In Depth Interpret the Structural Changes of Lignin and Formation of Diketones During Acidic Deep Eutectic Solvent Pretreatment

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Contents

1. Materials and Methods

1.1 Chemicals and Materials

Choline chloride (ChCl), lactic acid (LA) and oxalic acid (OA) were purchased from Sigma-Aldrich. All the chemicals used were analytical grade. The poplar (Triploid of *Populus tomentosa* Carr.) was ground to pass through a 0.8 mm size screen and was extracted with toluene-ethanol (2:1 v/v) in a Soxhlet extractor for 6 h. The extracted poplar sawdust was milled for 1 h in a 500 mL ZrO_2 bowl with mixed balls. The ball-milled poplar was pretreated by 1% sodium hydroxide with a solid–to-liquid ratio of 1:15 (g/mL) at 75 ^oC for 3 h. The purification procedure was performed according to the method of Sun *et al.*[1]

1.2 DES Preparation

Acidic DESs were prepared: choline chloride-lactic acid (ChCl-LA) and choline chloride-oxalic acid (ChCl-OA) with molar ratios of 1:2 and 1:1, respectively. The mixtures were carried out at 60 ^oC and stirred at 200 rpm until a clear liquid was formed. The obtained DESs were cooled at room temperature in a desiccator with silica gel for avoiding moisture absorption.

1.3 Analysis Methods

(1) Measurement of the solvatochromic parameters

Stock solutions of the Kamlet-Taft dyes (Nile red, 4-nitroaniline and N, N-diethyl-4nitroaniline) were firstly dissolved in methanol with a concentration of 1.0×10^{-3} mol L^{-1}.[2-4] The solution was transferred by a micropipette into quartz cells with 10 mm light-path length. Each time 0.5 mL dye solution was added into a 5 mL centrifuge tube. The methanol was carefully removed by vacuum drying at 40 ^oC for 48 h. Then 2 g DES was added and the solution carefully mixed with shaking. The absorption spectra were recorded with UV-Vis spectroscopy at room temperature in the wavelength range of 250-800 nm. Deionized water was measured for background substrate. The Kamlet-Taft parameters were determined using the following equations:

$E_{NR} = 28591 / \lambda_{NR}$

$\pi^* = 0.314\times(27.52- \nu_{NEt2})$

$\beta = 11.134-0.358\ \nu_{NH2} - 1.125\ \pi^*$

$\alpha = (19.9657-1.0241\ \pi^*- \nu_{NR}) /1.6078$

$\nu = 1 / (\lambda_{max}\times10^{-4})$

(2) NMR Analysis

All NMR spectra of lignin were recorded on a Bruker AVIII 400 MHz (Bruker, Germany) spectrometer at 25 °C. The liquid was solvent in 0.2 mL DMSO-d_6. The peak (2.49 ppm) of DMSO-d_6 was used as internal standard. The ^{1}H NMR spectra were acquired according to previous papers.[5,6] For the 2D-HSQC NMR, 30 mg of lignin was dissolved in 0.5 mL of DMSO-d_6. 2D-HSQC NMR spectra were acquired as previously.[7] The data was processed using standard Bruker Topspin-NMR software and the DMSO was used as an internal standard (δ_C/δ_H 39.5/2.49). For ^{31}P NMR, 20 mg lignin was dissolved in a solvent mixture composed of anhydrous pyridine and deuterated chloroform, cyclohexanol as an internal standard, chromium (III) acetylacetonate solution as relaxation reagent and 2-chloro-1, 3, 2-dioxaphospholane as phosphitylating reagent. ^{31}P NMR spectra were conducted according to previous literature.[8] For the quantitative ^{13}C NMR experiments, 140 mg of lignin was dissolved in 0.5 mL of DMSO-d_6. 20 μL of chromium (III) acetylacetonate (0.01 M) was added as a relaxation agent for the quantitative ^{13}C spectrum to reduce the relaxation delay. The quantitative ^{13}C NMR spectra were recorded according to a previous paper.[9]

(3) FTIR Analysis

The chemical information of AL and the regenerated lignin was characterized by FTIR spectrophotometer (Bruker Tensor Ⅱ). Dried samples with KBr were ground in agate mortar and pelletized. All spectra were tested with the scanning range from 4000 to 400 cm^{-1} at 4 cm^{-1} resolution.

(4) GPC Analysis

Prior to molecular weight determination, the lignin fractions were acetylated according to the previous research.[10] The weight-average (M_w) and number-average (M_n) molecular weights of the acetylated lignin were determined by gel-permeation chromatographic (GPC) (Agilent 1200, USA) with an ultraviolet detector (UV). The different molecular weight polystyrene standards were used to calibrate at UV absorbance of 254 nm. 4.0 mg of the acetylated lignin was dissolved in 2.0 mL of tetrahydrofuran (THF) and then filtered through a 0.45 μm organic filter. The column was a PL-gel 3 μm mixed-E 300×7.5 mm and eluted with THF at a flow rate of 0.5 mL min^{-1} at ambient temperature.

(5) TG Analysis

The devolatilization behavior of lignin was conducted by a Netzsch STA 409 thermogravimetric (TG) analyzer with a heating rate of 20 ^{o}C min^{-1} from 25 to 900 ^{o}C.

