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Sequential Extraction and Characterization of Lignin-Derived Compounds from Thermochemically Processed Biorefinery Lignins

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Supporting Information

ABSTRACT: As the most abundant source of renewable aromatic compounds on the planet, lignin is an attractive feedstock for producing a range of chemicals and products that are currently derived from petroleum. Despite its great potential, separation of lignin depolymerization products remains one of the main obstacles toward cost-effective lignin valorization. Two lignin-rich streams, residues from enzymatic hydrolysis of the dilute acid and alkaline-pretreated corn stover, were depolymerized via pyrolysis using induction heating and catalytic transfer hydrogenolysis (CTH), respectively. Differences in phenolic compounds from gas chromatography−mass spectrometry and gel permeation chromatography analyses suggest that both pretreatment conditions and lignin depolymerization methods affected the product distribution. CTH lignin oils contain less polar compounds as compared to pyrolysis lignin oils, probably due to saturation of the derived compounds as a result of the reductive chemistry. The resulting liquid oils were subjected to sequential liquid−liquid extraction using a series of solvents with different polarities: hexane, petroleum ether, chloroform, and ethyl acetate. Sequential extraction fractionated lignin-derived oil into groups of different compounds depending on the solvent polarities. This study provides a better understanding of how the lignin source and processing method affect the depolymerization products and provides a possible way to fractionate lignin-derived compounds.

1. INTRODUCTION

Lignin, which accounts for 15−25% of plant biomass, is one of the three major components, apart from cellulose and hemicelluloses, in the lignocellulosic matrix. Lignin is a three-dimensional amorphous polymer consisting of mainly methoxylated phenylpropane units. Lignin plays biological roles essential to the life of vascular plants such that it is responsible for the plants' rigid structure and water transport due to its hydrophobic nature.[1] Lignin is formed by the polymerization of three major monomers: *p*-coumaryl (H), coniferyl (G), and sinapyl (S) alcohols, while different plants have different ratios of these three monolignols.[2] For example, softwood lignin is primarily composed of G lignin units, while hardwood lignin and lignin in herbaceous consist mainly G and S units.[2]

As the most abundant source of renewable aromatic compounds on earth, lignin is gaining interest as a feedstock in replacing petroleum-based chemicals and products. It is however an underutilized natural resource due to its structural heterogeneities.[3] In its raw form, lignin has limited uses such as biodispersants, epoxy resins for circuit boards, adhesives, wood panel products, and cement additives.[4] Currently, most of the lignin is burned to produce heat and power. However, the breakdown of lignin could provide desirable chemical functionalities that are not achievable when lignin is a polymer. Once lignin polymers are broken down, low-molecule weight compounds become suitable for further upgrading to fuel and chemicals.[5]

Several lignin depolymerization pathways are being developed, including pyrolysis, catalytic oxidation, catalytic transfer hydrogenolysis (CTH), ionic liquid-based catalysis, and biological depolymerization. Pyrolysis and CTH are the two common thermochemical breakdown methods. Pyrolysis is the breakdown of large molecules into smaller ones by the application of heat in the absence of oxygen. During pyrolysis, lignin is heated to temperatures between 160 and 900 °C where cleavage of the ether (C−O) and C−C linkages takes place.[6] Lignin pyrolysis produces a range of pyrolytic aromatic compounds in the oil form in addition to gas products and residual char. The yield and composition of pyrolytic oil are influenced by many factors, including the lignin type and operation conditions.[7,8] CTH is an attractive alternative to traditional hydrogenation. With CTH using alcohol as a hydrogen donor, as compared to gaseous hydrogen in traditional hydrogenation, CTH is safer. During CTH of lignin, hydrogen-donating solvents, such as formic acid, methanol, ethanol, isopropyl alcohol (IPA), tetralin, and so forth, release hydrogen molecules at elevated temperatures usually with the help of catalysts. The hydrogen is transferred in situ for hydrogenation reactions between the lignin bonds, causing them to breakdown and thus leading to lignin depolymerization.[9] IPA remains a popular choice as hydro-

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gen-donating solvent due to its relative low cost and easy subsequent separation from the reaction mixture.[10]

Lignin depolymerization products are usually a mixture of aromatic compounds. In order to find the best use of these compounds, it is necessary to investigate a separation method that is cost effective and efficient in recovering specific aromatic compounds. Several separation techniques are being investigated, including chromatography, evaporation, and membrane filtration.[11−13] However, many of these techniques can be costly because of the use of multiple membranes for different molecular weight separations or the use of expensive chromatography columns. Liquid−liquid extraction (LLE), commonly known as solvent extraction and partitioning, is a method to separate compounds based on their relative solubilities in two different immiscible liquids and is relatively cost-effective because the solvents can be recovered and reused.[14] The two liquid phases usually have different polarities or solubilities for specific molecules, so the compounds partition into two phases depending on the polarities/solubility of the molecules.

