# Chemical Transformations of Poplar Lignin during Cosolvent Enhanced Lignocellulosic Fractionation Process

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**S** Supporting Information

**ABSTRACT:** Converting lignocellulosic biomass to biofuels and bioproducts is significantly hindered by the innate recalcitrance of biomass to chemical and biological breakdown, and it usually requires a pretreatment stage in order to improve conversion yields. A promising novel pretreatment named Cosolvent Enhanced Lignocellulosic Fractionation (CELF) involving dilute acid treatment of biomass in a THF−water mixture was recently developed to overcome biomass recalcitrance. Detailed elucidation of physicochemical structures of the fractionated lignin that is precipitated from CELF pretreatment of hardwood poplar, also called CELF lignin, reveals transformations in its molecular weights, monolignol composition, and hydroxyl group content. Isolated CELF lignin revealed dramatic reductions in its molecular weight by up to ∼90% compared with untreated native lignin. Furthermore, CELF lignin's β-O-4 interunit linkages were extensively cleaved after CELF pretreatment as indicated by a semiquantitative HSQC NMR analysis. This is further evidenced by a ^{31}P NMR analysis showing a significant decrease in aliphatic OH groups due to the oxidation of lignin side chains, whereas the content of total phenolic OH groups in CELF lignin significantly increased due to cleavage of interunit linkages. In conclusion, the CELF process generated a uniquely tunable and highly pure lignin feedstock of low content aryl ether linkages, low molecular weight, and high amount of phenolic hydroxyl groups, suitable for its development into fuels, chemicals, and materials.

**KEYWORDS:** Biomass recalcitrance, Cosolvent Enhanced Lignocellulosic Fractionation, Antioxidant, Lignin valorization
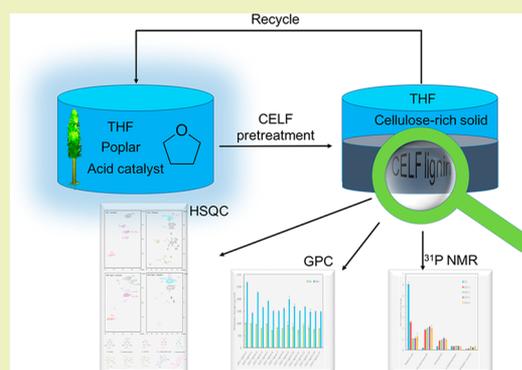
## INTRODUCTION

Lignocellulosic biomass represents a promising sustainable platform that is an alternative to fossil resources.[1] Long-term driving forces including environment concerns and energy security have promoted worldwide biofuel development.[2] Nevertheless, there are still significant challenges associated with biofuel commercialization. Biomass recalcitrance represents one of the critical challenges for biological biorefining of biomass; therefore, it is vital to develop cost-effective pretreatment techniques to enhance sugar-release performance through enzymatic hydrolysis. Over the past decade, various pretreatment technologies have been developed to overcome biomass recalcitrance, and each pretreatment has its own target;

however, all have been proven to change the plant-cell-wall structures.[3,4] Lignin, a three-dimensional cross-linked polyphenolic polymer, has been considered as the most recalcitrant component of the three primary biopolymers present in plant cell walls for the conversion of biomass to biofuels.[5] With its rich polyaromatic structure, lignin has significant potential to serve as a sustainable feedstock for the production of renewable chemicals and fuels.[6] Thus, many pretreatments have been

developed and modified with an effort of removing or at least redistributing lignin across the plant cell wall.[7]

Organosolv pretreatment is a process that usually involves addition of organic solvents such as ethanol, methanol, or acetone as modifiers or cosolvents to water in order to first solubilize and fractionate lignin from biomass as a "lignin-first" approach.[7] Recently, tetrahydrofuran (THF) has been identified as a new multifunctional solvent for pretreatment and fractionation of biomass to obtain higher yields of sugars and other fuel precursors as well as improving the overall utilization of biomass for conversion to fuels. Traditionally, THF has been used to dissolve acetylated lignin for molecular-weight analysis in biomass research.[8] Notably, THF can be considered as a renewable solvent as it can be manufactured from maleic anhydride, 1,4-butanediol, or furfural that are catalytically produced from C5 sugars.[9−11] THF is also inherently biodegradable; thus, it is not expected to be environmentally persistent.[12] Cosolvent Enhanced Lignocellulose Fractionation (CELF) pretreatment technology was developed as a new-generation pretreatment that involves reacting biomass with dilute acid in mixtures of THF−$H_2O$.[13−15] At reaction temperatures of 170 °C or higher, CELF pretreatment could produce high yields of fuel precursors including furfural (FF), 5-hydroxymethylfurfural (5-HMF), and levulinic acid (LA) directly from biomass.[13] For example, employing dilute $FeCl_3$ in CELF reaction of hardwood chips resulted in coproduction yields of >90% FF and >50% 5-HMF simultaneously.[16] At milder temperatures below 160 °C, CELF can be used to achieve nearly complete recovery (>95%) of fermentable C5 and C6 sugars from biomass if the pretreated solids were subsequently treated using enzymes at extremely low dosages.[14] Combining CELF pretreatment with simultaneous saccharification and fermentation (SSF) dramatically improved ethanol fermentation yields at high biomass solids loadings.[17] In all cases, between 85 and 95% of the acid insoluble "Klason" lignin can be removed from biomass during CELF pretreatment, resulting in the precipitation of a very clean lignin product, also called CELF lignin, from the liquid phase after recovery of the THF by low-temperature vacuum distillation.[13,14,16]