(6) GC-MS Analysis

To analyze the depolymerized products after the DES pretreatment, the extract solution was directly sampled for analysis without any further treatment other than the addition of 1.5 mL methyl alcohol. The extraction solution was analyzed with gas chromatography-mass spectrometer (GC-MS) (Shimadu GCMS-QP2010SE) equipped with a SH-Rxi-5Sil MS capillary column (30 m×0.25 mm). Helium was used as the carrier gas with a constant column flow of 1 mL min^{-1}. The injection temperature and the detection temperature were maintained at 250 and 290 ^{o}C, respectively. Sensitivity factors of the products were obtained based on the effective carbon number and according to the previous papers due to lack of commercial standards. [11,12]

(7) ATR-FTIR analysis

Attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectra ranging from 650 to 4000 cm^{-1} were measured to detect chemical structure changes of the liquid fraction by Nicolet 6700 spectroscopy equipped with ATR component with a resolution of 4 cm^{-1}.

2. Tables and Figures

Table S1 Kamlet-Taft parameters of ChCl-LA and ChCl-OA.

DES	π^*	α	β	α-β
ChCl-LA	1.03	1.34	0.54	0.80
ChCl-OA	0.98	1.41	0.31	1.10

Table S2 The NMR assignments of major signals in 2D-HSQC NMR spectra of AL and the regenerated lignin samples.

Label	δ_C/δ_H (ppm)	Assignments
B_β	53.1/3.46	C_β–H_β in phenylcoumaran substructures (B)
C_β	53.5/3.05	C_β-H_β in β–β' (resinol) substructures (C)
-OCH_3	56.4/3.70	C-H in methoxyls
A_γ	59.5/3.35-3.80	C_γ-H_γ in β–*O*–4' substructures (A)
A'_γ	63.0/4.36	C_γ-H_γ in γ-acylated β–*O*–4' (A')
B_γ	62.3/3.76	C_γ-H_γ in phenylcoumaran substructures (B)
C_γ	71.0/3.79-4.18	C_γ-H_γ in β–β' resinol substructures (C)
A_α	71.6/4.86	C_α-H_α in β–*O*–4' linked to S units (A)
A'_β(G)	80.8/4.62	C_β-H_β in β–*O*–4' linked to G (A')
A_β(G/H)	83.9/4.29	C_β–H_β in β–*O*–4' substructures linked to G and H units (A)
C_α	84.9/4.64	C_α-H_α in β–β' resinol substructures (C)
A_β(S)	85.9/4.11	C_β-H_β in β–*O*–4' linked to S units (A)
B_α	86.8/5.48	C_α-H_α in phenylcoumaran substructures (B)
$S_{2,6}$	104.0/6.72	$C_{2,6}$-$H_{2,6}$ in syringyl units (S)
$S'_{2,6}$	106.3/7.21	$C_{2,6}$-$H_{2,6}$ in oxidized S units (S′)
G_2	111.0/6.99	C_2-H_2 in guaiacyl units (G)
G_5	114.8/6.68	C_5-H_5 in guaiacyl units (G)
G_6	119.1/6.80	C_6-H_6 in guaiacyl units (G)
$PB_{2,6}$	131.2/7.67	$C_{2,6}$-$H_{2,6}$ in p-hydroxybenzoate substructures (PB)

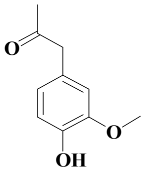
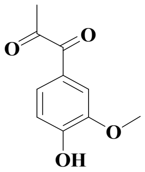
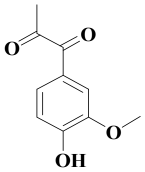
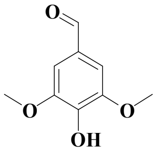
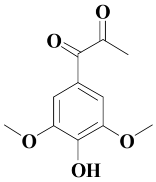
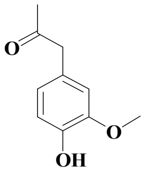
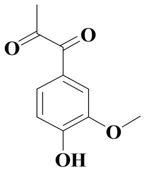
Table S3 The quantificational results of lignin by 2D-HSQC NMR spectra (results expressed per 100Ar).

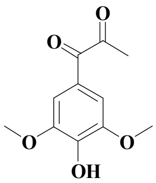
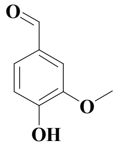
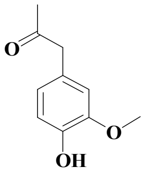
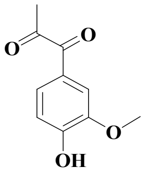
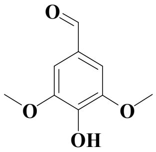
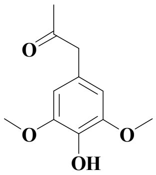
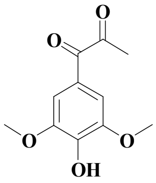
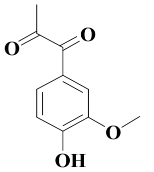
Samples	T(°C)[a]	Lignin interunit linkage[b]		
		β-*O*-4'	β-β'	β-5'
AL	-	62.0	12.5	1.5
LA-80	80	26.0	-	-
LA-100	100	12.8	-	-
LA-120	120	-	-	-
OA-80	80	2.7	11.1	-
OA-100	100	-	-	-
OA-120	120	-	-	-

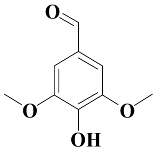
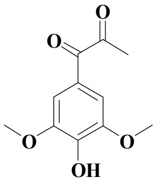
[a] Processing temperature.

