Effects of Fenton Oxidation on Structural Changes of Lignin from Steam-exploded Poplar

Cheng Pan,[a] Lan Yao,[a] Haitao Yang,[a,*] Lanfeng Hui,[b] and Zhong Liu [b,*]

Pretreatment of steam-exploded poplar by Fenton oxidation was used to effectively biodegrade lignin. The structure of lignin samples (L_S from steam-exploded poplar and L_{SF} from samples further treated by Fenton oxidation) obtained by enzymatic hydrolysis during pretreatment were characterized and compared. The results showed that the demethoxy reaction occurred in the process of Fenton oxidation. GPC results indicated that the weight average molecular weight (M_w) of L_{SF} did not change significantly, indicating that that there was no significant condensation during Fenton oxidation pretreatment. Heteronuclear single quantum coherence nuclear magnetic resonance (HSQC NMR) results suggested that the proportion of the three-unit basic structural units of poplar (H, G, and S) were significantly changed during Fenton oxidation process, and more lignin S units were removed than lignin G units. The content of *β-O-4* linkages was lower in L_{SF} (74.0%, as a fraction of *β-O-4* + *β-5* + *β-β*) than in L_S (78.2%), which indicated that *β-O-4* linkages were destroyed to a certain extent during Fenton oxidation process, and the content of total lignin interunit linkages including *β-O-4*, *β−β*, and *β-5* linkages over total lignin aromatic subunits (Ar%) in L_{SF} was higher, reaching 49.9%.

Keywords: Poplar; Steam explosion; Fenton oxidation; Lignin

Contact information: a: Hubei Provincial Key Laboratory of Green Materials for Light Industry, Hubei University of Technology, Wuhan 430068, China; b: Tianjin Key Laboratory of Pulp and Paper, Tianjin University of Science and Technology, Tianjin 300457, China;
* Corresponding authors: mglz@tust.edu.cn; pphtyang1979@aliyun.com

INTRODUCTION

Poplar is a fast-growing and high yield tree species. It has the characteristics of wide adaptability, long annual growth period, and fast production speed. Poplar resources are abundant in southern and northern China, and they are used generally in the pulp and paper industry. Although cellulose-rich biomass has great potential as bioenergy raw material, its application remains to be studied. As one of the important components of lignocellulosic materials, the structure of lignin is quite complex. Pretreatment is an indispensable link in the biological conversion process for bioenergy.

Steam explosion is an effective pretreatment technology with the attractive features of high efficiency and low pollution. Steam explosion has been applied to modify the chemical-physical properties of wheat bran (Sui *et al.* 2018). High quality cellulose preparation is isolated from rice straw by steam explosion and ionic liquid (Jiang *et al.* 2011). Compared with other pretreatment methods, steam explosion is a much more environmentally friendly method that is widely used for many biomasses (Chen and Liu 2015). The process generally avoids the use of potential pollutants. During the steam explosion process, the pretreated material becomes porous and accessible to be effective

for oligosaccharides productions from many materials (Villa-Lerma *et al.* 2013; Chiyanzu *et al.* 2014).

After steam explosion treatment, the structure of poplar wood changes from compact to fluffy, and the specific surface area of fibers increases, which is conducive to the adsorption in the process of Fenton treatment. Fenton oxidation is a rapid, green, and effective method for the degradation of organic compounds. Fenton oxidation exhibits excellent performance in lignin degradation. The use of Fenton chemistry as a feasible pretreatment method can improve the bioavailability of cellulose in microbial biofuel conversion (Kato *et al.* 2014; Seesuriyachan *et al.* 2015; Zhou *et al.* 2017). Hemicellulose and lignin are removed by a two-step method of dilute hydrochloric acid followed by Fenton oxidation, resulting in very high sugar recovery with a low enzyme load (Li *et al.* 2016). After Fenton oxidation treatment, the lignin fragments remain on the surface of fiber bundle, and the modified lignin can be easily extracted using hot alkaline solution (Yan *et al.* 2018). HO· (hydroxyl radical) is a strong oxidant that degrades organic compounds (Bhange *et al.* 2015). It is speculated that steam explosion following by a Fenton oxidation treatment is an effective method to remove lignin and separate cellulosic fibers, having a beneficial effect on the subsequent enzymatic hydrolysis process. Observing the modification of lignin in the Fenton pretreatment process not only can go deep into understand the separation mechanism of lignin, but also provide guidance for how to use lignin to produce high value-added products. Consequently, it is of great significance to clarify the structural characteristics of lignin during steam explosion and Fenton oxidation pretreatment.

This study examined the pretreatment of poplar *via* steam explosion and Fenton oxidation. Fenton oxidation was carried out on steam-exploded poplar to realize lignin degradation. The structural changes of lignin after pretreatment were studied. The structural features of L_S and L_{SF} were characterized by spectroscopic analyses including Fourier transform infrared (FT-IR) spectrometry, gel permeation chromatography (GPC), and 2D heteronuclear single quantum coherence nuclear magnetic resonance (HSQC NMR). Based on these results, the chemical characteristics of L_S and L_{SF} were extensively compared. The structural changes of lignin during steam explosion and Fenton oxidation pretreatment can provide valuable information for elucidating the pretreatment delignification process.