LLE has been applied to fractionate the bio-oil water extract recovered from pyrolysis of lignocellulosic biomass, e.g., switchgrass.[15] In such a process, a series of organic solvents including ethyl acetate, chloroform, petroleum ether, and hexane were used to extract groups of lignin-derived compounds. Results show that each of these solvents has different extraction efficiencies for different compounds based on the solvent polarity.[15] For example, chloroform has high extraction efficiency for furans, phenolics, and ketones. Ethyl acetate, on the other hand, has high extraction efficiency for organic acids. In another study, enzymatic hydrolysis lignin derived from steam explosion pretreatment of corn stover was fractionated by sequential extraction with dichloromethane, ethyl acetate, and *n*-butyl alcohol.[16] Results show that the molecular weight of the recovered fraction decreased, and the total phenolic and methoxyl concentrations increased following each extraction steps. Those lignin fractions exhibited improved antioxidant performance as determined by 2,2-diphenyl-1-picryl-hydrazyl-hydrate-based free-radical scavenging assay.[16]

Despite previous works on sequential extraction of lignin-derived compounds, it is not clear whether the same protocols can be applied for lignin sources from different biomass feedstocks and fractionated using different pretreatment methods. Furthermore, as lignin depolymerization methods greatly influence the product distribution, it is necessary to investigate whether the depolymerization method affect the extraction performance. Thus, we reported here the first comparative study on the sequential extraction and characterization of lignin-derived compounds from two biorefinery lignins processed using two thermochemical depolymerization methods. We aim to fractionate the lignin-derived liquid oils using a prescribed sequence of solvents and characterize extracted streams. To achieve this, we characterized the liquid oils resulted from pyrolysis, using induction heating and CTH of two lignin streams, with a difference in pretreatment chemistry. We then studied the fractionation of compounds that were sequentially extracted from lignin pyrolysis and CTH oils using organic solvents of different polarities. This study provides a better understanding of how the lignin source and processing method affect the depolymerization products and provides avenues to fractionate lignin-derived compounds toward novel uses for this underutilized natural resource.

2. MATERIAL AND METHODS

2.1. Materials. Two lignin samples used for this study were generously provided by National Renewable Energy Laboratory (NREL). Two pretreatment methods: dilute acid (DA) and alkali (AL) were used to pretreat corn stover at NREL.[17,18] The DA pretreatment was conducted at 175 °C, 30 g H_2SO_4/kg of dry biomass, 30% solids loading, and a residence time of 8 min. The AL pretreatment used 0.07 g NaOH/g biomass with a solid/liquid ratio of 1:12 at 92 °C for 2 h. The AL-pretreated biomass slurry was then disk-refined at 200 kW h/ODMT. The pretreated biomass was hydrolyzed with cellulase and hemicellulase enzymes, and the solid residues retained as the lignin streams for this study. Enzymatic hydrolysis was conducted at 15% solid loading, 64 mg CTec2, and 8 mg HTec2 per gram of cellulose following DA pretreatment, while at 48 mg CTec2 and 12 mg HTec2 per gram of cellulose following AL pretreatment for 36 h.[18] The two lignin samples were denoted as "DA" and "AL" lignins as shorthand notation for the lignin-rich residues collected after DA and alkaline pretreatment followed by enzymatic hydrolysis.

Upon receiving the DA and AL lignin samples, they were washed with deionized water to remove any soluble components, dried in a convection oven at 105 °C overnight and then grounded using a mortar and pestle. Composition of the lignin samples was determined using the procedure described by NREL.[19] Following the two-stage acid hydrolysis, the amount of monomeric sugars, glucose and xylose, was measured by Dionex UltiMate 3000 HPLC (Dionex Corporation, Sunnyvale, CA, USA) equipped with a refractive index detector and a Biorad Aminex HPX-87H column, using 5 mM H_2SO_4 as the mobile phase at a flow rate of 0.4 mL/min and a column temperature of 50 °C. The organic solvents, hexane, petroleum ether, chloroform, ethyl acetate, IPA, and palladium on activated charcoal (Pd/C), were purchased from Sigma-Aldrich.

2.2. Lignin Deconstruction. *2.2.1. Pyrolysis.* Pyrolysis of DA and AL lignin samples was performed at Louisiana State University (LSU). Approximately, 7.5 g of each pretreated sample was added directly to a custom pipe reactor (SS-316, 20″ length, 3/8″ i.d., 1/2″ o.d.). The biomass was distributed over the 8.0″ length of the reactor. Pieces of cotton were loaded into both ends of the reactor to retain biomass within the reactor. The reactor was then placed inside a horizontal induction coil (6 turns, 9″ length, 2.0″ i.d.) such that the biomass inside the reactor was positioned within the coil. This assured that all of the biomass was within the coil region and heated uniformly. One end of the reactor was attached to a N_2 source by way of high-temp tubing, and the flow rate was regulated by a MC-50SLPM-D Mass Flow Controller (Alicat Scientific, Tucson, Arizona). The other end of the reactor was attached to a cold trap collection system using high-temperature plastic tubing (∼2.5″, 0.2″ i.d.). The tubing was attached to a 1/8″ steel tube that traveled through the interior and ended at the bottom of a collection vial. The cold trap collection system consisted of this collection vial placed inside a larger beaker containing both dry ice and acetone. Gaseous products produced by the pyrolysis of biomass entered the cold trap system through the 1/8″ pipe connected to the reactor.

Prior to each pyrolysis experiment, N_2 gas flowed through the custom-designed reactor at a rate of 0.45 L/min for a minimum of 20 min in order to purge the system of any excess O_2. When the purging session was completed, the N_2 flow rate was adjusted to 0.15 L/min, and the induction heater was turned on. The PID portion of the IR2 SUPERMETER was programmed to the desired set point temperature (500 °C) and was configured to send voltage changes to the HFI model induction heater (RDO Induction LLC, Washington, NJ) in order to increase or decrease the power of the induction heater such that the reactor would be stabilized at 500 °C. The experiment ran for 50 min during which time gaseous products condensed into the cold trap system. The masses collected before and after the experiment were used to determine the liquid oil yield. The solid residue was weighed for the solid yield, and the gas yield was estimated by subtracting liquid and solid yields from the starting lignin mass.

