

Insights into Structural Changes of Lignin toward Tailored Properties during Deep Eutectic Solvent Pretreatment

Zhu Chen, Xianglan Bai, Lusi A, Hanwen Zhang, and Caixia Wan*

Cite This: *ACS Sustainable Chem. Eng.* 2020, 8, 9783–9793

Read Online

ACCESS |



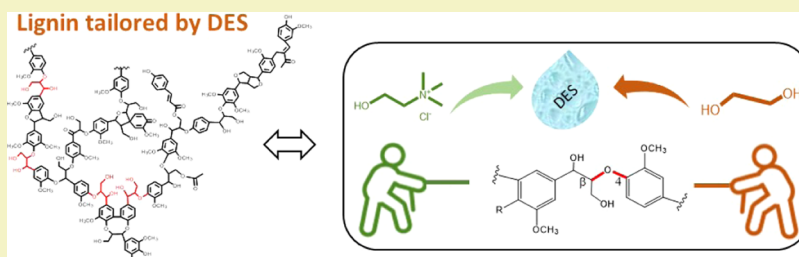
Metrics & More



Article Recommendations



Supporting Information



ABSTRACT: Biomass pretreatment enabling extraction of lignin with desirable properties (especially well-preserved β -O-4 linkages) while ensuring improved cellulose digestibility remains a challenge for sustainable biorefinery. This study aimed to address this challenge using a deep eutectic solvent (DES) comprising choline chloride and ethylene glycol (ChCl/EG) for switchgrass fractionation. Extensive characterization of extracted DES lignin was performed to understand how the lignin properties, including purity, molecular weight, thermal stability, and abundance of the β -O-4 linkages, were changed in the DESs under varied pretreatment conditions. Finely tuning the solvent system would steer DES-based biomass fractionation toward synchronizing the production of uncondensed lignin and digestible pulp. Appealing lignin properties, such as high-purity (91.4%), well-preserved β -O-4 linkages, and high volatility similar to that of cellulolytic enzyme lignin (CEL), is obtainable in this designer solvent system. At least 65% lignin can be removed, with the highest delignification reaching near 90% within 30 min. Overall, this versatile and tunable DES system is promising for lignocellulosic biomass processing and would fit into different biorefinery configurations toward value-added valorization of lignocellulosic biomass.

KEYWORDS: lignocellulosic biomass, lignin, deep eutectic solvent, pretreatment, biorefinery

INTRODUCTION

Lignocellulosic biomass, primarily composed of cellulose, hemicellulose, and lignin, is one of the most abundant renewable feedstocks to substitute petroleum sources for the production of fuels, chemicals, and materials. In a conventional biorefinery, first and foremost is cellulose conversion, which entails biomass fractionation to reduce the inherent recalcitrance of lignocellulosic biomass prior to sugar release. Industrially implemented pretreatments are typically based on acid, alkali, or water (steam), featuring low-cost and high-sugar output.^{1,2} However, lignin is often generated with poor quality and undesirable properties, leading to low valorization potential. From both economic and environmental perspectives, lignin valorization is considered essential for the development of sustainable biorefinery.^{3,4} In this context, the major challenge lies in efficient extraction of lignin with desirable properties while ensuring cellulose digestibility.⁵

Lignin-first biomass fractionation has recently been proposed to extract lignin with high reactivity and uncondensed structure toward high-yield aromatic monomer production.⁶ The preservation of alkyl-aryl ether bonds in extracted lignin would favor lignin depolymerization and

maximize its valorization potential for platform and specialty chemicals, especially aromatics.⁷ However, fractioning lignin from lignocellulosic biomass breaks down not only glycosidic side chains but also phenolic alkyl-aryl ether bonds (e.g., β -O-4). As a result, lignin is solubilized and partially depolymerized. Reactive intermediates resulting from the cleavage of β -aryl ether bonds can undergo the coupling reactions to form C-C bonds, yielding condensed lignin structure with high breakdown energy.⁸ Active stabilization of ether bonds in lignin via formaldehyde during pretreatment was reported to prevent lignin condensation, which led to near theoretical monomer yields via catalytic depolymerization.⁷ Similarly, alcohols (e.g., methanol, ethanol, and butanol) were reported to react with benzylic carbocation ($C\alpha$) formed during organosolv fractionation of lignocellulosic biomass

Received: March 23, 2020

Revised: May 22, 2020

Published: June 4, 2020



Table 1. Pretreatment Conditions and DES Compositions

pretreatment	DES ^a	water ^b (wt %)	acid ^c (wt %)	reaction temperature (°C)	reaction time (min)	pulp code ^d	lignin code
R1	ChCl/EG (1:2)	0	0.5	110	30	P1	L1
R2	ChCl/EG (1:2)	0	1.0	110	30	P2	L2
R3	ChCl/EG (1:2)	0	0.5	130	30	P3	L3
R4	ChCl/EG (1:2)	0	1.0	130	30	P4	L4
R5	ChCl/EG (1:2)	0	0.5	150	30	P5	L5
R6	ChCl/EG (1:2)	0	1.0	150	30	P6	L6
R7	ChCl/EG (1:2)	20	1.0	110	30	P7	L7
R8	ChCl/EG (1:2)	20	1.0	130	30	P8	L8
R9	ChCl/EG (3:2)	20	0.5	110	30	P9	L9
R10	ChCl/EG (3:2)	20	1.0	110	30	P10	L10
R11	ChCl/EG (3:2)	10	0.5	110	30	P11	L11
R12	ChCl/EG (3:2)	10	1.0	110	30	P12	L12

^aRatios in parenthesis stand for the molar ratios of ChCl to EG. ^bWater content refers to that in the DESs prior to pretreatment. ^cConcentrated sulfuric acid (98 wt %) was added to neat DESs prior to pretreatment. ^dPulp is the cellulose-rich solid residues recovered after the pretreatments.

under mild conditions and thus minimize C–C bond formation.^{9,10} γ -Valerolactone as a polar aprotic organic solvent was also reported to generate high-purity lignin with well-preserved β -O-4 ether bonds.³ While these solvent systems enable the extraction of uncondensed lignin, side reactions between cellulose/hemicellulose and solvent/active stabilization agents are often observed. This complicates the overall processes since post-treatment is required to realize the effective conversion of cellulose/hemicellulose into sugars or other products. Direct aromatic monomer production from raw lignocellulosic biomass not only gives rise to poor cellulose digestibility but also has many other complicated issues, such as catalyst recycling and cellulose-rich solid recovery.^{11–14} Instead of one-pot lignin extraction/depolymerization for monomer production, sequential organosolv fractionation was reported to tailor lignin chemistry and reactivity,¹⁵ but process complexity is a common issue with sequential process configuration.

As designer solvents, deep eutectic solvents (DESs) are emerging for biomass pretreatment and lignin solubilization/fractionation.^{16–19} Among various DESs, polyol-based DESs (i.e., glycerin (Gly), ethylene glycol (EG)) at the presence of a small amount of acids (e.g., Brønsted acid) appear to be the most effective for lignin extraction and improving cellulose digestibility even at high solid loadings. Our prior study reported that choline chloride (ChCl)/Gly added with H₂SO₄ (0.9 wt %) was able to remove 76.6% lignin while generating cellulose pulp with 89% glucose yield.¹⁷ Xia et al.²⁰ also reported that the ChCl/Gly pretreatment at the presence of Lewis acid (i.e., AlCl₃) showed near-complete lignin removal after 4 h pretreatment at 120 °C. Our work on other polyol-based DESs also showed that acidified ChCl/EG removed 87% of lignin and 82% of xylan from switchgrass when the pretreatment was conducted at 130 °C for 30 min.¹⁹ Due to the synergy between acid and DES, acidified polyol-based DESs outcompete either acid solution only or neat polyol-based DESs. More strikingly, this category of DESs is capable of preserving β -aryl ether bonds.^{17,19} Our prior studies also implied that water present in such DESs can affect lignin properties, especially abundance of β -aryl ether bonds.¹⁸ Such intriguing capabilities of polyol-based DESs make them have great potential to overcome the aforementioned limitations of conventional and lignin-first biomass fractionations. Moreover, diverse combinations of DES constituents allow tuning their solvent characteristics for the production of tailor-made lignin

suitable for not only monomer production but also other value-added applications (e.g., thermoplastics, antioxidants, and carbon fibers).

The main objective of this study was to understand: (1) how lignin chemistry and structure were modified toward desirable properties by the ChCl/EG pretreatment and (2) how lignin extraction impacted biomass recalcitrance and cellulose digestibility. To answer these questions, DES lignin extracted from switchgrass was evaluated for detailed chemistry, molecular weight distribution, thermal stability, and monomer production as a function of pretreatment conditions and ChCl/EG compositions (i.e., water content, the ratio of ChCl/EG). The results provided insights into chemical and structural changes of lignin in response to a varied ratio of DES components and pretreatment conditions. The present work will serve as a valuable guidance on realizing the production of both valorizable lignin and highly digestible pulp using a tunable DES solvent system.

■ EXPERIMENTAL SECTION

Feedstocks. Switchgrass was collected from the South Farm at the University of Missouri in Columbia, Missouri. It was air-dried, ground through 2 mm screen, and stored in an airtight container. Raw switchgrass contained 34.5% cellulose, 23.5% xylan, and 20.4% lignin. Hydrolytic enzymes (Cellic CTec2 and HTec2) were kindly provided by Novozyme (Franklinton, NC). All of the chemicals were purchased from Fisher Scientific (Hampton, NH).

