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Understanding the structural changes of lignin in poplar following steam explosion pretreatment

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Abstract: To understand the modifications of lignin in the steam explosion process (209°C, 7 min), lignin samples in native poplar (L_p), steam explosion solid residue (L_s) and steam explosion liquid (L_l) were separated and studied. The lignin samples (L_p , L_s and L_l) were characterized and analyzed by size exclusion chromatography (SEC), Fourier transform infrared spectroscopy (FTIR), and nuclear magnetic resonance [carbon 13 nuclear magnetic resonance (^{13}C -NMR) and heteronuclear single quantum coherence or heteronuclear single quantum correlation experiment NMR (HSQC) NMR] analysis. The results revealed that the pretreatment induced reductions in amounts of β -O-4', β - β' , and spirodienone structure, and increases in the syringyl/guaiacyl (S/G) ratio from 1.14 (L_p) to 1.70 (L_s) and 1.86 (L_l). The HSQC NMR spectra also gave more information about the predominance of G and S units, and only small amounts of p-hydroxyphenyl (H) units. Moreover, SEC demonstrated the depolymerization and repolymerization of lignin, which were the main reasons for the increase in the average molecular weight of L_s and the decrease in average molecular weight of L_l , respectively. In brief, after steam explosion treatment, the lignin structure changed, but the backbone structure was not noticeably modified.

Keywords: average molecular weight, lignin structure, NMR, size exclusion chromatography, steam explosion

Introduction

Lignocellulosic biomass has received increasing attention from researchers due to its ability to convert to fuels and common chemicals (Ragauskas et al. 2006; Himmel et al. 2007; Alonso et al. 2010). However, given the natural

recalcitrance toward destruction and bioconversion of lignocellulosic materials, what is required of this situation is pretreatment of biomass materials (Mosier et al. 2005; Ragauskas et al. 2006), which can improve the accessibility of enzymes and chemical reagents, and realize the high-value transformation of biomass resources (Ding et al. 2012). To date, four types of pretreating methods; physical, chemical, biological and combinatorial (physiochemical and biochemical) pretreatment methods, have been studied (Kumar et al. 2009; Silveira et al. 2015). In general, physical pretreating methods can reduce particle size and crystallinity of lignocellulose and thus increase the specific surface and decrease the degree of polymerization, but this process is not economically feasible because of high energy consumption (Alvira et al. 2010). Note that chemical pretreatment methods, mainly including alkaline pretreatment, acid pretreatment, ozonolysis, organosolv pretreatment and ionic liquid pretreatment, can break the structure of lignocellulosic biomass, and increase the internal surface area, which could result in the improvement of the conversion efficiency and product yield (Liew et al. 2014). As for chemical pretreatment methods, dilute acid pretreatment is the most widely used method, but it has some limitations, such as requiring high quality requirement of equipment, high pretreatment temperature and pressure, formation of fermentation inhibitors, and having carbohydrate degradation that is difficult to control (Chen et al. 2017). Meanwhile, biological pretreatment is low-energy and eco-friendly (Chen et al. 2017). However, this pretreatment process is generally time-consuming, which leads to low-efficiency industrial operations (Liew et al. 2014). Note that the combinatorial pretreatment method seems to be more effective than other methods (Liew et al. 2014). Among them, steam explosion, a physicochemical method, has been regarded as a highly efficient pretreatment method for biomass (Silveira et al. 2015; Chen et al. 2017).

Steam explosion pretreatment combines mechanical forces and chemical effects due to the instantaneous decompression and hydrolysis of acetyl groups present in hemicellulose, respectively. Compared with other pretreatment methods, this process reveals several attractive features, such as environmental friendliness, lower cost investment, fewer chemical reagent hazards, and complete sugar recovery (Avellar and Glasser 1998; Chen et al.

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2017). Under the action of high temperature steam, most hemicellulose and part of lignin are degraded, and the fiber bundle structure is softened. The degraded hemicellulose can be recovered by subsequent washing, and the cellulose-rich material remains.

