# GLASS TRANSITION OF OXYGEN PLASMA TREATED ENZYMATIC HYDROLYSIS LIGNIN

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This study investigated the effect of oxygen plasma treatment on the glass transition temperature of enzymatic hydrolysis lignin (EHL) derived from the production of bio-ethanol. Differential scanning calorimetry (DSC) was used to obtain the glass transition temperature ($T_g$) of EHL. The results showed that the $T_g$ value of EHL under different heating rates ranged from 160 to 200 °C, and there was a strong linear correlation between heating rate and $T_g$. The $T_g$ value of oxygen plasma treated EHL decreased when compared with the untreated samples. The apparent $T_g$ of the untreated sample was 168.2 °C, while the value of the treated sample was 161.5 °C. Distinct chain scission and introduction of oxygen-based functional groups on the surface of EHL were detected by XPS analysis. These changes may occur mainly on the bulky side chain and thus enhance molecular mobility of EHL. This indicates that oxygen plasma treatment can modify the structure and improve the reactivity of EHL efficiently.

*Keywords: Enzymatic hydrolysis lignin (EHL); Oxygen plasma treatment; Glass transition temperature; Differential scanning calorimetry (DSC)*

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## INTRODUCTION

Bio-ethanol, as a form of renewable energy that can be produced from lignocellulosic materials, has recently received considerable attention with increasing environmental and energy concerns. Both cellulose and hemicellulose in lignocellulosic materials can be converted to simple sugars by enzymatic hydrolysis. The hydrolysis products can be subsequently fermented to ethanol, while lignin is a barrier to enzymatic saccharification of carbohydrates. Therefore, enzymatic hydrolysis lignin (EHL) is considered as a waste product in cellulosic ethanol processes. It is expected that a future lignocellulosic ethanol industry will generate large quantities of EHL (Zhu and Pan 2010). Value-added utilization of EHL can help offset the cost of bio-ethanol production, boost the economic viability of the bio-ethanol industry, and also provide a source of renewable materials.

Lignin is a highly branched polymer which contains a variety of functional groups; it is capable of undergoing a large number of modification reactions. Tremendous efforts have been made during the last 50 years to develop high-value lignin products (Lora and Glasser 2002; Kumar *et al.* 2009). Since both hydroxyl and aldehyde groups are abundant in lignin, it has been used as a natural binder for making bio-composite products (Anglès *et al.* 2001; Velásquez *et al.* 2003; Dam *et al.* 2004; Zhou *et al.* 2011). Compared with formaldehyde-based resins, its environmental advantage could lead to a bright future for manufacturing bio-composites with lignin as a natural binder. However, bulky substituents on the aromatic ring, such as methoxy, diminish the available positions for polymerization and lower the reactivity of lignin. Therefore, ways to improve the reactivity of lignin has been the focus of attention.

Various physical (ultrasonic processing and thermal treatment), chemical (acid or alkali treatment), and biological (enzymatic processes) methods, and their combinations for lignin modification have been reported (Ren and Fang 2005; Crestini *et al.* 2006; Qiu and Chen 2008; Brosse *et al.* 2010). A low temperature plasma treatment has been widely used for modifying polymeric materials over the past decades. Desired outcomes such as enhanced wettability, superior adhesion characteristics, and improved chemical reactivity can be achieved by plasma treatment. Few studies have investigated modifying lignin by plasma treatments (Toriz *et al.* 2004; Sahin 2009; Titova *et al.* 2010; Klarhoefer *et al.* 2010). It is believed that plasma treatment is an effective method to modify the structure of lignin and implant reactive functional groups to lignin.

The objective of this work was to investigate the effect of oxygen plasma treatment on the grass transition temperature of EHL using differential scanning calorimetry (DSC), so that the sufficient data could be applied to optimize pressing parameters in the manufacture of bio-composites with EHL as a natural binder. To better understand the effects of plasma treatment on the thermo characteristic of EHL, the chemical changes of treated EHL were also evaluated using X-ray photoelectron spectroscopy (XPS).

