

Structural changes of lignins in natural *Populus* variants during different pretreatments

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Abstract: In the present study, three leading pretreatment technologies including dilute acid (DA), liquid hot water (LHW), and organosolv pretreatments (OS) were applied on two *Populus* natural variants with different recalcitrance. The structural features of the isolated lignins were analyzed accordingly. All the studied pretreatments reduced the molecular weights of the lignins. Aliphatic OH was reduced while phenolic OH was increased in all pretreated lignins. HSQC analysis revealed that pretreatment influenced the lignin composition and relative distribution of inter-unit linkages. The lignin S/G ratio was found to increase during DA pretreatment, while it was decreased after LHW and OS pretreatment. LHW pretreatment also resulted in much less cleavage of β -O-4 linkage than the other two pretreatments. These results offered guidelines on appropriate selection of biomass and pretreatment technology in the future biorefinery process.

Keywords: lignin; recalcitrance; natural *Populus* variants; pretreatment; S/G ratio

1. Introduction

As a potential substitute for fossil fuel, bioethanol generated from renewable lignocellulosics has attracted substantial attentions (Ragauskas et al., 2006). Lignocellulosic materials are mainly composed of cellulose, hemicellulose, and lignin. The characteristics of these components, in particular cellulose and lignin, significantly influence cellulase performance on biomass during the following enzymatic hydrolysis process (Yang et al., 2016; Zhang et al., 2012). Various pretreatment technologies have been employed in the past decades to increase the cellulose conversion to glucose by reducing hemicellulose/lignin content and altering biomass-related characteristics in the plant cell wall (Yao et al., 2010; Yang et al., 2016; Meng et al., 2015).

Removal of non-cellulosic components during pretreatments has been shown to dramatically increase the performance of cellulase (Yang et al., 2016; Zhang et al., 2012). Several studies indicate that lignin is probably the most recalcitrant component in woody biomass such as poplar (Demartini et al. 2013; Yoo et al., 2017b). Besides cellulose, hemicellulose can also be converted into fermentable sugars, and its efficient utilization could further increase the total yield of liquid fuel (Boboescu et al., 2018). Previous studies showed that lignin interfered with cellulase by acting as a physical barrier, binding to cellulase non-productively, and deactivating cellulase (Yoo et al., 2017a). Similarly, lignin showed detrimental effects on cellulase performance due to the non-productive adsorption of cellulase onto lignins (Ko et al., 2015).

Dilute acid (DA) and liquid hot water (LHW) pretreatments are two widely studied pretreatment methods. They are known for their ability to effectively remove hemicellulose, while delignification during these acidic types of pretreatments is typically limited. In fact, an increase of lignin content after DA pretreatment has been reported due to the formation of pseudo lignin under severe acid pretreatment conditions (Yao et al., 2010; Hu et al., 2013). The formation of spherical lignin droplets on the cell wall surface of LHW pretreated biomass was also observed, and the authors reported that the alternations of lignin structure were mainly due to condensation reactions (Ko et al., 2015). Hu and his coworkers reported that poplar lignin was only partially degraded during DA pretreatment (Hu et al., 2013). Similarly, lignin isolated from DA pretreated switchgrass exhibited a slight decrease of β -O-4 ether linkage (Samuel et al., 2010). Our previous studies have concluded that hydroxyl groups, phenolic compounds, condensed aromatics, and S/G ratio of lignin play significant role in the binding between Cellobiohydrolases I (CBH) and lignin from dilute acid pretreated biomass (Yao et al., 2017; Yao et al., 2018a; Yao et al., 2018b). A study on cellulase adsorption to lignin from LHW pretreated corn stover also reported that LHW pretreatment altered the structural features of lignin and subsequently the adsorption behavior of lignin toward cellulase accordingly (Lu et al., 2016).

Organosolv pretreatment (OS) is a well-known delignification method employing a diverse set of organic solvents with acid as a catalyst (Salapa et al., 2017). The OS pretreated poplar showed extremely high cellulose hydrolysis yield in

our previous study (Yao et al., 2018c). The OS lignin has high-purity and is rich in functionality; thus it could be used in many applications such as polyurethanes synthesis and antioxidant agent (Pan et al, 2006a; Sadeghifar et al., 2017; Yao et al., 2018d).