EXPERIMENTAL

Materials

Poplar was steam exploded at 213 °C for 5 min. The compositional analysis of steam exploded poplar on a dry basis was carried out as described (Xu *et al.* 2006) to determine the glucan (71.7%), xylan (4.0%), and lignin (13.0%). All standards and chemical compounds, including xylose, glucose, arabinose, cellulose, and protease, were chromatography grade and procured from Sigma-Aldrich (Shanghai, China). Sulfuric acid, ferrous sulfate heptahydrate, and hydrogen peroxide solution were analytical grade and procured from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

Fenton Oxidation Treatment

The 10 g of steam exploded poplar on dry basis was added to 500 mL of $FeSO_4.7H_2O$ solution (10 g/L) for 1 h. Wet fibers with 50% liquid (*w/w*) were transferred

into 500 mL of H_2O_2 (30%) solution (100 mL/L) for 1 h of FO activation. After being washed to neutral pH, the Fenton oxidation pretreated pulp was dried for sequential treatment and further analysis (Yan *et al.* 2018).

Lignin Extraction and Purification

The steam-pretreated poplar and Fenton oxidation pretreated pulp was milled and screened to pass 40 mesh and be retained on 60 mesh. The powders were extracted with absolute ethanol for 6 h. The L_S and L_{SF} were isolated by enzymatic hydrolysis (100 FPU/g substrate) of steam-pretreated poplar and Fenton oxidation poplar for 72 h, respectively. During the hydrolysis, fresh buffer and cellulase were used every 24 h. The residue was treated with protease for 24 h and recovered by centrifugation.

This crude lignin was further purified according to the purification method of milled wood lignin (MWL). The lignin-enriched residues were purified by dissolving in acetic acid-water (9:1, *v/v*) and precipitated in deionized water. The recovered solid (crude lignin) was dried, dissolved in 1,2-dichloroethane/ethanol (2:1, *v/v*), and precipitated in ethyl ether. The precipitated lignins (L_S and L_{SF}) were recovered by centrifugation, dried under vacuum at 40 °C, and stored in a desiccator (Lan *et al.* 2018).
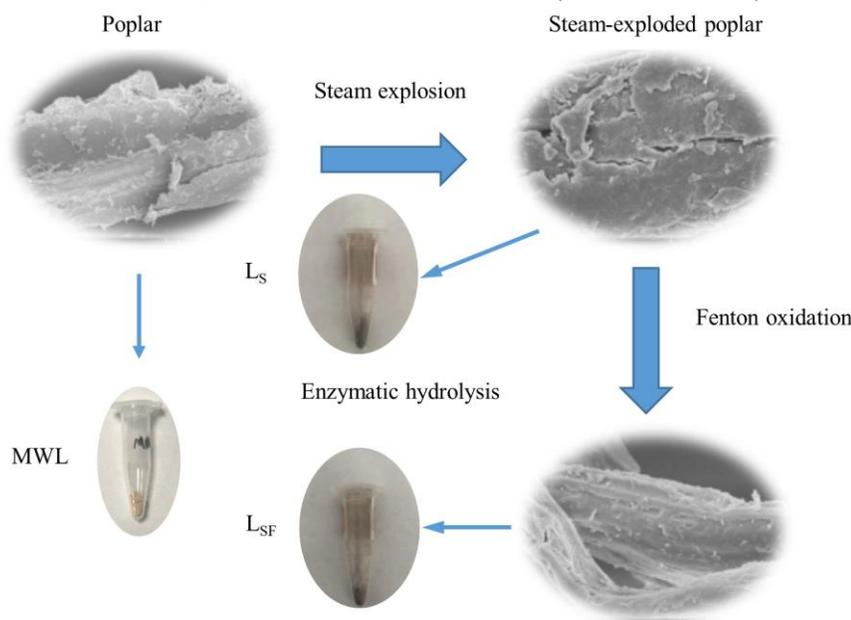
Fig. 1. Pretreatment and lignin extraction process

Lignin Characterization

The Fourier transform infrared spectrometer (Bruker, Karlsruhe, Germany) was operated in the wavelength range from 4000 cm^{-1} to 400 cm^{-1}. Lignin extracted from steam exploded poplar and Fenton oxidation pretreated pulp was milled and roughly mixed with KBr (1:100, *w/w*), and then pressed into a wafer for FT-IR measurement.

Thermogravimetric analysis (TGA) was conducted in a thermogravimetric simultaneous thermal analyzer. The samples of lignin were heated from room temperature to 800 °C at 10 °C/min, in a nitrogen atmosphere. The initial quantity of all samples was 5 to 10 mg.

Approximately 50 mg of lignin samples dissolved in DMSO-d6 was characterized by two dimensional (2D) ^{1}H-^{13}C heteronuclear single quantum coherence (HSQC) NMR conducted at 298 K with a Bruker Advance III 500-MHz spectrometer. The spectral width

was 11 ppm in ^{1}H with 2,048 data points and 190 ppm in ^{13}C with 256 data points (96 scans and 1 s interscan delay).