## EXPERIMENTAL

### Materials

EHL was extracted from corn stover residues, which was derived from the production of bio-ethanol in the pilot plant in Hei Longjiang Province, China, according to Liu and Cheng (2007) with 3-wt% NaOH solution. The reaction was conducted at 65 °C with gentle stirring for 1h, and the solution was kept at this temperature for an additional half an hour. Then the mixture was filtered, and the alkaline filtrate and wash water were collected and acidified to pH 3 by the dropwise addition of 2-wt% dilute $H_2SO_4$ solution. The acidified mixtures were then heated in a water bath at 70 °C for 30 minutes under continuous stirring. The precipitated lignin fractions were separated from the mixtures and centrifuged, then washed with water until neutral. The final products were dried in a vacuum oven at 60 °C for 24 h and screened to an average diameter of 20 μm. Typical physical and chemical characteristics of EHL are: free-flowing brown powder; solid content 96.5%; moisture content (MC) 3%; elemental content C 57.3%, H 5.9%, O 34.1%; OMe content 9.2%; and ash content 6.7%.

## Oxygen Plasma Treatment of EHL

Plasma treatments of EHL were carried out in a plasma reactor (HD-1B, made in Jiangsu, P.R. China), as shown in Fig. 1, with a radio frequency (RF) of 13.56 MHz. Powdery lignin was dispersed in a glass container to maximize surface exposure of the samples for uniform treatments. The system was evacuated to a base pressure of 0.1 to 0.3 mTorr, and then oxygen was fed directly into the chamber. Such a cycle was repeated five times to remove volatile contaminants. By operating the gas feeding system valves, a pressure of 1.3 to 1.5 mTorr and a steady-state flow rate were maintained. A RF magnetron sputtering unit was used to produce oxygen plasma. The input power was set at 200 W and sustained for a period of 3 min. At the end of the reaction, the chamber was pressurized, and the samples were removed and stored under dry conditions for later analysis.

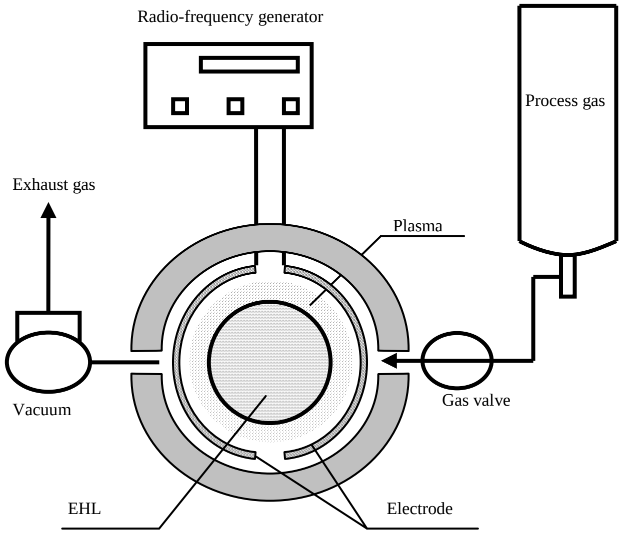


**Fig. 1.** The schematic diagram of RF plasma reactor

## DSC Measurement

A differential scanning calorimeter (Mettler toledo DSC823e, Switzerland) with high purity nitrogen (flow rate 10 mL/min) as the carrier gas was used to evaluate the thermokinetic behaviors of the EHL. Sample mass was *ca*. 12 mg. In order to eliminate the thermal history of the sample, two step scans were conducted. Firstly, the samples were heated in DSC from 25 $^{o}$C to 100 $^{o}$C, held at this temperature for 5 min, and cooled down to 25 $^{o}$C naturally. At the second scan, the samples were heated from 25 $^{o}$C to 250 $^{o}$C at five different heating rates (5 $^{o}$C/min, 10 $^{o}$C /min, 15 $^{o}$C/min, 20 $^{o}$C/min, and 25 $^{o}$C/min). Because the thermal history of the samples was removed during the first heating run, the 2nd heating run was used for analysis of glass transition temperature ($T_g$). To verify the $T_g$ values, the untreated samples were annealed at a glass transition temperature region for 4 h to investigate the enthalpy relaxation of EHL. For each group, five replicates of untreated and treated EHL samples were scanned.