[b] Abundances of different interunit linkages are expressed as per 100 aromatic units.

Table S4 Identified compounds, chemical structure, and retention time using GC-MS technique.

Samples	Compound name	Structure	Retention time (min)
L-80	Guaiacylacetone		17.260
	Guaiacyldiketone		18.080
L-100	Guaiacyldiketone		18.050
	Syringaldehyde		19.180
	Syringyldiketone		21.335
L-120	Guaiacylacetone		17.220
	Guaiacyldiketone		18.050

	Syringyldiketone		21.325
O-100	Vanillin		15.512
	Guaiacylacetone		17.200
	Guaiacyldiketone		18.040
	Syringaldehyde		19.100
	Syringylacetone		20.595
	Syringyldiketone		21.315
O-120	Guaiacyldiketone		18.050

	Syringaldehyde		19.090
	Syringyldiketone		21.325

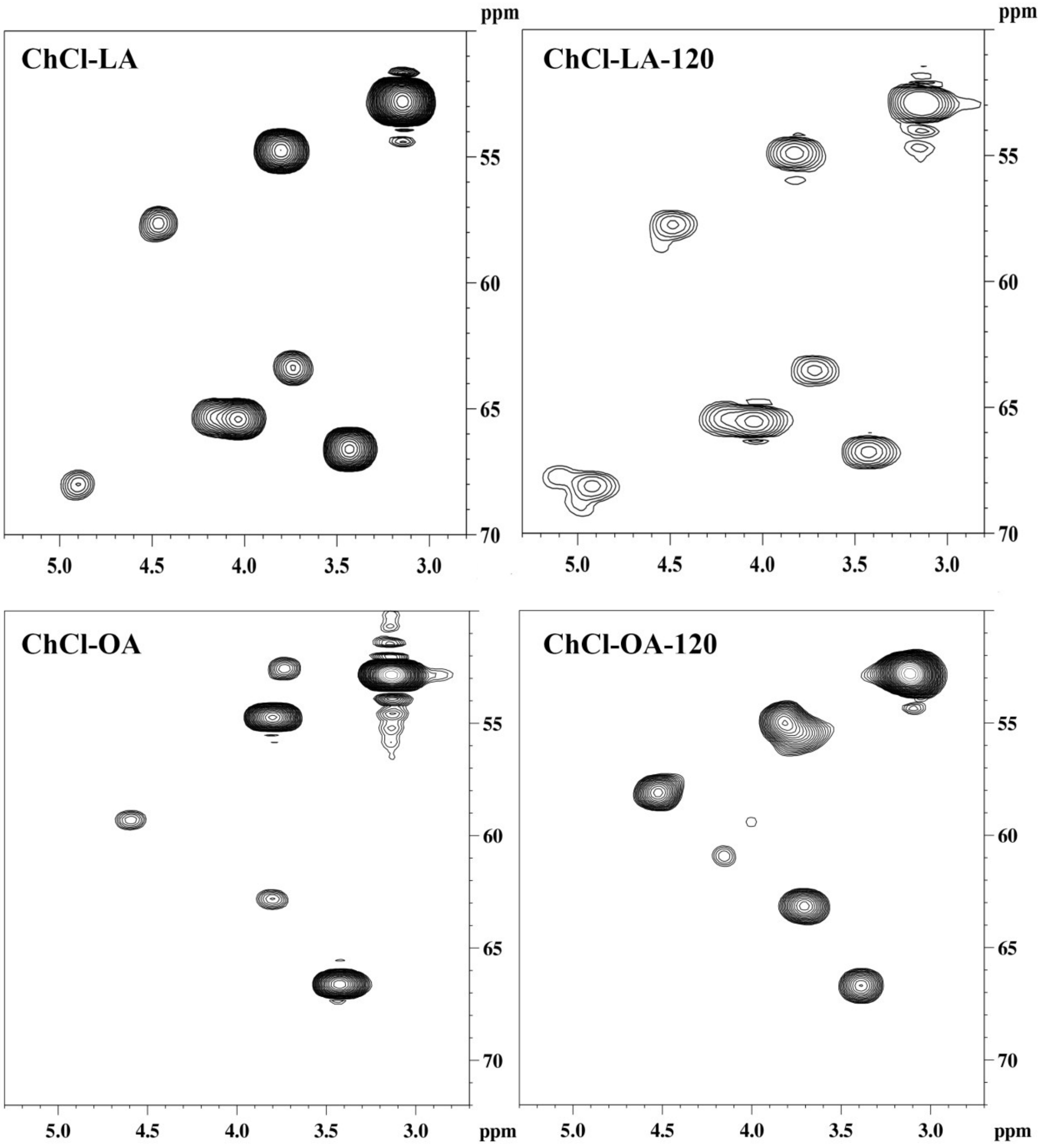


Fig. S1 2D-HSQC NMR spectra of the original DESs (ChCl-LA and ChCl-OA) and the pretreated DESs (ChCl-LA-120 and ChCl-OA-120) at 120 °C for 6 h.

