

Communication

Improving bamboo pulp delignification by grapheme-oxide-immobilised laccase-mediator system

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Abstract: A laccase mediator system (LMS), i.e. Lac/ABTS, was used for bamboo pulp delignification. Both laccase and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were then immobilised on graphene oxide (GO) to obtain GO-immobilised LMS, i.e. Lac/ABTS/GO. Lac/ABTS was also comparatively studied. The delignification selectivity and pulp properties achieved were better with Lac/ABTS/GO than Lac/ABTS in the catalytic delignification of bamboo pulp. The scanning electron micrograph of Lac/ABTS/GO-treated pulp showed a positive change in morphology and integrity of the pulp. UV resonance Raman spectroscopy also confirmed the better catalytic selectivity of Lac/ABTS/GO. Further, gel permeation chromatography, ³¹phosphorus nuclear magnetic resonance spectra and Fourier-transform infrared spectra of residual lignin samples from the treated pulp indicated an increase in the degradation and oxidation of lignin by Lac/ABTS/GO.

Keywords: laccase-mediator system (LMS), grapheme-oxide-immobilised LMS, bamboo pulp, bio-delignification, residual lignin

INTRODUCTION

Although the main raw material for pulp worldwide is wood, China has been plagued by a long-term shortage of wood resources. Thus, replacement of wood with fast-growing bamboo is urgent for sustainable development of the pulp and paper industry in China. The cellulose content of

bamboo is 40-50%. Thus, bamboo can be used as a supplement to wood as an ideal raw material for pulping [1]. The process of pulp delignification requires the removal of pulp lignin, followed by fibre separation [2]. Structurally, lignin consists of different interunit linkages, including mainly β -O-4, α -O-4, 4-O-5, β -5, β -1, β - β and 5-5 (Figure 1), the β -O-4 bonds being the most abundant substructures. The β -O-4, α -O-4 and 4-O-5 ether units are easy to degrade, while β -5, β -1, β - β and 5-5 carbon-carbon links are refractory. The complex chemical structure of lignin makes it highly resistant to chemical and enzymatic degradation [3], especially bamboo pulp lignin which has a high content of condensed units [4].

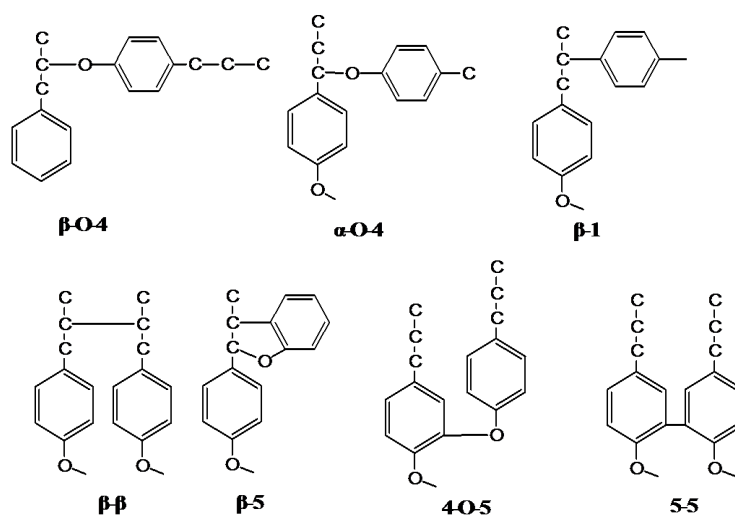


Figure 1. Interunit linkages of lignin

Thus, the removal of lignin is a primary requirement in the bamboo pulp industry. The chemical delignification method is widely used in bamboo pulp bleaching and is becoming non-sustainable. In recent years as a part of the commitment to mitigating pollution from the pulp and paper industry, novel bio-based pulp delignification techniques are under development as an option of reducing the environmental impact of pulping. Various enzymes such as xylanase, lignin peroxidase, manganese peroxidase and more recently laccases (EC 1.10.3.2) have been found to possess high lignolytic potential. Laccase has outstanding redox ability to attack and degrade lignin in conjunction with laccase-mediator system (LMS), by which a wide range of electron-rich phenolic and non-phenolic aromatic substrates could be oxidised into phenoxy radicals with a concomitant reduction of oxygen to water through the catalysis of the laccase's copper ions [5, 6]. Further, laccase has a distinct advantage of the biological approach and is more readily available and easier to manipulate than lignin peroxidase and manganese peroxidase [7, 8]. Liu et al. discovered that the main reactions of laccase oxidation are the degradation of β -O-4', 5-5' and 4-O-5' bonds [9]. Further development in this field is associated with immobilisation of laccase to improve its activity and stability for increasing lignin degradation. In Gou et al.'s study [10] the storage and thermal stability of the immobilised laccase (immobilised on Cu^{2+} -modified recyclable magnetite nanoparticles) increased by 70% and 24.1% respectively compared to those of free laccase at 65°C, pH 4.5; the immobilised laccase activity remained 50% after 6 cycles [10].

