

# Fractionation of herbaceous biomass using a recyclable hydrotropic p-toluenesulfonic acid (p-TsOH)/choline chloride (ChCl) solvent system at low temperatures

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## ARTICLE INFO

### Keywords:

Acid hydrotropic fractionation  
Herbaceous biomass  
p-TsOH/ChCl  
2D NMR  
Lignin condensation

## ABSTRACT

Lignin valorization is significantly influenced by the degrees of lignin condensation measured by the amount of cleaved  $\beta$ -O-4 ether linkages and interunit C-C linkages formed during biomass fractionation. To minimize the cleavage of aryl ether and formation of C-C linkages, a p-toluenesulfonic acid (p-TsOH)/choline chloride (ChCl) solvent system with hydrotropic properties was evaluated. The ChCl was chosen to control the H<sup>+</sup> concentration of the p-TsOH/ChCl solvent system. Compared with p-TsOH/H<sub>2</sub>O, the degradation of  $\beta$ -O-4 was kept in a similar rate during p-TsOH/ChCl fractionation; however, the formation of C-C linkage was slower. Under 75/25 p-TsOH/ChCl mass ratio at 80 °C for 20 min, 76.6 % of miscanthus lignin, with  $\beta$ -O-4 content of 30.9 %, and 88.9 % of wheat straw lignin, with  $\beta$ -O-4 content of 33.6 %, can be dissolved. In addition, the low polar and layered structure of hydrotropic p-TsOH/ChCl system has a strong ability to dissolve lignin, which resulted in lignin with larger particle size and lower surface charge. The results also indicated that lignin from p-TsOH/ChCl solvent system has great potential for lignin depolymerization to aromatics for valorization.

## 1. Introduction

Lignin is a renewable aromatic polymer from nature consisting of phenylpropanoid units. Compared with polysaccharides, lignin has higher carbon and lower oxygen content (Zhu and Zhuang, 2012). Notably, the high energy density and aromatic nature of lignin are beneficial for commercial production of biofuels and biochemicals (Rinaldi et al., 2016). Commercial pulping or existing biorefinery strategies aimed primarily at the valorization of polysaccharides often use harsh reacting conditions (150–180 °C for 2–4 h) with strong acid or base for delignification resulting in a highly condensed lignin with substantial  $\beta$ -O-4 cleavage and C-C bond formation. Valorization of commercial technical lignin is very difficult because of lignin condensation (Schutyser et al., 2018), and dissolved lignin from pulping is often used as fuel for heat and chemical recovery in pulp mills (Cao et al., 2018).

As a major component in lignocelluloses, lignin valorization is a

necessity for improving the economics of sustainable biorefineries (Rinaldi et al., 2016; Ponnusamy et al., 2019). Considering the aromatic nature of lignin, one of the most alluring uses is the exploitation of lignin as a resource for low-molecular-weight aromatic chemicals. Fractionation, depolymerization, and upgrading toward targeted chemicals are the three major steps to determine the realization of a successful lignin-to-chemical valorization chain (Schutyser et al., 2018). During the fractionation and depolymerization processes, controlling  $\beta$ -O-4 cleavage and minimizing C-C condensation is critically important to maintain the reactivity of the fractionated lignin (Deuss et al., 2017). Several fractionation processes with mild conditions have been investigated but with unpromising results. For example, organosolv (e.g., ethanol or formic acid) was developed as a promising lignin solvent system with high efficiency (Aziz and Sarkanen, 1989). Lignin yield > 70 % was achieved but with low  $\beta$ -O-4 content of approximately 3–10 % (Zhang et al., 2016). Similarly, certain ionic liquids (ILs) were found to have good selectivity in solubilizing lignin.

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Approximately 70–80 % of lignin can be dissolved from lignocelluloses under atmospheric pressure (Lan et al., 2011; Zhang et al., 2015), but this also results in lignin with low  $\beta$ -O-4 content in addition to difficulties in economic IL recovery. Deep eutectic solvents (DESs) as an inexpensive system, was recently applied for lignin extraction with high yields of 80–90 % at 140 °C (Alvarez-Vasco et al., 2016; Liu et al., 2017; Kumar et al., 2018). However, DESs also suffer from difficulties in limiting  $\beta$ -O-4 cleavage and C-C bond condensation.

Hydrotropic salts (such as sodium xylenesulfonate) have been exploited for wood pulping but were found effective only at high temperatures of 150–160 °C for 10–12 h (Procter, 1971). Despite their inefficiency in delignification, hydrotropic salts also have been used to fractionate wood for biorefinery applications (Mou et al., 2014). In comparison, acid hydrotropes such as *p*-toluenesulfonic acid (*p*-TsOH) (Bian et al., 2017; Chen et al., 2017; Ma et al., 2018a) and maleic acid (Cai et al., 2020) can rapidly and almost completely dissolve lignin at atmospheric pressure and temperatures below the boiling point of water. For example, using aqueous *p*-TsOH fractionation, approximately 90 % of hardwood lignin can be extracted at 80 °C for 20 min using aqueous *p*-TsOH solution at 80 wt% (Chen et al., 2017). However, a certain degree of  $\beta$ -O-4 cleavage and lignin condensation occurred during batch *p*-TsOH fractionation at moderate to high reaction severities (Cheng et al., 2019; Wang et al., 2019) because a certain amount of H<sup>+</sup> ion was released during lignin dissolution in aqueous *p*-TsOH, which can facilitate  $\beta$ -O-4 cleavage and C-C condensation. The dissolved lignin can be easily precipitated in the form of lignin nanoparticles (LNPs) (Ma et al., 2018a), which have attracted great interest for a variety of applications.

