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Hydrothermal Degradation of Enzymatic Hydrolysis Lignin in Water-Isopropyl Alcohol Co-Solvent

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The effect of hydrothermal conditions on enzymatic hydrolysis lignin (EHL) degradation in water-isopropyl alcohol co-solvent and optimal conditions were investigated. The yields and reactivity toward formaldehyde of degraded enzymatic hydrolysis lignin (DEL) were determined. The optimal conditions of temperature, time, and ratio of solids to liquids were 250 °C, 60 min, and 1:10 (w/v), respectively. The EHL and DEL were characterized by gel permeation chromatography (GPC), Fourier transform infrared spectroscopy (FT-IR), ^{1}H nuclear magnetic resonance (^{1}H NMR), thermal gravity (TG), and differential scanning calorimetry (DSC) analyses. The results revealed that the molecular weight and polydispersity of DEL were lower than that of EHL. Although the fundamental structure of lignin before and after hydrothermal degradation was retained, the ether (β-O-4, α-O-4, *etc.*) content decreased, while that of hydroxyl (phenolic and aliphatic) increased. The DTG_{max} and T_g values shifted from 334 and 117 °C to 304 and 105 °C, respectively.

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INTRODUCTION

Lignin is the second most abundant biomass resource in nature, after cellulose. It widely exists in the cell walls of plants. It is a polymer with a complicated, amorphous, and three-dimensional net-like structure, primarily composed of three alcohol units, coniferyl, p-coumaryl, and sinapyl (Tejado *et al*. 2007). Furthermore, these units are randomly assembled with ether bonds (β-O-4, α-O-4, and 4-O-5) as well as C-C bonds (Yáñez-S *et al*. 2014). In fact, lignin is a renewable aromatic compound, so its degradation is a potential method for providing phenolic compounds. Because of its natural phenolic structure, it can be used to partially replace phenol for synthesizing lignin-phenol-formaldehyde (PF) resin (Jin *et al*. 2010; Moubarik *et al*. 2013; Zhang *et al*. 2013).

However, both the high molecular weight and low reactivity of lignin limit its applications at the industrial level because they result in low substitution rates, which are crucial in replacing phenol for synthesizing PF resin. Hence, many methods have been adopted to activate lignin, such as hydroxymethylation (Yu *et al*. 2012), demethylation (Ibrahim *et al*. 2011), phenolation (Du *et al*. 2014), sulfonation (Ouyang *et al*. 2009), and degradation, including hydrothermal (Kang *et al*. 2013), thermal (Brebu and Vasile 2010), and catalytic degradation (Zhang *et al*. 2014), as well as biodegradation (Chen *et al*. 2012). Among them, hydrothermal degradation plays a key role in lignin degradation because water and alcohol as environmentally friendly co-solvents are usually applied during the hydrothermal process. As can be seen from previous studies (Ye *et al*. 2012), in which gas

chromatography–mass spectrometry (GC–MS) equipment was used, the reaction conditions have a significant influence on the degradation of enzymatic hydrolysis lignin. The molecular weight decline and the formation of phenolic compounds indicate that ether bonds in lignin can be easily degraded under hydrothermal conditions (Yong and Matsumura 2012).

Previous studies have indicated that water-ethanol co-solvent can be widely applied in hydrothermal degradation and biomass liquidation. On the one hand, because of its lower dielectric constant (ε) when compared with that of pure water, this co-solvent could dissolve relatively high-molecular weight products (Cheng *et al*. 2010). On the other hand, because of its lower critical temperatures (T_c) and pressures (P_c), it can easily achieve a critical state and promote yield (Wu *et al*. 2014). By comparison of two parameters, including dielectric constants and critical values, isopropyl alcohol ($\varepsilon = 19.92$, $T_c = 508.2$ K, $P_c = 4.8$ MPa) may have better or more suitable properties for this reaction than ethanol ($\varepsilon = 24.30$, $T_c = 513.8$ K, $P_c = 6.1$ MPa). Liquefaction of biomass in water-isopropyl alcohol co-solvent has been reported in recent years (Yuan *et al*. 2007), but the hydrothermal degradation of lignin in water-isopropyl alcohol co-solvent has rarely been investigated.