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2.2.2. Catalytic Transfer Hydrogenolysis. CTH was performed using a Parr Reactor (Series 4560 Mini Reactor, Moline, IL) at a set temperature of 270 ± 5 °C for 1 h. Prior to the reaction, the Parr reactor was preheated for 45 min to reach the set temperature, which was not included in the reaction time. In a typical reaction, 15.7 g of IPA and 2 g of the lignin were added to the reactor. Subsequently, 0.2 g (10 wt %) of the Pd/C catalyst was added to the reactants.[10] After the reaction, the reactor was quenched rapidly to 100 °C by forced air and then to room temperature in an ice bath to prevent undesirable secondary reactions. The contents in the reactor were then recovered by rinsing with acetone. Separation of solid residues and liquid was carried out by centrifuging at 4000 rpm for 10 min, and the liquid fraction was dried in a vacuum oven at room temperature for 48 h to remove acetone and IPA. The gas (not collected) yield was estimated by subtracting liquid and solid yields from the starting lignin mass.

2.3. Sequential Extraction. Sequential extraction was performed to separate the lignin-derived compounds based on polarity. The oil collected from pyrolysis or CTH was first dissolved in isopropanol and then diluted with water to a water–isopropanol ratio of 80:20 v/v. Four solvents with different polarities were used: from the most polar to least polar, ethyl acetate, chloroform, petroleum ether, and hexane.[20] Based on a previous study, the order from the least polar to polar was hexane, petroleum ether, chloroform, and ethyl acetate.[15] The flow chart of this extraction protocol is shown in Figure 1. Sequentially extracted fractions were dried in a vacuum oven at room temperature for at least 48 h or until all the solvents were evaporated.

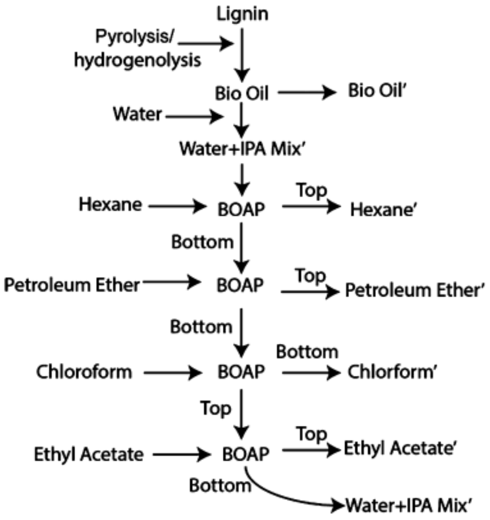

Figure 1. Sequential extraction flow chart (BOAP stands for bio-oil aqueous phase).

2.4. Characterization of Lignin-Derived Compounds. *2.4.1. Gas Chromatography–Mass Spectrometry.* Sequentially extracted fractions from pyrolysis and CTH were analyzed by Agilent 7890B GC coupled with a 5977B MS via HP-5MS (60 m × 0.32 mm) capillary column. The temperature program started at 40 °C with a holding time of 6 min and increased to 240 °C at 4 °C min^{-1} with a holding time of 7 min; finally, the temperature was raised to 280 °C at 20 °C min^{-1} with a holding time of 8 min. Helium was used as a carrier gas with a flow rate of 1.2 mL min^{-1}. Sequentially extracted oil samples were dissolved in 1 mL of dichloromethane and analyzed in gas chromatography–mass spectrometry (GC–MS) for phenolic compounds. The compounds were identified via the mass spectra from the National Institute of Standards and Technology (NIST) library. Because of the lack of chemical standards for most of the GC–MS identified compounds, products were reported as percentage of the total identified compounds using the relative peak area divided by the total peak area.

2.4.2. Gel Permeation Chromatography. The molecular weight distribution (MWD) of the raw lignin, the pyrolysis and CTH liquid oils, and the sequentially extracted fractions were determined using gel permeation chromatography (GPC).[21] GPC analysis was performed using an Ultimate 3000 HPLC system (Dionex Corporation, Sunnyvale, CA, USA) equipped with an ultraviolet (UV) detector. Separation was achieved in a mobile phase of tetrahydrofuran at a flow rate of 0.5 mL min^{-1}, using a Mixed-D PLgel column (5 μm particle size, 300 mm × 7.5 mm i.d., and a linear molecular weight range of 200–400 000 μm, Polymer Laboratories, Amherst, MA) at 50 °C. The weight-average molecular weight (M_w) and the number-average molecular weight (M_n) from the elution profile of materials were calibrated using a polystyrene standard kit (product no. 48937, Sigma-Aldrich). Absorbance of eluting materials was detected at 280 nm (UV).

2.4.3. Fourier Transform Infrared Spectroscopy. Fourier transform infrared spectroscopy (FTIR) spectra were obtained for the two lignin samples along with a commercial kraft lignin (Sigma-Aldrich) using a FTIR spectrometer (Thermo-Nicolet Nexus 670 FTIR, Waltham, MA). All spectra were generated over accumulative 64 scans with a resolution of 4 cm^{-1} in the range of 700–4000 cm^{-1}. A spectrum was generated without any sample (blank) to account for background noise.

2.5. Statistical Analysis. All experiments were conducted in duplicate or triplicate, and the data are presented as means and standard deviations. The statistical analysis, t-tests, were performed by SAS 9.4 (SAS Institute, Cary, NC, US), with a significance level of $P < 0.05$ for all the data obtained from experiments.