### XPS Analysis

X-ray photoelectron (XPS) spectroscopy was conducted to investigate the changes of elemental distributions and surface functionality of EHL after oxygen plasma treatment. Measurements were performed on a K-α using an Al K*α* (1480 eV) X-ray source operated at 100 W under a vacuum of $5\times10^{-7}$ mbar. To average out the heterogeneity of the sample, survey scans and high-resolution regional spectra were recorded from at least three measurement points in each sample.

## RESULTS AND DISCUSSION

### Glass Transition Temperature of EHL

Figure 2 shows a DSC thermogram of the untreated EHL sample. As a typical amorphous polymer, EHL has a distinct stage of glass transition starting around 150 $^{o}$C. The $T_g$ of untreated EHL samples, indicated by an arrow as the medial point of the step change, was observed around 170 $^{o}$C at the heating rate of 10 $^{o}$C/min. The glass transition is a characteristic of the viscoelastic behavior of amorphous polymers. At temperatures below the transition, lignin is stiff and brittle. When lignin is thermally processed in the transition region, the stiffness of lignin decreases, and it exhibits rubber-like elasticity as a result of chain entanglements. If the material is not cross-linked, and provided that thermal degradation does not occur, further increase in temperature will eventually result in rubbery flow as the entanglements begin to slip. If cross-linking is present, such flow cannot occur (Irvine 1985). With regard to lignin, both dehydration and non-reversible reactions will take place within the lignin matrix with further increase in temperature above its glass transition. The non-reversible reactions might be caused by the formation of new ether-type linkages between phenylpropane units of lignin (Guigo *et al.* 2009). If lignin is used as a natural binder for bio-composites, a higher hot-pressing temperature in the composites is necessary in order to reach temperatures above the glass transition point of lignin to cure and bond the fibers. It has been shown that improved properties of fiberboards with EHL as a natural binder were obtained when the hot-pressing temperature was above the $T_g$ value of EHL (Zhou *et al.* 2011). Lower $T_g$ values of EHL samples imply that the temperature during hot-pressing can be decreased and it can also be optimized based on the $T_g$ value.

Glass transition temperature of various kinds of lignin has been investigated by different techniques over the past few decades (Hatakeyama and Hatakeyama 2010). The $T_g$ values are found in a wide temperature range depending on plant species, isolation methods, and post treatments (Hatakeyama *et al.* 1982; Glasser and Jain 1993; Jain and Glasser 1993; Hatakeyama and Quinn 1999; Laborie *et al.* 2004; Li and Sarkanen 2005). As shown in Fig. 2, the $T_g$ value ranged from 160 to 200 $^{o}$C at the different heating rates. These values were much higher than that of industrial hydrolysis lignin from 75 to 90 $^{o}$C, as reported by Hatakeyama *et al*. (2010), based on EHL obtained as a by-product from the production of bio-ethanol (Hatakeyama *et al.* 2010). This is probably due to the different plant species and the different hydrolysis conditions. Moreover, the molecular motion of isolated lignin was confirmed at a temperature higher than that of lignin *in situ* by the above result (Salmén 1984; Hatakeyama 1992), which was expected as molecular weight is related glass transition temperature (Blanchard 1974).

Since amorphous polymers show enthalpy relaxation when glassification slowly takes place, EHL samples were annealed at 165 °C for 4 h, cooled slowly, and then heated at the rate of 10 °C/min. By annealing, enthalpy relaxation is identified as an endothermic shoulder peak in DSC curves (Hatakeyama *et al.* 2010). The enthalpy relaxation of EHL is visible as indicated by an arrow in Fig. 2. It indicates that lignin is a typical amorphous polymer having a broad spectrum of molecular higher order structure which coaggregates by annealing (Hatakeyama *et al.* 2010).