In this work, enzymatic hydrolysis lignin (EHL) was degraded in isopropyl alcohol-water (50/50, v/v) co-solvent under various hydrothermal conditions. From the degradative lignin yield and reactivity toward formaldehyde, the optimal hydrothermal conditions were obtained. To reveal information concerning the lignin groups, Fourier transform infrared spectroscopy (FT-IR) and ^{1}H nuclear magnetic resonance (^{1}H NMR) characterizations were performed. The molecular weight and polydispersity were obtained by gel permeation chromatography (GPC) analysis, and the thermodynamic properties of lignin were investigated using thermal gravity (TG) and differential scanning calorimetry (DSC) analyses.

EXPERIMENTAL

Materials

Bio-ethanol production residue (ER) was obtained from the fermentation of cornstalks. The cornstalks were harvested from Zhaodong County, Heilongjiang Province, China. After air drying, the cornstalks were cut into 5 cm to 10 cm sections and then treated by stream explosion at 1.7 MPa and 205 °C for 6 min. In the following fermentation process, the cellulose of the pretreated cornstalks was used as the raw material for conversion into bio-ethanol using the SSF (simultaneous saccharification and fermentation) method (Eckard *et al*. 2013). Additionally, EHL was purified from ER using an alkali-solution and acid-isolation method (Guo *et al*. 2013); all chemicals used were analytical-grade reagents.

Pretreatment of ER

EHL was obtained by pretreatment of ER *via* the alkali-solution and acid-isolation method, whereby 20 g of ER was dissolved in 200 mL of 1 M sodium hydroxide solution; this mixture was thoroughly stirred. Then, the undissolved solid residue was separated from the solution by centrifugation at 8000 rpm for 10 min. The resulting solution was collected and acidified to a pH value of 2 using diluted hydrochloric acid solution, after which lignin was precipitated out. Then, it was collected by centrifugation and washed carefully with distilled water until the pH value of the solution was greater than 4. The product was dried

in a vacuum oven at 0.1 MPa and 50 °C for 12 h to remove water, and finally the EHL product was purified from ER.

Hydrothermal Degradation of EHL

All the lignin degradation experimental tests were carried out in a 50-mL hydrothermal reactor at a temperature range from 150 to 300 °C. To prepare a uniform mixture, a set proportion (1:5, 1:10 and 1:15, w/v in g/mL) of lignin and water-isopropyl alcohol (1:1, v/v) co-solvent was mixed and sonicated for 20 min. This was transferred into a hydrothermal reactor, securely sealed, and kept at the desired temperature for a set amount of time. The reactor was rapidly cooled down to room temperature using a water bath after the hydrothermal process finished. The product in the reactor was all collected and rinsed with acetone and methanol, and the mixture was filtered using a Buchner funnel under vacuum. The organic solvents (isopropyl alcohol, acetone, and methanol) in the filtrates were recovered with an evaporator under vacuum at 40 °C, and water was further removed at 60 °C. The remaining product was further dried in a vacuum oven at 0.1 MPa and 50 °C for 12 h, denoted the obtained solid residue (SR), and then dried at 105 °C overnight. DEL yield rate and residue rate were calculated with Eqs. 1 and 2, respectively. The loss was calculated with Eq. 3.

$$\text{DEL yield rate (\%)} = \frac{m_{DEL}}{m_{EHL}} \times 100 \tag{1}$$

$$\text{Residue rate (\%)} = \frac{m_{residue}}{m_{EHL}} \times 100 \tag{2}$$

$$\text{Loss (\%)} = (1 - \text{DEL yield rate} - \text{Residue rate}) \times 100 \tag{3}$$

where m_{DEL}, m_{EHL}, and $m_{residue}$ are the masses of the DEL, EHL and residue samples, respectively.

Reactivity towards Formaldehyde (Formaldehyde Value)

Exactly 1.00 g of lignin and 10 mL of 0.5 M sodium hydroxide solution were painstakingly mixed in a test tube with a rubber plug, and then 1.00 mL of 37% formaldehyde solution was loaded. The test tube was sealed and kept at 80 °C with continuous shaking for 2 h (Vázquez *et al*. 1997). After this step, the solution was used to determine the free formaldehyde content using the hydroxylamine hydrochloride method in accordance with ISO 11402-2004 (2004). The reactive formaldehyde content was the difference between the initial and free formaldehyde contents.