In the present study, we use choline chloride (ChCl) to construct a novel *p*-TsOH/ChCl solvent system to decrease lignin condensation during solubilizing wood lignin. As an H-bonding acceptor (HBA), ChCl can easily liquefy *p*-TsOH (H-bonding donor, HBD) to form a novel deep eutectic solvent (DES). Because of the H-bonding formation, the H<sup>+</sup> concentration in *p*-TsOH/ChCl system is lower than that in an aqueous *p*-TsOH/H<sub>2</sub>O system at the same *p*-TsOH concentration. ChCl has a strong ability to form H-bonding, which facilitates lignin dissolution. Furthermore, ChCl, as a quaternary ammonium salt, plays a catalytic conversion role to decrease the surface energy of solute in solid phase and promote lignin dissolution (Shen et al., 2019). To demonstrate the novel *p*-TsOH/ChCl solvent system for lignin dissolution with low lignin condensation, we applied this solvent to fractionate wheat straw (a most important annual agricultural residue in the world) and miscanthus (a perennial rhizomatous grass widely distributed in the world). The solubilized lignin was characterized and compared with lignin derived from aqueous *p*-TsOH fractionation. The goal of this study is to develop viable solvent systems that can produce near-native-state lignin for complete valorization of all major components of lignocelluloses.

## 2. Materials and methods

### 2.1. Materials

Both air-dried wheat straw and miscanthus (~3 cm in length) were provided by a pulp mill in Jiangsu, China, and sealed in plastic bags and stored in a refrigerator at 4 °C before use. The samples were ground in a Wiley mill (FZ102, Tianjin Taisite, China), and the fractions between 20 and 80 mesh were collected for fractionation and chemical composition analysis. Solvent extraction was applied using benzene-ethanol (2:1, v/v) in a Soxhlet apparatus for 8 h before compositional analysis. Results were reported in weight percentages based on oven dried (OD) weight of as-received materials. All chemicals were analytical grade and purchased from Aladdin® Reagent Co., Ltd. (China).

### 2.2. Fractionation using *p*-TsOH/ChCl solvent

The *p*-TsOH/ChCl solvent was prepared by adding the required amounts of ChCl and *p*-TsOH into a 150 mL flask for a desired concentration. 38 mL of the prepared solvent was heated in a flask to the desired temperature, followed by adding 2 g (oven-dry weight) of 20–80 mesh Wiley-milled raw materials. Fractionations were carried out in an oil bath in a range of conditions with *p*-TsOH concentrations between 50 and 80 wt % and temperatures between 50 and 90 °C. At the end of the preset reaction time ranging 5–120 min, 60 mL hot water around 80 °C was added into the flask to dilute the acid concentration to terminate reaction. Water insoluble lignocellulosic solids (WIS) were separated from the spent liquor using vacuum filtration. After separation, the WIS was washed with deionized (DI) water by vacuum filtration at room temperature until the filtrate became neutral, and the spent liquor was collected to precipitate lignin as lignin nanoparticles (LNPs) as described previously (Ma et al., 2018a). The chemical compositions of all resulting washed WISs were analyzed.

### 2.3. Preparation of dissolved lignin

The dissolved lignin can be easily precipitated by diluting *p*-TsOH in the spent liquor to below the minimal hydrotropic concentration of 11.5 wt% (Chen et al., 2017). The precipitated lignin was dialyzed in a dialysis bag with a cut-off molecular weight of 3500 Da to remove ChCl, *p*-TsOH and other low molecular substances. The dialyzed lignin was centrifuged at 5000 rpm for 10 min. The *p*-TsOH/ChCl lignin (PCL) was then freeze-dried and weighed to calculate lignin recovery. The mill wood lignin (MWL) of miscanthus was isolated according to Björkman's method (Björkman, 1954). The aqueous *p*-TsOH/H<sub>2</sub>O lignin (AHL) samples were prepared with the same procedure at the same fractionation conditions except using H<sub>2</sub>O instead of ChCl.

### 2.4. NMR spectroscopic analysis

75 mg lignin were dissolved into 1 mL DMSO-*d*<sub>6</sub> solution. The NMR analyses were conducted on a Bruker AVANCE III spectrometer operating at 400.13 and 100.61 MHz for <sup>1</sup>H and <sup>13</sup>C, respectively. The NMR data were processed using the TopSpin 2.1 software. 2D <sup>1</sup>H-<sup>13</sup>C HSQC spectra were acquired using spectral widths of 12 ppm for <sup>1</sup>H and 220 ppm for <sup>13</sup>C with a 2-s relaxation delay, 1 K data points, 256 t1 increments, and 32 transients. A semiquantification method was applied to obtain relative quantities of various lignin chemical structures based on per 100 Ar units (del Rio et al., 2012; Gu et al., 2015; Huang et al., 2016). As illustrated, the total aromatic areas are defined as,  $I_{C9} = 0.5 \times I_{S2,6} + 0.5 \times I_{S'2,6} + I_{S \text{ condensed}} + I_{G2} + I_{G'2} + 0.5 I_{H2,6}$ . The contents of internal linkages were calculated as  $X = 100 \times I_x / I_{C9}$  ( $x = A_\beta, B_\alpha, C_\alpha, D_\alpha, E_\alpha, \text{PhGlc}, A', \gamma\text{-Est and BE}$ ). Contents of aromatic units are relative (%),  $S = 100 \times 0.5 \times I_{S2,6+S'2,6+S \text{ condensed}} / I_{C9}$ ,  $G = 100 \times I_{G+G'} / I_{C9}$ , and  $H = 100 \times 0.5 \times I_{H2,6} / I_{C9}$ . The S/G ratio is defined as,  $S/G = I_{S+S'+S \text{ condensed}} / I_{G+G'}$ .

### 2.5. Analytical methods

Chemical compositions of WISs were analyzed using the protocol by NREL (Sluiter et al., 2008). The Klason lignin (KL) content was determined as the ash free WIS residue after acid hydrolysis. The acid soluble lignin (ASL) was analyzed by absorbance of the acid hydrolysate at 205 nm in a UV-vis (TU-1810, Puxi, Beijing, China) spectrometer. The monomeric sugars were quantitatively measured with high performance liquid chromatography (HPLC, Agilent 1200 Series, Santa Clara, CA) equipped with a refractive index detector. The ash content was determined by burning lignocellulosic samples at 575 °C. Zeta potential and particle size of LNPs were measured using a Zeta potential analyzer (Brookhaven Instruments Company, Holtsville, NY). Morphology of lignin was observed using AFM (Nanoscope IIIa, Veeco

**Table 1**Chemical compositions of p-TsOH/ChCl and p-TsOH/H<sub>2</sub>O fractionated wheat straw (the data for miscanthus were listed in Table S1) under different conditions.