Gel permeation chromatography (GPC) was conducted to determine the molecular weights of each lignin fraction. Prior to the analysis, lignin samples were acetylated. GPC was performed on an Agilent 1200 HPLC system (Agilent Technologies, Inc., Santa Clara, CA, USA) equipped with Waters Styragel columns (HR1, HR4, and HR5; Waters Corporation, Milford, MA, USA) (Kumar *et al.* 2013).

RESULTS AND DISCUSSION

FT-IR Analysis

The FT-IR spectra of lignin fractions (L_S) and (L_{SF}) separated from pulp treated by steam explosion and Fenton oxidation as well as MWL are shown in Fig. 2. The FT-IR spectra showed a strong absorption at 3427 cm^{-1}, which can be attributed to the hydroxyl groups of phenolic and aliphatic structures stretching; the C-H stretching and asymmetric vibrations of CH$_3$ and CH$_2$ absorption appear at 2935 cm^{-1} (Guo *et al.* 2014). Signals at 1594, 1508, and 1423 cm^{-1} derived from the skeletal and stretching vibrations of benzene rings were characteristic peaks of lignin. As a result, the peaks could act as a standard for detecting the variation of other absorption bands. According to the lignin classification system of Faix, a semiquantitative lignin classification system based on FT-IR spectra, the intensity ratio of 1508/1463 cm^{-1} decreased from steam explosion pretreatment to Fenton oxidation pretreatment, indicating that the content of S units in these types of lignin fractions increased accordingly (Faix *et al.* 1990). The peak of the aromatic C-H deformation appeared at 1031 cm^{-1}. The absorption at 833 cm^{-1} was due to the C-H out-of-plane deformation of C2 and C6 in the S unit (Hage *et al.* 2009).
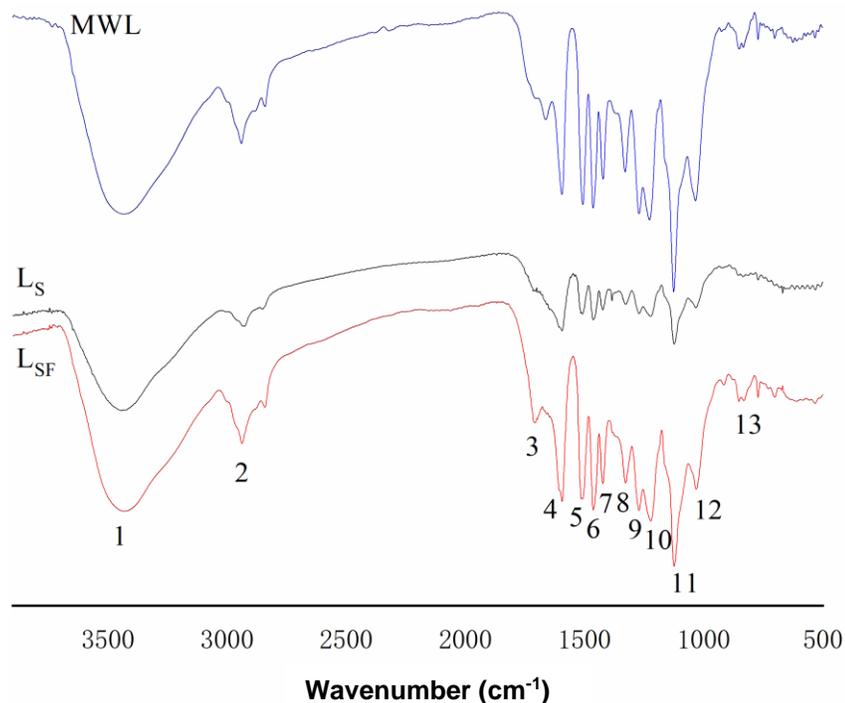
Fig. 2. FT-IR spectra of lignin fractions

The relative intensities of various cross peaks, calculated as the ratio of the intensity of the signal to that at 1508 cm^{-1} (Guo *et al.* 2014) are shown in Table 1. The content of total hydroxyl groups was decreased with Fenton oxidation treatment compared to L_S, suggesting that the lignin that already contains massive acid groups could be oxidized to lignin fragments (Yan *et al.* 2018). The intensities of the aromatic ring in L_S fractions were higher than that of L_{SF} fractions, which indicated that lignin did not undergo condensation during Fenton oxidation. The absorption peak at 1706 cm^{-1} corresponded to non-conjugated carbonyl stretching discovered in the spectrogram of L_S and L_{SF} and indicated that lignin fraction contained a significant amount of carbonyl groups. The characteristic peak of guaiacyl (G) rings could be found in the spectra at 1271 cm^{-1} and 1221 cm^{-1}. The signals at the wavenumbers of 1327 cm^{-1} and 1123 cm^{-1} were assigned to the presence of 5-condensed syringyl (S) units and C-H in-plane deformation of S units (Lan *et al.* 2018). The intensity ratios of S/G in L_S were higher than in L_{SF} and MWL indicated that the removal of the methoxy reaction happened during Fenton oxidation treatment.

