Extraction, recovery, and characterization of lignin from industrial corn stover lignin cake

Villő Enikő Bécsy-Jakab, Anthony Savoy, Brian K. Saulnier, David B. Hodge

Extraction, Recovery, and Characterization of Lignin from Industrial Corn Stover Lignin Cake

Villő Enikő Bécsy-Jakab[1], Anthony Savoy[1†], Brian K. Saulnier[1], Sandip K. Singh[1‡], David B. Hodge[1,2,*]

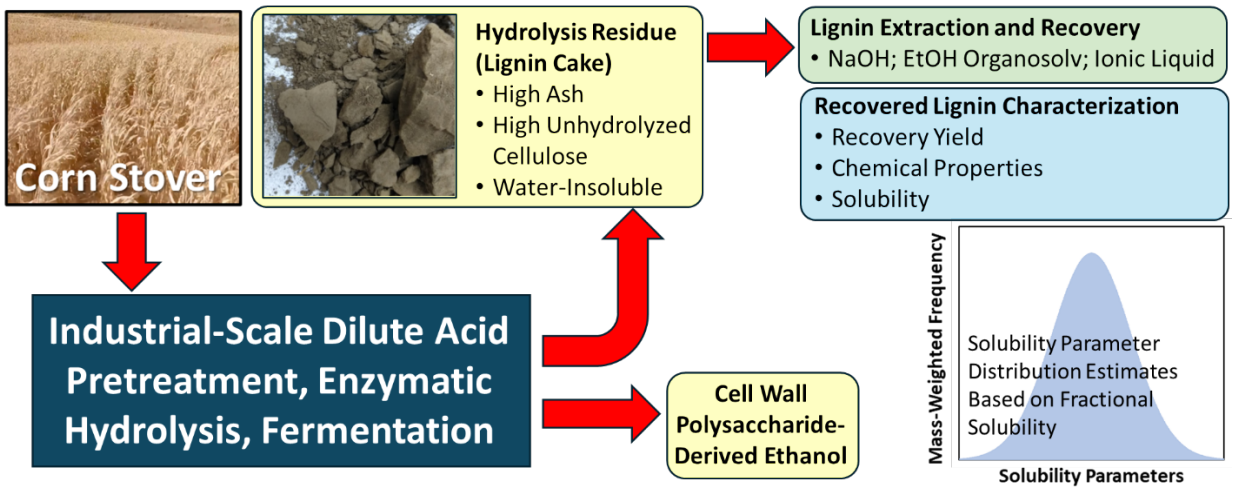
[1] Department of Chemical & Biological Engineering, Montana State University, Bozeman, Montana 59717, United States

[2] Division of Sustainable Process Engineering, Luleå University of Technology, Luleå, Sweden

[†] Current affiliation: Washington State University, Voiland School of Chemical Engineering and Bioengineering, Pullman, Washington, United States

[‡] Current affiliation: University of Kansas, Department of Chemical & Petroleum Engineering, Lawrence, Kansas, United States

* Corresponding author: david.hodge3@montana.edu



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 an 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 a 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 (Jungmeier et al., 2013). 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 (Laurichesse & Avérous, 2014; Sun et al., 2018). 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 (Davis et al., 2013). 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 (Ragauskas et al., 2014). 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) (Brienza et al., 2023) 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 (Schutyser et al., 2018). 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 (Deuss et al., 2015) 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 (Saulnier et al., 2023).

Approaches for lignin recovery from biorefinery lignin cake can include extraction using lignin-solubilizing solvents (Meyer et al., 2018) or potentially include features of delignifying pretreatments such as organosolv pretreatments (Pan et al., 2006; Thoresen et al., 2020), alkali pretreatments (Saulnier et al., 2023), ionic liquids (George et al., 2015), or deep eutectic solvents (DESs) (Wang & Lee, 2021). 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 (Meyer et al., 2018). 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) (Meyer et al., 2018). 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% (Zhang et al., 2019).. 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 (Cabrera et al., 2016). 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 ($[C_3SO_3HMIM][HSO_4]$) 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 (Martin, 2021) 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 [C_3SO_4HMIM][HSO_4]. 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 H_2SO_4/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 (Singh & Dhepe, 2019). 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 (Sluiter et al., 2008). 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 (Saulnier et al., 2023). 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 (Singh et al., 2019). ^{13}C and $^{1}H–^{13}C$ correlation 2D heteronuclear single-quantum coherence (HSQC) NMR spectra of ethanol organosolv extracted lignins at different temperatures was performed as in previous work (Singh et al., 2019).

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 1A**, 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 (Martin, 2021) 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 (Argyropoulos et al., 2023; Jassal et al., 2022).

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 (He et al., 2014) 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 (Martin, 2021). 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 (Martin, 2021). 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. 1A**) is one potential route to adding value to the process.