Characterization of Lignin

GPC analysis

To enhance the solubility of lignin in organic solvents for gel permeation chromatography (GPC) analysis and ^{1}H NMR characterization, samples were acetylated prior to the analysis. The acetylation process was carried out according to the following procedures: lignin was dissolved in a pyridine/acetic anhydride (1:2, v/v) mixture and acetylated in the dark at room temperature for 72 h. The resulting solution was added dropwise with stirring into diethyl ether to precipitate the acetylated lignin. This product was collected by centrifugation and vacuum-dried at 50 °C for 48 h. Just before GPC

analysis, the acetylated products were dissolved in tetrahydrofuran (THF) at a concentration of 1 mg/mL. This analysis was conducted on a JASCO LC-1500 apparatus (Jasco, Japan) with a UV detector. The monitoring wavenumber was set at 280 nm, while the THF-eluted flow rate was 1 mL/min; using polystyrene as a standard substance, a standard curve was drawn (Tejado *et al*. 2007).

FT-IR analysis
The FT-IR spectra of the lignin samples were collected using a Thermo Fisher Scientific Nicolet 6700 FT-IR instrument (USA) in the attenuated total reflection (ATR) mode over a wavenumber range from 500 to 4000 cm^{-1} and with a resolution of 4 cm^{-1}.

^{1}H NMR analysis
Before the ^{1}H NMR analysis was performed, the lignin samples were also acetylated to improve their solubility in dimethyl sulfoxide-d$_6$ (DMSO-d$_6$). The ^{1}H NMR analysis of acetylated lignin samples was conducted using a Bruker 400 MHz spectrometer (Bruker, Germany) at a frequency of 100 MHz with an acquisition time of 0.001 s.

Thermal analysis
The thermal behaviors of the lignin samples were determined using a NETZSCH STA 409 TGA (Germany) and a TA Q20 DSC instrument (USA) in which the flow rate of N$_2$ was 50 mL/min. For the TGA analysis, the temperature was increased from room temperature to 800 °C at a heating rate of 10 °C/min; for DSC analysis, the temperature was increased from 40 to 250 °C at the same heating rate.

RESULTS AND DISCUSSION

Optimization of Degradation Conditions
The impacts of various temperatures, times, and ratios of solid to liquid on the yield are illustrated in Table 1. Because this study aimed as much as possible at synthesizing a partial phenol replacement for PF resin, the reactivity of the product with formaldehyde was also used to discuss the effects.

Table 1. Effect of Hydrothermal Conditions on Yield and Reactivity toward Formaldehyde of DEL

No.	Temperature (°C)	Time (min)	Solid to liquid ratio	Yield (%)	Residue rate (%)	Loss (%)	Formaldehyde value (g/g)
1	150	60	1:10	50.76	44.22	5.02	0.2168
2	200	60	1:10	43.91	43.33	12.76	0.2371
3	250	60	1:10	64.38	24.57	11.43	0.2992
4	300	60	1:10	8.32	39.32	52.36	0.2110
5	250	30	1:10	52.84	41.12	6.04	0.2880
6	250	90	1:10	46.12	40.80	13.08	0.3170
7	250	120	1:10	42.63	42.22	15.15	0.2090
8	250	60	1:5	30.13	68.75	1.12	0.2634
9	250	60	1:15	39.80	43.35	16.85	0.2774

Effect of degradation temperature

Temperature is a vital factor for lignin degradation. The yield rate and reactivity value toward formaldehyde of DEL formed at 150, 200, 250, and 300 °C for 60 min with a set solid to liquid ratio (w/v=1:10) are shown in Table 1. When temperature increased from 150 to 250 °C, the yield rate and reactivity toward formaldehyde of DEL increased from 50.76% and 0.2168g/g to 64.38% and 0.2992 g/g, respectively. The yield rates of DEL increased with temperature until reaching a maximum at 250 °C. Various studies (Yuan *et al*. 2010; Cheng *et al*. 2012) of lignin have shown that the β-O-4 bond is readily ruptured at temperatures ranging from 200 to 300 °C (degradative reaction of lignin in various solvents). However, when the temperature was increased from 250 to 300 °C, the yield rate and reactivity toward formaldehyde of DEL considerably reduced, and residue rate largely increased. This indicated a possible repolymerization reaction between intermediates and oligomer products formed by C–C linkages or cross-linking with the side chains of the oligomer products (Yuan *et al*. 2010). Therefore, the optimized temperature for lignin degradation was 250 °C.