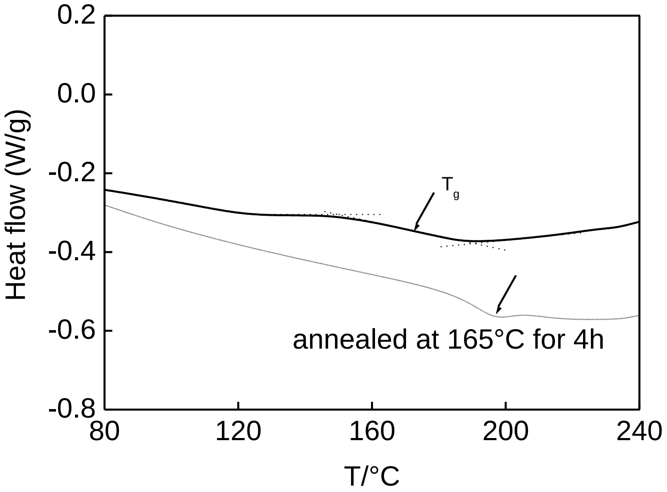
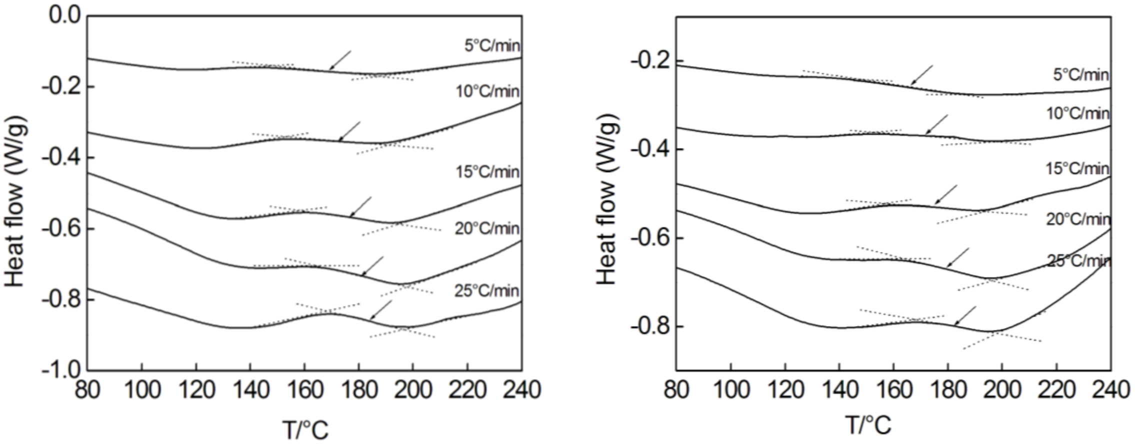