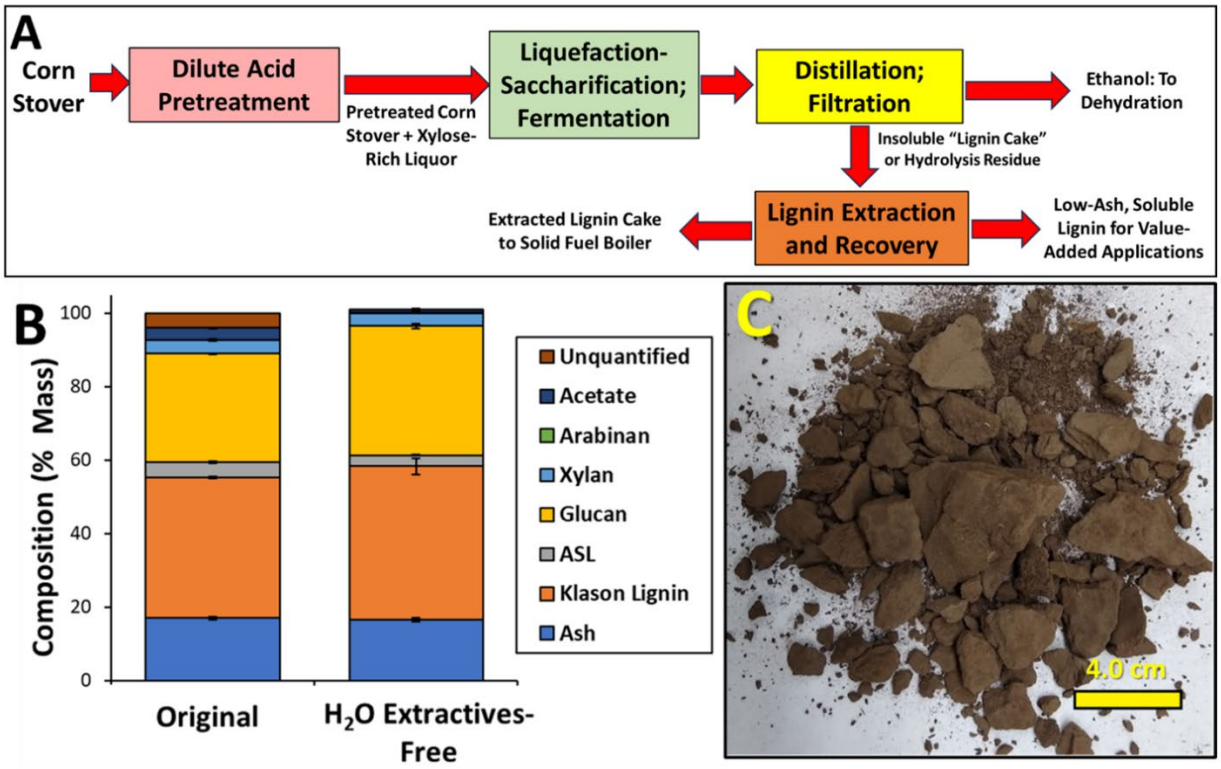


Figure 1. Simplified overall process flow diagram depicting **(A)** extraction and recovery of lignin at the "back end" of the process, (**B**) lignin cake composition on an original and water extractives-free basis, and (**C**) image of the as-received lignin cake used in this work. ASL: Acid-soluble lignin. Error bars represent data range for duplicate measurements.

The lignin cake used in this work (**Fig. 1B** and **1C**) 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_2SO_4 Loading (mg H_2SO_4/g biomass)**	**NaOH Loading (mg NaOH/g)**	**Solid Loading (g/mL)**	
Ethanol:H_2O 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_3SO_4HMIM][HSO_4] in 80:20 EtOH:H_2O	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 [C_3SO_4HMIM][HSO_4].

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 (Cabrera et al., 2016), which is critical for many applications such as polyurethane resins or carbon fibers (Gosselink, 2011). 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 (Deuss et al., 2015). 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 (Shuai & Saha, 2017; Sturgeon et al., 2014). 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 (Shuai et al., 2016) achieved high cleavage of β-aryl ethers without repolymerization.