3. RESULTS AND DISCUSSION

3.1. Compositional Analysis and Characterization of Raw Lignin Samples. A composition analysis was conducted to examine the makeup of the DA and AL lignin samples prior to thermochemical depolymerization. The results are shown in Table 1. While both the lignin and xylan content for the DA and AL lignin samples were not significantly different, the glucan content was found to be significantly different, 27.5 and 18.6%, respectively. Composition analysis on raw corn stover indicated glucan, xylan, and lignin contents of roughly 36, 20, and 19%, respectively.[22] During DA pretreatment, most of the hemicellulose (xylan) is solubilized where lignin and cellulose remain in solids, but in AL pretreatment, a large portion of lignin and hemicellulose is solubilized, and cellulose remains in solids.[23] Lignin can precipitate on cellulose during DA pretreatment thus blocking enzyme access to cellulose due to steric hindrance.[23] In a previous work, results show that AL pretreatment outperformed DA pretreatment in terms of higher enzymatic hydrolysis sugar yields from four types of biomass feedstocks.[24] This could explain the significantly higher glucan content in the residue after enzymatic hydrolysis of DA-pretreated corn stover as compared to AL-pretreated corn stover. Large portions of glucan and xylan in the biomass-

Table 1. Composition of DA- and AL-Pretreated Lignin Samples[a]

	lignin	glucan	xylan	total	other
DA lignin	62.83 ± 1.81^{a}	27.54 ± 0.24^{a}	6.04 ± 0.02^{a}	96.41 ± 2.07	3.60 ± 2.07
AL lignin	58.91 ± 5.67^{a}	18.63 ± 1.68^{b}	7.58 ± 0.71^{a}	85.12 ± 8.06	14.88 ± 8.06

[a] Letter differences amongst each lignocellulose fraction indicates differences at 95% confidence where values are mean ± SE ($n = 2$), using a t-test.

derived lignin samples are likely caused by incomplete enzymatic hydrolysis.[25] The carbohydrate impurities in the DA and AL lignin samples could affect the lignin depolymerization oil yields as compared to pure lignin.[8,26] However, any further purification step on the lignin samples would add extra cost in chemicals and energy. Thus, in this study, the lignin samples were used as received because they represent the real lignin samples that one could recover from a biorefinery.

To gain more insights into the compositional and chemical features of the DA and AL lignin samples, they were compared to commercial kraft lignin and examined by FTIR (Figure 2).

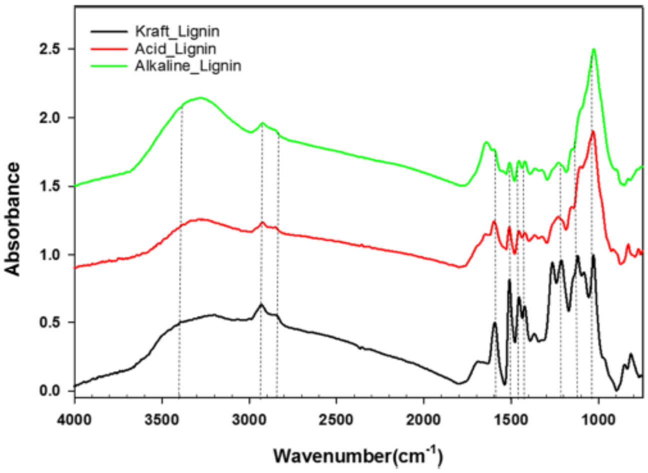

Figure 2. FTIR spectra of DA and AL lignins as compared to a commercial kraft lignin.

The aliphatic and aromatic O−H groups are visible at an absorption band of 3400 cm^{-1}, which can be seen in all three lignin samples as a result of water removal during drying.[27] The band at 2930 and 2840 cm^{-1} can be designated with the vibrations of C−H from the CH_2 and CH_3 groups.[28] The C═C peaks of aromatic skeletal vibrations at 1595 and 1510 cm^{-1}[29] were significantly lower in intensity for DA and AL lignins as compared to the kraft lignin. Significant decreases in peak intensity at 1420 cm^{-1} (C−H aromatic ring) for DA and AL lignins were noticed when compared to the kraft lignin, demonstrating possible cleavage of the CH_2 and CH_3 groups. Additionally, a decrease in peak intensity at 1460 cm^{-1} corroborates the possible removal of CH groups. The band at 1220 cm^{-1} is akin to C−C, C−O, and C═O stretching guaiacyl[30,31] showing a decrease in intensity when DA and AL lignins were compared to the kraft lignin.[32] The band at 1110 cm^{-1} assigned to aromatic C−H deformation in syringyl (García et al., 2012) showed a decrease in intensity from DA and AL lignins when compared to the kraft lignin. The peak at 1050 cm^{-1} refers to C−O vibrations of the crystalline cellulose region. With DA and AL lignins having a main peak at 1050 cm^{-1} indicates that both of these lignin samples have a higher cellulose content than the kraft lignin.[33] These FTIR results are in agreement with the composition analysis results that were obtained from DA and AL lignins. Both DA and AL lignin sources were much less pure than the kraft lignin. The higher impurity is probably contributing to the decrease in oil yield, seen below, from the thermochemical depolymerization of these two lignin streams.

