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Letter

Structural changes of lignin in the jute fiber treated by laccase and mediator system

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ABSTRACT

To study the structural changes of lignin in the jute fiber treated with laccase and mediator system (LMS), lignins from the control and LMS-treated jute fiber were isolated and characterized by gel permeation chromatography (GPC), elemental analysis, measurement of phenolic hydroxyl group content, FTIR and ^{1}H NMR. The results showed that the molecular weights of the lignin from LMS-treated jute fiber were lower than those of the lignin from the control jute fiber. The contents of phenolic hydroxyl group, aliphatic hydroxyl group and methoxy group of the lignin from LMS-treated jute fiber decreased, while the content of carboxyl group increased.

1. Introduction

The jute fiber lignin is composed of p-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) units with a H/G/S composition of 2:32:66 and a S/G ratio of 2.1 [1]. The lignin content in jute fiber is up to ca. 16%, which resulted in the coarseness and rigidity of the fiber. Therefore, jute fibers are mainly used to make the low-grade goods such as package fabrics and bags [2,3]. The removal of lignin from jute fiber is proved to be a key step in the manufacturing of high-value textile products. In the traditional process, the lignins in jute fibers are eliminated mainly in degumming using some chemical products, which often cause severe environmental pollution. In order to overcome the disadvantages of chemical degumming, enzymatic degumming has been attracted a great deal of attention.

Laccases (benzenediol:oxygen oxidoreductases, EC 1.10.3.2) are a widespread group of multi-copper enzymes [4]. It has been reported by many researchers that laccase can degrade or polymerize the phenolic compounds in lignin. Furthermore, when laccase is used in the presence of a mediator, such as 2,2′-azinobis(3-ethylbenzthiazoline-6-sulphonate) (ABTS) [5], 1-hydroxybenzotriazole (HOBT) [6] and 2,2′,6,6′-tetramethylpiperidine-*N*-oxyl (TEMPO) [7], it can further degrade the nonphenolic subunits of lignin. The mediators have high redox potential values and can produce radicals which transfer electrons from lignin to the enzyme, which finally reduces oxygen to water [8–10]. The use of a laccase–mediator system (LMS) is one of the promising possibilities as environmentally benign processes for pulp biobleaching [11,12], enzymatic pulping [13] and old newspaper deinking [14] because of its ability to delignify.

Bio-degumming refers to the enzymatic removal of the non-cellulosic matters such as waxes, pectins and lignins from the surface of bast fiber, which endows the fiber with better hydrophilicity in favor of subsequent processes. Laccase is also a promising enzyme for the degumming of bast fibers to remove lignins because of its ability of delignification. Ren et al. reported that the lignin content of linen fibers treated by laccase was decreased from 4.4% to 2.3% [15]. Liu et al. investigated the degumming of jute fibers with laccase and pectinase [16]. They found that the complex enzyme showed better removing effect for lignin. In our previous work, the degumming of linen/cotton fabric with pectinase, cellulose, xylanase and laccase were investigated [17]. The results showed that laccase treatment was the best way to remove lignins from linen/cotton fabric, but it still had a big gap compared to the traditional process. These results indicated that degumming of bast fibers with laccase is a feasible method.

Nevertheless, the mechanism of lignin oxidation during bast fiber degumming with LMS is not well established. Degumming of bast fibers with LMS may be improved if the fundamental chemical reactions contributing to this process are well understood. In this study, the jute fibers were treated by LMS, and then the lignins were extracted from it with dioxane/water solution. The structure changes of lignins from the control and LMS-treated jute fibers were characterized by GPC, elemental analysis, FTIR and ^{1}H NMR. We hope the results will provide useful references to the degumming of jute fibers with LMS.

2. Materials and methods

2.1. Materials

Jute fiber was supplied by Changshu Aocun Longtai weaving Co., Ltd. Laccase from *Trametes Versicolor* with an activity of 5.43 U/mg was supplied by Sigma. One unit of laccase activity was defined as the amount of enzyme converting 1 μmol of catechol per minute in 50 mM sodium citrate buffer (pH 6) at 25 °C using catechol as substrate. 2,2′-azinobis(3-ethylbenzthiazoline-6-sulphonate) (ABTS) provided by Sigma was used as a mediator.

2.2. Treatment of the jute fibers with LMS

The reaction solution (300 mL) contained jute fibers (13 g), laccase (total activity 160 U), ABTS (10 mg), sodium acetate buffer

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(0.05 M, pH 6). The mixtures were shaken and bubbled air at 25 °C for 6 h. After the enzymatic reaction, the jute fibers were washed several times with water, and air-dried.

2.3. Isolation of lignins from the jute fibers

The corresponding residual lignins in the control and LMS-treated jute fibers, L_c and L_t, were isolated using a method as described by Evtuguin et al. with slight modification [18].

The control and LMS-treated jute fibers were ground to 40 mesh fractions, refluxed with ethanol–benzene (1:2, v/v) solvent for 6 h, and then dried at room temperature. These fractions were refluxed with dioxane–water (9:1, v/v) solution containing 0.2 M HCl at 90 °C for 60 min. The liquid phase was decanted after the mixture was cooled to room temperature. The solid residue was subjected to the next extraction as described above, and then decanted the liquid phase. The two portions of the liquid phases were mixed, and concentrated to around 60 mL by vacuum evaporation at 40 °C. The lignins were precipitated from dioxane solution by dilution into cold water (about 800 mL). The precipitate was separated by centrifugation, followed by being washed with water and freeze-dried.