Sample label <sup>a</sup>	Water insoluble solids (WIS) % <sup>b</sup>						
	WIS Yield	Glucan	Xylan	Arabinan	KL	ASL	Ash
Untreated Wheat straw	100	35.8(100)	17.3(100)	3.1(100)	18.1(100)	2.4(100)	6.0(100)
p-TsOH/ChCl fractionation							
P50T60t10C	77.0	45.4(93.4)	12.7(56.8)	2.1(56.5)	13.0(60.6)	1.9(49.5)	8.8(91.6)
P50T80t20C	68.2	49.8(90.6)	11.1(44.0)	1.7(40.8)	10.6(41.8)	1.6(39.0)	9.0(83.1)
P75T70t10C	56.1	58.4(87.6)	10.7(34.9)	1.4(27.3)	9.6(37.5)	1.7(30.0)	9.8(74.4)
P75T80t20C	52.8	62.7(88.4)	4.9(15.2)	0.9(17.0)	3.4(10.4)	0.8(15.1)	9.8(70.6)
P50T80t120C	62.1	50.1(83.1)	2.7(9.7)	0.5(10.6)	3.5(12.5)	0.7(22.1)	8.8(73.8)
P75T80t60C	70.7	43.2(81.6)	2.3(9.5)	0.2(4.7)	3.0(17.8)	0.7(27.9)	8.3(79.9)
p-TsOH/H <sub>2</sub> O fractionation							
P50T60t10H	74.3	46.4(92.1)	11.8(51.0)	2.1(55.0)	12.7(48.7)	1.8(50.4)	8.7(87.8)
P50T80t20H	62.9	52.9(88.8)	10.8(39.5)	1.8(40.1)	10.6(33.6)	1.4(38.6)	8.5(72.3)
P75T70t10H	53.1	62.4(88.6)	10.2(31.4)	1.2(22.5)	8.0(24.4)	1.6(30.8)	9.8(70.7)
P75T80t20H	47.4	67.6(85.5)	5.4(14.8)	0.9(15.1)	2.8(7.7)	0.9(15.3)	10.4(67.2)
P50T80t120H	62.0	48.6(80.5)	3.2(10.7)	0.4(8.7)	2.2(7.8)	0.7(23.7)	8.6(72.1)
P75T80t60H	72.1	41.6(80.1)	1.7(7.2)	0.3(7.6)	2.3(9.7)	0.6(23.7)	8.4(82.1)

<sup>a</sup> PxxTyytzzA: xx stands for p-TsOH concentration in wt%, yy stands for temperature in degree C, zz stands for time in min; A = C = p-TsOH/ChCl, A = H = p-TsOH/H<sub>2</sub>O.

<sup>b</sup> Number in parentheses are component retained (yields) in WISs.

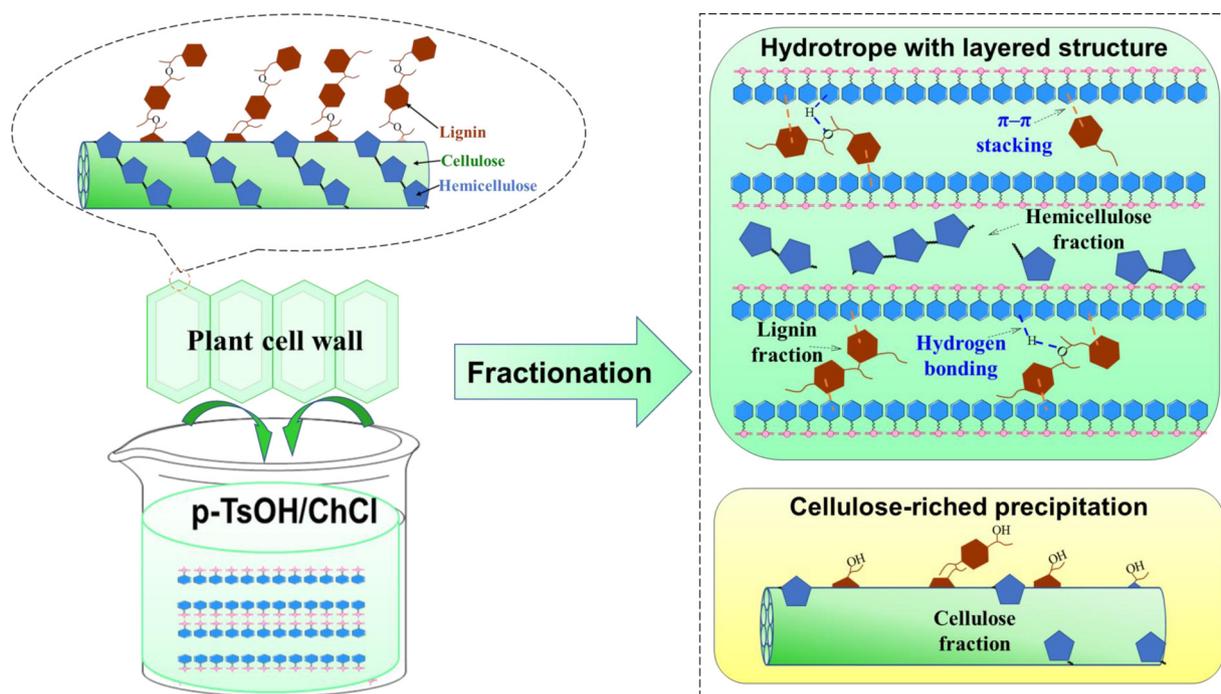


Fig. 1. The potential mechanism of herbaceous plants cell wall fractionated in p-TsOH/ChCl system.

Instruments Inc., Santa Barbara, CA). The images were scanned in multimode in air using a commercial silicon-tapping probe (NP-S20, Veeco Instruments, Plainview, NY) with a spring constant of 0.32 N/m, a resonance frequency of 273 kHz and settings of 512 pixels/line.