With all of the potential applications of lignin as renewable sources for production of fuels, materials, and chemicals, understanding its fundamental characteristics is of significant importance.[6] During dilute acid or aqueous hydrothermal pretreatments, lignin deposits typically onto cellulose surface as droplets.[18−20] On the other hand, it has been reported that lignin adopts extended coil configurations during the CELF process and is preferentially solvated by THF as determined by all-atom molecular-dynamics (MD) simulation.[21] A further replica exchange MD simulation study by Smith et al. showed that lignin was a coil regardless of being in the miscible or immiscible temperature region, suggesting CELF pretreatment may be performed at lower temperatures and pressures.[22] It was reported that CELF pretreatment increased S lignin fraction and reduced G lignin in solid residue for poplar and corn stover, and in fact, CELF pretreatment almost completely removed corn stover G units.[23] However, the detailed structural characterization of CELF lignin which is the lignin dissolved in THF, accounting for over 80% of lignin in raw material, has not been reported prior to this paper to the best of our knowledge. In this study, the structure of poplar lignin extracted from CELF pretreatment at different conditions was determined following well-established methodologies including GPC and NMR, and the results were compared to the native (untreated)

lignin to determine the exact effect of CELF process on the chemical structure of lignin.

## ■ MATERIALS AND METHODS

**Feedstocks and Chemicals.** Poplar (*Populus trichocarpa × deltoides*) was provided by National Renewable Energy Laboratory (NREL, Golden, CO) and size reduced in a laboratory mill (Model 4, Arthur H. Thomas Company, Philadelphia, PA) to obtain less than a 1 mm particle size. The composition of untreated poplar was measured to be ∼45% glucan, ∼14% xylan, and ∼22% Klason lignin using NREL laboratory analytical procedure. Ferric chloride was purchased from Sigma-Aldrich (St. Louis, MO) and reagent grade THF was purchased from Fisher Scientific (Pittsburgh, PA). All the chemicals used in this study were used as received without any further purification.

**Cosolvent Enhanced Lignocellulosic Fractionation Pretreatment.** CELF pretreatments at different severities were performed in a 1 L Parr reactor (236HC Series, Parr Instruments Company, Moline, IL) equipped with 6-blade impellers operating at 200 rpm driven by an electric motor. The Parr reactor was heated using a 4 kW fluidized sand batch (Model SBL-2D, Cole-Parmer, Staffordshire, U.K.), and the temperature was measured by an in-line thermocouple (Omega, K-type).[13] Biomass and acid catalyst ($FeCl_3$ or $H_2SO_4$) loadings based on the total mass of the reaction mixture and anhydrous mass were 5 and 1 wt %, respectively. After pretreatment, the reactor was quenched in a water bath at room temperature. The hydrolysate was then separated from the solids using vacuum filtration through a glass fiber filter paper (Fischer Scientific, Pittsburgh, PA). The CELF pretreatment conditions applied in this work are shown in Table 1.

**Table 1. List of Pretreatment Conditions Applied in This Work for CELF Process of Poplar**

| sample | temperature (°C) | time (min) | catalyst loading (M) | THF:water (v:v) |
|---|---|---|---|---|
| 1 | 160 | 15 | 0.1 $H_2SO_4$ | 1:1 |
| 2 | 180 | 20 | 0.1 $H_2SO_4$ | 4:1 |
| 3 | 170 | 30 | 0.1 $FeCl_3$ | 3:1 |
| 4 | 170 | 60 | 0.1 $FeCl_3$ | 3:1 |
| 5 | 180 | 20 | 0.1 $FeCl_3$ | 1:1 |
| 6 | 180 | 20 | 0.1 $FeCl_3$ | 2:1 |
| 7 | 180 | 20 | 0.1 $FeCl_3$ | 3:1 |
| 8 | 180 | 20 | 0.1 $FeCl_3$ | 4:1 |
| 9 | 180 | 20 | 0.1 $FeCl_3$ | 5:1 |
| 10 | 180 | 20 | 0.1 $FeCl_3$ | 6:1 |
| 11 | 180 | 20 | 0.1 $FeCl_3$ | 7:1 |
| 12 | 180 | 20 | 0.05 $FeCl_3$ | 4:1 |
| 13 | 180 | 20 | 0.15 $FeCl_3$ | 4:1 |
| 14 | 180 | 15 | 0.1 $FeCl_3$ | 3:1 |
| 15 | 180 | 30 | 0.1 $FeCl_3$ | 3:1 |