DES Synthesis, Pretreatment, and Lignin Recovery. The DESs were synthesized according to the method described in our prior study.¹⁹ Briefly, a neat DES was composed of ChCl and EG with a molar ratio of 1:2 or 3:2. It was synthesized by mixing ChCl (predried at 80 °C) and EG in a sealed glass bottle at 60 °C with continuous stirring (200 rpm) until a clear liquid was obtained and then stored in a desiccator prior to use. Aqueous ChCl/EG was prepared by adding 10 or 20 wt % water to a neat DES. An acidified DES contained either 0.5 or 1.0 wt % H₂SO₄, which was prepared by adding concentrated H₂SO₄ (98 wt %) to a neat or aqueous ChCl/EG DES. The pretreatment conditions and solvents tested are listed in Table 1. In a typical run, 5 g of switchgrass was mixed with 45 g of acidified neat/aqueous ChCl/EG in a 250 mL sealed glass bottle reactor and then heated in a preheated oil bath for 30 min with continuous stirring at 200 rpm. After pretreatment, the bottle was removed from the oil bath and cooled to below 50 °C. The pretreatment slurry was then added with 100 mL of acetone–water mixture (50:50, v/v) and vacuum-filtered. The solid residue was collected, washed with 50 mL of acetone–water mixture twice, and then stored at –20 °C for further use. The filtrate collected from slurry separation and solid washing was combined, and acetone was

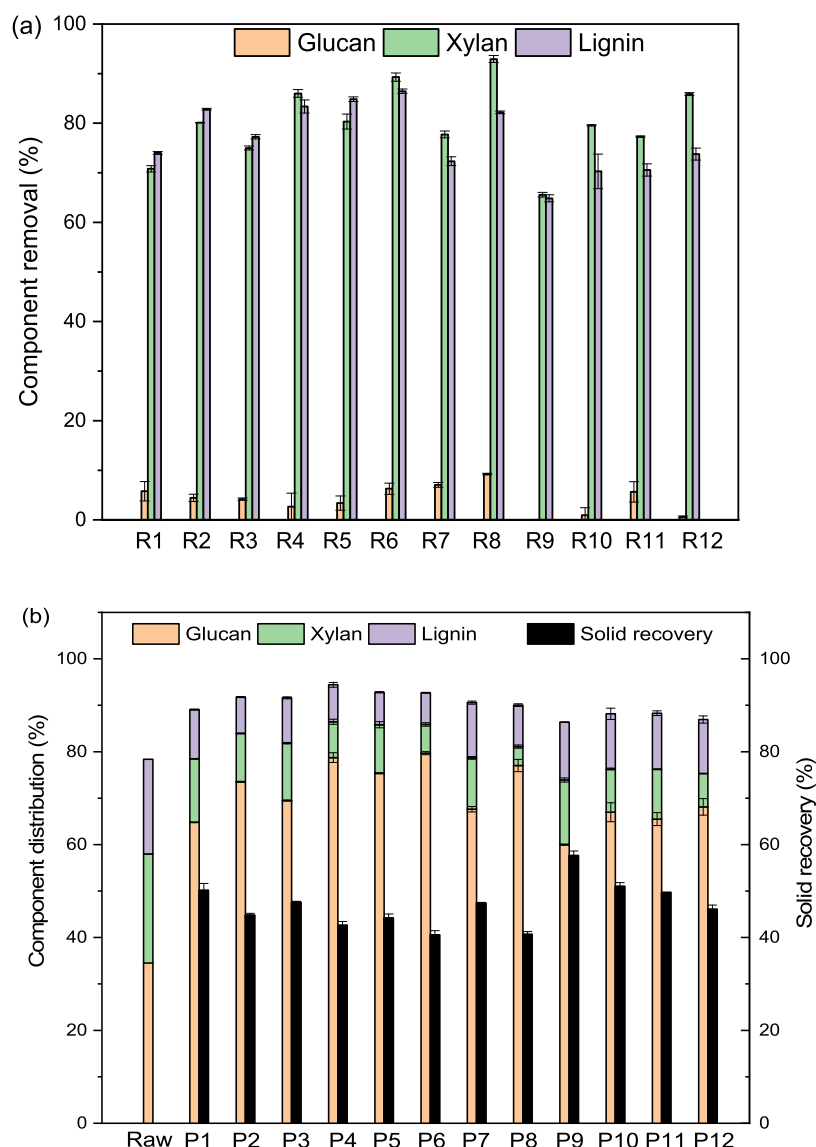


Figure 1. Switchgrass component distribution and component removal upon pretreatment: (a) major component removal and (b) major component distribution.

removed under reduced pressure. Lignin precipitated out upon acetone removal was collected via centrifugation at 12 000g for 5 min, then washed with deionized (DI) water four times, and further dried at 45 °C for 12 h. The dried lignin was stored in a desiccator at room temperature prior to further use.

Analytical Methods. Biomass composition was determined following the standard NREL analytical procedure.²¹ Lignin purity and monomeric sugar concentrations were determined according to our prior study.¹⁹ The detailed procedures were described in Supporting Information (SI).

Two-dimensional heteronuclear single quantum coherence (2D HSQC) NMR spectra were acquired on a Bruker AVIII 800 MHz spectrometer using Bruker supplied pulse sequence named hsqcetgp at 50 °C throughout the NMR acquisition.¹⁹ The spectral width was 15.4 ppm for ¹H and 170.0 ppm for ¹³C. A total of 2048 (¹H) complex points were acquired with 256 (¹³C) time increments. The ¹J_{CH} was set to 145 Hz, which is the average one-bond C–H coupling constant. The number of scans was 48, and the repetition delay was 1.5 s. The ¹³C dimension was zero-filled to 2048 points before the data was subjected to a sine-squared window function (sifted 90°) and Fourier transformation. The chemical shift axes were calibrated with respect to the solvent signals (2.49 ppm for residual proton and 39.5 ppm for ¹³C). Semiquantification of the β–O–4 linkages was

performed using a method as described in a previous study.²² Both cellulosytic enzyme lignin (CEL) and lignin extracted during the pretreatment were examined using 2D HSQC NMR. The method used for the preparation of cellulosytic enzyme lignin (CEL) was described in detail in SI.

As detailed in SI, the lignin samples were acetylated to increase their solubility in tetrahydrofuran (THF) prior to gel permeation chromatography (GPC) analysis. A Dionex Ultimate 3000 series high-performance liquid chromatography (HPLC) equipped with a Shodex refractive index (RI) detector and diode array detector (DAD) was used to perform GPC analysis. Two Agilent PLgel 3 μm 100 Å 300 × 7.5 mm² (p/n PL1110-6320) were connected in series. THF with a flow rate of 1 mL/min at 25 °C was used as the eluent. The GPC column was calibrated with polystyrene standards with a molecular weight ranging from 162 to 45 120 g/mol. An ultraviolet wavelength of 254 nm was used to detect lignin molecules.

A Mettler Toledo thermogravimetric analysis (TGA) system (TGA/DSC 1 STAR^e system) was used to pyrolyze lignin samples. Each time, approximately 20 mg of the sample was pyrolyzed in nitrogen flow from room temperature to 105 °C at 10 °C/min and then hold for 40 min to remove the moisture. The sample was then continuously pyrolyzed to 900 °C.

Fast pyrolysis of lignin was conducted using a Frontier Lab Tandem micropyrolyzer (Tx-3050 TR) with an autosampler. The configuration of the pyrolyzer can be found elsewhere.²³ Briefly, the pyrolyzer consists of two furnaces connected in series. During the tests, a sample cup containing approximately 500 μg of lignin was pyrolyzed in the first furnace at 500 $^{\circ}\text{C}$ using helium as the carrier gas. The vapor then passed the second furnace kept at 350 $^{\circ}\text{C}$ prior to entering online Agilent 7890B GC/MS-FID. In the gas chromatography (GC), helium with a flow rate of 156 mL/min was used as the purge gas and the split ratio at the front inlet was set at 50:1. The temperature of the GC oven was kept at 40 $^{\circ}\text{C}$ for 3 min and then gradually increased to 280 $^{\circ}\text{C}$ at a heating rate of 6 $^{\circ}\text{C}/\text{min}$. Finally, the GC was held at 280 $^{\circ}\text{C}$ for 3 min. The aromatic monomers were identified by the mass spectrometry (MS) and then quantified by the flame ionization detector (FID). The columns used in the MS and FID were Phenomenex ZB 1701 (60 m $\times$ 0.250 mm $\times$ 0.250 μm). Calibration curves were created by injecting the authentic chemicals into the GC. The pyrolysis tests were triplicated for each lignin sample, and the averaged results were reported.