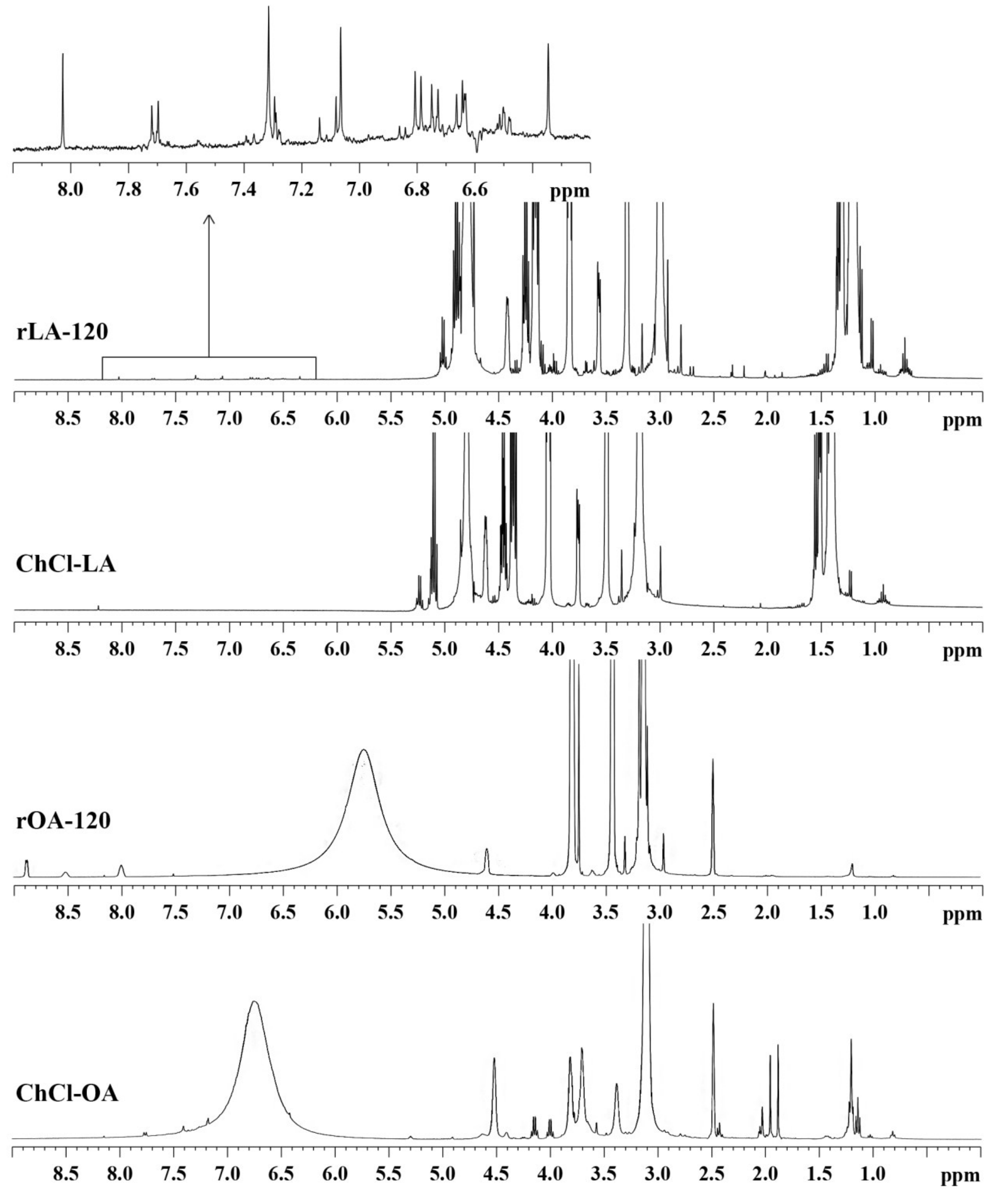


Fig. S2 ^{1}H NMR spectra of original DESs (ChCl-LA and ChCl-OA) and the recovered liquids (rLA-120 and rOA-120) pretreated at 120 °C for 6 h.