**Fig. 2.** DSC heating curve of EHL and enthalpy relaxation of EHL annealed at 165 °C for 4h

## Effect of Oxygen Plasma Treatment on Glass Transition Temperature of EHL

Figure 3 presents the DSC thermograms of one group of untreated and oxygen plasma treated EHL samples under different heating rates. It can be seen that there was a clear step change being detected and the size of the step change increased with increasing heating rate for all samples. The $T_g$ values, indicated by an arrow, increased with increasing heating rate for all samples and ranged from 160 to 200 °C. Because of the lag in heat conduction and sample relaxation, the characteristic temperature ($T$) of thermal properties was delayed due to the high heating rate. Theoretically, if all experimental conditions are constant, such as the heat conduction of the pan and sample thickness, the heating rate can only change the kinetics of the thermal behavior. In that case, the relationship between $T$ and heating rate should be linear (Liu *et al.* 2009). The $T_g$ was plotted as a function of heating rate in Fig. 4 based on the measured data from Fig. 3. It shows a strong linear correlation between heating rate and $T_g$ for both untreated and treated samples. The intercept was assumed as an apparent $T_g$ of samples, which represents $T_g$ when the heating rate is extremely slow and approaching 0 °C/min (Park and Wang 2005; Liu *et al.* 2009). The apparent $T_g$ of the untreated sample was 168.2 °C, while the value of the treated one was 161.5 °C. From Fig. 4, it is obvious that $T_g$ values of oxygen plasma treated samples were lower than that of untreated samples at the different heating time. Molecular motion of lignin is characterized by phenylpropane units in the molecular chain; bulky side chain and slight cross-linking establish intra- and intermolecular chains. Rigid groups in the main chain and cross-linking restrict molecular motion and have the effect of increasing the glass transition temperature. In contrast, bulky side chains enhance molecular mobility of lignin through the local mode relaxation (Hatakeyama *et al.* 2010). The above results indicate that the bulky side chains or cross-linking of EHL might be changed; even the ring might be opened during oxygen plasma treatment, since the $T_g$ values of treated samples decreased. Therefore, to better understand the effects of plasma treatment on the thermokinetic behavior of EHL, X-ray photoelectron (XPS) analysis of EHL samples was conducted.



(a) Untreated samples (b) Treated samples

**Fig. 3.** DSC curves of untreated and oxygen plasma treated EHL at different heating rates

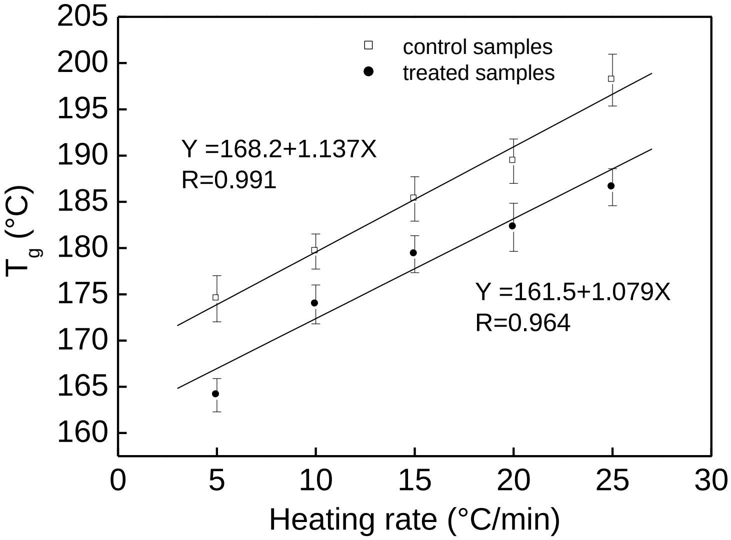
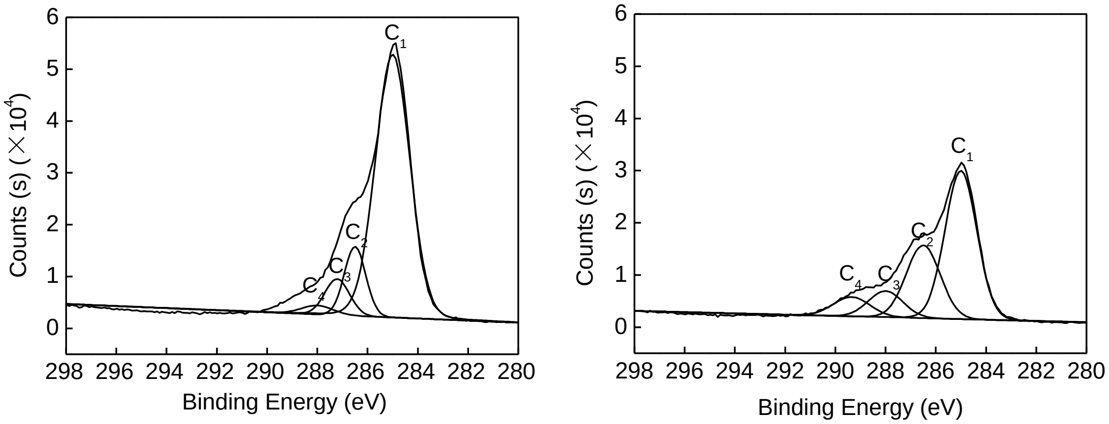