In addition, during the steam explosion process, organic acids, such as formic acid, levulinic acid and acetic acid, would be generated. Among them, acetic acid is the main component, and it is derived from the hydrolysis of acetyl groups in hemicellulose (Weil et al. 1997). Silverstein et al. found that when cotton stalk was pretreated with dilute acid, lignin was degraded, and a small amount of lignin (2–4%) was removed, of which the degree of lignin removal was proportional to the pretreatment strength (Silverstein et al. 2007). In addition, under acidic conditions, the main impacts on lignin were the decrease in the content of β -O-4' structures and increase in the content of C-C condensed structures (Li et al. 2007). Samuel et al. found that during the dilute acid treatment of switch grass, 36% of the β -O-4' bond in lignin structure was broken, and the ratio of S/G was also decreased, which was mainly because the β -O-4' bond in the straight chain of S-type lignin was more easily broken (Samuel et al. 2010). Therefore, the structure of lignin could be modified by steam explosion treatment. A few studies were conducted by some researchers, and relevant conclusions were drawn. Li et al. discovered that after steam explosion treatment, the condensation and depolymerization reactions of poplar lignin proceeded (Li et al. 2007). Based on previous studies, the changes in poplar lignin properties before and after steam explosion treatment were analyzed in this work.

The structure types of lignin are heterogeneous, composed of H, G, and S units (Kim et al. 2008). This study was to investigate the impact of steam explosion on the structure and molecular weight of lignin by using size exclusion chromatography (SEC), Fourier transform infrared spectroscopy (FTIR), heteronuclear single quantum coherence or heteronuclear single quantum correlation experiment nuclear magnetic resonance (HSQC NMR) and carbon 13 (^{13}C)-NMR. After pretreatment, the lignin fractions in steam-exploded solid residue (L_s) and steam-exploded liquid (L_l) were isolated to further evaluate the effects of steam explosion on lignin properties. Lignin in native poplar (L_p), which can be considered representative of native lignin, was also isolated for comparison. Understanding the structural information of lignin and the reaction mechanism during the steam explosion process will help to evaluate the utilization of steam-exploded products, including solid residue and steam-exploded liquid, and then effectively improve the utilization of biomass.

Materials and methods

Material

Poplar chips were obtained from Shandong Sun Paper Co., Ltd. (Shandong, China), with an average size of 30 mm $\times$ 30 mm $\times$ 5 mm. The poplar chips were washed repeatedly and oven-dried at 50°C. Compositional analysis of poplar was determined by the National Renewable Energy Laboratory (NREL) procedure (Sluiter et al. 2008), the results of which showed that the contents of cellulose, hemicellulose and lignin were 44.1%, 19.1% and 27.8%, respectively.

Pretreatment of poplar

A total of 0.4 kg dry weight of poplar chips was placed into the reaction chamber (Hebi Gentle, Henan, China), and high-pressure steam was introduced to reach the reaction temperature of 209°C. After 7 min of exposure to the steam with the temperature held constant, poplar chips were sharply exploded into the collecting device, resulting in two kinds of products: steam-exploded solid residue (SES) and steam-exploded liquid (SEL).

Lignin isolation

L_p and L_s were isolated and refined by the enzymatic-mild acidic hydrolysis method (Jääskeläinen et al. 2003; Guerra et al. 2006). This isolation process could give the isolated lignin the characteristics of higher yield, higher purity and minor structural changes (Guerra et al. 2006; Wang et al. 2016). Lignin was isolated according to the procedure shown in Figure 1.

Extractives were first removed by 8-h extraction using ethanol ($\geq 99.7\%$) (Macklin, Shanghai, China). The extractive-free samples were washed repeatedly by deionized water and air-dried, followed by milling in a Pulverisette 7 planetary ball mill (Fritsch, Germany) at the speed of 20 rpm for 2 h. In the enzymatic-mild acidic hydrolysis process, cellulase and hemicellulase (Novozymes, Denmark) were first added to the milled pulp to degrade cellulose and hemicellulose. The slurry was vibrated in a DH-2102 shaker incubator (Daohan, Shanghai, China) for 48 h at 40°C, followed by centrifugation. The sediment was washed with acidic water (pH=2), and then freeze-dried to obtain crude lignin. Afterwards, under a nitrogen atmosphere, crude lignin was refluxed for 2 h in a dioxane–water solution (dioxane:water=85:15, v/v). The obtained solution was filtered, and the solid was washed repeatedly with dioxane–water solution. The filtrate was neutralized and concentrated at 40°C. Finally, the concentrated solution was added to acidic water (pH=2) in which the purified lignin could be obtained by standing, centrifuging and freeze-drying.