Table 1. Signal Assignment and Relative Intensities in FTIR Spectra of Lignin Samples*

	Assignment	Wavenumber (cm^{-1})	L_S	L_{SF}	MWL
1	Hydroxyl groups	3427	2.59	1.06	1.05
2	C-H stretching	2938	1.20	0.72	0.69
3	Unconjugated carbonyl group	1706	0.63	0.61	0.46
4	Benzene rings	1594	1.28	1.01	0.95
5	Benzene rings	1508	1.00	1.00	1.00
6	Benzene rings	1463	1.09	1.06	1.02
7	Benzene rings	1423	0.93	0.92	0.87
8	Syringyl rings	1327	0.84	0.92	0.83
9	Guaiacyl rings	1271	0.99	1.06	1.05
10	Guaiacyl rings	1221	1.03	1.11	1.06
11	Syringyl rings	1123	1.49	1.34	1.44
12	Aromatic C-H deformation	1031	0.89	0.95	0.98
13	C-H out-of-plane vibrations	833	0.36	0.40	0.19
* The relative intensity was calculated as the ratio of the intensity of the signal to the intensity of the band at 1508cm^{-1}					

Molecular Weight Distributions of Lignin by GPC

The results of weight-average (M_w), number-average molecular weights (M_n), and polydispersity of lignin samples are summarized in Table 2.

Table 2. Weight-Average (M_w) and Number-Average (M_n) Molecular Weights and Polydispersity Indexes (M_w/M_n) of the Lignin Samples

Lignin samples	M_w	M_n	PDI (M_w/M_n)
MWL	12811	6942	1.9
L_S	127818	30201	4.2
L_{SF}	114789	19485	5.9

Both L_S (127818 g/mol) and L_{SF} (114789 g/mol) had higher M_w compared with MWL from untreated poplar (12811 g/mol). This is possibly due to the lignin repolymerization. Lignin interunit linkages were extensively cleaved during steam explosion pretreatment. The increased M_w of the lignin (L_S and L_{SF}) with the two-step pretreatment indicated that some repolymerization reactions probably occurred during

steam explosion pretreatment. The molar mass of lignin remained rather constant during Fenton oxidation, indicating that no polycondensation reaction occurred.

TGA

To further characterize the lignin products, the mass loss and rate of mass loss curves were obtained through thermogravimetric analysis, and the results are shown in Fig. 3. The lignin samples exhibited a major weight loss peak from 280 to 400 °C. When the temperature reached 800 °C, the residual weight of L_{SF} and L_S were 27.1% and 21.5%, respectively, while the residue of MWL was 19.7%. This variation can be attributed to repolymerization reactions probably occurred during the pretreatment process, resulting in relatively good thermostability.

The DTG curves provide information about the mass loss rate of the lignin samples. The main peak of MWL appeared at approximately 286.7 °C with a slightly weaker peak at approximately 422.5 °C. The maximum degradation temperatures of L_{SF}, L_S and MWL were 327.6 °C, 303.2 °C, and 286.7 °C, respectively. Fig. 3b shows that the highest loss rate of MWL was 0.34% per °C, compared with 0.25% per °C for L_S, and 0.29% per °C for L_{SF}. In addition, the maximal mass loss rate of MWL occurred earlier and was higher than that of L_{SF} and L_S obtained after pretreatment. This suggests that the lignin structure was changed during the pretreatment process, after a certain degree of degradation and condensation (Wang *et al.* 2018).
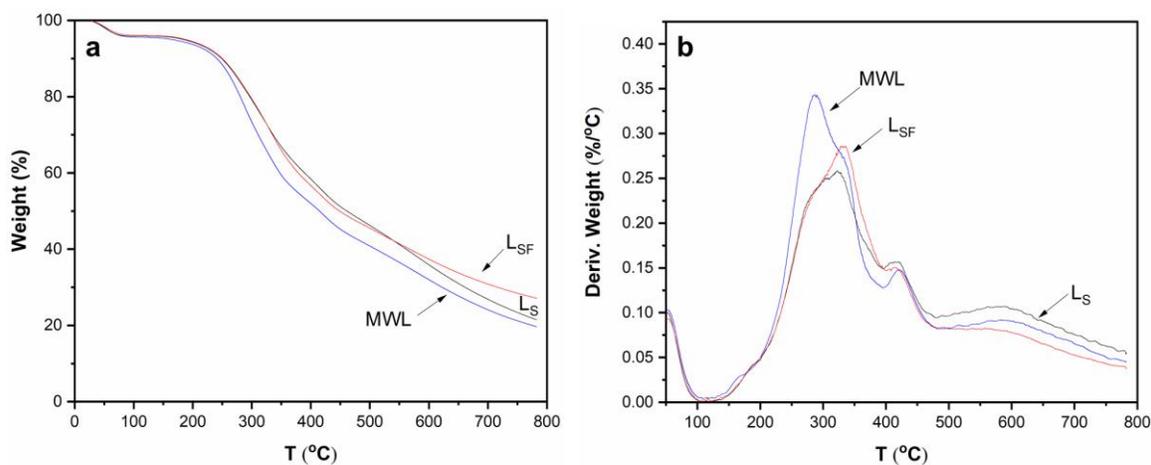
Fig. 3. TG (a) and DTG (b) curves of lignin fractions

2D HSQC NMR Characterization

HSQC is an efficient technology in elucidating lignin subunit composition and lignin interunit linkage distribution without deconstruction the cell wall. The lignin samples were characterized by 2D HSQC NMR to determine the structural differences of the extracted lignin (Mansfield *et al.* 2012). To make the spectra comparable, all lignins were extracted by enzymatic hydrolysis (Lan *et al.* 2018). The spectra were divided into two regions: the side-chain region and the aromatic/unsaturated regions. The key chemical structures and units are presented in detail in Fig. 4.

