day. To my family, for the foundation of support and belief in me that made this journey possible. To my cat Zserbó, for the quiet comfort and companionship you provided during countless late nights. To Kelsey, whose guidance and kindness helped me navigate through difficult times with resilience and clarity. To Jen, for your mentorship, empathy, and the countless hours you spent helping me find my strength.

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ABSTRACT

The biorefinery concept shows great promise for renewable energy and bioproducts. However, achieving economic viability remains a key challenge, particularly in realizing high-value applications for lignin. In this dissertation we investigated three key aspects of processing that affect lignin valorization: extraction, recovery, and solubilization. In Chapter Two, lignin was extracted from biorefinery hydrolysis residue using organosolv, alkaline, and ionic liquid methods and recovery yields were determined for each approach. Recovered lignin solubility was then screened with a range of organic solvents, and the fractional solubility of lignin was successfully predicted by using a novel method, which assumes lignin exhibits a distribution of solubility parameters, given its polydispersity. In Chapter Three, the alkaline extraction of lignin from corn stover and hybrid poplar was scaled and implemented to a 20-L scale by prioritizing the purity of lignin and throughput of lignin production. Lignin was recovered from the pretreatment black liquor by precipitation. The final lignin products were sent to collaborators for research on lignin fractionation and potential high-value applications. Due to inconsistent physical properties, especially color, we conducted a variable screening to determine which recovery condition impacts the physical properties of lignin. The filtering temperature had a significant impact on lignin recovery. In Chapter Four, the filtering temperature during lignin recovery was investigated in detail. A transition temperature was identified for lignin recovery marking a change in processability and color, from dark, slow-filtering lignin to light, easily filterable lignin. Chemical and physical differences between dark and light lignins were identified, such as particle morphology, surface roughness, chemical composition, and solubility. Finally, we proposed a theoretical framework based on colloid science to explain the observed differences by the kinetics of lignin aggregation.

INTRODUCTION

General Introduction

The shift towards renewable energy and materials is becoming increasingly critical as reliance on fossil fuel becomes unsustainable. A recent study estimates that approximately 350 million tons of biomass per year could be additionally utilized in the United States alone, nearly doubling the current bioenergy usage [1]. Bioenergy is the energy derived from organic materials, including plant and animal waste, which can be converted into usable forms of energy such as heat, electricity, or biofuels. It is not only one of the largest source of renewable energy, but also the sole provider of liquid transportation fuels. In response to climate change and energy security concerns, many governments are enacting legislation to support renewable energy initiatives, including bioenergy. This growing interest has positioned bioenergy as a focal point in contemporary energy research, as researchers and policymakers seek to mitigate carbon dependency and transition towards more sustainable alternatives [2]. A promising strategy of biomass utilization is the biorefinery approach (see Figure 1), which parallels the crude oil refinery concept by converting biomass into an assortment of high-value products. This figure combines relevant processing steps and materials into a general biorefinery scheme and illustrates how my research on lignin processing contributes to the development of high-value lignin-based products.

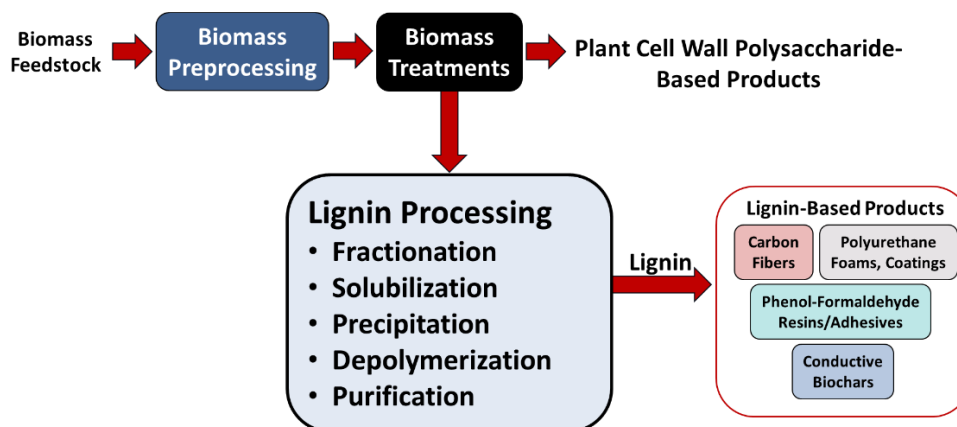


Figure 1. Biorefinery scheme with lignin processing for added value.

In this chapter, we will explore four key aspects of lignin valorization. We begin with lignin solubility, which is discussed in the paper presented in Chapter Two. Next, we will address lignin extraction methods, detailed as a scale-up process in Chapter Three. This will be followed by a discussion on lignin isolation through precipitation, which spans Chapters Three and Four.

Lignocellulosic Biomass

Lignocellulosic biomass is a major source of bioenergy and biobased products, forming the feedstock for many biorefining strategies. A typical biorefining process involves four core stages: feedstock handling (harvest, transportation, and storage), pretreatment, enzymatic hydrolysis, and fermentation [3]. Pretreatment is a key step discussed in detail in this dissertation, a scheme shown which disrupts the complex structure of the plant matrix to increase the availability of sugars (see Figure 2). Ash, which can occur as intrinsic (naturally part of the plant structure) or extrinsic (introduced during harvesting or processing), is also released during pretreatment. However, ash typically represents a smaller fraction of biomass compared to the primary components, such as cellulose, hemicellulose, and lignin. This is followed by enzymatic hydrolysis, where cellulose and

hemicellulose are broken down to simple sugars, and finally, fermentation, where these sugars are converted into fuels and chemicals [4, 5].

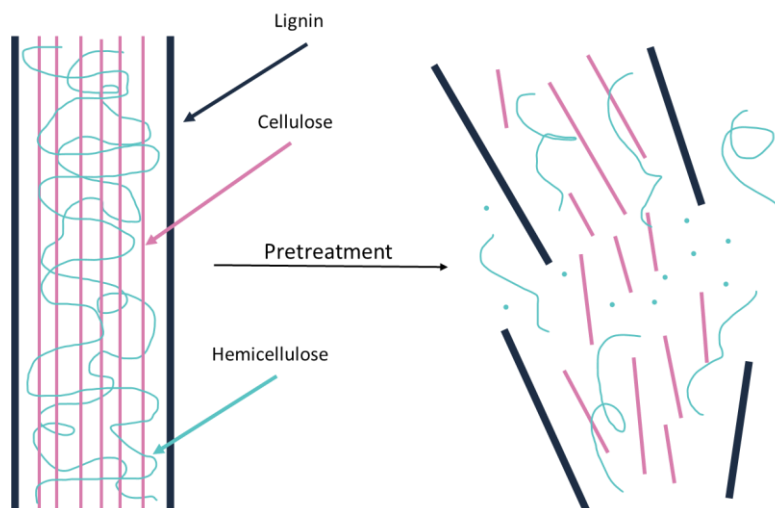


Figure 2. Pretreatment scheme. Recreated from [6].

Lignocellulosic biomass includes plant materials such as wood, agricultural residues and grasses. These abundant, non-food plant resources hold potential for sustainable energy production [7, 8]. The principal components of lignocellulosic biomass are polysaccharides (cellulose and hemicellulose) and lignin. While cellulose and hemicellulose are currently the primary focus of biorefinery processes, cost-effectively integrating lignin utilization to its full potential remains challenging [3]. This is due to lignin's irregular structure, its high variability across plant species, and its distinct method-specific modification during pretreatment. Its low solubility in many commercially used solvents further complicates efforts to convert lignin into homogenous products with high yield and selectivity [9]. As a result, current process designs utilize lignin primarily as solid fuel [10]. From an economic perspective, extracting more value from lignin is critical, as lignin represents 15-25% of the mass entering a lignocellulose biorefining process [3], and around 40% of biomass' energy content.

Lignin is the second most abundant biopolymer on Earth after cellulose, and the most abundant resource of aromatic compounds. It is a complex aromatic polymer that acts as the “glue” in the plant matrix, providing hydrophobicity to aid water transport and prevent water loss in plant cell walls, pathogen resistance, and structural support [11]. Lignin is generated in large quantities as a byproduct of the pulp and paper industry, as it is removed because its quality to impart brown color and brittleness to paper. Kraft pulping is one common pulping method, during which “Kraft lignin” is generated in surplus as a byproduct, utilized only by burning it for its heating value [12]. Similarly, in the biorefinery, lignin is produced as a byproduct in quantities that exceed its utilization as solid fuel, resulting in a significant surplus. The mentioned lignin generating industrial processes are summarized in Figure 3 with their respective final products.

In this work, two promising biomass feedstocks were investigated: hybrid poplar and corn stover. Poplar is of interest because it is a fast-growing hardwood native to the Northern Hemisphere, often cultivated as a hybrid and recognized as one of North America’s fastest growing trees. It is well suited for bioenergy and biobased product applications with a composition of cellulose content ranging from 42% to 49%, hemicellulose from 16% to 23%, and lignin from 21% to 29% [13]. Corn stover, the agricultural residue from the major cereal crop, is a readily available source of herbaceous biomass for bioethanol production. The residue contains around 36-45% cellulose, 25-30% hemicellulose and 17-20% lignin [14, 15]. In comparison, poplar, a woody biomass, has higher lignin and cellulose content, while corn stover, an herbaceous biomass, contains more hemicellulose and ash. This can be explained from a biomechanics perspective, as poplar, or trees in general, require more lignin to provide structural support, allowing them to bear their own weight and withstand environmental stresses such as wind [13].

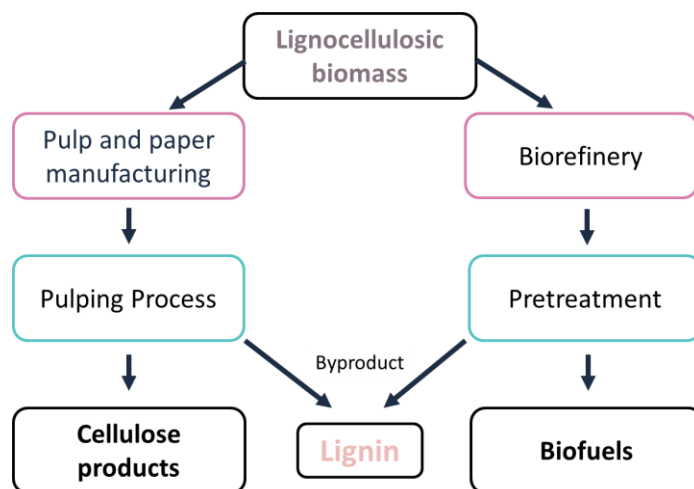


Figure 3. Lignin as a byproduct of pulp and paper and biorefinery industrial processes.

Despite its potential as a key aromatic resource in a bio-based economy, lignin is currently only utilized in vanillin production [16], or in commercial processes for low-value applications such as dispersing agents and adsorbents [17]. Thus, lignin valorization, which involves adding value to lignin in commercial processes, is an instrumental step for the global commercialization of biorefining. Additionally, the lignins from lignocellulose biorefining processes may offer novel opportunities for co-products as these lignins will exhibit unique properties relative to the traditional commercial Kraft lignin as a consequence of both the feedstock source (*i.e.*, grasses vs. hardwoods or softwoods) [18] and processing conditions.

Numerous recent advancements have been introduced to improve lignin utilization in the biorefinery, including bioengineering lignin, targeted feedstock selection [19], and lignin-first biorefining [20]. The latter focuses on stabilizing lignin during pretreatment to prevent condensation reactions, which would otherwise result in more recalcitrant lignin structures [21]. These developments contribute to enhancing the economic viability and sustainability of lignocellulosic biomass conversion processes.

Lignin Structure

The exact structure of naturally occurring lignin (also called native lignin or protolignin) in plants remains elusive due to its complex and heterogeneous nature. However, lignin is primarily composed of three types of monomeric units, known as monolignols, which are derived from p-coumaryl, coniferyl, and sinapyl alcohols. These monolignols undergo radical polymerization to form the phenylpropanoid units p-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) in the lignin polymer [11, 22]. Monolignols and lignin monomers are shown on Figure 4. The ratio of these subunits varies across plant species. For instance, hardwoods typically consist of a mix of G and S units, while softwoods are predominantly made up of G units. Herbaceous plants on the other hand contain similar proportions of G and S units but have a higher concentration of H units compared to dicots [11]. Recent studies have also identified a unique C-lignin structure in the seedcoat of a vanilla orchid, further expanding our understanding of lignin diversity [23].

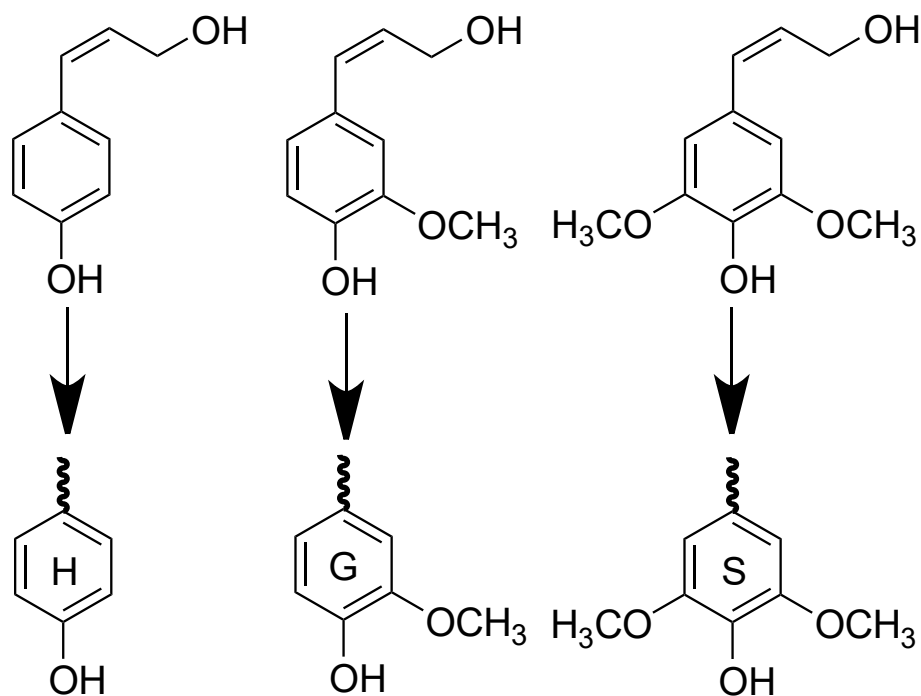


Figure 4. Monolignols at the top and the derived lignin monomer units at the bottom.

The lignin polymer can be characterized by a variety of linkages between its monomer units. The β -O-4 ether linkage is the most prevalent, accounting for approximately 50% of all linkages within the lignin structure. This linkage occurs between the β -carbon of one lignin monomer and the oxygen of another. Other significant bond types in lignin include β - β , where two monomers are connected through their β -carbons (a carbon-carbon bond), β -5, where the β -carbon of one monomer is bonded to the 5-position of another, and 5-5, a direct carbon-carbon linkage between two monomers. Additionally, unique structures like dibenzodioxocin are formed in some lignin types [9]. Due to its abundance, most depolymerization strategies target the β -O-4 linkage, aiming to break it down efficiently for subsequent conversion into valuable products.

Lignin Solubility

The solubility of lignin plays a pivotal role for effective pretreatment processes aimed at removing lignin from biomass and for its utilization across various industrial applications. Gaining insight into lignin solubility involves not only investigating its solubility behavior but also applying theoretical frameworks that describe and predict these interactions [24, 25]. Lignin's solubility, like that of any material, is fundamentally governed by its chemical structure and the solvent characteristics. As lignin can hydrogen bond with solvents, solvent properties like the polarity and hydrogen-bonding capacity of solvents significantly affect the solubility [26, 27]. Understanding these interactions and applying solubility parameters is useful for optimizing lignin's integration into biorefining processes and predicting its behavior in different solvent environments.

The Hildebrand and Hansen solubility parameters offer a theoretical framework for comparing the suitability of solvents for specific solutes. The Hildebrand theory focuses on

cohesive energy density, while the Hansen parameter expands on it by accounting for dispersive, dipole-dipole, and hydrogen-bonding interactions. By exploring these theoretical frameworks, we can better design and implement strategies for effective lignin solubilization [28].

Hildebrand Solubility Theory

The Hildebrand solubility theory is centered around the enthalpy change during solute-solvent interactions, quantified by the solubility parameter, which is the square root of the cohesive energy density. This parameter quantifies the nonpolar intermolecular interactions within a liquid, and is defined as:

$$\delta = \sqrt{\frac{E_c}{V_m}} = \sqrt{\frac{\Delta H_v - RT}{V_m}}, \quad (1)$$

where E_c is the cohesive energy [J/mol], and V_m is the molar volume [m³/mol], ΔH_v is the heat of vaporization [J/mol], R is the ideal gas constant 8.314 J/molK, and T is the temperature [K]. Cohesive energy is the opposite in sign but equal in magnitude to the potential energy of a volume unit of liquid, and it represents the energy required to separate a unit volume of liquid into individual molecules.

This theory aims to link the internal energy of mixing to the solubility parameters. If we are mixing two components, A and B, we can describe this relationship by the Hildebrand – Scatchard equation for the internal energy of mixing:

$$\Delta U^m = V\phi_A\phi_B(\delta_A - \delta_B)^2, \quad (2)$$

where V is the total volume defined as

$$V = n_A V_A + n_B V_B, \quad (3)$$

where φ_A and φ_B are the volume fractions defined as $\varphi_A = \frac{x_A V_A}{V_m}$ and $\varphi_B = \frac{x_B V_B}{V_m}$, with $x_A = \frac{n_A}{n_A + n_B}$ and $x_B = \frac{n_B}{n_A + n_B}$ as the mole fractions, where n_A and n_B are the amount of A and B component in moles.

Scatchard derived this equation by introducing the concept of cohesive energy, defined as:

$$c_{AA} = \frac{E_{c,A}}{V_A}, \quad (4)$$

where c_{AA} is the cohesive energy, $E_{c,A}$ is the cohesive energy density, and V_A is the molar volume for pure liquid component A.

Using the approach that the change in molar internal energy in a mixture can be expressed as:

$$\Delta U^m = \sum (\text{unlike interactions}) - \sum (\text{like interactions}), \quad (5)$$

and assuming some simplifications, this can be rewritten as:

$$\Delta U^m = V\varphi_A\varphi_B(c_{AA} + c_{BB} - 2c_{AB}), \quad (6)$$

where c_{AA} and c_{BB} are the cohesive energy densities for interactions between like molecules and c_{AB} is the cohesive energy density for interactions between unlike molecules.

Introducing the approximate relation between unlike molecules that:

$$c_{AB} = \sqrt{c_{AA}c_{BB}}, \quad (7)$$

the equation for the molar internal energy simplifies to:

$$\Delta U^m = V\varphi_A\varphi_B \left(\sqrt{\frac{E_{c,A}}{V_A}} - \sqrt{\frac{E_{c,B}}{V_B}} \right)^2, \quad (8)$$

then the previously introduced

$$\Delta U^m = V\varphi_A\varphi_B(\delta_A - \delta_B)^2, \quad (2)$$

using the solubility parameters.

The enthalpy of mixing can be expressed by rearranging the internal energy of mixing to:

$$\Delta H^m = \Delta U^m + p\Delta V, \quad (9)$$

where $p\Delta V$ is the pressure-volume work done during the mixing process, which is zero as we assume ideal solution behavior with no volume change during mixing. Inserting the Hildebrand – Scatchard equation into this gives:

$$\Delta H^m = V\varphi_1\varphi_2(\delta_A - \delta_B)^2. \quad (10)$$

Mixing is considered thermodynamically favorable when the Gibbs free energy of mixing is negative, indicating a spontaneous process and the dissolution of the solute. The Gibbs free energy of mixing [kJ/mol] is given by:

$$\Delta G^m = \Delta H^m - T\Delta S^m, \quad (11)$$

where ΔH^m is the enthalpy change of mixing [kJ/mol], T is the temperature [K], and ΔS^m is the entropy change of mixing [kJ/molK].

Plugging the already derived Hildebrand – Scatchard equation into the Gibbs free energy of mixing, we get:

$$\Delta G^m = V(\delta_A - \delta_B)^2\varphi_1\varphi_2 - T\Delta S^m. \quad (12)$$

If we look at this equation, both the ΔH^m term and the $T\Delta S^m$ term is always positive, as mixing is decreasing the system's order with a positive entropy and the temperature is always positive in Kelvin. For ΔG^m to be negative, the ΔH^m term needs to be small enough for the $T\Delta S^m$ term to keep the equation negative. This is why it is favorable to use a solvent with a close solubility parameter to the polymer, minimizing $(\delta_A - \delta_B)^2$. Using the Hildebrand solubility

parameters, the highest difference in solubility parameters where miscibility is still happening can be determined using the equation for the entropy of mixing as:

$$\Delta S^m = -nR(x_A \ln x_A + x_B \ln x_B), \quad (13)$$

where n is the total number of moles, R is the gas constant, and x_A and x_B are the mole fractions.

Polymer dissolution in a solvent is the result of solvent diffusion and chain disentanglement of the polymer in a thermodynamically compatible solvent. The calculation of the Hildebrand solubility parameter for a polymer is estimated based on the contributions of functional and atomic groups when the repeating unit is known. As the determination of monomer ratios is complicated and requires extensive experimentation, this calculation is proving to be difficult for lignin, a polymer without a regular repeating unit.

Hansen Solubility Theory

While the Hildebrand solubility parameter includes apolar interactions and does not account for polar interactions, the Hansen solubility theory provides a more comprehensive approach. Hansen's model incorporates three types of van der Waals intermolecular interactions: dispersive (also known as London dispersion forces), dipole – dipole interactions, and hydrogen bonding. These interactions vary in strength, but chemical bonds (ionic, covalent, metallic) are significantly stronger than hydrogen bonds, dipole – dipole interactions, and the weakest dispersive forces.

chemical bonds >>> hydrogen bonds >> dipole – dipole interactions > dispersion forces

For some polymers and solvents, hydrogen bonding plays a critical role and is a significant factor in solubility, resulting in inaccurate results when using the Hildebrand solubility parameter

which does not account for polar interactions. This is particularly relevant for lignin, a complex polydisperse material, as it dissolves best in highly polar solvents with good hydrogen-bonding capabilities. Forming hydrogen-bonded complexes between lignin and the solvent is crucial for overcoming intermolecular attractions among lignin molecules [24, 26].

Hansen's theory breaks down the total solubility parameter into contributions from non-polar (dispersion, d), polar (dipole – dipole, p), and hydrogen-bonding (h) effects:

$$\delta_T = \sqrt{\delta_d^2 + \delta_p^2 + \delta_h^2}. \quad (14)$$

Hansen's model also introduces the concept of the radius of interaction or the solubility sphere, which defines the limit for complete miscibility, providing a more nuanced understanding of solubility in various solvents.

$$R_{i,j} = \sqrt{4(\delta_{d,i} - \delta_{d,j})^2 + (\delta_{p,i} - \delta_{p,j})^2 + (\delta_{h,i} - \delta_{h,j})^2}. \quad (15)$$

Theoretical solubility requires $R_{i,j}$, the distance between the two solubility parameters, to be smaller than the solute's interaction radius, R_0 . This can be expressed using the relative energy difference, RED, defined as:

$$RED = \frac{R_{i,j}}{R_0}. \quad (16)$$

The Hildebrand model represents solubility in a single-dimensional framework, while the Hansen model expands this concept into three dimensions, shown in Figure 5. Hildebrand's solubility parameter considers only the total cohesive energy density, creating a single “dimension” of compatibility that simplifies solubility predictions but limits it to nonpolar substances. In contrast, Hansen solubility theory expands this concept into a three-dimensional space by incorporating dispersion, dipole – dipole, and hydrogen-bonding interactions separately. Each of

these components represent an axis in a three-dimensional solubility space, allowing for a more nuanced assessment of solubility.

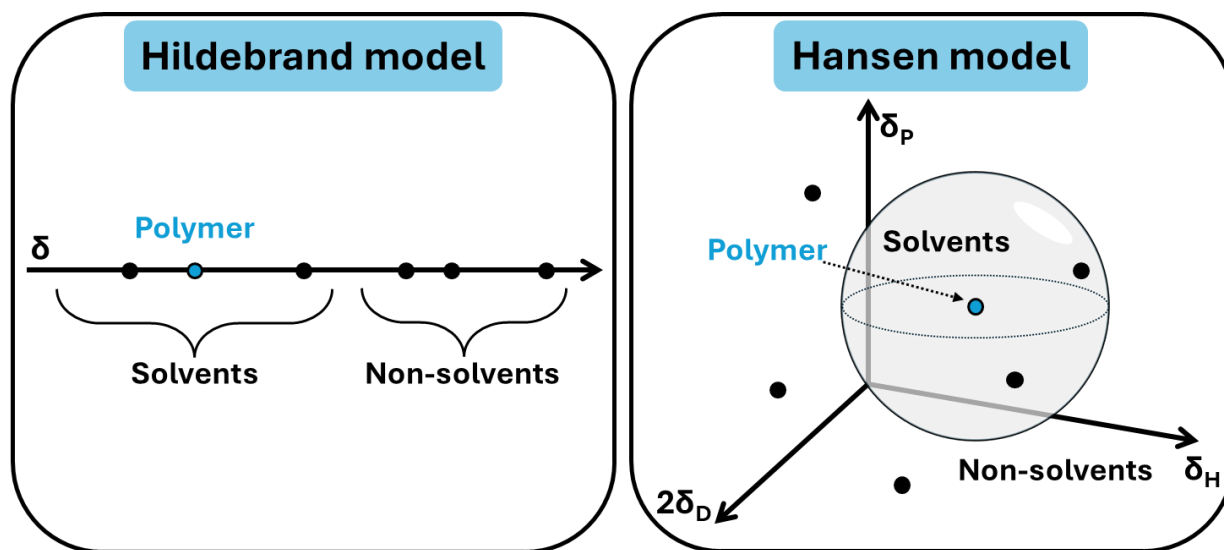


Figure 5. Visualization of the Hildebrand and Hansen solubility parameters. Recreated from [25].

Molecular Solubility from Simulations

Solubility simulations can provide an efficient screening tool for a wide range of solvents for lignin. Computational tools like LigninBuilder, which models the configuration of the lignin polymer in both water and organic solvents, streamline this process by using an input library [29, 30]. This input library was computer generated for a diverse set of biomass types [31]. Recent advancements include applying machine learning for designing solvents for lignin. The selected representative lignin in this study was a trimer of guaiacyl, G units connected via β -O-4 bonds [20]. Simulations, however, can be limited in scale by the computational demand to only using model lignin compounds, applying constraints to the size and may not include the complex interactions within lignin itself additionally to the solvent-lignin interactions. While these tools are valuable for initial screening, they cannot entirely replace experimental verification.

Fractional Solubility

In Chapter Two, our publication is presented discussing fractional lignin solubility. Our unique approach is shown in Figure 6, using the assumption that as lignin is polydisperse, its solubility parameter can be estimated as a normal distribution of solubility parameters from all the different subsets of lignin structures. This heterogeneity is what lignin fractionation processes take advantage of. By using the known solubility parameters of the investigated solvents and assuming a tolerance level for solubility we can determine the fractional solubility of lignin in a solvent by integrating the Gaussian distribution. Using already determined solubility parameters from literature and our experimental data in a range of organic solvents as fractional solubility, we will predict the mean of distributions for Hildebrand and Hansen lignin solubility parameters.

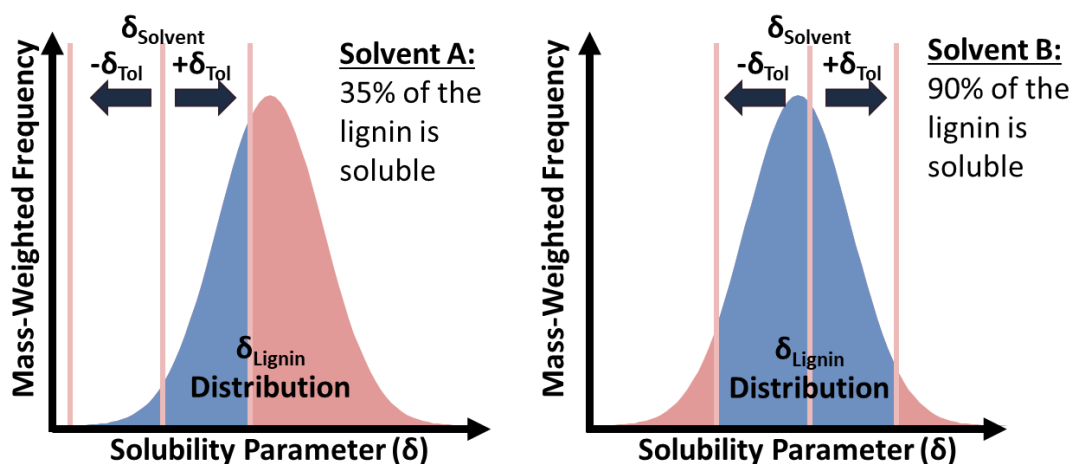


Figure 6. Fractional solubility approach.

Lignin Extraction

Pretreatment redistributes plant cell wall components by disrupting the plant cell wall structure and selectively solubilizing components. One common strategy is hydrolysis targeting the most common β -O-4 bonds in lignin [8]. As with most chemical processes, a catalyst is

frequently used for the process, resulting in acid (organosolv, dilute acid) or alkali (Kraft, alkali) catalyzed hydrolysis pretreatments [32]. Selected strategies pertaining to this dissertation are described in detail below.

Alkali Processes: Kraft Pulping and Alkaline Pretreatment

Kraft pulping is used in two thirds of pulp and paper manufacturing plants [33], where sodium hydroxide (NaOH) and sodium sulfide (Na_2S) is applied to wood chips under high pressure and high temperature (150 to 170 °C) to liberate fibers. Its predecessor, the soda pulping process omits sodium sulfide thereby decreasing its efficiency and pulp strength but avoiding sulfur. The alkali pretreatment uses the same principle as soda pulping, using sodium hydroxide for selectively solubilizing lignin and hemicellulose in the biomass and depolymerizing lignin. During the alkali pretreatment, both β -O-4 ether linkages of lignin and ester bonds between lignin and hemicellulose are cleaved. Due to the cleavage of β -O-4 ether linkages, phenolic hydroxyl group content of the lignin is increased. Demethoxylation (methoxy group leaving) of lignin and the formation of quinone methide intermediates is possible at high temperatures. Lignin recovered from this process is heterogeneous, exhibiting a diverse range of functional groups and structural units with a higher molecular weight compared to other treatments. The extensive modification to its structure is due to the cleavage of ether linkages and formation of new C-C bonds [12, 34, 35]. The use of high quantities of sodium hydroxide in alkali pretreatment results in higher ash content, as excess NaOH and its byproducts remain as residual inorganic compounds after the process. Alkali pretreatment is applied in all chapters of this dissertation.

Organosolv Pretreatment

Organosolv pretreatments use an organic solvent, usually ethanol (EtOH), for solvolysis reactions with lignin, cleaving β -O-4 ether linkages and forming ethoxy groups [36, 37]. Strong acids like sulfuric acid (H_2SO_4) can be used as a Lewis catalyst in this reaction to form an ethoxy group preventing recondensation reactions forming C-C bonds. This catalytic role is essential for promoting efficient lignin extraction while avoiding the formation of unwanted byproducts. A key advantage of this catalyst is that it is not consumed during the process, allowing for reuse. However, as the catalyst is homogeneous, it is difficult to recover and recycle.

The resulting lignin has a lower molecular weight than alkali lignin with less condensed structures and fewer C-C bonds, and higher purity with lower ash and polysaccharide content. Lignin's structure is also less modified using this method, closer to native lignin with a higher proportion of oxidized groups, and more phenolic hydroxyl groups to enhance solubility in polar organic solvents.

The ALCELL process is an organosolv-based pretreatment method that uses ethanol as a pulping agent to break down lignocellulosic biomass into its components, producing high-quality, fully bleachable pulp [38]. This process is advantageous over traditional kraft pulping due to its lower environmental impact and ability to operate on a smaller scale, making it suitable for a range of feedstocks, including hardwood and non-wood materials like bagasse. In comparison, the organosolv process applied in this dissertation similarly utilizes organic solvents, but with variations for milder pretreatment conditions.

Dilute Acid Pretreatment

Dilute acid pretreatments use a dilute solution of a strong acid at high temperatures. As with the other methods, dilute acid pretreatments disrupt the plant structure by targeting β -O-4 ether linkages. Those depolymerizing reactions are paired with repolymerization reactions, the formation of new C-C bonds through condensation reactions, significantly modifying the lignin structure [39, 40]. In Chapter Two we used the water-insoluble solid residue after dilute acid pretreatment and enzymatic hydrolysis of corn stover as the starting material for lignin extraction. Figure 7 shows the process this material came from, showcasing how our work fits into the larger biorefinery process.

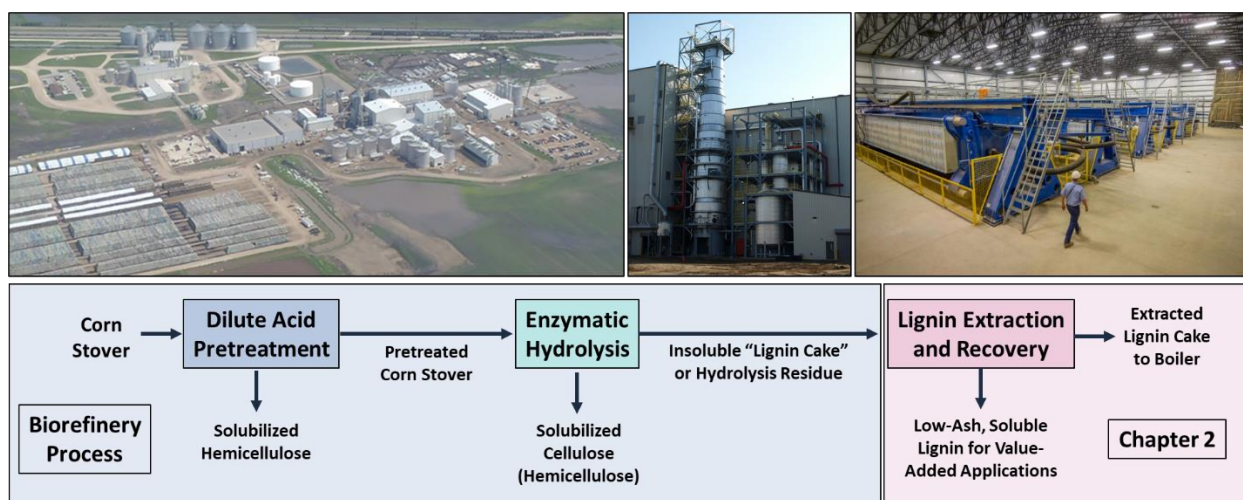


Figure 7. Biorefinery scheme of POET/DSM Project Liberty biorefinery (Emmetsburg, IA).

Lignin Precipitation

Alkaline pretreatments aim to selectively solubilize lignin from biomass to enhance the enzyme reactivity of the remaining polysaccharides, the solids resulting in pretreated biomass and “black liquor”, the liquid [41]. This process is used throughout all chapters, in a small scale for Chapter Two, as process scale-up for Chapter Three based on conditions from the group’s previous

work [42], and as the feedstock for Chapter Four. During industrial pulping, lignin is dissolved into black liquor, which must then be treated to recover lignin and chemicals to improve process efficiency, prevent equipment fouling, and waste management [43]. Additionally, recovering lignin enables its utilization in valuable applications, contributing to the overall sustainability and economic viability of the biorefining process [43].

In industrial settings, lignin recovery typically consists of three steps: 1) concentrating black liquor, 2) acidifying it to precipitate lignin, and 3) separating the lignin, generally via filtration. The acidification process involves coagulation or aggregation of lignin, which is intentionally induced for precipitation but needs to be minimized during pretreatment. To reverse lignin solubilization post-pretreatment, conditions such as pH, counterion concentration, and temperature are adjusted to those opposite to the original pretreatment settings. Recovery strategies vary based on biomass type and processing conditions. Besides acid precipitation, lignin can also be recovered using techniques such as flocculation, membrane filtration [44], adsorption or ion exchange. Industrial methods for lignin recovery include the LignoBoost and LignoForce processes, that use high filtration temperature, increased ionic strength, low pH, and membrane separation for improving precipitated lignin filterability from the black liquor [45, 46].

Influencing Variables

Temperature [47], pH [48], types and concentration of Na⁺ or other metal ions [44], anion type [49] and ionic strength [50] in the process are all important factors that govern lignin's coagulation and filtration behavior, impacting lignin precipitation and subsequent lignin recovery. Higher lignin concentration, higher ionic strength, lower pH, and lower temperatures increase precipitation yield [51], while xylan content impacts particle size, with higher xylan concentrations

shifting the size distribution toward smaller particles [48]. Regarding concentration and types of salt ions, higher sodium ion (Na^+) concentrations lead to a higher onset pH for precipitation and larger particle sizes. Salt type affects particle formation; sulfate ions (SO_4^{2-}) do not induce salting out, a phenomenon where solutes precipitate from solution due to reduced solubility in the presence of specific salts, unlike chloride ions (Cl^-) from NaCl [49]. Particle size control during recovery is important, as micro- and nanoparticles are challenging to recover due to their colloidal stability and low filterability [52]. Optimizing these parameters can significantly affect the efficiency and quality of lignin recovery. In Figure 8, some selected variables are shown that impact lignin precipitation, along with our implemented process steps. The parameters shown in blue are screened in Chapter Three, and the parameters in red are investigated in detail in Chapter Four. Our investigation into lignin recovery from hardwood and herbaceous alkaline black liquors focused on factors such as pH, acid type, drying temperature, and filtering temperature.

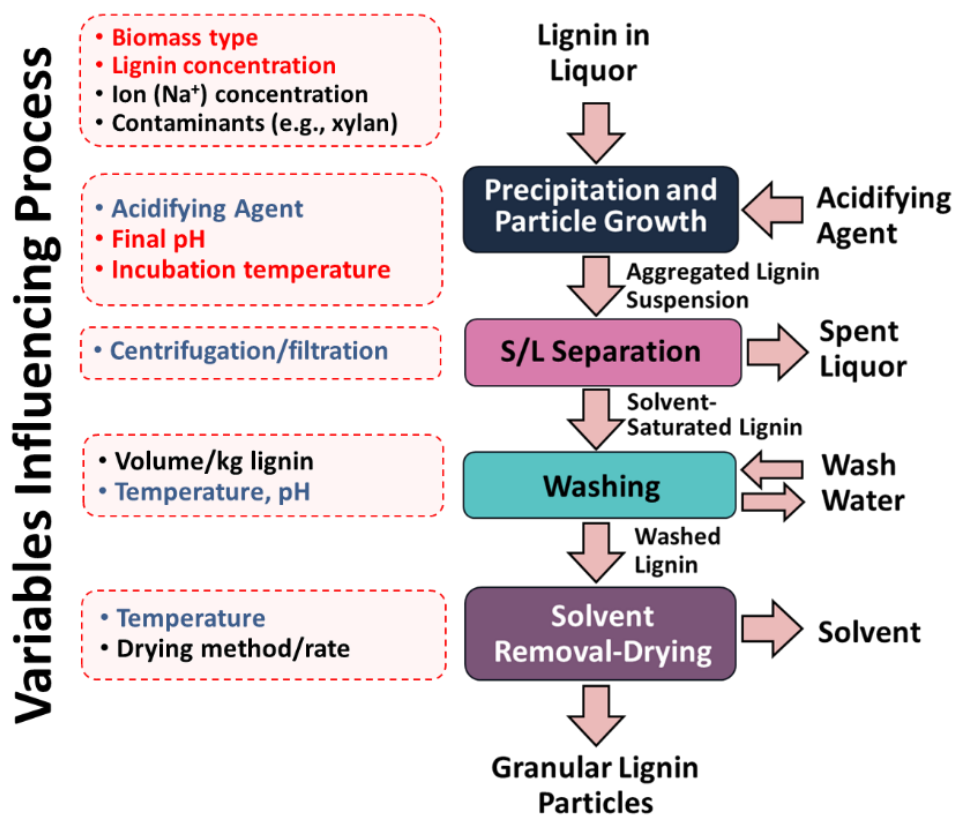


Figure 8. Overview of the variables influencing lignin precipitation and significant process steps used in Chapter Three. Variables in blue have been screened in Chapter Three, while variables in red are investigated in detail in Chapter Four.

A relevant patent [53] outlines a method to enhance the filterability of alkaline black liquors through acid precipitation at temperatures below 60 °C, followed by filtration above 50 °C. This approach allows for drying at temperatures up to 110°C while preventing the formation of undesirable lignins with low filterability. Under optimal conditions, filter cake solids can reach up to 35%, reducing drying needs [47]. An earlier investigation studied the filtration properties of lignin suspensions [47]; the filtration characteristics were shown to be strongly dependent on the precipitation conditions. Black liquor with a dry solids content of 30%, acidified with sulfuric acid to a pH below 10 at a precipitation temperature of 80°C gave lignin suspensions that were easily

filtered. A considerable increase in the average specific filtration resistance was noted at a modest temperature decrease of 10°C from the optimum of 80°C. If the temperature was raised above 80°C, however, larger clumps of lignin were formed that caused plugging problems in the equipment.

Lignin precipitated from black liquor by acidification often contains inorganic impurities, and its structure varies based on the degradation and condensation reactions during pretreatment [34, 35]. Comparisons between hardwood (sweetgum) and softwood (pine) show that softwoods generally offer better yield, purity and ease of separation for lignin recovery [54], whereas hardwoods (like poplar) often leave significant amounts of soluble lignin in black liquor after acidification. Soda lignins from non-wood feedstocks (like corn stover) differ in characteristics, such as low molecular weight, high intrinsic silica content, and more variability compared to wood lignins [55].

Thermodynamics of Aggregation: Stability of Colloidal Lignin Systems

Lignin precipitation can be regarded as the coagulation of a colloid [56, 57], governed by thermodynamics and kinetics. The Derjaguin–Landau–Verwey–Overbeek (DLVO) theory describes thermodynamic colloid stability as the interplay between attractive forces (such as van der Waals, hydrophobic interactions, π - π stacking [58]) and repulsive electrostatic forces (determined by the zeta potential). Two levels of aggregation have been identified in alkali lignin. One is intermolecular aggregation of polymer chains because of van der Waals attraction, and the other is π - π aggregation of aromatic groups in lignin because of non-bonded orbital interactions. π - π stacking of lignin occurs in the J-aggregation (head-to-tail conformation) showing a red spectral shift using UV-Vis spectroscopy [58].

As previously noted, pH, ionic strength, and temperature [50] significantly impact this balance due to the presence of weakly acidic groups on lignin. Aggregation and precipitation arise from the interplay of these thermodynamic forces and kinetic factors, with zeta (ζ) potential playing a key role in stability. The zeta potential can be measured to determine the stability of colloidal systems. A colloid particle in a liquid is surrounded by double liquid layers: an inner, strongly bound layer called Stern layer, and an outer diffuse layer where the ions are more loosely associated. The plane separating the mobile bulk solvent and the diffuse layer attached to the particle surface is called the slipping plane. The zeta potential is the electrical potential at the slipping plane. A high zeta potential promotes dispersion with dominating repulsive forces, while a low zeta potential leads to coagulation and sedimentation as the electrostatic repulsive barrier diminishes [59].

Figure 9 illustrates the key steps, attractive and repulsive forces during lignin coagulation in alkaline black liquor using representative G units in lignin. First, lignin is dissolved in the black liquor at high pH, in alkaline conditions, see left panel on Figure 9. In this step the phenolic groups on the lignin monomers are deprotonated with negative charges, forming the phenoxide group. As a result, there is significant electrostatic repulsion between these negatively charged groups and the lignin monomers. In this state, lignin forms a stable colloid solution in black liquor. Secondly, acid (an electrolyte) is added to the black liquor, and the pH decreases, see middle panel on Figure 9. In this step, the protonation of phenolic groups is initiated with a neutralization of negative charges on lignin, and the decrease of electrostatic repulsion between lignin monomers, destabilizing the solution, forming loose reversible aggregates. Thirdly, as more acid is added nearing low acidic pH around 2-3 and reaching the critical coagulation concentration (CCC), most

phenolic hydroxyl groups are protonated, attracting and forming hydrogen bonds with each other, see right panel on Figure 9. During this coagulation, the hydrophobicity of aggregates is increasing, becoming less soluble and heavy enough to settle, ultimately precipitating out of the solution. This is why larger molecular weight fractions precipitate out first from black liquor [33], as lower molecular weight fractions are more soluble, leading to the fractionation of lignin during precipitation.