Populus natural variants have been applied in many biomass recalcitrance studies (Meng et al., 2016; Thomas et al., 2017; Yoo et al., 2017b; Yao et al., 2018d). Many researchers have found that cellulose related characteristics were important to the digestibility of *Populus* (Meng et al., 2016; Hallac and Ragauskas, 2011; Hall et al., 2010). Furthermore, it was indicated that the features of lignin, such as lignin content, molecular weight, and S/G ratio were also correlated with sugar release to some extent. However, the exact influence of different pretreatment technologies on lignin structural changes of the same biomass feedstock was not directly compared in previous studies. In most of the current biorefinery systems, lignin samples are still significantly underutilized with the majority of technical lignins being burned for energy generation. Major challenges associated with the lignin valorization is the variability of heterogeneous lignin resources obtained from different fractionation or pretreatment processes. Thus it is essential to have a deep understanding of how these pretreatment processes alter the physicochemical structures of lignin. In the present study, two previously identified *Populus* natural variants with low sugar release (BESC 328) and high sugar release (BESC 131) after LHW pretreatment were selected as feedstocks (Meng et al., 2016; Thomas et al., 2017). DA, LHW, and OS pretreatments were applied on the two *Populous* natural variants, and lignins were

subsequently isolated from these untreated and pretreated *Populus* variants. Lignin samples were then analyzed and compared by FT-IR, GPC, ³¹P-NMR, and HSQC NMR techniques to elucidate the effect of various pretreatment methods on the structural features of lignin.

2. Materials and Methods

2.1 Materials

The natural variants, *Populus trichocarpa* ‘BESC-328’ and ‘BESC-131’ were obtained from Oak Ridge National Laboratory, TN. The samples were debarked, milled by Thomas-Wiley Laboratory Mill, and screened to 2 mm size. The screened *Populus trichocarpa* was Soxhlet-extracted by dichloromethane to obtain extractive-free *Populus* samples.

2.2 Pretreatment

Three different pretreatments were conducted on *Populus* samples using a stirred Parr 1 L reactor (Model 4571, Parr Instrument Co., IL, USA) as illustrated in a previous study (Yao et al., 2018c). All the pretreatment conditions were adopted from the literature. In brief, DA pretreatment was performed by employing 0.5% (w/w) dilute sulfuric acid, at a temperature of 170 °C for 10 min (Cao et al., 2012). LHW pretreatment was conducted using deionized water at a temperature of 180 °C for 44 min (Li et al., 2017). The solid to liquid ratio was kept at 1:20 for DA and LHW pretreatments. Organosolv (OS) pretreatment was performed with 65% aqueous ethanol, and 1.0 wt% sulfuric acid was used as the catalyst. The solid to liquid ratio was 1:8. The pretreatment was conducted at 180 °C for 60 min (Pan et al., 2006b).

2.3 Lignin isolation and purification.

The milled wood lignin (MWL) was extracted from native *Populus trichocarpa* according to a published procedure (Björkman, 1956). Lignins were isolated from the DA, LHW and OS pretreated *Populus trichocarpa* by an overloading cellulase hydrolysis (100 FPU/g cellulose) in sodium acetate buffer (pH 4.8) for 48 h. During the hydrolysis, cellulase and buffer were refreshed every 24 h. To remove the residual enzymes, protease treatment was conducted with 1 U/mL Pronase (Sigma Chemical Company, USA) at 37 °C in phosphate buffer (pH 7.4) for 24 h. The recovered solid residues were extracted by 96% dioxane in aqueous solution twice for 24 h. The collected solution was combined and rotary-evaporated at 40 °C under a reduced pressure to yield the crude lignin. The crude lignin was purified according to a previously published method (Yao et al., 2017).

2.4 Analytical methods

FT-IR spectroscopy (Spectrum One FTIR system, Perkin Elmer, Wellesley, MA) was obtained from 4000 to 500 cm^{-1} , signals were averaged and stored with 64 scans and a resolution of 2 cm^{-1} . For molecular weight analysis, gel permeation chromatography (GPC) analysis was conducted with acetylated lignin (Yao et al., 2017) on an Agilent 1200 HPLC system (Agilent Technologies, Inc., Santa Clara, CA) by employing Waters Styragel columns (HR1, HR4, and HR5; Waters Corporation, Milford, MA).

Two-dimensional (2D) ^1H - ^{13}C heteronuclear single quantum coherence (HSQC) NMR was conducted at 298 K with a Bruker Advance III 400-MHz spectroscopy

About 50 mg of lignin samples in DMSO- d_6 (0.4 mL) was characterized by a Bruker standard pulse sequence ('hsqcetgpsi2'), the spectral width was 11 ppm in F2 (^1H) with 2,048 data points and 190 ppm in F1 (^{13}C) with 256 data points (96 scans and 1 s interscan delay).

^{31}P NMR spectra were acquired after phosphorylation of lignin with TMDP (2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane) (Granata and Argyropoulos, 1995; Meng et al., 2019). The conditions for ^{31}P NMR spectra were as follows: a 90° pulse angle, 25s pulse delay, and 256 transients at ambient temperature.

3. Results and discussion

3.1. FTIR analysis

FTIR is commonly used to analyze the structural property of lignin. The main signals related to lignin structural features were assigned according to previous studies (Yang et al., 2016; Yao et al., 2018a). The signals at $\sim 3440\text{ cm}^{-1}$ and 2939 cm^{-1} represents OH stretching and asymmetric vibrations of saturated CH_2 in the side chain of lignin, respectively. The cross-peak at around 1710 cm^{-1} represents the unconjugated carbonyl stretching. The peaks near 1594 , 1506 and 1421 cm^{-1} which are assigned to the aromatic ring in lignin could be observed clearly in all lignin samples. Cross peaks at 1329 and 1124 cm^{-1} were from C-O vibration and C-H deformation of syringyl group, respectively. The guaiacyl ring related signals were also observed at around 1269 and 1226 cm^{-1} .