The separation process for L_l is relatively simple. After concentration, the pH of SEL was adjusted to 2, and then lignin precipitated, following by centrifugal separation. Finally, the L_l sample was obtained by washing repeatedly with acidic water (pH=2) and freeze drying.

Characterization of the isolated lignin samples

FTIR analysis: FTIR spectra were performed on VERTEX 70 Fourier transform spectrometer (Bruker Optics, Darmstadt, Germany). The

Figure 1: The flow chart of lignin isolating procedure.

Here, L_p is lignin from poplar, and L_s is lignin from steam-exploded solid residue, and L_l is lignin from steam-exploded liquid.

scanning range was from 4000 to 400 cm^{-1} at 4 cm^{-1} of resolution (16 scans per sample). Before scanning, transparent films were prepared with a ratio of sample to KBr 1:100 (w/w).

NMR spectroscopy: NMR spectra of lignin samples were recorded using a Bruker AVANCE III HD 600 MHz superconducting Fourier transform nuclear magnetic resonance spectrometer (Bruker, Darmstadt, Germany) at room temperature. For the collection of two-dimensional (2D) HSQC spectra, 50 mg of lignin sample was fully dissolved in 1 ml of dimethyl sulfoxide- d_6 (DMSO- d_6 , $\geq 99.8\%$) (Meryer, Shanghai, China) in an NMR tube. The spectral widths were 5000 Hz and 20 000 Hz for ^1H and ^{13}C dimensions, respectively, and pulse width was 90°. For the ^1H dimension, the number of sampling points was 1024 with a relaxation delay of 1 s. The number of scans was 64, and 256-time increments were always recorded in the ^{13}C dimension.

The ^{13}C -NMR experiment was also carried out in Bruker AVANCE III HD 600 MHz at room temperature. A total of 90 mg of lignin sample was dissolved in 0.5 ml of DMSO- d_6 . The parameters for data acquisition were as follows: 30° pulse width, 1 s relaxation delay and 30 000 scans. Data processing was performed using MestReNova software.

Molecular weight analysis: The molecular weight distributions of the L_p , L_s and L_l were analyzed using SEC on a 1260 infinity HPLC system (Agilent, Santa Clara, CA, USA) with a refractive index detector. Two columns were series-connected including PL-gel 5 μm 10³ Å

and PL-gel 3 μm Mixed-3. Approximately 10 mg of acetylated lignin sample was completely dissolved in 10 ml of tetrahydrofuran (THF $\geq 99.8\%$) (Meryer, Shanghai, China), and 10 μl of filtered solution in each run was injected. The temperature was maintained at 35°C and the flow velocity was 1.0 ml min^{-1} . The polystyrene-type standards with molecular weights in the range of 800–49 400 g mol^{-1} were used as standard compounds for molar mass calibration in SEC.

Results and discussion

FTIR analysis

The FTIR spectra of lignin samples, L_p , L_s and L_l , are shown in Figure 2, and the bands were assigned on the basis of the literature (Faix 1991; Sun et al. 2014; You et al. 2015). The most obvious difference in the FTIR spectra of the three lignin samples was that bands at 1652 cm^{-1} (conjugated carbonyl and carboxyl groups) in the FTIR spectra of L_s and L_l disappeared. These results indicated that the degradation of lignin fractions occurred

Figure 2: FTIR spectra of L_p , L_s and L_l .

Here, L_p is lignin from poplar, and L_s is lignin from steam-exploded solid residue, and L_l is lignin from steam-exploded liquid.

after steam explosion pretreatment (Sun et al. 2004). Compared with L_p , there were higher intensity peaks at 1715 cm^{-1} in FTIR spectra of L_s and L_l , which were assigned to nonconjugated carbonyl groups. This result may be due to the breakage of β -O-4' linkages to form unconjugated β -ketone (Marchessault et al. 1982; Sun et al. 2004). During the steam explosion process, organic acids were produced and generated a weak acidic conditions. Under acidic conditions, two reactions occurred, the breakage of β -O-4' linkages and condensation reaction, which would be occurred. In the former reaction, the Hibbert ketone structure would be produced together with a new carbonyl group (Li et al. 2007). In the fingerprint region, the bands at 1597 (1604), 1509 (1513), and 1419 cm^{-1} were associated with aromatic skeletal vibrations, meanwhile, the C-H stretching vibration of the aromatic ring emerged at 1462 cm^{-1} . There was no obvious change in these peaks at 1597 , 1509 , 1462 and 1419 cm^{-1} , demonstrating that the aromatic ring substructure of lignin was not destroyed during the steam explosion. The existence of peaks at 1326 cm^{-1} (S and condensed G units), 1268 cm^{-1} (G units) and 1126 (1119 cm^{-1} (typical aromatic C-H in-plane deformation vibrations for S units) indicated the features of GS-type lignin. It should be underlined that the band intensity at 1268 cm^{-1} was depressed in L_s and L_l , suggesting that there was less G type lignin content in the structure of L_s and L_l . In conclusion, although the spectra of L_p , L_s and L_l were rather analogous, the structures of lignin samples before and after the steam explosion were obviously different.