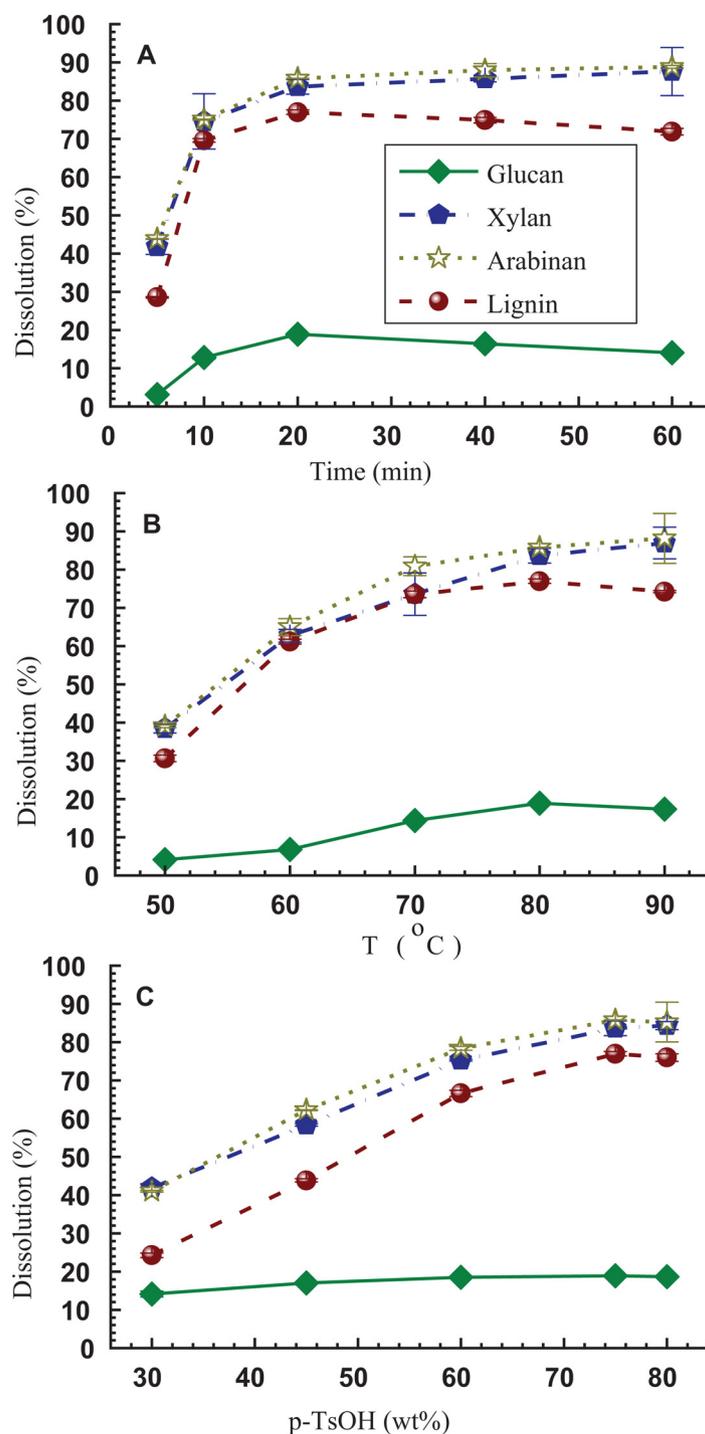
### 3. Results and discussion

#### 3.1. Component dissolution of herbaceous biomass by p-TsOH/ChCl

The chemical compositions of the fractionated WISs from wheat straw and miscanthus were listed in Tables 1 and S1, respectively. Both lignin and hemicellulose dissolutions were increased with the increase in reaction severity, whereas degradation of glucan was minimally affected. For example, when the wheat straw was fractionated under P75T80t20C, denoted as 75 wt% p-TsOH and 25 wt% ChCl at 80 °C for 20 min, lignin and xylan dissolutions were 89.5 % and 84.8 %,

respectively, with glucan retention of 82.8 %. Clearly, the p-TsOH/ChCl solvent system is highly selective in dissolving lignin and hemicelluloses rather than cellulose. The p-TsOH/ChCl is low polar with a layered structure at high concentrations (Srinivas et al., 1997). Lignin is easily dissolved in concentrated p-TsOH/ChCl solvent by similar polarity and weak interactions (such as hydrogen bonding and  $\pi$ - $\pi$  stacking). Because glycoside bonds are partially broken in acid hydrotrope, depolymerized hemicelluloses can also be dissolved. On the contrary, the strong polarity and large size of cellulose made it difficult to be dissolved by p-TsOH/ChCl as schematically shown in Fig. 1.

Dissolutions of hemicelluloses (xylan and arabinan) and lignin from wheat straw were rapid and reached maximal in only 20 min as clearly shown in Fig. 2A, consistent with previous studies using aqueous p-TsOH systems (Bian et al., 2017; Chen et al., 2017; Ma et al., 2018a, b). The slight decreases in lignin removal beyond 20 min were simply due to the condensation of the dissolved lignin that may well be



**Fig. 2.** Effects of reaction conditions on removal of lignin and carbohydrates from miscanthus (A. Time effects at p-TsOH concentration P = 75 wt % and 80 °C; B. Temperature effects at p-TsOH concentration P = 75 wt % for 20 min; C. p-TsOH concentration effects for 20 min at 80 °C.).

redeposited onto WISs, which was also observed previously (Chen et al., 2017). Condensation of dissolved lignin was also observed under similar severe conditions either using elevated temperatures (e.g., > 80 °C) (Fig. 2B) or elevated p-TsOH concentrations (e.g., > 80 wt%) (Fig. 2C). Compared with the aqueous p-TsOH system at the same temperature (80 °C) and p-TsOH concentration of 75 wt% and for the same period of time (20 min), the p-TsOH/ChCl system decreased lignin dissolution by approximately 15 %, from 88.2 % to 76.6 %, for miscanthus and 5%, from 93.6 % to 88.9 %, for wheat straw, with similar levels of hemicellulose dissolution and glucan retention for both miscanthus and wheat straw (Tables 1 and S1). This confirms that H<sup>+</sup>

concentration or acidity plays a role in lignin dissolution.