The relative intensities of signals calculated from the ratio of the signal intensity to that at 1506 cm^{-1} were presented in Table 1. It was found that the intensity of lignin

hydroxyl groups in the two *Populus* natural variants was dramatically decreased by all three pretreatments. As shown in Table 1, the content of the unconjugated carbonyl group was increased at different extent after each pretreatment, which was the most in OS pretreated *Populus* variant.

Although untreated BESC 328 presented more unconjugated carbonyl groups compared to BESC 131, more unconjugated carbonyl groups were observed in pretreated 131 lignins after all pretreatments, indicating that lignin in BESC 131 might be easier to be oxidized. C-O vibration of syringyl was all increased after pretreatments for all these *Populus* variants.

Table.1

3.2. Molecular weight distribution

Both depolymerization and repolymerization of lignin could occur during the acid-catalyzed pretreatment process, and the molecular weight distribution analysis could reflect variations of the two reactions occurring during pretreatments (Tolbert et al., 2015; Meng et al., 2018). Weight-average (M_w), number-average (M_n) molecular weights, and polydispersity indexes (M_w/M_n) of different lignin samples are summarized in Table 2. Results indicated that M_w of MWL was 10693 and 9161 g/mol for the BESC 328 and 131, respectively. Previous studies indicated that due to cleavage of β -O-4 linkages, the molecular weight of lignin could be decreased to various extents depends on the pretreatment conditions and biomass resources (Guo et al., 2015; Tolber et al., 2015). In this study, the molecular weight of lignin was dramatically decreased after DA, LHW, and OS pretreatments. Among the three

pretreatment methods, DA pretreatment resulted in a greater extent of lignin depolymerization (70 – 74%) than LHW (54 – 66%) and OS (44 – 52%) pretreatments. This suggested that acidolysis of β -O-4 linkages was the predominant reaction in the first 10 min of DA pretreatment, while longer pretreatment time in LHW and OS pretreatment led to some lignin repolymerization by acid-catalyzed condensation reactions. This is consistent with other studies that suggested lignin typically undergoes a greater extent of interlinkage cleavage than condensation at the early stage of acid pretreatment (Cao et al., 2012). In other DA pretreated *Populus* studies (Yao et al., 2018a; Samuel et al., 2014; Sun et al., 2015), the molecular weight of lignin after dilute acid or liquid hot water pretreatment was comparable with data obtained in this study.

DA and LHW pretreatments resulted in a greater extent of lignin depolymerization with BESC 131 than that with BESC 328, while OS pretreatment results similar extent of lignin depolymerization in two poplar variants as reflected by the lignin M_w . Furthermore, the PDI of lignins was also altered by different pretreatment methods, which was 2.55 and 2.29 for the MWL isolated from untreated BESC 328 and 131, respectively. After OS pretreatment, the molecular weight distribution of lignins became narrower than those of lignins in the untreated *Populus* variants. On the other hand, PDI of lignin was increased by the other two pretreatments. The changes in PDI of lignin upon different pretreatment might be again due to the different competition between lignin degradation and condensation reactions.

Table 2.

3.3 ³¹P NMR determination

Hydroxyl groups, especially phenolic hydroxyl, play a crucial role in lignin-cellulase interaction (Yao et al., 2018a; Pan, 2008). Thus in the present study, the amounts and distribution of various hydroxyl groups in untreated and pretreated lignin samples were quantitatively analyzed by ³¹P NMR. As shown in Fig. 1, the aliphatic OH signal was the dominant OH group in MWL ranging from 6-8 mmol/g in untreated *Populus* and accounting for 90-94% of the total hydroxyl group contents. The content of aliphatic hydroxyl group was significantly decreased after all three pretreatments. The aliphatic OH of lignin was reduced to 0.6-0.9 mmol/g by OS pretreatment, ~0.9 mmol/g by DA pretreatment, and 1.5-1.8 mmol/g by LHW pretreatment. The reduced aliphatic OH content was in part due to the loss of γ -OH as formaldehyde and α -OH groups to form ketone type of structures (Cao et al., 2012).

Phenolic hydroxyl groups including C₅ substituted, guaiacyl, and *p*-hydroxyl OH were also observed from the ³¹P NMR spectra. In particular, C₅ substituted OH was the most prominent among the phenolic hydroxyl groups. Both 131 and 328 *Populus* variants had higher guaiacyl OH contents than C₅ substituted OH in their MWL before pretreatments, while more C₅ substituted OH groups were found in the lignins after these pretreatments. Similarly, the amounts of syringyl hydroxyl groups were also reported to be increased after organosolv pretreatment (Guo et al., 2015) and dilute acid pretreatment (Sun et al., 2015). In this study, all three pretreatments increased phenolic hydroxyl group contents, which were mainly due to the cleavage

of aryl ether linkages in lignin (Wang et al., 2016). The content of carboxylic OH was also increased after pretreatments except for OS pretreatment for both *Populus* variants, which might be due to the hydrolysis of ester bonds or oxidation of aliphatic OH groups during acid-catalyzed pretreatments (Meng et al., 2018).