**CELF Lignin Isolation.** CELF lignin was isolated from the liquid product by room-temperature vacuum distillation according to literature procedures (Scheme 1).[16] Once the THF was removed from the aqueous phase, the precipitated sticky dark resinous solid lignin was rinsed with water and diethyl ether to remove nonlignin soluble impurities. The purified lignin was then dried at 40 °C for 15 h, and the dry weight was measured by a moisture content analyzer. The composition of isolated lignin was determined according to a

**Scheme 1. Schematic Diagram of CELF Lignin Isolation**
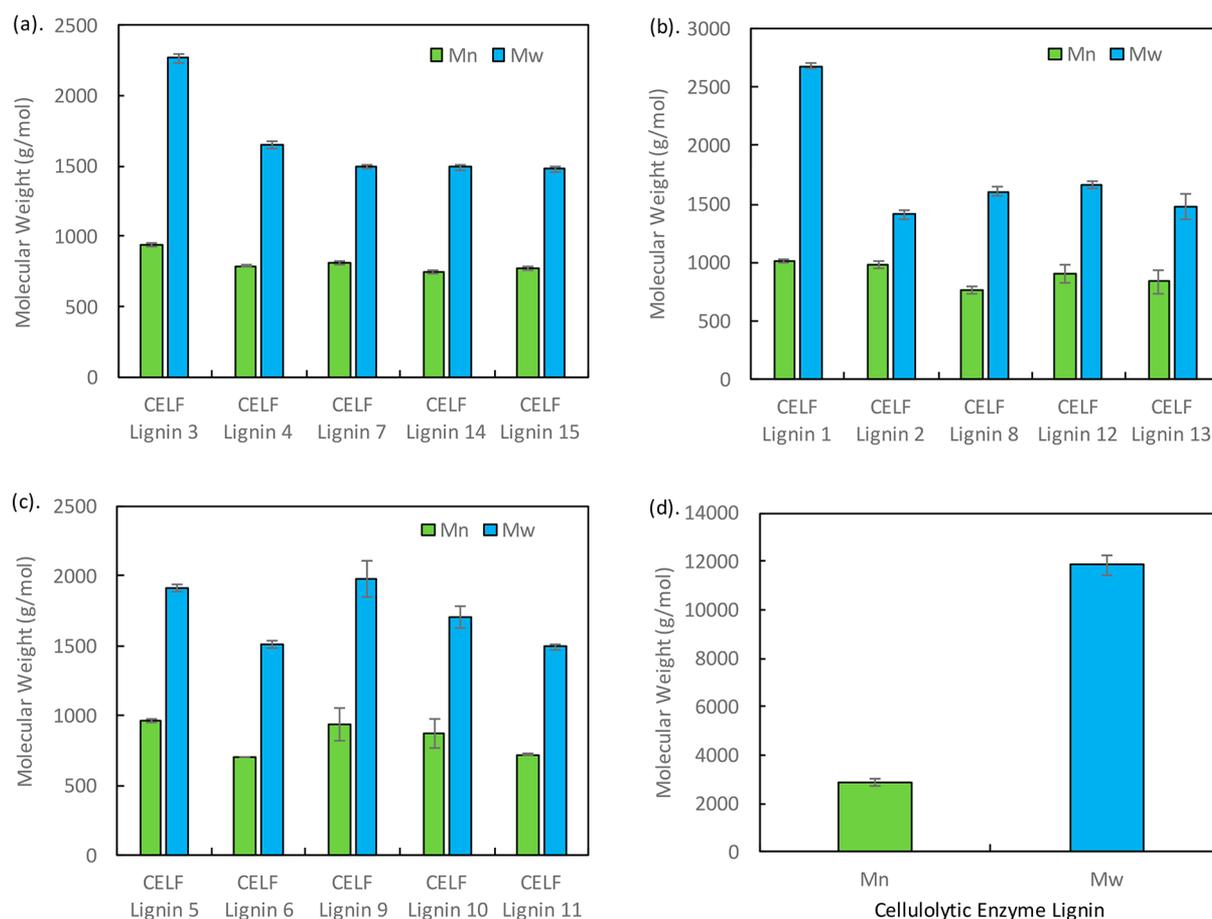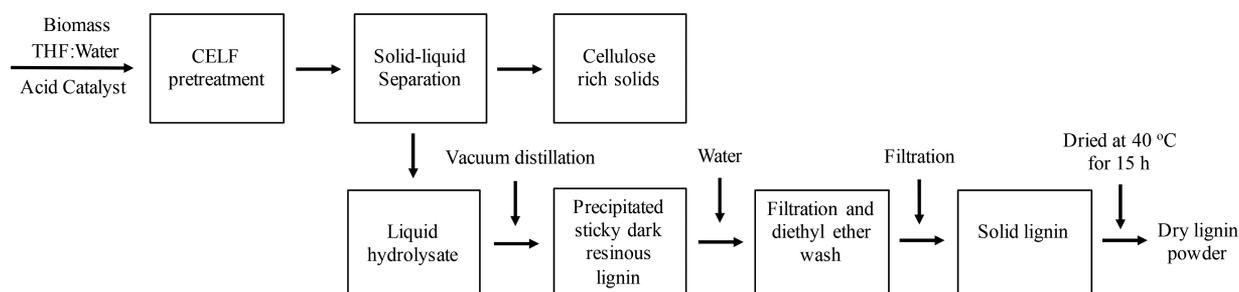




