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# LIQUEFACTION AND CHARACTERIZATION OF ENZYMATIC HYDROLYSIS LIGNIN WITH PHOSPHOTUNGSTIC ACID

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Enzymatic hydrolysis lignin (EHL) derived from bio-ethanol production is attracting increased attention for use as a substitute for petroleum-based phenol in phenolic resins. In this work, EHL was successfully liquefied into small phenolic compounds with phosphotungstic acid (PTA) as a catalyst in the presence of phenol. In addition, liquefied EHL was substituted for phenol to formulate a novolac bio-based phenolic resin (EHL-PF). The differences between EHL and liquefied EHL were studied by Fourier transform infrared (FT-IR) spectroscopy, gel permeation chromatography (GPC) and gas chromatography-mass spectrometry (GC-MS). Compared to the existing methods, liquefaction with the new catalyst provides a lower-molecular-weight product.

***Keywords***: enzymatic hydrolysis lignin, phosphotungstic acid, liquefaction, phenolic compounds

## INTRODUCTION

Lignocellulose biomass is the most abundant renewable resource available on Earth for chemical utilization. It is estimated that the annual plant growth in the world can produce up to 150 billion tons of lignin.[1] Enzymatic hydrolysis lignin (EHL) has a greater advantage over other types of industrial lignin. The extraction process from the wood fiber resources to the EHL is carried out under relatively mild conditions, so it is better to retain functional groups, such as phenolic hydroxyl groups.[2] It was considered to have the largest potential to substitute for petroleum-based phenol in the phenolic resin industry, considering the increasing concerns regarding the shortage of fossil resources and the environmental impact of petroleum-based products.[3] Fully utilizing EHL is a feasible method of reducing the current high bio-ethanol production costs and to help promote its large-scale development.

However, lignin is less reactive because of its large molecular structure, weight and size.[4] In recent decades, many efforts have been made to improve the reactivity of lignin.[5] Generally, the usage of lignin in phenolic resin is conducted by phenolation under acid conditions, including inorganic strong acids and organic weak acids, such as hydrochloric, sulfuric, oxalic and phosphoric acids.[6,7] However, strong acids cause serious corrosion of equipment, while the liquefaction effect of weak acids is inferior. Phosphotungstic acid (PTA), a type of solid heteropoly acid, is an excellent catalyst. It has been used for oxidative degradation of lignin and oxygen delignification of lignocellulosics.[8,9] Extensive research has demonstrated that PTA, due to its strong surface acidity, stable structure and thermal stability, can be used as an acid catalyst to overcome the above-mentioned defects of liquid acids.[10]

In the present work, EHL was liquefied using PTA in phenol and a novolak phenolic resin was synthesized by polymerization of liquefied EHL with formaldehyde. The effects of the structure of EHL were analyzed by GPC, FT-IR spectroscopy and GC-MS. The effects of the residue rate, combined phenol and molecular weight on PF and EHL-PF were investigated.

## EXPERIMENTAL

### Materials

EHL ($M_w$3107, $M_n$1288), from Shandong Longli Biotechnology Co. Ltd., was dried in an oven at 50 ºC for 24 h and then kept in a desiccator at room temperature until it was used. Phenol, PTA and formaldehyde (37% solution) were of technical grade and were purchased from Aladdin.

### Preparation and application of liquefied EHL

Liquefaction experiments were conducted at the same replacement percentages of phenol by EHL (wt/wt) 55%. A 100-mL four-neck flask provided with a thermocouple, mechanical stirrer and condenser was charged with 20 g of melted phenol and a calculated amount of PTA (2% of phenol). The system was heated to 100 ºC until the catalyst was totally dissolved. A measured amount of EHL powder was slowly added to the system, accompanied by continuous stirring. After adding EHL, the system temperature rose to 120 ºC. After 1.5 h, the reactor was cooled to room temperature.

The use of liquefied EHL as phenolic feedstock for phenolic resin synthesis was the main objective of this study. At a fixed molar ratio of phenol to formaldehyde of 1:0.85, the calculated amount of formaldehyde was poured into the above system. The mixture was heated slowly to 85-90 ºC, and then, the heater was turned off after boiling. Subsequently, the system temperature increased due to the exothermic reaction; the temperature was maintained before the reaction was complete. Then, the product was dehydrated in vacuum to remove residual volatiles.