Fig. 1.

3.4 HSQC NMR determination

Heteronuclear single-quantum-coherence (HSQC) is one of the most applied 2D NMR techniques in lignin structural analysis. In the present study, 2D HSQC NMR analysis was employed to compare the structural characteristic of each lignin sample. NMR spectra peaks were assigned according to the previously published literatures (Sun et al., 2015; Meng et al., 2016; Yang et al., 2016; Yao et al., 2018a).

Cross peaks associated with guaiacyl (G), syringyl (S), and *p*-hydroxybenzoate (PB) units were detected in the aromatic regions (δ_C/δ_H 160-90/8.0-5.5 ppm) of all the lignin samples, as shown in Fig.2. The semi-quantitative information for the distribution of lignin subunits and inter-linkages is presented in Table 3. The S/G ratio of MWL for native *Populus* BESC 131 and 328 was 2.1 and 2.5, respectively. Each pretreatment resulted in lignin with different S/G ratios. The S/G ratio in LHW and OS pretreated lignin was lower than that in MWL, suggesting that more S units might be removed during the LHW and OS pretreatment. It has been reported that OS pretreatment process selectively removed lignin fragments with a higher content of S unit and left G unit-rich lignin in the pretreated residue, subsequently resulting in a decreased S/G ratio (Guo et al., 2015; Yoo et al., 2017a). On the contrary, S/G ratio

was increased after DA pretreatments, which was also found in previous studies (Sun et al., 2015; Shi et al., 2018). This trend might be due to the fact that syringyl lignin was more stable than guaiacyl lignin during the acid pretreatment (Shi et al., 2018). In addition, it was reported that enhanced degradation of G lignin units was observed with the addition of acidic catalyst during chemical pretreatment process (Zhang et al., 2015).

Our results also indicated that contents of S and G lignin units are changed during these pretreatments. Previously, researchers have reported a strong correlation between lignin S/G ratio and biomass recalcitrance (Davison et al., 2006; Yoo et al., 2018). The majority of literatures concluded that lignin with more G unit tend to obtain a less glucose release (Guo et al., 2014; Yoo et al., 2018). However, some other researchers found that S/G ratio of biomass was negatively correlated with biomass digestibility after the pretreatments (Xu et al., 2012; Jiang et al., 2016; Meng et al., 2016). Furthermore, the condensation of S and G units was observed in all pretreated lignin samples (Table 3 and Figure 2). Specifically, the greatest extent of condensation was observed in DA pretreatment. It is reported that PB unit is normally involved in the acylation of lignin and selectively attaches at the γ -position of S units (Lu et al., 2015). Its content was significantly decreased during OS pretreatment by 52.8 % and 62.2 % in BESC 131 and 328, respectively. Compared to BESC 131, BESC 328 also had a larger extent of PB decrease after LHW pretreatment. The previous study on natural *Populus trichocarpa* variants concluded that lignin S/G

ratio showed negative correlations with *p*-hydroxybenzoate (PB) contents in native lignin (Yoo et al., 2018), which was also found in the present study.

The aliphatic region (δ_C/δ_H 50-90/2.5-6.0 ppm) mainly contained signals arising from β -O-4, β -5, and β - β lignin interlinkages. As shown in Table 3, it was found that in MWL isolated from untreated *Populus* natural variants, β -O-4 content was the most abundant inter-unit linkages, followed by β - β and β -5. After different pretreatments, the amount of β -O-4 over total lignin aromatic subunits was dramatically decreased. After DA pretreatment, it was decreased to 3.6 and 5.1% for BESC 131 and 328, respectively. The amount of β -O-4 was 17.2% and 12.5% after LHW pretreatment of BESC 131 and 328, indicating that much fewer ether linkages were cleaved during LHW pretreatment process. Similar to DA pretreatment, β -O-4 ether linkages were also dramatically cleaved during the acid-catalyzed OS pretreatment. The relative rupture of β -O-4 linkages was in agreement with the bond energy of different lignin linkages obtained by theoretical calculation (Shen et al., 2019). Overall, the relative contents of major lignin inter-unit linkages (β -O-4, β -5, and β - β) over total lignin aromatic subunits were 61.3 and 69.8% in untreated BESC 131 and 328, respectively. It was decreased dramatically after DA, LHW, and OS pretreatments. LHW pretreated *Populus* contained the most and DA pretreated ones had the least lignin inter-unit linkages among all the pretreated *Populus*.

Fig.2

Table 3.