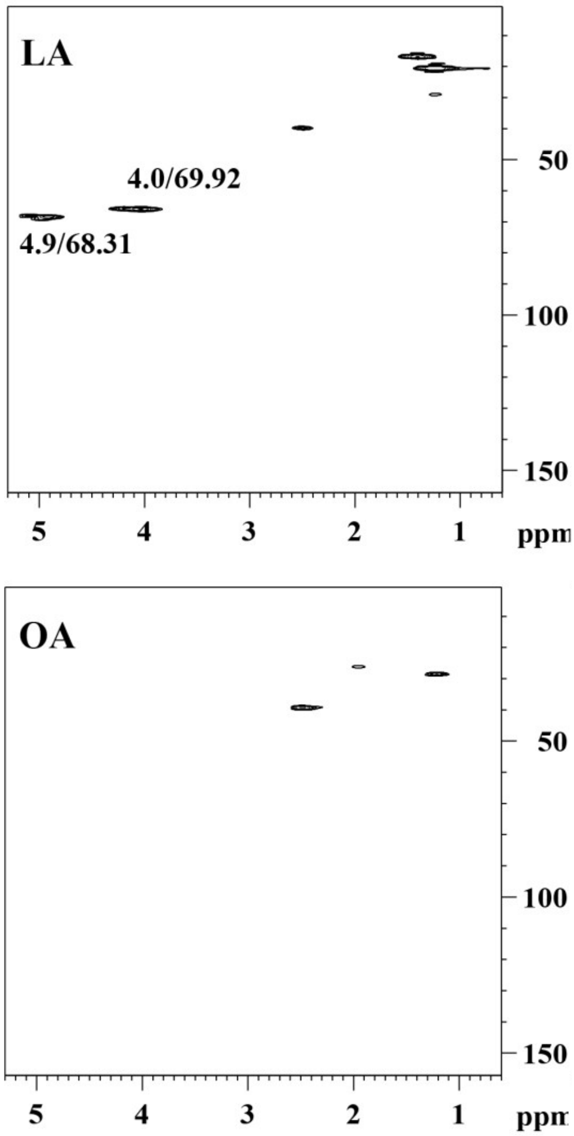


Fig. S3 2D-HSQC NMR spectra of LA and OA.

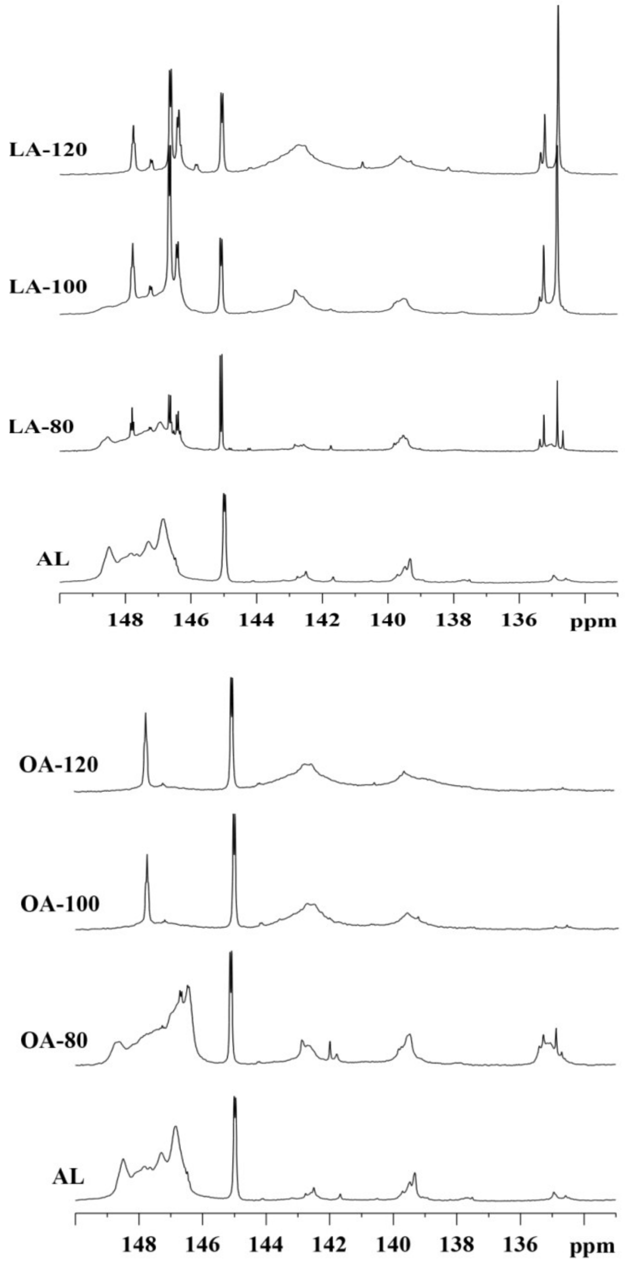


Fig. S4 ^{31}P NMR spectra of AL and regenerated lignin samples.

