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Chemical reactivity of alkali lignin modified with laccase

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ABSTRACT

The modification of alkali lignin with laccase was investigated. The structural change of lignin was analyzed. The sulfonation reactivity was measured by the content of sulfonic group. The results showed the sulfonation reactivity increased to some extent under the condition of atmosphere pressure, but decreased under the condition of 0.3 MPa oxygen pressure. The analysis of Fourier transform infrared spectroscopy (FTIR), nuclear magnetic resonance (NMR) and gel permeation chromatography (GPC) showed the cleavage of various ether linkages and demethylation took place in the structure of lignin to certain extent during modification with laccase, which contributed to the improvement of sulfonation reactivity. Under the condition of 0.3 MPa oxygen pressure, the ratio of *s/g* (guaiacyl/syringyl) increased after modification, which reduced the sulfonation reactivity of lignin. Simultaneously partial polymerization reaction, such as 4-O-5′, *β*-5, 5-5 and other reaction in the aromatic ring decreased the activity sites of C_2, C_5 and C_6. Abundant polymerization reaction of *α*-O increased steric hindrance of C_2 and C_6 in aromatic ring, resulting in low sulfonation reactivity of lignin.

1. Introduction

Lignin is a natural polymer found in biomass and one of the most abundant biomacromolecules, second only to cellulose in natural abundance [1]. Its structure is composed of three different types of phenolic precursor units (e.g. *p*-coumaryl-, coniferyl- and sinapyl-alcohols), which linked by ether and carbon–carbon bonds formed an irregular network biopolymer [2,3]. At present, lignin mostly comes from pulp and paper industry, where it usually serves as a fuel or for the recovery of inorganic cooking chemicals [4]. Only a limited amount is isolated from the spent pulping liquors and used in various specialty products such as biomaterials, fuels, biocides and biostabilisers, animal feed, health products and crops cultivations [5]. However, in many pulp mills, which do not install the recovery system, the black liquor is simply treated or discarded. In this case, the valuable chemical properties and functionality of lignin are not utilized. Thereby, the utilization of this renewable natural product, lignin, could have economic and environmental benefits [4].

Alkali lignin is one kind of technical lignin, coming from pulp and paper making industry. Its insolubility is the disadvantage limiting its applications. Thereby, alkali lignin must be modified before application. However the high-value-added application of lignin depended entirely on the structures of monolignols, the functional group in the aromatic ring and various linkages in lignin. The main chemical functional groups in lignin are the hydroxyl(phenolic or alcoholic),

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methoxyl, carbonyl and carboxylic groups. The abundant interunit linkages in all lignin are the β-aryl ether (β-O-4), 4-O-5(diaryl ether), 5-5′ (biphenyl), β-5 (phenylcoumaran), β-β, and β-1 structures [6]. The proportion of these groups and linkages depend on the genetic origin and the isolation processes employed [7]. During the violent isolation of alkali lignin, its various linkages may be broken or polymerized, which usually results in the decrease of chemical reactivity and hindering the further chemical modification [8]. Due to these reasons, the lignin must be activated before modification.

The current activation methods of alkali lignin could be either chemical or biological. The chemical methods including demethylation [9], hydroxymethylation [10,11], oxidation [12] and so on, have already used in the industry [11], but the biological one is still at the research stage. Compared to the chemical ones, the biological method has the advantage of mild reaction conditions and less chemical reagents, which could be the promising direction in future.

In this study, the wheat straw alkali lignin (WAL) was treated with laccase, then the treated wheat straw alkali lignin (TWAL) was modified by sulfonation reaction in order to increase its solubility and surface activity. The chemical reactivity of WAL was evaluated by the content of sulfonic group. Its structural change was also analyzed before and after treatment with laccase to understand the mechanisms of modification.