4. Conclusions

Lignin from pretreated natural *populous* variants showed different physiochemical properties. DA pretreatment resulted in the lowest M_w and the highest S/G. Lignin obtained after LHW pretreatment showed the most total and aliphatic OH among the three pretreatment methods. This kind of lignin could be used to form more complex aromatic compounds with additional functionality. The narrowest molecular weight distribution was found in OS pretreated residual lignin, which is favorable for lignin modification. The most amounts of inter-linkages were cleaved during DA pretreatment. Lignin with minimization of reactive C-O bonds in interunit lignin linkages could be applied for structural carbon-fiber production.

Acknowledgments

This manuscript has been authored, in part, by UT-Battelle, LLC under Contract No. DE-AC05-00OR22725 with the U.S. Department of Energy. This study was supported and performed as part of the BioEnergy Science Center (BESC) and the Center for Bioenergy Innovation (CBI). The BESC and CBI are U.S Department of Energy Bioenergy Research Centers supported by the Office of Biological and Environmental Research in the DOE Office of Science. The authors are also grateful for the support of the National Natural Science Foundation of China (No. 21978074, 31500496), key project of Hubei Provincial Department of Education (NO. D20161402) and Foundation of Hubei Provincial Key Laboratory of Green Materials for Light Industry (No. 201907B01, 201806A03).

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Fig. 1. ^{31}P -NMR analysis results of the distribution of different OH groups

Fig. 2. Aromatic regions ($\delta_{\text{C}}/\delta_{\text{H}}$ 160-90/8.0-5.5) and aliphatic regions ($\delta_{\text{C}}/\delta_{\text{H}}$ 90-45/6.0-2.0) in heteronuclear single quantum coherence (HSQC) spectra of the isolated lignin samples from *Populus* natural variants: (A) β -O-4 alkyl-aryl ethers; (A') β -O-4 alkyl-aryl ethers with acylated γ -OH; (B) phenylcoumarane; (C) resinols; (D) spirodienones; (X) xylan; (I) p-hydroxycinnamyl alcohol end groups; (PB) p-Hydroxybenzoate; (G) guaiacyl units; (S) syringyl units; (S') syringyl units with α oxidization ;(E) cinnamyl aldehyde end-groups

Table 1. Signal assignments and relative intensities in FTIR spectra of lignin samples.

Assignment	Wavenumber cm-1	131	131-DA	131-LH W	131-OS
Hydroxyl group	3440	1.07	0.64	0.67	0.53
C-H stretching	2939	0.73	0.77	0.78	0.79
Unconjugated carbonyl group	1710	0.53	1.25	1.15	2.24
Aromatic ring	1594	0.99	0.84	0.91	0.81
Aromatic ring	1506	1.00	1.00	1.00	1.00
C-H deformation	1462	1.03	1.18	1.27	1.39
Aromatic ring	1421	0.91	1.03	1.09	1.22
C-O vibration of syringyl	1329	0.86	0.93	0.97	0.97
Guaiacyl C-O units	1269	1.11	1.12	1.13	1.14
C-O vibration of guaiacyl	1226	1.14	1.78	1.76	2.39
Aromatic C-H deformation in syringyl	1126	1.49	1.21	1.47	1.76
C-O-C stretching	1036	1.10	1.31	1.56	1.32
Aromatic C-H deformation out of plane	830	0.25	0.25	0.34	0.25

Assignment	Wavenumber cm-1	328	328-DA	328-LH W	328-OS
Hydroxyl group	3440	1.14	0.67	0.63	0.54
C-H stretching	2939	0.65	0.86	0.72	0.79
Unconjugated carbonyl group	1714	0.62	1.06	0.85	1.48
Aromatic ring	1594	0.95	0.89	0.84	0.86
Aromatic ring	1506	1.00	1.00	1.00	1.00
C-H deformation	1462	1.02	1.28	1.10	1.39
Aromatic ring	1421	0.91	1.05	0.99	1.19
C-O vibration of syringyl	1329	0.88	1.01	0.89	0.99

Guaiacyl C-O units	1269	0.96	1.11	0.91	1.13
C-O vibration of guaiacyl	1226	1.00	1.71	1.53	2.51
Aromatic C-H deformation in syringyl	1124	1.33	1.36	1.72	1.51
C-O-C stretching	1036	0.96	1.15	1.15	1.21
Aromatic C-H deformation out of plane	830	0.45	0.24	0.29	0.27

Table 2. Weight-average (M_w), number-average (M_n) molecular weights and polydispersity indexes (M_w/ M_n) of lignin samples

Sample	M_n (g/mol)	M_w (g/mol)	PDI
328 MWL	4195	10693	2.55
131 MWL	3994	9161	2.29
328-DA	960	3134	3.26
131-DA	954	2372	2.49
328-LHW	1089	4904	4.50
131-LHW	991	3130	3.16
328-OS	3120	4959	1.59
131-OS	2745	4402	1.60