### 3.2. Chemical structures of dissolved lignin

MWL, PCL (under P75T80t20) and AHL (under P75T80t20) of wheat straw and miscanthus were analyzed by <sup>1</sup>H-<sup>13</sup>C 2D HSQC NMR spectroscopy to understand the structural features of lignin. 2D NMR of the side chain ( $\delta_C/\delta_H$  50–110/2.5–5.5 ppm) and the aromatic regions ( $\delta_C/\delta_H$  90–150/5.0–8.0 ppm) of MWLs, PCLs and AHLs are shown in Fig. 3. The main cross-signals of lignin assigned in 2D NMR spectra were listed in Table S2, and the observed signals were assigned

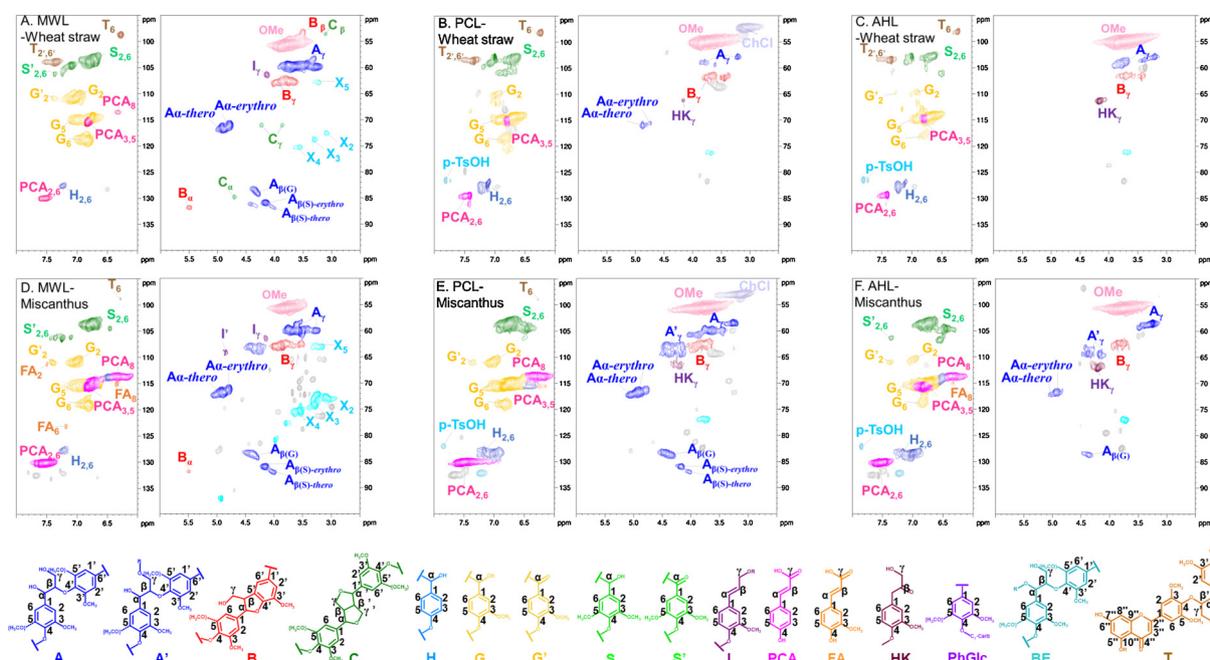


Fig. 3. Aromatic and side chain region in the 2D HSQC NMR spectra of the MWLs PCLs (under P75T80t20) and AHLs (under P75T80t20) from miscanthus and wheat straw.

Table 2

2D  $^{13}\text{C}$ - $^1\text{H}$  NMR HSQC spectral analyses of side chains and aromatic units present in lignin from batch p-TsOH fractionations.

Labels <sup>a</sup>	Yield, % on lignin in raw material		Lignin aromatic units, %					Lignin interunit linkages, %					C-C linkage <sup>b</sup> , %		LCC linkages, %	
	Delignification	Recovery <sup>c</sup>	S	S <sub>con</sub>	G	H	S/G	β-O-4	β-5	β-β	β-1	α-1	A'-v-Est	BE	PhGlc	
MWL-Wheat straw	24.7	15.6	41	2	55	4	0.74	58.3	5.2	5.3	3.1	1.9	8.0	20.9	3.5	0.2
P50T60t10CW	42.9	30.0	47	2	49	4	0.96	62.0	5.7	3.7	1.0	0.9	10.9	15.3	2.8	0.2
P50T80t20CW	58.6	39.3	53	8	42	5	1.25	46.4	5.0	6.3	1.0	0.6	9.4	21.8	5.0	0.1
P75T70t10CW	68.6	42.5	48	8	46	7	1.04	38.1	4.8	4.3	0.3	0.7	6.2	20.9	2.2	0.4
P75T80t20CW	88.9	51.6	68	20	26	7	2.63	33.2	2.2	4.5	1.1	1.2	5.7	21.3	3.7	0.2
P50T80t120CW	87.1	47.9	73	25	23	4	3.22	21.8	1.7	3.5	0.6	0.5	0.9	22.4	4.8	0.3
P75T80t60CW	87.1	47.9	66	3	31	4	2.14	20.5	1.3	3.4	1.0	1.0	2.4	22.1	3.4	0.6
P50T60t10HW	46.6	32.6	48	1	45	7	1.05	57.6	5.9	2.5	1.9	2.2	7.0	17.9	1.5	0.1
P50T80t20HW	62.6	41.9	53	9	43	4	1.22	40.8	6.3	3.7	1.4	0.4	7.2	22.5	1.8	0.0
P75T70t10HW	74.7	47.1	67	25	29	5	2.31	34.4	5.7	1.5	3.2	1.8	2.9	26.1	2.3	0.5
P75T80t20HW	93.6	53.4	65	30	30	5	2.16	20.6	3.2	0.9	1.3	1.3	2.1	27.3	0.8	0.3
P50T80t120HW	91.2	48.3	64	20	30	5	2.13	19.6	4.5	3.1	2.4	1.0	1.5	36.1	1.3	0.3
P75T80t60HW	89.5	44.8	65	5	31	5	2.09	5.8	1.6	2.2	1.0	0.7	1.1	48.7	0.0	0.4
MWL-Miscanthus	22.3	13.1	48	1	44	7	1.09	35.4	6.9	1.7	1.0	1.1	9.8	23.2	1.7	0.6
P75T80t20CM	77.0	53.3	59	5	27	14	2.14	30.9	2.6	1.8	0.5	0.2	9.0	14.4	1.5	0.2
P75T80t20HM	88.5	57.6	56	11	32	13	1.77	13.4	5.0	3.8	1.5	0.8	5.8	45.2	0.2	0.1

<sup>a</sup> PxxTyytzzAB: xx stands for p-TsOH concentration in wt%, yy stands for temperature in degree C, zz stands for time in min; A = C = p-TsOH/ChCl, A = H = p-TsOH/H<sub>2</sub>O; B = W = Wheat straw, B = M = Miscanthus.