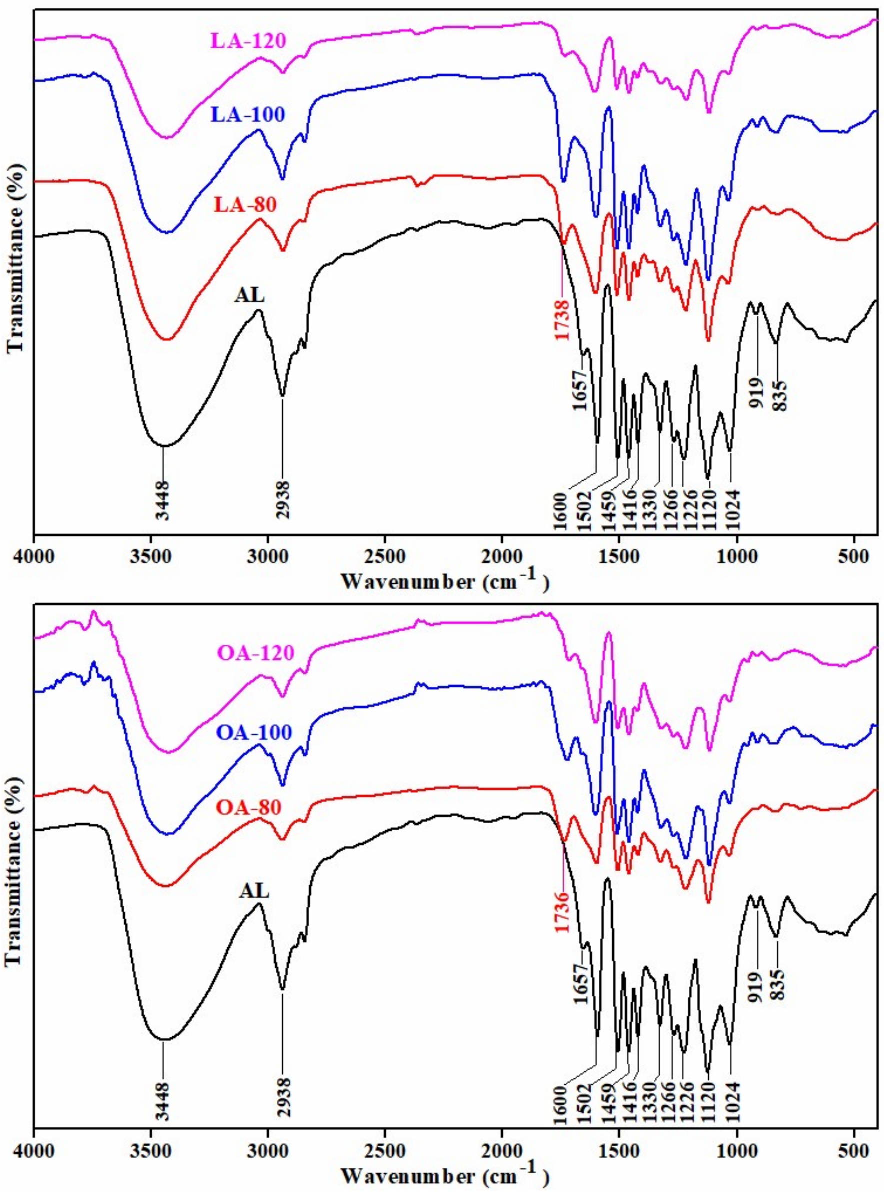


Fig. S5 FTIR spectra of AL and regenerated lignin samples.

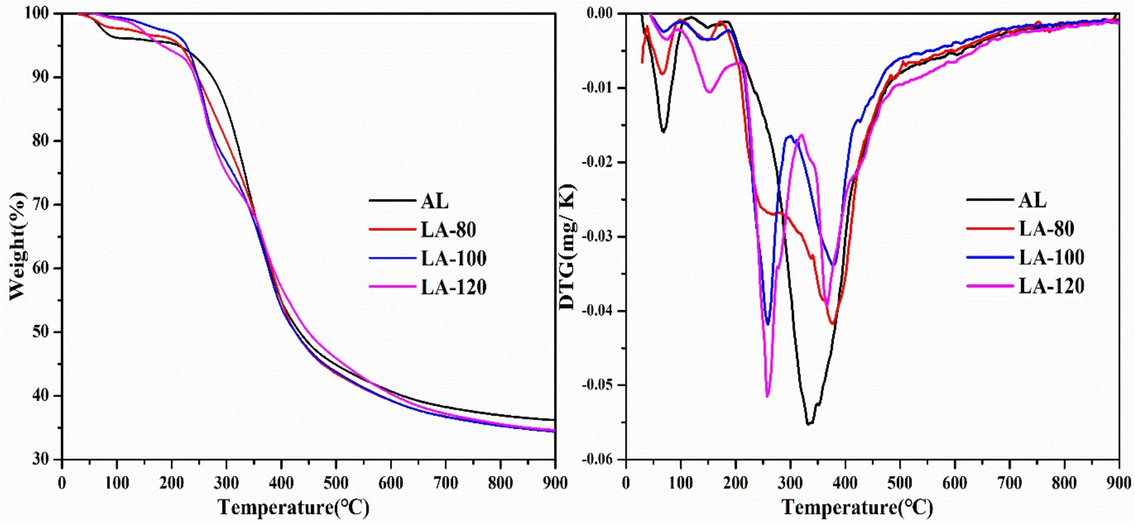


Fig. S6 TG and DTG curves of AL and regenerated lignin samples at heating rate of 20 ^{o}C min^{-1}.