RESULTS AND DISCUSSION

Lignin Solubilization. The compositional change of switchgrass in response to ChCl/EG composition and pretreatment condition is depicted in Figure 1. We first tested the effects of acid concentration and temperature on the removal of major cell wall components (R1–6 in Figure 1a). ChCl/EG (1:2) removed more than 70% lignin and xylan from switchgrass under an acidic condition, which only took 30 min at 110 $^{\circ}\text{C}$. With 0.5 wt % H_2SO_4 concentration, increasing the temperature from 110 to 150 $^{\circ}\text{C}$ led to an increase in lignin removal from 74.0 to 84.8% and an increase in xylan removal from 70.8 to 80.3% (R1, R3, and R5). A similar trend was observed with 1.0 wt % H_2SO_4 concentration (R2, R4, and R6). On the other hand, when the temperature was kept the same, a higher acid concentration led to more xylan and lignin removal across all of the tests (R1–6). Especially at a lower temperature (110 $^{\circ}\text{C}$), acid had more effects on lignin removal (R1 and R2). In contrast to lignin removal, xylan removal showed similar increases (9–11%) across the three temperatures (R1–6).

Water can tune the physicochemical properties of DESs (e.g., viscosity, basicity, and polarity) and improve the performance of DESs during biomass pretreatment.^{23–25} We thus investigated the effects of water addition on the pretreatment performance by adding 20 wt % water to ChCl/EG (1:2) (R7 and R8). When the temperature was 110 $^{\circ}\text{C}$, aqueous ChCl/EG (1:2) gave 77.7% xylan and 72.3% lignin removal, which were 2.4 and 10.5% lower than its counterpart with no water added (R7 vs R2). By increasing the temperature to 130 $^{\circ}\text{C}$, the switchgrass pretreated by aqueous ChCl/EG (1:2) demonstrated a slightly lower lignin removal than that by the neat ChCl/EG (1:2) pretreatment but 7% higher xylan removal (R8 vs R4). It appeared that water addition decreased delignification in ChCl/EG (1:2) during pretreatment. Water is not favorable to lignin dissolution as lignin–water interactions are not energetically favorable.²⁶ It has been reported that strong hydrogen-bond interactions between EG and free hydroxyl groups present in lignin enhance lignin dissolution.²⁷ Water could be deemed as a special “hydrogen-bond donor (HBD)”, which interacts with DESs via hydrogen-bonding interaction.²⁸ The presence of water may compete with the interaction between ChCl/EG and lignin. As a result, less lignin was consequently dissolved in the solvent system.

Finally, we investigated the effects of the increasing molar ratio of the hydrogen-bond acceptor (HBA) (i.e., ChCl) to HBD (i.e., EG) on the pretreatment performance (R9–12). Since ChCl/EG (3:2) was not able to maintain a stable liquid state at room temperature, 20% of water was added to this mixture before pretreatment. The lignin and xylan removal by 80% ChCl/EG (3:2) pretreatment (R10) were similar to that by 80% ChCl/EG (1:2) pretreatment under the same condition (1.0 wt % H_2SO_4 , 110 $^{\circ}\text{C}$) (R7). Reducing the water content from 20 to 10 wt % in aqueous ChCl/EG (3:2) resulted in a 5% higher xylan removal and 3.5% higher lignin removal (R12). Consistent with the results using neat ChCl/EG for pretreatment, when acid concentration was decreased from 1.0 to 0.5 wt % in 80 and 90% ChCl/EG (3:2), less xylan and lignin were removed (R10 vs R9 and R12 vs R11). It was interesting to note that under the same pretreatment condition (1.0 wt % H_2SO_4 , 110 $^{\circ}\text{C}$), 90% ChCl/EG (3:2) gave a 6% higher xylan removal but 9% lower lignin removal than 100% ChCl/EG (1:2) (R12 vs R2). A similar trend was observed with the pretreatments using 80% ChCl/EG (1:2) and 100% ChCl/EG (1:2) at 130 $^{\circ}\text{C}$ and 1.0 wt % H_2SO_4 (R8 vs R4). Thus, by adjusting the HBA/HBD ratio and water content in DESs, it is possible to control xylan and lignin removal. Resultant cellulose pulp may find their applications in cellulose-based functional materials requiring different amounts of lignin or xylan to tailor their properties.²⁹

Under all of the scenarios, more than 90% cellulose was preserved (Figure 1a), indicating that acidified ChCl/EG selectively solubilized lignin and hemicellulose. Due to significant removal of lignin and hemicellulose during pretreatment, the recovered solid residue showed an enriched cellulose content ranging from 60 to 80% (Figure 1b). The highest cellulose content of 79.7% was observed with the pulp P6 (100% ChCl/EG (1:2), 1.0 wt % H_2SO_4 , 150 $^{\circ}\text{C}$) followed by P4 (100% ChCl/EG (1:2), 1.0 wt % H_2SO_4 , 130 $^{\circ}\text{C}$) with 78.4% cellulose content. Residual lignin and xylan accounted for 13–16% of these pulp samples. It should be noted that yet-to-be-identified impurities in the pulp were significantly lower than that in raw switchgrass (only 5.6–7.3 vs 1.6%). This finding further corroborated that the proposed DES pretreatment can realize clean fractionation with highly selective removal of structural and nonstructural components over cellulose. As discussed in the supporting Results section in the SI, the cellulose pulp can be easily saccharified into fermentable sugars with more than 90% even at 20% high-solid loading (Figure S1). In terms of the solubilized hemicellulose fraction, our previous studies have demonstrated that it can be facilely upgraded into furfural with a high yield.^{19,30} Previous studies also reported that polyol-based DESs are biocompatible, and therefore, a one-pot process integrating pretreatment, enzymatic hydrolysis, and fermentation for biochemical production would be feasible upon conditioning of pretreatment slurry.^{17,31,32} In the future study, it is worthy of exploring such a one-pot process for cellulose conversion toward sustainable integrated biorefinery.

Lignin Purity. High-purity lignin is desired for many applications, such as polyurethane, adhesives, and epoxies.³³ As shown in Table 2, for the pretreatment using neat ChCl/EG (1:2), the purity of the lignin (L1, L3, and L5) increased from 78.7 to 87.2% upon temperature increase from 110 to 150 $^{\circ}\text{C}$ when acid was at 0.5 wt %. The increase in lignin purity could be partly attributed to more carbohydrate removal from lignin at a higher temperature (Table 2). With a higher acid (1.0 wt

Table 2. Composition and Purity of the Extracted Lignin

lignin code	lignin purity (%)	carbohydrates in lignin (%) ^a	EG in lignin (%) ^b
L1	78.66 ± 1.23	4.11 ± 0.45	7.17 ± 0.25
L2	82.94 ± 0.71	1.06 ± 0.17	7.12 ± 0.19
L3	84.62 ± 0.10	2.22 ± 0.03	6.98 ± 0.30
L4	88.35 ± 0.22	1.13 ± 0.34	6.58 ± 0.14
L5	86.90 ± 0.31	1.29 ± 0.09	7.54 ± 0.19
L6	87.22 ± 0.02	1.42 ± 0.37	6.76 ± 0.14
L7	86.46 ± 0.84	0.66 ± 0.00	5.76 ± 0.04
L8	91.36 ± 0.25	0.36 ± 0.00	4.34 ± 0.11
L9	81.52 ± 2.78	2.61 ± 0.25	2.98 ± 0.04
L10	87.02 ± 1.79	0.99 ± 0.04	3.07 ± 0.00
L11	86.93 ± 0.40	0.99 ± 0.07	3.67 ± 0.01
L12	90.42 ± 0.31	0.44 ± 0.01	3.60 ± 0.09

^aPercentages of the impurities as carbohydrates or EG in the recovered raw DES lignin.

%), lignin purity showed a similar increasing trend upon temperature increase from 110 to 130 °C, but further increasing temperature to 150 °C did not increase the purity. When the pretreatment temperature was kept at 110 or 130 °C, increasing acid concentration from 0.5 to 1 wt % led to an increase in lignin purity due, in part, to more carbohydrate removal with a higher acid concentration (Table 2). However, the acid effect on lignin purity diminished at a higher temperature (150 °C), and a similar carbohydrate content was shown for both lignin (L5 and L6). Interestingly, it was found that the lignin recovered from the neat ChCl/EG (1:2) pretreatment under all of the conditions contained an appreciable amount of EG (Table 2). As mentioned above, EG can react with many functional groups in depolymerized lignin. For example, EG can react with the carbonyl groups on depolymerized lignin to form acetals (Figures S3 and S4). Although such reactions introduced EG as an “impurity” to lignin, it could also stabilize reactive intermediates and prevent