3.2. Mass Balance of Oils and Sequential Extraction Fractions. A mass balance was attempted to determine what fractions were formed from DA and AL lignins after pyrolysis using induction heating and CTH. Induction heating offers a contactless heating method for pyrolysis that has several advantages over conductive heating methods such as rapid heating rates, precise temperature control, and high energy efficiency.[8,34] Table 2 shows the oil, solid, and gas percentages from the mass balance of the lignin streams. Pyrolysis produced significantly more lignin oil compared to CTH lignin for both the DA lignin (pyrolysis: 15.34 ± 0.96%; CTH: 8.27 ± 1.48%) and AL lignin (pyrolysis: 20.76 ± 5.67%; CTH: 9.92 ± 1.34%). Within pyrolysis and CTH treatments, the DA and AL lignins did not produce significantly different oil yields. Overall, the oil yields seen in this study were low relative to other papers for CTH and pyrolysis.[35] Reports have shown that the presence of glucan and xylan can suppress metal catalysts and inhibit lignin depolymerization into oils during CTH,[26] and that they produce more char and volatile gases during pyrolysis at the temperatures used in this investigation.[36] Thus, the liquid oil yields could be improved by purifying the lignin streams prior to lignin depolymerization.

A mass balance was performed on the sequentially extracted fraction of each lignin oil, which are presented in Figure 3. When examining CTH lignin oils, considerable differences in the mass fractions were seen. The majority of the mass was found in the hexane fraction (57.66%) of DA lignin oil; while most of the mass was found in the chloroform fraction (46.15%) of AL lignin oil. This observation indicates that it is possible that more nonpolar compounds were produced from DA lignin as compared to AL lignin. Results suggest that the source of lignin feedstocks can significantly affect the depolymerization products especially for CTH. This may be due to an increase in vinyl or alkyl groups in the lignin produced after DA pretreatment,[37] which could cause more nonpolar functional groups on the phenolic compounds produced with reductive chemistry during CTH.

Additionally, there is a general trend in each treatment's sequential extraction that hexane and chloroform extracted most of the lignin-derived compounds, compared to petroleum ether and ethyl acetate. Because hexane and petroleum ether have relatively similar polarities,[20] less compounds were caught in petroleum ether because hexane was used first. Similar results were found in a previous study that hexane and

Table 2. Oil, Solid, and Gas Products from Pyrolysis and CTH of DA and AL Lignins[a]

	pyrolysis			CTH		
	% oil	% solid	% gas	% oil	% solid	% gas
DA lignin	15.34 ± 0.96^{a}	60.35	24.31	8.27 ± 1.48^{b}	37.28 ± 0.53	54.46 ± 2.02
AL lignin	20.76 ± 5.67^{ad}	58.12	21.12	9.92 ± 1.34^{bd}	44.22 ± 1.51	45.87 ± 0.17

[a] Letter differences within and across treatments and the lignin type for the oil content indicate differences at 95% confidence where values are mean ± SE (n = 2), using a t-test; the % solid and % gas yields for pyrolysis samples were based on the combined mass of two runs.

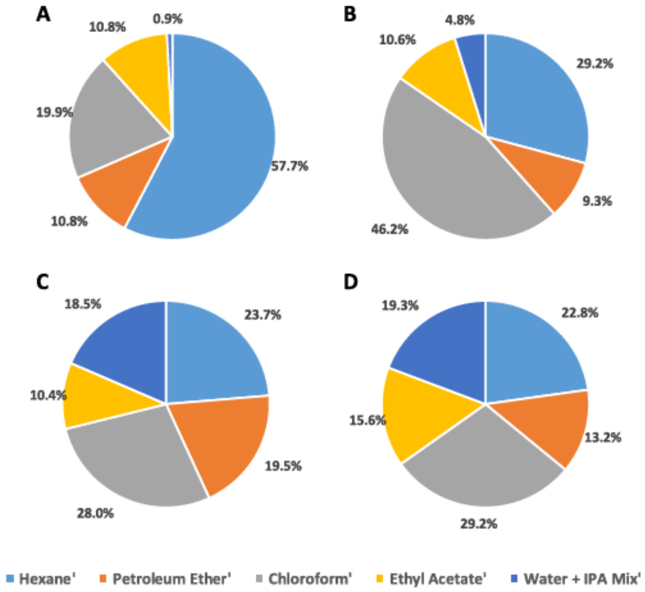

Figure 3. Mass fractions from different organic phases during sequential extraction of lignin oils from (A) CTH of the DA lignin, (B) CTH of the AL lignin, (C) pyrolysis of the DA lignin, and (D) pyrolysis of the AL lignin.

petroleum ether extracted similar fractions of compounds when used alone on pyrolytic oil from switchgrass.[15] Although ethyl acetate is relatively more polar than chloroform, chloroform is much more polar than petroleum ether or hexane. Therefore, when chloroform was used before ethyl acetate, it extracted most polar compounds and semipolar compounds. Overall, results suggest that the first nonpolar or polar solvent used during sequential extraction extracts a greater proportion of available compounds produced during lignin depolymerization.

Furthermore, when comparing CTH and pyrolysis, significantly higher portions of compounds remained in the water + IPA fraction for the pyrolysis lignin oils. Pyrolysis tends to generate more polar oxidation products such as organic acids, alcohols, furans, and hydroxyacetone, which all have a high distribution coefficient in water.[15] Conversely, as a reductive process, CTH increases aromatic ring saturation due to the high hydrogenation activity with Pd/C catalyst.[38] Less-polar compounds may have been produced during CTH that can be extracted by less polar solvents (hexane or petroleum ether), thus leading to a smaller portion of polar compounds remained in the water + IPA fraction. Taking together, results suggest that CTH is more sensitive to the lignin sources (DA and AL pretreatment) in terms of mass fractions during sequential extraction, while less variation was seen for pyrolysis. Depolymerization methods greatly affect the compounds produced and the mass fractionations in sequential extraction solvents due to the different chemistries of CTH and pyrolysis.