**Figure 1.** Weight-average ($M_w$) and number-average ($M_n$) molecular weights of CELF lignin and CEL lignin. (a) Effect of pretreatment time and temperature. (b) Effect of catalyst and its loading. (c) Effect of THF/water ratio. (d) Molecular weight of CEL. Sample information is presented in Table 1.

NREL analytical procedure.[24] All the CELF lignin samples were almost carbohydrate-free and presented a high total lignin content above 90%. The detailed composition analysis of CELF lignin are presented in Table S1 in Supporting Information.

**Cellulolytic Enzyme Lignin Isolation.** Cellulolytic enzyme lignin (CEL) was isolated from poplar according to a modified method.[25] In brief, poplar was Soxhlet-extracted with toluene/ethanol for 8 h. The extractives-free poplar was then ball-milled in a porcelain jar with ceramic balls via Retsch PM 100 (Newton, PA) at 600 rpm for 2.5 h followed by enzymatic hydrolysis in acetate buffer (pH 4.8, 50 °C) using Cellic CTec 2 cellulase and HTec 2 hemicellulase (1:1, mass ratio) as the enzymes (150 mg of protein loading/g of biomass) for 48 h. The solid residue was isolated by centrifugation and hydrolyzed again with freshly added buffer and enzymes at same loadings.

To remove any remaining cellulases, the lignin-rich residue was then treated with *Streptomyces griseus* protease (Sigma-Aldrich) at 37 °C overnight followed by a 10 min deactivation at 100 °C. After filtration, the solid residue was extracted twice with 96% (v/v) *p*-dioxane/water mixture at room temperature for 48 h. The extracts were combined, rotary evaporated, and freeze-dried to recover CEL.

**Lignin Molecular-Weight-Distribution Analysis.** The molecular-weight analysis was performed as previously described.[26] Oven-dried lignin samples (~5 mg) were acetylated with a 2.0 mL acetic anhydride/pyridine (1:1, v/v) mixture at room temperature. After 24 h, ethanol was added to the reaction and left for 30 min. The solvent was then subjected to rotary evaporation under reduced pressure. The whole process was repeated twice until all the acetic acid was removed
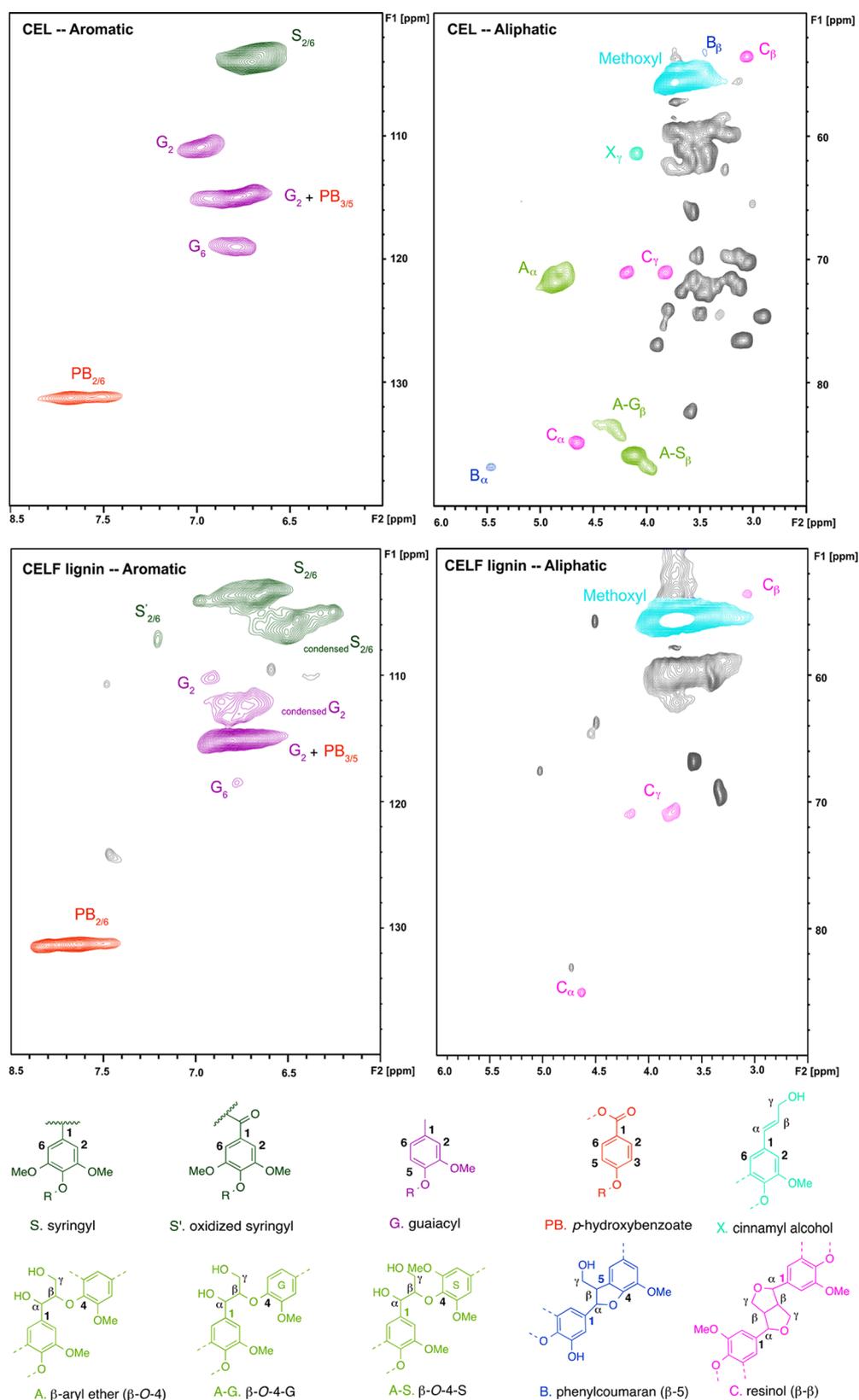
**Figure 2.** Representative HSQC NMR spectra of CEL (top) and CELF lignin sample 5 (bottom) including aromatic (left) and aliphatic (right) regions. S: syringyl; S′: oxidized syringyl.