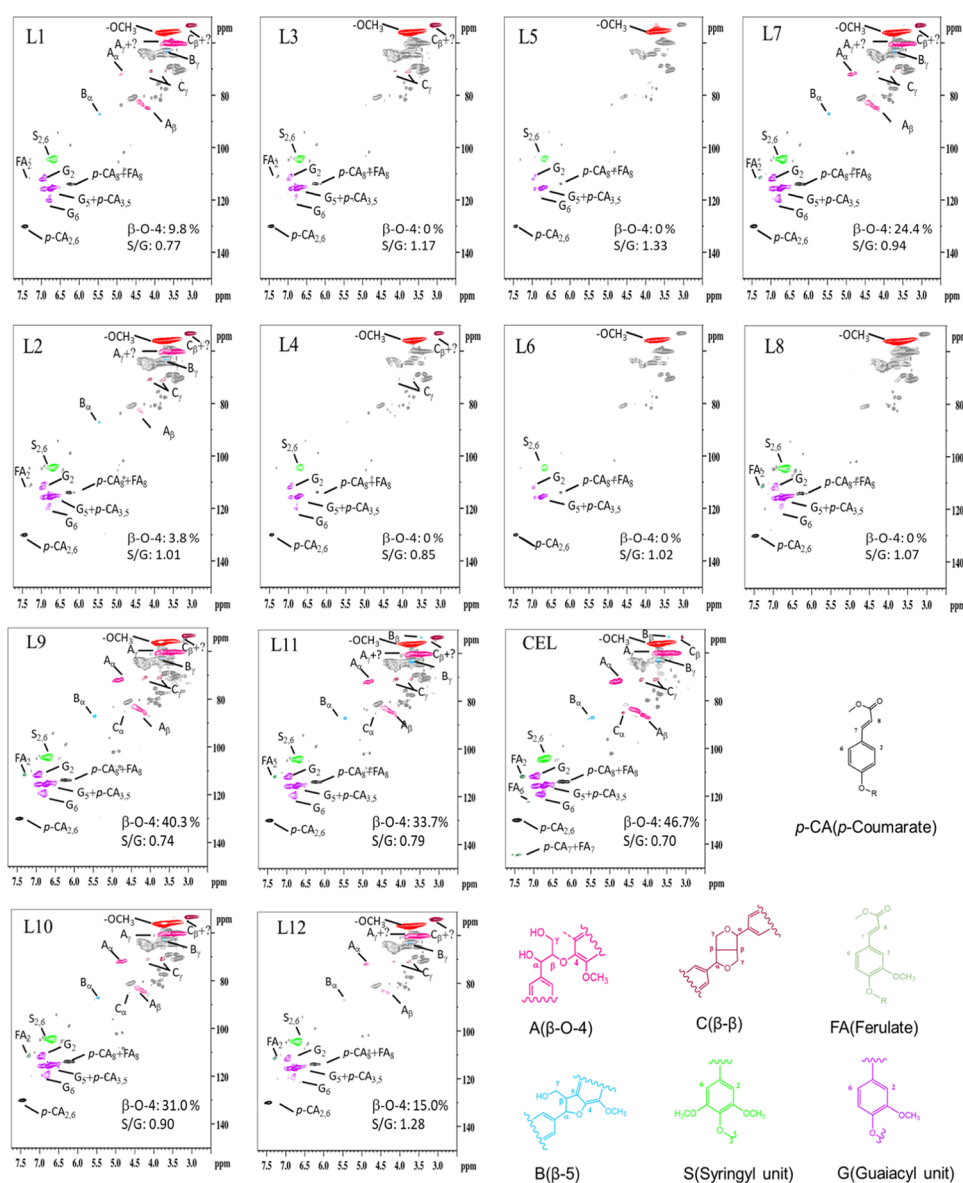


Figure 2. Two-dimensional HSQC NMR spectra of DES lignin. CEL: cellulolytic enzyme lignin; L1–12 represent the lignin samples listed in Table 1.

the repolymerization reactions of depolymerized lignin (Figure S4).³⁴

Water addition to ChCl/EG (1:2) reduced both EG and carbohydrate contents in the recovered lignin. As a result, the lignin (L7 and L8) from pretreatment at 110 and 130 °C using aqueous ChCl/EG (1:2) showed a purity of 86.46 and 91.36%, respectively, which were 3–4% higher than their counterparts (L2 and L4) from the pretreatment using neat ChCl/EG (1:2) (Table 2). The increase in lignin purity could be due to the reduced reactions between EG and lignin at the presence of water. Added to that, water addition reduced DES viscosity and facilitated the mass transfer, which, in turn, would also enhance lignin purity by dissolving more carbohydrates from lignin into DES.²³

Two-Dimensional HSQC NMR Analysis. To understand the structural and chemical changes of lignin in response to pretreatment condition and DES composition, cellulolytic enzyme lignin (CEL) and lignin extracted during the pretreatment were examined using 2D HSQC NMR. Main ¹³C–¹H cross signals in the HSQC spectra of switchgrass lignin were assigned according to the prior studies (Figure 2).^{35,36} For CEL, the β -O-4 units (A) were the major peak shown in the aliphatic regions, while the β -5 (B) and β - β (C) units were present at smaller contents. Compared with CEL, lignin recovered from the pretreatment using neat ChCl/EG (1:2) under different conditions demonstrated a varying extent of the cleavage of the β -O-4 linkages. Lignin extracted at 110 °C with 0.5 wt % acid showed the most preserved β -O-4 linkages (Figure 2 and Table S1), while a higher acid (1.0 wt %) at the same temperature led to more cleavage of the β -O-4 linkages. When the temperature was increased to 130 °C or above, the complete cleavage of the β -O-4 linkages in the lignin (L3–6) occurred despite the acid concentration used in the pretreatment. These results were in accordance with those from prior studies, which showed that the cleavage of the β -O-4 linkages was more significant under severer conditions.^{37,38}

When 20 wt % of water was added to ChCl/EG (1:2), the lignin (L7) obtained from the condition of 110 °C and 1.0 wt % H₂SO₄ showed a more CEL-like structure and a higher percentage of the β -O-4 linkages than the one (L2) from the neat ChCl/EG (1:2) pretreatment under the same condition (Figure 2 and Table S1). As illustrated in Figure S3, the acid-catalyzed cleavage of the β -O-4 linkages is initiated by protonation of the α -position, followed by dehydration and further breakdown of ether bonds. More preserved β -O-4 linkages in L7 could be largely due to the reduced catalytic activity of acidic proton upon water addition.³⁹ Increasing the temperature to 130 °C made proton more active and available in aqueous ChCl/EG (1:2), leading to complete cleavage of the β -O-4 linkages in lignin (L8). Such temperature effect was also found from the neat ChCl/EG (1:2) pretreatment as discussed above.

Interestingly, when increasing the molar ratio of ChCl/EG from 1:2 to 3:2, the recovered lignin (L10) demonstrated a much higher abundance of the β -O-4 linkages than L7 under the same pretreatment condition (110 °C, 1.0 wt % H₂SO₄) (31.0% for L10 vs 24.4% for L7) (Figure 2 and Table S1). Decreasing the acid concentration to 0.5 wt % at 110 °C for 80% ChCl/EG (3:2) resulted in lignin (L9) with an even higher abundance of the β -O-4 linkages (40.3%) (Figure 2 and Table S1). When the water content was reduced from 20 to 10 wt % for ChCl/EG (3:2), the abundance of the β -O-4

linkages of the lignin (L11 and L12) extracted at 110 °C with 0.5 and 1.0 wt % acid was decreased to 33.7 and 15.0%, respectively (Figure 2 and Table S1), but still much higher than that of both L1 and L7 extracted under the same conditions. It appeared that a higher ChCl content was beneficial to the preservation of the β -O-4 linkages. A prior study revealed that halide anions, such as Cl[−] and Br[−], can stabilize the carbocation formed.⁴⁰ Plausibly, one mechanism for better preservation of the β -O-4 linkages during pretreatment with a higher molar ratio of ChCl to EG is the enhanced stabilization of the β -O-4 linkages with the increased Cl[−] concentration (Figure S5). Further study is needed to understand and validate the stabilization mechanisms of the β -O-4 linkages in ChCl/EG with varying compositions.

The proposed aqueous ChCl/EG system had the comparable capability of delignification and preservation of the β -O-4 linkages with other pretreatment solvents, such as methanol and γ -valerolactone although the mechanisms involved may be different.^{9,41} The cleavage of the β -O-4 linkages led to the formation of reactive intermediates, which undergo the coupling to form C–C bonds with higher breakdown energy (Figure S3).⁸ The preservation of the β -O-4 linkages is critical for the high-yield production of aromatic monomers via lignin depolymerization.⁴² It should also be noted that the delignification and digestibility of cellulose pulp were not compromised significantly with better retention of the β -O-4 linkages during some pretreatments (Figures 1 and S1 and Table S1). For example, the pulp P1 and P11 resulting from the pretreatments at 110 °C with 0.5% acid using 100% ChCl/EG (1:2) and 90% ChCl/EG (3:2), respectively, gave similar glucose yields of about 80% and similar delignification of about 70% (Figure S1a), while L11 had 24% more β -O-4 linkages than L1 (Table S1). In contrast, for some other pretreatment (e.g., alkali pretreatment, alcohol pretreatment),^{43,44} the preservation of the more β -O-4 linkages is often at the expense of delignification efficiency as a much milder condition is required.

In the aromatic region, the cross signals for syringyl (S), guaiacyl (G), ferulate (FA), and *p*-coumarate (*p*-CA) units are predominant in CEL (Figure 2). Compared with CEL, the lignin (L1–6) recovered from the neat ChCl/EG (1:2) pretreatment demonstrated a shrinkage of contours for these units (Figure 2). The reduced intensity of the S and G peaks was more evident in L5 and L6 than in L1 and L2 (Figure 2). This was probably due to a more condensed structure formed in L5 and L6 under severer conditions as any condensation reaction occurring at 2-, 5-, and 6-positions could make this position lack of hydrogen and undetectable by the HSQC NMR.³⁸ The FA units were still visible in L1–3, but absent in L4–6 (Figure 2 and Table S1). In grass lignin, FA cross-links lignin and hemicellulose via the formation of ether and ester linkages.^{45,46} The disappearance of FA suggested the complete solubilization of FA in ChCl/EG under severer conditions.^{8,47} In terms of the lignin (L7) recovered from aqueous 80% ChCl/EG (1:2) pretreatment at 110 °C and 1.0 wt % H₂SO₄, the peaks for S and G showed a higher intensity than that in L2 extracted under the same condition. This is probably because in L7, the β -O-4 interunit linkages were less cleaved, which reduced the condensation reactions occurring at 2-, 5-, and 6-positions in the S and G units. Likewise, L9–11 with a higher extent of the β -O-4 linkages also demonstrated a higher peak intensity for the S and G units.