Effect of degradation time

The lignin degradation experiments were conducted at 250 °C, for 30 to 120 min, at a solid to liquid ratio of 1:10 (w/v). The yield rates of DEL and its formaldehyde values are illustrated in Table 1. When the degradation time was prolonged from 30 to 60 min, the yield rates of DEL increased from 52.84% to 64.38% and its reactivity toward formaldehyde also increased. However, when the degradation time was prolonged to 90 min, the reactivity toward formaldehyde reached a maximum (0.317 g/g), but the yield rate decreased considerably. When the reaction time was 120 min, both yield and reactivity toward formaldehyde noticeably decreased. This means that the degradation, decomposition, and condensation of lignin occurred simultaneously. The decomposition reaction was dominant at the initial time, while the condensation reaction dominated at longer residence times (Ye *et al*. 2012). As a consequence, the yield of solid residue increased continuously with increasing reaction time; thus, a reaction time of 60 min would be desirable for EHL degradation at 250 °C.

Effect of solid to liquid ratio

Using these results, lignin degradation experiments were conducted at 250 °C for 60 min with solid to liquid ratios (w/v) of 1:5, 1:10, or 1:15. The yield rates and reactivity toward formaldehyde of DEL are illustrated in Table 1. Undoubtedly, when the ratio of solid to liquid was changed from 1:5 to 1:10, the yield rate and reactivity toward formaldehyde of DEL were boosted. The alcohol-water co-solvent played an important role in the yield and composition of lignin. In fact, lignin and its derivative products (low molecular weight) showed good dissolution in the alcohol–water co-solvent, which can remarkably inhibit their repolymerization (Yuan *et al*. 2010; Ye *et al*. 2012). When the ratio of solid to liquid was 1:15, the yield rate and reactivity toward formaldehyde decreased. Previous research (Cheng *et al*. 2010) suggests that a higher solvent/solid ratio negatively affects the degradation process when using hot compressed ethanol-water co-solvent. This is likely due to the markedly enhanced formation of aqueous soluble products, which include low-molecular weight degradation products.

Thus, the EHL was degraded under optimal conditions as follows: a temperature of 250 °C, degradation time of 60 min, and solid to liquid ratio of 1:10 (w/v). The degraded EHL under the above conditions was shorter as DEL in the following text.

GPC Analysis

Figure 1 and Table 2 show the molar mass distribution of lignin before and after the hydrothermal degradation. Compared with the EHL molar mass curve, that of DEL slightly shifted toward the low-molar mass region, which indicated that EHL was degraded. Also, from Table 2 the M_n, M_w, and polydispersity values also varied noticeably; this supports the results as well. However, lignin with a lower molar mass possesses higher reactivity and thus can replace more phenol in lignin-PF resin production.
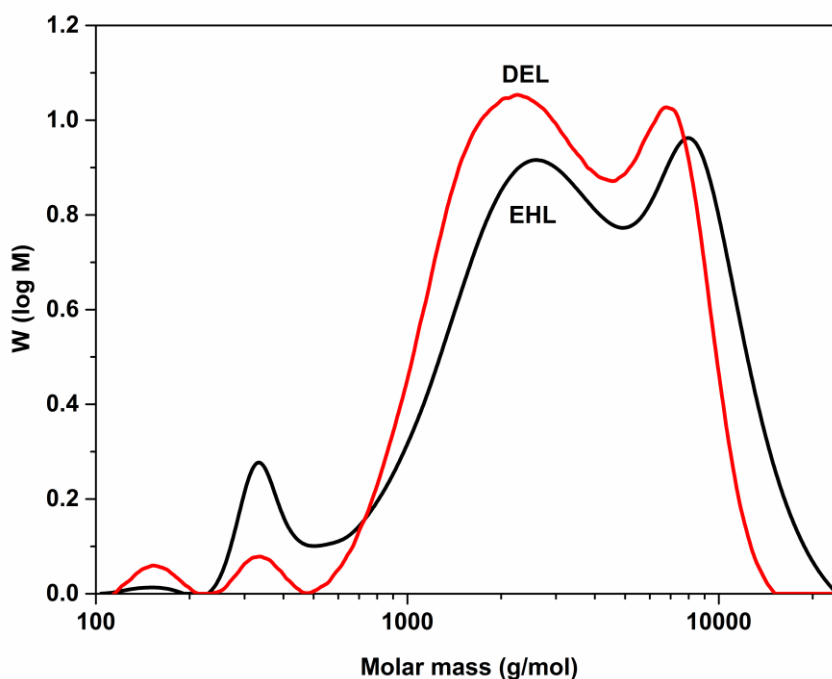