from the solution. The derivatized lignin was then dried at 45 °C overnight in a VWR 1400E vacuum oven and then dissolved in THF (1 mg/mL) overnight prior to the GPC analysis. The molecular-weight analysis was analyzed by an Agilent GPC

SECurity 1200 system equipped with four Waters Styragel columns (HR1, HR2, HR4, and HR6) and a UV detector (270 nm). Polystyrene narrow standards were used to prepare the calibration curve, and THF was used as the mobile phase at a

flow rate of 1.0 mL/min. The injection volume was set at 30 $\mu$L. The number-average and weight-average molecular weights were calculated using a WinGPC Unity software. While polystyrenes are widely used as standards to generate the relative calibration curve in GPC analysis, high-molecular-weight (MW) polystyrene standard and high MW lignin are not necessary eluted at the same elution volume, and the molecular weight should be taken with a measure of caution.

**Lignin NMR Characterization.** Two-dimensional $^{13}C-^1H$ HSQC and $^{31}P$ NMR experiments were both acquired with a Bruker Avance 400 MHz spectrometer according to a previously published study.[26] A standard Bruker pulse sequence was used under the following conditions: 210 ppm spectral width in F1 ($^{13}C$) dimension with 256 data points and 11 ppm spectral width in F2 ($^1H$) dimension with 1024 data points, a 90° pulse, a $^1J_{C-H}$ of 145 Hz, a 1.5 s pulse delay, and 32 scans. ~ 50 mg of dry lignin samples were dissolved in deuterated DMSO for HSQC experiments. Quantitative $^{31}P$ NMR spectra were acquired using an inverse-gated decoupling pulse sequence (Waltz-16), 90° pulse, 25 s pulse delay with 128 scans. Approximately 20.0 mg of lignin samples was dissolved in a solvent mixture of pyridine and deuterated chloroform (1.6/1.0, v/v, 0.50 mL). The lignin solution was then further derivatized with 0.075 mL of 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (TMDP). Chromium acetylacetonate and endo-*N*-hydroxy-5-norbornene-2,3-dicarboximide (NHND) were also added to the solution as relaxation agent and internal standard, respectively. All the data was processed using the TopSpin 2.1 software (Bruker BioSpin).

**Error Analysis.** Error bars shown in each figure represent standard error which is the standard deviation of the sampling distribution of a statistic, defined as the standard deviation divided by the square root of the sample size, that is, three independent assays unless otherwise specified.