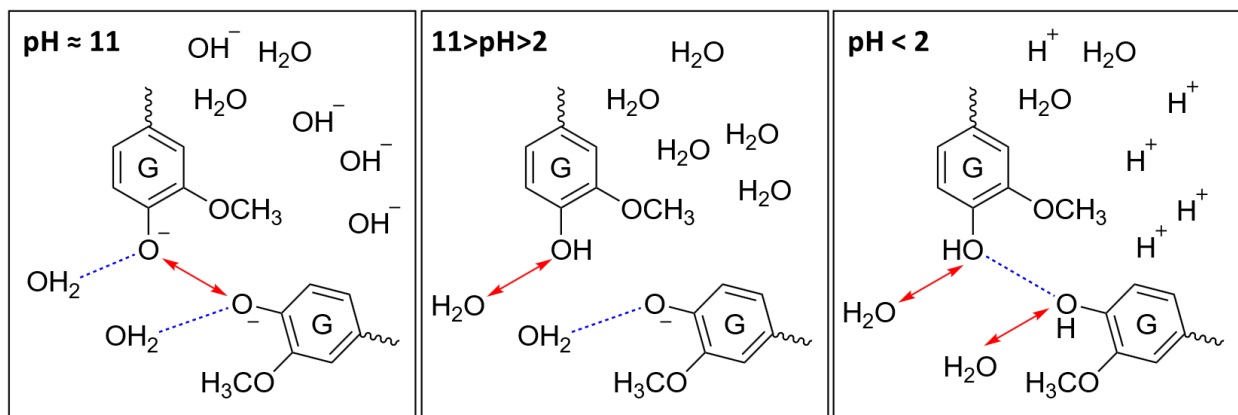


Figure 9. Lignin precipitation schematic, showing the initial stable colloid system as a result of the alkaline pretreatment (left panel), a transition state where the colloid is destabilized (middle panel), and the acidified liquor with lignin precipitation (right panel).

This colloid behavior of lignin was used for producing colloidal lignin particles (CLPs) [59], where they found that the zeta potential is strongly influenced by antisolvent pH and initial lignin concentration, with higher values leading to more negative zeta potentials. Particle color depended on the initial lignin concentration (higher concentrations result in lighter, more yellow particles) and antisolvent pH (higher pH darkens the particles). Additionally, increasing both lignin and ethanol concentrations enhanced hydrophobicity, while higher antisolvent pH and faster addition rates made the particles more hydrophilic.

Kinetics of Aggregation: Universality Laws in Coagulation

While the previously discussed thermodynamic principles regarding colloid systems determine the stability and whether aggregation occurs or not, the rate and mechanism by which particles aggregate define the kinetics of aggregation. Universality laws in coagulation describe how the rate and mechanism change under different conditions [60]. This means the kinetics of aggregation exhibits a universal behavior, independent of the chemical nature of the colloid particles. At both high and low temperatures, lignin remains insoluble and forms colloidal particles that aggregate and coagulate. The differences in the properties of these particles and the resulting lignin solids can be attributed to the universal two limiting regimes of aggregation: rapid aggregation limited only by diffusion and slow aggregation limited by the reaction rate at which bonds form between clusters.

Diffusion-Limited Cluster Aggregation (DLCA) involves the rapid formation of fractal structures with loose, complex, branched patterns. Aggregation in this case is limited by the diffusion of particles toward each other, leading to clusters growing as particles adhere based on proximity. As clusters become larger, their growth slows due to slower diffusion. Reaction-Limited Cluster Aggregation (RLCA) is characterized by less pronounced fractal patterns compared to DLCA. In RLCA, aggregation continues steadily as long as reactants are available, resulting in more compact cluster shapes. The growth of clusters is driven by the rate of chemical or physical reactions, rather than by diffusion. Light scattering methods can be used to distinguish between these regimes by determining the mass fractal dimension, the measure of how compact or dense a particle is, how efficiently it fills out a volume, and how its mass scales with increasing size. For DLCA, the fractal dimension is around 1.8, while it is around 2.1 for RLCA. Increasing fractal dimension means more compact structures.

Temperature effects both diffusion rates and reaction rates, thereby influencing which aggregation process dominates. This is the topic of Chapter Four, investigating temperature effects on final lignin particle properties.

Lignin Color Characterization

Color analysis can be quickly performed with a handheld colorimeter shown on Figure 10, which is a cost-effective, non-invasive, quantitative measure of color, usually used for color comparison for quality control in textile and paint manufacturing, along with dentistry and the food industry [61]. While a spectrophotometer measures the complete spectrum of light absorbed or transmitted by a sample across a wide range of wavelengths, a colorimeter does not provide a full spectrum, instead it measures the intensity in specific broad wavelength ranges, but can be used for quantifying lignin color [62, 63]. As discussed in Chapter Three, our primary goal is basic color comparison, where a colorimeter is more suitable due to its relative simplicity and lower cost. A colorimeter measures color by determining the tristimulus values of a sample (X , Y , Z), which represents the amount of red, green, and blue light absorbed or reflected by the object. Then these tristimulus values are converted to the CIELAB color space, which is designed to provide a system to mimic human color perception. The CIELAB color space expresses color using three values: L for lightness (0 to 100), and the chromatic a and b values (-128 to 128) for the four unique colors of human vision, shown on Figure 10. The a axis ranges from green to red and the b axis ranges from blue to yellow color.

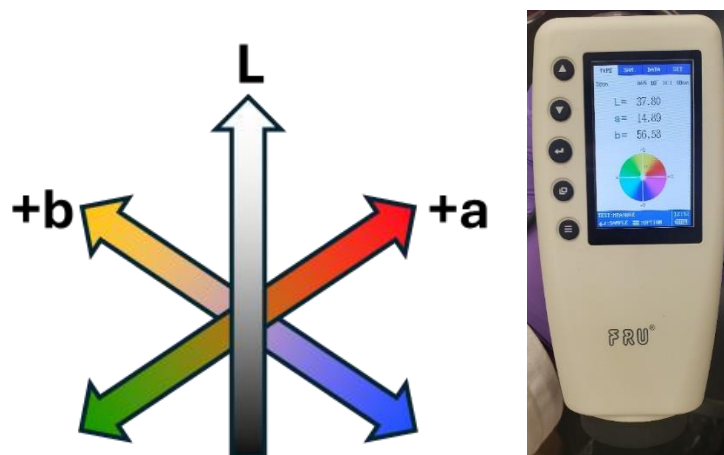


Figure 10. CIELAB color space (left) and the handheld colorimeter (right).

Differences in colors measured in the CIELAB color space can be expressed by the distance between two points in the color space, commonly denoted as

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}. \quad (17)$$

Another useful color measure is the Browning index (BI), commonly used to indicate the browning of sugar containing food products like produce [64], and it is defined as the brown color purity, calculated as:

$$BI = 100 \frac{X-0.31}{0.17}, \quad (18)$$

where

$$X = \frac{a(a + 1.75L)}{5.645L + a - 3.012b} \quad (19)$$

using empirically determined coefficients.

UV treatment of alkali lignin in tetrahydrofuran (THF) has been shown to result in lighter lignins, with decreases in the content of aromatic rings, methoxyl groups, and phenolic hydroxyl groups, while the content of carboxylic groups increases [65]. Additionally, the molar mass of

lignin decreased following UV exposure. Light scattering analysis revealed two peaks - representing aggregate and individual lignins - both of which decreased with UV treatment.

Aggregation significantly affects lignin color; less aggregated and conjugated lignins typically exhibit lighter colors. Lignins recovered at lower pH values display fewer conjugated structures, lighter colors, and lower bulk densities [66]. The micromorphology of lignin impacts its color performance as well. Acetylation and precipitation processes produce light-colored, low bulk density lignins that can serve as effective sunscreen additives, enhancing the SPF factor without significant staining on the skin [67]. For industrial applications, the color of lignin is affected by harsh delignification methods like Kraft pulping resulting in darker lignins, while organosolv processes yield lighter lignins. Stronger mineral acids used in the process lead to brighter lignins [68].

Variations in color are due to differences in how lignin absorbs, reflects, transmits, and scatters light [69, 70]. Mie scattering, which occurs when particle sizes are comparable to the wavelength of light, can make lignin appear lighter. This scattering effect is why clouds appear white, as water vapor particles scatter light equally across the spectrum. Color variations arise from both the physical properties of the surface (such as particle shape, size, and texture) and chemical composition [71]. Chromophores, which include conjugated structures involving quinonoid and side-chain double bonds, are examples of chemical differences that influence color [72]. Oxidation can lighten lignins, and studies have shown that overcoming lignin's dark color can be achieved by blocking or functionalizing free phenolic groups or through oxidation using O_2 , H_2O_2 , or hypochlorite [64]. Lignin bleaching and oxidation chemistry are key areas of research in improving lignin color [73], which irreversibly lightens lignin.

Fluorescence Microscopy, Raman Scattering

Microscopic imaging can provide a unique perspective to biomass and lignin samples. Due to the presence of fluorophores in the lignin structure (aromatic rings), lignin exhibits autofluorescence, so direct analysis using fluorescence microscopy is possible [74, 75]. It has been reported that this imaging can be used in a semi-quantitative method for lignin characterization, predicting the type of monolignol (lignin building block) that is present in the sample [76]. Confocal microscopy is also often used in plant cell wall research [74, 77], which is an imaging technique used to increase the optical resolution and contrast of an image by blocking out-of-focus light. A three-dimensional structure can be constructed with this technique by taking multiple two-dimensional images (slices) of a sample at different depths. Epifluorescence microscopy of lignin is introduced in Chapter Three, and it has a major role in Chapter Four with the addition of confocal microscopy. Raman scattering or the Raman effect is the inelastic scattering of photons (usually from a laser) that interacts with the molecular vibrations of the sample resulting in shifts of the energy of the laser photons, which gives information about the chemical bonds and structures in the sample, called Raman spectroscopy. Raman imaging techniques can be applied to investigate plant cell wall components to determine the spatial distribution of different components in plant cell walls. Mapping of aromatic rings, aldehyde and alcohol groups was demonstrated using stimulated Raman scattering (SRS) [78, 79]. Raman imaging is an advanced technique that can expand on our findings in Chapter Four.

Lignin Applications

Some processes where lignin can be used as a substitution for petroleum-derived chemicals are briefly introduced connected to work done in Chapter Two and Chapter Three. Here we provide more background information on these potential lignin applications.

Carbon fiber is made from the precursor polymer polyacrylonitrile (PAN). PAN production is complex and expensive, which makes the resulting carbon fiber expensive, limiting its applications. The automotive industry is a potential market for carbon fibers, but it is scarcely used due to this high cost. The estimated global demand for carbon fiber is 100,000 metric tons annually [80-82]. Phenol formaldehyde (PF) resins are created by a reaction of phenol (or phenol substitute) with formaldehyde. Phenol and phenol substitutes are toxic, and they are mainly produced from petroleum-derived feedstocks [11, 83]. They are used in many industries, including construction (for bonding plywood), automotive (brake linings) and woodworking. The market is expected to grow with the rising residential and commercial building demands [84]. Polyurethane (PU) is synthesized by reacting diisocyanates with terminal polyols. Polyols are relatively non-toxic, but they are produced from platform chemicals in a relatively cost-intensive process. PU is widely used in furniture, automobile seats, bedding, varnish, adhesives, foams, with the annual global consumption of 12 million metric tons [81, 82, 85-89]. Substituting PAN, polyols or a phenol with lignin opens new, innovative processes for the manufacturing of these widely used products, while significantly decreasing the toxicity and cost.

A novel application where the color of lignin is important is as a natural sunscreen additive. As lignin has chromophore functional groups it can absorb UV light in the range of 250-400 nm [90], and has synergistic effects with other sunscreen actives as an antioxidant [62, 91]. It can

prove to be a natural and renewable alternative to synthetic sunscreen actives [63]. Lignin color is important in other applications as well, like papermaking, cosmetics, plastics, paints, resins and composite materials [66].

Overview of Research Goals and Objectives

In Chapter Two, we are investigating the solubility of lignin using a novel fractional solubility approach in a range of organic solvents, using a biorefinery residue as lignin feedstock. This biorefinery residue is subjected to three different extraction methods for lignin recovery. In Chapter Three, a 20-L scale alkaline pretreatment method is developed and optimized to recover lignin for collaborators working with lignin purification and developing lignin-based products. A variable screening is conducted to determine which recovery condition impacts the observed variability in lignin physical properties. In Chapter Four, the identified filtering temperature from the variable screening in Chapter Three is closely investigated. The filtering temperature greatly impacts lignin color, exhibiting a transition threshold between dark and light lignin recovery. The different colored lignins are compared by their chemical and morphological properties.

References

1. Langholtz, M.H., *2023 Billion-Ton Report*. 2024: United States.
2. Zakzeski, J., et al., *The Catalytic Valorization of Lignin for the Production of Renewable Chemicals*. Chemical Reviews, 2010. **110**(6): p. 3552-3599.
3. Ragauskas, A.J., et al., *Lignin Valorization: Improving Lignin Processing in the Biorefinery*. Science, 2014. **344**(6185): p. 1246843.
4. Jungmeier, G., et al., *Biofuel-driven Biorefineries A selection of the most promising biorefinery concepts to produce large volumes of road transportation biofuels by 2025*. Report of IEA Bioenergy Task, 2013. **42**.

5. Calvo-Flores, F.G. and F.J. Martin-Martinez, *Biorefineries: Achievements and challenges for a bio-based economy*. Front Chem, 2022. **10**: p. 973417.
6. Hsu, T.A., M.R. Ladisch, and G.T. Tsao, *Alcohol from cellulose*. Chem. Technol.; (United States), 1980: p. Medium: X; Size: Pages: 315-319 2009-12-17.
7. Bajpai, P., *Pretreatment of Lignocellulosic Biomass*, in *Pretreatment of Lignocellulosic Biomass for Biofuel Production*, P. Bajpai, Editor. 2016, Springer Singapore: Singapore. p. 17-70.
8. Agbor, V.B., et al., *Biomass pretreatment: Fundamentals toward application*. Biotechnology Advances, 2011. **29**(6): p. 675-685.
9. Sun, Z., et al., *Bright Side of Lignin Depolymerization: Toward New Platform Chemicals*. Chemical Reviews, 2018. **118**(2): p. 614-678.
10. Davis, R., et al., *Process design and economics for the conversion of lignocellulosic biomass to hydrocarbons: dilute-acid and enzymatic deconstruction of biomass to sugars and biological conversion of sugars to hydrocarbons*. 2013, National Renewable Energy Lab.(NREL), Golden, CO (United States).
11. Laurichesse, S. and L. Avérous, *Chemical modification of lignins: Towards biobased polymers*. Progress in Polymer Science, 2014. **39**(7): p. 1266-1290.
12. Argyropoulos, D.D.S., et al., *Kraft Lignin: A Valuable, Sustainable Resource, Opportunities and Challenges*. ChemSusChem, 2023. **16**(23): p. e202300492.
13. Sannigrahi, P., A.J. Ragauskas, and G.A. Tuskan, *Poplar as a feedstock for biofuels: A review of compositional characteristics*. Biofuels, Bioproducts and Biorefining, 2010. **4**(2): p. 209-226.
14. Zabed, H.M., et al., *Towards the sustainable conversion of corn stover into bioenergy and bioproducts through biochemical route: Technical, economic and strategic perspectives*. Journal of Cleaner Production, 2023. **400**: p. 136699.
15. Wyman, C.E., et al., *Coordinated development of leading biomass pretreatment technologies*. Bioresource Technology, 2005. **96**(18): p. 1959-1966.
16. Fache, M., B. Boutevin, and S. Caillol, *Vanillin Production from Lignin and Its Use as a Renewable Chemical*. ACS Sustainable Chemistry & Engineering, 2016. **4**(1): p. 35-46.
17. Suhas, P.J.M. Carrott, and M.M.L. Ribeiro Carrott, *Lignin – from natural adsorbent to activated carbon: A review*. Bioresource Technology, 2007. **98**(12): p. 2301-2312.

18. Brienza, F., et al., *A guide to lignin valorization in biorefineries: traditional, recent, and forthcoming approaches to convert raw lignocellulose into valuable materials and chemicals*. RSC Sustainability, 2023.
19. Saulnier, B.K., et al., *Impact of dilute acid pretreatment conditions on p-coumarate removal in diverse maize lines*. Bioresource Technology, 2020. **314**: p. 123750.
20. König-Mattern, L., et al., *Machine learning-supported solvent design for lignin-first biorefineries and lignin upgrading*. Chemical Engineering Journal, 2024. **495**: p. 153524.
21. Abu-Omar, M.M., et al., *Guidelines for performing lignin-first biorefining*. Energy & Environmental Science, 2021. **14**(1): p. 262-292.
22. Arapova, O.V., et al., *Lignin as a Renewable Resource of Hydrocarbon Products and Energy Carriers (A Review)*. Petroleum Chemistry, 2020. **60**(3): p. 227-243.
23. Chen, F., et al., *A polymer of caffeyl alcohol in plant seeds*. Proceedings of the National Academy of Sciences, 2012. **109**(5): p. 1772-1777.
24. Schuerch, C., *The Solvent Properties of Liquids and Their Relation to the Solubility, Swelling, Isolation and Fractionation of Lignin*. Journal of the American Chemical Society, 1952. **74**(20): p. 5061-5067.
25. Venkatram, S., et al., *Critical Assessment of the Hildebrand and Hansen Solubility Parameters for Polymers*. Journal of Chemical Information and Modeling, 2019. **59**(10): p. 4188-4194.
26. Brown, W., *Solution properties of lignin. Thermodynamic properties and molecular weight determinations*. Journal of Applied Polymer Science, 1967. **11**(11): p. 2381-2396.
27. Sameni, J., S. Krigstin, and M. Sain, *Solubility of Lignin and Acetylated Lignin in Organic Solvents*. Bioresources, 2017. **12**: p. 1548-1565.
28. Le, H.Q., et al., *Solubility of Organosolv Lignin in gamma-Valerolactone/Water Binary Mixtures*. ChemSusChem, 2016. **9**(20): p. 2939-2947.
29. Vermaas, J.V., et al., *Automated Transformation of Lignin Topologies into Atomic Structures with LigninBuilder*. ACS Sustainable Chemistry & Engineering, 2019. **7**(3): p. 3443-3453.
30. Vermaas, J.V., M.F. Crowley, and G.T. Beckham, *Molecular Lignin Solubility and Structure in Organic Solvents*. ACS Sustainable Chemistry & Engineering, 2020. **8**(48): p. 17839-17850.
31. Dellon, L.D., et al., *Computational Generation of Lignin Libraries from Diverse Biomass Sources*. Energy & Fuels, 2017. **31**(8): p. 8263-8274.

32. Alonso, D.M., J.Q. Bond, and J.A. Dumesic, *Catalytic conversion of biomass to biofuels*. Green Chemistry, 2010. **12**(9): p. 1493-1513.
33. Marangon Jardim, J., et al., *A Quantitative Comparison of the Precipitation Behavior of Lignin from Sweetgum and Pine Kraft Black Liquors*. Bioresources, 2020. **15**: p. 5464-5480.
34. Gierer, J., *Chemical aspects of kraft pulping*. Wood Science and Technology, 1980. **14**(4): p. 241-266.
35. Chakar, F.S. and A.J. Ragauskas, *Review of current and future softwood kraft lignin process chemistry*. Industrial Crops and Products, 2004. **20**(2): p. 131-141.
36. Bajwa, D.S., et al., *A concise review of current lignin production, applications, products and their environmental impact*. Industrial Crops and Products, 2019. **139**: p. 111526.
37. Pan, X., et al., *Bioconversion of hybrid poplar to ethanol and co-products using an organosolv fractionation process: optimization of process yields*. Biotechnol Bioeng, 2006. **94**(5): p. 851-61.
38. Pye, E.K. and J.H. Lora, *The ALCELL process--a proven alternative to kraft pulping*. Tappi Journal, 1991. **74**: p. 113-118.
39. Lee, Y.Y., P. Iyer, and R.W. Torget, *Dilute-Acid Hydrolysis of Lignocellulosic Biomass*, in *Recent Progress in Bioconversion of Lignocellulosics*, G.T. Tsao, et al., Editors. 1999, Springer Berlin Heidelberg: Berlin, Heidelberg. p. 93-115.
40. Yan, L., et al., *A comprehensive mechanistic kinetic model for dilute acid hydrolysis of switchgrass cellulose to glucose, 5-HMF and levulinic acid*. RSC Advances, 2014. **4**(45): p. 23492-23504.
41. Mosier, N., et al., *Features of promising technologies for pretreatment of lignocellulosic biomass*. Bioresource Technology, 2005. **96**(6): p. 673-686.
42. 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.
43. Tuck, C.O., et al., *Valorization of Biomass: Deriving More Value from Waste*. Science, 2012. **337**(6095): p. 695-699.
44. Hubbe, M., et al., *Lignin recovery from spent alkaline pulping liquors using acidification, membrane separation, and related processing steps: A review*. BioResources, 2019. **14**: p. 2300-2351.
45. Tomani, P. *The Lignoboost Process*.

46. Kouisni, L., et al., *LignoForce System for the Recovery of Lignin from Black Liquor: Feedstock Options, Odor Profile, and Product Characterization*. ACS Sustainable Chemistry & Engineering, 2016. **4**(10): p. 5152-5159.
47. Ohman, F. and H. Theliander, *Filtration properties of lignin precipitated from black liquor*. TAPPI Journal, 2001. **6**: p. 3-9.
48. Sewring, T., et al., *Acid Precipitation of Kraft Lignin from Aqueous Solutions: The Influence of pH, Temperature, and Xylan*. Journal of Wood Chemistry and Technology, 2019. **39**(1): p. 1-13.
49. Sewring, T. and H. Theliander, *Acid precipitation of kraft lignin from aqueous solutions: the influence of anionic specificity and concentration level of the salt*. Holzforschung, 2019. **73**(10): p. 937-945.
50. Norgren, M., H. Edlund, and L. Wågberg, *Aggregation of Lignin Derivatives under Alkaline Conditions. Kinetics and Aggregate Structure*. Langmuir, 2002. **18**(7): p. 2859-2865.
51. Zhu, W. and H. Theliander, *Precipitation of Lignin from Softwood Black Liquor: An Investigation of the Equilibrium and Molecular Properties of Lignin*. BioResources, 2015. **10**.
52. Matsakas, L., et al., *Preparation of low carbon impact lignin nanoparticles with controllable size by using different strategies for particles recovery*. Industrial Crops and Products, 2020. **147**: p. 112243.
53. Abächerli, A. and F. Doppenberg, *Method for preparing alkaline solutions containing aromatic polymers*. 2001, Google Patents.
54. Jardim, J.M., et al., *A quantitative comparison of the precipitation behavior of lignin from sweetgum and pine kraft black liquors*. BioResources, 2020. **15**(3): p. 5464-5480.
55. 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**: p. 39-48.
56. Sundin, J., *Precipitation of Kraft Lignin under Alkaline Conditions*, in *Department of Pulp and Paper Chemistry and Technology*. 2000, Royal Institute of Technology: Stockholm.
57. Öhman, F., H. Wallmo, and H. Theliander, *An improved method for washing lignin precipitated from kraft black liquor-the key to a new biofuel*. Filtration, 2007. **7**(4): p. 309-315.

58. Deng, Y., et al., *Investigation of Aggregation and Assembly of Alkali Lignin Using Iodine as a Probe*. Biomacromolecules, 2011. **12**(4): p. 1116-1125.
59. Colucci, G., et al., *Development of colloidal lignin particles through particle design strategies and screening of their Pickering stabilizing potential*. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 2023. **666**: p. 131287.
60. Weitz, D.A., M.Y. Lin, and H.M. Lindsay, *Universality laws in coagulation*. Chemometrics and Intelligent Laboratory Systems, 1991. **10**(1): p. 133-140.
61. Park, J.-W., *Understanding of the color in composite resin*. J Korean Acad Conserv Dent, 2011. **36**(4): p. 271-279.
62. Lee, S.C., et al., *Lignin for white natural sunscreens*. International Journal of Biological Macromolecules, 2019. **122**: p. 549-554.
63. Zhang, H., et al., *Fabrication of Light-Colored Lignin Microspheres for Developing Natural Sunscreens with Favorable UV Absorbability and Staining Resistance*. Industrial & Engineering Chemistry Research, 2019. **58**(31): p. 13858-13867.
64. Lunadei, L., et al., *A multispectral vision system to evaluate enzymatic browning in fresh-cut apple slices*. Postharvest Biology and Technology, 2011. **60**(3): p. 225-234.
65. Wang, J., et al., *Reduction of lignin color via one-step UV irradiation*. Green Chemistry, 2016. **18**(3): p. 695-699.
66. Zhang, H., S. Fu, and Y. Chen, *Basic understanding of the color distinction of lignin and the proper selection of lignin in color-depended utilizations*. International Journal of Biological Macromolecules, 2020. **147**: p. 607-615.
67. Zhang, H., et al., *Micromorphology influence on the color performance of lignin and its application in guiding the preparation of light-colored lignin sunscreen*. ACS Sustainable Chemistry & Engineering, 2018. **6**(9): p. 12532-12540.
68. Ajao, O., et al., *Quantification and Variability Analysis of Lignin Optical Properties for Colour-Dependent Industrial Applications*. Molecules, 2018. **23**(2): p. 377.
69. Myers, T.L., et al., *Quantitative reflectance spectra of solid powders as a function of particle size*. Applied Optics, 2015. **54**(15): p. 4863-4875.
70. Li, Z., et al., *Effect of particle size, transparency and light intensity on the color of powder*. IOP Conference Series: Earth and Environmental Science, 2020. **545**: p. 012027.
71. Dilling, P. and P.T. Sarjeant, *Reduction of lignin color*. 1984, Google Patents.
72. Lin, S.Y., *Process for reduction of lignin color*. 1980, Google Patents.

73. Gierer, J., *Formation and involvement of superoxide ($O_2^{\cdot-}/HO_2^{\cdot-}$) and hydroxyl ($OH^{\cdot}$) radicals in TCF bleaching processes: A review*. 1997.
74. Baldacci-Cresp, F., et al., *A rapid and quantitative safranin-based fluorescent microscopy method to evaluate cell wall lignification*. Plant J, 2020. **102**(5): p. 1074-1089.
75. Maceda, A. and T. Terrazas, *Fluorescence Microscopy Methods for the Analysis and Characterization of Lignin*. Polymers, 2022. **14**(5): p. 961.
76. Escamez, S., et al., *Fluorescence Lifetime Imaging as an In Situ and Label-Free Readout for the Chemical Composition of Lignin*. ACS Sustainable Chemistry & Engineering, 2021. **9**(51): p. 17381-17392.
77. Singh, S.S., L.-T. Lim, and A. Manickavasagan, *Imaging and spectroscopic techniques for microstructural and compositional analysis of lignocellulosic materials: a review*. Biomass Conversion and Biorefinery, 2020.
78. Liu, B., et al., *Vibrational Fingerprint Mapping Reveals Spatial Distribution of Functional Groups of Lignin in Plant Cell Wall*. Analytical Chemistry, 2015. **87**(18): p. 9436-9442.
79. Guillon, F., et al., *Chapter Six - In situ imaging of lignin and related compounds by Raman, Fourier-transform infrared (FTIR) and fluorescence microscopy*, in *Advances in Botanical Research*, R. Sibout, Editor. 2022, Academic Press. p. 215-270.
80. Jin, J., et al., *Carbon Fibers Derived from Fractionated–Solvated Lignin Precursors for Enhanced Mechanical Performance*. ACS Sustainable Chemistry & Engineering, 2018. **6**(11): p. 14135-14142.
81. Frank, E., et al., *Carbon Fibers: Precursor Systems, Processing, Structure, and Properties*. Angewandte Chemie-International Edition, 2014. **53**(21): p. 5262-5298.
82. Kadla, J.F., et al., *Lignin-based carbon fibers for composite fiber applications*. Carbon, 2002. **40**(15): p. 2913-2920.
83. Arefmanesh, M., et al., *From acetone fractionation to lignin-based phenolic and polyurethane resins*. Industrial Crops and Products, 2022. **178**: p. 114604.
84. *Global Formaldehyde Market Size To Grow \$15.51 Billion By 2030 | CAGR 5%*. 2023; Available from: <https://www.globenewswire.com/en/news-release/2023/01/13/2588556/0/en/Global-Formaldehyde-Market-Size-To-Grow-15-51-Billion-By-2030-CAGR-5.html>.
85. Alinejad, M., et al., *Lignin-Based Polyurethanes: Opportunities for Bio-Based Foams, Elastomers, Coatings and Adhesives*. Polymers, 2019. **11**(7): p. 21.

86. da Silva, E.A.B., et al., *An integrated process to produce vanillin and lignin-based polyurethanes from Kraft lignin*. Chemical Engineering Research & Design, 2009. **87**(9A): p. 1276-1292.
87. Temples, S.C., et al., *Liquid–Liquid Equilibrium Compositions and Global Phase Behavior for Lignin–Acetone–Water Ternary at 25, 45, and 65 °C*. Industrial & Engineering Chemistry Research, 2019. **58**(50): p. 22619-22625.
88. Ding, J., et al., *Liquid–liquid equilibrium compositions and global phase behavior for the lignin–acetic acid–water system at 70 and 95 °C*. Fluid Phase Equilibria, 2018. **461**: p. 8-14.
89. Tindall, G.W., et al., *Fractionating and Purifying Softwood Kraft Lignin with Aqueous Renewable Solvents: Liquid–Liquid Equilibrium for the Lignin–Ethanol–Water System*. ChemSusChem, 2020. **13**(17): p. 4587-4594.
90. Sadeghifar, H. and A. Ragauskas, *Lignin as a UV Light Blocker—A Review*. Polymers, 2020. **12**(5): p. 1134.
91. Qian, Y., X. Qiu, and S. Zhu, *Lignin: a nature-inspired sun blocker for broad-spectrum sunscreens*. Green Chemistry, 2015. **17**(1): p. 320-324.

CHAPTER TWO

EXTRACTION, RECOVERY, AND CHARACTERIZATION
OF LIGNIN FROM INDUSTRIAL CORN STOVER
LIGNIN CAKE

Contribution of Authors and Co-Authors

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Contributions: writing, review, editing, investigation, conceptualization

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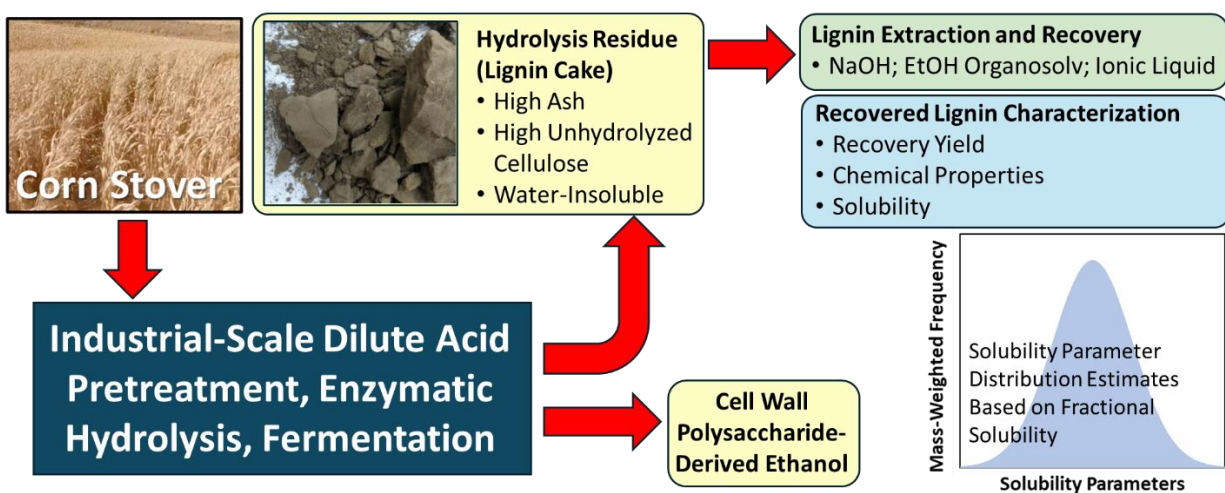


Figure 11. Visual abstract

Highlights

- Dilute acid pretreatment lignin cake is high in ash and glucan with poor solubility

- Three approaches for lignin extraction from lignin cake are compared
- Lignins low in contaminating polysaccharides can be recovered from the lignin cake
- NaOH extraction results in more than 80% lignin recovery
- A novel approach for estimating solubility parameter distributions is demonstrated

Abstract

Lignin utilization in value-added co-products is an important component of enabling cellulosic biorefinery economics. However, aqueous dilute acid pretreatments yield lignins with limited applications due to significant modification during pretreatment, low solubility in many solvents, and high content of impurities (ash, insoluble polysaccharides). This work addresses these challenges and investigates the extraction and recovery of lignins from lignin-rich insoluble residue following dilute acid pretreatment and enzymatic hydrolysis of corn stover using three extraction approaches: ethanol organosolv, NaOH, and ionic liquid. The recovered lignins exhibited recovery yields ranging from 30% for the ionic liquid, 44% for the most severe acid ethanol organosolv condition tested, and up to 86% for the most severe NaOH extraction condition. Finally, the fractional solubilities of different recovered lignins were assessed in a range of solvents and these solubilities were used to estimate distributions of Hildebrand and Hansen solubility parameters using a novel approach.

Keywords: Biorefining, solvent extraction, lignin recovery, dilute acid pretreatment, solubility parameters

1. Introduction

The carbon contained in the cell walls of plants (lignocellulose) has potential as a renewable feedstock for biorefining processes that can yield biobased fuels, chemicals, and materials. Plant cell walls are comprised primarily of polysaccharides (cellulose and hemicelluloses) and the complex aromatic biopolymer lignin. One lignocellulose biorefining strategy utilizes pretreatment followed by an enzymatic hydrolysis of the cellulose and remaining hemicellulose to facilitate the biological or catalytic conversion of the cell wall-derived sugars for fuels and chemicals [4]. Utilization of lignin in these approaches remains a challenge due to the irregular structure of the polymer, its modification during pretreatment processes, and the difficulty of converting lignins to sets of homogeneous products with high yields and selectivities [11, 92]. Due to the many challenges of utilizing the chemical functionality of lignin in higher value fuels and chemicals, many process designs propose utilizing lignin as solid fuel [10]. From both an atom efficiency perspective and an economic perspective, extracting more value from the lignin is critical for the economics of a lignocellulose biorefinery as 15% to 25% of the mass entering a lignocellulose biorefining process is comprised of lignin [3]. Additionally, the lignins from lignocellulose biorefining processes may offer novel opportunities for co-products as these lignins will exhibit unique properties relative to other commercial lignins (*e.g.*, sulfite and Kraft lignins) as a consequence of both the feedstock source (*i.e.*, grasses vs. hardwoods or softwoods) [18] and processing conditions.

Integrating lignin utilization into biorefining processes ultimately requires lignin solubilization, which can be performed at different stages in the process. Examples of different approaches include extraction of lignin during pretreatment in alkali and organosolv pretreatments

or incorporating a simultaneous lignin extraction and depolymerization [93]. Another approach is to utilize lignin extraction and recovery at the “back end” of a process that does not employ a lignin-solubilizing pretreatment. These processes such as an ethanol production process using aqueous acidic pretreatment produce a material known as “lignin cake”, or the water-insoluble hydrolysis residue at the end of the process. For a dilute acid pretreatment, key compositional features of this material include unreacted cellulose, a high content of acid-modified lignin, and high ash content. Furthermore, the lignin is substantially different from “native” lignins due to its modification during pretreatment. Acidic treatments of lignin can result in depolymerization and repolymerization with the introduction of new carbon-carbon bonds [94] that can impede further depolymerization and alter the solubility in many solvents. As an example, prior work subjected corn stover to a range of dilute acid pretreatment conditions and found that increasing dilute acid pretreatment severity reduced the extraction and recovery yield of lignins using NaOH and formic acid extraction [95].

Approaches for lignin recovery from biorefinery lignin cake can include extraction using lignin-solubilizing solvents [96] or potentially include features of delignifying pretreatments such as organosolv pretreatments [37, 97], alkali pretreatments [95], ionic liquids [98], or deep eutectic solvents (DESs) [99]. As examples of solvent extraction, a range of solvents were screened for extracting lignin from lignin cake derived from ammonia pretreatment and enzymatic hydrolysis of corn stover [96]. It was found that aqueous ethanol and acetic acid could yield 51% to 65% extractable solids, respectively, from the lignin cake at high lignin purities (*i.e.*, <5% total ash and polysaccharides) [96]. Other solvents, including aqueous acetone and γ -valerolactone could achieve high extraction yields but contained significant amounts (38% and 55%, respectively) of

contaminating polysaccharides. As an example of an acidic organosolv extraction from a hydrolysis residue, extraction of lignin from corn cob residue following furfural production from xylan was performed with acidic dioxane:water (85:15, v/v) to achieve lignin recovery yields ranging from 45% to 55% [100].. Ethanol extraction and recovery (and the associated lignin modification) was assessed for the hydrolysis residue of liquid hot water-pretreated wheat straw from the Inbicon A/S demonstration cellulosic biorefinery in Kalundborg, Denmark [101]. This work screened a range of temperatures and found that when a homogeneous acid catalyst (*p*-toluene sulfonic acid) was introduced along with a fatty alcohol as a capping agent to prevent repolymerization the lignin solubilization yields increased from 35% to 72%.

While lignins have potential value for bioproduct applications, utilization of lignin extracted from lignin cake introduces a unique set of challenges due to its poor solubility in many solvents and contamination with ash and unhydrolyzed polysaccharides. In this work approaches were investigated for extraction, recovery, and purification of lignin from an industrial corn stover lignin cake and assess the impacts of extraction conditions on lignin yields, purities, and properties. Specifically, the three different lignin extraction and recovery were employed: ethanol organosolv extraction, NaOH extraction, and extraction with the 1-methyl-3-(3-sulfopropyl)-imidazolium hydrogen sulfate ($[\text{C}_3\text{SO}_3\text{HMIM}][\text{HSO}_4]$) ionic liquid. Lignin chemical properties and recovery yields were assessed as a function of extraction conditions. Lignin chemical properties were characterized and solubility of recovered lignins in a range of organic solvents was tested. Finally, Hildebrand and Hansen solubility parameters for select recovered lignins were estimated by fitting distributions of these parameters to lignin fractional solubility data.

2. Material and Methods

2.1 Materials

Lignin cake was generated from dilute sulfuric acid pretreatment and enzymatic hydrolysis of corn stover at the POET-DSM Project Liberty demonstration scale facility in Emmetsburg, Iowa [102] in summer 2018 and was kindly provided by Dr. Tyler Jordison (POET LLC, Sioux Falls, South Dakota, USA). Lignin cake was dried at 50 °C for 11 days immediately after generation to a moisture content of 1.3% by mass. When water extractives-free material was used, the lignin cake was manually ground with a ceramic mortar and pestle then washed with 11 volumes of deionized water per unit mass of lignin cake in a Thermo Scientific Sorvall ST8 Centrifuge at 4500 rpm for 5-minute intervals for a total of 4 times. After the wash, the lignin was dried at room temperature in a fume hood for 24 hours, then stored in an airtight container at 4°C.

2.2 Extraction and Recovery of Lignin from Industrial Lignin Cake

Three general approaches for lignin extraction and recovery from lignin cakes were employed that include ethanol organosolv with and without added acid, aqueous NaOH extraction, and the ionic liquid [C₃SO₄HMIM][HSO₄]. For the ethanol organosolv lignin extraction, 2.0 g washed air-dried lignin cake was measured using a Mettler Toledo ME103E analytical balance into a reactor with 20.0 mL of 8:2 (vol/vol) EtOH-water solution corresponding to 10% (wt/vol) solids loading, with or without 0.5% (vol/vol) sulfuric acid (corresponding to 92 mg H₂SO₄/g biomass) together with a stir bar. Teflon-lined autoclave reactors in stainless steel shells were used for the reactions. Heating medium was silicone oil, heated by Corning PC-420D stirring hot plates.

The sealed reactor was placed in a preheated oil bath in duplicate. After 1 hour at the reaction temperature (120 °C, 150 °C, or 180 °C) the reactors were removed from the oil bath to

cool at room temperature for an hour. The reaction mixture was filtered using Whatman #42 55 mm ashless filter paper then washed with 25 mL of 70°C EtOH. The solids were dried at room temperature overnight. The solvent from the filtrate was evaporated using a Yamato Scientific RE200 rotary evaporator, using a Yamato Scientific BM500 water bath set at 40°C until a highly viscous gel was obtained. The gel was dried at room temperature overnight and washed the next day with 10 volumes of deionized water for 1 hour at room temperature. The washed solids were dried at room temperature overnight, weighed and subjected to subsequent characterization. Yields were determined gravimetrically.

For the NaOH extractions, 4.0 g (dry basis) washed air-dried lignin cake was placed into reactors reactor with 20.0 mL NaOH solution (1.0 M or 0.5 M), corresponding to 20% (wt/vol) solids loading and a stir bar. The sealed reactor was placed in a preheated oil bath in duplicate. After 1 hour at the reaction temperature (85 °C, 180 °C) the reactors were removed from the oil bath to cool at room temperature for an hour. The reaction mixture was vacuum filtered using Whatman 42 55 mm ashless filter paper. The solids were dried at room temperature overnight. The volume and pH of the filtrate was measured, then it was acidified with 96% sulfuric acid until the pH of the solution was below 2.0, then incubated overnight at 4 °C to precipitate the lignin fraction. The next day the precipitated solids were separated by filtration and washed with water until the pH of the filtrate was above 5. The washed solids were dried at room temperature overnight, weighed and characterized by the NREL/TP-510-42618. Yields were determined gravimetrically.

The 1-methyl-3-(3-sulfopropyl)-imidazolium hydrogen sulfate $[C_3SO_4HMIM][HSO_4]$ ionic liquid was synthesized as in prior work [103]. For the IL extraction, approximately 1.0 g of lignin (dry basis), 0.295 g of the IL, and 10 mL of a 3:1 v/v ethanol:water were added to a 40 mL

glass pressure tube (Ace Glass, 8648–29). The tube was capped and submerged in a 100 °C preheated oil bath for one hour while mixing using a stir bar. The reactor was cooled to room temperature and filtered (Whatman #42). The insoluble residue was washed with an additional 10 mL ethanol and dried in fume hood for 24 h. The residue mass was recorded following drying at 105 °C while the total mass of soluble solids in the filtrate was determined following drying in a rotatory evaporator. The yield of IL-soluble lignin was calculated from the mass of solids in the filtrate corrected for the mass of the IL used.

2.3 Solvent Screening for Lignin Solubility

Using 1:10 solid to solvent ratio, 300 mg recovered lignin was mixed in a test tube with 3.0 mL of the examined solvent. The test tube was sealed with a cap then put in the preheated oil bath for 1 hour at the predetermined temperature in duplicate. These temperatures were 70 °C (ethyl acetate and DMSO), 60 °C (THF and methanol), or 50 °C (acetone). The mixture was filtered using Whatman #42 55 mm ashless filter paper then washed using 30 mL of solvent. The remaining solids were air-dried overnight at room temperature.

2.4 Lignin Characterization

The comprehensive biomass analysis, including ash, carbohydrate, acid insoluble (Klason) lignin (AIL), and acid soluble lignin (ASL) content of the samples was performed in accordance with the two-step hydrolysis method of the NREL/TP-510-42618 [104]. The monomeric carbohydrate and acetyl content were measured from the filtrate by using an Agilent Technologies 1260 Infinity II HPLC equipped with a Bio-Rad Aminex® HPX-87H column operating with a 0.005 M sulfuric acid mobile phase at a flow rate of 0.6 mL/min. The acid soluble lignin content was measured by Thermo Scientific Biomate 3 UV-Vis spectrophotometer from the filtrate at a

wavelength of 320 nm. The content of *p*CA in samples was determined by alkaline saponification followed by HPLC analysis of solubilized *p*CA as outlined in prior work [95]. Gel permeation chromatography (GPC) was used to determine the number and weight average molar masses of the extracted lignins, with acetylated samples utilized for the analysis, as in previous work [105]. ^{13}C and ^1H - ^{13}C correlation 2D heteronuclear single-quantum coherence (HSQC) NMR spectra of ethanol organosolv extracted lignins at different temperatures was performed as in previous work [105].