### Characterization of liquefied fractions

The FT-IR spectra of lignin samples were obtained using a Nicolet iS10 IR spectrometer (Nicolet Co., USA) within the wavenumber range of 400 to 4000 $cm^{-1}$ and a resolution of 4 $cm^{-1}$.

GPC was performed at room temperature. Pre- and post-liquefaction EHL were dissolved in THF (0.5%, wt/wt) to determine $M_n$ and $M_w$ using a Waters 1550-2410 system with the following equipment: 1550 HPLC Pump, 2410 Refractive Index Detector, and Column Styragel HR1 and HR2. THF was used as the mobile phase at a flow rate of 1 mL/min, and the injection volume was 1 μL. The calibration curve was made using polystyrene standards at different molecular weights.

GC-MS (Shimadazu GC-2010 with Agilent Technologies 7890A GC system) measurements, with a HP-5MS column (30 m × 0.05 mm × 0.32 nm) and hydrogen flame ionization detector, were used to analyze the components of the liquefied fractions.

## RESULTS AND DISCUSSION

### Spectroscopy characterization

The spectra obtained by the FT-IR technique for the EHL and EHL liquefied products are shown in Figure 1. EHL was characterized by the presence of the bands at 1600-1700 $cm^{-1}$ (C=O vibration), 1450 $cm^{-1}$ (C=C vibration of aromatic ring), 1420 $cm^{-1}$ and 1330 $cm^{-1}$, 1250 $cm^{-1}$ (C-O vibration from aliphatic groups and methylol), 1123 $cm^{-1}$ (guaiacyl unit), 817 $cm^{-1}$ (phenol ortho-replacement) and 610 $cm^{-1}$ (C-H deformation outside the plane of the aromatic ring).[11,12] It was observed that the IR spectra of the EHL liquefied products were similar to that of EHL, implying the structural similarity of EHL. Nevertheless, compared with EHL, the peaks at 1600-1700 $cm^{-1}$ of the EHL liquefied products were stronger because there was degradation in the lignin molecules that led it to an increase. In addition, due to the enhancement of the peak at 750 $cm^{-1}$, we have reason to believe that phenol could react with EHL during the liquefying process.

The breaking and formation of chemical bonds of EHL in the liquefaction process, to some extent, reduced the molecular weight of EHL and reduced the reaction space steric hindrance, which made the lignin react more easily than other materials. The reactivity of lignin would greatly improve after reacting lignin with phenol because the phenolic hydroxyl group has high reactivity.

### Chromatography characterization

The weighted average molecular weight (Mw), the number average molecular weight (Mn), and the polydispersity index (Mw/Mn) for EHL, the liquefied EHL with oxalic acid[13] and PTA were listed in Table 1. The oxalic acid liquefied sample had an average molecular weight lower than that of the original simple and there was slightly lower polydispersity. Compared with the oxalic acid liquefied products, the PTA liquefied products had a lower average molecular weight, but had a higher molecular weight polydispersity. These results indicated that EHL liquefied with PTA as a catalyst had greater reactivity than with oxalic acid as a catalyst. In the liquefaction process, phenol was not only the medium of liquefaction, but also prevented the re-polymerization of the small liquefied fractions *via* combination with it. These results were also proved in the analysis of

CP.

## Reaction with formaldehyde

Under alkaline conditions, the ortho-position of EHL phenolic hydroxyl groups can react with formaldehyde, and the hydrogen in the ortho-position is methylated. Therefore, the reactivity of EHL can be judged by this reaction. Free formaldehyde was determined in accordance with GB/T 14074-2006.

EHL and its liquefied products were in reaction with formaldehyde, the consumption of formaldehyde during this reaction is shown in Figure 2. After liquefaction degradation, EHL and formaldehyde reactivity greatly improved. The consumption rate of formaldehyde is basically the same, the consumption being faster within the first 2 h, and slowing down after that. The amount of formaldehyde consumed by EHL after liquefaction degradation is 0.40 mol/100 g, which is close to twice that of the raw material before degradation. It can be noted that, after liquefaction, EHL can react with formaldehyde at a significantly increased rate, due to an increased number of active sites, which greatly improves the utilization of EHL.