Table 3. Semi-quantitative information of lignin samples in the HSQC spectra

Lignin substructure	131	131-DA	131-LHW	131-OS
	% ^a	% ^a	% ^a	% ^a
S	67.2	36.0	51.2	48.6
Condensed S	N/A	41.3	14.7	11.4
G	32.8	8.9	26.6	26.6
Condensed G	N/A	13.8	7.4	13.4
PB	21.2	10.3	11.2	10.0
S/G*	2.1	4.0	1.9	1.8
β -O-4	57.3	3.6	17.2	4.8
phenylcoumarane (β -5)	1.2	2.6	4.8	9.5
resinols (β - β)	2.8	0.3	3.1	1.2
Total linkages	61.3	6.5	25.1	15.5
Lignin substructure	328	328-DA	328-LHW	328-OS
	% ^a	% ^a	% ^a	% ^a
S	71.1	29.5	46.2	45.7
Condensed S	N/A	39.7	25.8	18.7
G	28.9	4.6	20.9	20.4
Condensed G	N/A	26.2	7.1	15.2
PB	14.8	7.2	5.4	5.6
S/G*	2.5	6.4	2.2	2.3

<i>β-O-4</i>	62.3	5.1	12.5	5.2
phenylcoumarane (<i>β-5</i>)	2.3	1.5	3.0	6.8
resinols (<i>β-β</i>)	5.2	1.9	2.3	4.7
Total linkages	69.8	8.6	17.8	16.6

Note. ^a Content (%) expressed as a fraction of S + G. *S/G is calculated based on non-condensed units.