## ■ RESULTS AND DISCUSSION

**Molecular-Weight Analysis.** Lignin molecular weight is an important index to evaluate the physical properties of lignin. The weight-average ($M_w$) and number-average ($M_n$) molecular weight of CELF lignin produced from hardwood poplar at different reaction conditions are presented in Figure 1 with samples numbered by the reaction conditions they were produced from. The $M_n$ and $M_w$ of native-like CEL isolated from untreated poplar were 2850 and 11850 g/mol, respectively. As shown in Figure 1, CELF lignin fraction had significantly lower $M_w$ and $M_n$ compared with CEL regardless of pretreatments conditions, suggesting that dramatic depolymerization of lignin had occurred during the CELF pretreatments. CELF lignin samples also showed relatively lower polydispersity index (PDI) compared with that of CEL (4.16). This indicated that CELF pretreatment led to relatively narrower molecular-weight distributions in soluble lignin. This is in agreement with other data reported that organosolv lignin mainly contains small-molecular-weight fractions compared to untreated native lignin.[27−30] In order to understand the effect of pretreatment conditions (temperature, time, THF/water ratio, acid catalyst, etc.) on molecular-weight distribution of CELF lignin, certain samples were further compared with each other. By comparing sample 7 with 14 and 15, it was concluded that extending the pretreatment time from 15 to 30 min at 180 °C did not have any significant impact on the lignin molecular weight, indicating that both depolymerization and condensation reactions were equally favored during this period.

However, extending the pretreatment time from 30 to 60 min, it was found to have a significant impact on the lignin molecular weight for CELF pretreatment performed at 170 °C, as GPC results indicated that 60 min pretreatment led to a ~27.1% decrease of lignin molecular weight compared with 30 min pretreatment (sample 3 vs 4). This suggested that cleavage reactions were favored over repolymerization under this particular condition. Given the fact that CELF lignin from trial 7, 14, and 15 had lower molecular weight than CELF lignin 3 and 4, it can be concluded that higher pretreatment temperature appeared to have a more dramatic effect on lignin molecular weight than longer pretreatment time. Comparison of sample 2 with 8 led to the conclusion that $H_2SO_4$ tended to generate lower-molecular-weight lignin than $FeCl_3$. Increasing pretreatment temperature from 170 to 180 °C (sample 3 vs 15) caused a ~34.8% decrease of lignin molecular weight, while increasing the $FeCl_3$ catalyst concentration from 0.05 to 0.15% (sample 8, 12, and 13) had a minimal effect on lignin molecular weight. Regarding the THF/water ratio, relatively lower-molecular-weight lignin was obtained using 2:1, 3:1, and 7:1 THF/water ratio; therefore, although the impact is significant, no obvious trend can be obtained (sample 5−11) for the impact of THF:water ratio, likely caused by competing solvent-solvent interactions and the unique phase behavior of THF-water. In conclusion, all lignin samples underwent significant degradation during CELF process regardless of pretreatment conditions; pretreatment temperature, acid catalyst, and THF/water ratio had an important effect on lignin molecular weight; pretreatment time had moderate effect depending on the pretreatment temperature applied; and $FeCl_3$ concentration had a negligible effect on CELF lignin molecular-weight distribution.

**HSQC NMR Analysis.** In an effort to further investigate changes in the lignin structure during CELF pretreatment, HSQC NMR analysis of selected CELF lignin samples was performed. The representative HSQC spectra of CEL and CELF lignin (sample 5, 180 °C, 20 min, 0.1 M $FeCl_3$, 1:1 THF/$H_2O$ ratio) are shown in Figure 2. The HSQC NMR spectra showed that CELF lignin demonstrated very different structural features compared with poplar CEL. The aromatic and aliphatic $^{13}C/^1H$ cross-peaks are assigned according to literature (Table S2).[25] As expected and consistent with literature, poplar CEL is primarily composed of syringyl (S) and guaiacyl (G) units along with considerable amounts of *p*-hydroxyphenyl benzoate (PB) units.[31] In aromatic regions, the cross peaks associated with S and G units were either reduced in intensity or significantly shifted to condensed form in CELF lignin. In particular, the peaks associated with condensed $G_2$ and $S_{2/6}$ were observed at $\delta_C/\delta_H$ 112.4/6.72 ppm and 106.5/6.51 ppm, respectively, which is consistent with literature.[32] In aliphatic regions, signals associated with methoxyl group and $\beta$-aryl ether ($\beta$-O-4) interlinkages appeared to be the most prominent ones in CEL. The presence of phenylcoumaran ($\beta$-5) and resinol subunit ($\beta-\beta$) in CEL were also confirmed by its C−H correlations. However, only a trace amount of $\beta-\beta$ interunit linkages was detected, and the remaining peaks for $\beta$-O-4 and $\beta$-5 were not apparent in CELF lignin as shown in Figure 2.