3.3. GC–MS Characterization of Oils and Sequential Extraction Fractions. The raw lignin-derived oil and sequential extraction fractions from each treatment and the lignin type were run on GC–MS, and the spectra are shown in Figures 4, 5, and S1, with relative percentages of compounds produced as shown in Table 3 and Table S1. As can be seen, CTH and pyrolysis produced a variety of monomeric phenolics. A similar range of compounds was found in previous studies as major lignin degradation products from

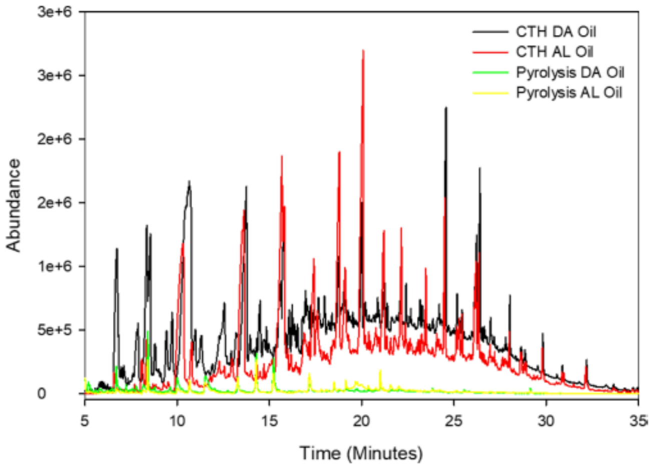

Figure 4. GC–MS chromatography of liquid lignin oils.

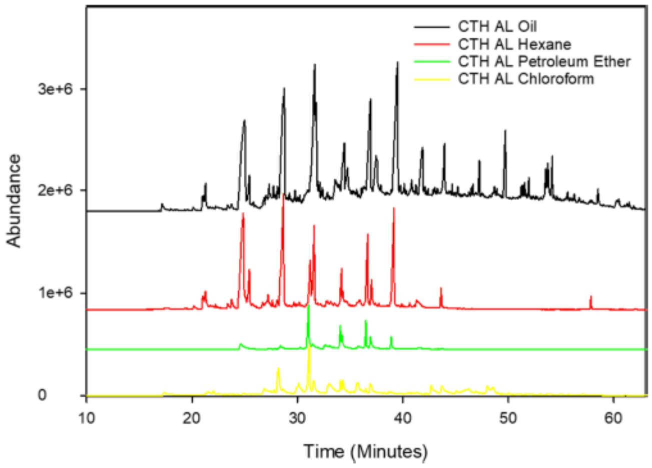

Figure 5. GC–MS spectra of the CTH AL oil and extracted fractions using different solvents.

the CTH of the sorghum lignin and from pyrolysis of the corn stover lignin.[39,40] However, there are major differences in the relative amounts of phenolic compounds in raw lignin-derived oils. As an example, when comparing the peak of 4-ethyl-phenol, it is observed from Table 3 and Table S1 that this compound represented ~20% of the total identified products (relative peak area) in CTH-DA lignin oil, while 4-ethyl-phenol only counted ~13% of the total identified products (relative peak area) in CTH-AL lignin oil. When comparing the peak of 4-ethyl-2-met-phenol, it was shown that the DA and AL lignin derived-oils from pyrolysis contain 6 and 14% of the total identified products (relative peak area), respectively.

Table 4 also shows the specific groups of compounds in each sequentially extracted fraction from the CTH-AL lignin-derived oil. Interestingly, it was seen that increasing polarity of the solvent led to a general increase in the number of oxygen groups (hydroxyl or ether) and less alkyl chains/groups on the phenolic rings in the extracted compounds. For example, the hexane fraction contains a high amount of 2-methoxy-4-propyl-phenol (~13%) with a long alkyl chain, while the chloroform fraction contains a high amount of 3-methoxy-1,2-benzenediol (~22%) with more polar hydroxyl groups. Furthermore, the hexane fraction of CTH–DA lignin-derived oil contains more 4-ethyl-phenol, which has a short nonpolar alkyl chain than any other monolignols, indicating that the DA lignins is highly

Table 3. Percentage of Compounds in Fractions Sequentially Extracted from CTH of the AL Lignin

time (min)	compound name	bio-oil′ (%)	hexane′ (%)	petroleum ether′ (%)	chloroform′ (%)
10.5	phenol, 4-ethyl-	12.04	23.68	9.53	
13.8	phenol, 4-ethyl-2-methoxy-	7.74	18.90	3.80	
16.0	phenol, 2,6-dimethoxy	13.15	5.85	33.23	31.47
17.6	phenol, 4-methoxy-3-(methoxymethyl)-	3.63	3.60	11.03	
19	benzene,1,2,3-trimet	6.82			
19.5	5-sec-butylpyrogallol	4.48			
20.3	3,5-dimethoxy-4-hydro	12.52			
25.4	creosol		4.54		
21.2	phenol, 2-methoxy-		2.29		
31.6	phenol, 2-methoxy-4-propyl-		8.78		
36.7	benzoic acid, 3,4-dimethoxy-		8.58		
37.2	4-ethoxy-3-methoxybenzyl alcohol		2.91		
39.2	benzeneacetic acid, 4-hydroxy-3,5-dimethoxy-		12.92		
32.6	4-ethylcatechol			2.32	
34.2	benzoic acid, 4-hydroxy-3-methoxy-			8.56	17.33
35.8	phenol, 5-methoxy-2,3-dimethyl-			2.80	
36.5	benzene, 1,2,3-trimethoxy-5-methyl-			12.86	
36.9	3-ethoxy-4-methoxybenzyl alcohol			9.74	
38.9	benzeneacetic acid, 4-hydroxy-3,5-dimethoxy-			6.12	
28.2	1,2-benzenediol, 3-methoxy-				22.21
29.9	1,2-benzenediol, 4-methyl-				4.53
31.5	phenol, 3,4-dimethoxy-				14.39
37.8	2-propanone, 1-(4-hydroxy-3-methoxyphenyl)-				4.01
42.7	ethanone, 1-(4-hydroxy-3,5-dimethoxyphenyl)-				6.07
	summary (>2% concentrations)	60.39	92.04	100.00	100.00