3. Results and Discussion

3.1 Characterization of Industrial Lignin Cake

In the first part of this work, the industrial lignin cake derived from the dilute acid pretreatment and enzymatic hydrolysis of corn stover was characterized for composition. A simplified process flow diagram for the major thermochemical and biological treatments that the corn stover undergoes prior to recovery as “lignin cake” is presented in **Figure 12A**, along with the placement of the lignin extraction and recovery at the “back end” of the process. When operating at nameplate capacity, the POET-DSM cellulosic ethanol plant could produce 150 tonnes/day of lignin cake [102] with the initial target application of a solid boiler fuel. Recovery yields of between 50% to 100% of this lignin with a selling price of US\$0.50/kg to US\$1.00/kg would add product value of US\$12.5M/yr to US\$50M/yr, which is approximately 5 to 10-fold greater than the value of the lignin as a solid fuel based on just the heating value [12, 106].

The lignin cake contains several classes of components that include (1) water-insoluble corn stover residue comprise of cell wall structural polymers, (2) inorganics derived from the intrinsic ash (*e.g.*, cell wall-associated silica phytoliths [107] in grasses), extrinsic ash (*e.g.*, soil

and dust that are collected during harvest and storage), or inorganics introduced into the process, (3) bound cellulase enzymes and yeast and from hydrolysis and fermentation, respectively, and (4) solubles that remain entrained in the lignin cake following filtration. The dilute acid pretreatment will primarily solubilize xylan and acetate esters in the hemicellulose as well as extractives, while the enzymatic hydrolysis solubilizes a significant fraction of the cellulose and unhydrolyzed xylan. Inorganics introduced to the process may include sulfate salts from the H_2SO_4 pretreatment, while additional pH adjustments prior to enzymatic hydrolysis and fermentation are via ammonia, which is recovered during anaerobic digestion of the stillage [102]. Insoluble solids pass through fermentation and the beer stripping stage of distillation and are recovered as “whole stillage” and are subjected to solid-liquid separation by first dewatering whole stillage in a decanter centrifuge followed by filtration to yield the lignin cake [102]. In the original design, the lignin cake is sent to a solid fuel boiler while the organics-rich filtrate is sent to anaerobic digestion. Introducing a lignin extraction and recovery step prior to lignin cake combustion (**Fig. 12A**) is one potential route to adding value to the process.

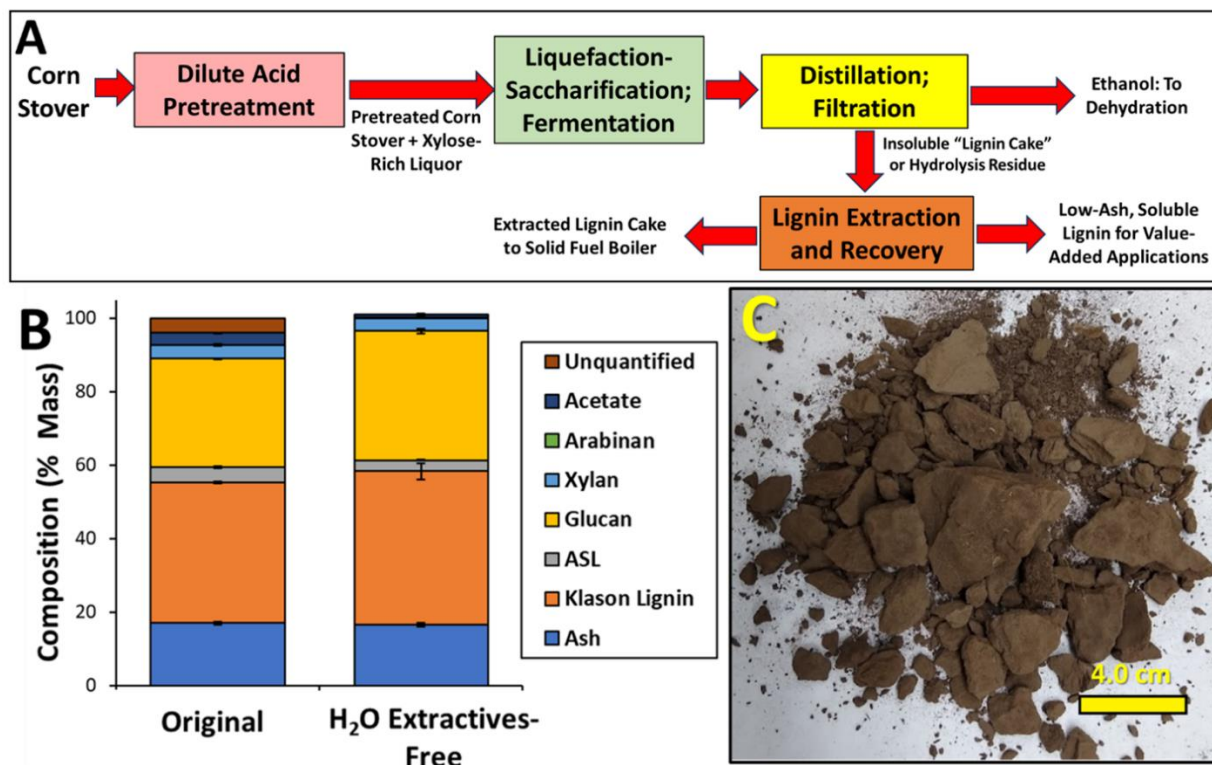


Figure 12. Simplified overall process flow diagram depicting (A) extraction and recovery of lignin at the “back end” of the process, (B) lignin cake composition on an original and water extractives-free basis, and (C) image of the as-received lignin cake used in this work. ASL: Acid-soluble lignin. Error bars represent data range for duplicate measurements.

The lignin cake used in this work (**Fig. 12B** and **12C**) was assessed and subjected to treatments on both an “as received” basis and following removal of water-soluble extractives. From the composition analysis, the original lignin cake is primarily comprised of acid-insoluble (Klason) lignin (38.4% or 41.7% for washed) with a significant fraction of non-lignin components including unhydrolyzed polysaccharides (*i.e.*, cellulose and a small amount of xylan), and ash (16.9%). Lignin cake may also contain hydrolytic enzymes bound to the biomass from the enzymatic hydrolysis stage. These enzymes would show up as Klason lignin. Following water extraction to remove water-soluble components, the composition remains relatively constant with minor decreases in acid-soluble lignin (ASL), acetate, and “unquantified”. This can be understood

as removing solubles that were entrained in the lignin cake (acetate, extractives, and unhydrolyzed oligosaccharides). The ash remains relatively unchanged indicating that the majority of this is water-insoluble inorganics, presumably extrinsic and intrinsic ash from the biomass. Critically, the majority of the mass of the lignin cake is comprised of “non-lignin” water-insoluble components (cell wall polysaccharides and ash) such that utilization of this lignin for chemical and materials applications requires a purification step.

Extraction Approach					Recovery Approach
Extraction Solvent	Temperature	H ₂ SO ₄ Loading (mg H ₂ SO ₄ /g biomass)	NaOH Loading (mg NaOH/g)	Solid Loading (g/mL)	
Ethanol:H ₂ O 80:20 (v/v)	120 °C	0		0.10	(1) Precipitation by concentration; Water washing; Filtration or (2) Water dilution; filtration
	120 °C	92			
	150 °C	0			
	150 °C	92			
	180 °C	0			
	180 °C	92			
NaOH	85 °C		100	0.20	Acidification to pH 2.0; Filtration
	85 °C		200		
	180 °C		100		
	180 °C		200		
30% (wt) Ionic Liquid [C ₃ SO ₄ HMIM] [HSO ₄] in 80:20 EtOH:H ₂ O	100 °C			0.10	Filtration; Solvent vaporization

Table 1. Summary of lignin extraction and recovery conditions.

This lignin purification and recovery can be achieved by selective solubilization from the lignin cake. Additionally, this solubilization step may entail modification of the lignin and/or selective solubilization to yield a subset of lignins with differing chemical properties (*e.g.*, lower molar mass) than the lignin in the original lignin cake. For this work, three general approaches for lignin extraction and recovery from lignin cakes were employed with conditions highlighted in

Table 1. These approaches include extractions using ethanol organosolv with and without added acid, aqueous NaOH extraction at extremes in temperature (85 and 180 °C) and two alkali loadings (100 and 200 mg/g), and the ionic liquid [C₃SO₄HMIM][HSO₄].

3.2 Ethanol Organosolv Lignin Extraction and Recovery

Ethanol was tested as a lignin extraction agent and the impacts of temperature, acid addition, and recovery approach on yield and lignin properties were assessed. Employing ethanol as a solvent may be advantageous in that ethanol is (1) produced on-site at cellulosic biorefineries and (2) is volatile and can therefore be recovered by distillation at potentially high yields. A further advantage of ethanol extraction of lignin is, relative to lignins recovered from aqueous NaOH, ethanol-recovered lignins are known to have low ash contents [101], which is critical for many applications such as polyurethane resins or carbon fibers [108]. Addition of acid improves extraction and recovery yields of lignin which can be hypothesized to be due to mechanisms including (1) additional lignin depolymerization to improve solubility, (2) alkylation of hydroxyl groups, presumably improving solubility, and (3) acid-catalyzed hydrolysis/depolymerization of recalcitrant cell wall polysaccharides to improve the release and solubilization of the lignin. It is well known that acidic chemistries result in cleavage of β -aryl ethers in lignin while the reactive intermediates are simultaneously capable of repolymerizing [94]. Moreover, because the repolymerization reactions have an activation energy that is at or below the energy required to form the intermediates, condensation of the enol ethers and carbonyls occurs spontaneously and cannot be prevented by minor changes to the reaction conditions [109, 110]. The use of “capping reagents” to trap the intermediates before they react with each other is one promising strategy to prevent significant repolymerization. For example, the use of homogeneous acid catalysts in an

organic solvent in the presence of the capping agent formaldehyde [111] achieved high cleavage of β -aryl ethers without repolymerization.

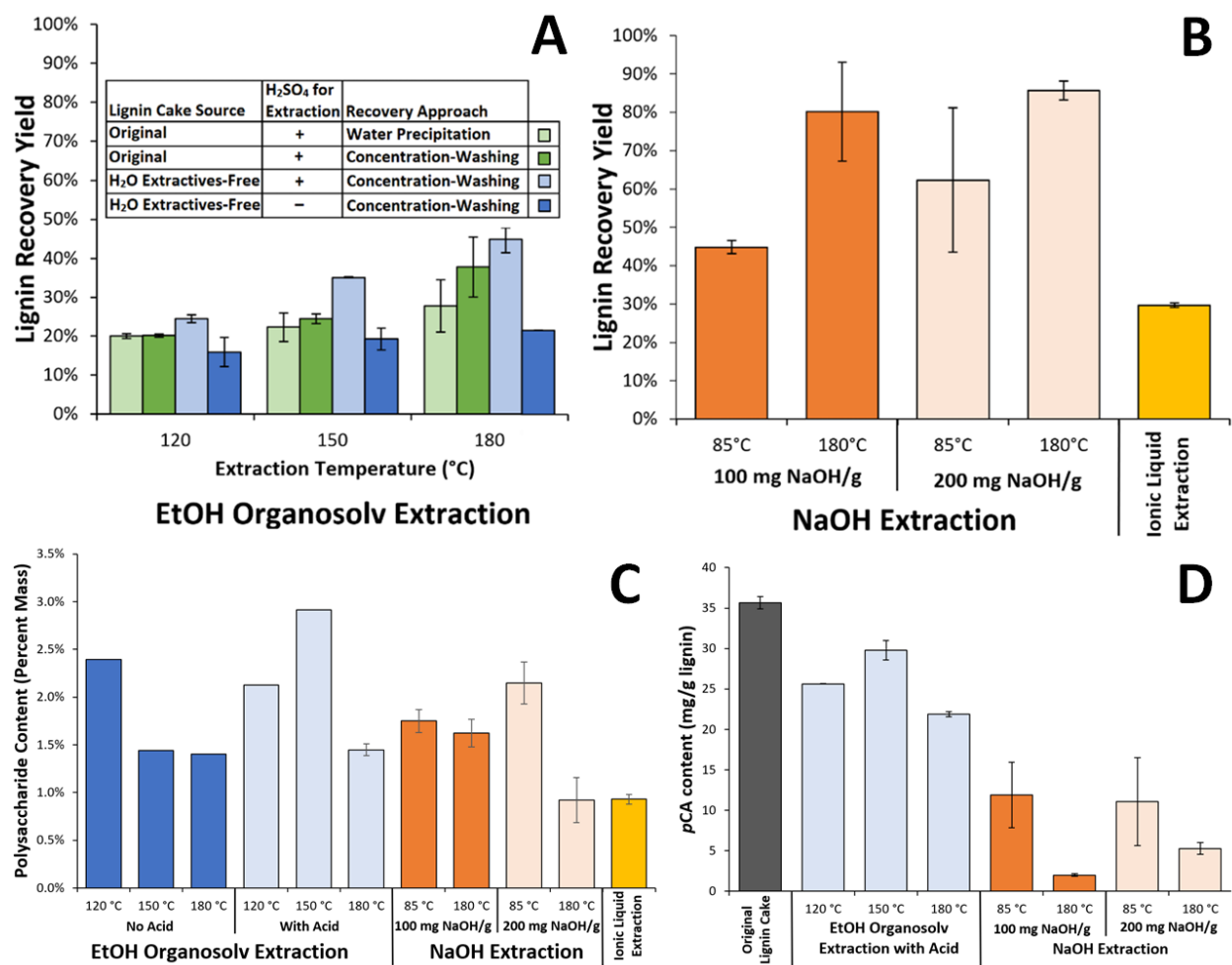


Figure 13. Recovery yields of lignins from (A) ethanol organosolv extraction of lignin cake at differing temperatures using two approaches for lignin recovery for raw lignin cake using dilute H₂SO₄ during the extraction and impact of acid during ethanol organosolv extraction using the water extractives-free lignin cake. Lignin recovery yields from lignin cake for (B) NaOH extraction and ionic liquid extraction. Composition of recovered lignins including (C) total polysaccharides (glucan, xylan, arabinan) and (D) p-coumarate content. Error bars represent data range for duplicate measurements.

In the first set of experiments, the impact of extraction temperature during acid-catalyzed ethanol organosolv extraction was performed using the unwashed lignin cake. Two approaches for

lignin recovery were compared: (1) direct precipitation by water dilution (*i.e.*, use of water as an antisolvent) and (2) concentration of the extraction liquor (initially 9.5 mg/mL lignin cake-derived solubles for the 120 °C condition up to 30.5 mg/mL for the 180 °C condition) to a viscous paste to precipitate the lignin followed by water washing. Rossberg et al. [112] compared lignin precipitation using water dilution versus concentration for acid ethanol organosolv pretreatment of hardwoods and found that the concentration resulted in lignins with higher aliphatic hydroxyl contents and lower aromatic hydroxyl contents. In the present work, the direct water precipitation from the organosolv black liquor resulted in 20% to 28% recovery yields (**Fig. 13A**), while the concentration-washing approach resulted in slightly higher recovery of lignins from the liquor (20% to 37.8%). Motivated by this, subsequent tests employed concentration of liquors first to maximize yields. The impact of the addition of dilute H₂SO₄ (92 mg/g biomass) during lignin extraction was next tested. The results show the general trends of increasing lignin recovery yields with increasing temperatures and significantly higher lignin yields (up to 44.9% yield) with the addition of acid (**Fig. 13A**). Prior work with ethanol organosolv extraction of hydrothermal pretreated wheat straw hydrolysis residue generated at the Inbicon A/S demonstration biorefinery in Kalundborg, Denmark using 50:50 EtOH:H₂O and recovery by precipitation by water dilution also found increasing lignin extraction yields with increasing extraction temperature from 100 to 200 °C (6% to 23%) [101].

3.3 Alkali and Ionic Liquid Extraction and Recovery of Lignins

NaOH and the ionic liquid [C₃SO₄HMIM][HSO₄] in 80:20 EtOH:H₂O were next assessed for their ability to extract and recover lignin from the lignin cake. Aqueous NaOH is an effective solvent for lignins and xylan as well as a facilitating cleavage of β-O-4 linkages within lignin at

elevated temperature. This depolymerization has the net effect of decreasing molar mass and increasing the phenolic hydroxyl content. These chemical modifications both improve the solubility of lignins in aqueous alkali and enable extraction of the lignin from the lignin cake. The results for alkali extraction show the trends that increasing alkali loading and extraction temperature increase the lignin extraction and recovery yields (**Fig. 13B**). Notably, substantially more lignin could be extracted using alkali than using the ethanol organosolv approach (**Fig. 13A**), with 89.6% lignin recovery yields using the highest extraction temperature (180 °C) and highest alkali loading (200 mg NaOH/g lignin cake). Prior work with the same dilute acid-pretreated corn stover lignin cake used in the present work was performed using 100 mg NaOH/g biomass to yield a lignin that was a suitable phenol replacement in phenol-formaldehyde resins with application as a wood adhesive [113]. Previous work investigated the impact of dilute acid pretreatment severity on the extraction of lignin from corn stover lignin cake using the same conditions of 200 mg NaOH/g lignin at 85 °C and obtained extraction yields ranging from 15.6% (for the high severity pretreatment) to 71.5% for the “medium” severity pretreatment [95]. Other work investigated the NaOH extraction of lignin from lignin cake derived from the dilute acid pretreatment and enzymatic hydrolysis of wheat straw [114]. It was determined that using alkali loadings of 4 to 200 mg NaOH/g biomass and temperatures up to 100 °C, that between 11% and 42% of the lignin could be recovered with temperature and alkali loading correlated with increasing lignin yields.

The utilization of aqueous NaOH as an extraction solvent can confer both process-derived challenges and benefits. One challenge is potentially the added cost of chemical inputs. Using an estimated NaOH cost of US\$800/tonne, the added cost of alkali for the highest lignin recovery yields (*i.e.*, >80%) at the lower alkali loading (*i.e.*, 100 mg NaOH/g lignin cake at 180 °C) are only

US\$0.026/kg lignin recovered. A second challenge to utilizing NaOH includes the introduction of inorganics that can accumulate in process water streams and become particularly problematic for facilities with a goal of achieving net zero discharge. A third challenge is the potential cleavage of *p*-coumarate (*p*CA) esters that may be an important reactive site for the synthesis of PF resins using lignin [95]. Advantages of utilizing NaOH are potentially high extraction and recovery yields and that if these lignins are used for PF resins, the aqueous NaOH can also be used as a solvent and catalyst during resin synthesis, reducing the number of separation and recovery unit operations [113]. Ionic liquids have shown promise as a solvent for lignin extraction and solubilization during pretreatments [115] as well as a catalyst for chemically altering solubilized lignins by, for example, facilitating depolymerization [116]. In the current work, the ionic liquid extraction performed at 100 °C gave a modest lignin recovery yield of 29.7% (**Fig. 13B**), which is similar to the results for the acid-catalyzed ethanol organosolv extraction at 120 °C (**Fig. 13A**).

3.4 Characterization of Recovered Lignins

In the next part of this work, the recovered lignins were subjected to characterization to assess how extraction and recovery of the lignins from the lignin cake impact recovered lignin purity (*i.e.*, content of contaminating polysaccharides), molar mass, and functional groups within the lignin. First, the lignins remaining in the lignin are already substantially altered relative to lignin in untreated corn stover and that increasing severity for dilute acid pretreatment results in increasingly “char-like” lignins with decreased extractability and solubility in many organic solvents [100]. Results for polysaccharide (primarily xylan) content (**Fig. 13C**) show that the recovered lignins contain only between 0.9% to 2.9% contaminating polysaccharides. Furthermore, these low polysaccharide contents only represent a yield of between 0.27% to 1.4%

of the total polysaccharide content of the original lignin cake. The likely explanation for this is that the xylan was largely solubilized during the dilute acid pretreatment and the enzymatic hydrolysis as the lignin cake only contained 3.6% xylan (**Fig. 12B**). The unhydrolyzed cellulose remaining in the lignin cake would be expected to remain insoluble during the lignin extractions.

Monocot lignins (such as corn stover) are notable for their significant inclusion of *p*-hydroxycinnamic acids, including *p*CA [117]. Lignin suitability as a phenol replacement in phenol-formaldehyde (PF) resins has been linked to higher *p*CA contents [113], as *p*CA is expected to exhibit higher reactivity with formaldehyde compared to other monomers in the lignin backbone as it should contain two reactive sites in the *ortho* position on the aromatic ring. Additionally, the *p*CA content is impacted by both the pretreatment step and the subsequent extraction and recovery steps [95], so for lignin applications such as PF resins it's important to identify conditions that can preserve the *p*CA-lignin ester bond.

The esterified *p*-coumarate (*p*CA) content of the original lignin cake and recovered lignins was next determined and several key results can be highlighted (**Fig. 13D**). First, the *p*CA content in the original lignin cake is 35.7 mg/g lignin and it can be expected that this is significantly lower than in the original untreated corn stover as the *p*CA-lignin ester bond is highly susceptible to acid-catalyzed hydrolysis although the exact conditions for dilute acid pretreatment and composition of the initial corn stover are not disclosed. Previous work determined a *p*CA content of 98.5 mg/g lignin in a commercial hybrid corn stover [95], suggesting that as much as 60% of the *p*CA has already been solubilized during the pretreatment. A second key result that can be highlighted is that for the conditions tested for the acidic ethanol organosolv lignin extraction, the majority (*i.e.*, 61.4% to 83.5%) of the *p*CA esters are preserved (**Fig. 13D**), indicating that these lignins may be

both recoverable at relatively high yields (**Fig. 13A**), high purities (**Fig. 13B**), and high *p*CA contents. The results for *p*CA contents for the NaOH extractions show that significantly less esterified *p*CA is retained in the recovered lignins (**Fig. 13D**), with only ~30% retained at the lower temperature extraction (85°C) and <15% retained for the higher temperature aqueous NaOH extraction (180°C). This is likely due to the high susceptibility of the *p*CA ester bond to base-catalyzed hydrolysis (saponification) and has been documented for aqueous NaOH extraction and recovery of lignins from dilute acid pretreated corn stover [95].

3.5 Solubility of Recovered Lignins

In the next portion of the work, the solubility of recovered lignins was assessed for a range of organic solvents with the goal of understanding how the solvent properties can be linked to the lignin's solubility. The solvents tested include polar protic (methanol) and polar aprotic (DMSO, ethyl acetate, acetone, THF) solvents at a lignin concentration of 100 mg lignin / mL solvent. The lignins included (Lignin 1) the acidic ethanol organosolv extraction from 180 °C for the raw lignin cake (**Fig. 13A**), this same condition (Lignin 2) using the water extractives-free lignin cake (**Fig. 13A**), and the most severe NaOH extraction and recovery condition (Lignin 3) using the water extractives-free lignin cake (200 mg NaOH/g, 180 °C; **Fig. 13B**).

It should be considered that the recovered lignins are not monodisperse in their properties and can exhibit distributions with respect to molar mass, chemical functionalities, and monomeric subunits, as well as contaminating polysaccharides with differing property distributions. The implication for this is that solubility of lignins is not a binary property (*i.e.*, soluble versus insoluble) but will be a percentage solubility out of the total lignin since a single solvent may be effective at solubilizing certain subset of lignin polymers from within these distributions. This

fractional solubility of lignins in different solvents is apparent in **Figure 14A**, from which several interesting trends can be observed. First, it can be observed that the NaOH-extracted lignins (Lignin 3) exhibited significantly lower solubilities in most of the solvents than the ethanol-extracted lignins. Potential reasons for these differences may be due to differences in the properties of the recovered lignins. These differences in properties can be derived from lignin modifications during the extraction processes. Furthermore, the lignins tested exhibit significant differences in the extraction and recovery yields, 44.9% for the ethanol organosolv extraction (**Fig. 14A**) and 89.6% for the NaOH extraction (**Fig. 14B**). As such, in addition to the structural changes the lignins undergo during extraction, they also represent different fractions of the original lignin within the lignin cake. Slight differences in the lignin solubilities can be observed for the ethanol-extracted lignins using the raw (Lignin 1) versus the water extractives-free (Lignin 2) lignin cake (**Fig. 14A**). Potential explanations for these differences may be due to minor contributions of the solubles to the extraction process that result in different extraction and recovery yields (*i.e.*, 37.8% versus 44.9%; **Fig. 13A**). These different yields may potentially result in lignins with slightly different properties and resulting solubilities.

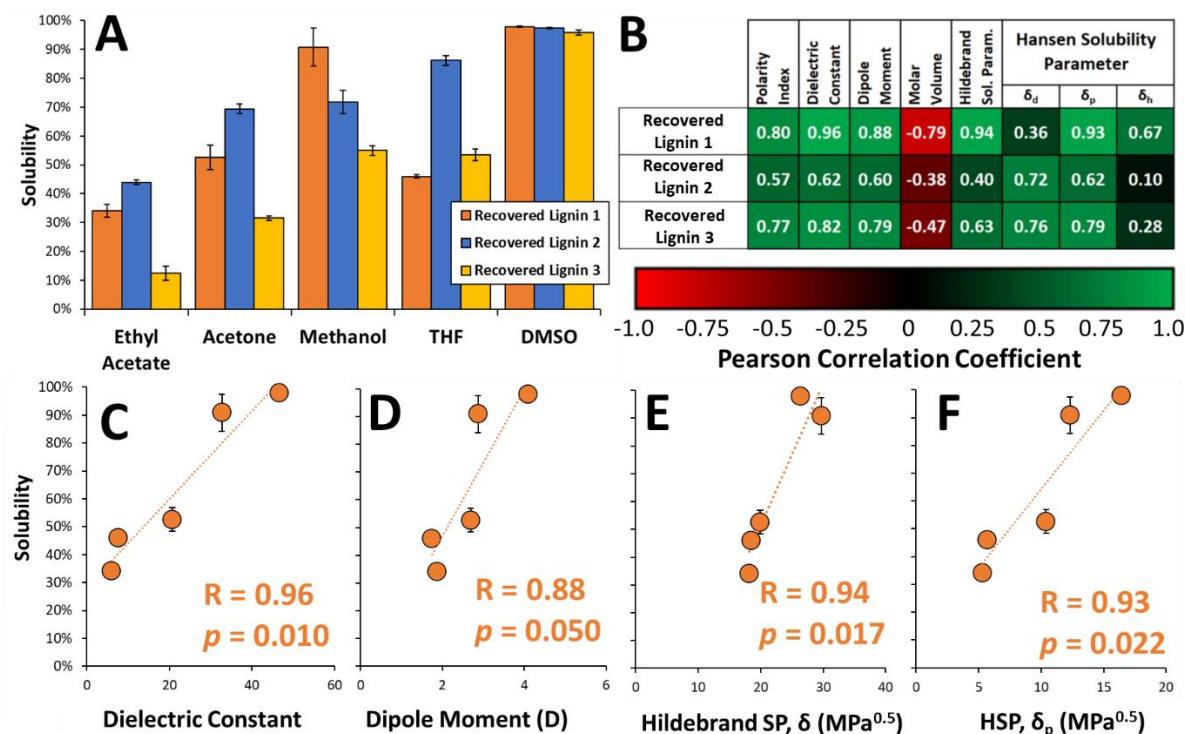


Figure 14. Solubility of acidic ethanol organosolv- and NaOH-extracted lignins in (A) various organic solvents, (B) Pearson correlation coefficient between lignin solubility and solvent properties, and (C through F) select significant correlations ($p \leq 0.05$) between solubility of the ethanol-extracted unwashed lignin cake (Lignin 1) and solvent properties. Error bars represent data range for duplicate measurements.

To further explore the relationship between lignin solubility and solvent properties, solubility was regressed against several solvent descriptors or properties that have been used to describe polymer-solvent interactions and predict solubility (**Fig. 14B**). The most notable trends are correlations between solubility and descriptors of solvent polarity including dielectric constant (**Fig. 14C**), dipole moment (**Fig. 14D**), and the dipole force interaction contribution (δ_p) to the Hansen solubility parameter (**Fig. 14F**). This trend of increasing lignin solubility with increasing polarity with a maximum solubility in DMSO has been observed in alkali-extracted lignins including a commercial softwood kraft lignin (“Indulin AT”) and a commercial non-wood soda lignin (“Protobind”) [27]. This result has also been predicted using molecular dynamics

simulations to show that model lignin oligomers exhibit their maximum polymer expansion and solvation in solvents with polarities similar to DMSO [30].

3.6 Estimation of Optimal Distribution for Hildebrand Solubility Parameters

Polymer dissolution in a solvent can be predicted by matching a solubility parameter (*e.g.*, Hildebrand or Hansen) to the solubility parameter of the solvent. In the current work, the Hildebrand solubility parameter is shown to be a strong linear function of the fractional solubility (**Fig. 14E**). A similar linear correlation was observed for the total Hansen solubility parameter (*i.e.*, the distance between the three components of the solubility parameter and the origin) for solvent-fractionated kraft lignin [118]. However, if a wider range of solvents were used to include high, these curves would be expected to go through a maximum and decrease rather than follow a linear trend [24].

One key feature of these results is that the lignins only exhibit partial solubility in most of the solvents and that these differences in solubility represent differences in the distribution of lignin chemical properties. This fractional solubility is the basis for “fractional precipitation” or “solvent fractionation” approaches to fractionating lignins based on differences in polymer properties within this distribution. Notably, prior work for relating lignin solubility to solubility parameters have used a binary soluble/insoluble assessment for the Hansen solubility parameter [119] or a qualitative assessment (insoluble, slightly, soluble, partially soluble, and soluble) for comparing to the Hildebrand solubility parameter [24]. Motivated by this, a framework was next developed for predicting Hildebrand and Hansen solubility parameters for lignins by assuming these parameters represent a range of values rather than a single value and result in the observed fractional solubility of lignins.

For the Hildebrand solubility parameter, the squared difference between the solubility parameter of solvent i ($\delta_{Solvent\ i}$) and the lignin (δ_{Lignin}) is a key term determining the magnitude and sign of the overall Gibbs free energy of mixing (ΔG_{mix}). A critical value for this difference ($\Delta\delta$) can be defined for a polymer solute in a solvent at thermodynamic equilibrium according to the Flory-Huggins solution theory [120, 121] that yields a negative or zero value for ΔG_{mix} and comprises the feasible region for polymer dissolution:

$$(\delta_{Lignin} - \delta_{Solvent\ i})^2 \leq (\Delta\delta)^2 \quad (\text{Eqn. 1})$$

Since a positive and negative solution satisfies this inequality, it is possible to rearrange this expression to yield lower ($\delta_{S_{i,L}}$) and upper bounds ($\delta_{S_{i,U}}$) for the feasible region:

$$\delta_{S_{i,L}} \leq \delta_{Lignin} \leq \delta_{S_{i,U}} \quad (\text{Eqn. 2})$$

Where:

$$\delta_{S_{i,U}} = \delta_{Solvent\ i} + \Delta\delta \quad (\text{Eqn. 3})$$

$$\delta_{S_{i,L}} = \delta_{Solvent\ i} - \Delta\delta \quad (\text{Eqn. 4})$$

Polymer solubility is typically treated as binary soluble/insoluble and the polymer solubility parameter is treated as a single value, which corresponds to either a single polymer or a population of polymers that is monodisperse and uniform for all chemical functionalities. However, for a heterogeneous population of polymers such as lignins, a typical set of lignins would be both polydisperse and exhibit substantial variability in the distribution of linkages, cross-linkages, monomers, and chemical functionalities. Consequently, many solvents will only solubilize a subset of lignins whose solubility parameters, as defined by their chemical functionalities, more closely match those of the solubility parameter of the solvent. To address this, it is assumed that a lignin population exhibits a δ_{Lignin} that is a distribution rather than single value.

Many probability density functions could be used, although as a demonstration of this approach, a normally distributed population of δ_{Lignin} described by the population mean ($\bar{\delta}_{Lignin}$) and standard deviation (σ_{Lignin}) is assumed. Using the analytical solution for the cumulative distribution function (CDF) of a Gaussian distribution, the probability that the value for δ_{Lignin} lies within the feasible region for solvent i is:

$$\Pr[\delta_{S_i,L} \leq \delta_{Lignin} \leq \delta_{S_i,U}] = \frac{1}{2} \left(\operatorname{erf} \left(\frac{\delta_{S_i,U} - \bar{\delta}_{Lignin}}{\sqrt{2}\sigma_{Lignin}} \right) - \operatorname{erf} \left(\frac{\delta_{S_i,L} - \bar{\delta}_{Lignin}}{\sqrt{2}\sigma_{Lignin}} \right) \right) \quad (\text{Eqn. 5})$$

Next, the values for $\bar{\delta}_{Lignin}$ and σ_{Lignin} that best fit the experimental data for all n solvents with solubility data for lignin were estimated. For this, a residual sum of squares (RSS) for the difference between the estimated fraction of soluble lignin polymers over the distribution of δ_{Lignin} ($\bar{\delta}_{Lignin}, \sigma_{Lignin}$) and the actual mass fraction of soluble lignin in solvent i (θ_i) were first determined:

$$RSS = \sum_{i=1}^n (\Pr[\delta_{S_i,L} \leq \delta_{Lignin} \leq \delta_{S_i,U}] - \theta_i)^2 \quad (\text{Eqn. 6})$$

A nonlinear optimization can be performed to determine the best estimates of $\bar{\delta}_{Lignin}$ and σ_{Lignin} that minimize RSS:

$$\min_{\bar{\delta}_{Lignin}, \sigma_{Lignin} \in \mathbb{R}} RSS \quad (\text{Eqn. 7})$$

Using this methodology, the values for $\bar{\delta}_{Lignin}$ and σ_{Lignin} that minimize the RSS for a given $\Delta\delta$ were estimated using the *fminsearch* function in MATLAB. The solution for these parameters is completely empirical.

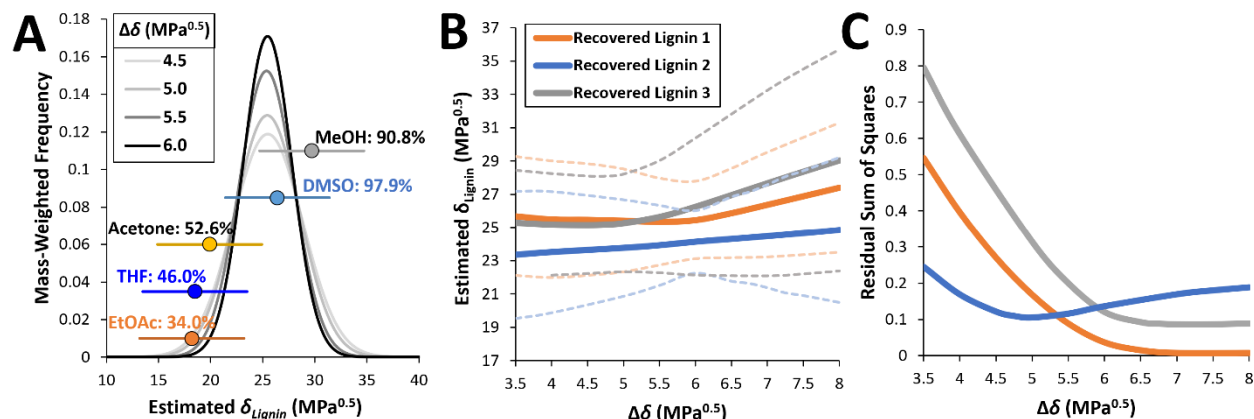


Figure 15. Examples of parameters fitted for Hildebrand solubility parameter (mean and standard deviation for δ_{Lignin}) demonstrating the impact of $\Delta\delta$ on (A) the estimated distribution of δ_{Lignin} , for Lignin 1, (B) the estimated mean ($\bar{\delta}_{Lignin}$, solid lines) and standard deviation (σ_{Lignin} , dashed lines) for a population of δ_{Lignin} for all three lignins, and (C) the residual sum of squares (RSS) for each of the three lignins.

Using this approach, populations of Hildebrand solubility parameters for the lignin (δ_{Lignin}) were estimated for a range of values for $\Delta\delta$ (Fig. 15). As an example to illustrate the results using this approach, δ_{Lignin} distributions for Lignin 1 are presented for a range of values of $\Delta\delta$ (Fig. 15A). Also shown on this plot are the estimated solubility ranges for each of the five solvents ($\delta_{Solvent} \pm \Delta\delta$) for a $\Delta\delta = 5.0$ MPa^{0.5} and the mass percent of the lignin that is soluble in a particular solvent to illustrate how the distribution for δ_{Lignin} matches the range of solubilities observed for each solvent (Fig. 15A). This approach is applied for each of the three recovered lignins and the estimated means ($\bar{\delta}_{Lignin}$) range from 23.4 to 29.0 MPa^{0.5} (Fig. 15B) which, not surprisingly, is close to the values for Hildebrand solubility parameters for DMSO (26.4 MPa^{0.5}) and methanol (29.7 MPa^{0.5}), which exhibit the highest fraction of the soluble lignins. In 1952 Schuerch [24] reviewed prior work that demonstrated the same trend of increasing solubility up to a maximum in the range of solvent Hildebrand solubility parameters of 22 (e.g., pyridine) to 29 MPa^{0.5} (e.g., ethylene glycol) for a diverse range of lignins that included a softwood kraft lignin, a mixed

hardwood soda lignin, a hardwood lignin derived from catalytic hydrogenolysis, an ethanol organosolv hardwood lignin, and “native” hardwood and softwood lignins. Other prior work estimated Hildebrand solubility parameters ranging from 26.2 to 27.4 MPa^{0.5} for a range of lignins using group contribution theory [27]. Increasing values $\Delta\delta$ result generally result in a decreased RSS (**Fig. 15C**) since a broad tail for the δ_{Lignin} is necessary to achieve partial solubility in solvents with $\delta_{Solvent} < 20.0$ MPa^{0.5}. The value for $\Delta\delta$ depends on a number of other factors including the polymer size, the polymer volume in solution, and the solvent size and suggested value for $\Delta\delta$ include 2.0 MPa^{0.5} [25] to 3.7 MPa^{0.5} [122].

3.7 Estimation of Optimal Distribution for Hansen Solubility Parameters

The Hildebrand solubility parameter has been used with success for non-polar, non-hydrogen-bonding solvents and has been shown to be 70–75% for nonpolar polymers, with a lower success rate for polar polymers [25]. The Hansen solubility parameter was developed in response to the limitations of the Hildebrand approach and break down the polymer-solvent interactions to include three components: energy of intermolecular dispersion forces (δ_D), dipole interactions (δ_P), and hydrogen bonding (δ_H) [122]. In the next part of the work, as a proof-of-concept, a mathematical framework for estimating population distributions of these three components is developed. Using the Hansen solubility parameter approach, the feasible region for solubility in solvent i can be described as a three-dimensional ellipsoid centered on $\delta_{Di}, \delta_{Pi}, \delta_{Hi}$ and with a radius of R_0 or $2R_0$ in the dispersion force (δ_D) dimension (see **Supplementary Information**) as recommend by Hansen (2007).

To determine values for Hansen solubility parameters for lignin that can fit within these feasible regions, a multivariate Gaussian distribution in three dimensions was assumed to represent

the distributions for the three components of the Hansen solubility parameter ($\delta_{D, Lignin}$, $\delta_{P, Lignin}$, $\delta_{H, Lignin}$) described by the population means ($\bar{\delta}_{D, Lignin}$, $\bar{\delta}_{P, Lignin}$, $\bar{\delta}_{H, Lignin}$) and standard deviations ($\sigma_{D, Lignin}$, $\sigma_{P, Lignin}$, $\sigma_{H, Lignin}$). This population comprises a three-dimensional ellipsoid with each axis assumed to be linearly independent. The probability that a multivariate population of lignin Hansen solubility parameters lies within the feasible region is:

$$\Pr[(\delta_{D_i,L} \leq \delta_{D,Lignin} \leq \delta_{D_i,U}) \cap (\delta_{P_i,L} \leq \delta_{P,Lignin} \leq \delta_{P_i,U}) \cap (\delta_{H_i,L} \leq \delta_{H,Lignin} \leq \delta_{H_i,U})] \quad (\text{Eqn. 8})$$

or:

$$\Pr[\delta_{D_i,L} \leq \delta_{D,Lignin} \leq \delta_{D_i,U}] \times \Pr[\delta_{P_i,L} \leq \delta_{P,Lignin} \leq \delta_{P_i,U}] \times \Pr[\delta_{H_i,L} \leq \delta_{H,Lignin} \leq \delta_{H_i,U}] \quad (\text{Eqn. 9})$$

where:

$$\delta_{D_i,U} = \delta_{D_i} + 2R_0 \quad (\text{Eqn. 10})$$

$$\delta_{D_i,L} = \delta_{D_i} - 2R_0 \quad (\text{Eqn. 11})$$

$$\delta_{P_i,U} = \delta_{P_i} + R_0 \quad (\text{Eqn. 12})$$

$$\delta_{P_i,L} = \delta_{P_i} - R_0 \quad (\text{Eqn. 13})$$

$$\delta_{H_i,U} = \delta_{H_i} + R_0 \quad (\text{Eqn. 14})$$

$$\delta_{H_i,L} = \delta_{H_i} - R_0 \quad (\text{Eqn. 15})$$

Using the same approach outlined in Eqns. 5, 6, and 7, values for means and standard deviations for the lignin Hansen solubility parameters can be determined. Using this methodology, the values for $\bar{\delta}_{D, Lignin}$, $\bar{\delta}_{P, Lignin}$, $\bar{\delta}_{H, Lignin}$, $\sigma_{D, Lignin}$, $\sigma_{P, Lignin}$, $\sigma_{H, Lignin}$ that minimize the RSS for a given R_0 were estimated using the *fminsearch* function in MATLAB.

Using this methodology, examples of three-dimensional parameter populations projected into two dimensions are presented in **Figure 16A** and **16B**. These results highlight that a wide range of solutions for each of the three sets of parameters can be obtained that minimize the RSS,

with a particularly wide range of solutions obtained for the dispersion force contribution (δ_D) at the largest value for R_0 . Notably, at R_0 values greater than $8.0 \text{ MPa}^{0.5}$ the minimum RSS is close to zero for a diverse set of non-unique solutions (**Fig. 16C**). Prior work has suggested appropriate values for R_0 from $2.0 \text{ MPa}^{0.5}$ [121] to $13.7 \text{ MPa}^{0.5}$ for an alkali lignin solubility study [119]. This same study, using a binary soluble/insoluble assessment for the lignin estimated Hansen solubility parameters of $\delta_{D, \text{Lignin}} = 21.9 \text{ MPa}^{0.5}$, $\delta_{P, \text{Lignin}} = 14.1 \text{ MPa}^{0.5}$, and $\delta_{H, \text{Lignin}} = 16.9 \text{ MPa}^{0.5}$, which are contained within the estimated sets of solutions for the present study (**Fig. 16A and 16B**). Overall, this was intended as a proof-of-concept for this approach and many other features could be incorporated such as different distribution functions.