Although a variety of studies have confirmed the potential of LMS for delignifying various pulps, several issues still remain to be solved before its efficient implementation. One of them is the stability of mediators, which is difficult to regulate in industrial conditions. Another concern is the

toxicity of the mediators or their degradation products [11]. A way to solve the above problems would be the immobilisation of the mediator together with laccase, in which both laccase and mediator were immobilised on one support, i.e. immobilised LMS [12]. In this study we prepared an immobilised LMS using graphene oxide (GO) as support [13] and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) as mediator (Lac/ABTS/GO). Its use in bamboo pulp delignification was described. The oxidative degradation of pulp lignin was studied. The molecular weight and structure of the residual lignin samples were characterised by gel permeation chromatography (GPC), ³¹P-phosphorus-nuclear magnetic resonance (³¹P-NMR) spectra and Fourier transform infrared (FTIR) spectra.

MATERIALS AND METHODS

Materials and Chemicals

Unbleached bamboo (*Dendrocalamus latiflorus* Munro) kraft pulp with kappa number of 21.8, brightness of 30.1% and viscosity of 1257 mL/g (cupric-ethylenediamine method) was obtained from our laboratory. Prior to the delignification treatment, the pulp was chelated with diethylenetriaminepentaacetic acid at 50°C for 30 min. and thoroughly washed with ultrapure water to remove the transition metal ions from the pulp fibres.

Laccase from *Trametes versicolor*, cellulase from *Aspergillus niger* (150 filter paper activity/g) and ABTS were provided by Sigma-Aldrich (USA). GO was obtained from Sinopharm Chemical Reagent Co. (China). Milli-Q-Plus ultrapure water was used in all trials.

Immobilisation of Laccase and Mediator

Laccase (30 mg) and ABTS (30 mg) were mixed in phosphate buffer solution (20 mL, 50 mM, pH 5) in a conical beaker. GO (7.5 g) was put into the mixture. The immobilisation was performed in a rotary shaker (160 rpm) at 25°C for 4 hr. The obtained biocatalyst was thoroughly washed with ultrapure water to wash away any loosely-bound laccase and ABTS from GO. This biocatalyst was designated as Lac/ABTS/GO. For Lac/ABTS, laccase and ABTS were mixed in sodium tartrate buffer solution (pH 5) in a conical beaker to obtain Lac/ABTS according to the conditions described in the next sub-section ('Bamboo pulp treatment with LMS and GO-immobilised LMS').

One activity unit of laccase was defined as 1 μmol of ABTS oxidised per min. in 0.1 M sodium acetate, pH 5, at 25°C. The immobilised quantity of laccase or mediator was determined through measuring the activity or content that remained in the solution with reversed-phase high performance liquid chromatography at completion of the adsorption procedure.

Bamboo Pulp Treatment with LMS and GO-immobilised LMS

The delignification of bamboo pulp was conducted with LMS (Lac/ABTS) according to the following procedure. Bamboo pulp in 50 mM sodium tartrate at pH 5 was bubbled with O₂ at 50°C for 8 hr with pulp consistency of 4%, mediator charge of 0.1 mmole ABTS/g pulp and laccase charge of 20U/g pulp. The delignification was also carried out with GO-immobilised LMS (Lac/ABTS/GO) following the above procedure. As control trials, bamboo pulp was treated under the same conditions in the absence of both laccase and ABTS.