2. Material and method

2.1. Modification of lignin with laccase

Crude laccase was supplied by Shanghai Denykem Co., Ltd, which was kept at the low temperature of 4 °C. The alkali lignin was supplied by Quanlin Paper Co., Ltd. in Shandong province, China, which was separated from wheat straw pulping black liquor using acid precipitation. The wheat straw was collected around Quanlin Paper Mill in Shandong region. The lignin purification process was as follows. Firstly, the lignin was dissolved in alkali solution, and then the impurity was separated from lignin solution by centrifugation. At last, the lignin solution was adjusted to pH 5.0 with hydrochloric acid for further experiment. The experiment was conducted in a 500 mL reaction vessel, which was agitated at 2.5 Hz under the temperature of 60 °C. After the treatment, the lignin was washed and freezingly desiccated. 0503, 0506, 05012 and 0524 represent the lignin samples treated for 3 h, 6 h, 12 h and 24 h under atmosphere pressure respectively; whilst O-0503, O-0506 and O-0509 represent those treated for 3 h, 6 h and 9 h under 0.3 MPa oxygen pressure respectively. The dosage of crude laccase for all samples is a mass fraction of 5% on the lignin.

About 25 mg of dry lignin was put in a 1:1 mixture of acetic anhydride/pyridine (2.00 mL) and stirred at room temperature for 24 h. Ethanol (25.0 mL) was added to the reaction mixture, leaving it for 30 min, and then removed by a rotary evaporator. The addition and removal of ethanol was repeated until all traces of acetic acid were removed from the sample. The residue was dissolved in chloroform (2.0 mL) and precipitated with diethyl ether (100.0 mL). The precipitate was centrifuged, washed with diethyl ether (3×), and dried under vacuum prior to the NMR analysis.

2.2. Sulfonation of lignin

10 g of lignin was dissolved in 100 mL of NaOH (concentration of 25 kg m^{-3}), and pH is 10.7. 0.5 g formaldehyde was added to the solution and reacted at 70 °C for 1.0 h. Then the temperature was raised to 90 °C; Added 2.5 g Na_2SO_3 to the solution and let them react for 2.5 h.

2.3. Sulfonic group content measurements

The potentiometric titration method was used to measure the sulfonic group content of the samples with an automatic potentiometric titrator (809 Titrando, Metrohm Corp.). Before titration, the lignosulfonate sample was ion-exchanged through anion exchange resin and cation exchange resin to remove the low molecule organic acid, inorganic salt and other impurities. The Sulfonic group content was calculated as follows:

$$\text{Sulfonic group content} = \frac{\text{Mole of sulfonic group}}{\text{Mass of dry lignin}}\left(\text{mo lg}^{-1}\right)$$

2.4. Fourier transform infrared spectroscopy (FTIR) analysis

FTIR spectra were recorded between 4000 and 400 cm^{-1}, using a Nexus spectrometer (Thermo Nicolet, Madison state, USA). Discs were prepared by firstly mixing 2 mg of dried sample with 200 mg of KBr (for spectroscopy) in an agate mortar. The resulted mixture was successively pressed at 10 MPa for 3 min. The spectra were normalized by Omnic software.

2.5. Liquid-state NMR analysis

The NMR spectra were recorded on a Bruker DRX-400 spectrometer using DMSO-d^6 as the solvent. Chemical shifts were referenced to TMS (0.0 ppm). The ^{13}C NMR spectra were recorded at 100.59 MHz using 5 mm-diameter tubes with the following parameters: 90° pulse angle; 12 s relaxation delay and 18,000 scans. The acetylated lignin was resolved in DMSO-d^6.

2.6. Molecular weight distribution analysis

The molecular weight distribution of lignin was determined by using aqueous gel permeation chromatography (GPC) which consisted of Waters 1515 Isocratic HPLP pump, Waters 2487 UV Absorbance Detector (Waters Corp., USA) and Ultrahydragel 120 and Ultrahydragel 250 columns. The mobile phase was 0.10 mol L^{-1} $NaNO_3$ solution with pH 10.7 and ran at a flow rate of 0.50 mL min^{-1}. The polystyrene sulfonate was used as the standard substance. Samples were filtrated by a 0.22 μm filter and analyzed in duplicate.

3. Results and discussion

3.1. Sulfonation reactivity of lignin

The scheme of sulfomethylation was shown as Fig. 1. The sulfonation reactivity of lignin was described by the content of sulfonic group. Table 1 showed the reactivity of modified