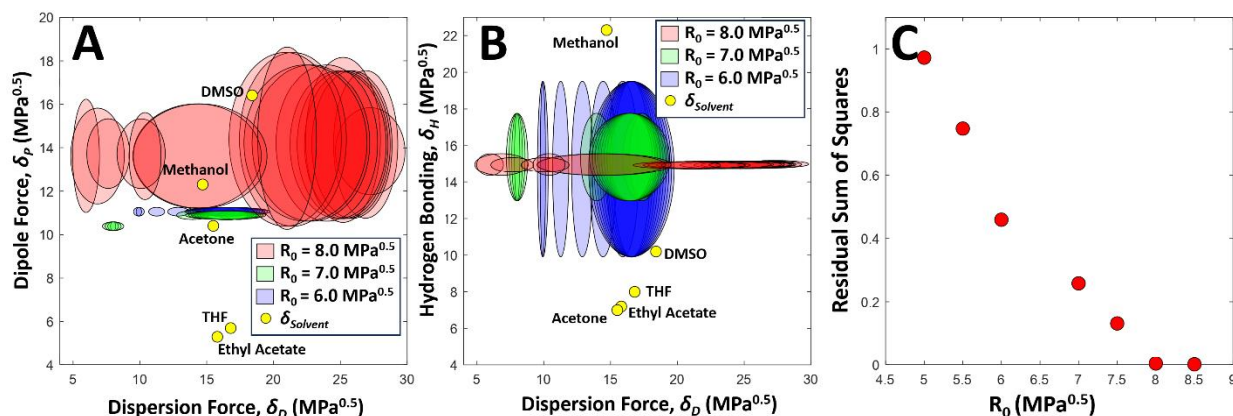


Figure 16. Graphical summary of select solutions resulting in comparable values for RSS for the estimated means ($\bar{\delta}_D, \text{Lignin}, \bar{\delta}_P, \text{Lignin}, \bar{\delta}_H, \text{Lignin}$) and standard deviations ($\sigma_D, \text{Lignin}, \sigma_P, \text{Lignin}, \sigma_H, \text{Lignin}$) for Lignin 1 as a function of size of feasible solubility region (R_0) for (A) δ_D versus δ_P , (B) δ_D versus δ_H , and (C) the impact of R_0 on the minimum RSS. The perimeter of the circles represents the standard deviations for the multivariate distributions for the Hansen solubility parameters.

4. Conclusions

In this work, lignin was extracted and recovered from industrial lignin cake derived from the dilute acid pretreatment and enzymatic hydrolysis of corn stover. Up to 84% recovery yields

of lignin from the lignin cake was possible using a relatively harsh NaOH extraction followed by lignin precipitation by acidification. Besides differences in recovery yields, lignins recovered using different approaches or extraction conditions exhibited slight differences in chemical composition, lignin properties, and solubility. Fractional solubility of lignins in different organic solvents could be successfully predicted by assuming lignins exhibited a distribution of solubility parameters due to their heterogeneity.

5. Supplementary Information

5.1 Supplemental Methods

5.1.1 Estimation of Optimal Distribution for Hildebrand Solubility Parameters. For the Hildebrand solubility parameter, the squared difference between the solubility parameter of solvent i ($\delta_{Solvent\ i}$) and the lignin (δ_{Lignin}) is a key term determining the magnitude and sign of the overall Gibbs free energy of mixing (ΔG_{mix}). A critical value for this difference ($\Delta\delta$) can be defined for a polymer solute in a solvent at thermodynamic equilibrium according to the Flory-Huggins solution theory [120, 121] that yields a negative or zero value for ΔG_{mix} and comprises the feasible region for polymer dissolution:

$$(\delta_{Lignin} - \delta_{Solvent\ i})^2 \leq (\Delta\delta)^2 \quad (\text{Eqn. 1})$$

Since a positive and negative solution satisfies this inequality, it is possible to rearrange this expression to yield lower ($\delta_{S_{i,L}}$) and upper bounds ($\delta_{S_{i,U}}$) for the feasible region:

$$\delta_{S_{i,L}} \leq \delta_{Lignin} \leq \delta_{S_{i,U}} \quad (\text{Eqn. 2})$$

Where:

$$\delta_{S_{i,U}} = \delta_{Solvent\ i} + \Delta\delta \quad (\text{Eqn. 3})$$

$$\delta_{S_i,L} = \delta_{Solvent\ i} - \Delta\delta \quad (\text{Eqn. 4})$$

Polymer solubility is typically treated as binary soluble/insoluble and the polymer solubility parameter is treated as a single value, which corresponds to either a single polymer or a population of polymers that is monodisperse and uniform for all chemical functionalities. However, for a heterogeneous population of polymers such as lignins, a typical set of lignins would be both polydisperse and exhibit substantial variability in the distribution of linkages, cross-linkages, monomers, and chemical functionalities. Consequently, many solvents will only solubilize a subset of lignins whose solubility parameters, as defined by their chemical functionalities, more closely match those of the solubility parameter of the solvent. To address this, it is assumed that a lignin population exhibits a δ_{Lignin} that is a distribution rather than single value. Many probability density functions could be used, although as a demonstration of this approach, a normally distributed population of δ_{Lignin} described by the population mean ($\bar{\delta}_{Lignin}$) and standard deviation (σ_{Lignin}) is assumed. Using the analytical solution for the cumulative distribution function (CDF) of a Gaussian distribution, the probability that the value for δ_{Lignin} lies within the feasible region for solvent i is:

$$\Pr[\delta_{S_i,L} \leq \delta_{Lignin} \leq \delta_{S_i,U}] = \frac{1}{2} \left(\text{erf} \left(\frac{\delta_{S_i,U} - \bar{\delta}_{Lignin}}{\sqrt{2}\sigma_{Lignin}} \right) - \text{erf} \left(\frac{\delta_{S_i,L} - \bar{\delta}_{Lignin}}{\sqrt{2}\sigma_{Lignin}} \right) \right) \quad (\text{Eqn. 5})$$

Next, the values for $\bar{\delta}_{Lignin}$ and σ_{Lignin} that best fit the experimental data for all n solvents with solubility data for lignin were estimated. For this, a residual sum of squares (RSS) for the difference between the estimated fraction of soluble lignin polymers over the distribution of δ_{Lignin} ($\bar{\delta}_{Lignin}, \sigma_{Lignin}$) and the actual mass fraction of soluble lignin in solvent i (θ_i) were first determined:

$$RSS = \sum_{i=1}^n (Pr[\delta_{S_i,L} \leq \delta_{Lignin} \leq \delta_{S_i,U}] - \theta_i)^2 \quad (\text{Eqn. 6})$$

A nonlinear optimization can be performed to determine the best estimates of $\bar{\delta}_{Lignin}$ and σ_{Lignin} that minimize RSS:

$$\min_{\bar{\delta}_{Lignin}, \sigma_{Lignin} \in \mathbb{R}} RSS \quad (\text{Eqn. 7})$$

Using this methodology, the values for $\bar{\delta}_{Lignin}$ and σ_{Lignin} that minimize the RSS for a given $\Delta\delta$ were estimated using the *fminsearch* function in MATLAB. The solution for these parameters is completely empirical. Feasible ranges for these distributions of solubility parameters could be estimated from group contribution approaches [4].

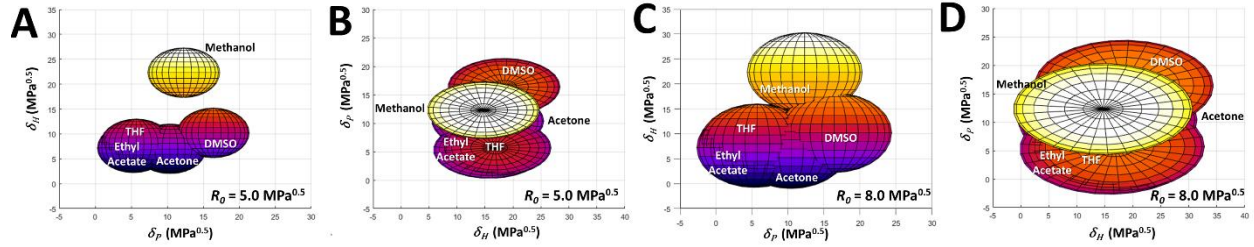


Figure 17. Feasibility regions in each of the three dimensions for the Hansen solubility parameter for the five solvents tested in this work using an R_0 of either $5.0 \text{ MPa}^{0.5}$ (A and B) or $8.0 \text{ MPa}^{0.5}$ (C and D).

5.1.2 Estimation of Optimal Distribution for Hansen Solubility Parameters. Using the Hansen solubility parameter approach, the feasible region for solubility in solvent i can be described as a three-dimensional ellipsoid centered on δ_{D_i} , δ_{P_i} , δ_{H_i} and with a radius of R_0 or $2R_0$ in the dispersion force (δ_D) dimension as recommend by Hansen (2007). For the five solvents tested in this work, these feasible regions projected into two dimensions will appear as in **Figure 18**.

To determine values for Hansen solubility parameters for lignin that can fit within these feasible regions, a multivariate Gaussian distribution in three dimensions was assumed to represent the distributions for the three components of the Hansen solubility parameter ($\delta_{D,Lignin}$, $\delta_{P,Lignin}$, $\delta_{H,Lignin}$) described by the population means ($\bar{\delta}_{D,Lignin}$, $\bar{\delta}_{P,Lignin}$, $\bar{\delta}_{H,Lignin}$) and standard deviations ($\sigma_{D,Lignin}$, $\sigma_{P,Lignin}$, $\sigma_{H,Lignin}$). This population comprises a three-dimensional ellipsoid with each axis assumed to be linearly independent. The probability that a multivariate population of lignin Hansen solubility parameters lies within the feasible region is:

$$\Pr[(\delta_{D_i,L} \leq \delta_{D,Lignin} \leq \delta_{D_i,U}) \cap (\delta_{P_i,L} \leq \delta_{P,Lignin} \leq \delta_{P_i,U}) \cap (\delta_{H_i,L} \leq \delta_{H,Lignin} \leq \delta_{H_i,U})] \quad (\text{Eqn. 8})$$

or:

$$\Pr[\delta_{D_i,L} \leq \delta_{D,Lignin} \leq \delta_{D_i,U}] \times \Pr[\delta_{P_i,L} \leq \delta_{P,Lignin} \leq \delta_{P_i,U}] \times \Pr[\delta_{H_i,L} \leq \delta_{H,Lignin} \leq \delta_{H_i,U}] \quad (\text{Eqn. 9})$$

Where:

$$\delta_{D_i,U} = \delta_{D_i} + 2R_0 \quad (\text{Eqn. 10})$$

$$\delta_{D_i,L} = \delta_{D_i} - 2R_0 \quad (\text{Eqn. 11})$$

$$\delta_{P_i,U} = \delta_{P_i} + R_0 \quad (\text{Eqn. 12})$$

$$\delta_{P_i,L} = \delta_{P_i} - R_0 \quad (\text{Eqn. 13})$$

$$\delta_{H_i,U} = \delta_{H_i} + R_0 \quad (\text{Eqn. 14})$$

$$\delta_{H_i,L} = \delta_{H_i} - R_0 \quad (\text{Eqn. 15})$$

Using the same approach outlined in Eqns. 5, 6, and 7, values for means and standard deviations for the lignin Hansen solubility parameters can be determined. Using this methodology, the values for $\bar{\delta}_{D,Lignin}$, $\bar{\delta}_{P,Lignin}$, $\bar{\delta}_{H,Lignin}$, $\sigma_{D,Lignin}$, $\sigma_{P,Lignin}$, $\sigma_{H,Lignin}$ that minimize the RSS for a given R_0 were estimated using the *fminsearch* function in MATLAB.

5.2. Supplemental Figures

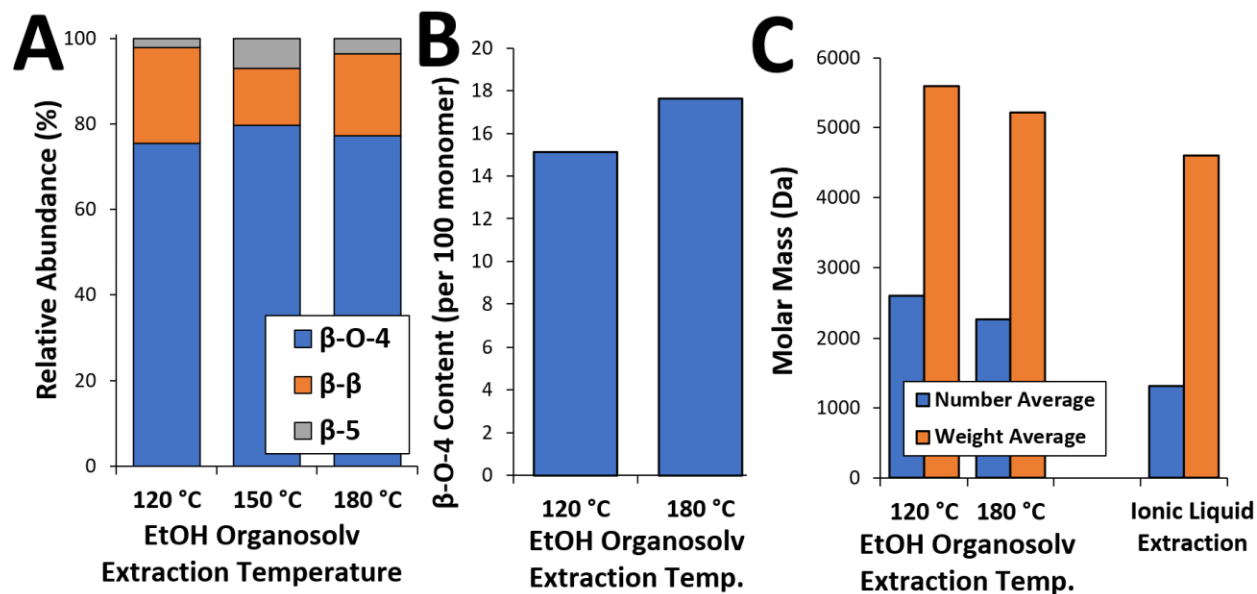


Figure 18. Properties of acidic ethanol organosolv-extracted (and IL-extracted) lignins as a function of extraction temperature for (A) relative linkage abundance as determined by 2D HSQC NMR, (B) β -O-4 content as determined by ^{13}C NMR, and (C) molar mass as determined by GPC of acetylated lignins.

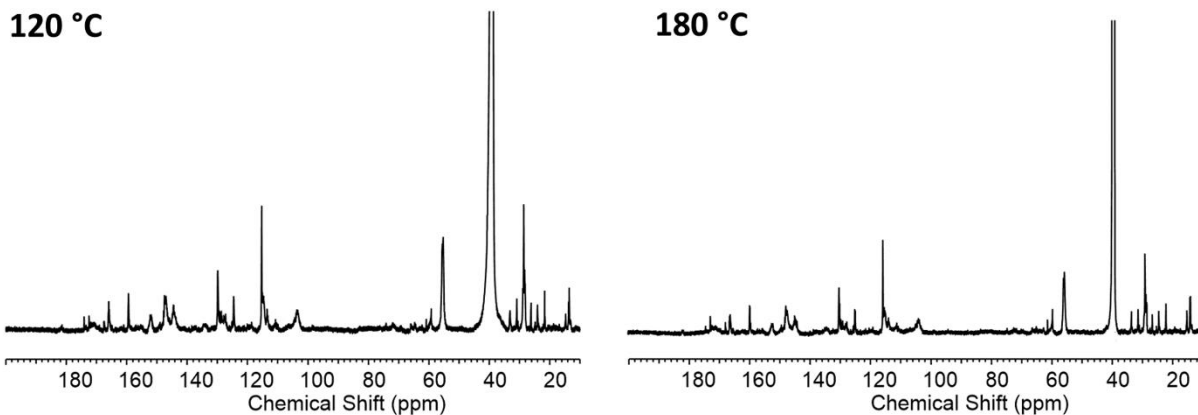


Figure 19. ^{13}C NMR spectra of ethanol-extracted lignin at 120 °C and 180 °C

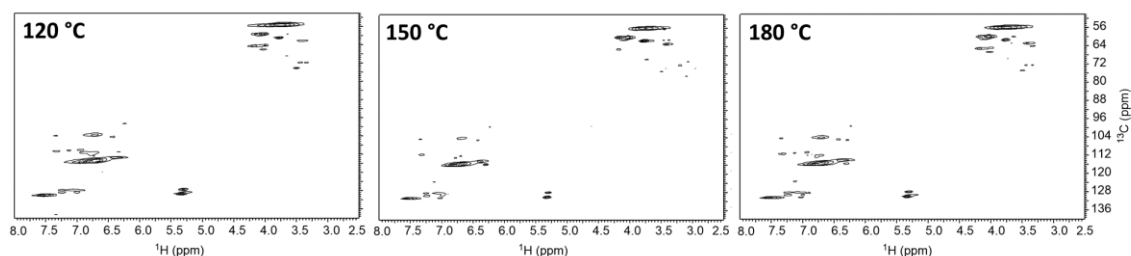


Figure 20. 2D HSQC NMR spectra of ethanol-extracted lignin at 120 °C, 150 °C, and 180 °C

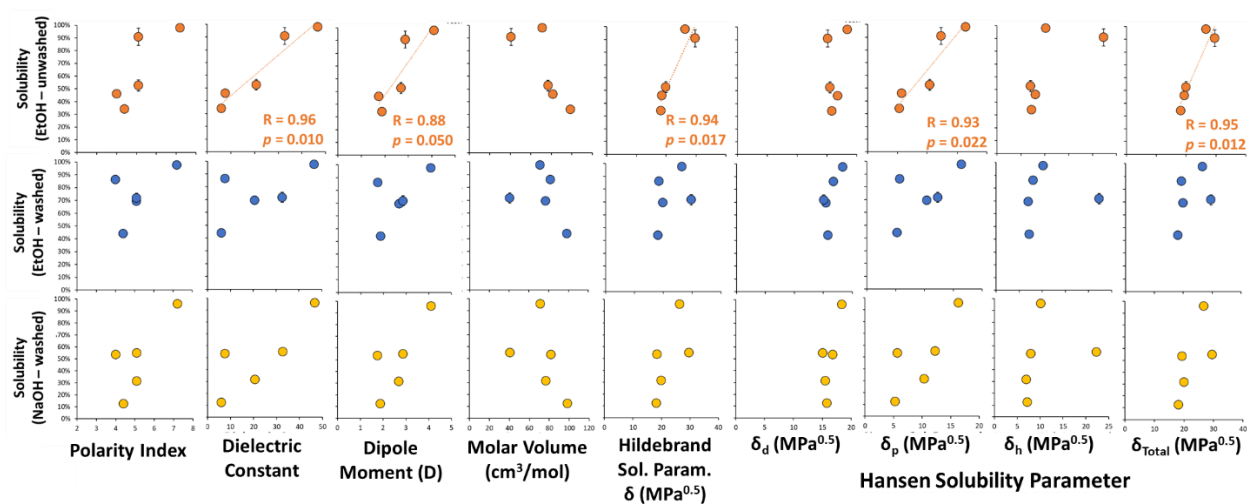


Figure 21. Correlation between lignin solubility and solvent properties

6. Supplemental References

1. Barton, A.F. 2017. *CRC handbook of solubility parameters and other cohesion parameters*. Routledge, Milton Park, UK.
2. Hansen, C.M. 2007. *Hansen solubility parameters: A user's handbook*. CRC Press, Boca Raton, FL.
3. Lindvig, T., Michelsen, M.L., Kontogeorgis, G.M. 2002. A Flory–Huggins model based on the Hansen solubility parameters. *Fluid Ph Equilib*, **203**(1), 247-260.

7. References

92. Sun, Z., et al., *Bright Side of Lignin Depolymerization: Toward New Platform Chemicals*. Chem Rev, 2018. **118**(2): p. 614-678.
93. Schutyser, W., et al., *Chemicals from lignin: an interplay of lignocellulose fractionation, depolymerisation, and upgrading*. Chemical Society Reviews, 2018. **47**(3): p. 852-908.
94. Deuss, P.J., et al., *Aromatic monomers by in situ conversion of reactive intermediates in the acid-catalyzed depolymerization of lignin*. J Am Chem Soc, 2015. **137**(23): p. 7456-67.
95. Saulnier, B.K., et al., *Effect of Dilute Acid Pretreatment and Lignin Extraction Conditions on Lignin Properties and Suitability as a Phenol Replacement in Phenol-Formaldehyde Wood Adhesives*. J Agric Food Chem, 2023. **71**(1): p. 592-602.
96. Meyer, J.R., et al., *Isolation of lignin from Ammonia Fiber Expansion (AFEX) pretreated biorefinery waste*. Biomass and Bioenergy, 2018. **119**: p. 446-455.
97. Thoresen, P.P., et al., *Recent advances in organosolv fractionation: Towards biomass fractionation technology of the future*. Bioresource Technology, 2020. **306**: p. 123189.
98. George, A., et al., *Design of low-cost ionic liquids for lignocellulosic biomass pretreatment*. Green Chemistry, 2015. **17**(3): p. 1728-1734.
99. Wang, W. and D.J. Lee, *Lignocellulosic biomass pretreatment by deep eutectic solvents on lignin extraction and saccharification enhancement: A review*. Bioresour Technol, 2021. **339**: p. 125587.
100. Zhang, X., et al., *Extraction and characterization of lignin from corncob residue after acid-catalyzed steam explosion pretreatment*. Industrial Crops and Products, 2019. **133**: p. 241-249.
101. Cabrera, Y., et al., *Purification of Biorefinery Lignin with Alcohols*. Journal of Wood Chemistry and Technology, 2016. **36**(5): p. 339-352.
102. Martin, J., *Project Liberty: Launch of an Integrated Bio-Refinery with Eco-Sustainable and Renewable Technologies. Conversion of Corn Stover Biomass to Bio-Ethanol, Final Report*. 2021: United States. p. Medium: ED; Size: 94 p.
103. Singh, S. and P. Dhepe, *Lignin Conversion Using Catalytic Ionic Liquids: Understanding the Role of Cations, Anions and Hammett Acidity Functions*. Industrial & Engineering Chemistry Research, 2019. **2019**.

104. Sluiter, A., et al., *Determination of structural carbohydrates and lignin in biomass, in: Laboratory Analytical Procedure (LAP)*. National Renewable Energy Laboratory, 2008.
105. Singh, S.K., et al., *Integrated Two-Stage Alkaline-Oxidative Pretreatment of Hybrid Poplar. Part 1: Impact of Alkaline Pre-Extraction Conditions on Process Performance and Lignin Properties*. Industrial & Engineering Chemistry Research, 2019. **58**(35): p. 15989-15999.
106. Jassal, V., et al., *Finding values in lignin: A promising yet under-utilized component of the lignocellulosic biomass*. Frontiers in Chemical Engineering, 2022. **4**.
107. He, H., et al., *Physiological and ecological significance of biomineralization in plants*. Trends Plant Sci, 2014. **19**(3): p. 166-74.
108. Gosselink, R., *Lignin as a Renewable Aromatic Resource for the Chemical Industry*. 2011.
109. Shuai, L. and B. Saha, *Towards high-yield lignin monomer production*. Green Chemistry, 2017. **19**(16): p. 3752-3758.
110. Sturgeon, M.R., et al., *A Mechanistic Investigation of Acid-Catalyzed Cleavage of Aryl-Ether Linkages: Implications for Lignin Depolymerization in Acidic Environments*. ACS Sustainable Chemistry & Engineering, 2014. **2**(3): p. 472-485.
111. Shuai, L., et al., *Formaldehyde stabilization facilitates lignin monomer production during biomass depolymerization*. Science, 2016. **354**(6310): p. 329-333.
112. Rossberg, C., et al., *Effect of process parameters in pilot scale operation on properties of organosolv lignin*. BioResources, 2019.
113. Kalami, S., et al., *Replacing 100% of phenol in phenolic adhesive formulations with lignin*. Journal of Applied Polymer Science, 2017. **134**(30): p. 45124.
114. Zoia, L., et al., *Valorization of Side-Streams from a SSF Biorefinery Plant: Wheat Straw Lignin Purification Study*. Bioresources, 2017. **12**: p. 1680-1696.
115. Singh, S., et al., *Understanding the impact of ionic liquid pretreatment on cellulose and lignin via thermochemical analysis*. Biomass & Bioenergy, 2013. **54**: p. 276-283.
116. Dutta, T., et al., *Survey of Lignin-Structure Changes and Depolymerization during Ionic Liquid Pretreatment*. ACS Sustainable Chemistry & Engineering, 2017. **5**(11): p. 10116-10127.
117. Hatfield, R.D., et al., *Grass lignin acylation: p-coumaroyl transferase activity and cell wall characteristics of C3 and C4 grasses*. Planta, 2009. **229**(6): p. 1253-67.

118. Duval, A., et al., *Solvent screening for the fractionation of industrial kraft lignin*. *Holzforschung*, 2016. **70**(1): p. 11-20.
119. Hansen, C.M. and A. Björkman, *The Ultrastructure of Wood from a Solubility Parameter Point of View*. *Holzforschung*, 1998. **52**(4): p. 335-344.
120. Barton, A., *CRC handbook of solubility parameters and other cohesion parameters, second edition*. 2017. 1-739.
121. Lindvig, T., M. Michelsen, and G. Kontogeorgis, *A Flory–Huggins model based on the Hansen solubility parameters*. *Fluid Phase Equilibria*, 2002. **203**: p. 247-260.
122. Hansen, C., *Hansen Solubility Parameters: A User's Handbook, Second Edition*. 2012. 546.

CHAPTER THREE

SCALE UP OF LIGNIN PRODUCTION FROM
CORN STOVER AND HYBRID POPLARIntroduction

This chapter focuses on the development and troubleshooting of a high-throughput 20-L scale lignin production using an alkaline pretreatment method for a project involving multiple research groups (left panel of Figure 22). The goal was to provide high purity lignin, concentrated liquor, and lignin cake (the water insoluble hydrolysis residue), to collaborators for further research in lignin fractionation ALPHA process [123-125], and lignin-based products like carbon fibers [126, 127], activated carbon [128], and polyurethane foams [129]. The produced lignins also contributed to biochar research at Montana State University [130]. Co-authored papers include Refs. [123-125, 129, 130], and our work has been acknowledged in Refs. [126, 127]. To address the fluctuating physical properties of the recovered lignins, we conducted a variable screening to identify the recovery temperature as a key factor contributing to these inconsistencies investigated in detail in Chapter Four.

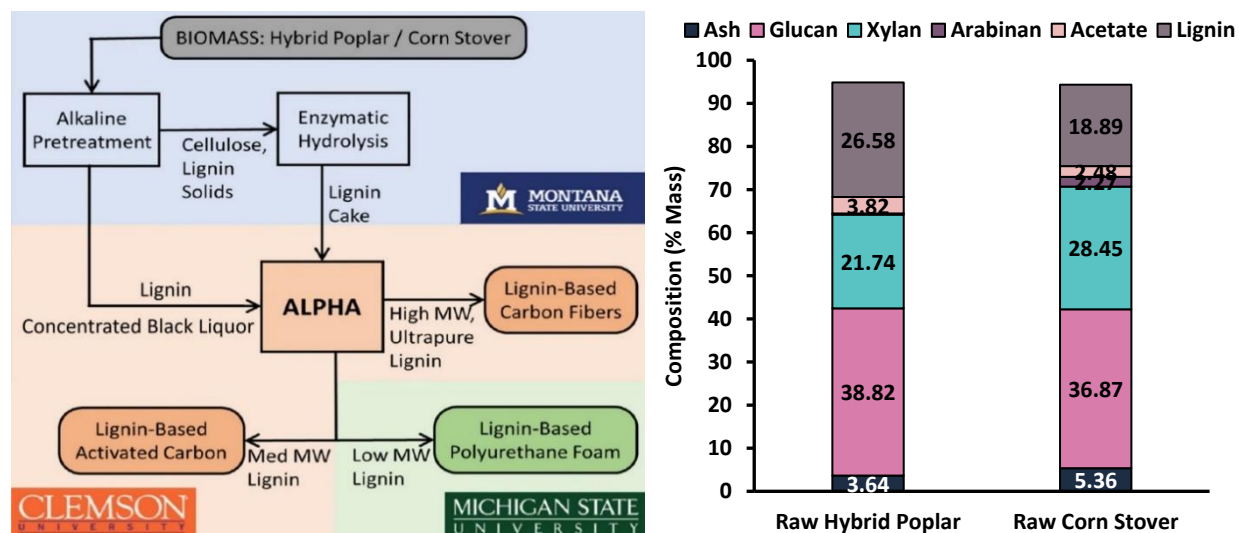


Figure 22. Project summary (left panel), and composition of raw biomass (right panel).

Corn stover and hybrid poplar were used for the alkaline pretreatment as an herbaceous and a woody biomass type (left and middle panel on Figure 23). Notable differences in composition on a dry basis are shown on the right panel in Figure 22, with hybrid poplar containing more lignin than corn stover, 26.58 wt% to 18.89 wt%. Corn stover has higher ash content than hybrid poplar, 5.36 wt% and 3.64 wt%. It also has a higher xylan content than hybrid poplar, to 28.45 wt% and 21.74 wt% (right panel in Figure 22). The moisture content was also different for the two types of biomass, not shown on the figure. For raw hybrid poplar, the moisture content was around 4.4%, and it was 8.4% for raw corn stover. These compositional differences are well documented in the literature [13, 14] and in Chapter One. Due to these differences, the applied reaction conditions varied between the two types of biomass. As corn stover is an herbaceous biomass type with low bulk density and grass-like structure, less severe conditions were used than for the hybrid poplar, a hardwood, which was supplied as wood chips that required harsher conditions for efficient penetration and reaction. The initial conditions for the alkaline pretreatment were based on previous work from the group [131].



Figure 23. Hybrid poplar woodchips (left panel), corn stover (middle panel), and black liquor in a bucket (right panel).

Alkaline pretreatment was selected as the lignin extraction method due to its efficiency in disrupting the complex plant structure and solubilizing lignin and partially hemicellulose [132]. Cellulose remains intact without significant degradation, however, it becomes more accessible for subsequent enzymatic hydrolysis in the biorefinery due to swelling in the biomass fibers increasing the surface area. After the alkaline pretreatment, black liquor (right panel in Figure 23) and pretreatment biomass are obtained. Lignin is commonly recovered from black liquor by acidification [46], which induces the precipitation of lignin that makes it possible to recover lignin using solid-liquid separation methods such as filtration (membrane [44], filter press, ultrafiltration [133], etc.) or centrifugation.

Final Process

On Figure 24 the basic process diagram for lignin production is shown. The first step is the alkaline pretreatment in a laboratory digester using either hybrid poplar or corn stover as a feedstock. After the reaction, the black liquor is separated from the pretreated biomass. The pretreated biomass is either stored or further processed via enzymatic hydrolysis to produce lignin cake. The black liquor is either evaporated to make concentrated liquor, or for most cases, it is acidified to precipitate and recover lignin. Finally, the dried powder is sent to collaborators for further research.

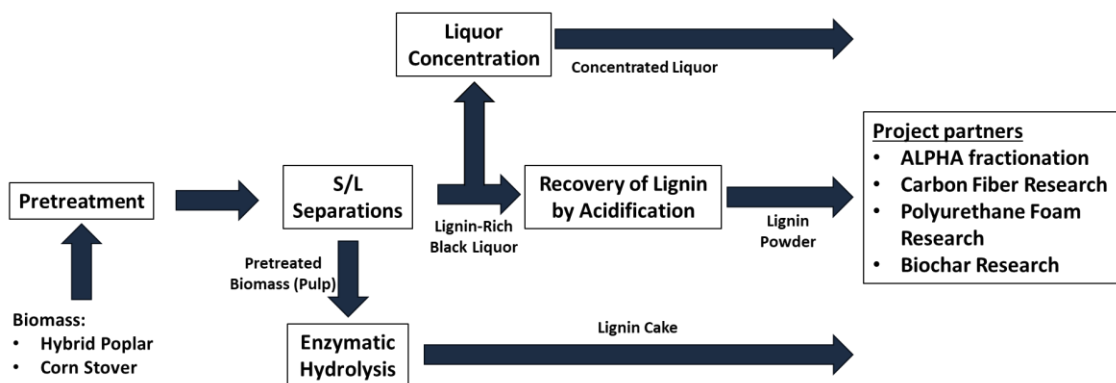


Figure 24. Lignin production process diagram.

Figure 25 shows the finalized process highlighting key equipment and detailed steps during lignin recovery not shown in the previous figure. This detailed view includes the first solid-liquid separation of black liquor from pretreated biomass using a press. After acidification, the lignin is separated from the acidified black liquor by centrifugation, after which it undergoes two washing cycles to remove impurities, each followed by centrifugation. To optimize throughput and purity or troubleshooting, each stage underwent multiple iterations, outlined below. Most iteration processes were developed using hybrid poplar, as corn stover lignin production was only necessary for the second half of the project.

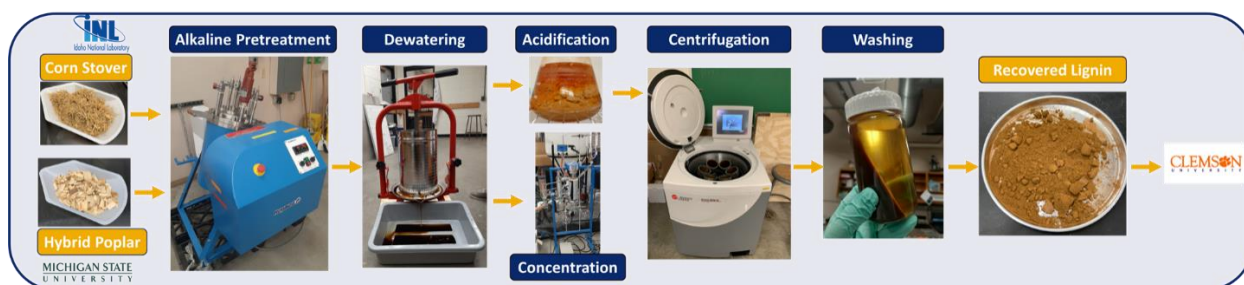


Figure 25. Final flowchart for lignin production.

Process Development and Optimization

Scale

A laboratory digester was used for conducting alkaline pretreatment. This reactor setup is capable of easily switching between two modes by switching the "lid" on top (left panel in Figure 26). The first setup uses four smaller reactors with separate chambers for indirect heating inside intermediate chambers. This setup enables venting of the steam in the intermediate chamber immediately after the reaction, thereby cooling it down fast. The second setup, which was used mostly in this project, uses the big 20-L reactor chamber (right panel in Figure 26). This chamber can only be air-cooled overnight, as only the hazardous black liquor could be vented to release pressure and heat due to the lack of an intermediate chamber.

Originally, four smaller reactors were used for the digester with a 1.5 L capacity each. That setup would enable us to investigate different reaction conditions and enable faster cooldown times. That alone was a significant jump from the previous 20-mL scale used in Chapter Two, however, because of the large lignin demand of the collaboration, we needed to drastically increase the reaction scale a further three-fold (the individual headspace for four reactors is higher than for one big chamber) and produce lignins from only one set of conditions for both biomass types. To achieve this, we switched from the four-reactor setup to the 20-L digester chamber. This switch increased the downtime between batches as the reactor needed to cool down overnight.

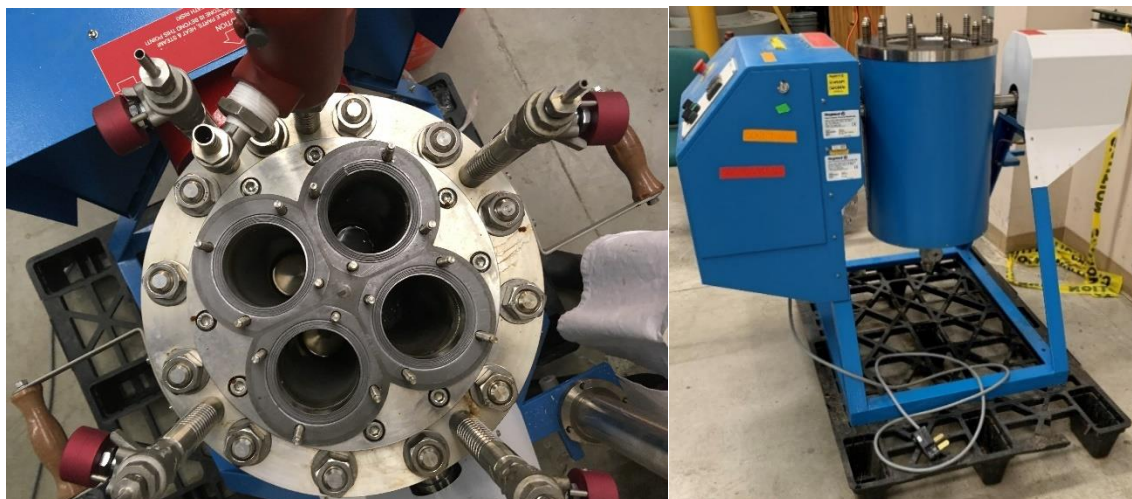


Figure 26. Digester with four reactor chamber lid (left panel), and the digester from the side without the lid (right panel).

Scaling up the process also necessitated additional large-scale equipment, such as larger containers and mixing apparatuses, to accommodate increased material volumes and ensure efficient handling. It also required significant manual labor to prepare solutions and biomass, transfer them to the digester, load and unload, and then transfer them back to the processing laboratory, press them multiple times, then acidify. Due to this, eight undergraduate researchers and two visiting elementary school teachers contributed to this project with great care and dedication.

Corn Stover Liquid to Solid Ratio

The liquid loading needed to change for corn stover due to its porous structure that absorbs significantly more liquid than the wood chips. The five to one ratio for hybrid poplar was adjusted to seven, then finally to nine as the biomass was not mixing sufficiently at seven to one ratio and it burnt on the side of the digester (see Figure 27).

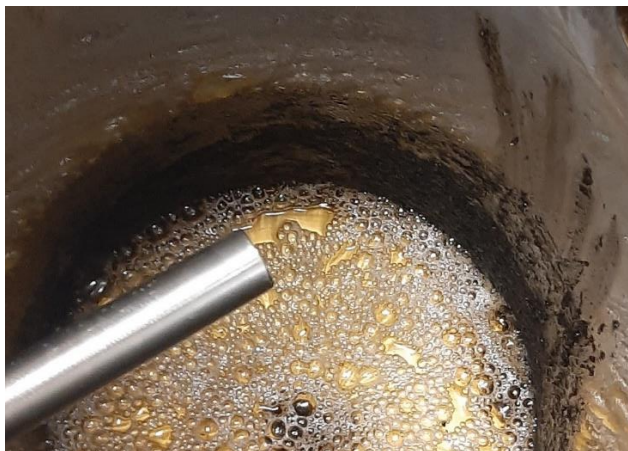


Figure 27. Corn stover burnt on the bottom of the reactor chamber due to low liquid loading.

Solid – Liquid Separations: Black Liquor and Pretreated Biomass

During the initial iteration, biomass was separated from black liquor with a small sieve from a sieve tower (left panel in Figure 28). This method was slow and bothersome because of the limited size of the sieve and the high risk of spillage. To improve this, we introduced brewer's mesh bags for more effective separation. As there was no press available to efficiently extract the liquor, separation relied on gravity, with the mesh bags left hanging overnight, periodically mixed, and manually pressed to aid extraction.



Figure 28. Separation using sieves (left panel), separation using the apple cider press and a mesh bag (right panel).

Due to the inefficiency of this initial method, two lignin fractions (“A” and “B”) were recovered. Fraction “A” lignin was obtained directly from the alkaline pretreatment black liquor, while fraction “B” lignin was collected by soaking the pretreated biomass in water to release residual lignin adhered to the biomass surface. After the mesh bag had drained fully, and no further liquid could be extracted by hand pressing, the biomass was submerged in water until fully covered and left to soak overnight. The following day, this soaking liquid was processed similarly to the “A” lignin, involving acidification, centrifugation, and washing steps, to recover “B” lignin. This “B” lignin made up roughly 30% of all recovered lignin, but greatly prolonged the recovery time.

Until the summer of 2020 “B” lignin was recovered, then the apple cider press (right panel in Figure 28) arrived in September, which removed much more liquid from the biomass than the original method, making “B” lignin redundant. The apple cider press is a basket press with a cylindrical drum, which uses a screw mechanism to manually force down a pressing plate and hold

the solids in the mesh bag. Using this final iteration, we managed to recover around 12 L of the 13.6 L initial liquid, achieving around 88% liquid recovery, greatly improving on the previous recovery which was around 8.5 L of black liquor.

Acidification Method

Initial plans included the acidification of black liquor using carbon dioxide, which took hours without reaching the targeted $\text{pH} < 2$, and there was significant foaming due to carbon dioxide that needed to be controlled with an ice bath to increase the solubility of carbon dioxide in the liquor (see Figure 29). As this proved to be slow and inefficient even at the smaller scale, we changed the acidifying agent to concentrated sulfuric acid.

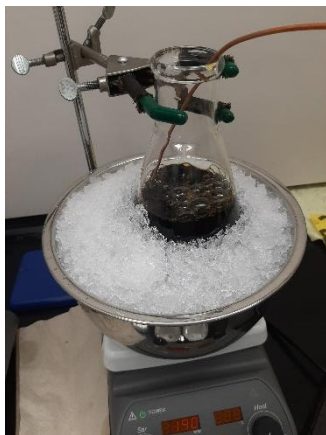


Figure 29. Acidification using carbon dioxide.

Solid – Liquid Separations: Precipitated Lignin from Acidified Black Liquor

Initially, the plan was to use benchtop laboratory vacuum filtration to separate the precipitated lignin from the acidified black liquor (left panel in Figure 30), however, the recovery was unsuccessful due to the slow filtration, clogging, and eventual tearing of all trialed filter papers with different pore sizes (Whatman 1004150 20-25 μm , Whatman 1440110 8 μm , Fisherbrand 098035E 1-5 μm , and Fisherbrand glass fiber 09804110A 1.6 μm among others). A pressure filter

was purchased to speed up the process, but still, we could not find a suitable filter medium for it (cloth, paper, metal mesh, and a combination of each). Filtering would eventually stop because of clogging, or the equipment would leak along a connection. As this hindered the progress of the entire collaboration, we decided to shift to centrifugation instead of filtering for this separation.

The first centrifuge used for this separation had a total capacity of 1.5-L, with six 250 mL centrifuge tubes (middle panel in Figure 30). With a black liquor recovery of around 8.5 L out of the initial 13.6 L liquid loaded, the centrifuge needed to be run six times just for the initial separation, with around 30 minutes necessary for each run, without counting cleanup and decanting. This time was multiplied with additional washing steps. The separation step was improved four-fold with the purchase of a 6-L high-capacity centrifuge (right panel in Figure 30).



Figure 30. Original filtration setup (left panel), 250 mL centrifuge tube for the 1.5 L centrifuge after lignin separation (middle panel), and the 6 L high-capacity centrifuge (right panel).

Washing and Purity

In an industrial pulping or pretreatment process, ash from reaction chemicals is efficiently recovered and recycled, resulting in around 2-4 wt% low ash content lignin instead of the 36 wt% without recovery [134]. Our process does not include a chemical recovery step, resulting in extremely high ash contents for the recovered lignins without washing. What makes washing challenging is that lignin yield decreases and centrifugation times increase after additional washing steps, making centrifugation times longer, especially for corn stover lignin because of small particles retained in the liquid phase. During centrifugation, the separated lignin has a very high moisture content because there are no drying effects during centrifugation, and additional washing steps introduce more and more liquid around the particles.

Drying Methods

Careful control of the drying method proved to be essential for consistent lignin physical characteristics. Following centrifugation, the recovered lignin is a pudding-like substance with high moisture content (see Figure 31). Vacuum drying proved unsuitable for the initial drying step due to the closed environment of the vacuum oven, which prevented efficient ventilation of evaporated water. This led to water recondensing on the lignin surface, forming undesirable pools. Therefore, an initial air-drying stage was implemented to remove the bulk of the moisture, followed by a second stage in a vacuum oven to ensure thorough and controlled final drying.



Figure 31. Lignin recovered from centrifugation before drying.

Training and Safety

Given the addition of personnel working with large-scale equipment and hazardous materials, it was crucial to conduct risk assessments to ensure safety and compliance with standard practices. Most personnel working in production had no prior experience working in a laboratory, especially at this scale. Therefore, they gained valuable experience and knowledge about operational procedures, safety, and handling the equipment and materials at the 20-L scale, all to maintain a safe working environment.

The safety measures were primarily built around administrative controls, ensuring that all personnel receive proper training. First-time laboratory users underwent mandatory online or in-person training, followed by close supervision during initial tasks. As they gained experience, they transitioned to independent work with continued access to the supervisor for questions and assistance. If possible, multiple personnel were present to share the physical demands of the work. Standard operating procedures (SOPs) were provided, shown in Appendix A.

Personnel working during production got familiar with all aspects of laboratory safety measures. The following personal protective equipment (PPE) was provided: heavy duty long

chemical resistant gloves, disposable aprons, lab coats, face shields, eye protection, and N95 masks for fine powder protection. Signage and proper labeling of all materials and containers was essential for working in a shared laboratory, whether for storage of black liquor and lignin, or for in progress tasks like drying the recovered lignin over days. To facilitate the handling of large, heavy materials, physical aids like trolleys were used to move the 20-kilogram batches between processing locations, while ergonomic supports, such as chairs, were provided for those working long hours.

