

Enzymatic Treatments to Improve Mechanical Properties and Surface Hydrophobicity of Jute Fiber Membranes

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Fiber membranes prepared from jute fragments can be valuable, low cost, and renewable. They have broad application prospects in packing bags, geotextiles, filters, and composite reinforcements. Traditionally, chemical adhesives have been used to improve the properties of jute fiber membranes. A series of new laccase, laccase/mediator systems, and multi-enzyme synergisms were attempted. After the laccase treatment of jute fragments, the mechanical properties and surface hydrophobicity of the produced fiber membranes increased because of the cross-coupling of lignins with ether bonds mediated by laccase. The optimum conditions were a buffer pH of 4.5 and an incubation temperature of 60 °C with 0.92 U/mL laccase for 3 h. Laccase/guaiacol and laccase/alkali lignin treatments resulted in remarkable increases in the mechanical properties; in contrast, the laccase/2,2'-azino-bis-(3-ethylthiazoline-6-sulfonate) (ABTS) and laccase/2,6-dimethoxyphenol treatments led to a decrease. The laccase/ guaiacol system was favorable to the surface hydrophobicity of jute fiber membranes. However, the laccase/alkali lignin system had the opposite effect. Xylanase/laccase and cellulase/laccase combined treatments were able to enhance both the mechanical properties and the surface hydrophobicity of jute fiber membranes. Among these, cellulase/laccase treatment performed better; compared to mechanical properties, the surface hydrophobicity of the jute fiber membranes showed only a slight increase after the enzymatic multi-step processes.

Keywords: Natural fiber; Fiber membrane; Laccase; Mediator; Xylanase; Cellulase

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INTRODUCTION

In recent decades, enzymatic technology has been widely employed in the processing of lignocellulose-based materials, which include wood, fiberboards, plant fibers, and pulp (Hüttermann *et al.* 2001; Kudanga *et al.* 2008, 2010; Nyanhongo *et al.* 2011; Zhou *et al.* 2013a; Kalia *et al.* 2014; Nasir *et al.* 2015). The enzymatic processes have the merits of substrate specificity, low cost, eco-friendliness, and mild operating conditions. Laccases (EC 1.10.3.2, multi-copper oxidoreductases) are the most investigated enzymes in this field. They catalyze the mono-electronic oxidation of phenols or amines to reactive radical species and simultaneously reduce dioxygen to water in a redox reaction (Riva 2006).

Lignin is a three-dimensional aromatic polymer with three structural units, guaiacyl, syringyl, and *p*-hydroxyphenyl, linked together in an irregular manner (Rio *et al.*

2009). The phenolic sites of lignin macromolecules can be oxidized to phenoxy radicals by laccase (Lahtinen *et al.* 2009). These reactive radicals can then undergo covalent coupling to initiate the polymerization of lignins; while the degradation of lignin can occur simultaneously, mediated by the radical-induced cleavage of covalent bonds or aromatic rings. Previous research has shown that the molecular weight of lignin can be increased by laccase alone (Mattinen *et al.* 2008; Kim *et al.* 2009; Zhou *et al.* 2013b) or in the presence of some phenolic compounds (Chandra *et al.* 2004; Elegir *et al.* 2007; Liu *et al.* 2009) in response to the enhanced cross-coupling. However, the depolymerization of lignin is dominant in laccase/mediator systems. These mediators can be synthetic mediators (Bourbonnais *et al.* 1997; Camarero *et al.* 2004) such as 2,2'-azino-bis-(3-ethylthiazoline-6-sulfonate) (ABTS) and 1-hydroxybenzotriazole (HBT), as well as natural mediators with higher redox potential (Camarero *et al.* 2007; Fillat *et al.* 2010; Barneto *et al.* 2012) such as syringaldehyde (SA) and acetosyringone (AS), which can be activated by laccase to oxidize the non-phenolic units dominating the lignin structure. In addition, laccase-oxidized (radical-containing) phenols or non-oxidized amines of foreign interest can also be grafted to radicalized lignins or lignocellulosic surfaces to produce engineered materials with novel functions (Lund and Ragauskas 2001; Elegir *et al.* 2008; Witayakran and Ragauskas 2009; Pei *et al.* 2013; Reynaud *et al.* 2014).