Table 4. MWD of Raw Lignin and the Lignin Oils Derived from CTH and Pyrolysis and Their Sequentially Extracted Fractions[a]

		M_n (g/mol)	M_w (g/mol)	PDI
raw lignin	DA	1525.4	3483.2	2.28
	AL	1701.1	4347.1	2.56
CTH DA	bio-oil	389.4	1181.6	3.03
	hexane	501.9	1018.1	2.03
	petroleum ether	620.7	1035.2	1.67
	chloroform	454.2	1047.6	2.31
	ethyl acetate	452.6	1218.4	2.69
	water + IPA mix	ND	ND	ND
CTH AL	bio-oil	597.0	1157.1	1.94
	hexane	579.2	946.1	1.63
	petroleum ether	540.4	831.6	1.54
	chloroform	527.4	933.0	1.77
	ethyl acetate	379.5	657.1	1.73
	water + IPA mix	ND	ND	ND
pyrolysis DA	bio-oil	468.9	822.7	1.75
	hexane	576.6	821.1	1.42
	petroleum ether	525.2	850.8	1.62
	chloroform	519.8	896.4	1.72
	ethyl acetate	390.8	925.5	2.37
	water + IPA mix	ND	ND	ND
pyrolysis AL	bio-oil	513.9	874.0	1.70
	hexane	608.9	854.5	1.40
	petroleum ether	578.8	844.8	1.46
	chloroform	553.5	904.5	1.63
	ethyl acetate	466.9	836.6	1.79
	water + IPA mix	ND	ND	ND

[a] ND = not determined.

alkylated. Generally speaking, results support the hypothesis that sequential extraction can be used to extract specific groups of compounds based on polarity from lignin-derived oils. Thus, it is possible to fractionate lignin depolymerization using sequential extraction to channel groups of compounds that have a specific property or targeted functionality.

3.4. MWDs of Oils and Sequential Extraction Fractions. High-molecular weight phenolic compounds remain undetected in GC–MS analysis due to their low volatility. Hence, to better understand changes in the molecular weight distribution, the weight-average molecular weight (M_w) and number-average molecular weight (M_n) of the untreated lignin, the raw lignin-derived oils, and the oil fractions recovered from the sequential extraction are shown in Table 4. In addition, the MWD profiles are illustrated in Figure 6. Comparing the MWD profiles of the unreacted AL and DA lignins with CTH and pyrolysis lignins, the MWD curves shifted to later retention times (corresponds to lower M_w as shown in Figure 6A,B), indicating lignin depolymerization via CTH and pyrolysis. This is confirmed by M_w and M_n values for both DA and AL lignins as shown in Table 4. The much lower M_w values of pyrolysis lignin-derived oils suggest a greater degree of depolymerization during pyrolysis as compared to CTH. It has been reported that during pyrolysis, alkyl chains and aromatic rings are disrupted to a range of small phenolic compounds.[36] CTH promotes reductive aryl ether bond cleavage of the lignin in the presence of hydrogen as the reducing agent.[38] The M_w and M_n values reported in this study corroborate other works for pyrolysis of switchgrass and CTH of the wheat straw lignin from soda pulping.[15,41] Although GPC can give a rough estimation of the MWD, quantification of lignin oligomers is very challenging due to the lack of chemical standards. The GPC results suggest that on average, the lignin oligomers are in the range of trimers to