NMR spectroscopy

^{13}C -NMR spectroscopy

To better understand the changes in the lignin structure after steam explosion treatment, ^{13}C -NMR technology was adopted to analyze the structures of L_p , L_s and L_l , and the corresponding spectra are presented in Figure 3. Signal assignments were based on previous studies (Capanema et al. 2010; Yuan et al. 2011a).

In the aromatic region from 162 to 103 ppm , the S units were observed by peaks at 152.6 ppm (C_3/C_5 , etherified) and 147.3 ppm (C_3/C_5 , nonetherified), 138.6 ppm (C_4 , etherified), 135.3 and 134.7 ppm (C_1 , etherified), and 104.7 ppm (C_2/C_6). The signals of the G units emerged at 149.5 ppm (C_3 , etherified), 147.3 ppm (C_4 , etherified), 145.8 ppm (C_4 , nonetherified), 135.3 and 134.7 ppm (C_1 , etherified), 119.6 ppm (C_6), 115.7 ppm (C_5) and 111.7 ppm (C_2). Additionally, the H units had two signals at 128.2 ppm (C_2/C_6) and 120.6 ppm (C_1). The signals of the G, S and H units in the ^{13}C -NMR spectra of all lignin samples were obvious, indicating that all the backbone structures of L_p , L_s and L_l were all GSH-type, and only the signal intensities of linkages in the three units were different, which showed that steam explosion did not pronouncedly modify the "core" of the lignin structure. In addition, the signals of p-hydroxybenzoic acid could be clearly found in the aromatic region, mainly including 166.1 ppm ($\text{C}=\text{O}$), 162.5 ppm (C_4), 131.9 ppm (C_2/C_6) and 114.9 ppm (C_3/C_5), but there were relatively weak in

Figure 3: ^{13}C -NMR spectra of L_p , L_s and L_l .

Here, L_p is lignin from poplar, and L_s is lignin from steam-exploded solid residue, and L_l is lignin from steam-exploded liquid.

L_l spectra. The existence of these signals suggested that there was a certain amount of p-hydroxybenzoic acid in lignin samples before and after the steam explosion.

In the sidechain region, the peaks of β -O-4' emerged at 72.7 ppm (C_α), 85.1 ppm (C_β) and 60.3 ppm (C_γ). It was noteworthy that the intensities of these three signals in the spectra of L_s and L_l were all lower than that of L_p , which may result from the breakage of β -O-4' during the process of the steam explosion. The strong signal at 56.0 ppm was caused by $-\text{OCH}_3$ in S and G units. Meanwhile, two weak signals at 53.8 ppm (β - β) and 53.3 ppm (β -5') also emerged.

HSQC NMR spectroscopy

HSQC NMR spectroscopy can separate overlapping signal peaks in either ^1H or ^{13}C NMR spectra, thus providing more important structural information of lignin. In terms of lignin structure, the sidechain (δ_C/δ_H 50–90/2.5–6.0) and the aromatic regions (δ_C/δ_H 90–160/6.0–8.0) of the HSQC NMR spectra of L_p , L_s and L_l are presented in Figure 4. The main substructures of lignin identified, including side chain linkages and aromatic lignin units, are depicted in Figure 5. The HSQC NMR signal assignments of lignin were based on previous studies (Ralph et al. 2004; Ibarra et al. 2007; Rencoret et al. 2009, 2015; Villaverde et al. 2009; Yuan et al. 2011b; Sun et al. 2014).