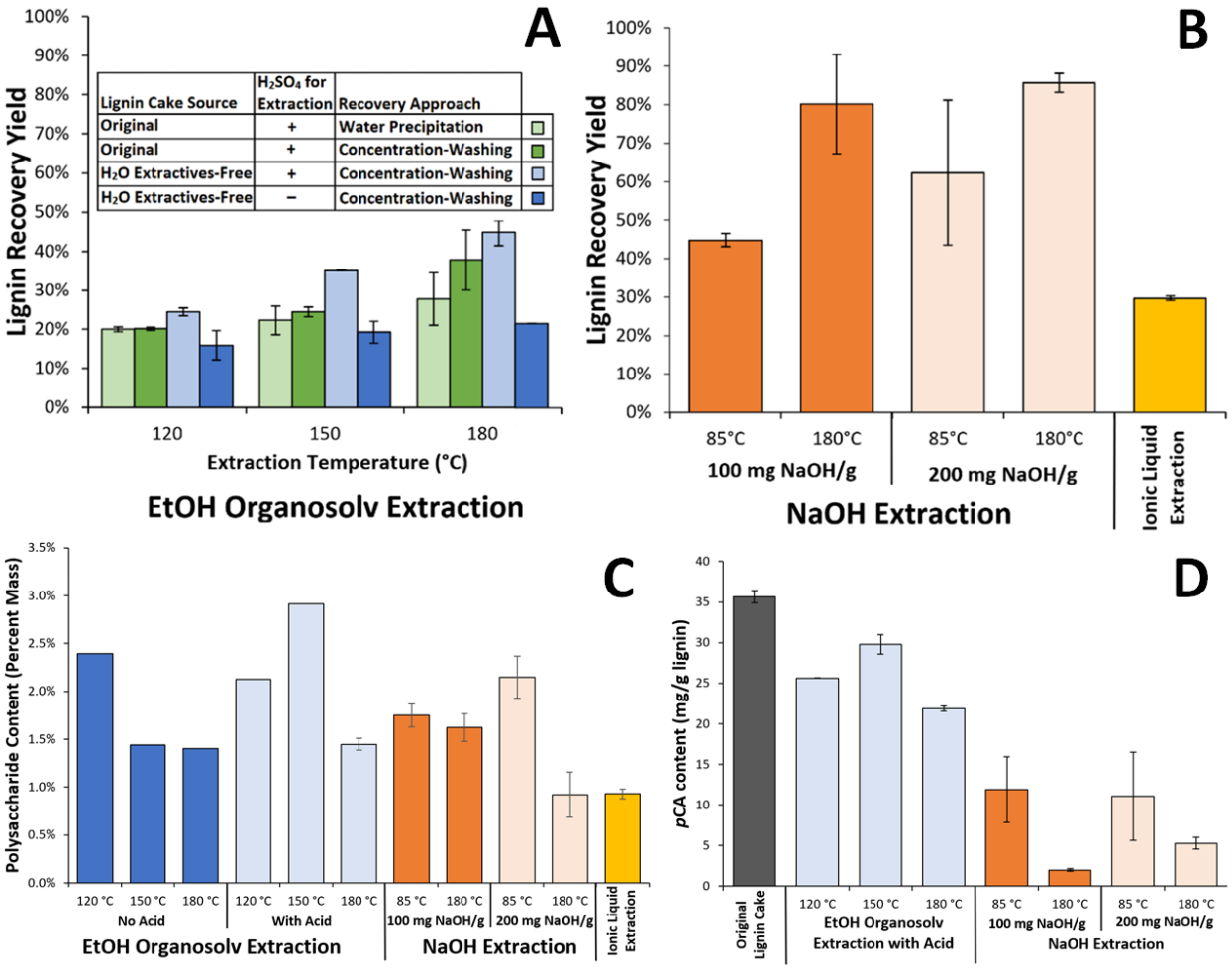


Figure 2. Recovery yields of lignins from (**A**) ethanol organosolv extraction of lignin cake at differing temperatures using two approaches for lignin recovery for raw lignin cake using dilute H_2SO_4 during the extraction and impact of acid during ethanol organosolv extraction using the water extractives-free 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. (Rossberg et al., 2019) 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. 2A**), 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 H_2SO_4 (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. 2A**). Prior work with ethanol organosolv extraction of hydrothermal pretreated wheat straw hydrolysis residue generated at the Inbicon A/S demonstration biorefinery in Kalundsborg, Denmark using 50:50 EtOH:H_2O 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%) (Cabrera et al., 2016).

3.3 Alkali and Ionic Liquid Extraction and Recovery of Lignins

NaOH and the ionic liquid [C_3SO_4HMIM][HSO_4] in 80:20 EtOH:H_2O 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. 2B**). Notably, substantially more lignin could be extracted using alkali than using the ethanol organosolv approach (**Fig. 2A**), 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 (Kalami et al., 2017). 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 (Saulnier et al., 2023). Other work investigated the NaOH extraction of lignin from lignin cake derived from the dilute acid pretreatment and enzymatic hydrolysis of wheat straw (Zoia et al., 2017). 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 (Saulnier et al., 2023). 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 (Kalami et al., 2017). Ionic liquids have shown promise as a solvent for lignin extraction and solubilization during pretreatments (Singh et al., 2013) as well as a catalyst for chemically altering solubilized lignins by, for example, facilitating depolymerization (Dutta et al., 2017). In the current work, the ionic liquid extraction performed at 100 °C gave a modest lignin recovery yield of 29.7% (**Fig. 2B**), which is similar to the results for the acid-catalyzed ethanol organosolv extraction at 120 °C (**Fig. 2A**).