<sup>b</sup> C-C linkage =  $100 * (I_{B\alpha} + I_{C\alpha} + I_{D\alpha} + I_{E\alpha}) / (I_A + I_{A'} + I_{A''} + I_{B\alpha} + I_{C\alpha} + I_{D\alpha} + I_{E\alpha})$ , the integral value of each signal in 2D HSQC NMR.

<sup>c</sup> Lignin recovery, % =  $100 * \text{the weight of freeze-dried lignin after fractionation and dialysis} / \text{the weight of lignin in raw material}$ .

according to literature (del Rio et al., 2012).

Compared with MWLs (Fig. 3a and d) and PCLs (Fig. 3b and e), the attenuation of A<sub>β(G)</sub> and disappearance of A<sub>β(S)</sub>, A<sub>α-erythro</sub> and B<sub>α</sub> in the side chain region of AHLs (Fig. 3c and f) demonstrated that more β-O-4 linkages were cleaved by p-TsOH/H<sub>2</sub>O than by p-TsOH/ChCl under the same condition of P75T80t20. The signals of Hibbert's ketones (HK) in AHLs of 2D NMR were much stronger than that in PCLs but were not present in the MWL spectra. There are two main approaches to break β-O-4 linkages during acidic delignification: Hibbert's ketone route (Li et al., 2018) and retro-Prins reaction route (Lancefield et al., 2017). The contours of S<sub>2,6</sub> units were substantially shrunk in the aromatic region, and the new S-condensed signals that appeared in δ<sub>C</sub>/δ<sub>H</sub> 106.5/6.48 ppm implied that β-O-4 linkages cleaved and condensed in the acid hydrotropic system. Moreover,

β-O-4 cleavage and recondensation reactions are more pronounced for AHLs than for PCLs with Hibbert's ketone route being the dominant degradation pathway. In addition, the signals (e.g., δ<sub>C</sub>/δ<sub>H</sub> 68–82/2.9–4.0 ppm) from carbohydrates could be seen in MWLs but were not observable in PCLs and AHLs, suggesting p-TsOH fractionation produced very pure lignin. This is in agreement with our previous studies (Chen et al., 2017; Wang et al., 2019; Cheng et al., 2019). Lignin with higher β-O-4 aryl ether and lower C-C condensed linkages had been repeatedly reported to facilitate subsequent valorization through depolymerization (Rinaldi et al., 2016; Jiang et al., 2018; Wang et al., 2019). Less condensed lignin also results in a lower glass transition temperature (Wang et al., 2019; Cheng et al., 2019), which facilitates compounding for producing lignin-based composites.

Table 2 listed the semiquantitative data of lignin structure expressed

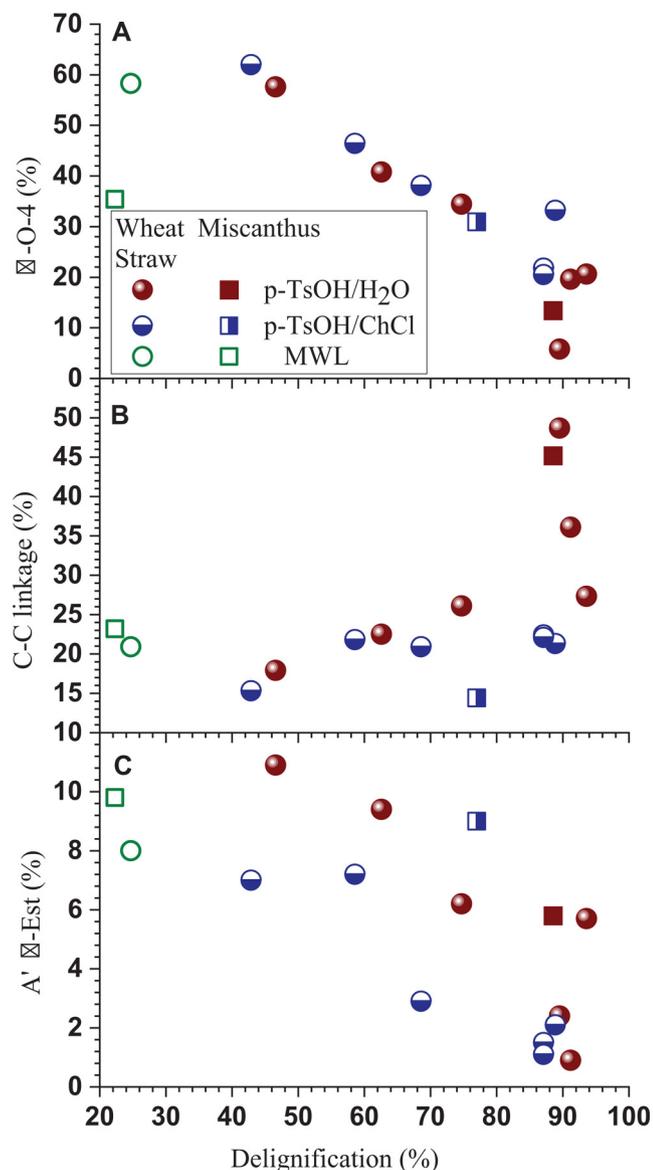


Fig. 4. Correlation between delignification and  $\beta$ -O-4, C-C, A' $\gamma$ -Est linkages of the resultant lignin from p-TsOH fractionation.