A semiquantitative analysis using volume integration of contours in HSQC spectra was further performed to calculate the monolignol compositions (such as S/G ratio) and relative abundance of lignin interunit linkages (such as $\beta$-O-4, $\beta-\beta$, and $\beta$-5). For monolignol compositions of S, G, and PB calculation,

$S_{2/6}$, oxidized and condensed $S_{2/6}$, $G_2$, condensed $G_2$, $PB_{2/6}$ contours were used with $G_2$ and condensed $G_2$ integrals doubled. The $C_\alpha$ signals were used for contour integration for the calculation of all the interunit linkages. Table 2 presents the

**Table 2. Semiquantitative Analysis of Lignin Subunits and Interunit Linkages in Poplar CEL and CELF Lignin Samples**[a]

| lignin samples | CEL | CELF 1 | CELF 2 | CELF 5 | CELF 8 |
|---|---|---|---|---|---|
| Lignin Subunits (%) | | | | | |
| syringyl | 60.9 | 56.8 | 44.3 | 54.2 | 52.4 |
| guaiacyl | 39.1 | 43.2 | 55.7 | 45.8 | 47.6 |
| *p*-hydroxybenzoate | 14.3 | 13.6 | 17.5 | 17.7 | 14.0 |
| S/G ratio | 1.6 | 1.3 | 0.8 | 1.2 | 1.1 |
| Interlinkages (%) | | | | | |
| $\beta$-O-4 | 61.1 | 9.2 | ND | ND | ND |
| $\beta-\beta$ | 4.7 | 1.9 | 0.04 | 0.2 | 0.07 |
| $\beta$-5 | 5.4 | 1.8 | ND | ND | ND |

[a]Content (%) expressed as a fraction of S+G. ND: below detection limit.

relative contents of lignin subunits as well as interunit linkages for poplar CEL and several selected CELF lignin samples. The relative content of total S units (including oxidized/condensed units) decreased while the content of total G units (including condensed units) increased in CELF lignin samples causing a decrease of S/G ratio. The change in PB content upon pretreatment was found to depend on the applied CELF pretreatment conditions. Table 2 also confirms the dramatic cleavage of $\beta$-O-4 which is probably the most prominent change in lignin structure following CELF.

The observed scission of $\beta$-O-4, $\beta-\beta$, and $\beta$-5 interunit linkages are in part agreement with those observed for conventional organosolv lignin using ethanol, methanol or acetone. It was reported that organosolv lignin isolated from ethanol pretreated *Miscanthus × giganteus* (190 °C and 60 min) lignin had a ~ 55% decrease of $\beta$-O-4 compared with the untreated milled wood lignin (MWL).[30] Similarly, Hallac et al. reported that ethanol organosolv pretreated *Buddleja davidii* (195 °C and 60 min) had a ~57% decrease in the content of $\beta$-O-4 compared to MWL.[27] The acid-catalyzed cleavage of $\beta$-O-4 interunit linkages has been recognized as the major mechanism of lignin depolymerization during acidic organosolv pretreatments.[33] However, this type of depolymerization is usually accompanied by repolymerization or condensation reactions, especially under acidic conditions.[34] Thus, cleavage of lignin interunit linkages is generally incomplete as there are competing solubilization and condensation pathways through the carbocation intermediate. Herein, we reported a near complete removal of the common lignin interunit linkages by an acid-catalyzed organosolv pretreatment using THF as the organic solvent at relatively mild temperature (180 °C). The reason that THF can preferentially solvate lignin is that the THF/water mixture can form a "theta" solvent system, in which the solvent−lignin and lignin−lignin interactions are approximately equivalent in strength, leading to the lignin adopting Gaussian random-coil conformations.[21] Lignin in this type of coil conformation would not self-aggregate; therefore, it can be more easily broken down into smaller molecular fractions. The reduction of $\beta$-5 could be attributed to the formation of stilbenes through the loss of γ-methylol groups as formaldehyde.[35]

**$^{31}$P NMR Analysis.** Lignin OH groups are important characteristics associated with lignin's negative role in enzymatic hydrolysis of lignocellulosics.[36] Quantitative $^{31}$P NMR analysis was used to determine the amount of various types of hydroxyl groups in selected CELF lignin samples, and results are compared to that of the CEL (Figure 3). The
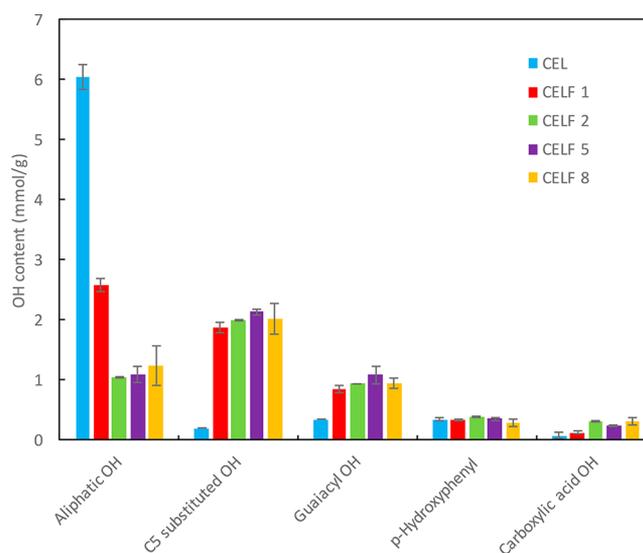