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 (Zhang et al., 2019). Results for polysaccharide (primarily xylan) content (**Fig. 2C**) 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. 1B**). 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 (Hatfield et al., 2009). Lignin suitability as a phenol replacement in phenol-formaldehyde (PF) resins has been linked to higher *p*CA contents (Kalami et al., 2017), 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 (Saulnier et al., 2023), 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. 2D**). 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 (Saulnier et al., 2023), 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. 2D**), indicating that these lignins may be both recoverable at relatively high yields (**Fig. 2A**), high purities (**Fig. 2B**), 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. 2D**), 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 (Saulnier et al., 2023).

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. 2A**), this same condition (Lignin 2) using the water extractives-free lignin cake (**Fig. 2A**), 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. 2B**).

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 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 3A**, 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. 2A**) and 89.6% for the NaOH extraction (**Fig. 2B**). 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. 3A**). 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. 2A**). These different yields may potentially result in lignins with slightly different properties and resulting solubilities.

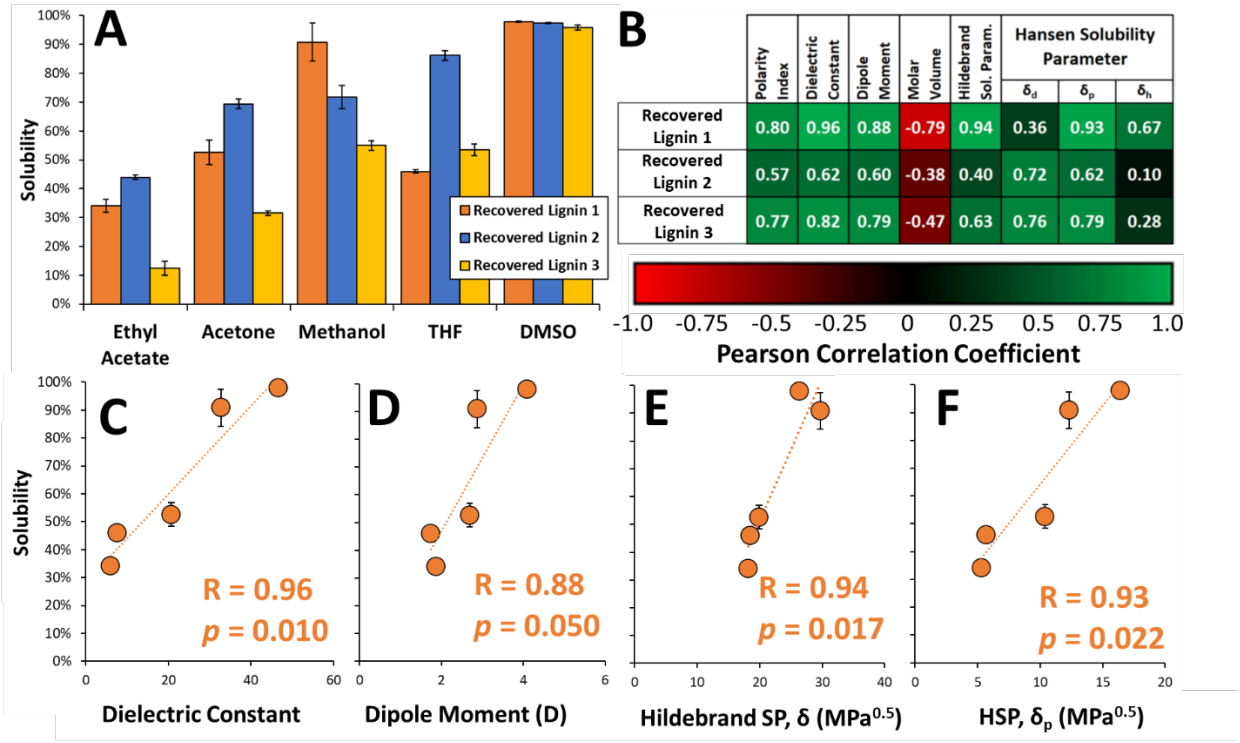


Figure 3. Solubility of acidic ethanol organosolv- and NaOH-extracted lignins in (**A**) various organic solvents, (**B**) Pearson correlation coefficient between lignin solubility and solvent properties, and (**C** through **F**) select significant correlations ($p \leq 0.05$) between solubility of 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. 3B**). The most notable trends are correlations between solubility and descriptors of solvent polarity including dielectric constant (**Fig. 3C**), dipole moment (**Fig. 3D**), and the dipole force interaction contribution (δ_P) to the

Hansen solubility parameter (**Fig. 3F**). 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") (Sameni et al., 2017). 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 (Vermaas et al., 2020).