Fig. 1. GPC elution curves for EHL and DEL

Table 2. Molecular Weights and polydispersity of Lignin

Samples	M_n (g/mol)	M_w (g/mol)	Polydispersity
EHL	2716	5070	1.87
DEL	2430	3942	1.62

FT-IR Analysis

The FT-IR spectra of EHL and the depolymerized product (DEL) formed under the optimized conditions are shown in Fig. 2. The peaks at approximately 3300 cm^{-1} are a result of the stretching vibration of aromatic and aliphatic O-H. The peaks at 2932 cm^{-1} can be attributed to asymmetric and symmetric vibrations of methyl or methylene groups, respectively, and the one at 2843 cm^{-1} can be ascribed to the C-H vibration of CH$_3$O-. The band assignments are given in Table 3 (Tejado *et al*. 2007). As can be seen in Fig. 2, the spectra of EHL and DEL showed some similarities in peak positions, which confirms that hydrothermal degradation of EHL did not break the fundamental structure of lignin groups. However, the peak at 1030 cm^{-1} decreased greatly. Considering the GPC analysis result, some ether linkages might have been cleaved during the degradation process. Further study with ^{1}H NMR would be needed to confirm this.

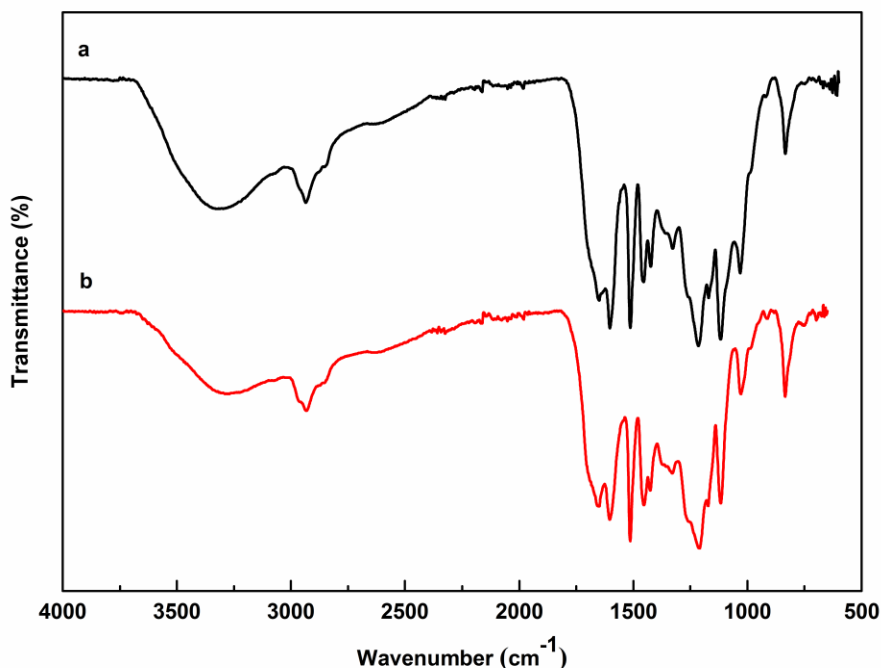**Fig. 2.** FT-IR spectra of lignin: (a) EHL; (b) DEL