In the aromatic region of HSQC spectra, signals belonging to syringyl (S) and guaiacyl (G) were labeled (Kim *et al.* 2008; Hallac *et al.* 2010; del Río *et al.* 2012; Li *et*

al. 2016; Lan *et al.* 2018; Lei *et al.* 2018; Ning *et al.* 2018). As shown in Fig. 4 (a1,b1), S units were observed as a prominent signal in L_S and L_{SF} for the $C_{2,6}/H_{2,6}$ correlation at δ_C/δ_H 104.48/6.71 ppm, while the signals at δ_C/δ_H 106.95/7.34 ppm were originated from the oxidized syringyl (S') units. G units were also observed with the correlations for C_2/H_2 at δ_C/δ_H 111.52/6.92 ppm, C_5/H_5 at δ_C/δ_H 115.79/6.81 ppm, and C_6/H_6 at δ_C/δ_H 119.19/6.77 ppm, respectively. The presence of these chemical shifts may be due to interactions with other functional groups formed during pretreatment on the altered side chain in the G unit and in the aromatic rings of the S units. Moreover, the signal of the H unit was found at δ_C/δ_H 129.15/7.23 ppm in the spectra of L_S. Furthermore, cross signals appearing in the spectra at δ_C/δ_H 116.2/6.76 ppm ($PB_{3/5}$) and $\delta_C/\underline{\delta}_H$ 131.76/7.67 ppm ($PB_{2/6}$) originated from p-hydroxy benzoate unit (PB). The semiquantitative analysis of HSQC NMR spectra is shown in Table 3. The relative contents of lignin S units in L_S was higher than in L_{SF}, indicating that more S units were removed than G units during Fenton oxidation pretreatment. As a result, the S/G ratio of L_{SF} was much lower than L_S. Most of the PB units were retained during the pretreatment process. The distinction of the absorption peak intensities between L_S and L_{SF} indicated that the proportion of the three-unit basic structural units of poplar differed during the Fenton oxidation process, which was in accordance with the FTIR analysis.

Table 3. Quantitative Information of Three Lignin Samples in the HSQC Spectra

Lignin Substructure	L_S		L_{SF}	
	%	%	%	%
S	75.48		59.32	
G	24.52		40.68	
PB	4.19		4.23	
S/G	3.08		1.46	
β-O-4	37.21 [a]	78.16 [b]	37.05 [a]	73.99 [b]
β-5	3.60	7.58	4.42	9.08
β-β	6.79	14.26	8.47	16.92
[a]Amount of specific functional group was expressed as a percentage of $S_{2,6}/2+G_2$ (Sette *et al.* 2011). [b]Amount of specific functional group was expressed as a fraction of *β-O-4 + β-5 + β-β*.				