**Figure 3.** Quantification of different hydroxyl group contents (mmol/g) of CEL and CELF lignin samples determined by $^{31}$P NMR spectra.

chemical shifts and integration regions are summarized in Table S3. The aliphatic OH group is the dominant OH type in poplar CEL which accounts for ∼87% of the total free OH groups, and CELF pretreatment resulted in a decrease of the content of aliphatic OH group and an increase of the content of total phenolic OH group. Among phenolic OH groups, $C_5$-substituted OH was observed as the most prominent OH type in all the CELF lignin samples. The amount of carboxylic acid group in CELF lignin is also higher than that in CEL, which could be due to the hydrolysis of ester bonds or oxidation of aliphatic OH groups during CELF pretreatment.[29,30] The dramatic increase of the content of phenolic OH could be a result of $\beta$-O-4 interunit linkages scission as evidenced by the HSQC analysis. The significant decrease of aliphatic hydroxyl groups could be attributed to the loss of γ-methylol group as formaldehyde and OH groups on $C_{\alpha,\beta}$ or the whole side chain in general to from stilbene structures ultimately.[27] CELF lignin 1 had the highest amount of aliphatic OH (2.57 mmol/mg) and lowest amount of total phenolic OH (3.06 mmol/mg), possibly because of its low pretreatment severity. An increase in catalyst loading and THF/water ratio were found to have a negligible effect on the lignin OH content. As free phenolic groups are essential for antioxidant activity of lignin, CELF lignin might be suitable for application as antioxidants.[37]

**Potential Pathways for CELF Lignin Valorization.** Lignin is the most abundant renewable aromatic polymer on earth, offering great potential for high value-added applications in polymeric materials and chemical development.[38] Organosolv lignin is usually sulfur-free, rich in functionality, and has limited carbohydrate contamination.[39] Following the depolymerization of lignin via CELF process to yield CELF lignin, a solvent-based extraction or solute-based precipitation technique can be applied to further extract the low-molecular-weight

components from the high-molecular-weight components. The low-molecular-weight components of CELF lignin could be a preferable candidate as a nonfuel precursor for incorporation in polymeric materials such as lignin-based polyurethanes. On the other hand, a catalytic oxidative or reductive fragmentation could be applied on the cross-linked high-molecular-weight fraction of CELF lignin to yield viable fuel, chemical, and pharmaceutical precursors. In addition, CELF lignin could also be considered as a favorable lignin for structural carbon fiber production because of its low content of reactive C−O bonds in interunit linkages such as $\beta$-O-aryl ether.[40]

## ■ CONCLUSIONS

Sustainable pretreatment techniques need to be developed to extract lignin with high yield, quality, and purity for an integrated biorefinery. Poplar was subjected to a Cosolvent Enhanced Lignocellulosic Fractionation process, and a new type of lignin stream named CELF lignin was recovered and characterized for its physicochemical structural features. Results showed that CELF lignin presented a decreased molecular weight, aryl ether interunit linkages, aliphatic hydroxyl content and increased free phenolic and carboxylic acid hydroxyl groups compared with the untreated native lignin. The scission of $\beta$-O-4 ether interunit linkages in CELF lignin is the major mechanisms of lignin depolymerization during CELF pretreatment. CELF process provides a potential value-added lignin stream to the biorefinery process, and it could also serve as a unique tool to study lignin chemistry in biomass pretreatments.

## ■ ASSOCIATED CONTENT

### Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acssusche-meng.8b01028.

> Composition analysis of CELF lignin samples, assignments of lignin $^{13}$C−$^1$H correlation signals observed in the HSQC spectra of lignin samples, typical chemical shifts and integration regions for lignin samples in $^{31}$P NMR spectra, weight-average ($M_w$) and number-average ($M_n$) molecular weights of CELF lignin and CEL lignin, and quantification of different hydroxyl group contents (mmol/g) of CEL and CELF lignin samples determined by $^{31}$P NMR spectra (PDF)

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### Author Contributions

The experiments were performed through contributions of all authors. All authors have approved the final version of the manuscript.

### Notes

The authors declare no competing financial interest.

## ■ ACKNOWLEDGMENTS

## ■ ABBREVIATIONS

CEL:cellulolytic enzyme lignin
CELF:cosolvent enhanced lignocellulosic fractionation (CELF)
FF:furfural
G:guaiacyl
GPC:gel permeation chromatography
HMF:5-hydroxymethylfurfural
LA:levulinic acid
MD:Molecular-dynamics
MWL:milled wood lignin
$M_w$:weight-average molecular weight
$M_n$:number-average molecular weight
NMR:nuclear magnetic resonance
NHND:endo-$N$-hydroxy-5-norbornene-2,3-dicarboximide
NREL:National Renewable Energy Laboratory
PB:$p$-hydroxyphenyl benzoate
PDI:polydispersity index
S:syringyl
SSF:simultaneous saccharification and fermentation
TMDP:2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane

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