Emergency preparedness was ensured by familiarizing personnel with engineering controls such as pressure release valves, and safety showers and eyewash stations were pointed out to all personnel. Given the hazardous nature of the materials involved, including the alkaline solution, black liquor, and concentrated sulfuric acid, strict behavioral and hygiene practices were enforced. These practices included regular handwashing, single usage of gloves, and safe waste disposal.

Materials and Methods

Materials

Hybrid poplar (NM6, *Populus nigra* x *P. maximowiczii*) was obtained from Michigan State University. It was harvested in January 2020 from less than 5-year-old trees with stem diameters of 15.5 to 20.3 cm, and were debarked, chipped, and dried to approximately 5% moisture content. Commercial hybrid corn stover was obtained from DuPont via Idaho National Laboratory. The corn stover was harvested in October 2014 in Story County, Iowa, and was subjected to milling first with Vermeer BG480 bale grinder fitted with a 2-inch sieve, followed by further milling in a Bliss Hammermill fitted with a 1-inch sieve. The alkaline cooking solution was prepared using on-site DI water and NaOH pellets from Millipore Sigma.

Alkaline Pretreatment

Corn stover and hybrid poplar was subjected to delignification in a 20-L stainless-steel tumbling pulp digester (RegMed model AU/E-20 with a TP-2027 lid, São Paulo, Brazil). The biomass moisture content was determined using an Ohaus MB90 moisture balance. Then the biomass was loaded into the digester and mixed with the NaOH solution. The temperature ramping was 2.5°C/min until the target temperature, which was maintained for the reaction time. **Table 1** shows the different conditions used, and part of this method has been published in [129]. After the reaction, the digester was air-cooled until atmospheric pressure was restored and the temperature fell below 60°C, then unloaded. The black liquor was separated from the pretreated biomass using a 120-micron nylon CONIE mesh filter bag and a MacIntosh Apple Cider Press with a 5-gallon stainless-steel basket. The pretreated biomass was airdried and stored until further analysis, while the black liquor was acidified or stored at 4°C in a refrigerator. Detailed Standard Operating Procedures (SOPs) for the alkaline pretreatment of both corn stover and hybrid poplar are provided in Appendix A.

Biomass type	mg NaOH/g biomass loading	Liquid – solid ratio	Reaction t [hr]	Reaction T [°C]	Starting biomass [kg]	V of NaOH solution [L]	Approx. V of black liquor [L]	Original pH of black liquor	Approx. H₂SO₄ used [mL]
Hybrid Poplar	180	5:1	3	150	3	13.7	12	13	200
Corn Stover	130	9:1	0.5	140	2	16	14	11	185

Table 2. Reaction conditions and black liquor properties for alkaline pretreatment

Lignin Precipitation

Black liquor was acidified to below 2 with 96% H₂SO₄ to precipitate the lignin, before it was placed in the 4 °C fridge overnight to let it settle. Then lignin was separated from the liquid with

centrifugation at 8000 rpm in a Beckman Coulter Avanti JXN-6 centrifuge until the phases separated, resulting in clear liquid and gel-like lignin. After the first centrifugation cycle the lignin was washed two times with a 1:5 wet lignin to deionized water ratio, using centrifugation to separate the lignin from the washing liquid. Lastly, the lignin was airdried at room temperature, then dried in a vacuum oven at 40 °C for a few days. The dry lignin was weighed and characterized according to [104], as in Chapter Two.

Lignin Cake Preparation

Lignin cake was prepared from the alkaline pretreated hybrid poplar biomass followed by enzymatic hydrolysis. Wood pulp (left panel in Figure 32, corn stover also shown on middle panel in Figure 32) was first washed by loading pulp and DI water into a blender and blending on low speed. This was repeated until the pH of the liquid was below 8. Then the mixture was pressed to remove the water and then air-dried to 5% moisture content. For enzymatic hydrolysis, washed wood pulp was added to a 5-gallon bucket along with DI water at 10% (wt/vol) solid loading. Cellulase enzyme blend (Cellic CTec2, Novozymes A/S, Bagsværd, Denmark) was loaded at 25 mg protein/g glucan, along with a 0.05 M Na-citrate buffer (pH 5.0), and tetracycline at 0.8% of the sample weight. After mixing, the bucket was covered and placed in an incubator for 72 hours at 50 °C, and manually stirred every 2 hours (right panel on Figure 32). pH was measured and adjusted back to 5 using sulfuric acid if necessary. Following enzymatic hydrolysis, the resulting lignin cake was washed by adding 4 L of DI water to the solids, centrifuging for 2 hours at 8000 rpm, decanting the supernatant, and then repeating 3 times. Finally, the lignin cake was airdried.



Figure 32. Pretreated biomass of hybrid poplar (left panel) and corn stover (middle panel), and hybrid poplar during enzymatic hydrolysis (right panel).

Black Liquor Evaporation

Black liquor concentration was carried out using a Pope Scientific Inc. Wiped-Film Still (right panel in Figure 33), equipped with an Edwards RV8 rotary vane vacuum pump and a MKS Series 275 vacuum gauge. The black liquor was fed in 1-liter increments and passed through multiple times until evaporation stopped. The solid content of hybrid poplar black liquor increased from 10.4% to 40%, while corn stover black liquor's solid content rose from 5.6% to 30% (left panel in Figure 33).

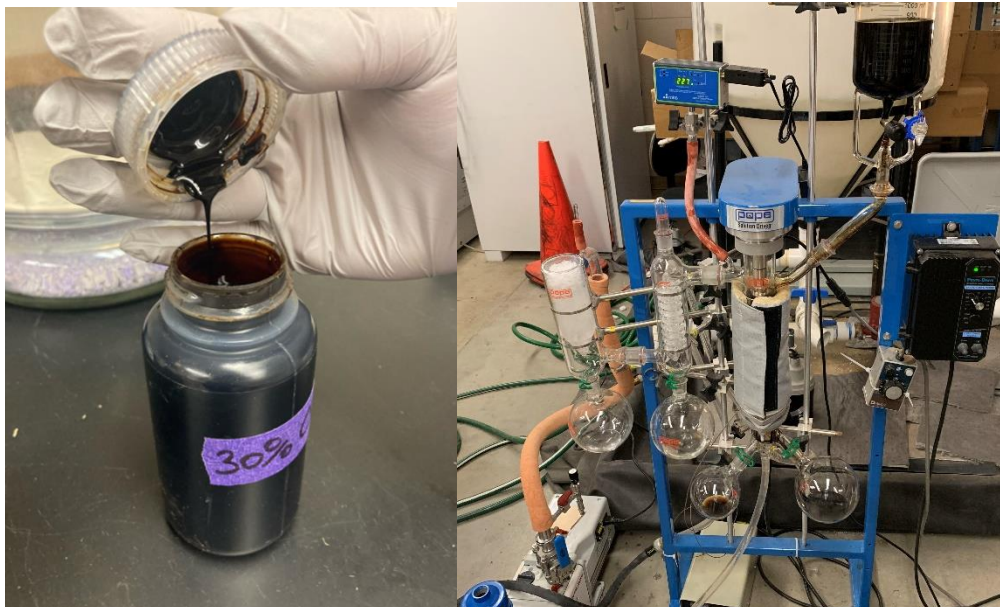


Figure 33. Concentrated black liquor (left panel), the wiped-film evaporator still (right panel).

Lignin Oxidation for Variable Screening

After alkaline pretreatment, the separated black liquor was homogenized using a mixing paddle for a minimum of 5 minutes. For each sample, 50 mL of black liquor was heated to 75°C in a round-bottom flask within an oil bath. Once the temperature reaches 75°C, the liquor was oxidized for 1 hour by bubbling air through it. After oxidation, the liquor was cooled to below 40°C and then acidified with concentrated sulfuric acid to achieve a pH below 2. The acidified black liquor was then reheated to 75°C and maintained at this temperature for 10 minutes. While still hot, the liquid was immediately filtered through Whatman #40 filter paper using a Büchner funnel and washed with 50 mL of 75°C deionized water. The samples were then air-dried for at least 24 hours.

Fourier-Transform Infrared Spectroscopy

Fourier-transform infrared spectroscopy (FTIR) measurements are collected using a Thermo Fisher Nicolet iS50 FTIR spectrometer equipped with a Smart iTX attenuated total reflectance (ATR) accessory fitted with a diamond crystal, as in [130]. Spectra are collected over the wavenumber range of 400 cm^{-1} to 4000 cm^{-1} with a resolution of 1 cm^{-1} . Data are filtered in MATLAB with the "*smoothdata*" function using the Savitzky-Golay filter and a moving window of approximately 32 cm^{-1} .

Folin-Ciocalteu Analysis

The phenolic hydroxyl content is measured using the Folin-Ciocalteu method [135]. First the Folin-Ciocalteu reagent is diluted 10-fold, then sodium carbonate solution is prepared at 0.115 g/mL concentration. The standard solution is prepared with vanillic acid from 0 to 1.0 mg/mL to generate the standard curve (see Figure 34). For the sample solution, lignin powder is dissolved in 70% (v/v) ethanol. A 0.1 mL aliquot of the sample or standard is mixed with 5 mL of the Folin-Ciocalteu solution and incubated for a few minutes. Then, 3.5 mL of the sodium carbonate solution is added, stirred, and the mixture is incubated at room temperature for 2 hours. The absorbance is measured with a Thermo Scientific Biomate 3 UV-Vis spectrophotometer at 760 nm .



Figure 34. Folin-Ciocalteu calibration set with increasing concentration from left to right, and one lignin sample on the far right.

Total Fluorescence Intensity

The total fluorescence intensity of epifluorescence microscopy images (not taking the particles into account) are determined using the histogram data in ImageJ/Fiji. The unweighted intensity values are extracted and averaged across the entire image. The resulting data is then thresholded using the same threshold previously applied for particle size distribution analysis. The equation used for calculating the average area weighted intensity is: $\frac{\sum_{i=n}^{255} ih_i}{\sum_n^{255} h_i}$, where n is the selected threshold, and h_i is the number of pixels in each bin. Detailed Standard Operating Procedures (SOPs) for the microscope imaging are provided in Appendix B.

Lignin Lightness

Lignin samples were subjected to color measurements using an FRU WR18 handheld colorimeter, with the measurements conducted in triplicate. Prior to the measurements, the colorimeter underwent calibration using the manufacturer's calibration standards, including a blackboard and a whiteboard. To measure the lignin samples, the colorimeter's measurement window was placed in close contact with each sample to ensure accurate readings. Thorough cleaning of the colorimeter was carried out between measurements. The color data, represented by the L, a, and b values, were recorded within the CIELAB color space, where L represents the lightness, ranging from 0 to 100. These measurements utilized a 4.0 mm caliber measuring window, a standard D65 light source using a 10° CIE standard observer, and an 8/d illumination system with a photodiode array sensor. This method was published previously [136].

Process Metrics

Figure 35 shows the cumulative lignin production over the course of the project. Poplar feedstock was switched from “Poplar 1” to “Poplar 2” after the first few initial batches due to limited availability of “Poplar 1”. Lignin in liquor means lignin is not recovered as a powder and only black liquor is produced for research detailed in Chapter Four. The initial capacity of the reactor quickly increased three-fold from four 1.5 L chambers to one 20 L chamber. The final addition was a high-performance 6-liter centrifuge to maximize throughput. Concentrated liquor and lignin cake were also generated. To maximize throughput, only one set of conditions was used, one for hybrid poplar and one for corn stover biomass. The process of one run is 5 full workdays after the iterations explained in detail above. With the drying time and necessary analyses, it becomes a 2-week process. The total weight of the lignin generated is around 20 kg, as seen in Figure 35, with more than 14 kg of lignin powder supplied for the project over 58 batches. The reason for the high demand for lignin is that the ALPHA process requires large quantities of lignin as it has been shown in a recent paper using lignin from this chapter [123], that 20 wt% of our produced lignin is lost during ALPHA fractionation. For the carbon fiber application, only 8-40 wt% of lignin fractionated from the ALPHA process is utilized [126]. For the PU foam application, the yield of lignin after ALPHA fractionation is 68.7 wt% [129].

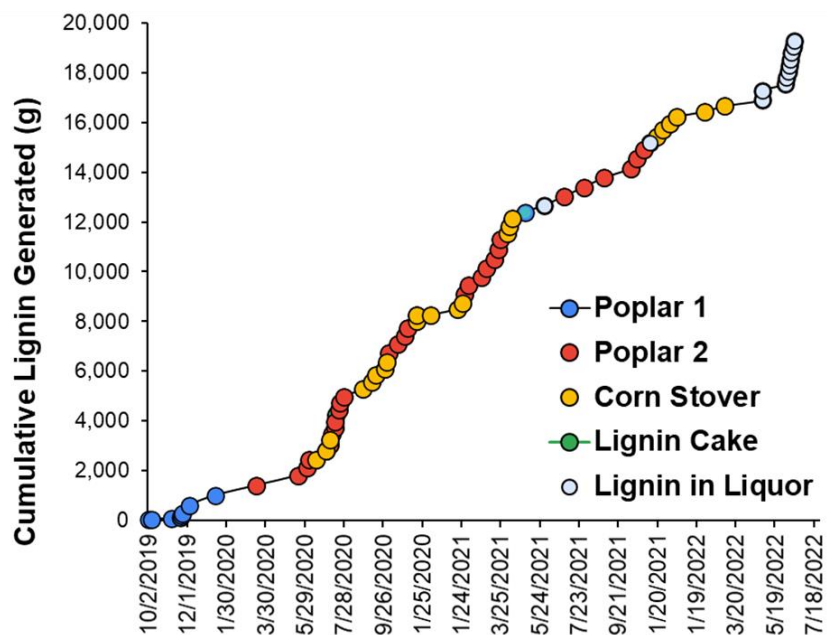


Figure 35. Lignin generated over the course of the project.

The lignin yield showed high variability during the first iteration steps but evened out for the final process (left panel on Figure 36). The first few batches had low yields, around 100 g, during the optimization of the lignin recovery from acidified black liquor step (filtering). “B” lignin was produced until the summer of 2020, then the apple cider press was purchased. Corn stover has lower lignin yields, as the amount of starting material is lower and corn stover has a lower lignin content. The composition and moisture content of hybrid poplar and corn stover was discussed previously (right panel on Figure 22). The theoretical maximum lignin yield from 3 kg of hybrid poplar is around 760 g, and it is around 350 g for 2 kg corn stover. For the final iteration the lignin yield was around 45% for hybrid poplar and around 70% for corn stover, accounting for impurity content. Xylan and ash content were established as descriptors of lignin quality, and both decreased to an acceptable level for the hybrid poplar, to less than half of the initial numbers (see middle and right panel on Figure 36), due to the implementation of a two-cycle rigorous washing

procedure. For corn stover, xylan content stayed consistently high, more than 10% even after washing, but ash content was successfully lowered.

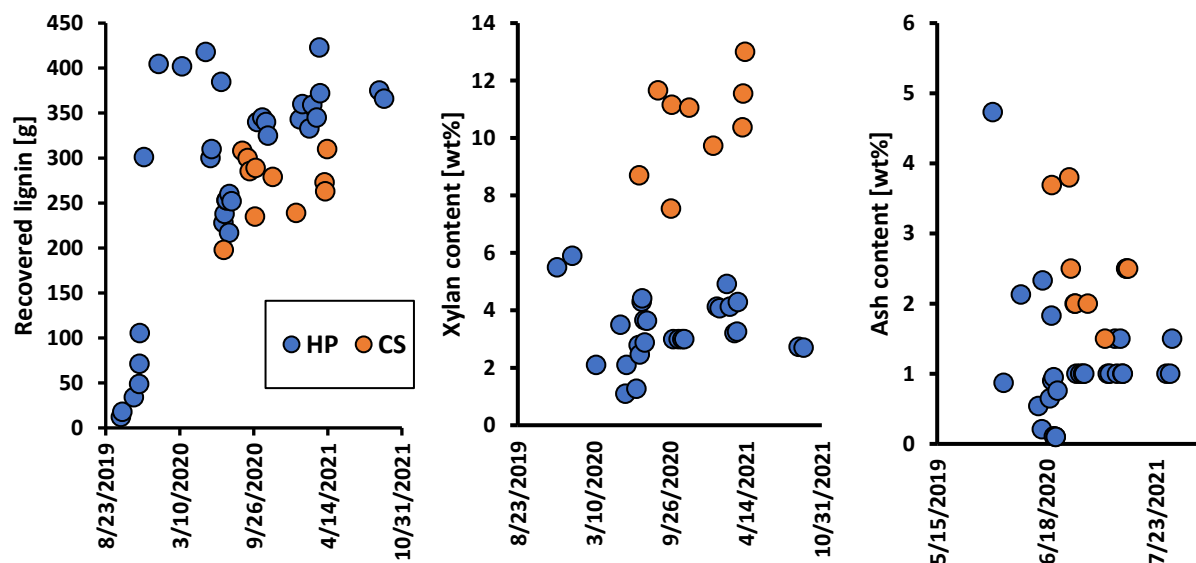


Figure 36. Lignin yield, xylan and ash content during production.

Reactor Profiles

In Figure 37 the temperature profile during the reaction for selected batches is shown, with a steady climb to reach the reaction temperature at around an hour mark and staying constant after. As the digester is a tumbling reactor without additional mixing, overheating by the maximum of 10 °C happened occasionally due to the digester's inconsistent temperature measurements. Table 3 shows the scale, biomass type and reaction conditions for each batch. The temperature profile is nearly identical for both types of biomass.

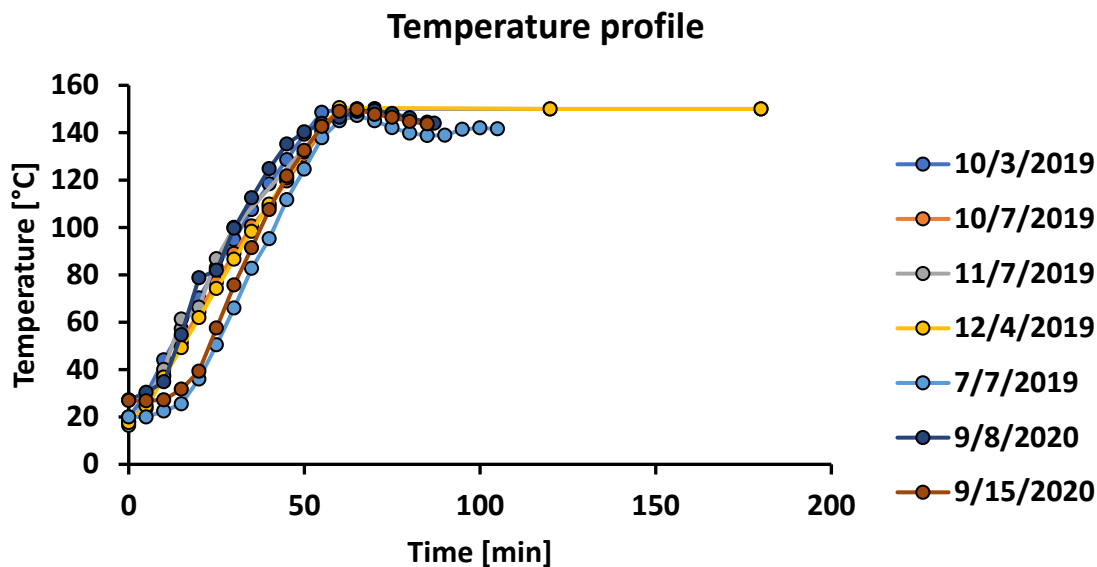


Figure 37. Temperature profile of selected reactions.

In Figure 38 we see the pressure profiles of these batches, with some reaching the maximum of 7 psig, but generally staying around 5 psig after the one-hour mark. After the initial climb, the pressure decreases, which corresponds to the manual temperature control to keep the reactor from overheating. There is no significant difference between the two biomass types.

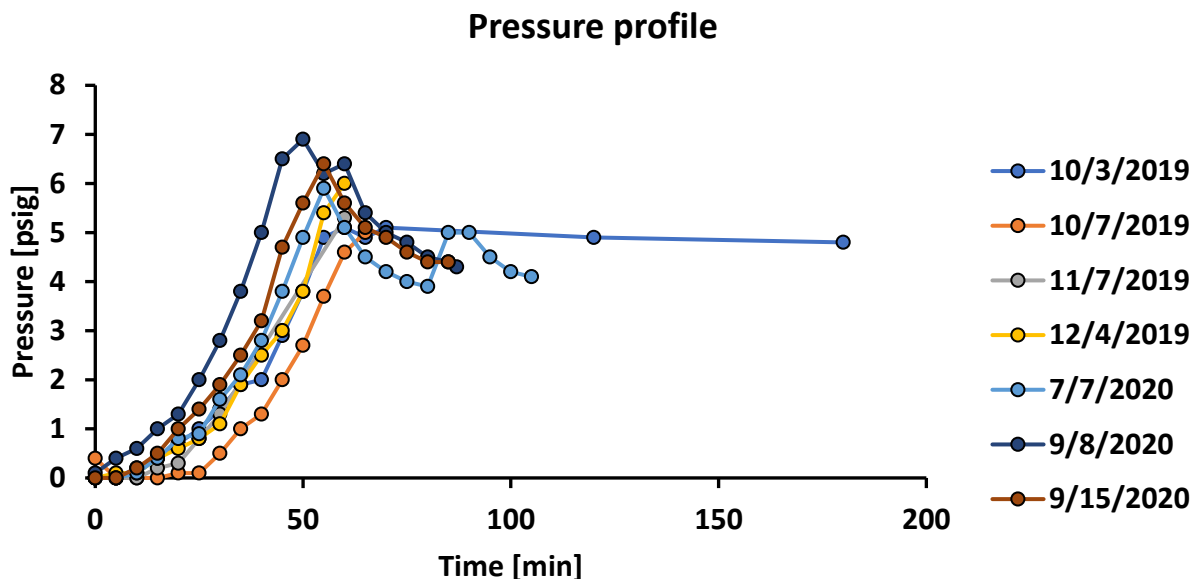


Figure 38. Pressure profile of selected reactions.

Date	10/3/2019	10/7/2019	11/7/2019	12/4/2019	7/7/2020	9/8/2020	9/15/2020
Reactor setup	4 reactors	4 reactors	4 reactors	20 L	20 L	20 L	20 L
Liq-sol ratio	4:1	5:1	5:1	5:1	9:1	9:1	9:1
NaOH loading	18%	18%	18%	18%	13%	13%	13%
Biomass type	HP	HP	HP	HP	CS	CS	CS
Reaction time	3 hrs	3 hrs	3 hrs	3 hrs	30 min	30 min	30 min
Reaction T	150°C	150°C	150°C	150°C	140°C	140°C	140°C

Table 3. Conditions for reaction profiles.

It is important to note that the specified reaction times refer to the active heating phase of the reaction. However, due to the slow cooldown rate of the digester as approximately 4.78°C/hr (shown in Figure 39), meaning the reaction effectively extends beyond the defined heating period. This slow cooling rate required overnight cooling of the digester before it could be safely unloaded. Table 4 shows the batch conditions for this cooldown profile over 25 hours. Even 24 hours after the heating is turned off, the reactor is 46 °C, requiring caution during unloading.

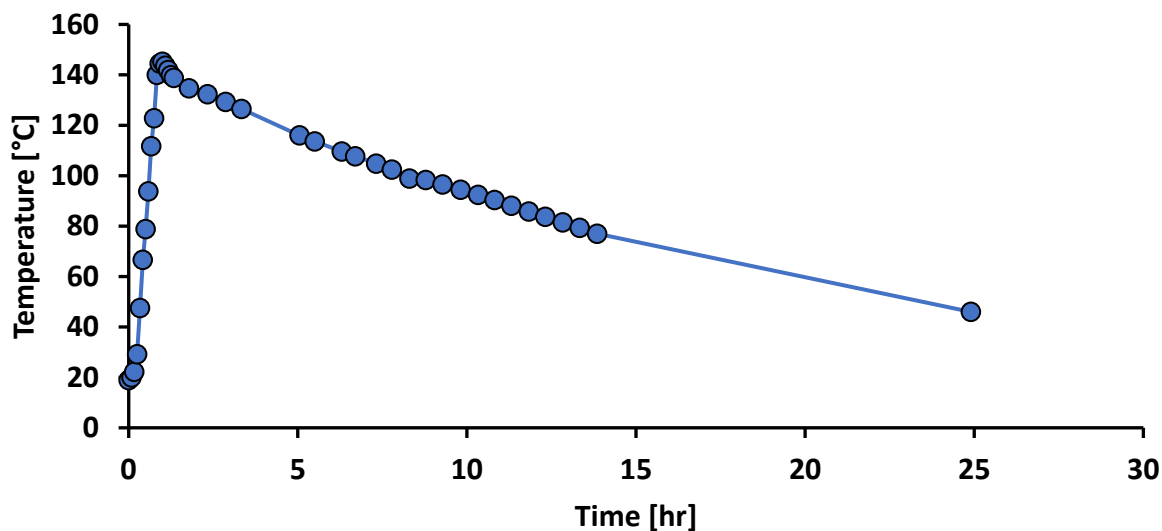


Figure 39. Cooldown profile of the digester.

Date	Reactor setup	Liquid-solid ratio	NaOH loading	Biomass type	Reaction time	Reaction T
9/29/2020	20 L	9:1	13%	CS	30 mins	140°C

Table 4. Conditions for cooldown profile.

Inconsistent Physical Properties

During production, we encountered variability in the final lignin color and texture from both types of biomass (see Figure 40). These inconsistencies persisted even within samples from the same biomass and using the same recovery method. Variations in color can affect the reproducibility of research for collaborators and reportedly impacted how well the lignins went into solution. However, color variability did not significantly impact lignin purity, as shown in Table 5. All lignins met the established purity, with an ash content below 1.5% and xylan content below 5%.

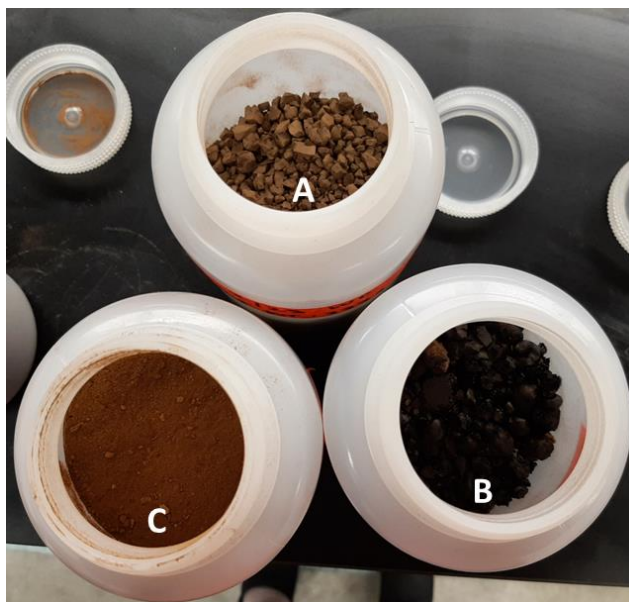


Figure 40. Color and texture inconsistencies for hybrid poplar lignins

	A 02/23/2021	B 03/03/2021	C 03/15/2021
Ash	<1.5%	<1.5%	<1.0%
Xylan	3.21%	4.92%	4.13%

Table 5. Impurity content of shown batches.

Variable Screening

Building on insights from the literature, we conducted a variable screening to identify factors contributing to the variability in physical lignin properties in our bench-scale lignin recovery from hardwood and herbaceous alkaline black liquors. We investigated variables such as solid-liquid separation types (filtering and centrifugation), final pH, acid used for precipitation, drying temperature, and filtering temperature. This variable screening does not provide a comprehensive evaluation of each variable's full impact on the process. Our focus was on physical properties, particularly color, as it was observed to be highly inconsistent for our lignin production.

Figure 41 shows how as the acidification progresses, the color and general appearance of black liquor is changing. In the initial steps of acidification lignin globules are forming in the dark black liquor (left panel in Figure 41). Then, there is noticeable foaming as more and more acid is added (middle panel in Figure 41). This foaming did not produce foul smelling gases, which would indicate sulfur containing gases like H_2S or SO_2 . Rather, it is probably a result of localized overheating of the black liquor with added sulfuric acid, or the precipitating lignin acting as a surfactant promoting bubble formation. Finally at $\text{pH} < 2$ most of the lignin is precipitated out, resulting in light brown acidified black liquor (right panel in Figure 41),



Figure 41. Start of precipitation forming globular lignin particles (left panel), during precipitation significant foaming observed (middle panel), and final acidified black liquor (right panel).

Solid-Liquid Separation Method

Filtering was quicker, and it removed significantly more ash using the same amount of washing liquid than centrifuging, while promoting drying. It would be advantageous to use filtering as a recovery method for larger scales, but for the production centrifuging was used because of large scale filtering challenges. No color difference was observed. Comparing the composition of the recovered lignins (top panels on Figure 42), centrifuging consistently resulted

in higher impurity contents. This can be explained by the different separation mechanisms. During filtration, the washing liquid is in direct contact with the material, continuously removing impurities with fresh water to flow through. During centrifugation, there is less contact between the washing liquid and the solute, and the impurities are rather redistributed than removed. The impurities can remain in or near the compacted solid. The ash content was also measured for the dry solids from the filtrate or centrifuge liquid, showing the same trend, filtering removing more impurities than centrifuging (bottom left panel on Figure 42). Comparing the recovered lignin mass, there are slightly different, with more recovered during filtration because of the effective entrapment of lignin due to the pore size of the filter while during centrifugation small particles can stay dispersed in the liquid.

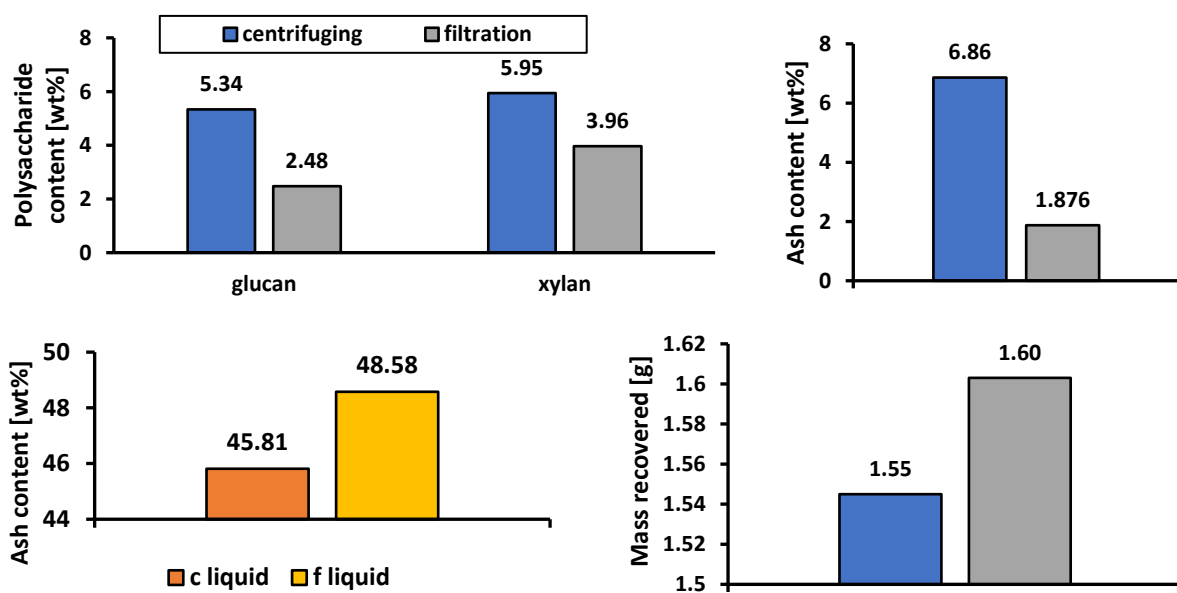


Figure 42. Impurity content of different separation methods.

Drying Temperature

The initial idea was that the color difference is because of some kind of oxidizing reaction during drying, but the drying method was always consistent and gentle. First, the samples were airdried for 2 days then vacuum dried for 2 more days at 50°C. Drying lignin samples at different temperatures shows how the color only changes at a high temperature, 105°C (see Figure 43), which was never reached for production. Darkening at that temperature is expected as oxidation with air, thermal decomposition of lignin, and Maillard reaction of carbohydrate impurities are all possible. This finding indicates that color variability is a result from a variable earlier in the process.

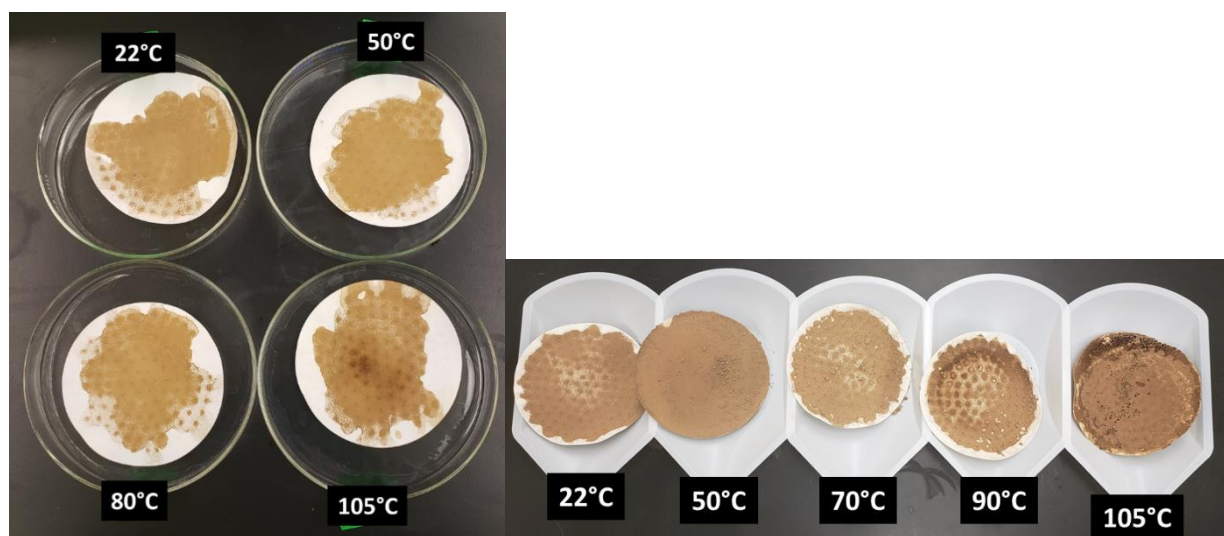


Figure 43. Lignins dried at different temperatures.

Oxidation Length

The additional oxidation step decreased the pH of the original black liquor (left panel of Figure 44) and significantly increased the mass yield only for the final eight-hour oxidation length (right panel of Figure 44). The pH decrease is minor, from the original black liquor pH of 11.45 to the final 10.78 after eight hours.

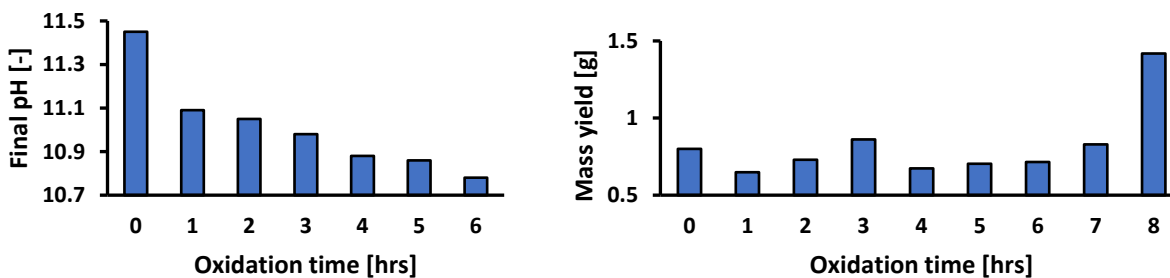


Figure 44. pH and mass yield of oxidation experiments.

Impurity content did not stay consistent throughout the oxidation process (see Figure 45). For xylan content, the results range from 4.3% to 6.6%, with an outlier at the final eight-hour mark with 8.7% xylan content. Ash content shows the same trend of minor changes from 1.4% to 3.8% for most oxidation length, with the same outlier at the final eight-hour mark with an 8.5% ash content.

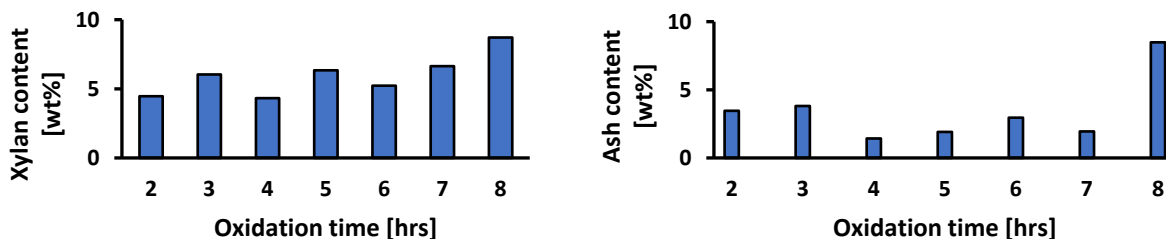


Figure 45. Impurity content after oxidation experiments.

A slight darkening effect was observed starting at seven hours of oxidation (see Figure 46). Due to the nature of the experimental setup (air bubbling through black liquor), the oxidation was probably inefficient and inconsistent, only resulting in significant changes in visual appearance, mass yield and impurity content at the final eight-hour oxidation.

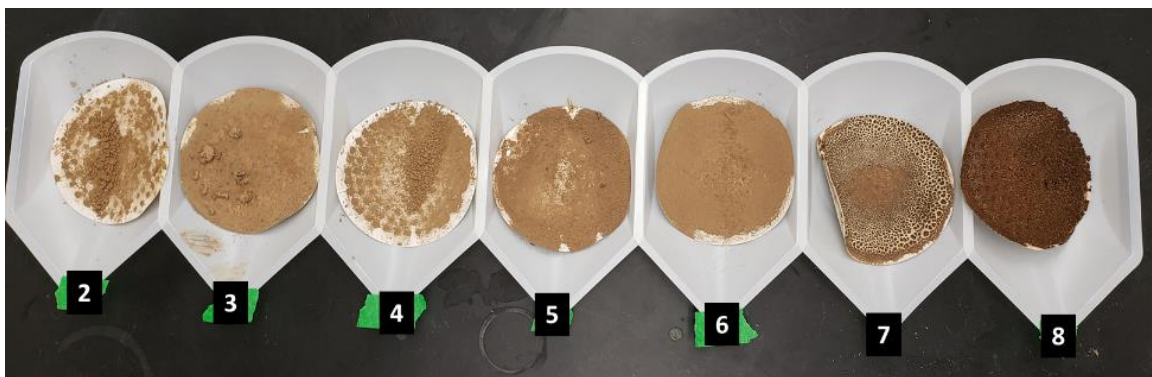


Figure 46. Recovered lignins from oxidation times.

Acidifying Agent and Precipitation pH

We compared acidifying agents for the whole pH range. The well-established trend of increasing lignin yield with decreasing pH was observed for both types of acids (top panel on Figure 47). A significant jump in yield happened at around pH five for both types of acids. Sulfuric acid precipitation yielded slightly more lignin, but the difference is only around 5%.

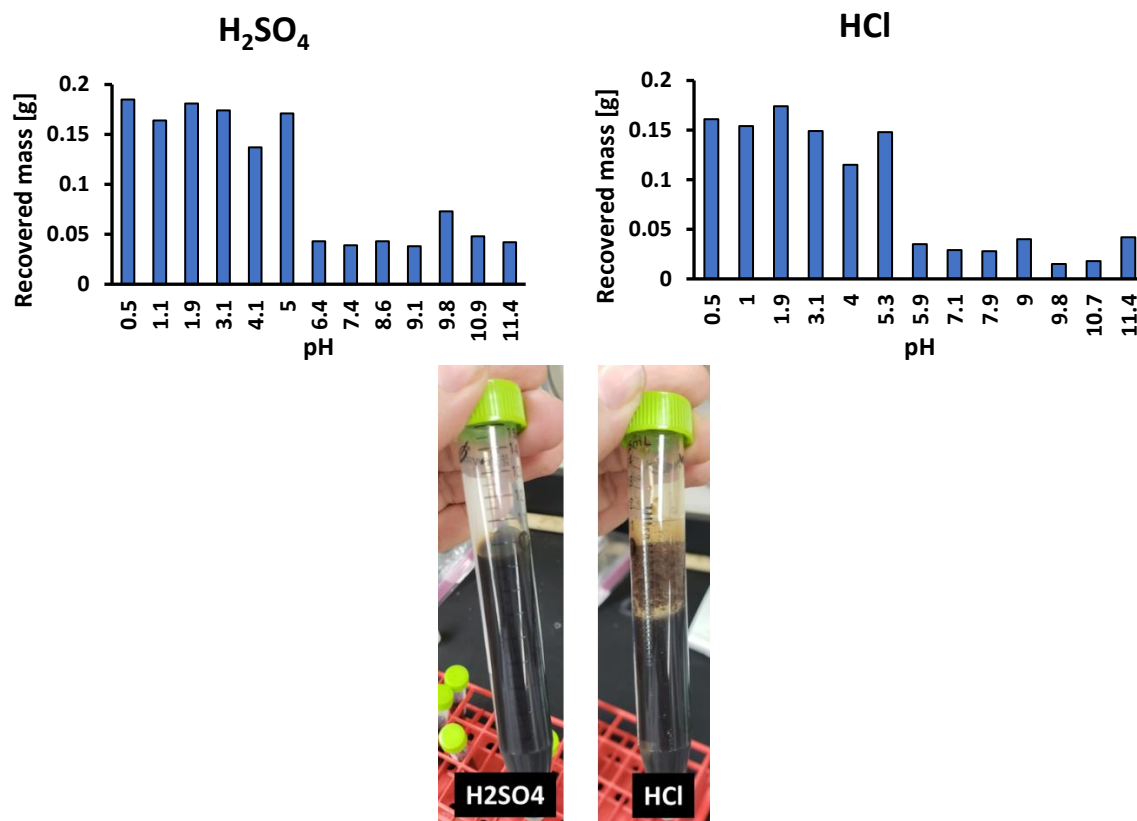


Figure 47. Mass yield of lignin for different pH levels using sulfuric acid (top left panel) or hydrochloric acid (top right panel). More bubbling during hydrochloric acid precipitation (bottom right panel).

Hydrochloric acid generated significantly more foam and gas compared to sulfuric acid (bottom panel on Figure 47). Both sulfuric and hydrochloric acid are strong acids, however, sulfuric acid is diprotic while hydrochloric acid is monoprotic. This difference may contribute to the foaming, as hydrochloric acid dissociates completely in one step, while the second deprotonation of sulfuric acid is happening from the already deprotonated hydrogen sulfate ion, which is a much weaker acid than the hydrochloric acid. Figure 48 shows the recovered solids from the original black liquor up until the final 0.5 pH. It is difficult to determine color for samples above pH 5 as the quantity of solids is not enough, but below 5 the lignin is becoming lighter with

lower pH levels. The color difference is less pronounced than during production, but variability in the final pH during production is still a likely factor.

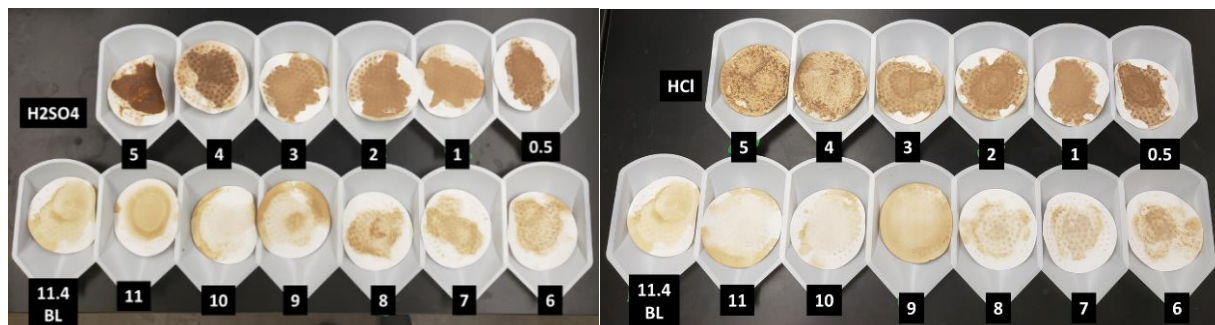


Figure 48. Lignins recovered at different pH levels using sulfuric acid (left panel) and hydrochloric acid (right panel).