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. 3E**). 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 (Duval et al., 2016). 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 (Schuerch, 1952).

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 (Hansen & Björkman, 1998) or a qualitative assessment (insoluble, slightly, soluble, partially soluble, and soluble) for comparing to the Hildebrand solubility parameter (Schuerch, 1952). 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

461 the Flory-Huggins solution theory (Barton et al., 2017; Lindvig et al., 2002) that yields a
462 negative or zero value for ΔG_{mix} and comprises the feasible region for polymer dissolution:

463 $$\left(\delta_{Lignin} - \delta_{Solvent\ i}\right)^2 \leq (\Delta\delta)^2 \quad \text{(Eqn. 1)}$$

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

466 $$\delta_{S_i,L} \leq \delta_{Lignin} \leq \delta_{S_i,U} \quad \text{(Eqn. 2)}$$

467 Where:

468 $$\delta_{S_i,U} = \delta_{Solvent\ i} + \Delta\delta \quad \text{(Eqn. 3)}$$

469 $$\delta_{S_i,L} = \delta_{Solvent\ i} - \Delta\delta \quad \text{(Eqn. 4)}$$

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

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

484 Next, the values for $\bar{\delta}_{Lignin}$ and σ_{Lignin} that best fit the experimental data for all *n* solvents with
485 solubility data for lignin were estimated. For this, a residual sum of squares (RSS) for the
486 difference between the estimated fraction of soluble lignin polymers over the distribution of

$\delta_{Lignin}(\bar{\delta}_{Lignin}, \sigma_{Lignin})$ and the actual mass fraction of soluble lignin in solvent i (θ_i) were first determined:

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

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.

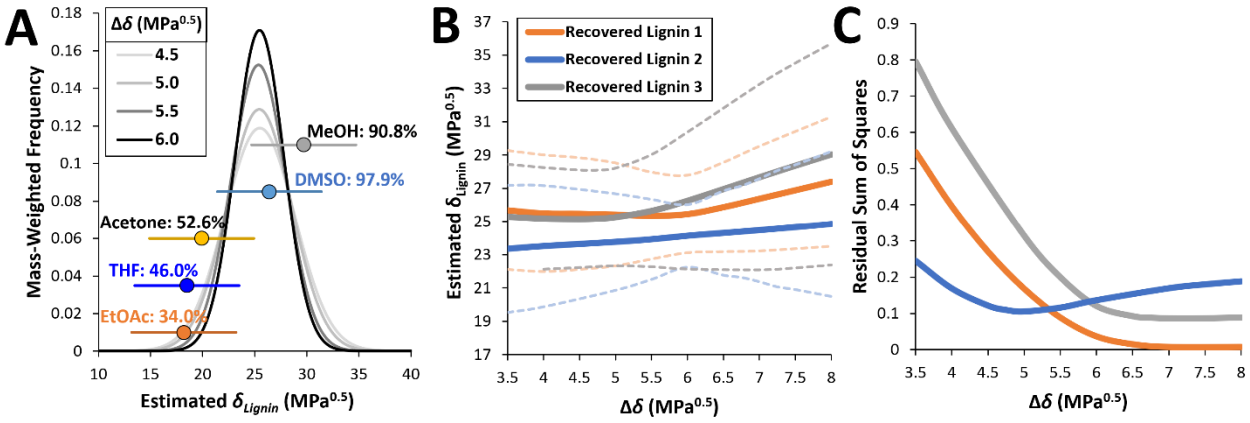


Figure 4. Examples of parameters fitted for Hildebrand solubility parameter (mean and standard deviation for δ_{Lignin}) demonstrating the impact of $\Delta\delta$ on (**A**) the estimated distribution of δ_{Lignin}, for Lignin 1, (**B**) the estimated mean ($\bar{\delta}_{Lignin}$, solid lines) and 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. 4**). 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. 4A**). 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. 4A**). 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. 4B**) 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 (Schuerch, 1952) 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 (Sameni et al., 2017). Increasing values $\Delta\delta$ result generally result in a decreased RSS (**Fig. 4C**) 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 $MPa^{0.5}$ (Venkatram et al., 2019) to 3.7 $MPa^{0.5}$ (Hansen, 2007).