In the aliphatic region of the lignin HSQC NMR spectra, correlation signals of the methoxyl groups (OMe) were at δ_C/δ_H 56.29/3.75 ppm, which are obviously the most prominent units in this region, and major interunit linkages including *β-O-4* aryl ether (A), phenylcoumaran (*β-5*) (B), and resinol (*β-β*) (C) were the main type of connections in L_S and L_{SF}. The C-H correlations in *β-O-4* linkages of structure were observed from α, β, and γ positions at approximately δ_C/δ_H 72.44/4.88 ppm (A_α), δ_C/δ_H 86.54/4.13 ppm (*β* position of *β-O-4* linked to S unit ($A_{\beta(S)}$), δ_C/δ_H 84.25/4.32 ppm (*β* position of *β-O-4* linked to G unit ($A_{\beta(G)}$), and δ_C/δ_H 60.17/3.40 ppm (A_γ), respectively. Phenylcoumaran (B) was resolved in all lignin structure with C_α-H_α, C_β-H_β, and C_γ-H_γ correlations at δ_C/δ_H 87.56/5.44 ppm (B_α), 53.72/3.74 ppm (B_β), and 63.41/3.71 ppm (B_γ), respectively. The presence of resinol substructure was confirmed by C_α-H_α correlations at around δ_C/δ_H 85.54/4.66 ppm (C_α), C_β-H_β correlations at around 54.19/3.07 ppm (C_β), and C_γ-H_γ correlations at around δ_C/δ_H 71.64/3.81 ppm and δ_C/δ_H 71.58/4.19 ppm (C_γ). The content of total lignin interunit linkages including *β-O-4*, *β−β*, and *β-5* linkages over total lignin aromatic subunits (Ar%) in the L_{SF} was 49.9%. The presence of a signal at δ_C/δ_H 64.2/3.30 ppm in all lignin fractions is ascribed to the methylene in α-ethoxylated *β-O-4* (A'), suggesting the occurrence of α-ethoxylation during the pretreatment process (Guo *et al.* 2015).
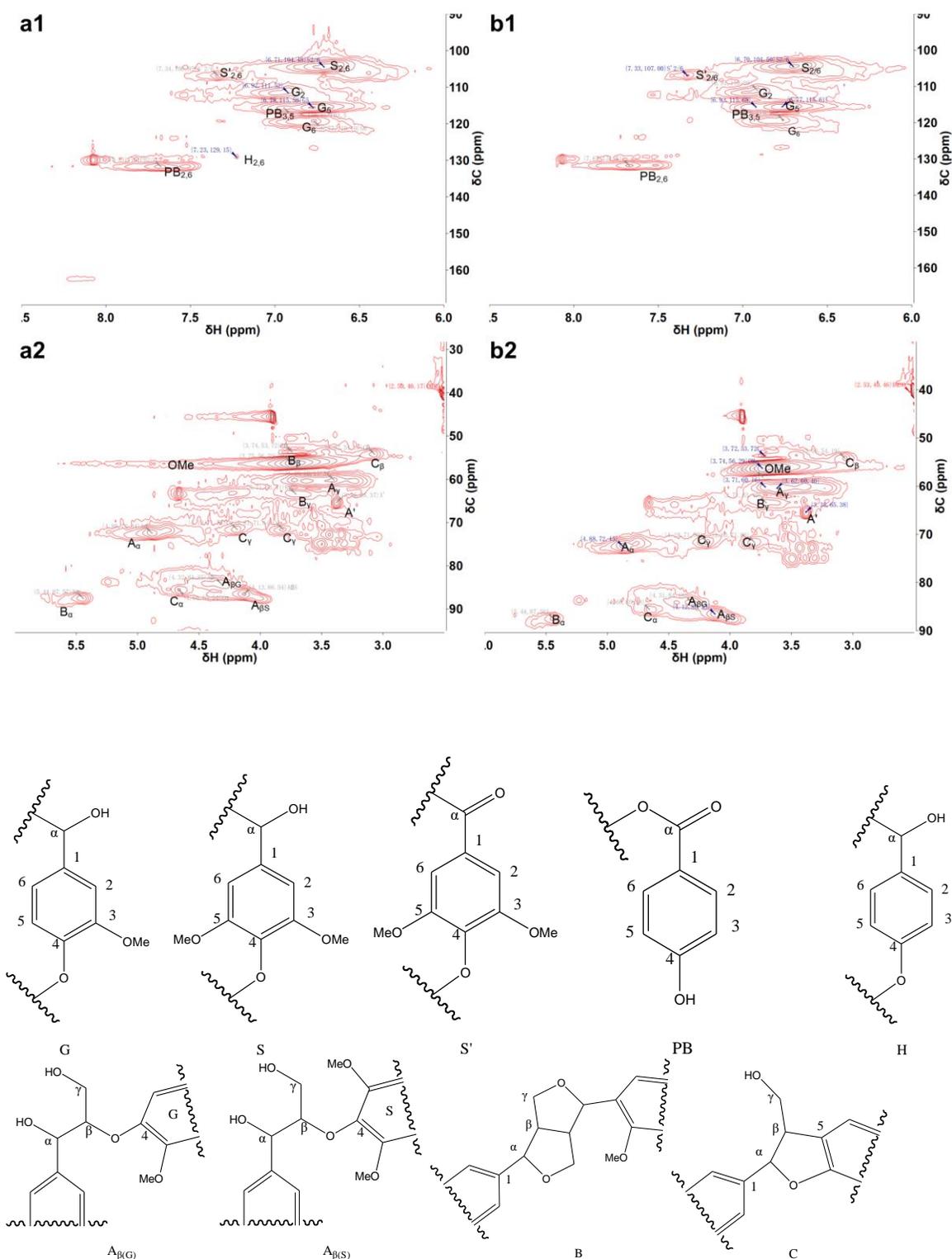
Fig. 4. Aromatic/unsaturated ($δ_C/δ_H$ 90-160/6-8.5) and side chain ($δ_C/δ_H$ 30-90/2.5-6) regions in the 2D HSQC NMR spectra of L_S (a1, a2) and L_{SF} (b1, b2). (S) syringyl units; (S') the oxidized syringyl units; (G) guaiacyl units; (H) p-hydroxyphenyl units; (PB) phydroxybenzoate substructures; ($A_{β(G)}$) *β-O-4* linked to G unit; ($A_{β(S)}$) *β-O-4* linked to S unit; (B) phenylcoumaran (*β-5*); (C) resinol (*β-β*).