Washing Medium and Ratio

Washing the recovered lignin using low pH water is common practice to remove as much impurities as possible by being more effective at solubilizing impurities like metal ions than neutral pH water. It can also improve the color of the recovered lignin. Our results indicate that washing the filter cake with pH=2 DI water introduces more variability and decreases the recovery yield of lignin (top panels on Figure 49). For the impurity content, our results suggest the opposite of the well-established improvement. Washing with acidic water resulted in slightly higher impurity contents for all washing ratios. There was no observed color difference for different washing ratios or media.

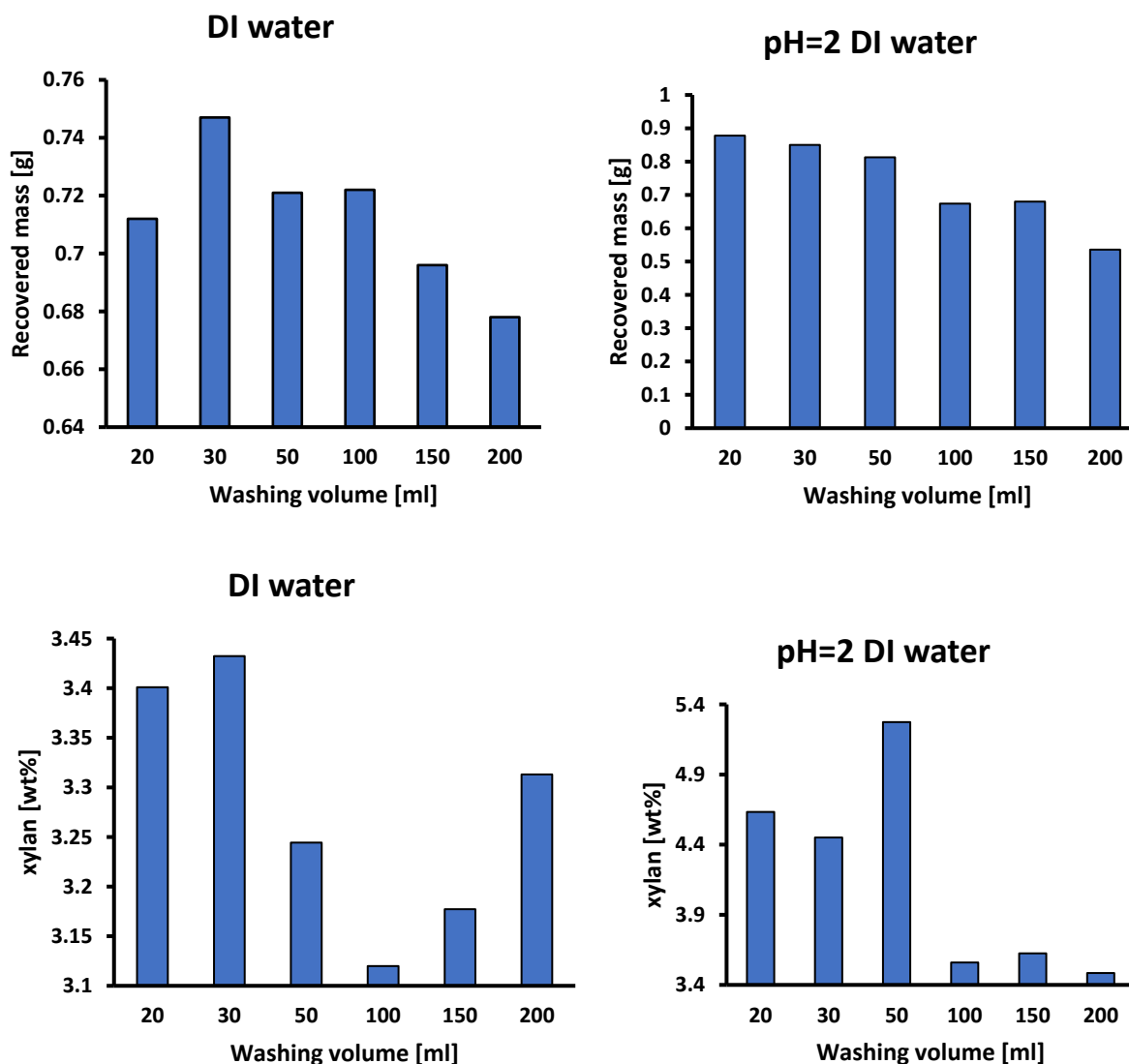


Figure 49. Comparison between different washing mediums.

Reheating after Precipitation, Filtering Temperature

An additional reheating step after precipitation for the filtration was the final investigated variable. It became immediately clear that this reheating step has a great impact on the lignin color (left panel on Figure 51). Different reheating temperatures resulted in two different textures and colors. There was a distinct transition temperature after which the lignin sample consistently becomes significantly lighter, more powdery. The additional reheating step after acidification also

greatly improved the filtration time and ash content, while only slightly decreasing the recovered mass (see Figure 50).

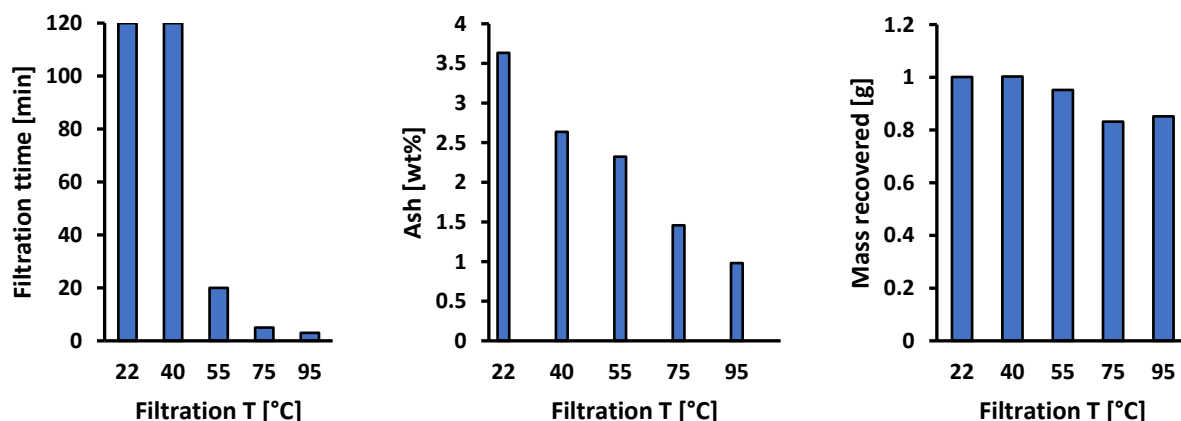


Figure 50. Preliminary filtration temperature experiments. Filtration time (left panel), ash content (middle panel), and recovered mass (right panel) using different filtration temperatures.

The first transition temperature identified was for corn stover lignin between 55 and 75 °C (left panel on Figure 51). The filtrate also changed in appearance at different filtering temperatures (middle and right panel on Figure 51), becoming cloudy at high temperatures indicating lignin dissolution back into the liquid. After the initial experiment, the final investigated filtering temperature was adjusted to 80 °C because of the increasing evaporation at higher temperatures.



Figure 51. Color differences for corn stover samples filtered at different temperatures (left panel), and comparison between filtrate appearance for room temperature and high temperature showing cloudiness for high temperature filtration (middle and right panel).

This experiment was conducted multiple times using the corn stover and the hybrid poplar black liquor for accurate validation of the phenomenon and determination of the transition temperature. Corn stover gave the same results as the preliminary experiments with a transition temperature between 70 – 75 °C (left panel on Figure 52). Hybrid poplar exhibits a transition temperature as well, but at a lower temperature, between 35 – 45 °C (right panel on Figure 52). This phenomenon, along with the differences in properties of lignins with varying colors, is explored in detail in Chapter Four.

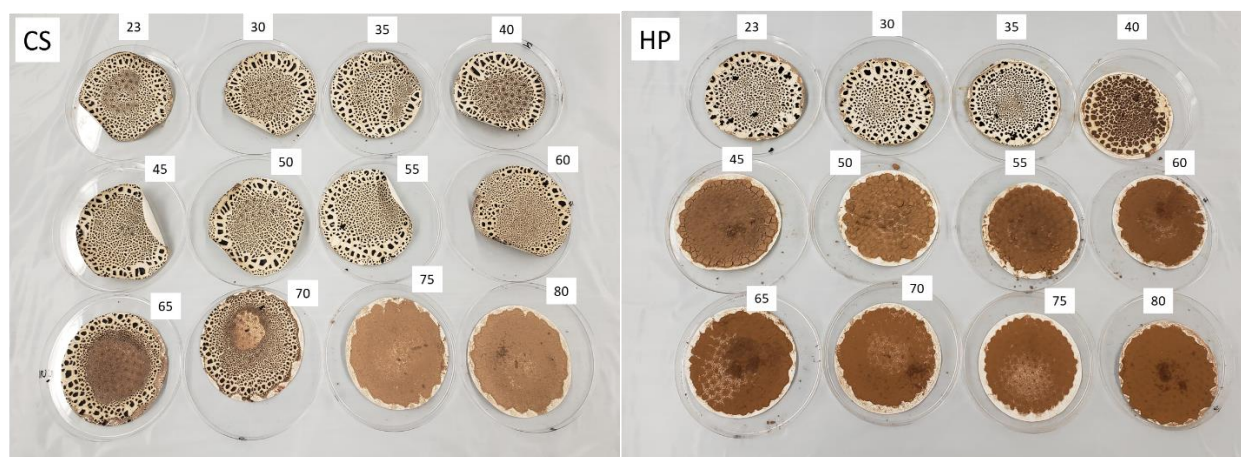


Figure 52. Lignins recovered from detailed filtration temperature experiments using corn stover (left panel) and hybrid poplar black liquor (right panel).

Preliminary Microscopy Results

Preliminary epifluorescence microscope images were taken of lignin samples recovered at different filtering temperatures (see Figure 53). Recently published work [136] from the group used fluorescence imaging to compare average fluorescence intensities of lignin particles. Epifluorescence imaging can also be implemented for measuring particle size distribution. A method was developed and applied in Chapter Four. Apart from visual comparison, intensity and particle size provides more opportunities for comparison of light and dark colored lignin samples.

The lignin samples are not subjected to milling or grinding, only transferred to the microscope slide. To the naked eye, the dark colored lignin is noticeably more “crystalline” (left panels on Figure 53), the particles are glossy and hard, while the light-colored lignin is more powdery and crumbles easily (right panels on Figure 53). Dark samples exhibit more distinct, well-defined individual particles with smooth and sharp surfaces. Light samples appear to form aggregates of particles, with a significant amount of smaller, fragmented particles scattered throughout. However, these images are not suitable for precise particle size distribution analysis due to the large number of particles and the lack of clear boundaries. These preliminary images only provide qualitative insight into differences rather than quantitative data. This is why in Chapter Four, lignin samples were resuspended in excess water to investigate separated individual particles. Lignin is insoluble in water, so it does not interfere with the particle morphology.

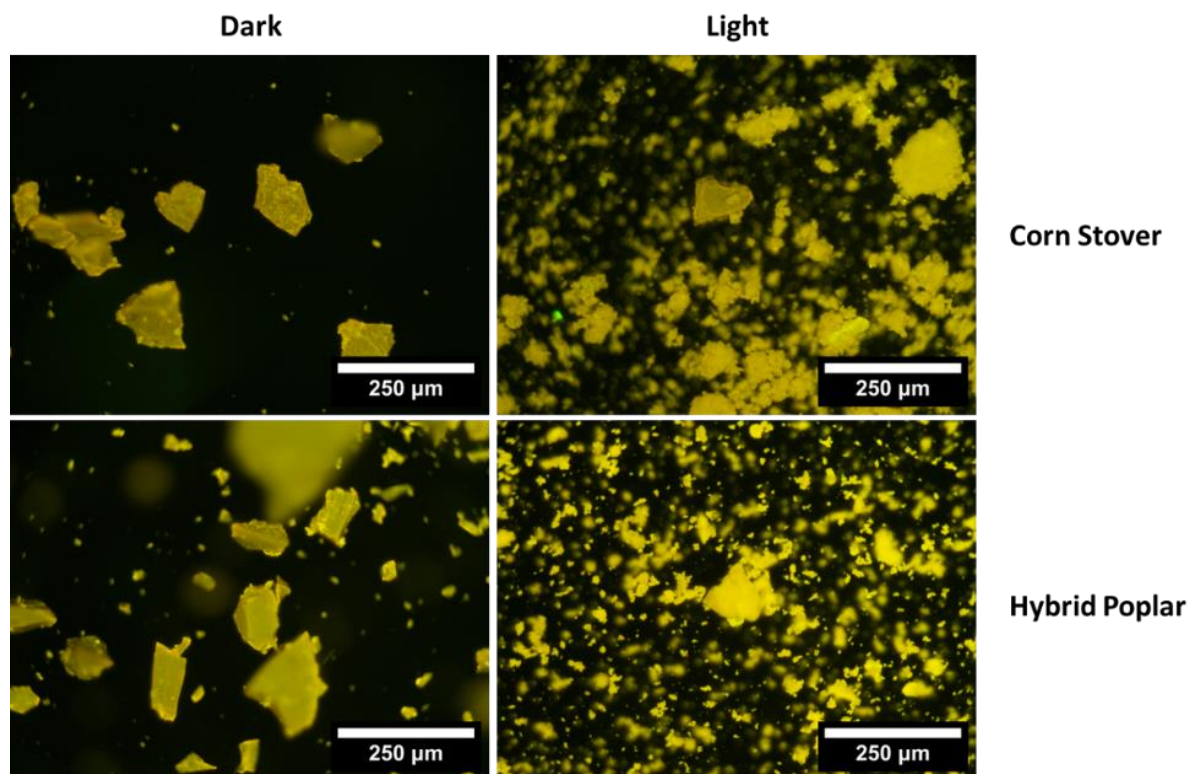


Figure 53. Preliminary epifluorescence microscope images. Both corn stover (top panels) and hybrid poplar (bottom panels) were analyzed at low filtering temperatures (23 °C, dark samples, left panels) and high filtering temperatures (75 °C for corn stover in top right panel, 45 °C for hybrid poplar in bottom right panel) using 10x magnification.

Preliminary trials showed that using the bright field illumination for imaging is ineffective for lignin due to the compact particles being completely opaque in standard lighting. However, this method could be used for the imaging of raw corn stover (top panel in Figure 54) and hybrid poplar (bottom panel in Figure 54), showing cellular structures of both types of biomass, using 4x magnification. Corn stover, comprising of various anatomical fractions of the corn plant, exhibits a range of particle structures. The particles range from semi-opaque dense rods to semi-translucent sheets to translucent layers, possibly corresponding to decreased lignification in the respective particles. In comparison, hybrid poplar wood chips are much more uniform, showing lignified dense rods with more translucent layers along the edges. More thorough imaging of plant tissue is

typically done through cross-sectional slices of the plant rather than the side views like those shown here. Thin cross-sectional slices also eliminate the issue of limited microscope depth of field, which often causes parts of the image to appear clear while others remain blurry due to the thickness of the particles.

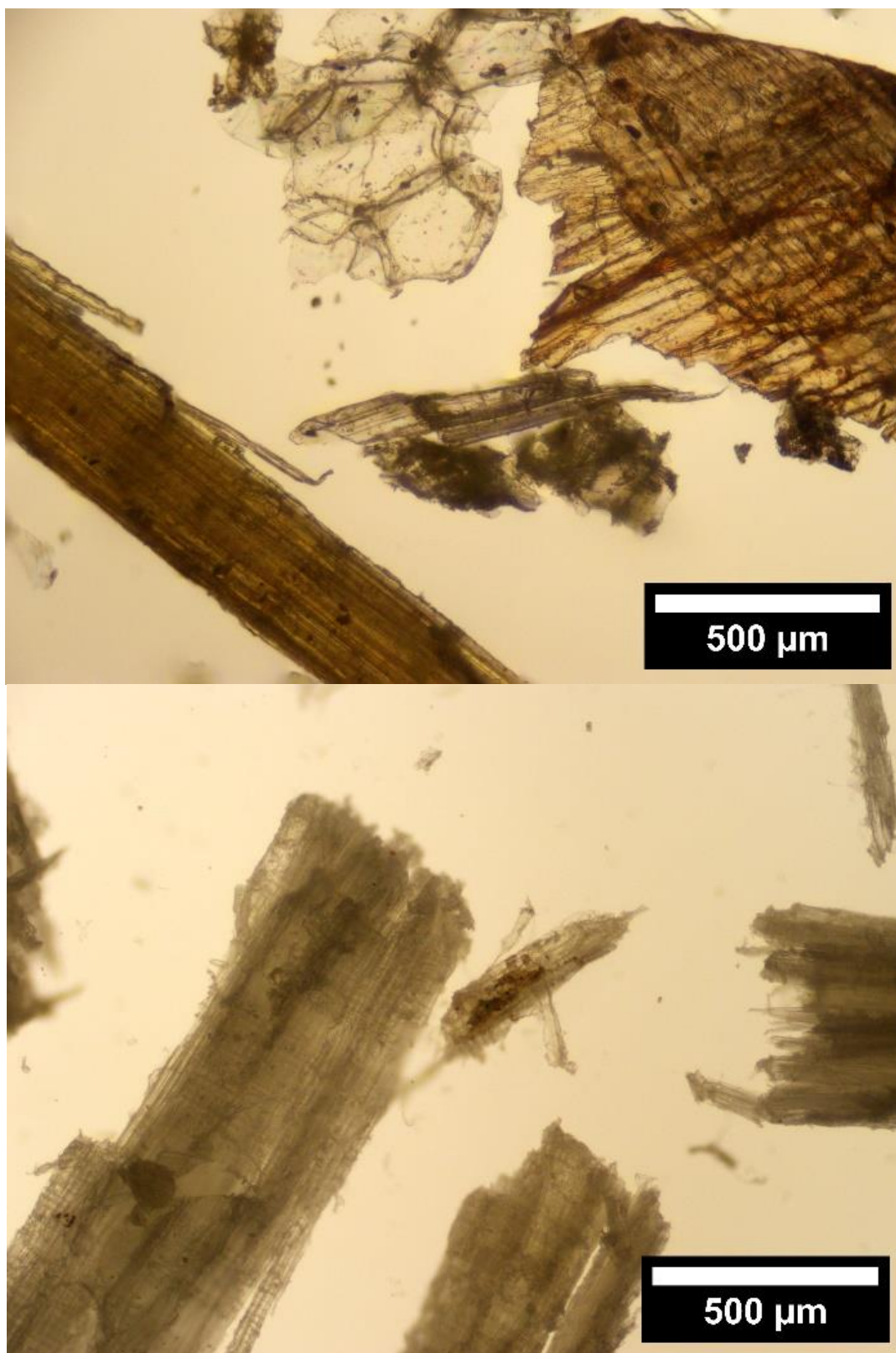


Figure 54. Brightfield images of corn stover (top panel) and hybrid poplar (bottom panel).

CHAPTER FOUR

IMPACT OF PROCESSING HISTORY AND RECOVERY
CONDITIONS ON LIGNIN COAGULATION,
FILTERABILITY, COLOR, AND
CHEMICAL PROPERTIES

Contribution of Authors and Co-Authors

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Manuscript Information

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Impact of Processing History and Recovery Conditions on Lignin Coagulation, Filterability, Color, and Chemical Properties

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Abstract

Inconsistent physical properties in recovered lignins pose a substantial challenge for advancing high-value applications and biorefinery efficiency. This study examines how controlled temperature during lignin precipitation and filtration can impact these properties, with the aim of achieving more homogeneous lignin recovery from acidified pretreatment liquors of corn stover and hybrid poplar. Our findings highlight a transition temperature at which we observe distinct changes in lignin filterability, chemical, and physical properties. Considering our observations, we propose a correlation between reaction-limited and diffusion-limited cluster aggregation (RLCA and DLCA) regimes and the resulting lignin properties. Findings from this study provide insights into optimizing lignin recovery by refining process parameters, ultimately enhancing lignin's suitability for commercial applications.

KEYWORDS: Lignin, corn stover, pretreatment, acidification, color

Introduction

Transitioning to renewable energy is crucial for reducing our reliance on fossil fuels. Bioenergy is the largest renewable energy source, offering a sustainable alternative [2]. Biorefineries process biomass to produce a variety of valuable products [4, 5]. A key resource in this transition is lignocellulosic biomass, an abundant, non-food feedstock which includes agricultural residues and woody crops [7, 8]. The primary components of lignocellulosic biomass—cellulose, hemicellulose, and lignin—are utilized in a biorefinery with differing priorities. While cellulose and hemicellulose are primarily utilized to high-value biobased products [3], lignin, which comprises 10-30% of biomass [18], remains underutilized and only used for its heating value as solid fuel [10, 12]. Due to its complex aromatic structure and low solubility in many solvents, producing consistent, high-quality lignin-based products is challenging [9, 11]. In addition to lignin's variable chemical structure, its ratio and monomer composition vary depending on the type of biomass. For instance, woody biomass like poplar contains 20-30% lignin [13], while herbaceous biomass like corn stover, an agricultural residue left after harvesting, has 15-20% lignin and higher hemicellulose content [14, 15]. Understanding these differences is crucial for refining lignin recovery methods and achieving consistent lignins across biomass types. Despite these challenges, lignin holds immense potential for value-added applications, including polyurethane foams [129, 137], carbon fibers [126, 127], biochars [130].

Consistent lignin recovery is necessary for the economic viability of biorefineries, improving process efficiency while supporting waste management and chemical recovery [43]. This reliability is also critical for producing lignin with the desirable properties needed for high-value applications. In industrial settings, lignin is typically recovered after pretreatment, a process

designed to improve the accessibility of cellulose and hemicellulose for further processing [41]. In this study we used alkali pretreatment, which is one common method, wherein lignin is solubilized from the biomass into the black liquor. The typical industrial process for recovering lignin from black liquor involves three main steps: concentrating the liquor, acidifying it to precipitate lignin, and filtering the precipitated lignin.

Precipitation and subsequent recovery is governed by factors such as temperature [47], pH [48], types and concentration of Na^+ or other metal ions [44], anion type [49] and ionic strength [50]. These factors influence lignin's coagulation and filtration behavior and final properties. Higher lignin concentration, higher ionic strength, lower pH, and lower temperatures increase precipitation yield [51]. Particle size control during recovery is also important, as micro- and nanoparticles are challenging to recover due to their colloidal stability and low filterability [52]. A patented method [53] improves filterability of alkaline black liquors by acidification at temperatures below 60 °C, and filtration above 50 °C. This approach allows drying at temperatures up to 110 °C while preventing the formation of undesirable "black" lignins with low filterability. Under optimal conditions, filter cake solids can reach up to 35%, reducing drying needs [47]. Advanced recovery technologies like LignoBoost [45] and LignoForce [46] have been introduced to optimize this process, along with innovations like the pilot-scale Sequential Liquid-lignin Recovery Process (SLRP) [33, 138].

During lignin precipitation, the goal is to destabilize the colloidal system [56, 57], causing the particles to aggregate into larger clusters. There are two universal limiting regimes of colloid aggregation: diffusion-limited cluster aggregation (DLCA) and reaction-limited cluster aggregation (RLCA) [60, 139-142]. Previous studies demonstrated that these aggregation regimes

are applicable to lignin, [50, 143], but the direct influence of temperature on these mechanisms in lignin precipitation has yet to be thoroughly examined.

Color changes can serve as a macroscopic indicator of microscopic differences in lignin properties, like how processing conditions during pretreatment and chemical treatments impact the color of lignin [66]. Handheld colorimeters provide a quick, non-invasive method for comparing apparent lignin color [68]. Lignin color can also serve as a quality indicator for various applications [62, 63, 67]. Microscopic imaging techniques, such as fluorescence microscopy, offer valuable insights into biomass [74, 144]. Lignin's natural autofluorescence facilitates straightforward analysis without fluorescent dyes, usually employed for plant tissues [75]. These techniques are particularly useful for characterizing lignin to understand how processing conditions and the type of biomass impact its particle properties.

Despite significant advances in lignin recovery techniques, the production of homogeneous lignins with consistent physical properties remains a persistent challenge. This inconsistency hinders the development of reliable, high-value applications for lignin and impedes the overall efficiency of biorefinery operations. While various factors influencing lignin properties have been studied, the specific role of temperature in determining the final characteristics of recovered lignin has not yet been thoroughly investigated. This gap in understanding presents a significant opportunity to improve the consistency and quality of recovered lignin. This study explores the effects of temperature and solid content on lignin properties and processability, focusing on lignin recovered from hybrid poplar and corn stover black liquors. Notably, our research is the first to link cluster aggregation regimes to lignin particle properties after precipitation using color assessment and microscopy techniques.

Experimental

Biomass

Hybrid poplar (NM6, *Populus nigra* x *P. maximowiczii*) was obtained from the Michigan State University Upper Peninsula Forestry Innovation Center (Escanaba, Michigan). Poplar was harvested in January 2020 from less than 5-year-old trees with stem diameters of 15.5 to 20.3 cm and were debarked, chipped, and dried to approximately 5% moisture content. A commercial hybrid corn stover was obtained from DuPont via Idaho National Laboratory. The corn stover was harvested in October 2014 in Story County, Iowa and was subjected to milling first with Vermeer BG480 bale grinder fitted with a 2-inch sieve, followed by further milling in a Bliss Hammermill fitted with a 1-inch sieve.

Alkaline Pretreatment

Corn stover was subjected to delignification in a 20-L stainless-steel tumbling pulp digester (RegMed model AU/E-20 with a TP-2027 lid, São Paulo, Brazil). The biomass moisture content was determined using an Ohaus MB90 moisture balance (Parsippany, NJ). Then, 2.0 kg of biomass (dry basis) was loaded into the digester and mixed with a NaOH solution to achieve a liquor to biomass ratio of 9:1, with an alkali loading of 130 mg NaOH/g biomass. The temperature was ramped up at a rate of 2.5°C/min until it reached the target temperature of 140°C, which was maintained for 30 minutes. Hybrid poplar was subjected to a similar delignification in the same equipment, but with 3.0 kg of biomass, a liquor-to-biomass ratio of 5:1 and an alkali loading of 180 mg NaOH/g biomass. The temperature ramping was identical, but the target temperature was 150°C, maintained for 3 hours. A similar method was used previously [129].

After the reaction, the digester was air-cooled until atmospheric pressure was restored and the temperature fell below 60°C, at which point it was unloaded. The black liquor was separated from the pretreated biomass using a 120-micron nylon CONIE mesh filter bag and a MacIntosh Apple Cider Press with a stainless-steel basket (Pleasant Hill Grain, Nebraska, US). The pretreated biomass was airdried and stored for further analysis, while the black liquor was stored at 4°C in a refrigerator.

Biomass	NaOH loading	Liquid – solid ratio	Reaction time [hr]	Reaction T [°C]	Starting biomass [kg]	Approx. volume for NaOH solution [L]	Approx. volume of black liquor [L]	Approx. original pH of black liquor	Approx. H2SO4 used [mL]
WC	18%	5:1	3	150	3	13.7	12	13	200
CS	13%	9:1	0.5	140	2	16	14	11	185

Table 5. Summary of alkaline pretreatment conditions.

Black Liquor Concentration

Black liquor concentration is carried out using a Pope Scientific Inc. Wiped-Film Still, equipped with an Edwards RV8 rotary vane vacuum pump, an MKS Series 275 Mini-Convector vacuum gauge, BriskHeat Corporation XtremeFLEX heating tape, a Penta KB Power Penta-Drive DC motor, and a Glas-Col PL312 power control unit. The black liquor obtained from the alkaline pretreatment process is fed into the evaporator in 1-liter batches. The solid content of the hybrid poplar black liquor increased from 10.4% to 40%, while the corn stover black liquor's solid content rose from 5.6% to 30%.

GPC

The number-average and weight-average molar masses of the extracted lignins are determined by gel permeation chromatography (GPC) following the method described in [105]. Acetylated lignin samples are analyzed using an Agilent 1260 HPLC system equipped with a Waters Styragel HR 4 column (7.8×300 mm, Milford, MA, USA). HPLC-grade tetrahydrofuran (THF, Millipore Sigma) serves as the mobile phase at a flow rate of 0.5 mL/min. The system operates with UV detection at 280 nm and a column temperature of 35 °C. Monodisperse polystyrene standards ranging from 484 to 65,000 Da (Readycal, Fluka Analytical) are used for reference.

Lignin and Biomass Characterization

The comprehensive analysis of biomass, pretreated biomass and recovered lignin, including ash, carbohydrate and acid insoluble (Klason) lignin (AIL) content, is performed according to the two-step hydrolysis method outlined in NREL/TP-510-42618 [104]. Monomeric carbohydrate content is quantified from the hydrolysis filtrate using an Agilent Technologies 1260 Infinity II HPLC system equipped with a Bio-Rad Aminex® HPX-87H column, with 0.005 M sulfuric acid as mobile phase at a flow rate of 0.6 mL/min.

Color change temperature/ Lignin Precipitation

Corn stover or hybrid poplar black liquor is homogenized for a minimum of 5 minutes using a metal mixer attachment on a drill, and 310 mL of the liquor is transferred into a 500 ml Erlenmeyer flask. The sample is then acidified to below pH 2 with concentrated sulfuric acid, causing the temperature to increase from the storage temperature of 4°C but remaining below 23°C. Heating is achieved with an oil bath, and 50 mL samples are taken at 5°C intervals from

room temperature up to 80°C Each sample is filtered through Whatman #40 filter paper in a Büchner funnel and washed with 100 mL of deionized water at the same temperature as the sample. Finally, the solids are removed from the filter paper and left to airdry over 2 days. Temperature, filtering time, final mass and color are recorded. Due to availability of the concentrated liquor, the procedure is modified to a smaller sample size. 20 mL of the high viscosity 30% concentrated corn stover liquor is diluted with 40 mL deionized water, and 30 mL of the 40% concentrated hybrid poplar liquor is diluted with 30 mL deionized water, resulting in 12.9% and 21.0% solid contents respectively, and stored in a refrigerator overnight at 4°C. Then the liquors are acidified, heated and filtered similarly to the dilute black liquor, except using a smaller 5 mL sample size and 25 mL washing water for effective filter cake forming. The recovered solids are airdried overnight.

Solubility

Following the methodology outlined in our previous work [145], we investigated the solubility differences between light and dark lignin samples. A 1:10 (w/v) solid-to-solvent ratio was employed, where 300 mg of lignin was dissolved in 3 mL of solvent, with ethyl acetate and dimethyl sulfoxide selected to represent the two extremes of solubility and solvent polarity reported previously. The mixture was incubated in a sealed test tube for 1 hour at 70°C. Following incubation, the undissolved solids were filtered using Whatman #42 55 mm filter paper, washed with 30 mL of fresh solvent, and air-dried overnight at room temperature.

Colorimetry

Lignin color is determined following our previously published method [136], using an FRU WR18 colorimeter. Color data is recorded in the CIELAB color space, with L* ranging from 0 to 100, and a* and b* values ranging from -128 to 128. The measurement is taken through a 0.4 mm

diameter window, using a D65 standard light source, a 10° CIE standard observer, and an 8/d illumination system with a photodiode array sensor.

Transition Temperature

The transition temperature was determined through linear interpolation using the measured lightness values of the samples isolated at different temperatures. First, a transition lightness parameter was calculated as the average of the lowest and highest measured lightness values, which serves as a threshold for distinguishing whether a sample is classified as dark or light. The transition temperature is defined as the point at which the interpolation crosses this transition lightness parameter. The exact temperatures from interpolation can be seen in the Supplementary Materials, in Figure 66.

Fluorescence Microscopy

For the microscope slide preparation, a sample was taken from homogenized black liquor. Then it was acidified with concentrated sulfuric acid to the predetermined pH and diluted 1:100 (v/v) with deionized water. A 1 μ L aliquot of the diluted sample is pipetted onto a microscope slide making 10 drops and allowed to dry. No observed change in particle size during drying according to a video recording during the drying process. Powder lignin samples from different filtering temperatures are suspended in DI water at a 1:100,000 (w/w) ratio, and 10 μ L of the suspension is pipetted onto a slide to dry. Imaging is performed using an OMAX EPI-Fluorescence Trinocular Microscope (M837ZFLR-C180U3) with an 18MP camera (A35180U3), a mercury lamp, a blue excitation filter (410-490 nm), and a 515 nm barrier filter. Images are captured at 10x magnification using the ToupLite software.

Particle Size Distribution

Image processing is performed using ImageJ/Fiji software, starting with standard preprocessing steps (excluding images containing artifacts like biomass particles or the edge of the sample drop, calibration, thresholding to binary, etc.). Particle sizes are then determined with the software, excluding small artifacts (<9 pixels) and edge-bound particles. For powder samples, heavily aggregated large particle images are excluded. Particle counts and areas are extracted and processed using a Python/Numpy script to generate particle size distributions, normalized histograms, fit Gaussian curves, and to calculate mean and variance.

Confocal Microscopy

The confocal microscopic images were obtained using an LSCM Leica Stellaris DMI-8 confocal microscope (Leica Microsystems). The CLSM images were analyzed using Fiji software and MATLAB. The lignin particle contour extraction was done using the ImageJ macro, Surfcut [146]. The average roughness (Ra), and the root-mean-square roughness (Rq) were obtained as described in [144].

Results and Discussion

Alkaline Pretreatment and Initial Observations

Prior to this work, we produced alkaline black liquors from both corn stover and hybrid poplar using a standardized alkaline pretreatment process, forming a basis for all subsequent experiments in this study. This setup enabled us to examine how recovery conditions influence lignin properties, addressing prior observations of inconsistent lignin color and texture. Previous work indicated that some batches yielded “dark” and char-like lignins with poor solubility and

extended filtration times. Drawing on findings from patent literature [53], we conducted experiments to test the effect of reheating the liquor after precipitation and observed that temperature had a pronounced impact. As shown in Figure 55, temperature variations led to distinct differences in the color and physical characteristics of lignin recovered from each biomass, identifying it as a critical variable. Dark lignins, isolated at lower temperatures, appeared as cohesive, clumped particles with smooth surfaces that subtly reflect light, giving them a sheen despite their darker color. In contrast, light lignins, recovered at higher temperatures, were less cohesive, powdery, and crumbled easily. Interestingly, Andeme et al. [147] reported a different trend, finding that lignin isolated from mixed hardwood black liquor yielded darker colors at high temperatures. Helander et al. observed that undesirable, rigid, large lignin particles were formed at high temperature (70 °C) precipitation [148]. Research by Zhang et al. [66] attributes the dark color of lignin to conjugated structures formed during the harsh Kraft pulping process. They also emphasize that while drying can affect color, it is not the dominant factor; rather, aggregation plays a crucial role, as less aggregated lignin with lower bulk density tends to be lighter. In order to lighten already recovered lignins, Wang et al. [65] demonstrated that lignin can be lightened using UV irradiation in tetrahydrofuran (THF), where quinones are photo-oxidized into aliphatic acid structures following disaggregation and depolymerization. As an alternative to harsh conditions, Cheng et al. [149] used deep eutectic solvents to isolate lignin directly from biomass, resulting in lighter lignin.



Figure 55. Variability in color and texture of lignins recovered from corn stover (A,B) and hybrid poplar (C,D) at 23°C (A,C) and elevated temperatures (75°C for corn stover B, 45°C for hybrid poplar D).

Impact of pH on Precipitation

Figures 56 and 57 illustrate the effect of pH on the solids recovered from corn stover and hybrid poplar alkaline pretreatment liquors. Acid precipitation of pulping liquor has long been proven as an efficient lignin separation method [150], with lower pH resulting in higher lignin yields, as confirmed by our results. To demonstrate lignin aggregation and precipitation we used epifluorescence microscopy to visualize lignin across the pH scale. Fluorescence microscopy is a common technique for plant tissue component analysis [76, 79, 151] or model lignin compound analysis [152], but it is seldom utilized for imaging lignin precipitation. Figure 56 shows selected images to highlight the differences between solids in original black liquor (pH $\approx$ 11) and in acidified black liquor (pH $<$ 2). The original, high-pH black liquors (Figures 57A and 57C) contain fine, dispersed, fluorescent particles from biomass. After acidification to a pH below 2 (Figures 57B and 57D), distinct morphological changes occur as larger lignin particles appear. This shift from a dispersed state to larger, more cohesive particles shows how acidification promotes lignin aggregation, facilitating its separation from the solution. This aggregation enables subsequent solid-liquid separation steps.

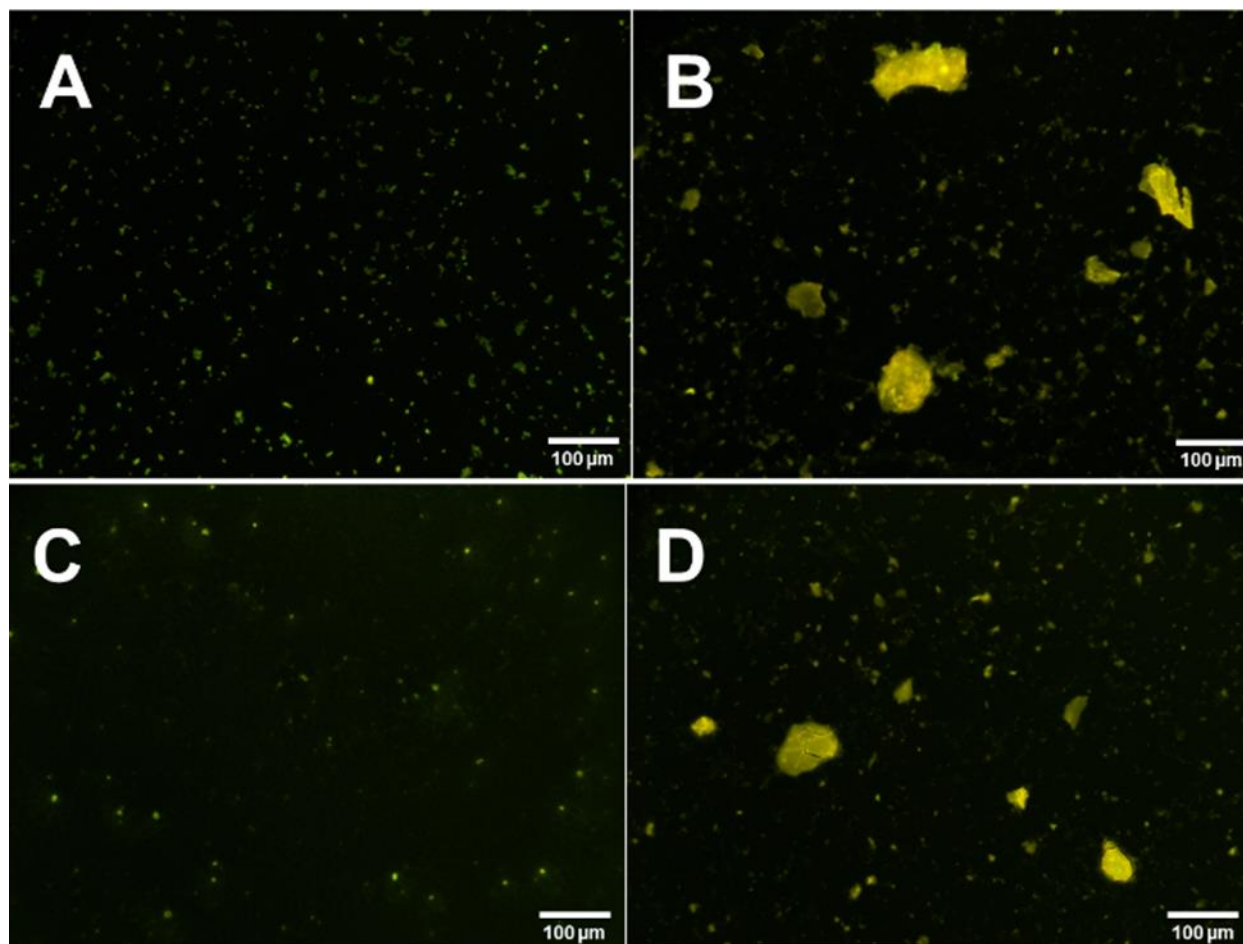


Figure 56. Epifluorescence microscopy images of black liquor solids from corn stover (**A,B**) and hybrid poplar (**C,D**) at 10x magnification: (**A**) and (**C**) show solids from original, unacidified black liquor ($\text{pH} \approx 11$), (**B**) and (**D**) show solids from acidified black liquor ($\text{pH} < 2$). The scale bar is 100 μm , magnification is 10x.

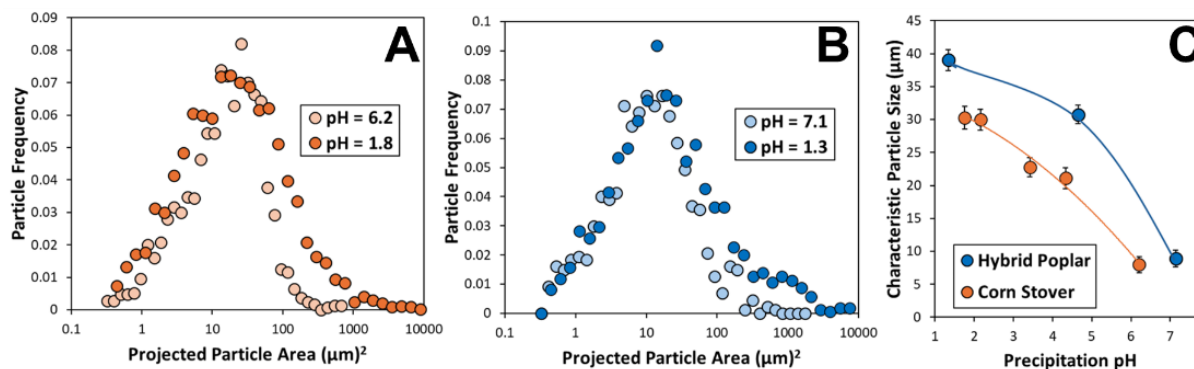


Figure 57. Particle size distribution of acidified black liquors from corn stover (A) and hybrid poplar (B), with characteristic particle sizes at different pH levels (C).

We used the microscopy images to determine the particle size distribution and quantify the characteristic particle size across the pH range. In our work the characteristic particle size is defined as the area-weighted mean of the particle size distribution. Figure 57 presents selected particle size distributions at different pH levels during lignin precipitation, at a middle point around neutral pH, and at the final pH. In both corn stover and hybrid poplar black liquors, the particle size distribution display a normal distribution. At lower pH, a characteristic "tail" extends toward larger particle sizes, indicating the presence of larger, aggregated particle formation as the pH decreases. The characteristic particle size quantifies the average size of these particles, around 10 μm for the neutral and 30 μm for corn stover and 40 μm for hybrid poplar at the final pH, showing slightly larger particles for the hybrid poplar.

Together these results emphasize the role of pH in controlling lignin particle size and morphology. Acidification initiates lignin precipitation, with lower pH values promoting the formation of larger lignin particles. This controlled precipitation process facilitates lignin recovery and suggests potential for adjusting pH to optimize separation techniques based on the targeted lignin characteristics.

Impact of Filtration Temperature on Filtration Time, Lignin Yield, and Lignin Color

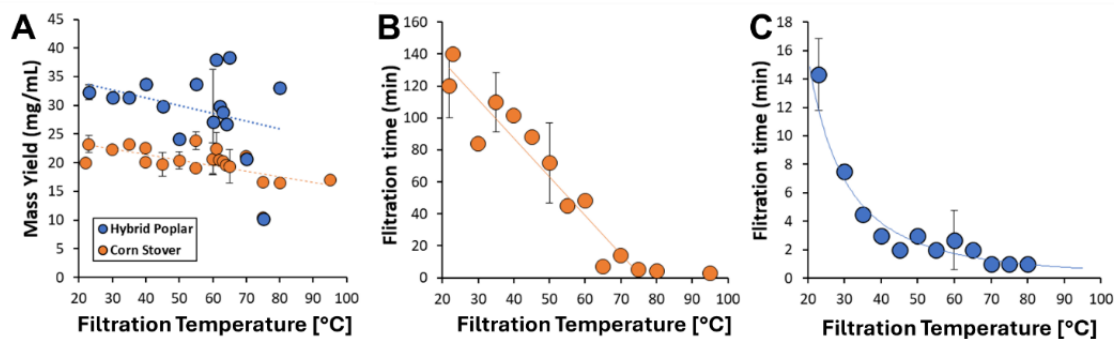


Figure 58. Impact of liquor filtration temperature on recovered mass yield (A) and filtration time for (B) corn stover and (C) hybrid poplar.