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 (Venkatram et al., 2019). 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) (Hansen, 2007). 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 $\delta_{D_i}, \delta_{P_i}, \delta_{H_i}$ 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 ($\overline{\delta}_{D,\,Lignin}$, $\overline{\delta}_{P,\,Lignin}$, $\overline{\delta}_{H,\,Lignin}$) 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:

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

or:

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

where:

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

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

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

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

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

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

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 $\overline{\delta}_{D,\,Lignin}$, $\overline{\delta}_{P,\,Lignin}$, $\overline{\delta}_{H,\,Lignin}$, $\sigma_{D,Lignin}$, $\sigma_{P,Lignin}$, $\sigma_{H,Lignin}$ 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 5A** and **5B**. 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 $MPa^{0.5}$ the minimum RSS is close to zero for a diverse set of non-unique solutions (**Fig. 5C**). Prior work has suggested appropriate values for R_0 from 2.0 $MPa^{0.5}$ (Lindvig et al., 2002) to 13.7 $MPa^{0.5}$ for an alkali lignin solubility study (Hansen & Björkman, 1998). This same study, using a binary soluble/insoluble assessment for the lignin estimated Hansen solubility parameters of $\delta_{D,\,Lignin}$ = 21.9 $MPa^{0.5}$, $\delta_{P,\,Lignin}$ = 14.1 $MPa^{0.5}$, and $\delta_{H,\,Lignin}$ = 16.9 $MPa^{0.5}$, which are contained within the estimated sets of solutions for

the present study (**Fig. 5A** and **5B**). Overall, this was intended as a proof-of-concept for this approach and many other features could be incorporated such as different distribution functions.

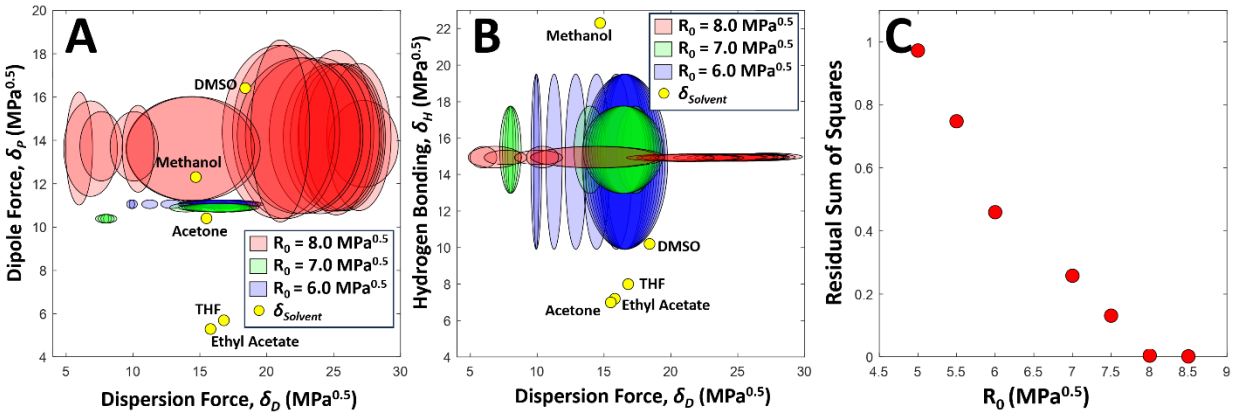


Figure 5. Graphical summary of select solutions resulting in comparable values for RSS for the estimated means ($\bar{\delta}_{D,\,Lignin}$, $\bar{\delta}_{P,\,Lignin}$, $\bar{\delta}_{H,\,Lignin}$) and standard deviations ($\sigma_{D,\,Lignin}$, $\sigma_{P,\,Lignin}$, $\sigma_{H,\,Lignin}$) for Lignin 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

E-supplementary data for this work can be found in e-version of this paper online.