Molecular Weight Distributions of Lignin. The molecular weight distributions of the lignin extracted under different pretreatment conditions are presented in Figure 3.

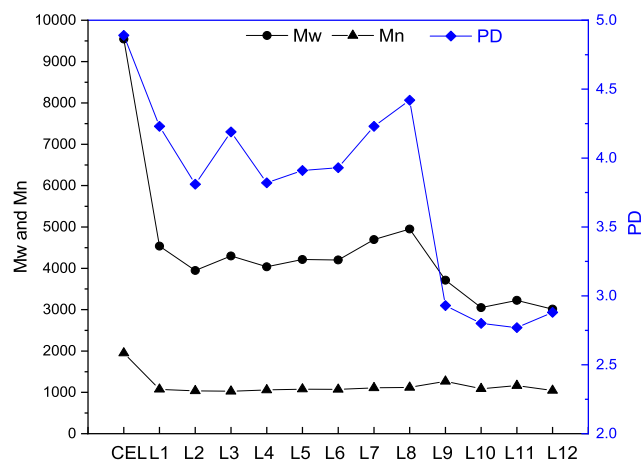


Figure 3. Molecular weight distribution of DES lignin. CEL: cellulytic enzyme lignin; L1–12 represent the lignin samples listed in Table 1.

Compared to CEL, ChCl/EG-extracted lignin had reduced weight average molecular weights (M_w), attributed to partial depolymerization of lignin during delignification. The M_w of the ChCl/EG-based lignin was found to correlate with the severity of the pretreatment conditions. Among the lignin extracted using the neat ChCl/EG (1:2) pretreatments (L1–6), L1 (110 °C, 0.5 wt % H_2SO_4) had the highest M_w , due to its mildest pretreatment condition and a lower degree of lignin fragmentation. Increasing pretreatment temperature or acid concentration lowered the M_w of the extracted lignin. The polydispersity (PD) of the lignin also decreased with decreasing M_w , indicating their narrower molecular distributions. The effect of acid concentration was more pronounced when the pretreatment temperature was below 150 °C. When the pretreatment temperature increased to 150 °C, no significant differences were found between L5 (150 °C, 0.5 wt % H_2SO_4) and L6 (150 °C, 1 wt % H_2SO_4) in terms of their M_w 's and PDs.

Compared to L2 (110 °C, 1.0 wt % H_2SO_4) and L4 (130 °C, 1.0 wt % H_2SO_4), their respective counterpart lignin (L7 and L8) extracted using aqueous 80% ChCl/EG (1:2) pretreatments had both increased M_w and PD. Their higher M_w and PD could result from the net effects of lignin depolymerization and repolymerization in aqueous ChCl/EG (1:2), which was more complex than that in the neat DES. Water can play a dual role in lignin transformation in aqueous ChCl/EG. On the one hand, due to less reactive acidic proton in aqueous DES compared to a neat DES environment, water addition can weaken the acid-catalyzed bond cleavages involved in delignification and lignin depolymerization, leading to lesser degrees of delignification and lignin depolymerization as well as limited cleavage of the β -O-4 bonds (Tables 2 and S1). On the other hand, water may prevent EG from stabilizing depolymerized lignin (Figure S4),³⁴ making such lignin fragments repolymerize into a condensed lignin structure (Figure 2 and Table S2), which was also suggested by the TGA profiles and pyrolysis results of L7 and L8.

The M_w and PD of L10 (80% ChCl/EG (3:2), 110 °C, 1.0 wt % H_2SO_4) were both lower than those of L7 (80% ChCl/

EG (1:2), 110 °C, 1.0 wt % H_2SO_4) as a result of the higher molar ratio of ChCl/EG during the pretreatment. Considering that L10 preserved a much higher content of the β -O-4 linkages than L7, this result is somehow unexpected. L9 (80% ChCl/EG (3:2), 110 °C and 0.5 wt % H_2SO_4) also had lower M_w and PD than L1 (100%ChCl/EG (1:2), 110 °C and 0.5 wt % H_2SO_4), despite that the presence of water in ChCl/EG reduces the reactivity of acid protons for depolymerization. Both M_w and PD were substantially lower with L9 and L10, suggesting that the pretreatments with a higher molar ratio of ChCl/EG not only increased the extent of lignin depolymerization, but also promoted selective bond cleavages to produce more homogeneous lignin rich in the β -O-4 linkages. As described above, Cl^- ions in the solvent could stabilize the carbocation formed during depolymerization and thus preserve more β -O-4 linkages and protect depolymerized lignin fragments from repolymerization to some extent (Figure S5). As a result, less condensed lignin structure could be obtained. Compared to L9, both M_w and PD decreased in L11 (90% ChCl/EG (3:2), 110 °C and 0.5 wt % H_2SO_4), suggesting that lignin depolymerization was enhanced with the acidified ChCl/EG (3:2) with a lower water content due to higher reactivity of acidic protons. On the other hand, the content of the β -O-4 linkages decreased in L11 compared to L9, implying that the presence of more water was beneficial in increasing the content of the β -O-4 linkages in the lignin. As the acid concentration increased to 1.0 wt % in ChCl/EG (3:2), the water content had minor effects on M_w and PD of the extracted lignin (L10 vs L12).

Thermal Stability and Volatility of Lignin. Thermal stability and volatility of lignin can be evaluated by TGA analysis. In general, higher thermal stability and higher solid residue are preferred if lignin is used in composite materials and other biobased carbon applications. On the other hand, higher volatility and lower solid residue are desired if lignin is used as a precursor feedstock of biofuels and chemicals. The results of TGA analysis are given in Figure 4. CEL showed the highest volatility and produced the lowest amount of solid residue upon pyrolysis up to 900 °C (Figure 4a). The abundant aryl ether linkages in CEL are susceptible to thermal decomposition, which contribute to its low thermal stability and high volatility. In contrast, all of the neat ChCl/EG lignin (L1–6) showed increased thermal stability and decreased volatility due to their more thermally recalcitrant structures. The formation of free-radical intermediates is an important step in lignin depolymerization. Due to the intrinsic deficiency of hydrogen in lignin, the reactive free radicals could not be stabilized effectively. Instead, radical couplings lead to the formation of various inter-/intra C–O and interunit C–C bonds that require high dissociation energies.⁴⁸ Generally speaking, lignin extracted at higher temperatures or using a higher acid concentration had higher thermal stability and lower volatility. For example, L4 (130 °C, 1.0 wt % H_2SO_4) showed higher thermal stability than L2 (110 °C, 1.0 wt % H_2SO_4), and the similar trend was observed with L2 (110 °C, 1.0 wt % H_2SO_4) and L1 (110 °C, 0.5 wt % H_2SO_4). However, some exceptions were noticed. Especially at 150 °C, the temperature became a determining factor, evidenced by the similar TGA profiles with L5 (150 °C, 0.5 wt % H_2SO_4) and L6 (150 °C, 1.0 wt % H_2SO_4).

As discussed above, water addition could affect both lignin depolymerization and repolymerization in ChCl/EG. We thus also looked into the thermal property of lignin extracted by

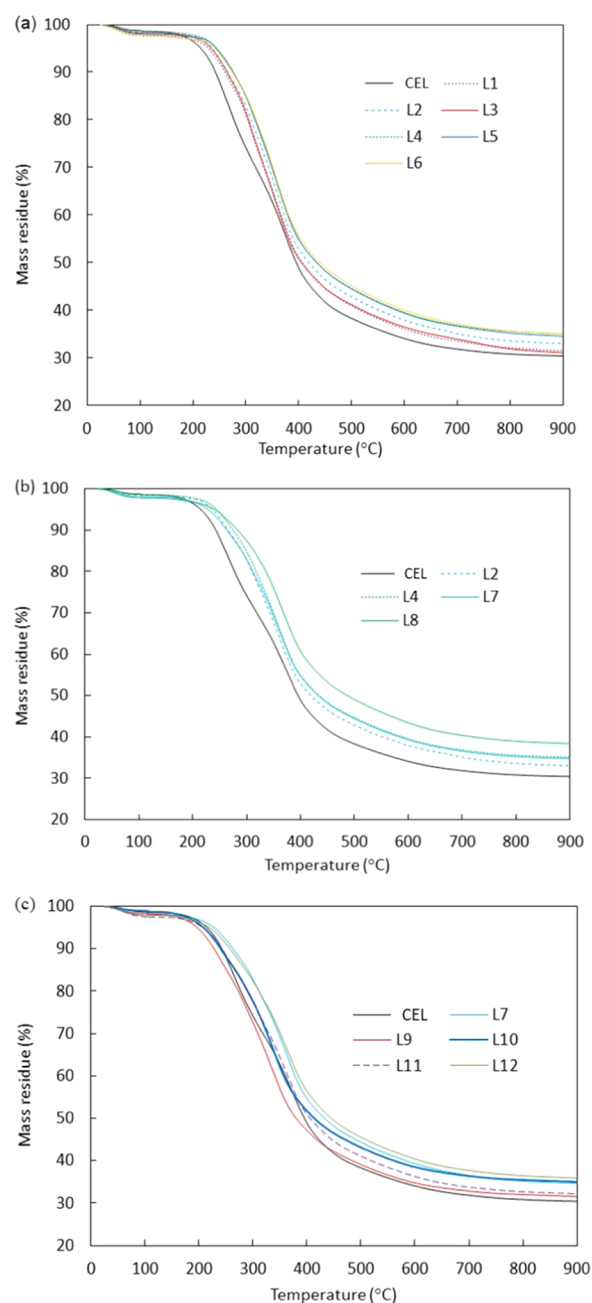


Figure 4. Thermal stability and volatility profile of DES lignin via TGA analysis. CEL: cellulytic enzyme lignin; L1–12 represent the lignin samples listed in Table 1.