The crude lignins were further purified according to the method of Lundquist et al. [19].

2.4. Acetylation of lignins from the jute fibers

The L_c and L_t were acetylated using a method proposed by Sarwar Jahan et al. [20]. Lignin of 100 mg was added in 9 mL of pyridine–acetic anhydride solution (1:2, v/v) and kept for 72 h in dark. The solution was poured into a 10-fold volume of an ice-water bath where the acetylated lignins were recovered as a precipitate, which were further purified by successive washing with water and dried under vacuum. The acetylated lignins were used for ^{1}H NMR analysis.

2.5. Estimation of molecular weight

The number-average molecular weight (M_n) and weight-average molecular weight (M_w) of L_c and L_t were determined by GPC. The GPC equipment used was a Waters 1515 Isocratic HPLC Pump (Waters Corporation, Milford, USA), with a Waters 2414 Refractive Index Detector (Waters Corporation, Milford, USA) and a GPC KD-802 Packed Column (Shodex, Japan).

The lignin samples were dissolved in N,N-dimethylformamide (DMF) and 20 μL solution was injected into the HPLC column. The test was operated at 35 °C and eluted with DMF at a flow rate of 1.5 mL/min. The molecular weight was calibrated with a polystyrene standard.

2.6. Chemical analysis

C, H and N elements of L_c and L_t were determined using a Vario ZLIII elemental analyzer. The percentage of oxygen was calculated by subtracting the C, H and N contents from 100%. Methoxyl group contents were calculated according to ^{1}H NMR spectra. Phenolic hydroxyl group contents were determined by an ultraviolet spectrophotometer [21].

2.7. Analysis by FTIR

FTIR spectra were recorded on a Nicolet iS10 FTIR spectrometer. The lignin samples were embedded in KBr pellets in the concentration of ca. 1 mg/200 mg KBr. The spectra were recorded in the absorption band mode in the range from 4000 to 500 cm^{-1}.

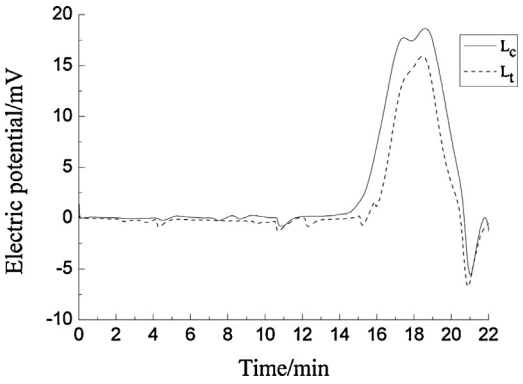

Fig. 1. Molecular weight distribution curve of L_c and L_t.

2.8. Analysis by ^{1}H NMR

The ^{1}H NMR spectra of 20 mg acetylated lignins solved in 0.5 mL chloroform ($CDCl_3$) were recorded, using tetramethylsilane (TMS) as the internal standard in a Bruker Avance 400 spectrometer with an operating frequency at 400 MHz.

3. Results and discussion

3.1. Molecular weight distribution of the jute fiber lignins

The molecular weight distribution curves of L_c and L_t were shown in Fig. 1. The values of the weight-average (M_w) and number-average (M_n) molecular weights of L_c and L_t were calculated from the curves, and the polydispersity (M_w/M_n) was given in Table 1. As can be seen from Table 1, the M_w and M_n were 34,130 and 24,177 for L_t, respectively, and the polydispersity value was 1.412. Comparing with L_c, M_w, M_n and polydispersity of L_t were decreased. The similar outcome was also obtained by Fu et al. in studies on the degradation of residual lignin in kraft pulp by laccase and mediator system [22]. This result meant that the lignins in jute fibers could be degraded by laccase and mediator system into smaller fragments.

3.2. Chemical analysis

Table 2 summarizes the results from C, H, N, O, methoxyl and phenolic hydroxyl analyses of L_c and L_t, together with the approximate C_9 formula calculated therefrom [23,24]. The L_t contained a high percentage of oxygen. It is in agreement with the change of oxygen content of residual lignin in the biobleaching of pulp with LMS reported by Balakshin et al. [25]. It may be the result of the oxidation of the LMS treatment. The methoxyl content was calculated according to ^{1}H NMR spectra. Although it is an approximate calculation method, the variation tendency of methoxy group content in lignin before and after LMS treatment could be observed through this result. As can be seen from Table 2, the methoxyl content in L_t was lower than that in L_c, which suggested that lignin demethylation took place during the LMS treatment. This result was compatible to earlier report of Bourbonnais and Paice

Table 1
Weight-average (M_w), number-average (M_n) molecular weights, and polydispersity (M_w/M_n) of jute fiber lignins.

Lignin	M_w	M_n	M_w/M_n
L_c	39,105	25,059	1.561
L_t	34,130	24,177	1.412