Jute fiber, an abundant lignocellulosic bioresource, occupies the second place in the world production of natural fibers, behind cotton (Cao *et al.* 2012). Fiber membranes made from jute can be low cost, light weight, biodegradable, and renewable, with a variety of applications such as packing for bags, geotextiles, filters, and composite reinforcements (Mohanty *et al.* 2000; Chattopadhyay and Chakravarty 2009; Abdullah *et al.* 2011). However, the connection between natural fiber fragments is normally weak, and thus the fiber membranes are loose, which remarkably limited their utilizations. In order to improve the application properties of the fiber membranes, chemical crosslinking agents are traditionally used; but they are more or less harmful to the environment. In this paper, green biotechnology, *i.e.*, laccase-facilitated self-coupling of lignin macromolecules on the lignocellulosic jute fibers was employed. With some natural mediators of low redox potential such as guaiacol, the enzymatic cross-coupling can be extended to larger scales.

Alkali lignin is a by-product of alkali-based pulping, with an annual production of 50 million tons (Gosselink *et al.* 2004), but its technological application is largely limited; only 2% is used commercially as dispersing surfactants or binding agents. Therefore, the replacement of natural compounds with alkali lignin as a mediator to assist laccase is of great interest. In addition, xylanase and cellulase have been incorporated to pretreat jute fragments, aimed at exposing more lignins on the surface by hydrolyzation of the other components and expanding the effects of laccase-mediated coupling, although several studies have reported the involvement of xylanase in the laccase/mediator-facilitated bleaching of pulps (Valls *et al.* 2010; Thakur *et al.* 2012).

In the present work, fiber fragments ground from jute fabrics were treated by laccase, laccase/mediator systems, or multi-enzyme synergisms before being used to prepare jute fiber membranes. Laccase from *Aspergillus*, mediators (ABTS, 2,6-dimethoxyphenol (DMP), guaiacol (G), alkali lignin (AL)) and other enzymes (xylanase, cellulase) were applied. Then, the mechanical and surface hydrophobic properties of the jute fiber membranes were investigated. Finally, the chemical structure and the surface morphology of the jute fiber membranes were characterized by Fourier transform infrared spectroscopy (FT-IR) and scanning electron microscopy (SEM), respectively.

EXPERIMENTAL

Materials and Reagents

The 100% raw jute fabrics (427 g/m²), with a 7/7 (warp/weft) cm⁻¹ fabric density, were supplied by Longtai Weaving Co. Ltd. (Changshu, China). Laccase (Denilite II in graininess) was produced by the *Aspergillus* species and purchased from Xianhua Biotechnology Co. Ltd. (Shanghai, China). Xylanase JNM-5 was provided by Meixinda Biotechnology Co. Ltd. (Huzhou, China). Cellulase 989N was provided by Novozymes (Shanghai, China). Alkali lignin (AL) and 2,2'-azino-bis-(3-ethylthiazoline-6-sulfonate) (ABTS) were supplied by Sigma-Aldrich (Shanghai, China). Guaiacol (G) and 2,6-dimethoxyphenol (DMP) were obtained from TCI Co. Ltd. (Shanghai, China). All other chemicals used in this study were commercially available and of analytical grade.

Activity Assay of Laccase, Cellulase, and Xylanase

The activity of laccase was measured using a UV-1800 UV/Vis spectrophotometer (Shimadzu, Japan) by monitoring the oxidation of ABTS ($\epsilon_{420} = 36,000 \text{ M}^{-1} \times \text{cm}^{-1}$) as the substrate at 420 nm in a pH 4.5 acetate buffer at 60 °C. The enzyme activity was expressed in units defined as micromoles of ABTS oxidized per minute (Childs and Bardsley 1975).

The cellulase activity was measured using 1% (w/v) carboxymethylcellulose (CMC) as the substrate. The amount of generated glucose in a pH 5.0 acetate buffer at 60 °C was measured by a UV/Vis spectrophotometer at 546 nm using 3,5-dinitrosalicylic acid (DNS) as the color indicator. One unit of cellulase activity was defined as the amount of enzyme that produced 1.0 μmol of reducing sugar from the substrate per minute (Kondo *et al.* 1994).