6. References

1. Argyropoulos, D.D., Crestini, C., Dahlstrand, C., Furusjö, E., Gioia, C., Jedvert, K., Henriksson, G., Hulteberg, C., Lawoko, M., Pierrou, C. 2023. Kraft Lignin: A Valuable, Sustainable Resource, Opportunities and Challenges. *ChemSusChem*, e202300492.
2. Barton, A.F. 2017. *CRC handbook of solubility parameters and other cohesion parameters*. Routledge, Milton Park, UK.
3. Brienza, F., Cannella, D., Montesdeoca, D., Cybulska, I., Debecker, D.P. 2024. A guide to lignin valorization in biorefineries: Traditional, recent, and forthcoming approaches to convert raw lignocellulose into valuable materials and chemicals. *RSC Sus*, **2**, 37-90.
4. Cabrera, Y., Cabrera, A., Jensen, A., Felby, C. 2016. Purification of biorefinery lignin with alcohols. *J Wood Chem Technol*, **36**(5), 339-352.
5. Davis, R., Tao, L., Tan, E., Biddy, M., Beckham, G., Scarlata, C., Jacobson, J., Cafferty, K., Ross, J., Lukas, J. 2013. 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. NREL/TP-5100-60223
6. Deuss, P.J., Scott, M., Tran, F., Westwood, N.J., de Vries, J.G., Barta, K. 2015. Aromatic monomers by in situ conversion of reactive intermediates in the acid-catalyzed depolymerization of lignin. *J Am Chem Soc*, **137**(23), 7456-7467.
7. Dutta, T., Isern, N.G., Sun, J., Wang, E., Hull, S., Cort, J.R., Simmons, B.A., Singh, S. 2017. Survey of Lignin-Structure Changes and Depolymerization during Ionic Liquid Pretreatment. *ACS Sus Chem Eng*, **5**(11), 10116-10127.
8. Duval, A., Vilaplana, F., Crestini, C., Lawoko, M. 2016. Solvent screening for the fractionation of industrial kraft lignin. *Holzforschung*, **70**(1), 11-20.
9. George, A., Brandt, A., Tran, K., Zahari, S.M.N.S., Klein-Marcuschamer, D., Sun, N., Sathitsuksanoh, N., Shi, J., Stavila, V., Parthasarathi, R. 2015. Design of low-cost ionic liquids for lignocellulosic biomass pretreatment. *Green Chem*, **17**(3), 1728-1734.
10. Hansen, C., Björkman, A. 1998. The ultrastructure of wood from a solubility parameter point of view. *Holzforschung*, **52**, 335-344.
11. Hansen, C.M. 2007. *Hansen solubility parameters: A user's handbook*. CRC Press, Boca Raton, FL.

12. Hatfield, R.D., Marita, J.M., Frost, K., Grabber, J., Ralph, J., Lu, F., Kim, H. 2009. Grass lignin acylation: p-coumaroyl transferase activity and cell wall characteristics of C3 and C4 grasses. *Planta*, **229**(6), 1253-1267.
13. He, H., Veneklaas, E.J., Kuo, J., Lambers, H. 2014. Physiological and ecological significance of biomineralization in plants. *Trend Plant Sci*, **19**(3), 166-174.
14. Jassal, V., Dou, C., Sun, N., Singh, S., Simmons, B.A., Choudhary, H. 2022. Finding values in lignin: A promising yet under-utilized component of the lignocellulosic biomass. *Front Chem Eng*, **4**, 1059305.
15. Gosselink, R.J.A. 2011. Lignin as a renewable aromatic resource for the chemical industry. PhD Thesis. Wageningen University, Wageningen, Netherlands.
16. Jungmeier, G., Hingsamer, M., Van Ree, R., Joergenson, H., Wellisch, M., Stichnothe, H., Rauch, R., de Jong, E., De Bari, I. 2013. 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 42.
17. Kalami, S., Arefmanesh, M., Master, E., Nejad, M. 2017. Replacing 100% of phenol in phenolic adhesive formulations with lignin. *J Appl Polym Sci*, **134**(30), 45124.
18. Laurichesse, S., Avérous, L. 2014. Chemical modification of lignins: Towards biobased polymers. *Prog Polym Sci*, **39**(7), 1266-1290.
19. 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.
20. Martin, J. 2021. Project Liberty: Launch of an Integrated Bio-Refinery with Eco-Sustainable and Renewable Technologies. Final Technical Report. POET-DSM Project Liberty, LLC. https://doi.org/10.2172/1866610
21. Meyer, J.R., Waghmode, S.B., He, J., Gao, Y., Hoole, D., da Costa Sousa, L., Balan, V., Foston, M.B. 2018. Isolation of lignin from Ammonia Fiber Expansion (AFEX) pretreated biorefinery waste. *Biomass Bioenerg*, **119**, 446-455.
22. Nielsen, J.B., Jensen, A., Schandel, C.B., Felby, C., Jensen, A.D. 2017. Solvent consumption in non-catalytic alcohol solvolysis of biorefinery lignin. *Sus Energ Fuel*, **1**(9), 2006-2015.
23. Pan, X., Gilkes, N., Kadla, J., Pye, K., Saka, S., Gregg, D., Ehara, K., Xie, D., Lam, D., Saddler, J. 2006. Bioconversion of hybrid poplar to ethanol and co-products using an

organosolv fractionation process: optimization of process yields. *Biotechnol Bioeng*, **94**(5), 851-61.