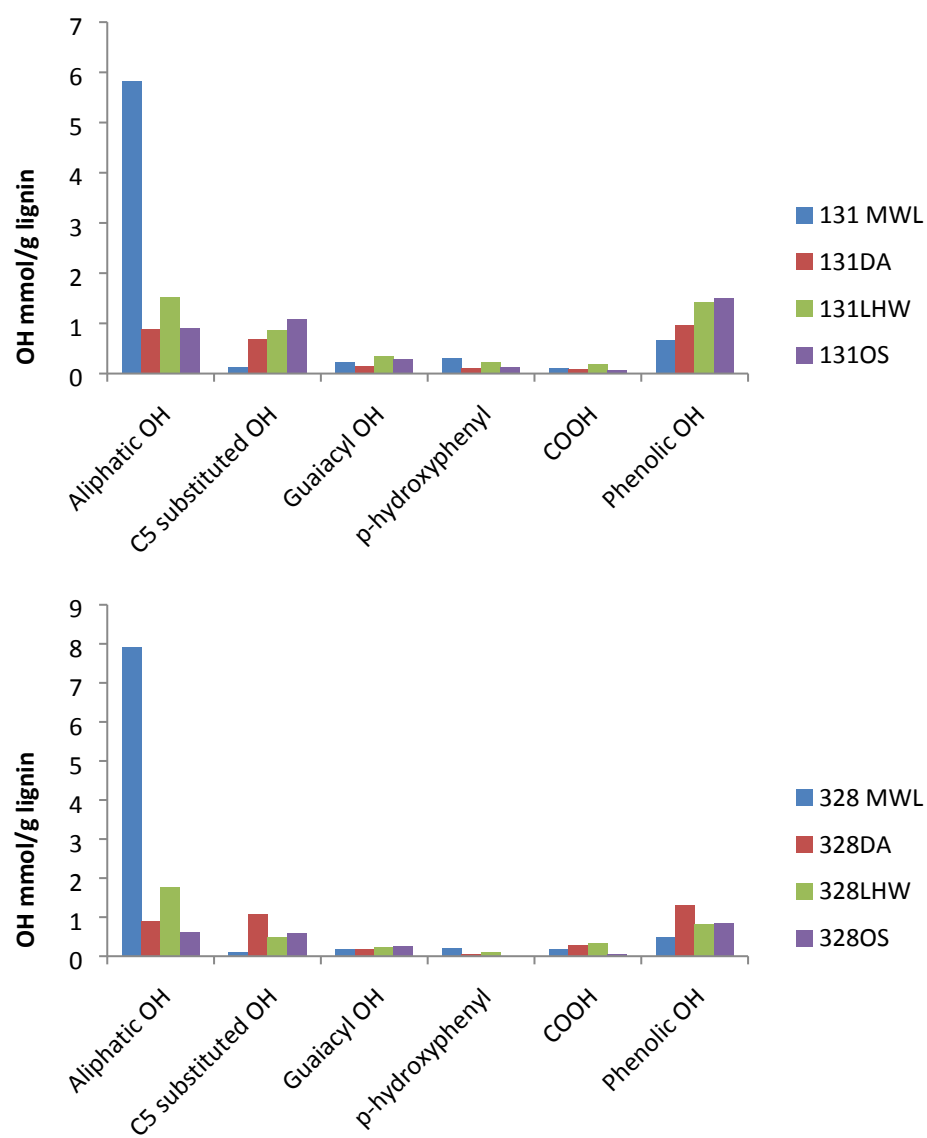
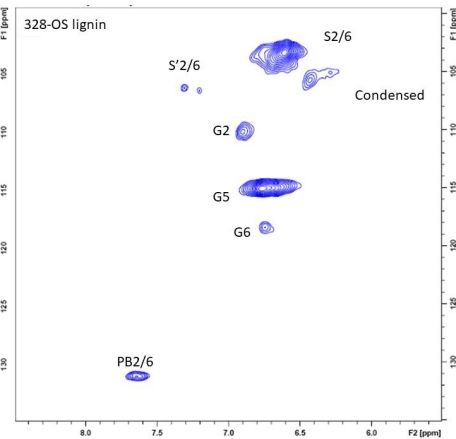
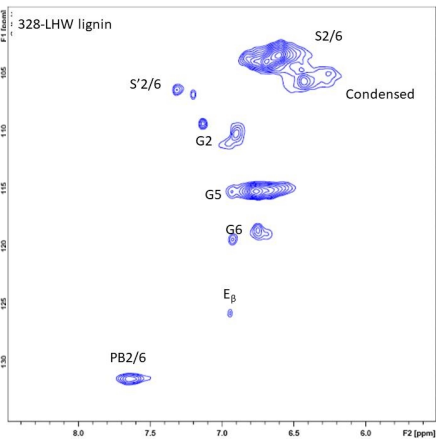
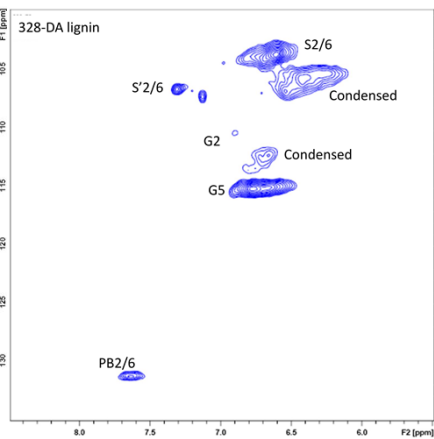
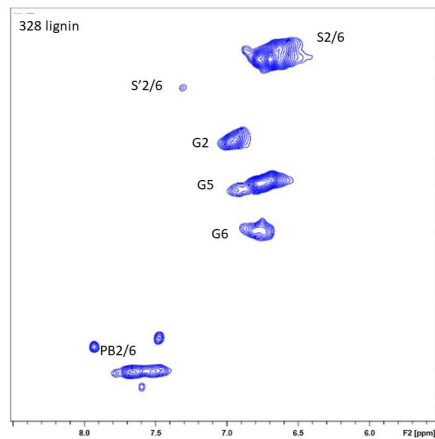
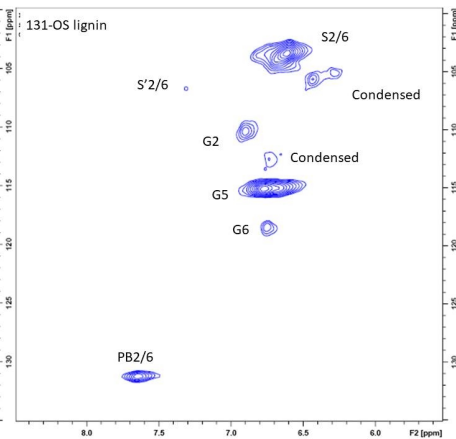
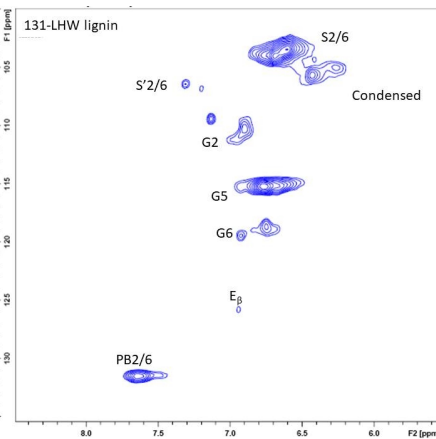
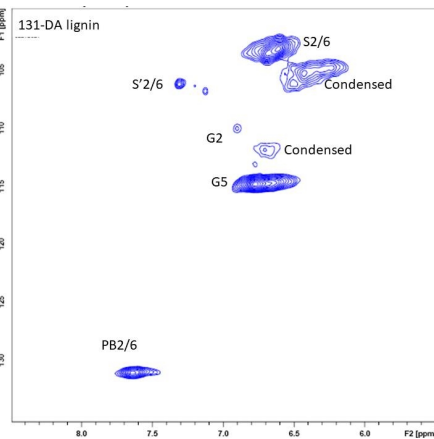
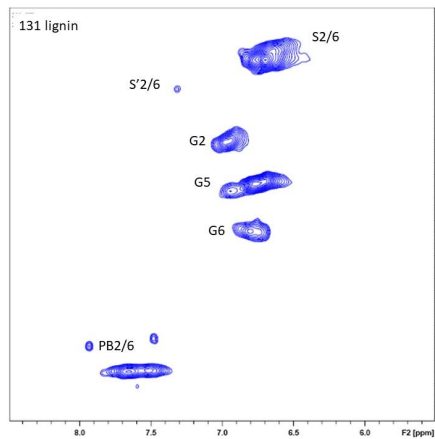