Quantification of lignin recovery yields demonstrated that yields of lignin from liquors decreased slightly over the temperature range of 23 to 95 °C for both corn stover and hybrid poplar lignins (**Fig. 4**), though others reported a more pronounced decline for softwood black liquor [51]. This decrease can be attributed to the increased solubility of lignin, xylan, and ash at elevated temperatures. Due to the alkaline pretreatment and subsequent precipitation, process ash is present in significant quantities, in the form of sodium sulfate (Na_2SO_4), derived from the alkaline agent sodium hydroxide (NaOH) and the acidifying agent sulfuric acid (H_2SO_4). Sodium sulfate's solubility increases around 3.5-fold in pure water from room temperature to 32.38 °C [153]. Herbaceous biomass like corn stover is known to contain a large amount of hemicellulose in the form of arabinoxylan, consisting of a xylan backbone with arabinose side chains. Lignin's low solubility in water is a property that allows it to act as an antisolvent in certain processes [154], making water effective for lignin precipitation, especially under acidic conditions. Xylan and lignin have limited solubility in water, but are more soluble in hot water [155, 156].

Figure 58B and 58C show the established trend in the literature [157, 158] that filtration time decreases with higher filtration temperatures, attributed to lower filtering resistance. Corn stover filtration time appears to follow a linear trend on this large scale, and hybrid poplar follows a more exponential trend, as it is closer to the final filtering time of a couple of minutes even at the lowest temperatures/room temperature. Lignin from the corn stover and the hybrid poplar both exhibited a transition from slow-filtering to quick-filtering (Fig. 58). Interestingly, the transition was at a significantly different temperatures for the corn stover (~ 70 °C) versus hybrid poplar (~ 40 °C). Additionally, the filtering times for the corn stover lignins for the “slow-filtering” lignins were substantially longer for the corn stover lignins (40-160 min) relative to the hybrid poplar lignins (4-14 min).

Findings from Öhman and Theliander et al. [158] emphasize the importance of maintaining low pH (< 3.75) and washing procedures in improving lignin yield, reducing plugging during filtration, and reducing final sodium content. Uneven washing and plugging problems can be a reason for the long filtering times for corn stover.

Other findings from Öhman and Theliander et al. [159] emphasize the importance of high temperature for effective lignin filtration.

Henneman et al. [160] investigate how the characteristics of the filter cake, particularly the particle size distribution and fine particle concentration, affect filtration rates. They conclude that maintaining high particle uniformity and optimizing the conditions before filtration can significantly improve performance. In our work hybrid poplar exhibits a narrower particle size distribution suggesting more uniform particles, which explains the faster filtration times. Conversely, wide particle size distributions like corn stover result in lower filter cake porosity and

higher filter cake resistance. Heating can also enhance particle uniformity, which aligns with our observation that higher temperatures lead to decreased filtration times.

We performed apparent color analysis of the isolated lignins using a commercial colorimeter, which measures light intensity in specific wavelength ranges and are useful for quick, cost-effective, non-invasive color comparison [62, 63, 147]. Color serves as a macroscopic indicator of microscopic differences in lignin [63, 67], with the observed color changes correlating to structural variations in lignin particles that reflect aggregation processes influencing their physical properties. Lignin's color is also influenced by process variables; harsher processing conditions, such as those used in Kraft pulping, result in darker lignin, while milder processes yield lighter lignin [66].

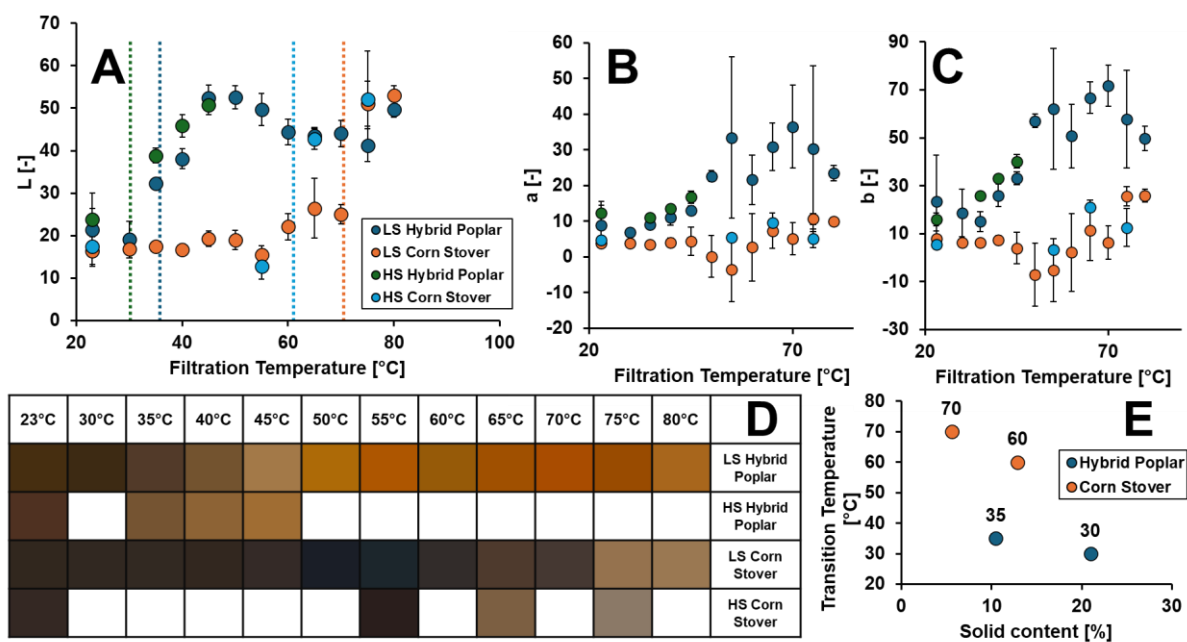


Figure 59. CIELAB color parameters for lignins recovered at different precipitation temperatures and solid contents for (A) lightness with transition temperatures indicated, (B) a parameter, (C) b parameter, (D) reconstituted colors, and (E) transition temperatures for different solid contents. LS – Low Solids HS – High Solids.

We measured lignin color in the CIELAB colorspace as a function of the incubation or filtration temperature. The a and b parameters are important to determine the hue, but our goal was to compare the recovered lignins by their lightness parameter, L . The lightness parameter exhibits a similar transition temperature where the lightness parameter jumps from low to high lightness values to that observed during filtration for both biomass types (**Fig. 59A**). For the other parameters, hybrid poplar lignins have higher a and b values (**Fig. 59B,C**), subsequently more saturated color than corn stover lignins (**Fig. 59D**). Additionally, we examined two solid content levels of black liquors, denoted as HS (high solids) and LS (low solids), finding that the transition temperature decreases as solid content increases (**Fig. 59E**). The transition temperatures were determined using interpolation, which for corn stover are 71 °C at 5.57% solids and 62 °C at 12.89% solids, while for hybrid poplar, it is 35 °C at 10.44% solids and 30 °C at 20.96% solids.

Here we propose a theoretical explanation for the observed shift in transition temperature between the different solid contents. Higher solid content means increased lignin concentration, increasing the likelihood of particle interactions and aggregation, reducing the energy needed to reach a point where lignin particles aggregate. Higher solid content also means increased ionic strength, that can reduce the repulsive forces between particles to promote aggregation. Higher solid content favors DLCA at lower temperatures, as the diffusions and collisions are easier with higher concentrations, so the system requires less kinetic energy (lower temperature) to facilitate aggregation. Conversely if we discuss lower solid contents, RLCA is favored as particles are farther apart and the aggregation is more influenced by the rate of reaction, so particles require higher temperatures to overcome the repulsive forces and aggregate.

Properties of Recovered Lignins

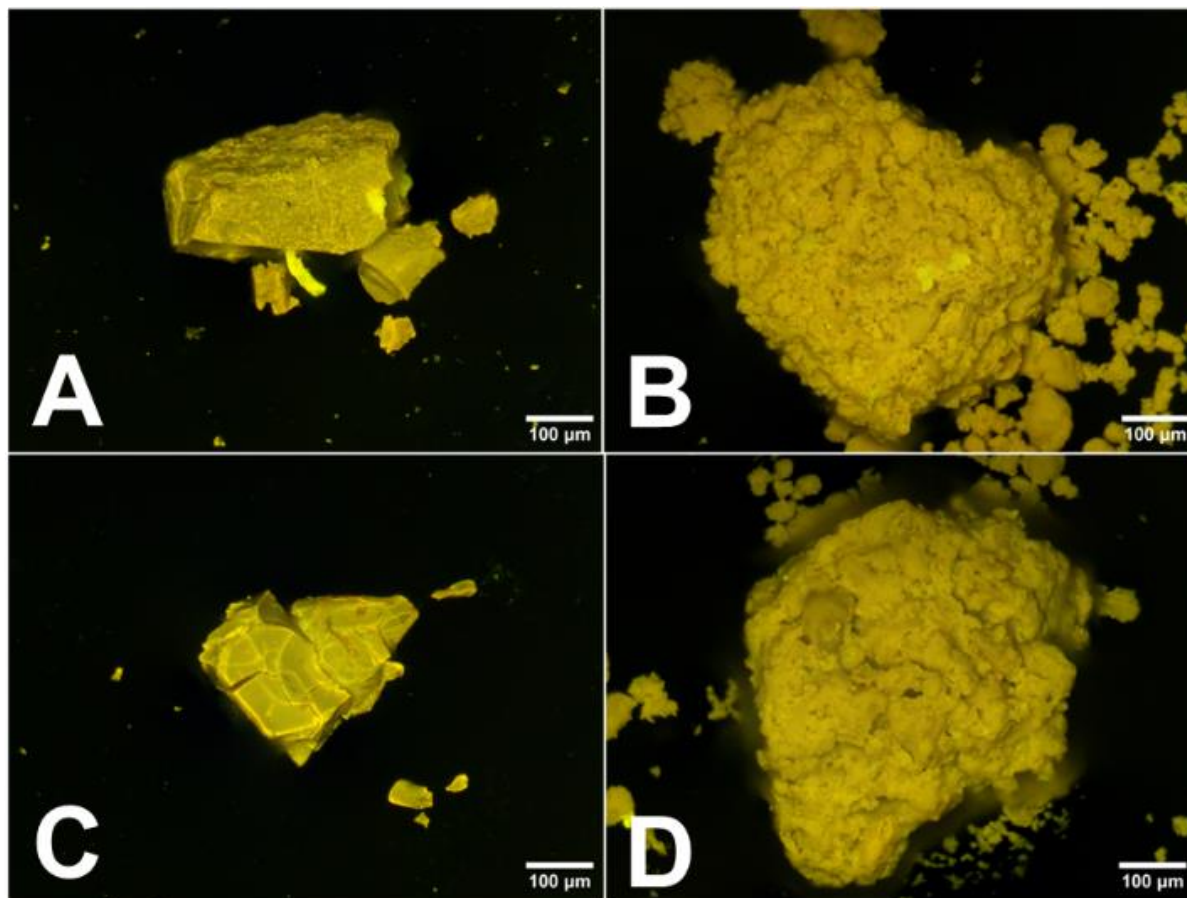


Figure 60. Epifluorescence microscopy images of recovered lignin powder of corn stover (A,B) and hybrid poplar (D,E), using 23°C (A,D) and high temperature (75°C for corn stover B, 45°C for hybrid poplar E) at 10x magnification, and particle size distribution (C,F).

In order to show the morphological differences between the recovered lignins, we used epifluorescence microscopy, with Figure 60A and Figure 60C showing the hard to filter dark lignin isolated at low temperature (23°C for both types of biomass) and Figure 60B and Figure 60D showing the quick filtering light colored lignin isolated at high temperatures (75°C for corn stover, 45°C for hybrid poplar). Figure 60A and Figure 60B shows the corn stover samples while Figure 60C and Figure 60D shows the hybrid poplar samples. The same morphological differences

between different colored lignins are observed for both types of biomass. The dark lignins exhibit individual particles with smooth surfaces, which expands on previously shown observations of brittle particles with a slight sheen. The light lignins exhibit textured, aggregated particles, which expands on previously shown observations of powdery matte particles. A possible explanation for these differences can be proposed using universal aggregation mechanisms from colloid science. At low temperatures when the RLCA dominates, the resulting particles are more compact and can result in smooth surfaces at a larger scale, however at high temperature with the DLCA dominating the particles become looser and more branched that can result in a more aggregated appearance at larger scale. It should be noted that the 10x magnification used for these images is not capable of directly imaging cluster aggregation, and is not sufficient to prove this theory, but it can provide more insights on the morphological particle differences.

Prior work showed differences in particle size distributions of kraft lignins precipitated at pH 9.7 at different temperatures with 80 °C showing a broad distribution of particle diameters centered at 25 μm while at 70 °C a narrow distribution centered on 18 μm was observed [159]. Three lignins from different sources generated from acidification of kraft black liquors had different particle size distributions [161].

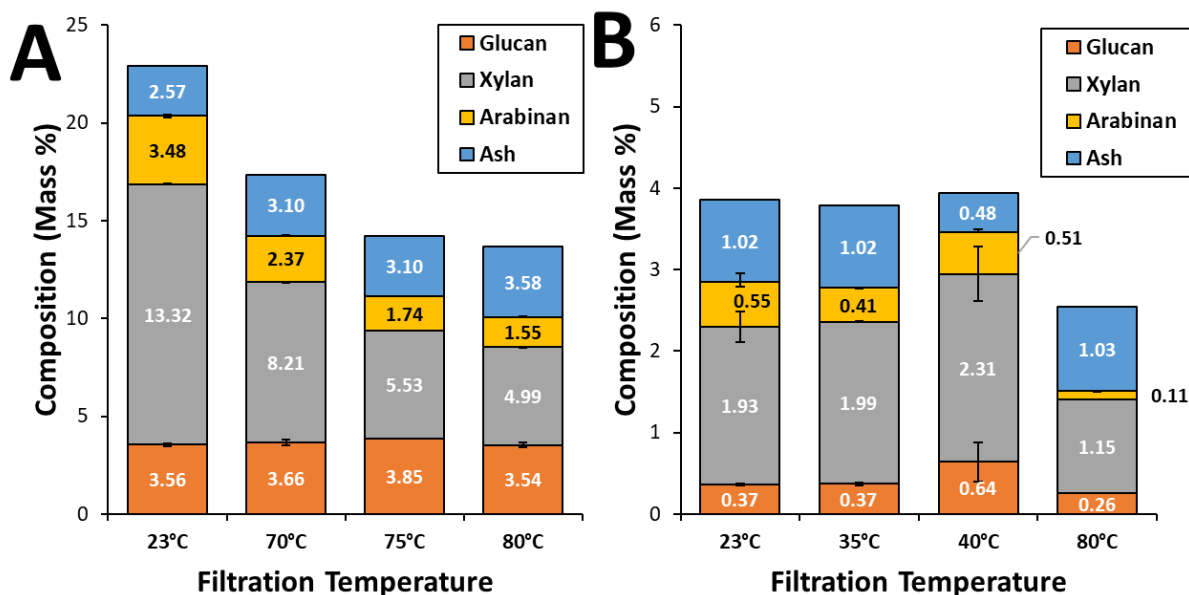


Figure 61. Differences in impurity composition as a function of filtration temperature for (A) corn stover and (B) hybrid poplar.

Lignin filtered at higher temperatures shows less impurities in the recovered lignin for both biomass types (**Fig. 61**). There are significantly more impurities in corn stover lignin (xylan, ash) because of inherent differences between corn stover and hybrid poplar biomass. Glucan and ash content is relatively stable across different filtering temperatures, the biggest change is in xylan and ash content, especially for corn stover. For corn stover, with increasing temperature the impurity content decreased 2.6-fold from 22.93% to 13.66% comparing the room temperature filtration to the highest temperature observed, 80°C (**Fig. 61A**). For hybrid poplar the decrease is smaller, from 3.87% to 2.55%. As explained previously related to the decreasing mass yields, the solubility of impurities in lignin increases with increasing temperature, especially close to room temperature [153, 155], therefore decreasing the impurity content of the isolated lignin.

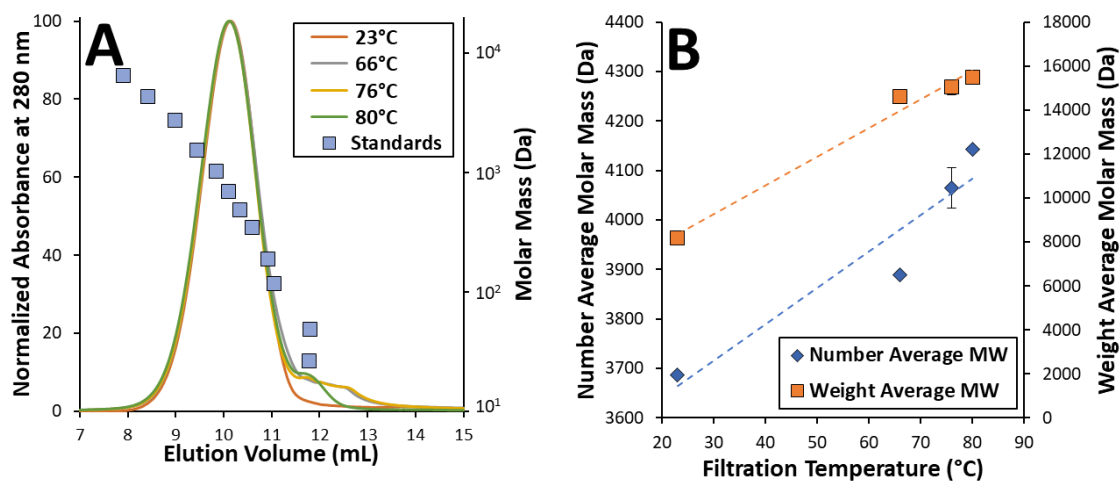


Figure 62. Molar mass distributions for recovered corn stover lignins.

Molar mass characterization of recovered corn stover lignins demonstrated the trend of increasing number and weight average MW with increasing recovery temperature (Fig 62.). Sameni et al. [162] precipitated non-wood soda lignins (Protobind 1000) in DCM and found that molar mass impacted particle size.

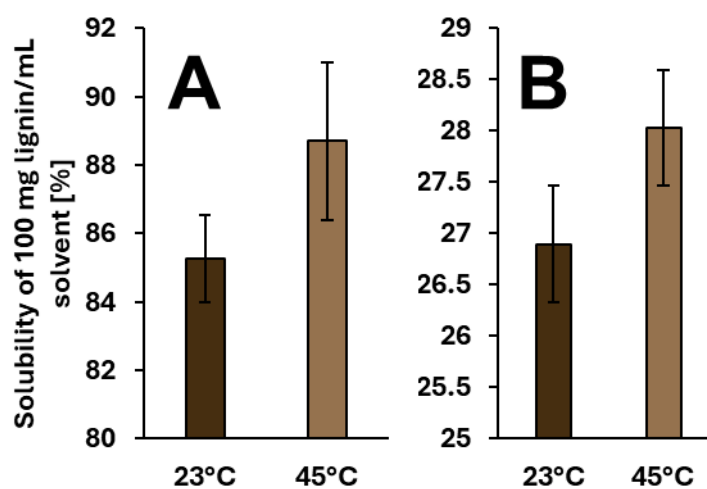


Figure 63. Solubility of recovered hybrid poplar lignins in dimethyl sulfoxide (A) and ethyl acetate (B).

We investigated the difference between the solubility of light and dark hybrid poplar lignin. In **Fig. 63A** the solubility in dimethyl sulfoxide, and in **Fig. 63B** in ethyl acetate is shown. The average solubility of lignin is notably different in these two types of solvents, as dimethyl sulfoxide is a well-known, effective lignin solvent with 85-89% mass soluble observed here, while ethyl acetate is ineffective for lignin dissolution, with 27-28% soluble mass measured. Lignins isolated at low temperature (23°C) are dark samples, and they are light if isolated at high temperatures (45°C). For both solvents the light samples are more soluble. It has been shown before that higher phenolic hydroxyl group content and lower molecular weight lignins are more soluble [27, 156], although in this work higher molecular weight samples proved to be slightly more soluble. These consistent variations can be attributed to differences in impurity content, surface area, functional group abundance, or molecular weight distribution. This is consistent with previous observations, as the lighter lignins have lower impurity content and a more textured surface, both promoting dissolution.

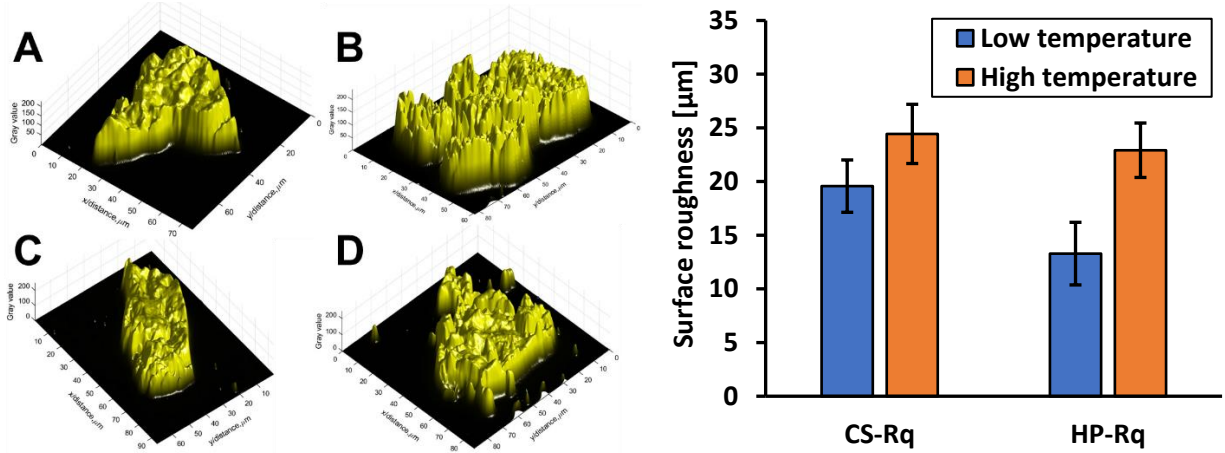


Figure 64. Surface plots of corn stover (A,B) and hybrid poplar (C,D) lignin particles at low (23°C for both A and C) and high (75°C for corn stover B, 45°C for hybrid poplar D) temperatures obtained using confocal laser scanning microscopy (CLSM) at 40x magnification. Roughness parameters (E) for average roughness (Ra) and root-mean-square roughness (Rq) measured on CLSM images for the particles shown in A-D. Standard deviations are shown, n=6. The t-test p values for all the datasets were <0.05.

Surface plots from confocal laser scanning microscopy show the surface morphology of the particles investigated (Fig. 64). Fig. 64A and Fig. 64B show corn stover samples, Fig. 64C and Fig. 64D hybrid poplar samples. The left side of both biomass type (Fig. 64A,C) show lignins isolated at low temperature (23°C), and the right side (Fig. 64B,D) show the high temperature lignins (75°C for corn stover B, 45°C for hybrid poplar D). The surface plots show similar differences as the epifluorescent microscopy images (Fig. 60), smoother surface for the dark lignin and more ridges for the light lignin. This difference is quantified using the Ra and Rq roughness values, with higher values signifying rougher surfaces. These values use a mean height around which the profile deviations are measured. Ra is the arithmetic average and Rq is the root mean square average. Both biomass types and both roughness parameters exhibit significant differences between dark and light samples, with the light samples being more rough. Again, consistent

differences are observed between different colored samples, and the trend observed in **Fig. 64** reinforced using a higher magnification.

Based on the extensive differences presented above, we suggest a theoretical framework as an explanation for them, shown on **Fig. 65** as a visual representation. As mentioned before, lignin aggregation in black liquor is a destabilized colloidal system. Universal cluster aggregation mechanisms in colloid science have been investigated and implemented for a wide range of materials like silica [163, 164], polystyrene, gold [139], proteins [165], and lignin. In DLCA (**Fig. 65** bottom panel), particle aggregation is limited by diffusion, resulting in branched structures, while in RLCA (**Fig. 65** top panel), aggregation is limited by the reaction rate, forming denser clusters. Temperature influences which mechanism dominates: lower temperatures typically favor RLCA, producing more compact particles that clog filters and increase filtering resistance (**Fig. 65** middle of top panel). Higher temperatures promote DLCA, leading to more open, rougher aggregates that allow easier filtration (**Fig. 65** middle of bottom panel). This study observed distinct “transition” temperatures, correlating with shifts in lignin color, particle size, and surface roughness for corn stover and hybrid poplar-derived black liquors. Higher solid content lowered the transition temperature, with corn stover consistently exhibiting a higher transition threshold than hybrid poplar. This transition indicates a shift between aggregation mechanisms, producing either darker, smoother, brittle, hard to filter particles at lower temperatures or lighter, rougher, easy to filter particles at higher temperatures.

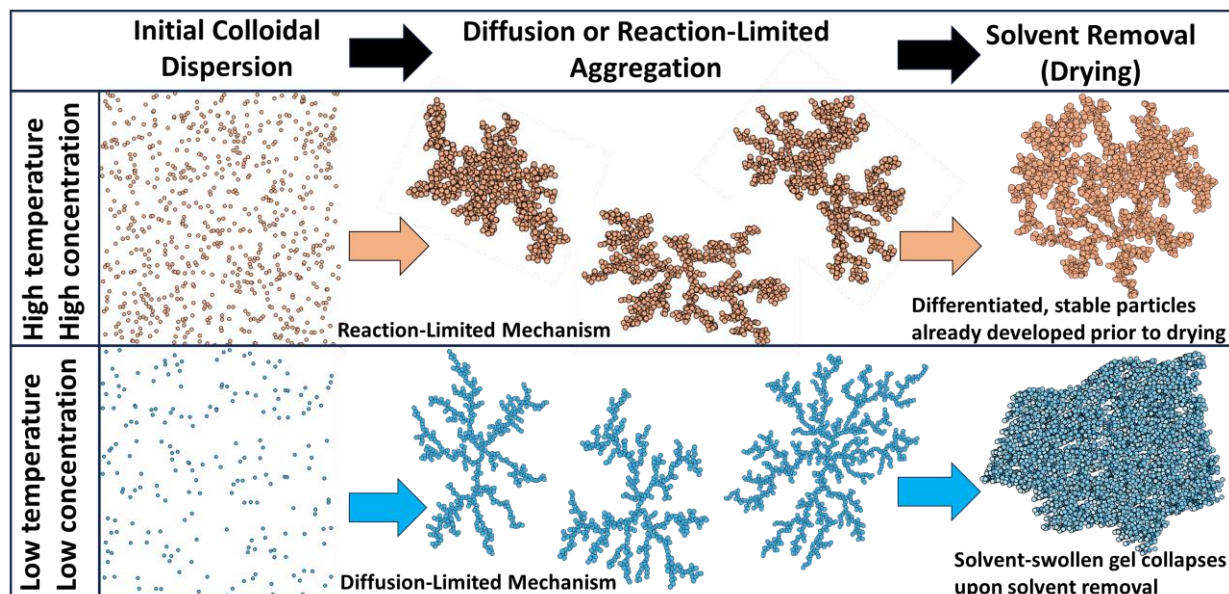


Figure 65. Schematic showing the difference between the two aggregation regimes.

Conclusions

We investigated lignin isolation from in-house alkaline pretreatment identifying transition temperatures across four types of liquor corresponding to two biomass types and two solid contents. We used epifluorescence and confocal fluorescence microscopy to quantify particle properties such as size distribution during precipitation and surface roughness, and we measured apparent lignin color and filtering time. Precipitation below the transition temperature produced slow-filtering, dark, brittle lignins with smoother particle surfaces, while precipitation above this threshold yielded fast-filtering, lighter, powdery lignins with rougher particle surfaces. The identified transition temperatures correspond to marked shifts in physical properties. For corn stover, the transition temperature is 71 °C at 5.6% solids and 62 °C at 12.9% solids, while for hybrid poplar, it is 35 °C at 10.4% solids and 30 °C at 21.0% solids. Increased solid content in

both biomass types led to a lower transition temperature; corn stover consistently showed greater transition temperatures than hybrid poplar.

We propose that the transition temperature signifies a shift between two universal regimes of particle aggregation: Reaction-Limited Cluster Aggregation (RLCA) and Diffusion-Limited Cluster Aggregation (DLCA). One theory is that DLCA dominates at low temperatures, resulting in slowly filtering particles because of a solvent-swollen gel structure that collapses upon drying. On the other hand, RLCA dominates at high temperatures, resulting in distinct particles with a rougher surface, reflecting more light, contributing to lighter colored particles. Another, more widely supported explanation in the literature suggests that slow RLCA behavior at low temperatures produces more compact, smooth particles that can clog the filter and increase the resistance hindering the filtration. In contrast, higher temperatures favor DLCA, leading to rougher, lighter particles that filter more easily.

This theoretical framework can be validated by conducting transmission electron microscope (TEM), light scattering, or turbidity experiments to measure fractal dimensions of aggregates to validate the RLCA and DLCA behavior. Colloid chemistry models can be used in computational simulations, either as a standalone approach or to complement experimental studies.

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Supplementary Materials

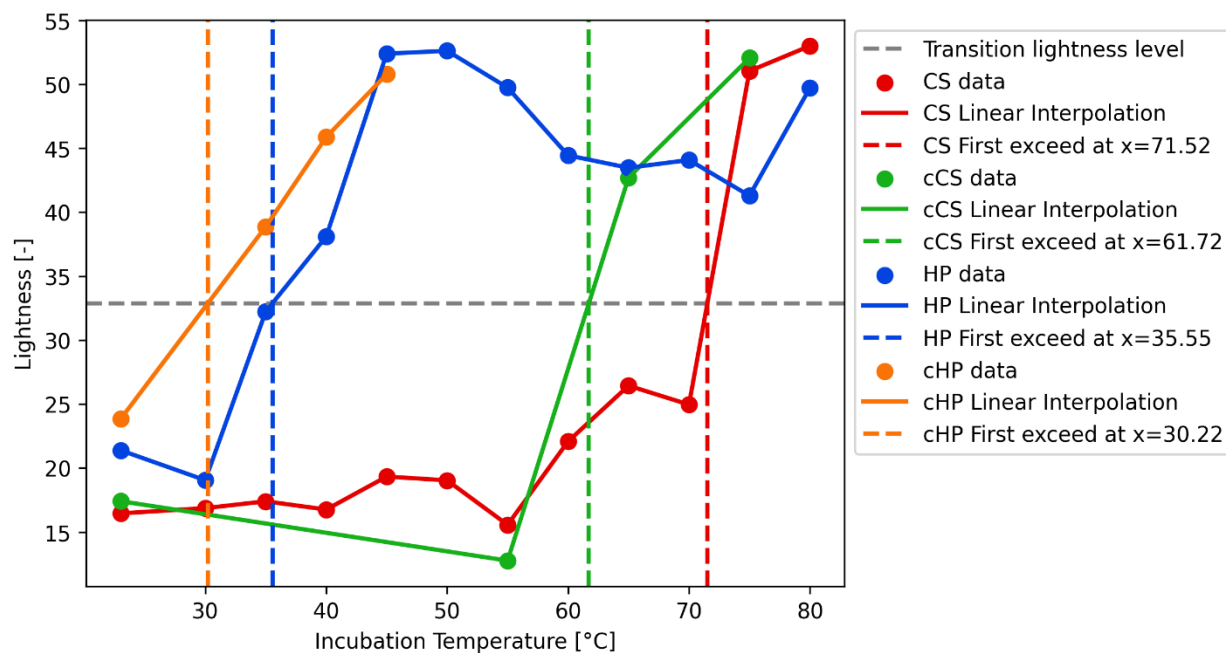


Figure 66. Linear interpolation to determine transition temperature.

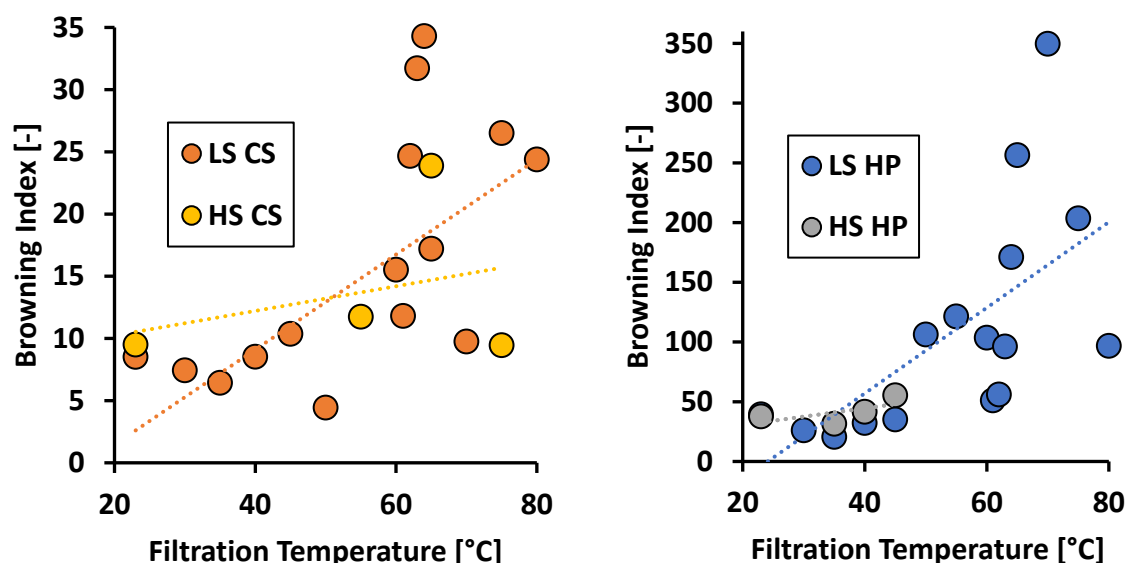


Figure 67. Browning index of lignins recovered from both types of biomass.

References

123. Tindall, G., et al., *Ultraclean hybrid poplar lignins via liquid–liquid fractionation using ethanol–water solutions*. MRS Communications, 2021. **11**(5): p. 692-698.
124. Tindall, G.W., et al., *Liquefying Lignins: Determining Phase-Transition Temperatures in the Presence of Aqueous Organic Solvents*. Industrial & Engineering Chemistry Research, 2021. **60**(47): p. 17278-17282.
125. Lynn, B., et al., *Fractionation and purification of a high-impurity, alkaline-pretreated, corn stover lignin with simple renewable solvents*. Separation and Purification Technology, 2024. **342**: p. 126924.
126. Kanhere, S.V., et al., *Carbon fibers derived from liquefied and fractionated poplar lignins: The effect of molecular weight*. iScience, 2022. **25**(12): p. 105449.
127. Kanhere, S.V., et al., *Carbon fibers derived from environmentally benign, ethanol-fractionated corn-stover lignin*. RSC Sustainability, 2024. **2**(8): p. 2357-2366.
128. Wu, C., et al., *The Role of Lignin Molecular Weight on Activated Carbon Pore Structure*. Molecules, 2024. **29**(16): p. 3879.

129. Henry, C., et al., *Fractionated and purified hybrid poplar lignins as a polyol replacement in rigid polyurethane/polyisocyanurate foams*. Journal of Applied Polymer Science, 2023. **140**(45): p. e54648.
130. Kane, S., et al., *Role of sodium sulfate in electrical conductivity and structure of lignin-derived carbons*. Journal of Analytical and Applied Pyrolysis, 2024. **181**: p. 106600.
131. Bär, J., et al., *Deconstruction of hybrid poplar to monomeric sugars and aromatics using ethanol organosolv fractionation*. Biomass Conversion and Biorefinery, 2018. **8**(4): p. 813-824.
132. Bali, G., et al., *The effect of alkaline pretreatment methods on cellulose structure and accessibility*. ChemSusChem, 2015. **8**(2): p. 275-9.
133. Toledano, A., et al., *Lignin separation and fractionation by ultrafiltration*. Separation and Purification Technology, 2010. **71**(1): p. 38-43.
134. Sameni, J., et al., *Thermal Characteristics of Lignin Residue from Industrial Processes*. Bioresources, 2014. **9**: p. 725-737.
135. Singleton, V.L. and J.A. Rossi, *Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents*. American Journal of Enology and Viticulture, 1965.
136. Dülger, D.N., et al., *Scale-Up of Two-Stage Cu-Catalyzed Alkaline-Oxidative Pretreatment of Hybrid Poplar*. Biomass Conversion and Biorefinery, 2023.
137. Henry, C., et al., *Studying the Suitability of Nineteen Lignins as Partial Polyol Replacement in Rigid Polyurethane/Polyisocyanurate Foam*. Molecules, 2022. **27**(8): p. 2535.
138. Fitzgerald, C.L. and M.C. Thies, *Continuous recovery of high-purity Kraft lignin from black liquor via simultaneous, liquid-phase acidification and purification*. Industrial Crops and Products, 2022. **184**: p. 115084.
139. Lin, M.Y., et al., *Universality in colloid aggregation*. Nature, 1989. **339**(6223): p. 360-362.
140. Lin, M.Y., et al., *Universal reaction-limited aggregation*. Physical review. A, 1990. **41**: p. 2005-2020.
141. Lin, M.Y., et al., *Universal diffusion-limited colloid aggregation*. Journal of Physics: Condensed Matter, 1990. **2**(13): p. 3093.
142. Meakin, P., *Fractal aggregates*. Advances in Colloid and Interface Science, 1987. **28**: p. 249-331.

143. Norgren, M., et al., *Aggregation of kraft lignin derivatives under conditions relevant to the process, part I: phase behaviour*. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 2001. **194**(1): p. 85-96.
144. Crowe, J.D., et al., *Xylan Is Critical for Proper Bundling and Alignment of Cellulose Microfibrils in Plant Secondary Cell Walls*. Frontiers in Plant Science, 2021. **12**.
145. Enikő Bécsy-Jakab, V., et al., *Extraction, recovery, and characterization of lignin from industrial corn stover lignin cake*. Bioresource Technology, 2024: p. 130610.
146. Erguvan, Ö., et al., *ImageJ SurfCut: a user-friendly pipeline for high-throughput extraction of cell contours from 3D image stacks*. BMC Biology, 2019. **17**(1): p. 38.
147. Andeme Ela, R.C., et al., *Understanding the Effect of Precipitation Process Variables on Hardwood Lignin Characteristics and Recovery from Black Liquor*. ACS Sustainable Chemistry & Engineering, 2020. **8**(37): p. 13997-14005.
148. Helander, M., et al., *Fractionation of Technical Lignin: Molecular Mass and pH Effects*. BioResources, 2013. **8**.
149. Cheng, J., et al., *Low-chromophore lignin isolation from natural biomass with polyol-based deep eutectic solvents*. Green Chemistry, 2024. **26**(14): p. 8298-8314.
150. Uloth, V. and J. Wearing, *Kraft lignin recovery: acid precipitation versus ultrafiltration. I: Laboratory test results*. Pulp & Paper Canada, 1989. **90**(9): p. 67-71.
151. Kitin, P., et al., *Direct fluorescence imaging of lignocellulosic and suberized cell walls in roots and stems*. AoB PLANTS, 2020. **12**(4).
152. Radotić, K., et al., *Component analysis of the fluorescence spectra of a lignin model compound*. Journal of Photochemistry and Photobiology B: Biology, 2006. **83**(1): p. 1-10.
153. Bharmoria, P., et al., *Temperature-Dependent Solubility Transition of Na₂SO₄ in Water and the Effect of NaCl Therein: Solution Structures and Salt Water Dynamics*. The Journal of Physical Chemistry B, 2014. **118**(44): p. 12734-12742.
154. Ortega-Sanhueza, I., et al., *Preparation and Characterization of Lignin Nanoparticles from Different Plant Sources*. Polymers, 2024. **16**(11): p. 1610.
155. Fahmi, A., *Natural Xylan Production From Corncobs (Zea Mays L.) With Extraction Method*. KnE Social Sciences, 2019. **3**(18).
156. Evstigneev, E.I., *Factors affecting lignin solubility*. Russian Journal of Applied Chemistry, 2011. **84**(6): p. 1040-1045.
157. Hough, W.J., *Process of treating spent alkaline pulping liquors*. 1910: United States.

158. Öhman, F., H. Wallmo, and H. Theliander, *A novel method for washing lignin precipitated from kraft black liquor – Laboratory trials*. Nordic Pulp & Paper Research Journal, 2007. **22**(1): p. 9-16.
159. Öhman, F.T., H., *Filtration properties of lignin precipitated from black liquor*. TAPPI J, 2007. **6**(7): p. 3-9.
160. Hennemann, M., M. Gastl, and T. Becker, *Influence of particle size uniformity on the filter cake resistance of physically and chemically modified fine particles*. Separation and Purification Technology, 2021. **272**: p. 118966.
161. Richardson, B., *Kraft lignin as a fuel for the rotary lime kiln*. 1993.
162. Sameni, J., et al., *Preparation and characterization of biobased microspheres from lignin sources*. Industrial Crops and Products, 2018. **117**: p. 58-65.
163. Tang, P., D.E. Colflesh, and B. Chu, *Temperature effect on fractal structure of silica aggregates*. Journal of Colloid and Interface Science, 1988. **126**(1): p. 304-313.
164. Seljelid, K.K., et al., *Growth kinetics and structure of a colloidal silica-based network: in situ RheoSAXS investigations*. The European Physical Journal Special Topics, 2024.
165. Vétier, N., et al., *Effect of Temperature and Aggregation Rate on the Fractal Dimension of Renneted Casein Aggregates*. Journal of Dairy Science, 2003. **86**(8): p. 2504-2507.

CHAPTER FIVE

CONCLUSION

To effectively utilize lignin in high-value applications and improve the economic viability of biorefineries, we investigated lignin extraction, recovery, and solubility across diverse feedstocks, including corn stover, hybrid poplar, and an industrial biorefinery residue. Since consistent lignin properties are essential for its industrial application, we examined the variability in physical lignin properties post recovery to address inconsistencies.

In Chapter Two, lignin was extracted and recovered from industrial lignin cake, the biorefinery residue, derived from the dilute acid pretreatment and enzymatic hydrolysis of corn stover. Recovery yields of up to 84% were achieved using alkali extraction followed by lignin precipitation by acidification. Besides differences in recovery yields, lignins recovered using different extraction methods and conditions exhibited slight differences in lignin properties and solubility. Fractional solubility of lignins in different organic solvents could be successfully predicted using a novel approach by assuming lignins exhibited a distribution of solubility parameters due to their heterogeneity.

In Chapter Three, hybrid poplar and corn stover was subjected to alkaline pretreatment for lignin extraction. The reaction was first scaled up to 1.5 L-scale, then three-fold to 20-L scale as a result of process optimization and troubleshooting. A total of approximately 20 kg of lignin was generated during the project, with over 14 kg produced as lignin powder. Additional lignin fractions were recovered as concentrated liquor and lignin cake, distributed across 58 production batches. The produced lignin was utilized by collaborators for research in lignin fractionation and lignin-based polyurethane foams, carbon fibers and activated carbon [123-129]. During lignin

recovery, physical inconsistencies were observed that we investigated by conducting a variable screening determining that filtering temperature is a significant variable impacting the color of recovered lignins.

In Chapter Four, we investigated lignin recovery from in-house alkaline pretreatment from Chapter Three identifying transition temperatures across four types of liquor corresponding to corn stover and hybrid poplar at two solid contents. Precipitation below the transition temperature produced slow-filtering, dark, brittle lignins with smoother particle surfaces, while precipitation above this threshold yielded fast-filtering, lighter, powdery lignins with rougher particle surfaces. The identified transition temperatures correspond to marked shifts in physical properties. Increased solid content in both biomass types led to a lower transition temperature, and corn stover consistently showed greater transition temperatures than hybrid poplar. We propose that the transition temperature signifies a shift between two universal regimes of particle aggregation mechanisms: Reaction-Limited Cluster Aggregation (RLCA) and Diffusion-Limited Cluster Aggregation (DLCA). This theoretical framework can be validated in the future by applying light scattering techniques to measure fractal dimensions of aggregates to validate the RLCA and DLCA behavior.