24. Ragauskas, A.J., Beckham, G.T., Biddy, M.J., Chandra, R., Chen, F., Davis, M.F., Davison, B.H., Dixon, R.A., Gilna, P., Keller, M., Langan, P., Naskar, A.K., Saddler, J.N., Tschaplinski, T.J., Tuskan, G.A., Wyman, C.E. 2014. Lignin Valorization: Improving Lignin Processing in the Biorefinery. *Science*, **344**(6185), 1246843.
25. Rossberg, C., Janzon, R., Saake, B., Leschinsky, M. 2019. Effect of process parameters in pilot scale operation on properties of organosolv lignin. *BioRes*, **14**(2), 4543-4559.
26. Sameni, J., Krigstin, S., Sain, M. 2017. Solubility of lignin and acetylated lignin in organic solvents. *BioRes*, **12**(1), 1548-1565.
27. Saulnier, B.K., Siahkamari, M., Singh, S.K., Nejad, M., Hodge, D.B. 2023. 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*, **71**(1), 592-602.
28. Schuerch, C. 1952. The Solvent Properties of Liquids and Their Relation to the Solubility, Swelling, Isolation and Fractionation of Lignin. *J Am Chem Soc*, **74**(20), 5061-5067.
29. Schutyser, W., Renders, T., Van den Bosch, S., Koelewijn, S.F., Beckham, G.T., Sels, B.F. 2018. Chemicals from lignin: an interplay of lignocellulose fractionation, depolymerisation, and upgrading. *Chem Soc Rev*, **47**(3), 852-908.
30. Shuai, L., Amiri, M.T., Questell-Santiago, Y.M., Héroguel, F., Li, Y., Kim, H., Meilan, R., Chapple, C., Ralph, J., Luterbacher, J.S. 2016. Formaldehyde stabilization facilitates lignin monomer production during biomass depolymerization. *Science*, **354**(6310), 329-333.
31. Shuai, L., Saha, B. 2017. Towards high-yield lignin monomer production. *Green Chem*, **19**(16), 3752-3758.
32. Singh, S., Varanasi, P., Singh, P., Adams, P.D., Auer, M., Simmons, B.A. 2013. Understanding the impact of ionic liquid pretreatment on cellulose and lignin via thermochemical analysis. *Biomass Bioenerg*, **54**, 276-283.
33. Singh, S.K., Dhepe, P.L. 2019. Lignin conversion using catalytic ionic liquids: understanding the role of cations, anions, and hammett acidity functions. *Ind Eng Chem Res*, **58**(47), 21273-21284.

34. Singh, S.K., Savoy, A.W., Yuan, Z., Luo, H., Stahl, S.S., Hegg, E.L., Hodge, D.B. 2019. Integrated Two-Stage Alkaline-Oxidative Pretreatment of Hybrid Poplar. Part 1: Impact of Alkaline Pre-Extraction Conditions on Process Performance and Lignin Properties. *Ind Eng Chem Res*, **58**(35), 15989-15999.
35. Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., Crocker, D. 2008. Determination of structural carbohydrates and lignin in biomass. NREL/TP-510-42618
36. Sturgeon, M.R., Kim, S., Lawrence, K., Paton, R.S., Chmely, S.C., Nimlos, M., Foust, T.D., Beckham, G.T. 2014. A mechanistic investigation of acid-catalyzed cleavage of aryl-ether linkages: implications for lignin depolymerization in acidic environments. *ACS Sus Chem Eng*, **2**(3), 472-485.
37. Sun, Z., Fridrich, B., de Santi, A., Elangovan, S., Barta, K. 2018. Bright Side of Lignin Depolymerization: Toward New Platform Chemicals. *Chem Rev*, **118**(2), 614-678.
38. Thoresen, P.P., Matsakas, L., Rova, U., Christakopoulos, P. 2020. Recent advances in organosolv fractionation: Towards biomass fractionation technology of the future. *Biores Technol*, **306**, 123189.
39. Venkatram, S., Kim, C., Chandrasekaran, A., Ramprasad, R. 2019. Critical assessment of the Hildebrand and Hansen solubility parameters for polymers. *J Chem Inf Model*, **59**(10), 4188-4194.
40. Vermaas, J.V., Crowley, M.F., Beckham, G.T. 2020. Molecular Lignin Solubility and Structure in Organic Solvents. *ACS Sus Chem Eng*, **8**(48), 17839-17850.
41. Wang, W., Lee, D.-J. 2021. Lignocellulosic biomass pretreatment by deep eutectic solvents on lignin extraction and saccharification enhancement: A review. *Biores Technol*, **339**, 125587.
42. Zhang, X., Zhu, J., Sun, L., Yuan, Q., Cheng, G., Argyropoulos, D.S. 2019. Extraction and characterization of lignin from corncob residue after acid-catalyzed steam explosion pretreatment. *Ind Crop Prod*, **133**, 241-249.
43. Zoia, L., Salanti, A., Tolppa, E.-L., Ballabio, D., Orlandi, M. 2017. Valorization of side-streams from a SSF biorefinery plant: Wheat straw lignin purification study. *BioRes*, **12**(1), 1680-1696.