Pulp Lignin Isolation

Residual lignin was isolated from pulp sample using the method suggested by Argyropoulos et al. [14]. Delignified pulp (2 g) was extracted with acetone for 24 hr to remove the extractive. Then the pulp was hydrolysed with cellulase of 300 filter paper activity/g pulp in acetic acid/sodium acetate buffer solution (pH 4.8, 80 mL) at 50°C for 48 hr. The residue (6 g) obtained during the enzymatic hydrolysis was then acidolysed with 0.10 M HCl in 150 mL dioxane/water (85:15 v/v) at 95°C for 2 hr under nitrogen. After that the mixture was filtered to obtain the filtrate, which was neutralised with NaHCO₃ and vacuum concentrated. The concentrated solution was then added dropwise to water to precipitate lignin. The crude lignin was fully washed with n-hexane and freeze-dried. Finally, the lignin sample was further purified via dissolving in 1,2-dichloroethane:ethanol (2:1) and precipitating in diethyl ether. The yield of the isolated lignin samples was ~65%, the content of sugar in the lignin sample was ~0.4%, and nitrogen content was ~0.4%.

Testing of Pulp Properties

Pulp capillary viscosity and kappa number were determined according to the methods of the Technical Association of the Pulp and Paper Industry (TAPPI), viz. TAPPI T 230 om-08, TAPPI T 236 om-06, TAPPI T 222 om-06 and TAPPI T 437 om-08. Physical properties, i.e. tensile index, tear index and burst index, were determined according to TAPPI T494, TAPPI T414 and TAPPI T403 om 97 respectively [15].

Delignification selectivity was then obtained by calculation according the following equations [16]:

$$DL = (K_1 - K_2)/K_1 \times 100 \%$$

$$VDR = (V_1 - V_2)/V_1 \times 100 \%$$

$$DS = DL/VDR$$

where DL = delignification rate (%), K_1 = kappa number of bamboo pulp before delignification, K_2 = kappa number of bamboo pulp after delignification, VDR = viscosity decreasing rate (%), V_1 = viscosity of bamboo pulp before delignification, V_2 = viscosity of bamboo pulp after delignification, and DS = delignification selectivity.

Characterisation of Residual Lignin

Handsheets of pulps were prepared and pressed with a KBr press. Sheet samples were then characterised with UV resonance Raman spectroscopy (UVRR) (Renishaw 1000, Renishaw Co., UK) at the excitation wavelengths of 244 nm and 257 nm. The residual lignin was characterised as $I_{1600}(\text{lignin})/I_{1092}(\text{cellulose})$ [17].

SEM Analysis of Pulp

Scanning electron micrographs (SEMs) were obtained for pulp samples (gold-coated) at 5 kV with a Tescan Mira scanning electron microscope (Tescan Co., Czeck Republic).

Molecular Weight Distribution of Lignin

Molecular weight distribution of lignin was analysed with gel permeation chromatography (GPC) (Agilent 1100, Agilent Technology Co., USA). Prior to analysis, the lignin was acetylated in

pyridine with acetic anhydride [18]. The acetylated lignin was dissolved in tetrahydrofuran to achieve a concentration of 1 mg/mL and the solution was filtered through a 0.45- μm membrane.

³¹P-NMR Analysis of Lignin

Quantification of lignin hydroxyl groups was carried out with ³¹P-NMR (Bruker DRX500 NMR spectrometer using cyclohexanol as the internal standard after lignin (25 mg) was derivatised with 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (110 mL) in a mixture of pyridine and deuterated chloroform (1.6:1 v/v, 0.6 mL) [19, 20]. Conditions used for ³¹P-NMR determination were: inverse gated decoupling sequence = 300 transients, relaxation delay between pulses = 5 sec., and pulse width = 90°.

FTIR Analysis of Lignin

FTIR analysis of lignin samples (2-5 mg) in potassium bromide pellets was carried out with a Bruker VERTEX-70 IR spectrophotometer in the spectral range of 4000-400 cm^{-1} .

RESULTS AND DISCUSSION

Bamboo Pulp Delignification

The catalytic efficiency was assessed in terms of delignification selectivity and pulp properties (Figure 2). The results show that delignification selectivity and values for pulp properties (tensile index, tear index and burst index) all increase with laccase catalysis compared to control, with Lac/ABTS/GO giving the best results. SEMs of pulp samples also show positive effects of the catalytic treatment on pulp morphology and integrity compared to the control (Figure 3). The pulp surfaces of the Lac/ABTS/GO-treated sample also exhibit further prominent changes in the arrangement of the fibrils (Figure 3c). This can be explained by the fact that the immobilisation of Lac and ABTS on GO increases the tolerance and adaptability of Lac and ABTS [21, 22].