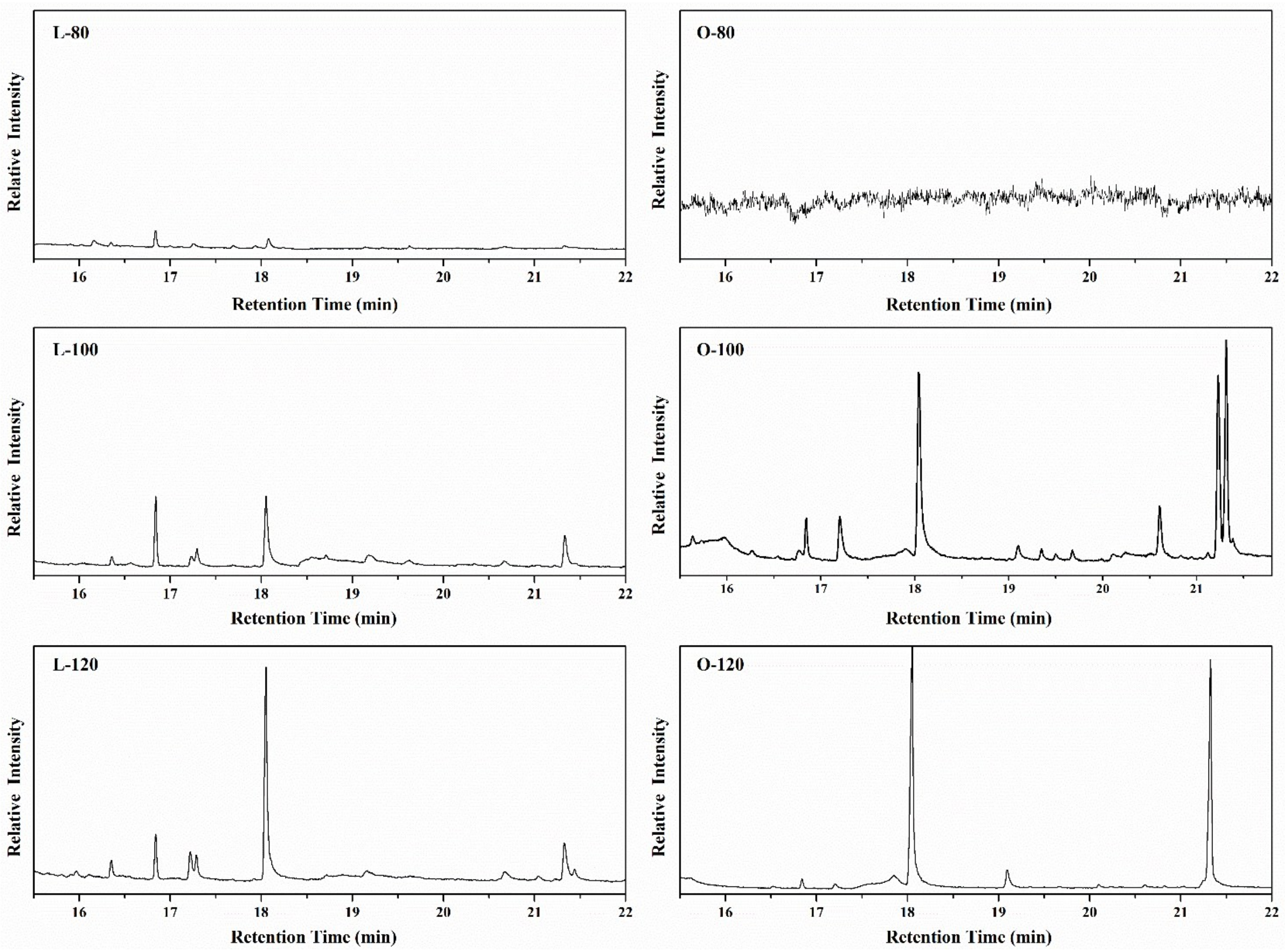


Fig. S7 Gas chromatogram of lignin oil obtained after DES pretreatment.