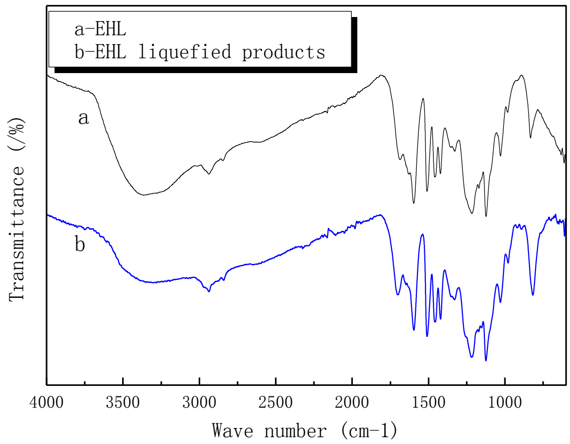

Figure 1: FTIR spectra of (a) EHL and (b) EHL liquefied products

Table 1
Mw, Mn and Mw/Mn of EHL and its liquefied products analysed by GPC

| Sample | $M_n$ | $M_w$ | $M_w/M_n$ |
|---|---|---|---|
| EHL before liquefaction | 1288 | 3107 | 2.41 |
| Oxalic acid liquefied | 655 | 1448 | 2.21 |
| PTA liquefied | 371 | 1261 | 3.40 |

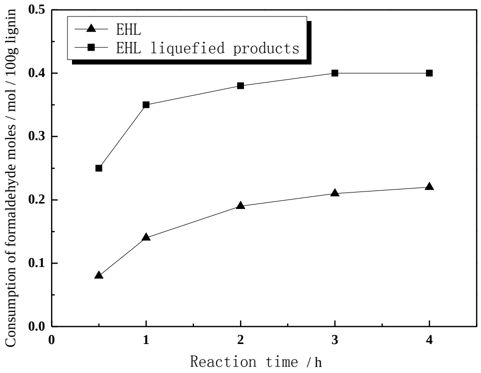

Figure 2: Formaldehyde consumption of EHL and liquefied products

Table 2
Main peaks of corresponding compounds detected by GC-MS

| Order | Appearance time (min) | Peak area (%) | Reference |
|---|---|---|---|
| 1 | 7.567 | 58.15 | Phenol |
| 2 | 7.762 | 12.55 | Phenol |
| 3 | 7.807 | 17.62 | Phenol |
| 4 | 13.100 | 0.19 | 2-Methoxy-4-vinylphenol |
| 5 | 21.986 | 0.64 | 4,4'-Ethylidenediphenol |
| 6 | 22.816 | 1.16 | 4,4'-Ethylidenediphenol |
| 7 | 23.045 | 0.10 | 4,4'-(2,2-Dichloroethylidene)diphenol |
| 8 | 23.514 | 0.66 | 3,3-Dimethyl-5-phenyl-4-phenylethynyl-phenol |

Table 3
Properties of PF and EHL-PF

|  | Free phenol (wt%) | Softening point (ºC) | Gelation time (s) | Flowing distance (cm) |
|---|---|---|---|---|
| PF | 3.15 | 91.5 | 77 | 6.8 |
| EHL-PF | 1.77 | 124.6 | 92 | 1.3 |

Table 2 provides a summary of the GC-MS results of the products that can be dissolved in methanol after degradation with PTA as a catalyst. The data are expressed as the percent yield of each compound from the total compounds identified. As observed, there were different types of small molecules of phenolic compounds, except phenol, which proved that the previous evaluation was correct. This likely results from the fact that the presence of PTA could cause protonation of the benzyl hydroxyl group and dehydration at the α-carbon to yield carbonium ions.

## EHL-PF characterization

The properties of PF and EHL-PF are presented in Table 3. Compared with the properties of the petroleum-based phenol novolac phenolic resin, the softening point and gelation time in EHL-PF of 124.6 °C and 92 s, respectively, were higher than those in PF, 91.5 °C and 77 s, respectively. This difference is due to the more rigid benzene-ring content of EHL-PF, which also leads to a shorter flowing distance, almost one-fifth for 55% LPF *versus* PF. It should be noted that free phenol (1.77%) in 55% LPF was much lower than its counterpart (3.15%) in the PF, which is beneficial for environmental protection and molding compound preparation.

## CONCLUSION

Characterization of pre- and post-liquefaction EHL has been performed, and the results were related to their molecular structures and reaction mechanisms. FT-IR spectroscopy revealed that phenol could react with EHL during the liquefying process and that the basic structure of the EHL was constant. GPC and GC-MS showed that EHL can be liquefied into small fractions in the presence of phenol, as catalyzed by PTA. The properties of EHL-PF showed that EHL can compete with synthetic modifiers for more traditional applications. We can conclude that PTA is a new type of catalyst, which is efficient and also helps protect the environment.

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