Fig. 1. ^{31}P -NMR analysis results of the distribution of different OH groups



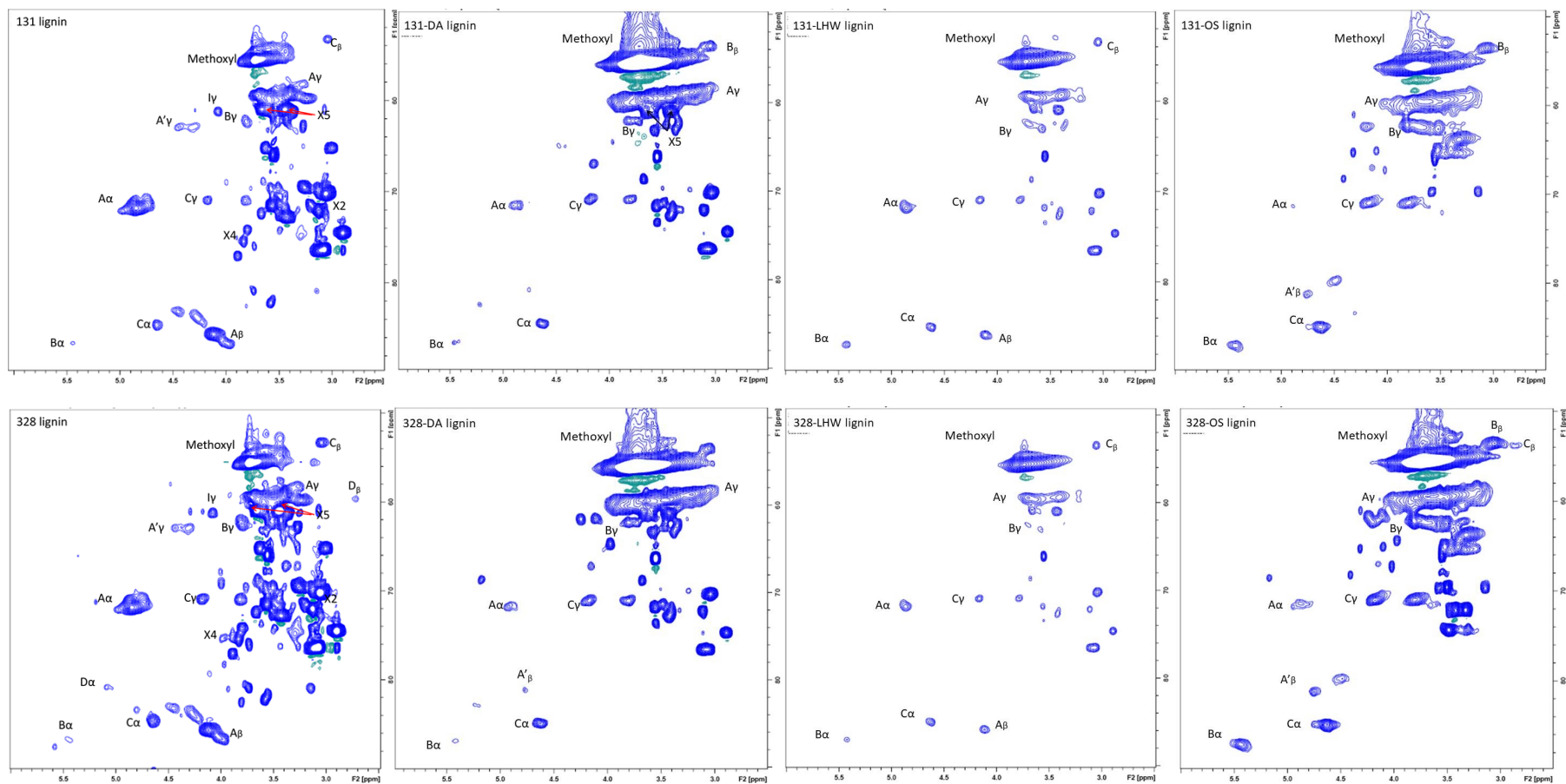


Fig. 2. Aromatic regions (δ_C/δ_H 160-90/8.0-5.5) and aliphatic regions (δ_C/δ_H 90-45/6.0-2.0) in heteronuclear single quantum coherence (HSQC) spectra of the isolated lignin samples from *Populus* natural variants: (A) β -O-4 alkyl-aryl ethers; (A') β -O-4 alkyl-aryl ethers with acylated γ -OH; (B) phenylcoumarane; (C) resinols; (D) spirodienones;(X)xylan; (I) *p*-hydroxycinnamyl alcohol end groups; (PB) *p*-Hydroxybenzoate; (G) guaiacyl units; (S) syringyl units; (S') syringyl units with α oxidization ;(E) cinnamyl aldehyde end-groups