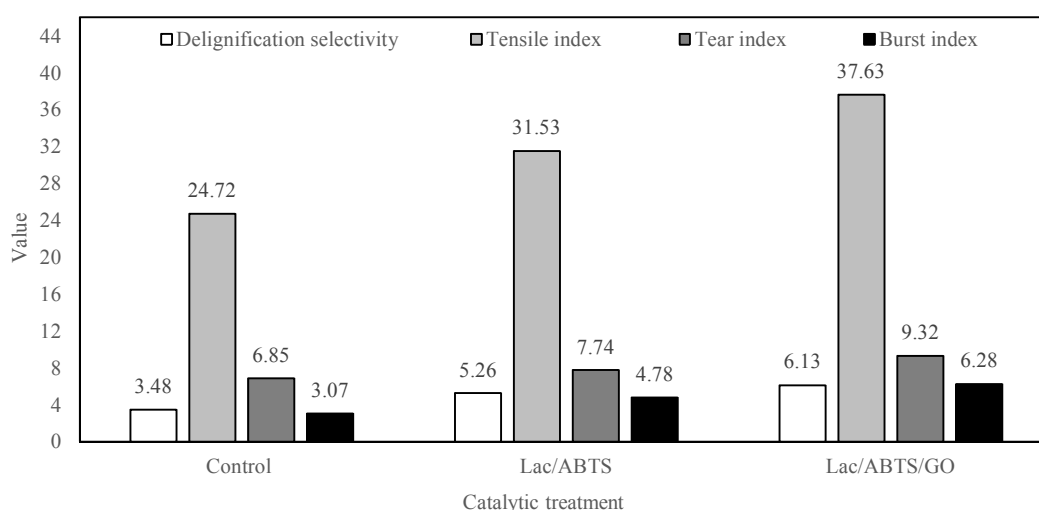


Figure 2. Delignification selectivity and pulp properties

Bamboo Pulp Lignin Oxidation

Pulp samples were characterised with UVRR, which is a powerful tool for pulp lignin analysis [23], and the results are listed in Table 1. The values of $I_{1600}(\text{lignin})/I_{1092}(\text{cellulose})$ of the

pulp indicate that lignin is effectively degraded by the catalysts, and to a larger extent by Lac/ABTS/GO than Lac/ABTS.

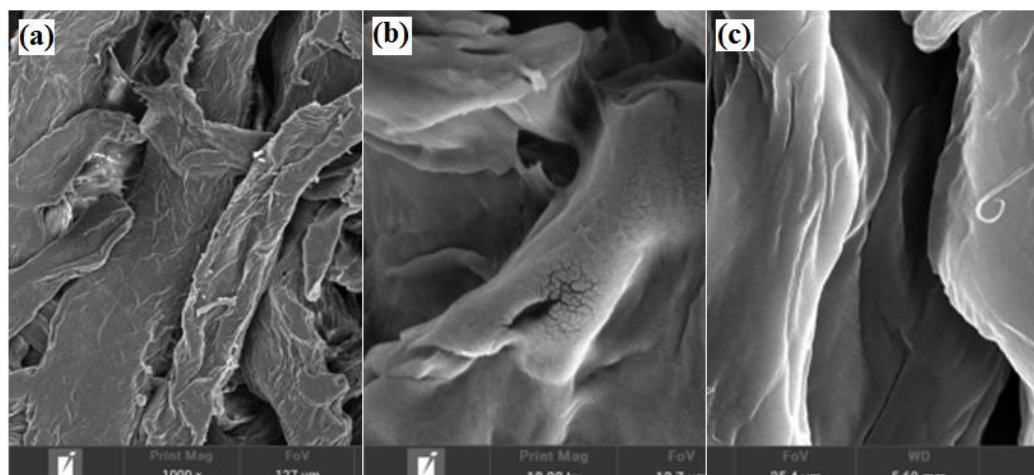


Figure 3. SEMs of pulp samples: (a) control, (b) Lac/ABTS, (c) Lac/ABTS/GO

Table 1. $I_{1600}(\text{lignin})/I_{1092}(\text{cellulose})$ of pulp and molecular weight and hydroxyl content of lignin