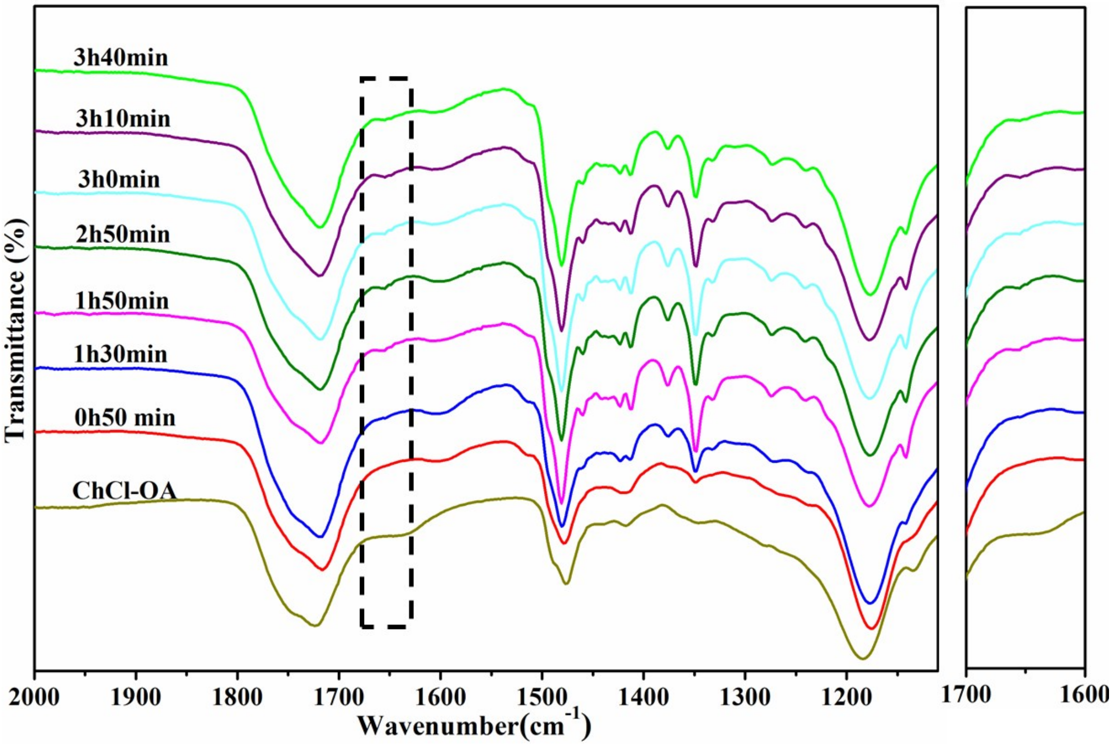
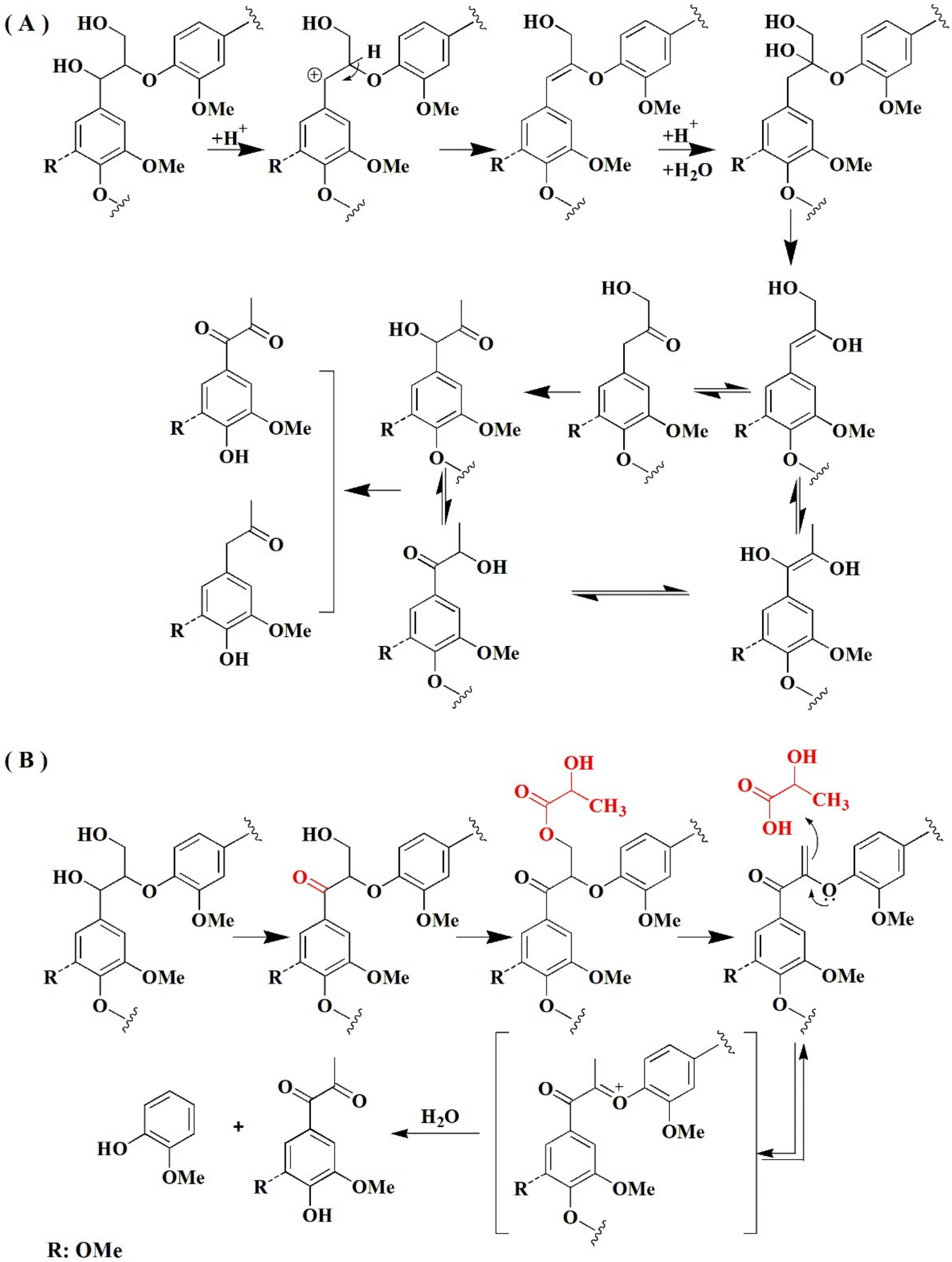


Fig. S8 ATR-FTIR spectra of liquid fractions treated during ChCl-OA pretreatment at 120 °C at different times.



R: OMe

Fig. S9 Possible routes of the cleavage of β-*O*-4' linkages during DES pretreatment.

3. References

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