aqueous ChCl/EG. It was found that lignin extracted by aqueous ChCl/EG (1:2) (L7 and L8) had increased thermal stability and decreased volatility than their counterpart lignin (L2 and L4) extracted by neat ChCl/EG (1:2) (Figure 4b). Considering that L7 had a higher amount of thermally decomposable β -O-4 linkages than L2 (Table S1), the higher thermal stability of L7 could be due to promoted repolymerization of lignin fragments by water addition during delignification by ChCl/EG (1:2).

Similar to water effect, the ChCl/EG molar ratio in the pretreatment solvent also played a significant role in tailoring the lignin properties. Figure 4c shows their effects on the thermal stability of lignin. L10 (80% ChCl/EG (3:2), 110 °C, 1.0 wt % H_2SO_4) had significantly lower thermal stability and

higher volatility than L7, the counterpart lignin derived from the 80% ChCl/EG (1:2) pretreatment. Reducing acid concentration in 80% ChCl/EG (3:2) to 0.5 wt % further increased the volatility and lowered the thermal stability of the extracted lignin (L9), leaving a low amount of the solid residue (31.5%) that was comparable to that from CEL (30.4%). It was also found that reducing water content in ChCl/EG (3:2) from 20 to 10 wt % lowered the volatility of the extracted lignin (L11 and L12). The result suggested that the higher molar ratio of ChCl/EG could counteract possible water-induced lignin condensation as proposed above for aqueous ChCl/EG (1:2), leading to a less condensed lignin structure. Systematic investigation of the role of individual aqueous DES constituents in the future study would help optimize DES compositions for tailoring lignin thermal property in a more sophisticated way.

Lignin Monomer Production via Fast Pyrolysis.

During fast pyrolysis, lignin is rapidly decomposed to produce phenolic monomers and oligomers. While both monomers and oligomers can be used for polymer synthesis or catalytically deoxygenated to produce biofuels, monomers are more preferred for their applicability. The phenolic monomers produced from lignin pyrolysis were quantified by GC/MS. The detailed compositions of identified phenolic monomers are summarized in Table S2. The yields of total phenolic monomers and two major monomers (vinylphenols) are presented in Figure 5 for a better illustration of the process—

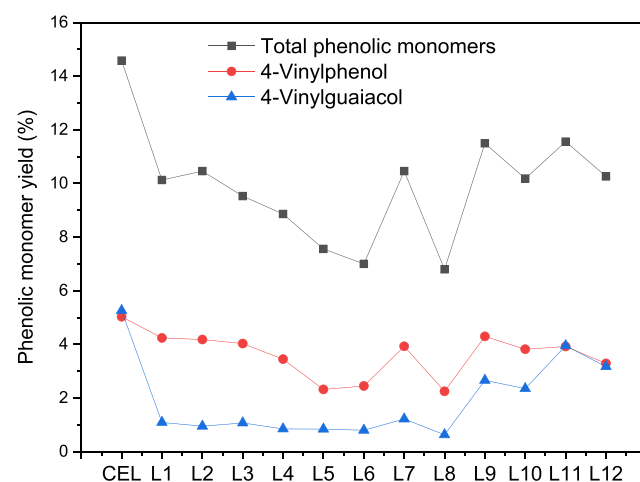


Figure 5. Yields of total and major phenolic monomers from DES lignin via fast pyrolysis. CEL: cellulytic enzyme lignin; L1–12 stand for the lignin samples listed in Table 1.

property relationship. Compared to the monomer yields, the phenolic monomer compositions were affected to a lesser degree by the lignin extraction condition. Aryl-ether bonds, methoxyl C–O and alkyl, chain C–C bonds are preferentially cleaved during pyrolysis to form various phenolic monomers. Two vinylphenols, i.e., 4-vinylphenol and 2-methoxy vinylphenol, were the major monomers, which is typical for herbaceous lignin upon pyrolysis due to the presence of ferulic acid.²³ The yield of total monomers produced from CEL was 14.58%, which is the highest. The yields of phenolic monomers were evidently lower with the lignin extracted using the ChCl/EG pretreatments. The lower monomer yields correspond to the decreased volatility of the lignin compared to CEL, as previously shown in Figure 5. The increasing temperature

during the neat ChCl/EG (1:2) pretreatment was detrimental to the monomer production from the extracted lignin (L1–6). The effect of acid concentration was less significant when the pretreatment temperature was up to 130 °C. However, the monomer yield became noticeably lower with L6 (1.0 wt % H_2SO_4) in comparison to L5 (0.5 wt % H_2SO_4) as the pretreatment temperature increased to 150 °C. The lower monomer yield is usually associated with the aromatically condensed structure of lignin. While the acidified ChCl/EG pretreatment promoted delignification, the acid also catalyzes repolymerization and condensation of lignin enhanced by high pretreatment temperatures.

For lignin extracted using the acidified ChCl/EG (1:2) pretreatments, the effect of water addition was not obvious when the pretreatment temperature was 110 °C, evidenced by the similar monomer yields obtained from pyrolysis of L7 (80% ChCl/EG (1:2), 1.0 wt % H_2SO_4) and L2 (100% ChCl/EG (1:2), 1.0 wt % H_2SO_4). As described above, L7 had a lower volatility than L2 possibly attributed to water-induced lignin repolymerization during the ChCl/EG (1:2) pretreatment. The repolymerized lignin fragments could have an increased content of interunit C–C bonds and other stable C–O bonds, and thus, they become more difficult to be depolymerized. On the other hand, L7 also retained a higher content of the β –O–4 linkages than L2, thus still enabling a comparable yield of total monomers. In contrast, the monomer yield was noticeably lower for pyrolysis of L8 (80% ChCl/EG (1:2), 130 °C, 1.0 wt % H_2SO_4) compared to pyrolysis of L4 (100% ChCl/EG (1:2), 130 °C, 1.0 wt % H_2SO_4) since the β –O–4 linkages were absent in L8.

L10 (80% ChCl/EG (3:2), 110 °C, 1.0 wt % H_2SO_4) and L7 (80% ChCl/EG (1:2), 110 °C, 1.0 wt % H_2SO_4) gave similar monomer yields despite a higher β –O–4 content in L10 than that in L7 due to the increased ChCl/EG molar ratio used for lignin extraction. However, a closer comparison of the monomer yields revealed that L7 tended to produce the phenolic monomers with shorter side chains (e.g., phenol, guaiacol, cresol), whereas L10 tended to produce the phenolic monomers with longer side chains (e.g., 2,6-dimethoxy-4-allylphenol, 4'-hydroxy-3',5'-dimethoxy-acetophenone). The yield of 4-vinylguaiacol was also higher with L10 than that with L7. This result suggests that L10 has a more spread-out molecular structure than L7, thus more resembling CEL than L7. Considering that L10 had a higher volatility upon pyrolysis and phenolic oligomers usually account for a significant fraction of lignin-derived bio-oil,⁴⁹ it is possible that L10 also produced higher amounts of phenolic oligomers in addition to the monomers. However, the oligomers are not detectable by GC/MS. When the acid concentration decreased from 1.0 to 0.5 wt % in aqueous ChCl/EG (3:2), the extracted lignin (corresponding to L9 and L11) both produced higher monomer yields upon pyrolysis compared to the respective counterpart lignin extracted using the higher acid pretreatments (corresponding to L10 and L12). Unexpectedly, total monomer yields were similar with the lignin extracted using 80% ChCl/EG (3:2) pretreatment and 90% ChCl/EG (3:2) pretreatment using the same pretreatment temperature and acid concentration (i.e., L9 vs L11, L10 vs L12) despite that the lignin extracted using 90% ChCl/EG (3:2) pretreatments (L11 and L12) had a lower volatility and lower contents of the β –O–4 linkages. The results also showed that while the yields of the most monomer species decreased from pyrolysis of L11 or L12, the decreased monomer yields were counterbalanced

by the increased yield of 4-vinylguaiacol produced from L11 or L12 compared to that from L9 or L10. In fact, the yields of 4-vinylguaiacol were constantly higher in pyrolysis of the lignin extracted using the ChCl/EG (3:2) pretreatments compared to other lignin extracted either using neat or aqueous ChCl/EG (1:2) pretreatments. The yields of 4-vinylphenol were comparable to those of 4-vinylguaiacol during pyrolysis of the lignin (L11 and L12) extracted using the ChCl/EG (3:2) pretreatments, which was also observed with the pyrolysis of CEL. On the other hand, the lignin extracted using the neat or aqueous ChCl/EG (1:2) pretreatments produced significantly lower yields of 4-vinylguaiacol than 4-vinylphenol upon pyrolysis. Thus, it is likely that lignin was demethoxylated more severely during the ChCl/EG (1:2) pretreatments compared to the ChCl/EG (3:2) pretreatments. Overall, the lignin extracted using aqueous ChCl/EG (3:2) better preserved the native lignin structure than the lignin extracted using neat ChCl/EG (2:1) or aqueous ChCl/EG (2:1), thus producing higher yields of the monomers. Noteworthy, fully characterizing the pyrolysis products (including phenolic oligomers and light gases in addition to monomers) in the future study could be helpful in better understanding the role of the ChCl/EG molar ratio and water content in the biomass pretreatment in terms of delignification pathways and structures of extracted lignin.