After the pretreatment process, the aryl ether linkages in L_S and L_{SF} were 37.2% and 37.0% of the total lignin aromatic subunits (Ar%), respectively. The relative contents of phenylcoumaran and resinol units were increased in lignin fractions during the pretreatment process. The cleavage of interunit linkages during pretreatment was observed clearly in this study. As a whole, the content of *β-O-4* linkages was lower in L_{SF} than in L_S, and more lignin S units were removed than lignin G units. This result indicated that *β-O-4* linkages were destroyed to a certain extent during Fenton oxidation process. The results show that the characteristics of lignin change with the selection of pretreatment methods and steps, which can be used as a thread for the development of lignin.

CONCLUSIONS

1. A pretreatment method including steam explosion and Fenton oxidation was studied in poplar. Structural changes of lignin samples from poplar were studied after steam explosion and Fenton oxidation pretreatment. The structural changes in lignin samples (L_S and L_{SF}) obtained by enzymatic hydrolysis showed that demethoxylation occurred during Fenton oxidation pretreatment.

2. The M_W of L_{SF} did not change significantly, compared with L_S, indicating that the lignin condensation reaction mainly arose in the steam explosion process, but not in the Fenton oxidation process. The maximal mass loss rate of MWL occurred earlier, and was higher than that of L_{SF} and L_S obtained after pretreatment. The distinction of the absorption peak intensities in the 2D HSQC NMR spectra of L_S and L_{SF} indicated that the proportion of the basic structural units of pretreated poplar differed during the Fenton oxidation process, which was in accordance with the FTIR analysis.

3. The content of *β-O-4* linkages was lower in L_{SF} (74.0%, as a fraction of *β-O-4* + *β-5* + *β-β*) than in L_S (78.2%), and the content of S units was lower in L_{SF} (59.3%, as a percentage of S+G) than in L_S (75.5%). Thus, *β-O-4* linkages were destroyed to a certain extent during the Fenton oxidation process. L_{SF} showed relatively higher contents of phenylcoumaran and resinol units after the Fenton oxidation pretreatment, and this information can be a clue to developing lignin valorization strategies.

ACKNOWLEDGMENTS

REFERENCES CITED

Bhange, V. P., William, S., Sharma, A., Gabhane, J., Vaidya, A. N., and Wate, S. R. (2015). "Pretreatment of garden biomass using Fenton's reagent: Influence of Fe^{2+} and H_2O_2 concentrations on lignocellulose degradation," *Journal of Environmental Health Science and Engineering* 13(1), 12. DOI: 10.1186/s40201-015-0167-1

Chen, H. Z., and Liu, Z. H. (2015). "Steam explosion and its combinatorial pretreatment

refining technology of plant biomass to bio-based products," *Biotechnology Journal* 10(6), 866-885. DOI: 10.1002/biot.201400705

Chiyanzu, I., Brienzo, M., García-Aparicio, M. P., and Görgens, J. F. (2014). "Application of endo-β-1,4,d-mannanase and cellulase for the release of manno-oligosaccharides from steam-pretreated spent coffee ground," *Applied Biochemistry and Biotechnology* 172(7), 3538-3557. DOI: 10.1007/s12010-014- 0770-0

Del Río, J. C., Rencoret, J., Prinsen, P., Martínez, Á. T., Ralph, J., and Gutiérrez, A. (2012). "Structural characterization of *wheat straw* lignin as revealed by analytical pyrolysis, 2D-NMR, and reductive cleavage methods," *Journal of Agricultural and Food Chemistry* 60(23), 5922-5935. DOI: 10.1021/jf301002n

Faix, O., Fortmann, I., Bremer, J., and Meier, D. (1990). "Thermische Abbauprodukte des Holzes. Gaschromatographische Trennung und massenspektrometrische Charakterisierung Monomerer Polysaccharid-abbauprodukte; Gas chromatographic separation and mass spectrometric characterization of polysaccharide derived products," *Holz Als Roh Und Werkstoff* 48(7-8), 281-285. DOI: 10.1007/BF02626519

Guo, F., Shi, W., Sun, W., Li, X., Wang, F., Zhao, J., and Qu, Y. (2014). "Differences in the adsorption of enzymes onto lignins from diverse types of lignocellulosic biomass and the underlying mechanism," *Biotechnology for Biofuels* 7(1), 38. DOI: 10.1186/1754-6834-7-38

Guo, Y., Zhou, J., Wen, J., Sun, G., and Sun, Y. (2015). "Structural transformations of triploid of *Populus tomentosa* Carr. lignin during auto-catalyzed ethanol organosolv pretreatment," *Industrial Crops and Products* 76, 522-529. DOI: 10.1016/j.indcrop.2015.06.020

Hage, R. E., Brosse, N., Chrusciel, L., Sanchez, C., Sannigrahi, P., and Ragauskas, A. (2009). "Characterization of milled wood lignin and ethanol organosolv lignin from *miscanthus*," *Polymer Degradation and Stability* 94(10), 1632-1638. DOI: 10.1016/j.polymdegradstab.2009.07.007

Hallac, B. B., Pu, Y., and Ragauskas, A. J. (2010). "Chemical transformations of *Buddleja davidii* lignin during ethanol organosolv pretreatment," *Energy and Fuels* 24(4), 2723-2732. DOI: 10.1021/ef901556u