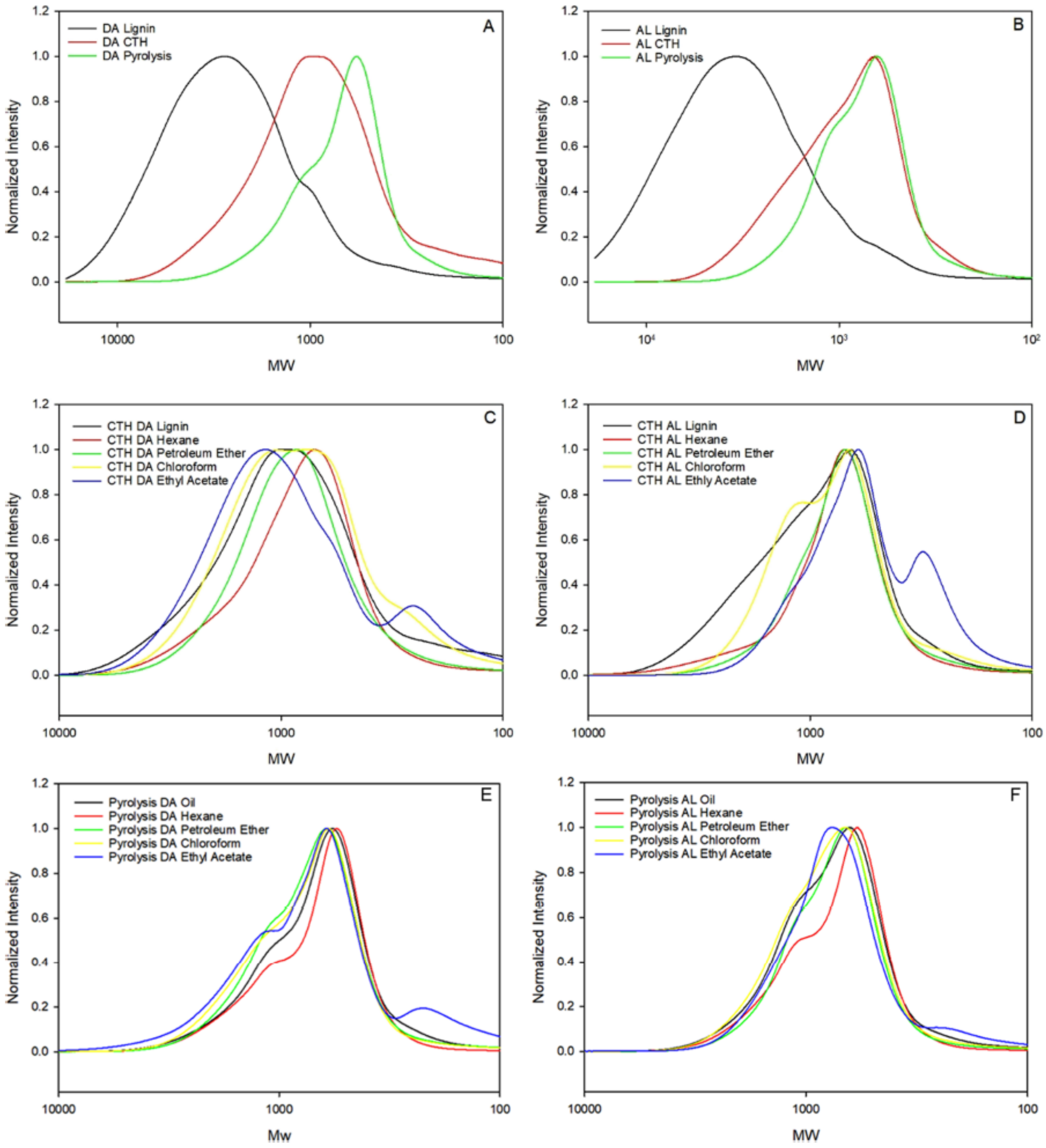

Figure 6. GPC spectra of raw and thermochemically processed (A) DA and (B) AL lignins, (C) DA lignin's sequentially extracted fractions, (D) AL lignin's sequentially extracted fractions resulted from CTH, (E) DA lignin's sequentially extracted fractions from pyrolysis, and (F) AL lignin's sequentially extracted fractions resulted from pyrolysis.

tetramers. The rough ratio of monomers in the bio-oil is about 15−20% for this work which is in general agreement with the ratios reported in previous studies.[8,42]

When comparing the raw lignin-derived oils with the sequential extraction fractions, in general, the raw lignin-derived oils have similar or in some cases slightly higher M_w values and significantly higher polydispersity index (PDI) values. Results also indicate that the sequential extraction fraction has a narrower size range of compounds, suggesting that compounds in the raw lignin oil were separated to more specific groups. This corroborates the GC−MS results that each fraction contained fewer compounds as compared to the compounds in the raw lignin oil. When the different sequential extraction fractions were compared, the oil fractions from CTH and pyrolysis of the DA lignin have slightly higher M_w and PDI values in the ethyl acetate fractions. Conversely, lower M_w with slightly higher PDI values was observed for the ethyl acetate fractions from CTH and pyrolysis of the AL lignin. These results suggest that ethyl acetate extracted not only larger compounds but also a wider size range of compounds, which can be seen by the presence of multiple peaks in the ethyl acetate fraction for both pyrolysis and CTH oil fractions (Figure 6C,E). Taking together, both the source of lignins (DA or AL lignin) and the depolymerization method affect the products' MW distribution as well the sequential extraction performances.

4. CONCLUSIONS

From the composition analysis and FTIR results, both DA and AL lignin samples contain a large portion of polysaccharides.

The sugar impurities in the lignin samples probably contributed to the low oil yields during pyrolysis and CTH. Differences in phenolic compounds from GC−MS and GPC analyses suggest that both pretreatment conditions (DA or AL pretreatment) and lignin depolymerization methods (pyrolysis and CTH) affect the product distribution. Results show that CTH lignin oils contain less polar compounds as compared to pyrolysis lignin oils, probably due to saturation of the derived compounds as a result of the reductive chemistry. Pyrolysis appeared to be more effective in reducing the average molecular weight than CTH. Depending on the polarity preferences, the sequential extraction process separated lignin-derived oil into groups of different compounds based on solvent polarities. This study demostrates the potential of using sequential extraction for fractionating lignin depolymerization products into groups of compounds with a specific property or functionality.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.energy-fuels.9b00376.

> GC−MS spectra of CTH and pyrolysis oil and extracted fractions using different solvents and percentage of compounds in fractions sequentially extracted from DA and AL lignins (PDF)

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Notes

The authors declare no competing financial interest.

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