CONCLUSIONS

This study demonstrated that acidified ChCl/EG was highly effective for lignin and hemicellulose dissolution while preserving most cellulose during switchgrass fractionation. The lignin properties, including molecular weight, abundance of ether bonds, condensation extent, and thermal stability, were tunable by controlling the pretreatment conditions and DES compositions (e.g., water content, the molar ratio of HBA and HBD). More strikingly, extraction of lignin with the properties suitable for valorization did not sacrifice the cellulose digestibility, and the highest sugar yield reached over 90% after enzymatic hydrolysis at 20% solid loading. In short, this versatile DES system has a great potential to serve as a platform solvent system for lignocellulosic biomass fractionation and lignin extraction.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acssuschemeng.0c01361>.

Experimental section; supporting results for digestibility of pretreated switchgrass; quantification of the β –O–4 linkages, S/G ratios, and ferulic acid in lignin (Table S1); yields of the phenolic monomers from DES lignin via fast pyrolysis (Table S2); digestibility of pretreated switchgrass (Figure S1); photos of pretreatment switchgrass (Figure S2); illustration of the acid-catalyzed cleavage of the β –O–4 linkages in lignin (Figure S3); proposed reaction pathway of EG with the depolymerized lignin fraction under acidic conditions (Figure S4); proposed mechanism for stabilization of the carbocation intermediate by Cl^- in the acid-catalyzed cleavage of the β –O–4 linkages (Figure S5) (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Caixia Wan – Department of Biomedical, Biological, and Chemical Engineering, University of Missouri, Columbia, Missouri 65211, United States; orcid.org/0000-0001-5490-7490; Phone: +1 573 884 7882; Email: wanca@missouri.edu; Fax: +1 573 884 5650

Authors

Zhu Chen – Department of Biomedical, Biological, and Chemical Engineering, University of Missouri, Columbia, Missouri 65211, United States

Xianglan Bai – Department of Mechanical Engineering, Iowa State University, Ames, Iowa 50011, United States; orcid.org/0000-0001-6849-2687

Lusi A – Department of Mechanical Engineering, Iowa State University, Ames, Iowa 50011, United States; orcid.org/0000-0001-8362-3120

Hanwen Zhang – Department of Biomedical, Biological, and Chemical Engineering, University of Missouri, Columbia, Missouri 65211, United States

Complete contact information is available at:

<https://pubs.acs.org/10.1021/acssuschemeng.0c01361>

Author Contributions

The manuscript was written through the contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work is partially supported by the National Science Foundation (Grant nos. 1605034, 1706046, and 1933861). The authors also thank Dr. Yuan Xue for his contribution to the sample analysis.

■ REFERENCES

- (1) Sun, S.; Sun, S.; Cao, X.; Sun, R. The role of pretreatment in improving the enzymatic hydrolysis of lignocellulosic materials. *Bioresour. Technol.* **2016**, *199*, 49–58.
- (2) Kumar, P.; Barrett, D. M.; Delwiche, M. J.; Stroeve, P. Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production. *Ind. Eng. Chem. Res.* **2009**, *48*, 3713–3729.
- (3) Alonso, D. M.; Hakim, S. H.; Zhou, S.; Won, W.; Hosseinaei, O.; Tao, J.; Garcia-Negron, V.; Motagamwala, A. H.; Mellmer, M. A.; Huang, K.; et al. Increasing the revenue from lignocellulosic biomass: Maximizing feedstock utilization. *Sci. Adv.* **2017**, *3*, No. e1603301.
- (4) 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.; et al. Lignin valorization: improving lignin processing in the biorefinery. *Science* **2014**, *344*, No. 1246843.
- (5) Cao, S.; Pu, Y.; Studer, M.; Wyman, C.; Ragauskas, A. J. Chemical transformations of Populus trichocarpa during dilute acid pretreatment. *RSC Adv.* **2012**, *2*, 10925–10936.
- (6) Huang, Y.; Duan, Y.; Qiu, S.; Wang, M.; Ju, C.; Cao, H.; Fang, Y.; Tan, T. Lignin-first biorefinery: a reusable catalyst for lignin depolymerization and application of lignin oil to jet fuel aromatics and polyurethane feedstock. *Sustainable Energy Fuels* **2018**, *2*, 637–647.
- (7) Shuai, L.; Amiri, M. T.; Questell-Santiago, Y. M.; Héroguel, F.; Li, Y.; Kim, H.; Meilan, R.; Chapple, C.; Ralph, J.; Luterbacher, J. S. Formaldehyde stabilization facilitates lignin monomer production during biomass depolymerization. *Science* **2016**, *354*, 329–333.
- (8) Mittal, A.; Katahira, R.; Donohoe, B. S.; Pattathil, S.; Kandemkavil, S.; Reed, M. L.; Biddy, M. J.; Beckham, G. Ammonia pretreatment of corn stover enables facile lignin extraction. *ACS Sustainable Chem. Eng.* **2017**, *5*, 2544–2561.
- (9) Luo, H.; Abu-Omar, M. M. Lignin extraction and catalytic upgrading from genetically modified poplar. *Green Chem.* **2018**, *20*, 745–753.
- (10) Lancefield, C. S.; Panovic, I.; Deuss, P. J.; Barta, K.; Westwood, N. J. Pre-treatment of lignocellulosic feedstocks using biorenewable alcohols: towards complete biomass valorisation. *Green Chem.* **2017**, *19*, 202–214.
- (11) Sagues, W. J.; Bao, H. X.; Nemenyi, J. L.; Tong, Z. H. Lignin-First Approach to Biorefining: Utilizing Fenton's Reagent and Supercritical Ethanol for the Production of Phenolics and Sugars. *ACS Sustainable Chem. Eng.* **2018**, *6*, 4958–4965.
- (12) Huang, X. M.; Zhu, J. D.; Koranyi, T. I.; Boot, M. D.; Hensen, E. J. M. Effective release of lignin fragments from lignocellulose by Lewis acid metal triflates in the lignin-first approach. *ChemSusChem* **2016**, *9*, 3262–3267.
- (13) Van den Bosch, S.; Renders, T.; Kennis, S.; Koelewijn, S. F.; Van den Bossche, G.; Vangeel, T.; Deneyer, A.; Depuydt, D.; Courtin, C. M.; Thevelein, J. M.; Schutyser, W.; Sels, B. F. Integrating lignin valorization and bio-ethanol production: on the role of Ni-Al₂O₃ catalyst pellets during lignin-first fractionation. *Green Chem.* **2017**, *19*, 3313–3326.
- (14) Li, S.; Li, W. Z.; Zhang, Q.; Shu, R. Y.; Wang, H. Z.; Xin, H. S.; Ma, L. L. Lignin-first depolymerization of native corn stover with an unsupported MoS₂ catalyst. *RSC Adv.* **2018**, *8*, 1361.
- (15) Liu, Z. H.; Hao, N.; Shinde, S. D.; Pu, Y.; Kang, X.; Ragauskas, A.; Yuan, J. S. Defining lignin nanoparticle properties through tailored lignin reactivity by sequential organosolv fragmentation approach (SOFA). *Green Chem.* **2019**, *21*, 245–260.
- (16) Alvarez-Vasco, C.; Ma, R.; Quintero, M.; Guo, M.; Geleynse, S.; Ramasamy, K. K.; Wolcott, M.; Zhang, X. Unique low-molecular-weight lignin with high purity extracted from wood by deep eutectic solvents (DES): a source of lignin for valorization. *Green Chem.* **2016**, *18*, 5133–5141.
- (17) Chen, Z.; Reznicek, W. D.; Wan, C. Deep eutectic solvent pretreatment enabling full utilization of switchgrass. *Bioresour. Technol.* **2018**, *263*, 40–48.
- (18) Li, W.; Amos, K.; Li, M.; Pu, Y.; Debolt, S.; Ragauskas, A. J.; Shi, J. Fractionation and characterization of lignin streams from unique high-lignin content endocarp feedstocks. *Biotechnol. Biofuels* **2018**, *11*, No. 304.
- (19) Chen, Z.; Bai, X.; A, L.; Wan, C. High-solid lignocellulose processing enabled by natural deep eutectic solvent for lignin extraction and industrially relevant production of renewable chemicals. *ACS Sustainable Chem. Eng.* **2018**, *6*, 12205–12216.
- (20) Xia, Q.; Liu, Y.; Meng, J.; Cheng, W.; Chen, W.; Liu, S.; Liu, Y.; Li, J.; Yu, H. Multiple hydrogen bond coordination in three-constituent deep eutectic solvents enhances lignin fractionation from biomass. *Green Chem.* **2018**, *20*, 2711–2721.
- (21) Sluiter, A.; Hames, B.; Ruiz, R.; Scarlata, C.; Sluiter, J.; Templeton, D.; Crocker, D. *Determination of Structural Carbohydrates and Lignin in Biomass*, Technical Report NREL/TP-510-42618; National Renewable Energy Laboratory, 2008; pp 1–16.
- (22) Wen, J.-L.; Yuan, T.-Q.; Sun, S.-L.; Xu, F.; Sun, R.-C. Understanding the chemical transformations of lignin during ionic liquid pretreatment. *Green Chem.* **2014**, *16*, 181–190.
- (23) Zhou, S.; Xue, Y.; Sharma, A.; Bai, X. Lignin Valorization through Thermochemical Conversion: Comparison of Hardwood, Softwood and Herbaceous Lignin. *ACS Sustainable Chem. Eng.* **2016**, *4*, 6608–6617.
- (24) Hou, X.-D.; Feng, G.-J.; Ye, M.; Huang, C.-M.; Zhang, Y. Significantly enhanced enzymatic hydrolysis of rice straw via a high-performance two-stage deep eutectic solvents synergistic pretreatment. *Bioresour. Technol.* **2017**, *238*, 139–146.
- (25) Soares, B.; Tavares, D. J.; Amaral, J. L.; Silvestre, A. J.; Freire, C. S.; Coutinho, J. A. P. Enhanced solubility of lignin monomeric model compounds and technical lignins in aqueous solutions of deep eutectic solvents. *ACS Sustainable Chem. Eng.* **2017**, *5*, 4056–4065.