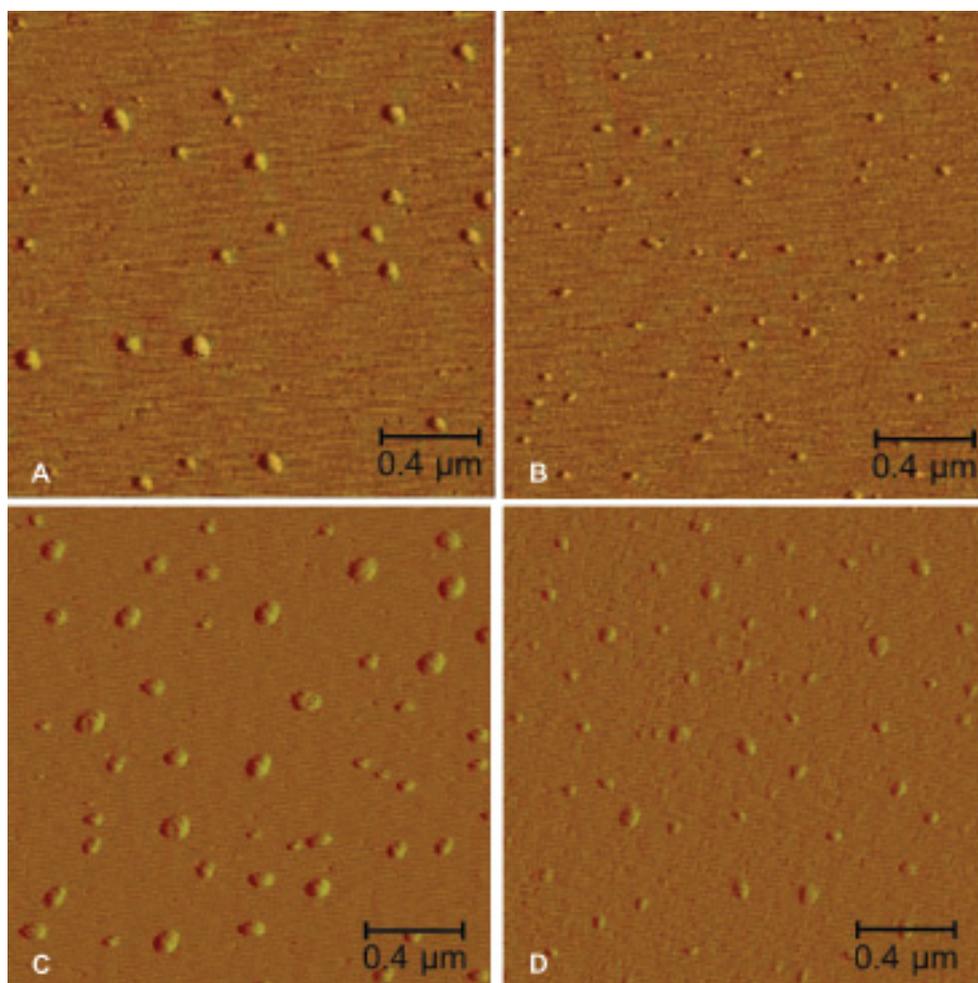
as per 100 Ar units based on proportions of side chains and aromatic units from 2D NMR peak areas (Figs. S1–S16). The  $\beta$ -O-4 bond was the major linkage in dissolved lignin samples for both wheat straw and miscanthus. The  $\beta$ -O-4 content in PCL obtained with ChCl from wheat straw was decreased from 62 % to 21 % as delignification was increased from 43 % to approximately 90 %, which was smaller than the decrease from 58 % to 6% in AHL obtained using aqueous p-TsOH. More specifically,  $\beta$ -O-4 contents in AHLs were lower than that in PCLs, whereas the yield of AHL was higher than that of PCL under the same condition. This is also reflected from the amount of calculated C-C linkages (Table 2), which increased from 18 % to 49 % for AHLs, greater than the increase from 15 %–22 % for PCLs. It could be due to the fact that higher  $H^+$  concentration or acidity in the p-TsOH/ $H_2O$  system led to the increase in  $\beta$ -O-4 cleavage and lignin fragmentation, which improved delignification but increased the extent of condensation reactions. In other words, the higher  $\beta$ -O-4 content and less lignin condensation observed in the p-TsOH/ChCl system were achieved with the sacrifice of delignification. Deuss et al. (2017) and Cheng et al. (2019) also reported that delignification and the  $\beta$ -O-4 content are closely related, with a high lignin dissolution usually

concurring with low  $\beta$ -O-4 content and vice versa.

S/G ratio of PCL and AHL increased with fractionation severity, as illustrated in Table 2, and then decreased at very high severities. The increase in S/G ratio is because the S unit lignin is relatively easier to dissolve than the G unit lignin. The linkage of A' $\gamma$ -Ester (A' $\gamma$ -Est) was semiquantitatively calculated in Table 2. It was confirmed that grass lignin is acylated at the  $\gamma$ -position by p-coumarate (Balakshin et al., 2011; Wen et al., 2013). The A' $\gamma$ -Est was disrupted readily in both p-TsOH/ChCl and p-TsOH/ $H_2O$  fractionations, which facilitated lignin dissolution. The degradation of A' $\gamma$ -Est in p-TsOH/ $H_2O$  fractionation was significantly faster than that in p-TsOH/ChCl fractionation. Lignin-carbohydrate complex (LCC) was believed to link lignin with polysaccharide through covalent bonds. It was prone to breaking during acid or alkaline cooking (Balakshin et al., 2011). Two major LCC linkages, benzyl ether (BE) and phenyl glycoside (PhGlc), were also calculated in Table 2. The content of BE linkages decreased in p-TsOH/ $H_2O$  fractionation, whereas it had no obvious change in p-TsOH/ChCl fractionation. The above results indicated that the ester and ether bonds were easy to break in the p-TsOH/ $H_2O$  solvent with high  $H^+$  concentration or acidity. Because more A' $\gamma$ -Est and BE linkages were broken in p-TsOH/ $H_2O$  system, AHL had higher yield, higher purity and lower carbohydrate content than PCL. The PhGlc showed its stability in both p-TsOH/ChCl and p-TsOH/ $H_2O$  fractionations. This implied that the hydrotropic solvents have little effect on glycoside; therefore, a large amount of cellulose can be retained in fractionated residue.