In conclusion, this dissertation contributes to lignin valorization by applying different methods of extraction using a range of feedstocks and scales. The solubility and consistent physical properties of recovered lignins were investigated in detail by applying a novel approach for both. By modeling lignin's solubility parameters as distributions rather than fixed values, this method accounted for the material's inherent polydispersity, offering more accurate predictions. A 20-L scale alkaline pretreatment method was implemented and optimized to supply high-purity lignin

to collaborators working on a wide range of value-added lignin applications. A transition temperature during lignin recovery linked to differences in physical properties like color and particle morphology was determined, and a novel theoretical framework for these differences was proposed.

While this dissertation provides valuable insights into lignin extraction, recovery, and solubility, there are still significant opportunities for further research. For Chapter Two, a more robust dataset of fractional solubility measurements across a wider range of solvents could improve the estimation of lignin solubility parameters. Expanding this dataset would help refine the current model and offer better predictions for lignin solubility across different industrial and biorefinery solvents. In Chapter Three, a key area for future work is improving the consistency of lignin products, particularly with respect to physical properties. Although lignin recovery was scaled up to a 20-L process, challenges with variability in product characteristics suggest that refining process parameters and ensuring greater reproducibility in lignin recovery could be beneficial. Lastly, for Chapter Four, further investigation using light scattering or similar techniques would allow for the validation of the RLCA and DLCA models. These advanced methods could help confirm the theoretical explanations for the observed color and particle morphology differences and solidify the connection between processing history, aggregation behavior, and final lignin properties.

CUMULATIVE REFERENCES CITED

1. Langholtz, M.H., *2023 Billion-Ton Report*. 2024: United States.
2. Zakzeski, J., et al., *The Catalytic Valorization of Lignin for the Production of Renewable Chemicals*. Chemical Reviews, 2010. **110**(6): p. 3552-3599.
3. Ragauskas, A.J., et al., *Lignin Valorization: Improving Lignin Processing in the Biorefinery*. Science, 2014. **344**(6185): p. 1246843.
4. Jungmeier, G., et al., *Biofuel-driven Biorefineries A selection of the most promising biorefinery concepts to produce large volumes of road transportation biofuels by 2025*. Report of IEA Bioenergy Task, 2013. **42**.
5. Calvo-Flores, F.G. and F.J. Martin-Martinez, *Biorefineries: Achievements and challenges for a bio-based economy*. Front Chem, 2022. **10**: p. 973417.
6. Hsu, T.A., M.R. Ladisch, and G.T. Tsao, *Alcohol from cellulose*. Chem. Technol.; (United States), 1980: p. Medium: X; Size: Pages: 315-319 2009-12-17.
7. Bajpai, P., *Pretreatment of Lignocellulosic Biomass*, in *Pretreatment of Lignocellulosic Biomass for Biofuel Production*, P. Bajpai, Editor. 2016, Springer Singapore: Singapore. p. 17-70.
8. Agbor, V.B., et al., *Biomass pretreatment: Fundamentals toward application*. Biotechnology Advances, 2011. **29**(6): p. 675-685.
9. Sun, Z., et al., *Bright Side of Lignin Depolymerization: Toward New Platform Chemicals*. Chemical Reviews, 2018. **118**(2): p. 614-678.
10. Davis, R., et al., *Process design and economics for the conversion of lignocellulosic biomass to hydrocarbons: dilute-acid and enzymatic deconstruction of biomass to sugars and biological conversion of sugars to hydrocarbons*. 2013, National Renewable Energy Lab.(NREL), Golden, CO (United States).
11. Laurichesse, S. and L. Avérous, *Chemical modification of lignins: Towards biobased polymers*. Progress in Polymer Science, 2014. **39**(7): p. 1266-1290.
12. Argyropoulos, D.D.S., et al., *Kraft Lignin: A Valuable, Sustainable Resource, Opportunities and Challenges*. ChemSusChem, 2023. **16**(23): p. e202300492.
13. Sannigrahi, P., A.J. Ragauskas, and G.A. Tuskan, *Poplar as a feedstock for biofuels: A review of compositional characteristics*. Biofuels, Bioproducts and Biorefining, 2010. **4**(2): p. 209-226.
14. Zabed, H.M., et al., *Towards the sustainable conversion of corn stover into bioenergy and bioproducts through biochemical route: Technical, economic and strategic perspectives*. Journal of Cleaner Production, 2023. **400**: p. 136699.

15. Wyman, C.E., et al., *Coordinated development of leading biomass pretreatment technologies*. Bioresource Technology, 2005. **96**(18): p. 1959-1966.
16. Fache, M., B. Boutevin, and S. Caillol, *Vanillin Production from Lignin and Its Use as a Renewable Chemical*. ACS Sustainable Chemistry & Engineering, 2016. **4**(1): p. 35-46.
17. Suhas, P.J.M. Carrott, and M.M.L. Ribeiro Carrott, *Lignin – from natural adsorbent to activated carbon: A review*. Bioresource Technology, 2007. **98**(12): p. 2301-2312.
18. Brienza, F., et al., *A guide to lignin valorization in biorefineries: traditional, recent, and forthcoming approaches to convert raw lignocellulose into valuable materials and chemicals*. RSC Sustainability, 2023.
19. Saulnier, B.K., et al., *Impact of dilute acid pretreatment conditions on p-coumarate removal in diverse maize lines*. Bioresource Technology, 2020. **314**: p. 123750.
20. König-Mattern, L., et al., *Machine learning-supported solvent design for lignin-first biorefineries and lignin upgrading*. Chemical Engineering Journal, 2024. **495**: p. 153524.
21. Abu-Omar, M.M., et al., *Guidelines for performing lignin-first biorefining*. Energy & Environmental Science, 2021. **14**(1): p. 262-292.
22. Arapova, O.V., et al., *Lignin as a Renewable Resource of Hydrocarbon Products and Energy Carriers (A Review)*. Petroleum Chemistry, 2020. **60**(3): p. 227-243.
23. Chen, F., et al., *A polymer of caffeyl alcohol in plant seeds*. Proceedings of the National Academy of Sciences, 2012. **109**(5): p. 1772-1777.
24. Schuerch, C., *The Solvent Properties of Liquids and Their Relation to the Solubility, Swelling, Isolation and Fractionation of Lignin*. Journal of the American Chemical Society, 1952. **74**(20): p. 5061-5067.
25. Venkatram, S., et al., *Critical Assessment of the Hildebrand and Hansen Solubility Parameters for Polymers*. Journal of Chemical Information and Modeling, 2019. **59**(10): p. 4188-4194.
26. Brown, W., *Solution properties of lignin. Thermodynamic properties and molecular weight determinations*. Journal of Applied Polymer Science, 1967. **11**(11): p. 2381-2396.
27. Sameni, J., S. Krigstin, and M. Sain, *Solubility of Lignin and Acetylated Lignin in Organic Solvents*. Bioresources, 2017. **12**: p. 1548-1565.
28. Le, H.Q., et al., *Solubility of Organosolv Lignin in gamma-Valerolactone/Water Binary Mixtures*. ChemSusChem, 2016. **9**(20): p. 2939-2947.

29. Vermaas, J.V., et al., *Automated Transformation of Lignin Topologies into Atomic Structures with LigninBuilder*. ACS Sustainable Chemistry & Engineering, 2019. **7**(3): p. 3443-3453.
30. Vermaas, J.V., M.F. Crowley, and G.T. Beckham, *Molecular Lignin Solubility and Structure in Organic Solvents*. ACS Sustainable Chemistry & Engineering, 2020. **8**(48): p. 17839-17850.
31. Dellon, L.D., et al., *Computational Generation of Lignin Libraries from Diverse Biomass Sources*. Energy & Fuels, 2017. **31**(8): p. 8263-8274.
32. Alonso, D.M., J.Q. Bond, and J.A. Dumesic, *Catalytic conversion of biomass to biofuels*. Green Chemistry, 2010. **12**(9): p. 1493-1513.
33. Marangon Jardim, J., et al., *A Quantitative Comparison of the Precipitation Behavior of Lignin from Sweetgum and Pine Kraft Black Liquors*. Bioresources, 2020. **15**: p. 5464-5480.
34. Gierer, J., *Chemical aspects of kraft pulping*. Wood Science and Technology, 1980. **14**(4): p. 241-266.
35. Chakar, F.S. and A.J. Ragauskas, *Review of current and future softwood kraft lignin process chemistry*. Industrial Crops and Products, 2004. **20**(2): p. 131-141.
36. Bajwa, D.S., et al., *A concise review of current lignin production, applications, products and their environmental impact*. Industrial Crops and Products, 2019. **139**: p. 111526.
37. Pan, X., et al., *Bioconversion of hybrid poplar to ethanol and co-products using an organosolv fractionation process: optimization of process yields*. Biotechnol Bioeng, 2006. **94**(5): p. 851-61.
38. Pye, E.K. and J.H. Lora, *The ALCELL process--a proven alternative to kraft pulping*. Tappi Journal, 1991. **74**: p. 113-118.
39. Lee, Y.Y., P. Iyer, and R.W. Torget, *Dilute-Acid Hydrolysis of Lignocellulosic Biomass*, in *Recent Progress in Bioconversion of Lignocellulosics*, G.T. Tsao, et al., Editors. 1999, Springer Berlin Heidelberg: Berlin, Heidelberg. p. 93-115.
40. Yan, L., et al., *A comprehensive mechanistic kinetic model for dilute acid hydrolysis of switchgrass cellulose to glucose, 5-HMF and levulinic acid*. RSC Advances, 2014. **4**(45): p. 23492-23504.
41. Mosier, N., et al., *Features of promising technologies for pretreatment of lignocellulosic biomass*. Bioresource Technology, 2005. **96**(6): p. 673-686.

42. 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.
43. Tuck, C.O., et al., *Valorization of Biomass: Deriving More Value from Waste*. Science, 2012. **337**(6095): p. 695-699.
44. Hubbe, M., et al., *Lignin recovery from spent alkaline pulping liquors using acidification, membrane separation, and related processing steps: A review*. BioResources, 2019. **14**: p. 2300-2351.
45. Tomani, P. *The Lignoboost Process*.
46. Kouisni, L., et al., *LignoForce System for the Recovery of Lignin from Black Liquor: Feedstock Options, Odor Profile, and Product Characterization*. ACS Sustainable Chemistry & Engineering, 2016. **4**(10): p. 5152-5159.
47. Ohman, F. and H. Theliander, *Filtration properties of lignin precipitated from black liquor*. TAPPI Journal, 2001. **6**: p. 3-9.
48. Sewring, T., et al., *Acid Precipitation of Kraft Lignin from Aqueous Solutions: The Influence of pH, Temperature, and Xylan*. Journal of Wood Chemistry and Technology, 2019. **39**(1): p. 1-13.
49. Sewring, T. and H. Theliander, *Acid precipitation of kraft lignin from aqueous solutions: the influence of anionic specificity and concentration level of the salt*. Holzforschung, 2019. **73**(10): p. 937-945.
50. Norgren, M., H. Edlund, and L. Wågberg, *Aggregation of Lignin Derivatives under Alkaline Conditions. Kinetics and Aggregate Structure*. Langmuir, 2002. **18**(7): p. 2859-2865.
51. Zhu, W. and H. Theliander, *Precipitation of Lignin from Softwood Black Liquor: An Investigation of the Equilibrium and Molecular Properties of Lignin*. BioResources, 2015. **10**.
52. Matsakas, L., et al., *Preparation of low carbon impact lignin nanoparticles with controllable size by using different strategies for particles recovery*. Industrial Crops and Products, 2020. **147**: p. 112243.
53. Abächerli, A. and F. Doppenberg, *Method for preparing alkaline solutions containing aromatic polymers*. 2001, Google Patents.
54. Jardim, J.M., et al., *A quantitative comparison of the precipitation behavior of lignin from sweetgum and pine kraft black liquors*. BioResources, 2020. **15**(3): p. 5464-5480.

55. 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**: p. 39-48.
56. Sundin, J., *Precipitation of Kraft Lignin under Alkaline Conditions*, in *Department of Pulp and Paper Chemistry and Technology*. 2000, Royal Institute of Technology: Stockholm.
57. Öhman, F., H. Wallmo, and H. Theliander, *An improved method for washing lignin precipitated from kraft black liquor-the key to a new biofuel*. Filtration, 2007. **7**(4): p. 309-315.
58. Deng, Y., et al., *Investigation of Aggregation and Assembly of Alkali Lignin Using Iodine as a Probe*. Biomacromolecules, 2011. **12**(4): p. 1116-1125.
59. Colucci, G., et al., *Development of colloidal lignin particles through particle design strategies and screening of their Pickering stabilizing potential*. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 2023. **666**: p. 131287.
60. Weitz, D.A., M.Y. Lin, and H.M. Lindsay, *Universality laws in coagulation*. Chemometrics and Intelligent Laboratory Systems, 1991. **10**(1): p. 133-140.
61. Park, J.-W., *Understanding of the color in composite resin*. J Korean Acad Conserv Dent, 2011. **36**(4): p. 271-279.
62. Lee, S.C., et al., *Lignin for white natural sunscreens*. International Journal of Biological Macromolecules, 2019. **122**: p. 549-554.
63. Zhang, H., et al., *Fabrication of Light-Colored Lignin Microspheres for Developing Natural Sunscreens with Favorable UV Absorbability and Staining Resistance*. Industrial & Engineering Chemistry Research, 2019. **58**(31): p. 13858-13867.
64. Lunadei, L., et al., *A multispectral vision system to evaluate enzymatic browning in fresh-cut apple slices*. Postharvest Biology and Technology, 2011. **60**(3): p. 225-234.
65. Wang, J., et al., *Reduction of lignin color via one-step UV irradiation*. Green Chemistry, 2016. **18**(3): p. 695-699.
66. Zhang, H., S. Fu, and Y. Chen, *Basic understanding of the color distinction of lignin and the proper selection of lignin in color-depended utilizations*. International Journal of Biological Macromolecules, 2020. **147**: p. 607-615.
67. Zhang, H., et al., *Micromorphology influence on the color performance of lignin and its application in guiding the preparation of light-colored lignin sunscreen*. ACS Sustainable Chemistry & Engineering, 2018. **6**(9): p. 12532-12540.

68. Ajao, O., et al., *Quantification and variability analysis of lignin optical properties for colour-dependent industrial applications*. *Molecules*, 2018. **23**(2): p. 377.
69. Myers, T.L., et al., *Quantitative reflectance spectra of solid powders as a function of particle size*. *Applied Optics*, 2015. **54**(15): p. 4863-4875.
70. Li, Z., et al., *Effect of particle size, transparency and light intensity on the color of powder*. *IOP Conference Series: Earth and Environmental Science*, 2020. **545**: p. 012027.
71. Dilling, P. and P.T. Sarjeant, *Reduction of lignin color*. 1984, Google Patents.
72. Lin, S.Y., *Process for reduction of lignin color*. 1980, Google Patents.
73. Gierer, J., *Formation and involvement of superoxide (O_2^-/HO_2^-) and hydroxyl ($OH^\cdot$) radicals in TCF bleaching processes: A review*. 1997.
74. Baldacci-Cresp, F., et al., *A rapid and quantitative safranin-based fluorescent microscopy method to evaluate cell wall lignification*. *Plant J*, 2020. **102**(5): p. 1074-1089.
75. Maceda, A. and T. Terrazas, *Fluorescence Microscopy Methods for the Analysis and Characterization of Lignin*. *Polymers*, 2022. **14**(5): p. 961.
76. Escamez, S., et al., *Fluorescence Lifetime Imaging as an In Situ and Label-Free Readout for the Chemical Composition of Lignin*. *ACS Sustainable Chemistry & Engineering*, 2021. **9**(51): p. 17381-17392.
77. Singh, S.S., L.-T. Lim, and A. Manickavasagan, *Imaging and spectroscopic techniques for microstructural and compositional analysis of lignocellulosic materials: a review*. *Biomass Conversion and Biorefinery*, 2020.
78. Liu, B., et al., *Vibrational Fingerprint Mapping Reveals Spatial Distribution of Functional Groups of Lignin in Plant Cell Wall*. *Analytical Chemistry*, 2015. **87**(18): p. 9436-9442.
79. Guillon, F., et al., *Chapter Six - In situ imaging of lignin and related compounds by Raman, Fourier-transform infrared (FTIR) and fluorescence microscopy*, in *Advances in Botanical Research*, R. Sibout, Editor. 2022, Academic Press. p. 215-270.
80. Jin, J., et al., *Carbon Fibers Derived from Fractionated–Solvated Lignin Precursors for Enhanced Mechanical Performance*. *ACS Sustainable Chemistry & Engineering*, 2018. **6**(11): p. 14135-14142.
81. Frank, E., et al., *Carbon Fibers: Precursor Systems, Processing, Structure, and Properties*. *Angewandte Chemie-International Edition*, 2014. **53**(21): p. 5262-5298.

82. Kadla, J.F., et al., *Lignin-based carbon fibers for composite fiber applications*. Carbon, 2002. **40**(15): p. 2913-2920.
83. Arefmanesh, M., et al., *From acetone fractionation to lignin-based phenolic and polyurethane resins*. Industrial Crops and Products, 2022. **178**: p. 114604.
84. *Global Formaldehyde Market Size To Grow \$15.51 Billion By 2030 | CAGR 5%*. 2023; Available from: <https://www.globenewswire.com/en/news-release/2023/01/13/2588556/0/en/Global-Formaldehyde-Market-Size-To-Grow-15-51-Billion-By-2030-CAGR-5.html>.
85. Alinejad, M., et al., *Lignin-Based Polyurethanes: Opportunities for Bio-Based Foams, Elastomers, Coatings and Adhesives*. Polymers, 2019. **11**(7): p. 21.
86. da Silva, E.A.B., et al., *An integrated process to produce vanillin and lignin-based polyurethanes from Kraft lignin*. Chemical Engineering Research & Design, 2009. **87**(9A): p. 1276-1292.
87. Temples, S.C., et al., *Liquid–Liquid Equilibrium Compositions and Global Phase Behavior for Lignin–Acetone–Water Ternary at 25, 45, and 65 °C*. Industrial & Engineering Chemistry Research, 2019. **58**(50): p. 22619-22625.
88. Ding, J., et al., *Liquid–liquid equilibrium compositions and global phase behavior for the lignin–acetic acid–water system at 70 and 95 °C*. Fluid Phase Equilibria, 2018. **461**: p. 8-14.
89. Tindall, G.W., et al., *Fractionating and Purifying Softwood Kraft Lignin with Aqueous Renewable Solvents: Liquid–Liquid Equilibrium for the Lignin–Ethanol–Water System*. ChemSusChem, 2020. **13**(17): p. 4587-4594.
90. Sadeghifar, H. and A. Ragauskas, *Lignin as a UV Light Blocker—A Review*. Polymers, 2020. **12**(5): p. 1134.
91. Qian, Y., X. Qiu, and S. Zhu, *Lignin: a nature-inspired sun blocker for broad-spectrum sunscreens*. Green Chemistry, 2015. **17**(1): p. 320-324.
92. Sun, Z., et al., *Bright Side of Lignin Depolymerization: Toward New Platform Chemicals*. Chem Rev, 2018. **118**(2): p. 614-678.
93. Schutyser, W., et al., *Chemicals from lignin: an interplay of lignocellulose fractionation, depolymerisation, and upgrading*. Chemical Society Reviews, 2018. **47**(3): p. 852-908.
94. Deuss, P.J., et al., *Aromatic monomers by in situ conversion of reactive intermediates in the acid-catalyzed depolymerization of lignin*. J Am Chem Soc, 2015. **137**(23): p. 7456-67.

95. Saulnier, B.K., et al., *Effect of Dilute Acid Pretreatment and Lignin Extraction Conditions on Lignin Properties and Suitability as a Phenol Replacement in Phenol-Formaldehyde Wood Adhesives*. J Agric Food Chem, 2023. **71**(1): p. 592-602.
96. Meyer, J.R., et al., *Isolation of lignin from Ammonia Fiber Expansion (AFEX) pretreated biorefinery waste*. Biomass and Bioenergy, 2018. **119**: p. 446-455.
97. Thoresen, P.P., et al., *Recent advances in organosolv fractionation: Towards biomass fractionation technology of the future*. Bioresource Technology, 2020. **306**: p. 123189.
98. George, A., et al., *Design of low-cost ionic liquids for lignocellulosic biomass pretreatment*. Green Chemistry, 2015. **17**(3): p. 1728-1734.
99. Wang, W. and D.J. Lee, *Lignocellulosic biomass pretreatment by deep eutectic solvents on lignin extraction and saccharification enhancement: A review*. Bioresour Technol, 2021. **339**: p. 125587.
100. Zhang, X., et al., *Extraction and characterization of lignin from corncob residue after acid-catalyzed steam explosion pretreatment*. Industrial Crops and Products, 2019. **133**: p. 241-249.
101. Cabrera, Y., et al., *Purification of Biorefinery Lignin with Alcohols*. Journal of Wood Chemistry and Technology, 2016. **36**(5): p. 339-352.
102. Martin, J., *Project Liberty: Launch of an Integrated Bio-Refinery with Eco-Sustainable and Renewable Technologies. Conversion of Corn Stover Biomass to Bio-Ethanol, Final Report*. 2021: United States. p. Medium: ED; Size: 94 p.
103. Singh, S. and P. Dhepe, *Lignin Conversion Using Catalytic Ionic Liquids: Understanding the Role of Cations, Anions and Hammett Acidity Functions*. Industrial & Engineering Chemistry Research, 2019. **2019**.
104. Sluiter, A., et al., *Determination of structural carbohydrates and lignin in biomass, in: Laboratory Analytical Procedure (LAP)*. National Renewable Energy Laboratory, 2008.
105. Singh, S.K., et al., *Integrated Two-Stage Alkaline-Oxidative Pretreatment of Hybrid Poplar. Part 1: Impact of Alkaline Pre-Extraction Conditions on Process Performance and Lignin Properties*. Industrial & Engineering Chemistry Research, 2019. **58**(35): p. 15989-15999.
106. Jassal, V., et al., *Finding values in lignin: A promising yet under-utilized component of the lignocellulosic biomass*. Frontiers in Chemical Engineering, 2022. **4**.
107. He, H., et al., *Physiological and ecological significance of biomineralization in plants*. Trends Plant Sci, 2014. **19**(3): p. 166-74.

108. Gosselink, R., *Lignin as a Renewable Aromatic Resource for the Chemical Industry*. 2011.
109. Shuai, L. and B. Saha, *Towards high-yield lignin monomer production*. Green Chemistry, 2017. **19**(16): p. 3752-3758.
110. Sturgeon, M.R., et al., *A Mechanistic Investigation of Acid-Catalyzed Cleavage of Aryl-Ether Linkages: Implications for Lignin Depolymerization in Acidic Environments*. ACS Sustainable Chemistry & Engineering, 2014. **2**(3): p. 472-485.
111. Shuai, L., et al., *Formaldehyde stabilization facilitates lignin monomer production during biomass depolymerization*. Science, 2016. **354**(6310): p. 329-333.
112. Rossberg, C., et al., *Effect of process parameters in pilot scale operation on properties of organosolv lignin*. BioResources, 2019.
113. Kalami, S., et al., *Replacing 100% of phenol in phenolic adhesive formulations with lignin*. Journal of Applied Polymer Science, 2017. **134**(30): p. 45124.
114. Zoia, L., et al., *Valorization of Side-Streams from a SSF Biorefinery Plant: Wheat Straw Lignin Purification Study*. Bioresources, 2017. **12**: p. 1680-1696.
115. Singh, S., et al., *Understanding the impact of ionic liquid pretreatment on cellulose and lignin via thermochemical analysis*. Biomass & Bioenergy, 2013. **54**: p. 276-283.
116. Dutta, T., et al., *Survey of Lignin-Structure Changes and Depolymerization during Ionic Liquid Pretreatment*. ACS Sustainable Chemistry & Engineering, 2017. **5**(11): p. 10116-10127.
117. Hatfield, R.D., et al., *Grass lignin acylation: p-coumaroyl transferase activity and cell wall characteristics of C3 and C4 grasses*. Planta, 2009. **229**(6): p. 1253-67.
118. Duval, A., et al., *Solvent screening for the fractionation of industrial kraft lignin*. Holzforschung, 2016. **70**(1): p. 11-20.
119. Hansen, C.M. and A. Björkman, *The Ultrastructure of Wood from a Solubility Parameter Point of View*. Holzforschung, 1998. **52**(4): p. 335-344.
120. Barton, A., *CRC handbook of solubility parameters and other cohesion parameters, second edition*. 2017. 1-739.
121. Lindvig, T., M. Michelsen, and G. Kontogeorgis, *A Flory–Huggins model based on the Hansen solubility parameters*. Fluid Phase Equilibria, 2002. **203**: p. 247-260.
122. Hansen, C., *Hansen Solubility Parameters: A User's Handbook, Second Edition*. 2012. 546.

123. Tindall, G., et al., *Ultraclean hybrid poplar lignins via liquid–liquid fractionation using ethanol–water solutions*. MRS Communications, 2021. **11**(5): p. 692-698.
124. Tindall, G.W., et al., *Liquefying Lignins: Determining Phase-Transition Temperatures in the Presence of Aqueous Organic Solvents*. Industrial & Engineering Chemistry Research, 2021. **60**(47): p. 17278-17282.
125. Lynn, B., et al., *Fractionation and purification of a high-impurity, alkaline-pretreated, corn stover lignin with simple renewable solvents*. Separation and Purification Technology, 2024. **342**: p. 126924.
126. Kanhere, S.V., et al., *Carbon fibers derived from liquefied and fractionated poplar lignins: The effect of molecular weight*. iScience, 2022. **25**(12): p. 105449.
127. Kanhere, S.V., et al., *Carbon fibers derived from environmentally benign, ethanol-fractionated corn-stover lignin*. RSC Sustainability, 2024. **2**(8): p. 2357-2366.
128. Wu, C., et al., *The Role of Lignin Molecular Weight on Activated Carbon Pore Structure*. Molecules, 2024. **29**(16): p. 3879.
129. Henry, C., et al., *Fractionated and purified hybrid poplar lignins as a polyol replacement in rigid polyurethane/polyisocyanurate foams*. Journal of Applied Polymer Science, 2023. **140**(45): p. e54648.
130. Kane, S., et al., *Role of sodium sulfate in electrical conductivity and structure of lignin-derived carbons*. Journal of Analytical and Applied Pyrolysis, 2024. **181**: p. 106600.
131. Bär, J., et al., *Deconstruction of hybrid poplar to monomeric sugars and aromatics using ethanol organosolv fractionation*. Biomass Conversion and Biorefinery, 2018. **8**(4): p. 813-824.
132. Bali, G., et al., *The effect of alkaline pretreatment methods on cellulose structure and accessibility*. ChemSusChem, 2015. **8**(2): p. 275-9.
133. Toledano, A., et al., *Lignin separation and fractionation by ultrafiltration*. Separation and Purification Technology, 2010. **71**(1): p. 38-43.
134. Sameni, J., et al., *Thermal Characteristics of Lignin Residue from Industrial Processes*. Bioresources, 2014. **9**: p. 725-737.
135. Singleton, V.L. and J.A. Rossi, *Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents*. American Journal of Enology and Viticulture, 1965.
136. Dülger, D.N., et al., *Scale-Up of Two-Stage Cu-Catalyzed Alkaline-Oxidative Pretreatment of Hybrid Poplar*. Biomass Conversion and Biorefinery, 2023.

137. Henry, C., et al., *Studying the Suitability of Nineteen Lignins as Partial Polyol Replacement in Rigid Polyurethane/Polyisocyanurate Foam*. Molecules, 2022. **27**(8): p. 2535.
138. Fitzgerald, C.L. and M.C. Thies, *Continuous recovery of high-purity Kraft lignin from black liquor via simultaneous, liquid-phase acidification and purification*. Industrial Crops and Products, 2022. **184**: p. 115084.
139. Lin, M.Y., et al., *Universality in colloid aggregation*. Nature, 1989. **339**(6223): p. 360-362.
140. Lin, M.Y., et al., *Universal reaction-limited aggregation*. Physical review. A, 1990. **41**: p. 2005-2020.
141. Lin, M.Y., et al., *Universal diffusion-limited colloid aggregation*. Journal of Physics: Condensed Matter, 1990. **2**(13): p. 3093.
142. Meakin, P., *Fractal aggregates*. Advances in Colloid and Interface Science, 1987. **28**: p. 249-331.
143. Norgren, M., et al., *Aggregation of kraft lignin derivatives under conditions relevant to the process, part I: phase behaviour*. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 2001. **194**(1): p. 85-96.
144. Crowe, J.D., et al., *Xylan Is Critical for Proper Bundling and Alignment of Cellulose Microfibrils in Plant Secondary Cell Walls*. Frontiers in Plant Science, 2021. **12**.
145. Enikő Bécsy-Jakab, V., et al., *Extraction, recovery, and characterization of lignin from industrial corn stover lignin cake*. Bioresource Technology, 2024: p. 130610.
146. Erguvan, Ö., et al., *ImageJ SurfCut: a user-friendly pipeline for high-throughput extraction of cell contours from 3D image stacks*. BMC Biology, 2019. **17**(1): p. 38.
147. Andeme Ela, R.C., et al., *Understanding the Effect of Precipitation Process Variables on Hardwood Lignin Characteristics and Recovery from Black Liquor*. ACS Sustainable Chemistry & Engineering, 2020. **8**(37): p. 13997-14005.
148. Helander, M., et al., *Fractionation of Technical Lignin: Molecular Mass and pH Effects*. BioResources, 2013. **8**.
149. Cheng, J., et al., *Low-chromophore lignin isolation from natural biomass with polyol-based deep eutectic solvents*. Green Chemistry, 2024. **26**(14): p. 8298-8314.
150. Uloth, V. and J. Wearing, *Kraft lignin recovery: acid precipitation versus ultrafiltration. I: Laboratory test results*. Pulp & Paper Canada, 1989. **90**(9): p. 67-71.

151. Kitin, P., et al., *Direct fluorescence imaging of lignocellulosic and suberized cell walls in roots and stems*. AoB PLANTS, 2020. **12**(4).
152. Radotić, K., et al., *Component analysis of the fluorescence spectra of a lignin model compound*. Journal of Photochemistry and Photobiology B: Biology, 2006. **83**(1): p. 1-10.
153. Bharmoria, P., et al., *Temperature-Dependent Solubility Transition of Na₂SO₄ in Water and the Effect of NaCl Therein: Solution Structures and Salt Water Dynamics*. The Journal of Physical Chemistry B, 2014. **118**(44): p. 12734-12742.
154. Ortega-Sanhueza, I., et al., *Preparation and Characterization of Lignin Nanoparticles from Different Plant Sources*. Polymers, 2024. **16**(11): p. 1610.
155. Fahmi, A., *Natural Xilan Production From Corncobs (Zea Mays L.) With Extraction Method*. KnE Social Sciences, 2019. **3**(18).
156. Evstigneev, E.I., *Factors affecting lignin solubility*. Russian Journal of Applied Chemistry, 2011. **84**(6): p. 1040-1045.
157. Hough, W.J., *Process of treating spent alkaline pulping liquors*. 1910: United States.
158. Öhman, F., H. Wallmo, and H. Theliander, *A novel method for washing lignin precipitated from kraft black liquor – Laboratory trials*. Nordic Pulp & Paper Research Journal, 2007. **22**(1): p. 9-16.
159. Öhman, F.T., H., *Filtration properties of lignin precipitated from black liquor*. TAPPI J, 2007. **6**(7): p. 3-9.
160. Hennemann, M., M. Gastl, and T. Becker, *Influence of particle size uniformity on the filter cake resistance of physically and chemically modified fine particles*. Separation and Purification Technology, 2021. **272**: p. 118966.
161. Richardson, B., *Kraft lignin as a fuel for the rotary lime kiln*. 1993.
162. Sameni, J., et al., *Preparation and characterization of biobased microspheres from lignin sources*. Industrial Crops and Products, 2018. **117**: p. 58-65.
163. Tang, P., D.E. Colflesh, and B. Chu, *Temperature effect on fractal structure of silica aggregates*. Journal of Colloid and Interface Science, 1988. **126**(1): p. 304-313.
164. Seljelid, K.K., et al., *Growth kinetics and structure of a colloidal silica-based network: in situ RheoSAXS investigations*. The European Physical Journal Special Topics, 2024.
165. Vétier, N., et al., *Effect of Temperature and Aggregation Rate on the Fractal Dimension of Renneted Casein Aggregates*. Journal of Dairy Science, 2003. **86**(8): p. 2504-2507.

APPENDICES

APPENDIX A

ALKALINE PRETREATMENT

Hybrid Poplar**Personal Protective Equipment (PPE):**

- General: Lab coat, gloves, eye protection
- Unloading and high-risk steps: heavy duty gloves, apron, face shield, (noise protection)

WC – woodchips, MC – moisture content, DI water - deionized water, BM – biomass, L – liter, BL – black liquor, RT – room temperature

Hazardous Materials and Procedures

- Dry BM fine particles during loading: Wear a mask and avoid unnecessary movement of the biomass that promotes dust particles in the air.
- NaOH solution preparation and loading: pH ~13; NaOH pellets are irritants, and mixing with water generates heat (~40°C). Transfer using smaller jugs and avoid spillage, use disposable apron.
- Black liquor and pretreated biomass unloading: pH ~11; Make sure to use a disposable apron. Exercise caution with the alignment of the bottom valve and the bucket, use heavy-duty gloves for scooping remaining biomass. The digester must be inverted carefully for unloading everything (2 people).
- cc. H₂SO₄: Extremely low pH (~ -1); inform lab members of use, WEAR PPE and proceed slowly to avoid spillage on anything (especially you)
- Centrifuge: Ensure weight difference between tubes is minimized (within a few grams for 1L tubes), avoid overfilling so they can comfortably close and seal

A. Prepping (1st day) [18% NaOH loading, 5:1 liquid:solid ratio, ~**4.4% WCMC**(measure!)]

(The exact amounts might change according to the changing MC of the WC)

1. 3 kg WC [known moisture and ash content]
2. ~13.6 L DI water [from CBE general lab]
3. ~516 g NaOH [SLOWLY add to water, stirring constantly until clear, pH is ~13!]

B. Loading the digester, reaction (2nd day)

1. Make sure the digester is clean, and CLOSE the bottom valve
2. Carefully transfer WC to digester
3. Carefully transfer NaOH solution to digester
4. Put on lid [valve CLOSED on lid], tighten the nuts REASONABLY
5. CHECK EQUIPMENT for leaks or loose parts
6. Plug in reactor, set temperature (150°C), start rotation and heating, log T and p every 5 mins [~1 hr to heat up, if it overheats by more than 5°C monitor closely, if still increasing switch off heating until it's back to normal]
7. Once reaction temperature is reached, start timer (3 hrs) [MONITOR for RAPIDLY INCREASING PRESSURE, leaks, weird noises, dripping]
8. Switch off, unplug, put on CAUTION tape, but make sure it is NOT TOUCHING the reactor body, as that is 150°C!

C. Unloading the digester, press (3rd day)

1. Check the pressure and temperature [pressure reading ≈ 0 and $T < 60^\circ\text{C}$]
2. Line bucket with mesh bag (optional: put press drum in bucket, mesh bag inside)
3. Put bucket under bottom valve, open

4. Close valve when dripping stops, open lid on top
5. Transfer WC and BL (pH ~12.5) to bucket [check the hole on the inside sidewall of the digester for stuck BM]
6. Rinse digester multiple times with tap water until clean (using extra bucket)
7. Upstairs use the press to extract as much BL as possible (~12 L), breaking up the filter cake multiple times between presses, clean press
8. Save a sample of the WC in a small Ziploc bag, label, keep in fridge

D. Acidification (3rd day)

1. Filter the BL again through a sieve (fits on top of a bucket), measure the volume, weight, pH, and solid content
2. Acidify BL until pH<2, ~320 ml cc. H₂SO₄, start with 300 ml, 10/5/1 ml additions
3. Label, put in fridge overnight

E. Centrifuging (4th-6th day)

1. Centrifuge the acidified, precipitated lignin [8000 rpm, 4°C, 2 hours, max. difference between centrifuge bottles is a few grams], until the **liquid is CLEAR**
2. Discard liquid IF CLEAR, wash solids with 5 L RT DI water (1:5 solid-water ratio) in the bucket, mix with drill mixer attachment for 2 mins
3. Centrifuge washed lignin (8000 rpm, 4 hours)
4. Wash lignin as step 2
5. Centrifuge washed lignin (8000 rpm, 4°C, 4 hours) and any opaque leftover liquid
6. Transfer solids to Al foil/plastic wrap lined metal pan, let it dry in hood for at least 48 hours, after 24 hours smash by hand until the texture is like sand

F. Drying (7th day)

1. Check lignin, put in vacuum oven at 50°C, CHECK REGULARLY, WIPE DOWN DOOR IF NECESSARY, if too wet dry under hood for one more day

G. Finishing up (8th-14th day)

1. Check regularly
2. When dry, determine **ash content**
3. If ash content is good (<3%), ship lignin (keep ~2 g each in fridge)
4. Composition analysis (3*0.1 g triplicate), **xylan content**
5. Document everything in the Google Sheet

Corn Stover**Personal Protective Equipment (PPE):**

- General: Lab coat, gloves, eye protection
- Unloading and high-risk steps: heavy duty gloves, apron, face shield, (noise protection)

CS – corn stover, MC – moisture content, DI water - deionized water, BM – biomass, L – liter, BL – black liquor, RT – room temperature

Hazardous Materials and Procedures

- Dry BM fine particles during loading: Wear a mask and avoid unnecessary movement of the biomass that promotes dust particles in the air.
- NaOH solution preparation and loading: pH ~13; NaOH pellets are irritants, and mixing with water generates heat (~40°C). Transfer using smaller jugs and avoid spillage, use disposable apron.

- Black liquor and pretreated biomass unloading: pH ~11; Make sure to use a disposable apron. Exercise caution with the alignment of the bottom valve and the bucket, use heavy-duty gloves for scooping remaining biomass. The digester must be inverted carefully for unloading everything (2 people).
 - cc. H_2SO_4 : Extremely low pH (~ -1); inform lab members of use, WEAR PPE and proceed slowly to avoid spillage on anything (especially you)
 - Centrifuge: Ensure weight difference between tubes is minimized (within a few grams for 1L tubes), avoid overfilling so they can comfortably close and seal
- A. Prepping (1st day) [13% NaOH loading, 9:1 liquid:solid ratio, ~**8.4% CS MC (measure!)**]
(The exact amounts might change according to the changing MC of the CS)
1. 2 kg CS [known moisture and ash content]
 2. ~16 L DI water [from CBE general lab]
 3. ~238 g NaOH [SLOWLY add to water, stirring constantly until clear, pH ~13!]
- B. Loading the digester, reaction (2nd day)
1. Make sure the digester is clean, and CLOSE the bottom valve
 2. Carefully transfer CS to digester
 3. Carefully transfer NaOH solution to digester
 4. Put on lid [valve CLOSED on lid], tighten the nuts REASONABLY
 5. CHECK EQUIPMENT for leaks or loose parts

6. Plug in reactor, set temperature (140°C), start rotation and heating, log T and p every 5 mins [~1 hr to heat up, monitor temperature and pressure closely, if T is rapidly increasing turn off heating around 130°C]
7. Once the reaction temperature is reached, start timer (30 mins) [MONITOR for RAPIDLY INCREASING PRESSURE, leaks, weird noises, dripping]
8. Switch off, unplug, put on CAUTION tape but make sure it is NOT TOUCHING the reactor body, as that is 140°C!

C. Unloading the digester, pressing (3rd day)

1. Check the pressure and temperature [pressure reading ≈ 0 and $T < 60^\circ\text{C}$]
2. Line bucket with mesh bag (optional: put press drum in bucket, mesh bag inside)
3. Put bucket under bottom valve, open the valve to collect BL
4. Close valve when dripping stops, open lid on top
5. Transfer CS and BL (pH ~ 12.5) to bucket [check the hole on the inside sidewall of the digester for stuck BM]
6. Wash digester multiple times with tap water until clean (using extra bucket)
7. Upstairs use the press to extract as much BL as possible (~ 14 L), breaking up the filter cake multiple times between presses, clean press
8. Save a sample of the CS in a small Ziploc bag, label, keep in fridge

D. Acidification (3rd day)

1. Filter the BL again through a sieve (fits on top of a bucket), measure the volume, weight, pH and solid content
2. Acidify BL until pH < 2 , ~ 185 ml H_2SO_4 , start with 150 ml, 10/5/1 ml additions

3. Label, put in fridge overnight

E. Centrifuging (4th-6th day)

1. Centrifuge the acidified, precipitated lignin [8000 rpm, 4°C, 2 hours, max. difference between centrifuge bottles is a few grams] until the **liquid is CLEAR**
2. Discard liquid IF CLEAR, wash solids with 5 L RT DI water (1:5 solid-water ratio) in the bucket, mix with drill mixer attachment for 2 mins
3. Centrifuge washed lignin (8000 rpm, 4°C, 4 hours)
4. Wash lignin as step 2
5. Centrifuge washed lignin (4 hours) and any opaque leftover liquid
6. Transfer solids to Al foil/plastic wrap lined metal pan, let it dry in hood for at least 48 hours, after 24 hours smash by hand until the texture is like sand

F. Drying (8th day)

1. Check lignin, put in vacuum oven at 50°C, CHECK REGULARLY, WIPE DOWN DOOR IF NECESSARY, if too wet dry under hood for one more day

G. Finishing up (9th-14th day)

1. Check lignin regularly
2. When dry, determine **ash content**
3. If ash content is good (<3%), ship lignin (keep ~2 g each in fridge)
4. Composition analysis (3*0.1 g triplicate), **xylan content**
5. Document everything in the Google Sheet

APPENDIX B

PARTICLE SIZE DISTRIBUTION ANALYSIS FROM
MICROSCOPY IMAGES

Sample Preparation

1. Black Liquor Preparation (Black Liquor)

The black liquor was homogenized and filtered through a 120-micron mesh bag.

2. pH Adjustment and Dilution (Black Liquor)

The homogenized black liquor (5 mL) was acidified with concentrated sulphuric acid.

After acidification, the sample was diluted 1:100 (v/v) with DI water.

3. Microscope Slide Preparation (Black Liquor)

1 μ L of the diluted sample was pipetted onto a microscope slide and allowed to dry over a few minutes. No change in particle size was observed during drying, as confirmed by video recording the process.

4. Microscope Slide Preparation (Powder Samples)

Previously recovered lignin powder samples were suspended in DI water at a 1:100,000 (w/w) ratio. 10 μ L of the suspension was pipetted onto a microscope slide and allowed to dry.

Microscopy

1. Microscopy Equipment:

Images were captured using an OMAX EPI-Fluorescence Trinocular Compound Microscope (M837ZFLR-C180U3) equipped with an 18MP camera (A35180U3) and a mercury lamp. A blue excitation filter (410-490 nm) and a barrier (emission) filter of 515 nm was used. Images were taken at 10x magnification using ToupLite software, with consistent resolution and camera settings.

Image Processing

1. Software and Initial Filtering

ImageJ/Fiji software was utilized for image processing. Images containing biomass particles or at the edge of the sample drop were excluded from further analysis.

2. Image Calibration and Conversion

A global scale was set for the 10x magnification images. RGB images were converted to 8-bit grayscale. The grayscale images were thresholded to binary using consistent thresholds within each condition. The thresholds were set subjectively, balancing the detection of all particles with accurate sizing while avoiding visual artifacts, and were standardized across similar sample types (e.g., corn stover and hybrid poplar black liquor).

3. Particle Analysis

The “Analyze Particles” command was applied to the binary images, excluding small artifacts (less than 9 pixels) and edge-bound particles, with no restrictions on particle circularity. For powder samples, heavily aggregated large particles were also excluded.

4. Data Extraction:

Particle data, including counts and areas, were extracted and saved for further analysis.

Data Analysis

1. Python Processing:

The extracted particle data were processed using a Python/Numpy script to generate

normalized histograms, fit Gaussian curves, and calculate the mean and variance of the particle size distributions.