In the sidechain region, the contours of methoxy groups (δ_C/δ_H 55.9/3.77) and sidechains in β -O-4'

substructures (A and A') are the most remarkable. The C_α - H_α correlations in β -O-4' substructures were observed at δ_C/δ_H 72.6/4.87 (A_α). The C_β - H_β linkages were found at 84.5/4.33 [$A_{\beta(G/H)}$] and 86.6/4.17 [$A_{\beta(S)}$] for β -O-4' substructures related to G/H and S units, respectively. The signals of C_γ - H_γ linkages in β -O-4' substructures could be detected at δ_C/δ_H 60.2/3.45–3.67 (A_γ). In addition, at δ_C/δ_H 63.8/4.37–4.51 (A'_γ), assigned to C_γ - H_γ linkages in γ -acylated β -O-4' substructures, a relatively strong signal in the sidechain of L_p could be found, but it was small in the spectrum of L_s , and even vanished in the structure of L_l . For β - β' resinol substructures (B), the peaks at δ_C/δ_H 85.6/4.70 (B_α), 54.1/3.09 (B_β), and 71.5/3.86 and 4.22 (B_γ) were assigned to C_α - H_α , C_β - H_β , and C_γ - H_γ , respectively. As for phenylcoumaran substructures (β -5', C), C_α - H_α , C_β - H_β and C_γ - H_γ linkages were detected at δ_C/δ_H 87.5/5.48 (C_α), 53.9/3.46 (C_β) and 63.3/3.75 (C_γ), respectively, where the C_γ - H_γ linkage peak overlapped with other peaks at δ_C/δ_H 62.8/3.75 (Yuan et al. 2011b). Weak signals for the spirodienone substructures (β -1', D) were identified with their C_α - H_α and C_β - H_β linkages at δ_C/δ_H 82.0/5.09 (D_α) and 60.5/2.78 (D_β), respectively, only in the sidechain of L_p . In addition, two weak signals at δ_C/δ_H 61.8/4.12 (E_γ) and 83.8/5.27 (F_β) appeared in the L_p spectrum only, which were ascribed to C_γ - H_γ linkages in the p-hydroxycinnamyl alcohol end groups (E) and C_β - H_β linkages in oxidized ($C_\alpha=O$) β -O-4' substructures (F), respectively. In brief, by comparing the spectral changes of lignin before and after the steam explosion, it can be found that the linkages of lignin have degraded to varying

Figure 4: Sidechain (left column) and aromatic regions (right column) in the HSQC NMR spectra: δ_C/δ_H 50–90/2.5–6.0 and δ_C/δ_H 100–150/5.5–8.5, respectively.

Here, L_p is lignin from poplar, and L_s is lignin from steam-exploded solid residue, and L_l is lignin from steam-exploded liquid.

degrees, and even disappeared, after the steam explosion, especially the C_γ-H_γ linkages in γ-acetylated β-O-4' substructures (A_γ), C_α-H_α linkages in phenylcoumaran substructures (C_α), C_α-H_α linkages in spirodienone substructures (D_α), C_β-H_β linkages in spirodienone substructures (D_β),

and C_γ-H_γ linkages in p-hydroxycinnamyl alcohol end groups (E_γ).

As for the aromatic regions of the lignin samples, signals of S, G and H units were found, implying that the lignin is GSH-type lignin. A remarkable signal emerging

Figure 5: The main substructures of lignin.

The main structures identified by 2D NMR spectra of L_p , L_s and L_L : (A) β -aryl-ether units with a free -OH at the γ -carbon (β -O-4'); (A') β -O-4' aryl ether linkages with acylated -OH at γ -carbon; (B) resinol substructures (β - β'); (C) phenylcoumaran substructures (β -5'); (D) spirodienone substructures (β -1'); (E) p-hydroxycinnamyl alcohol end groups; (F) oxidized ($C_\alpha=O$) β -O-4' substructures; (I) cinnamaldehyde end groups; (PB) p-hydroxybenzoate substructures; (H) p-hydroxyphenyl unit; (G) guaiacyl unit; (G') oxidized guaiacyl units with a α -ketone (S) syringyl unit; (S') oxidized syringyl units with a ketone at C_α . Here, L_p is lignin from poplar, and L_s is lignin from steam-exploded solid residue, and L_L is lignin from steam-exploded liquid.