Table 3. FT-IR Band Assignment for Lignin

Band (cm^{-1})	Vibration	Assignment
1650	st C=O	Conjugated C=O
1600	st C-C	Aromatic skeleton
1515~1505	st C-C	Aromatic skeleton
1460	δ C-H	Asymmetric in –CH$_3$ and –CH$_2$
1425	st C-C	Aromatic skeleton
1366	δ$_{ip}$ O-H	Phenolic OH
1328	st C-O	Syringyl (S)
1270~1267	st C-O	Guaiacyl(G)
1219	st C-O(H)+C-O(Ar)	Phenolic OH and ether
1115	δ$_{ip}$ Ar C-H	Syringyl (S)
1030	st C-O(H)+C-O(C)	First order aliphatic OH and ether
830	δ$_{op}$ Ar C-H	Ar
st : stretching vibration δ$_{ip}$: in-plane deformation vibration δ$_{op}$: out-of-plane deformation vibration.		

^{1}H NMR Analysis

The ^{1}H NMR spectra of lignin are illustrated in Fig. 3, and the attributions and integral areas of the peaks are listed in Table 4 (Moubarik *et al*. 2013). The signals at $\delta =$ 2.50 and 3.30 ppm are mostly from the solvent DMSO-d$_6$ and water. The chemical shifts of the DEL peak are similar to that of EHL and are in agreement with the results from FT-IR analysis. Although the fundamental structure of lignin was retained after hydrothermal degradation, the content of functional groups appeared to be noticeably different.
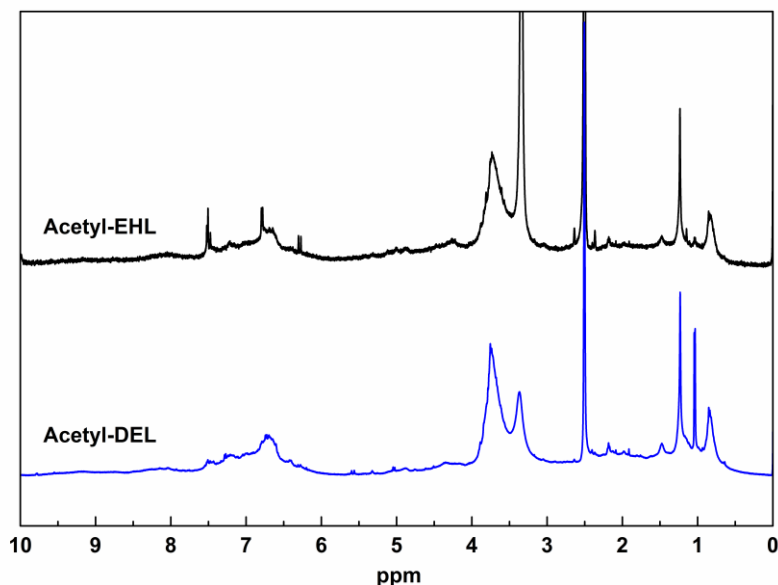
Fig. 3. ^{1}H NMR spectra of lignin

Table 4. Assignment of Signals in the ^{1}H NMR Spectra of Acetylated Lignin Samples

δ (ppm)	Assignment	Integral areas	
		Acetyl-EHL	Acetyl-DEL
8.00-6.00	Aromatic protons	1.00	1.00
6.00-4.00	H in β-O-4, β-β, β-1 and β-5 structures	0.67	0.40
4.00-3.50	Protons in methoxyl groups	0.86	0.82
2.40-1.60	Aliphatic and Aromatic acetate	0.36	0.46
< 1.60	H in methyl and methylene	0.58	0.72
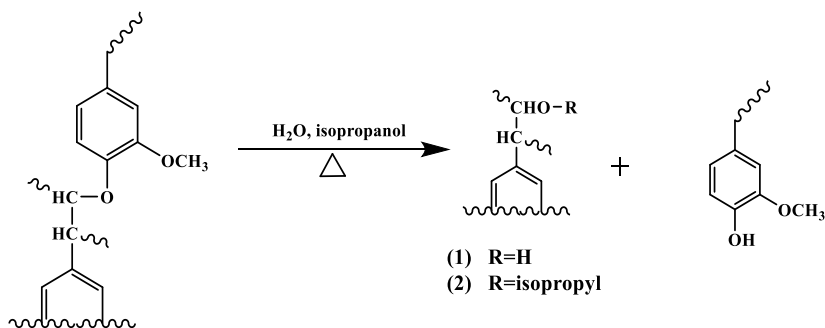
Scheme 1. Hydrothermal degradation mechanism for lignin in water-isopropyl alcohol co-solvent