Jiang, M., Zhao, M., Zhou, Z., Huang, T., Chen, X., and Wang, Y. (2011). "Isolation of cellulose with ionic liquid from steam exploded rice straw," *Industrial Crops and Products* 33(3), 734-738. DOI: 10.1016/j.indcrop.2011.01.015

Kato, D. M., Elía, Noelia, Flythe, M., and Lynn, B. C. (2014). "Pretreatment of lignocellulosic biomass using Fenton chemistry," *Bioresource Technology* 162, 273-278. DOI: 10.1016/j.biortech.2014.03.151

Kim, H., Ralph, J., and Akiyama, T. (2008). "Solution-state 2D NMR of ball-milled plant cell wall gels in DMSO-d6," *Bioenergy Research* 8(3), 576-591. DOI: 10.1007/s12155-008-9004-z

Kumar, R., Hu, F., Hubbell, C. A., Ragauskas, A. J., and Wyman (2013). "Comparison of laboratory delignification methods, their selectivity, and impacts on physiochemical characteristics of cellulosic biomass," *Bioresource Technology* 130(13), 372-381. DOI: 10.1016/j.biortech.2012.12.028

Lan, Y., Congxin, C., Geun, Y. C., Xianzhi, M., Mi, L., Ragauskas, A. J., Dong, C., Yang, H., and Yunqiao, P. (2018). "Insights of ethanol organosolv pretreatment on lignin properties of *Broussonetia papyrifera*," *ACS Sustainable Chemistry and Engineering* 6(11), 14767-14773. DOI: 10.1021/acssuschemeng.8b03290

Lei, W., Ran, Z., Jian, L., Li, G., Haiping, Y., and Fuying, M., *et al.* (2018).

"Comparative study of the fast pyrolysis behavior of ginkgo, poplar, and wheat straw lignin at different temperatures," *Industrial Crops and Products* 122, 465-472. DOI: 10.1016/j.indcrop.2018.06.038

Li, W., Liu, Q., Ma, Q., Zhang, T., Ma, L., and Jameel, H., *et al.* (2016). "A two-stage pretreatment process using dilute hydrochloric acid followed by Fenton oxidation to improve sugar recovery from *corn stover*," *Bioresource Technology* 219, 753-756. DOI: 10.1016/j.biortech.2016.08.025

Li, C., Guo, H., Zhang, B., Dai, T., Qi, Z., and Wang, A., et al. (2016). "Valorization of lignin to simple phenolic compounds over tungsten carbide: Impact of lignin structure," *ChemSusChem* 10(3), 523-532. DOI: 10.1002/cssc.201601326

Mansfield, S. D., Kim, H., Lu, F., and Ralph, J. (2012). "Whole plant cell wall characterization using solution-state 2D NMR," *Nature Protocols* 7(9), 1579-1589. DOI: 10.1038/nprot.2012.064

Ning, D., Xiaoqiang, S., Yetao, J., Bin, L., Xianhai, Z., Yong, S., Tang, X., Lei, T., and Lin, L. (2018). "Cooking with active oxygen and solid alkali facilitates lignin degradation in bamboo pretreatment," *Sustainable Energy and Fuels* 2, 2206-2214. DOI: 10.1039/C8SE00181B

Seesuriyachan, P., Kuntiya, A., Kawee-ai, A., Techapun, C., Chaiyaso, T., and Leksawasdi, N. (2015). "Improvement in efficiency of lignin degradation by Fenton reaction using synergistic catalytic action," *Ecological Engineering* 85, 283-287. DOI: 10.1016/j.ecoleng.2015.10.013

Sette, M., Wechselberger, R., and Crestini, C. (2011). "Elucidation of lignin structure by quantitative 2D NMR," *Chemistry – A European Journal* 17, 9529- 9535. DOI: 10.1002/chem.201003045

Sui, W., Xie, X., Liu, R., Wu, T., and Zhang, M. (2018). "Effect of *wheat bran* modification by steam explosion on structural characteristics and rheological properties of wheat flour dough," *Food Hydrocolloids* 84(NOV.), 571-580. DOI: 10.1016/j.foodhyd.2018.06.027

Villa-Lerma, G., González-Márquez, Humberto, Gimeno, M., López-Luna, Alberto, Bárzana, Eduardo, and Shirai, K. (2013). "Ultrasonication and steam-explosion as chitin pretreatments for chitin oligosaccharide production by chitinases of *Lecanicillium lecanii*," *Bioresource Technology* 146, 794-798. DOI: 10.1016/j.biortech.2013.08.003

Yan, S., Wei, J., Yuanming, Z., Haoxi, B., Guangting, H., and Ragauskas, A. J. (2018). "Isolation and characterization of cellulosic fibers from *kenaf bast* using steam explosion and Fenton oxidation treatment," *Cellulose*. DOI: 10.1007/s10570-018-1916-y

Zhou, J., Li Z., and Yu C. (2017). "Property of ramie fiber degummed with Fenton reagent," *Fibers and Polymers* 18 (10), 1891-1897. DOI: 10.1007/s12221-017-6489-0