- (26) Smith, M. D.; Mostofian, B.; Cheng, X.; Petridis, L.; Cai, C. M.; Wyman, C. E.; Smith, J. C. Cosolvent pretreatment in cellulosic biofuel production: effect of tetrahydrofuran-water on lignin structure and dynamics. *Green Chem.* **2016**, *18*, 1268–1277.
- (27) Sun, J.; Dutta, T.; Parthasarathi, R.; Kim, K. H.; Tolic, N.; Chu, R. K.; Isern, N. G.; Cort, J. R.; Simmons, B. A.; Singh, S. Rapid room temperature solubilization and depolymerization of polymeric lignin at high loadings. *Green Chem.* **2016**, *18*, 6012–6020.
- (28) Durand, E.; Lecomte, J.; Baréa, B.; Dubreucq, E.; Lortie, R.; Villeneuve, P. Evaluation of deep eutectic solvent–water binary mixtures for lipase-catalyzed lipophilization of phenolic acids. *Green Chem.* **2013**, *15*, 2275–2282.
- (29) Bian, H.; Chen, L.; Dai, H.; Zhu, J. Y. Integrated production of lignin containing cellulose nanocrystals (LCNC) and nanofibrils (LCNF) using an easily recyclable di-carboxylic acid. *Carbohydr. Polym.* **2017**, *167*, 167–176.
- (30) Chen, Z.; Wan, C. A novel deep eutectic solvent/acetone biphasic system for high-yield furfural production. *Bioresour. Technol. Rep.* **2019**, *8*, No. 100318.
- (31) Xu, F.; Sun, J.; Wehrs, M.; Kim, K. H.; Rau, S. S.; Chan, A. M.; Simmons, B. A.; Mukhopadhyay, A.; Singh, S. Biocompatible choline-based deep eutectic solvents enable one-pot production of cellulosic ethanol. *ACS Sustainable Chem. Eng.* **2018**, *6*, 8914–8919.
- (32) Gunny, A. A. N.; Arbain, D.; Nashef, E. M.; Jamal, P. Applicability evaluation of Deep Eutectic Solvents–Cellulase system for lignocellulose hydrolysis. *Bioresour. Technol.* **2015**, *181*, 297–302.
- (33) Lauberts, M.; Sevastyanova, O.; Ponomarenko, J.; Dizhbite, T.; Dobeles, G.; Volperts, A.; Lauberte, L.; Telysheva, G. Fractionation of technical lignin with ionic liquids as a method for improving purity and antioxidant activity. *Ind. Crops Prod.* **2017**, *95*, 512–520.
- (34) Deuss, P. J.; Scott, M.; Tran, F.; Westwood, N. J.; de Vries, J. G.; Barta, K. Aromatic monomers by in situ conversion of reactive intermediates in the acid-catalyzed depolymerization of lignin. *J. Am. Chem. Soc.* **2015**, *137*, 7456–7467.
- (35) Samuel, R.; Pu, Y.; Raman, B.; Ragauskas, A. J. Structural characterization and comparison of switchgrass ball-milled lignin before and after dilute acid pretreatment. *Appl. Biochem. Biotechnol.* **2010**, *162*, 62–74.
- (36) Samuel, R.; Foston, M.; Jiang, N.; Allison, L.; Ragauskas, A. J. Structural changes in switchgrass lignin and hemicelluloses during pretreatments by NMR analysis. *Polym. Degrad. Stab.* **2011**, *96*, 2002–2009.
- (37) Gschwend, F. J.; Malaret, F.; Shinde, S.; Brandt-Talbot, A.; Hallett, J. P. Rapid pretreatment of Miscanthus using the low-cost ionic liquid triethylammonium hydrogen sulfate at elevated temperatures. *Green Chem.* **2018**, *20*, 3486–3498.
- (38) Chen, L.; Dou, J.; Ma, Q.; Li, N.; Wu, R.; Bian, H.; Yelle, D. J.; Vuorinen, T.; Fu, S.; Pan, X.; Zhu, J. Rapid and near-complete dissolution of wood lignin at ≤ 80 °C by a recyclable acid hydrotrope. *Sci. Adv.* **2017**, *3*, No. e1701735.
- (39) Mellmer, M. A.; Sener, C.; Gallo, J. M. R.; Luterbacher, J. S.; Alonso, D. M.; Dumesic, J. A. Solvent effects in acid-catalyzed biomass conversion reactions. *Angew. Chem., Int. Ed.* **2014**, *53*, 11872–11875.
- (40) Enslow, K. R.; Bell, A. T. The role of metal halides in enhancing the dehydration of xylose to furfural. *ChemCatChem* **2015**, *7*, 479–489.
- (41) Luterbacher, J. S.; Azarpira, A.; Motagamwala, A. H.; Lu, F.; Ralph, J.; Dumesic, J. A. Lignin monomer production integrated into the γ -valerolactone sugar platform. *Energy Environ. Sci.* **2015**, *8*, 2657–2663.
- (42) Phongpreecha, T.; Hool, N. C.; Stoklosa, R. J.; Klett, A. S.; Foster, C. E.; Bhalla, A.; Holmes, D.; Thies, M. C.; Hodge, D. B. Predicting lignin depolymerization yields from quantifiable properties using fractionated biorefinery lignins. *Green Chem.* **2017**, *19*, 5131–5143.
- (43) Sun, S.-N.; Li, H.-Y.; Cao, X.-F.; Xu, F.; Sun, R.-C. Structural variation of eucalyptus lignin in a combination of hydrothermal and alkali treatments. *Bioresour. Technol.* **2015**, *176*, 296–299.
- (44) Zijlstra, D. S.; Lahive, C. W.; Analbers, C. A.; Figueirêdo, M. B.; Wang, Z.; Lancefield, C. S.; Deuss, P. J. Mild Organosolv lignin extraction with alcohols; The importance of benzylic alkoxylation. *ACS Sustainable Chem. Eng.* **2020**, *8*, 5119–5131.
- (45) Rodríguez, A.; Salvachúa, D.; Katahira, R.; Black, B. A.; Cleveland, N. S.; Reed, M.; Smith, H.; Baidoo, E. E.; Keasling, J. D.; Simmons, B. A.; et al. Base-catalyzed depolymerization of solid lignin-rich streams enables microbial conversion. *ACS Sustainable Chem. Eng.* **2017**, *5*, 8171–8180.
- (46) Brandt, A.; Chen, L.; van Dongen, B. E.; Welton, T.; Hallett, J. P. Structural changes in lignins isolated using an acidic ionic liquid water mixture. *Green Chem.* **2015**, *17*, 5019–5034.
- (47) Dutta, T.; Papa, G.; Wang, E.; Sun, J.; Isern, N. G.; Cort, J. R.; Simmons, B. A.; Singh, S. Characterization of Lignin Streams during Bionic Liquid-Based Pretreatment from Grass, Hardwood, and Softwood. *ACS Sustainable Chem. Eng.* **2018**, *6*, 3079–3090.
- (48) Shuai, L.; Saha, B. Towards high-yield lignin monomer production. *Green Chem.* **2017**, *19*, 3752–3758.
- (49) Scholze, B.; Hanser, C.; Meier, D. Characterization of the water-insoluble fraction from fast pyrolysis liquids (pyrolytic lignin): Part II. GPC, carbonyl groups, and ¹³C-NMR. *J. Anal. Appl. Pyrolysis* **2001**, *58–59*, 387–400.