		Control	Lac/ABTS	Lac/ABTS/GO
Pulp (UVR)	$I_{1600}(\text{lignin})/I_{1092}(\text{cellulose})$ of pulp	2.21	1.83	1.26
Lignin (GPC)	M_w^*	6107	4818	3754
	PD**	1.62	1.51	1.16
Lignin (^{31}P -NMR)	Aliphatic OH (mmol/g)	2.80	2.55	1.84
	Guaiacyl PhOH (mmol/g)	0.26	0.34	0.53
	<i>p</i> -Hydroxylphenyl PhOH (mmol/g)	0.18	0.24	0.37
	Syringyl PhOH (mmol/g)	0.20	0.28	0.36
	Free PhOH (mmol/g)***	0.64	0.86	1.26
	Condensed PhOH (mmol/g)	0.42	0.32	0.18
	Carboxyl OH (mmol/g)	0.17	0.29	0.42

*Weight-average molecular weight; **Polydispersity; ***Guaiacyl PhOH + *p*-hydroxyphenyl PhOH + syringyl PhOH

Additionally, the residual lignin present in the catalyst-treated pulp was also analysed by other methods [24], the results of which are reported in Table 1. The GPC data demonstrate that the molecular weight decreases from 6107 in the control lignin to 4818 in the Lac/ABTS lignin and finally to 3754 in the Lac/ABTS/GO lignin. This suggests that the bonds between lignin units such as β -O-4 bonds were effectively cleaved [25]. The polydispersity also decreases in a similar manner, indicating an efficient depolymerisation of bamboo pulp lignin by these biocatalysts [26].

Regarding the structural changes of the pulp lignin, a number of functional groups were quantitatively determined with ^{31}P -NMR (Table 1). The results show increasing oxidation of the pulp lignin with catalysis, indicating a decrease in aliphatic OH content of lignin [27] and an increased amount of lignin units containing carboxyl groups. In addition, the increasing content of free phenolic hydroxyl groups of lignin (guaiacyl PhOH, *p*-hydroxylphenyl PhOH and syringyl PhOH) is indicative of the occurrence of oxidative breaking of lignin ether bonds [28]. These

biocatalysts also positively promote the degradation of condensed substructures of the bamboo pulp lignin, resulting in a successive reduction of condensed PhOH in control, Lac/ABTS and Lac/ABTS/GO lignin samples. This is crucial to the deep removal of lignin from the bamboo pulp [29]. Finally, the findings are confirmed by FTIR spectra (Figure 4), which show a high carbonyl content and low benzene ring content of residual lignin in pulp treated with the two biocatalysts, with Lac/ABTS/GO giving the best results.

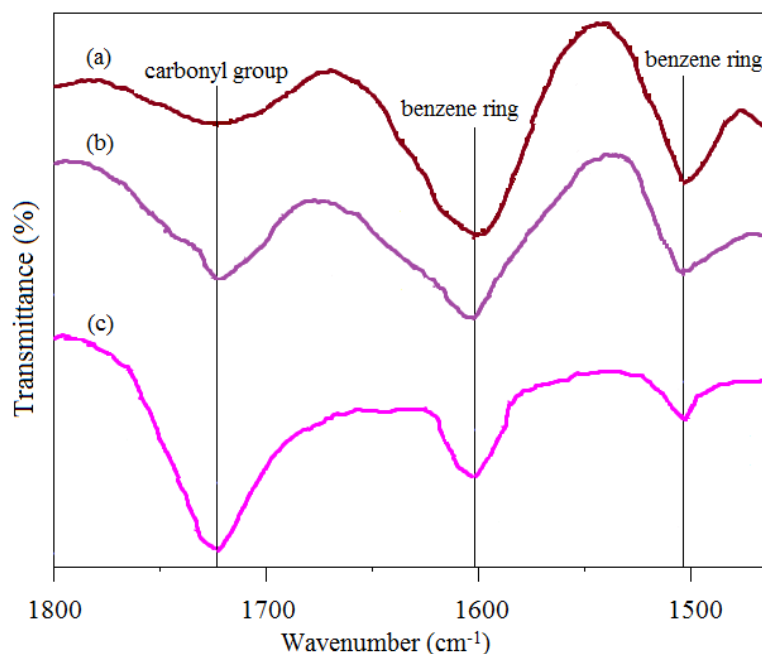


Figure 4. FTIR spectra of pulp residual lignin samples: (a) Control; (b) Lac/ABTS; (c) Lac/ABTS/GO

CONCLUSIONS

Maximum delignification of bamboo pulp is obtained when Lac/ABTS/GO catalytic system is used for delignification process, in which both laccase and ABTS are immobilised on GO. The process also improves key physico-chemical properties of pulp required for papermaking.

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