**Fig. 4.** The glass transition temperature ($T_g$) of samples as a function of heating rate

## Effect of Oxygen Plasma Treatment on Chemical Change of EHL

From the low-resolution XPS spectra results, the O/C atomic ratio was found to be 0.25 of the untreated samples and was increased notably to 0.40 after oxygen plasma treatment. Moreover, the deconvoluted peak areas of the untreated and treated EHL surfaces in the C1s spectra were significantly different as well (Fig. 5). The peak areas of oxygen-related functional groups concerning the peaks $C_2$ (corresponds to carbon atoms linked to single oxygen), $C_3$ (carbon atoms linked two non-carbonyl oxygen, or to single carbonyl oxygen), and $C_4$ (carbon atoms bonded to carbonyl and non-carbonyl oxygen) of the treated samples were increased by 126, 37, and 246%, respectively, compared with the untreated samples. On the other hand, a considerable decrease in $C_1$, which corresponds to carbon atoms only linked to carbon and/or hydrogen, such as C−C and C−H groups of plasma-treated lignin, was identified. This indicates that a great number of oxygen-related functional groups are attached to the surface of EHL after treatment (Dorris and Gray 1978a,b). During the oxygen plasma treatment, oxygen-based polar species with high energy (0 to 20 eV) as so-called oxygen plasma will be generated, such as oxygen free radicals and atoms, ionized oxygen molecules, ozone, and a number of other oxygen metastable states, while the bond energies of the atoms of organic structures are variable and less than the energy range of such species (Denes *et al.* 2005). Therefore, plasma can lead to polymer chain scission and promote the formation of free radicals and ions. As a result, fragmentation, isomerization, cross-linking, and the introduction of functional groups to the surface of polymer materials occur (Li *et al.* 2007). Considering the lignin structure, it has C-H, C-C, C=C, C-O, and C=O functionalities with bond energies less than 20eV (Lide 1995). Hence, oxygen plasma is intense enough to dissociate almost all chemical bonds involved in recovering lignin structures and to create free radical species. Meanwhile, oxygen-based polar species react with these free radical species by the generation of hydroxyl, carbonyl, and carboxyl groups on the surface of EHL. The XPS analysis results imply that the chain scission and the introduction of functional groups occur mainly on the bulky side chain. These changes enhance molecular mobility of EHL, so that the $T_g$ values decrease after oxygen plasma treatment.



(a) Untreated samples (b) Treated samples

**Fig. 5.** The high-resolution C1s XPS spectra of untreated and oxygen plasma treated EHL

## CONCLUSIONS

The $T_g$ values of isolated enzymatic hydrolysis lignin (EHL) under different heating rates ranged from 160 to 200 $^oC$. They were much higher than that of industrial hydrolysis lignin, which was also obtained as a by-product from the production of bio-ethanol.

The glass transition temperature ($T_g$) of EHL was decreased after being treated by oxygen plasma due to efficiently modified chemical structure of EHL. Distinct chain scission and introduction of oxygen-based functional groups were detected by XPS analysis. These changes may occur mainly on the bulky side chain and thus enhance molecular mobility of EHL.

Lower $T_g$ values of oxygen plasma treated EHL samples imply that the temperature of hot-pressing can be decreased and also be optimized based on the $T_g$ value so that good cross-linking between EHL and lignocellulosic fibers will probably be formed when EHL is used as an natural thermosetting binder.

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