at δ_C/δ_H 104.1/6.72 ($S_{2,6}$) was ascribed to $C_{2,6}$ - $H_{2,6}$ in S units, and meanwhile the signal of $C_{2,6}$ - $H_{2,6}$ in oxidized S units was also discovered at δ_C/δ_H 106.8/7.06 and 7.30 ($S'_{2,6}$). For G units, the signals were all strong, including C_2 - H_2 (δ_C/δ_H 111.6/6.99, G_2), C_5 - H_5 (δ_C/δ_H 115.3/6.75, G_5), and C_6 - H_6 (δ_C/δ_H 119.8/6.83, G_6). The C_2 - H_2 and C_6 - H_6 correlations in oxidized ($C=O$) G structures (G'_2 and G'_6) were discovered at δ_C/δ_H 111.4/7.51 and 118.9/6.07, respectively. Remarkably, it can be seen from Figure 4 that compared with L_p , a reduction emerged in the signal intensity of G'_2 and G'_6 in L_s and L_L , especially in L_L . In addition, a small signal

$C_{2,6}$ - $H_{2,6}$ aromatic correlation in H units emerged at δ_C/δ_H 128.2/7.20.

Moreover, there were other signals in the aromatic regions, including spirodienone substructures (D), p-hydroxycinnamyl alcohol end groups (E), cinnamaldehyde end groups (I), and p-hydroxybenzoate substructures (PB), of which a small peak for the C_2 - H_2 linkages of substructure D could be only detected in the aromatic regions of L_p at δ_C/δ_H 114.2/6.31 (D_2). Meanwhile, the contours related to the C_α - H_α and C_β - H_β linkages in substructures E could only be seen at δ_C/δ_H 129.2/6.44 (E_α) and 128.9/6.26

(E_β), respectively, in the spectrum of L_p . In addition, the contour at δ_C/δ_H 126.9/6.78 (I_β) was ascribed to cinnamaldehyde end groups. However, as to the PB substructures, the contours related to correlations of $C_{2,6}$ - $H_{2,6}$ were found at δ_C/δ_H 131.9/7.67 ($PB_{2,6}$). It can be seen from Figure 4 that there was a slight difference in $PB_{2,6}$ signals in the spectra of L_p , L_s and L_L , showing that the steam explosion had a relatively weak effect on such substances. In addition, it is noteworthy that the signal of C_7 - H_7 in the p-hydroxybenzoate substructures at δ_C/δ_H 144.7/7.56 (PB_7) was very weak in the aromatic region of L_p and meanwhile this signal was undetected even at a very low level in the aromatic region of L_s and L_L .

Quantification of lignin structures

Quantitative analysis of lignin structures is very important to understand the modification of lignin during the steam explosion process. Therefore, the different structural characters of L_p , L_s and L_L were quantified according to the method combining ^{13}C and HSQC NMR (Zhang and Gellerstedt 2007; Yuan et al. 2011a). Selecting a suitable signal as an internal standard reference was significant (Zhang and Gellerstedt 2007). The integration for the total aromatic carbons (163.0–103.6 ppm in ^{13}C NMR spectrum) was set as 600.0, so the structural units could be calculated by expressing per 100 Ar (Yuan et al. 2011b). The quantified results of lignin substructures in L_p , L_s and L_L are summarized in Table 1.

According to Table 1, the amount of β -O-4' bond was 43.2/100 Ar in L_p while this content dramatically decreased in the L_s (29.9/100 Ar) and L_L (12.9/100 Ar). All the data demonstrated that depolymerization of the lignin occurred during the steam explosion process, and as for L_L , which was obtained from the liquid product of steam explosion, the extent of this process was more remarkable, resulting in the dissolution of lignin from

solid fraction to liquid fraction during the steam explosion process. Similarly, with the steam explosion pretreatment, the amounts of β - β' and β -5' were also reduced from 10.8/100 Ar (L_p) to 8.15/100 Ar (L_s) and 4.80/100 Ar (L_L), and 7.38/100 Ar (L_p) to 7.23/100 Ar (L_s) and 3.46/100 Ar (L_L), respectively. It was noted that a low amount of β -1' in substructure D was detected in the structure of untreated L_p , whereas this substructure was not observed in structures of L_s and L_L . Interestingly, the total amounts of sidechain substructures in lignin structure dramatically decreased after steam explosion pretreatment, particularly in L_L which had dissolved in the steam-exploded liquid during the steam explosion process. It is noteworthy that there were substituents on C_α in the β -O-4', β - β' , β -5' and β -1' linkages, so the main reason for decreases in these four structures could be explained by the destructive effect of the C_α cationization on the binding bond (Wang et al. 2016). In addition, lignin is a polymer sensitive to high temperature and acidic environment, and under these conditions, depolymerization and repolymerization occur, as shown in Figure 6 (Li et al. 2007).