Whereas the integral area of DEL signals in β-O-4, β-β, β-1, and β-5 structures (Hauteville *et al*. 1986; Lundquist and Stern 1989) showed a decrease, the hydroxyl groups, including phenolic and aliphatic groups, presented an increase. This suggests that the β-O-4, β-β, β-1, and β-5 structures of EHL were ruptured during hydrothermal degradation treatment, while phenolic and aliphatic hydroxyl groups were generated (Zhou 2014). This

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is consistent with the results of GPC and FT-IR analyses, and the possible hydrothermal degradation mechanisms for lignin in water-isopropyl alcohol co-solvent are proposed in Scheme 1. The increase of the integral area of DEL signal in methyl and methylene established that Compound 2 was produced.

Thermal Behavior

TG analysis

      The TG and DTG curves of lignin are shown in Fig. 4. The maximum degradation temperature (DTG_{max}) and glass transition temperature (T_g) are given in Table 5. The TG curves reveals the weight loss of substances in relation to the temperature of thermal degradation, while the first derivative of that curve (DTG) presents the rate of weight loss at the same time.
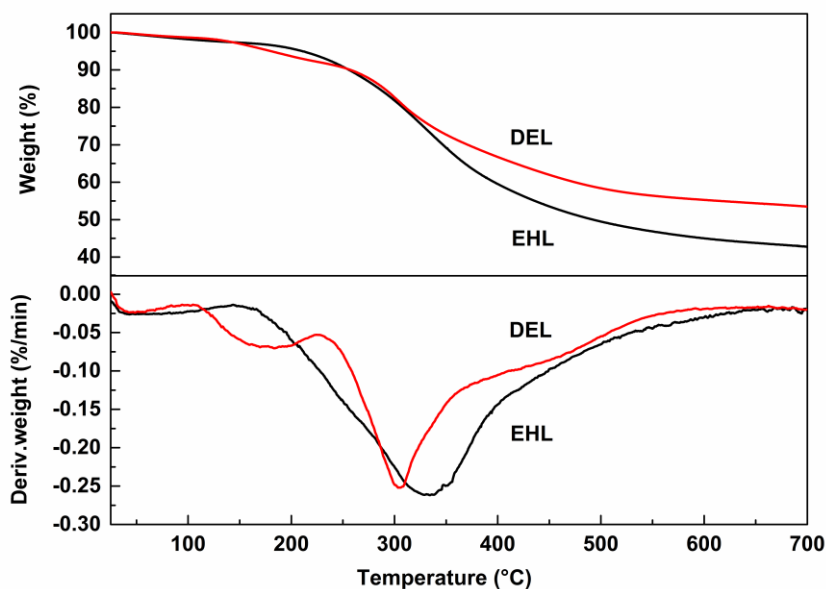

Fig. 4. TG and DTG curves of EHL and DEL

Table 5. T_g, DTG_{max}, and Non-Volatile Residues for EHL and DEL

Sample	DTG_{max} (°C)	Residue (%)	T_g (°C)
EHL	334	43	117
DEL	304	52	105

      According to previous studies (Liu *et al.* 2008), with respect to the TG/DTG graphs, the curves can be divided into three main regions. The first weight loss stage occurs at 25 to 120 °C because of the evaporation of absorbed water and dehydration reactions. The second one occurs at 120 to 330 °C and can be attributed to the pyrolysis of the carbohydrate components of the lignin, which were translated into volatile gases containing CO, CO_2, and H_2O. Additionally, phenols were the most essential volatiles in this stage. The final stage occurs at temperatures above 350 °C and can be attributed to the pyrolysis of ether, methoxyl, and methylene groups, which all were converted into volatile products, including CO, CO_2, CH_3OH, and CH_4. CO is primarily a gaseous product at a wide range of temperatures. Above 500 °C, the process is related to the pyrolysis of some aromatic

rings (Tejado *et al*. 2007). The DTG$_{max}$ of DEL was distinctly lower than that of EHL, and this was related to the molar mass of lignin as well as the different contents of phenols and ether linkages.