Fig. 4 illustrates the correlations between delignification and  $\beta$ -O-4, C-C, and A' $\gamma$ -Est linkage contents in the dissolved lignin during p-TsOH fractionations. When delignification was less than 80 %, the  $\beta$ -O-4 contents of PCLs and AHLs were in good linear negative correlation with the extent of delignification. And there was essentially no difference in  $\beta$ -O-4 content with respect to delignification between PCLs and AHLs (Fig. 4A). The C-C linkage content was around 22 % in PCL when the condition was 75 % p-TsOH and 80 °C for 60 min; however, it was about 49 % in AHL under the same condition. Although p-TsOH/ChCl fractionations couldn't slow down the rate of  $\beta$ -O-4 cleavage during delignification, they could prevent the formation of C-C bonding to a certain degree, especially under severe condition (Fig. 4B). The lower C-C linkage in PCL could be affected by the following two potential reasons: 1) the lower acidity of p-TsOH/ChCl decreased the probability of condensation reactions; 2) condensation reactions were replaced by other reactions. The signals of p-TsOH and ChCl were observed in the 2D NMR spectra of dissolved lignin, but further studies are needed to elucidate this difference. Fig. 4C shows that the A' $\gamma$ -Est linkage in PCL is greater than that in AHL under the same set of conditions. More carbohydrates remained in the PCL samples, resulting in differences in polarity, functional groups, and other physicochemical properties between PCLs and AHLs, which could have contributed to the difference in lignin solubility between p-TsOH/ChCl and p-TsOH/ $H_2O$  systems.

Comparing the results in this study with literature, Cheng et al. (2019) reported that a p-TsOH/ $H_2O$  fractionation run at 67 % acid concentration and 70 °C for 30 min dissolved 56 % of poplar wood lignin with 25 % of  $\beta$ -O-4 content. Shen et al. (2019) dissolved 88.5 % poplar wood lignin with a  $\beta$ -O-4 content of only 5.4 % using a lactic acid (LA)/ChCl system with 10:1 M ratio at 120 °C for 6 h.  $\beta$ -O-4 linkages were almost completely lost at 130 °C. These comparisons implied the following two factors: 1) The p-TsOH/ChCl solvent system can maintain the structural integrity (especially in controlling the formation of C-C linkages) of dissolved lignin at a relatively high lignin dissolution, advantageous for subsequent lignin valorization; and 2) the p-TsOH/ChCl system showed good performance in fractionation of herbaceous biomass at atmospheric pressure and low temperatures for a short period of time.



**Fig. 5.** Demonstration of fractionating colloidal LNPs. A: AFM image of wheat straw lignin fractionated by P75T80t20CW; B: AFM image of wheat straw lignin fractionated by P75T80t20HW; C: AFM image of Miscanthus lignin fractionated by P75T80t20CM; D: AFM image of Miscanthus lignin fractionated by P75T80t20HM.

**Table 3**

Particle size and Zeta potential of PCL and AHL.

Sample label	DLS diameter (nm)	Zeta potential (mV)
P75T80t20CW	63.3 ± 4.6	-36.8 ± 1.7
P75T80t20HW	44.5 ± 2.5	-40.3 ± 2.7
P75T80t20CM	119.7 ± 7.5	-29.6 ± 0.8
P75T80t20HM	68.6 ± 6.1	-34.5 ± 1.1

### 3.3. Morphologies of dissolved lignin in spent liquors

The morphologies of colloidal PCLs and AHLs from the respective supernatants after precipitating aggregated lignin particles through centrifugation of the diluted spent liquors were observed using AFM as shown in Fig. 5. The AHL and PCL nanoparticles have diameters of 20–80 nm and heights of 15–55 nm. It appears that the LNPs from the p-TsOH/ChCl system have a smaller diameter than those from aqueous p-TsOH system. The smaller LNP sizes of AHLs were also verified by dynamic light scattering (DLS) measurements as listed in Table 3. The surface charge of PCL is lower than that of the corresponding AHL under the same fractionation condition. In the p-TsOH/ChCl system, the introduction of Cl<sup>-</sup> with a lower surface charge (Table 3) increased the weak interactions among lignin, p-TsOH and ChCl by H bonding and  $\pi$ - $\pi$  stacking (Xia et al., 2018; Zhang et al., 2017), resulting in a larger particle size through greater aggregations.

## 4. Conclusions

p-TsOH/ChCl fractionation at 75 % p-TsOH and 25 % ChCl and 80 °C for 20 min dissolved 76.6 % of miscanthus lignin and 88.9 % of wheat straw lignin with  $\beta$ -O-4 content of 30.9 and 33.6 %, respectively. The H<sup>+</sup> concentration or acidity plays an important role in lignin dissolution. Compared with p-TsOH/H<sub>2</sub>O fractionation, the high  $\beta$ -O-4 content with low lignin condensation could be achieved by slightly sacrificing delignification with ChCl to control H<sup>+</sup> concentration. The result also indicated that PCL has different physicochemical properties from AHL, such as particle size, polarity, surface charge and functional groups. The p-TsOH/ChCl system showed a good performance in fractionation of herbaceous biomass at atmospheric pressure and low temperatures for a short period of time (20 min).

### CRediT authorship contribution statement

**Wangxia Wang:** Conceptualization, Methodology, Investigation, Writing - original draft. **Feng Gu:** Investigation, Validation, Formal analysis, Visualization, Data curation, Writing - original draft. **J.Y. Zhu:** Conceptualization, Project administration, Supervision, Writing - review & editing. **Kaiyong Sun:** Validation, Visualization, Writing - review & editing. **Zhaosheng Cai:** Resources, Writing - review & editing. **Shufeng Yao:** Validation, Writing - review & editing. **Wenjuan Wu:** Software, Writing - review & editing. **Yongcan Jin:** Resources, Writing - review & editing.

## Declaration of Competing Interest

JY Zhu is a co-inventor of Aqueous p-TsOH fractionation method.

## Acknowledgments

The authors are grateful for the financial support from the Natural Science Foundation of Jiangsu Provincial Key Lab of biomass energy and materials (Grants No. JSBEM201810), The National Natural Science Foundation of China (Grants No. 21908188 and 21801216), Natural Science Foundation of Jiangsu Province (Grants No. BK20181051 and BK20181052), Natural Science Foundation of Jiangsu Provincial Key Lab of Pulp & Paper science and Technology (Grants No. KL201915) and Natural Science Foundation of Jiangsu Provincial Key Lab of fine petrochemical Engineering (Grants No. KF1704).

## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.indcrop.2020.112423>.

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