In addition to the decrease in the amount of sidechain substructures, the increase in the S/G ratio was another important structural modification after steam explosion pretreatment (Table 1). As shown, the S/G ratios of L_p , L_s , and L_L were 1.14, 1.70, and 1.86, respectively. The reason for the increased S/G ratio was mainly due to the faster demethoxylation reaction in G units than in S units during the steam explosion (Wen et al. 2013; Sun et al. 2014).

Based on these results, it can be concluded that modification of lignin structure occurred during the steam explosion at high temperature under acidic conditions, but the "core" did not change pronouncedly, and remained GSH-type.

Molecular weight analysis

In the steam explosion process, depolymerization and repolymerization of lignin occurred, as shown in Figure 6, which may affect the molecular weight of lignin (Li et al. 2007).

SEC was preferentially applied to investigate the molecular weights of L_p , L_s and L_L , and the results are presented in Figure 7 and Table 2. As for L_s , a clear shift of the main peak towards a shorter retention time in the elution pattern curve was found, which indicated an increase in molecular weight. However, there was a shift of the main peak towards a longer retention time in the elution pattern curve of L_L , indicating a decrease in molecular weight.

Table 1: Quantificational results of the lignin by the combination of ^{13}C and HSQC NMR spectra (results expressed per 100 Ar).

Samples	L_p	L_s	L_L
β -Aryl-ether units (β -O-4', A)	43.2	29.9	12.9
Resinol (β - β' , B)	10.8	8.15	4.80
Phenylcoumaran (β -5', C)	7.38	7.23	3.46
Spirodienones (β -1', D)	1.21	ND	ND
S/G ratio ^a	1.14	1.70	1.86

^aS/G ratio obtained by the equation: S/G ratio = $0.5IS_{2,6}/IG_{2,6}$. ND, Not detected or not allowing an acceptable quantification.

Figure 6: Depolymerization of β -O-4' linkages and repolymerization of lignin fragments.

Figure 7: Size exclusion chromatography (SEC) of L_p , L_s and L_l . Here, L_p is lignin from poplar, and L_s is lignin from steam-exploded solid residue, and L_l is lignin from steam-exploded liquid.

Table 2: Average molecular weight and polydispersity of L_p , L_s , and L_l .

Samples	L_p	L_s	L_l
M_w (g mol ⁻¹)	2860	6250	2140
M_n (g mol ⁻¹)	1980	4090	1110
M_w/M_n	1.44	1.53	1.92

The weight-average (M_w), number-average (M_n) molecular weights, and polydispersity (M_w/M_n) of the lignin samples before and after steam explosion pretreatment are presented in Table 2. As shown, the M_w and M_n of L_p were 2856 g mol⁻¹ and 1978 g mol⁻¹, respectively, lower than that of L_s , extracted from the solid product of steam explosion.

This was possibly correlated with the predominance of repolymerization between lignin molecules in solid residue during the steam explosion process. Steam explosion pretreatment can also depolymerize lignin and disrupt cell walls, resulting in dissolved lignin (L_l) in the steam explosion liquid. As for L_l , the M_w and M_n were 2138 g mol⁻¹ and 1113 g mol⁻¹, respectively, lower than that of L_p . Moreover, the polydispersity of lignin increased from 1.44 (L_p) to 1.53 (L_s) and 1.92 (L_l), with increases of 6.25% and 33.3%, respectively.

Conclusion

During the steam explosion process, depolymerization and repolymerization took place in the lignin, which influenced the structure and average molecular weight of lignin, but the “core” of the lignin structure was not pronouncedly modified and remained GSH-type lignin. The SEC analysis provided evidence that the repolymerization was dominant in the reaction of lignin molecules in solid residue during the steam explosion process, while for lignin molecules in liquid, depolymerization dominated. ¹³C-NMR and 2D-HSQC NMR spectra illustrated that steam explosion pretreatment can lead to a reduction of the amounts of sidechain substructures in lignin structure and an increase in the ratio of S/G.

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