DSC analysis

In addition to TG analysis, DSC provides a supplementary way to study the properties of thermal transitions; it is a widely used method to determine the glass transition temperature (T_g) of amorphous polymers. Figure 5 shows the DSC scans of EHL and DEL, and the T_g data are listed in Table 5.

The DSC thermogram of EHL depicts an endothermic peak at 117 °C, while that of DEL is evident at 105 °C, and they both display the melting process of lignin (Murugan *et al*. 2008). The T_g value of DEL was visibly lower than that of EHL because of the cleavage of ester bonds and β-aryl-ether linkages (β-O-4 linkages) during hydrothermal degradation treatment, as well as its lower molecular weight. This is in agreement with the results of TG analysis and a recent study (Shao *et al*. 2009).
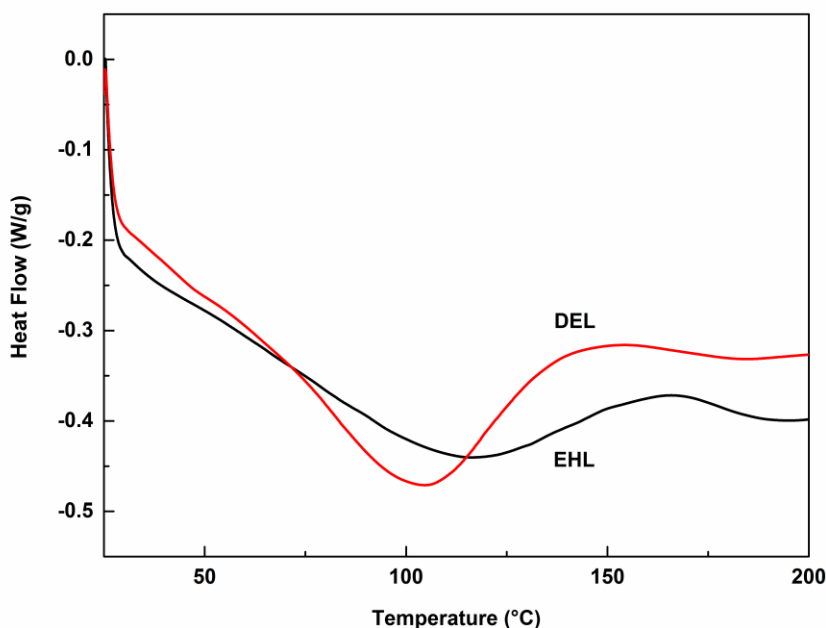

Fig. 5. DSC curves of lignin samples (EHL and DEL)

CONCLUSIONS

1. EHL was degraded in a water-isopropyl alcohol co-solvent under various degradation conditions. According to the yield and reactivity toward formaldehyde of degradative EHL, the optimal degradation conditions were defined as 250 °C, 60 min, and 1:10 (w/v), respectively. While the yield of the product at optimal conditions was 64.38%, the reactivity toward formaldehyde was 0.2992 g/g.

2. The M_n, M_w, and polydispersity of DEL decreased from 2716 g/mol, 5070 g/mol, and 1.87 to 2430 g/mol, 3942 g/mol and 1.62, respectively.

3. The FT-IR results indicated no substantial variation in the fundamental structure of lignin. Although ^{1}H NMR analyses revealed that the ether bonds of EHL (β-O-4, α-O-

4, *etc*.) were broken during the degradation process, the contents of phenolic and aliphatic hydroxyl groups increased.

4. The results of TG and DSC analyses implied that DEL had a lower T_g value than EHL; thus, DEL is more suitable for PF resin preparation.

ACKNOWLEDGEMENTS

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