The activity of xylanase was determined using 0.5% (w/v) xylan as the substrate. The amount of generated xylose in pH 5.0 acetate buffer at 60 °C was measured on a UV/Vis spectrophotometer at 540 nm using DNS as the color indicator. One unit of xylanase activity was defined as the amount of enzyme that produced 1.0 μmol of xylose from the substrate per minute (Knob and Carmona 2010).

Pretreatment of Jute Fabrics

The jute fabrics were desized by boiling with distilled water for 2 h. The iodine-potassium iodide method was used to examine whether the starch or polyvinyl alcohol (PVA) sizes were dislodged completely or not (Hu *et al.* 2010). Then, the jute was ground to 7- to 9-mm fibers using a XA-1A pulverizer (Yinhe Instrument Co., China) and incubated in distilled water at 10 wt.% for 3 h at room temperature. Afterwards, the defibrillation of the mixture was conducted on a ZQS7-PFI grinder (Machinery Equipment Co. in Shanxi University of Science and Technology, China) with a rotation speed of 1400 rpm for 7 min to cut fibers into 2-mm fragments. These jute fragments were dried later at 80 °C for further use.

Laccase Treatment of Jute Fragments

Jute fragments (4 g) were incubated in 400 mL of pH 4.5 phosphate buffer with 0.92 U/mL laccase at 60 °C for 3 h in a shaking bath. Then, the mixture was boiled for 20 min to inactivate the enzyme. The treated jute fragments were washed twice with distilled water and dried at 80 °C. Control samples without laccase followed the same treatment conditions.

Laccase/Mediator Treatment of Jute Fragments

Four mediators (5 mM) were used in the laccase/mediator treatment of jute fragments with laccase: ABTS, DMP, AL, and G. The other treatment conditions were the same as the laccase treatment above.

Xylanase/Laccase Combined Treatment of Jute Fragments

Jute fragments (4 g) were incubated in 400 mL of pH of 5.0 phosphate buffer with 0.50 U/mL xylanase at 60 °C for 1 h in a shaking bath. Afterwards, the mixture was boiled for 20 min to inactivate the xylanase. The treated jute fragments were dried by squeezing and then treated with laccase as mentioned above.

Cellulase/Laccase Combined Treatment of Jute Fragments

Jute fragments (4 g) were incubated in 400 mL of pH 5.0 phosphate buffer with 0.50 U/mL cellulase at 60 °C for 15 min in a shaking bath. After the inactivation of cellulase by boiling for 20 min, the treated jute fragments were dried by squeezing and then treated with laccase following the conditions above.

Preparation of Jute Fiber Membranes

Treated jute fragments (4 g) were incubated in 2 L of water and the mixture was stirred at 20,000 rpm to uniformly disperse the fragments. Then, the mixture was poured into a storage tube and the water was drawn out by a vacuum pump. The wet membrane was spread on a dryer and pressed at 105 °C for 12 min to obtain the dry jute fiber membrane. Membranes were conditioned for 24 h in a constant temperature and humidity environment (21 °C, 65% relative humidity (RH)) before measuring the mechanical properties and surface hydrophobicity.

Mechanical Measurements of Jute Fiber Membranes

Jute fiber membranes were cut into pieces 150 mm in length and 10 mm in width. Then, the tensile strength was determined on a DC-KZ300C computer-controlled tensile testing machine (Changjiang Papermaking Instrument Co. Ltd., China) with a testing length of 90 mm.

Other jute fiber membranes were cut into pieces of 63 mm (length) × 50 mm (width). Then, the tear strength was determined on a DCP-NPY1200 computer-controlled tear testing machine (Changjiang Papermaking Instrument Co. Ltd., China) in the range of 50 to 500 mN.

The burst strength of jute fiber membranes was determined on a DCP-SLY1000 computer-controlled burst testing machine (Changjiang Papermaking Instrument Co. Ltd., China). Five samples were tested for each treatment in each mechanical measurement and the results were averaged.

Surface Hydrophobicity Evaluation of Jute Fiber Membranes

The static contact angle of jute fiber membranes was determined 2 s after water drop deposition using a JC2000D4 contact angle meter (Zhongchen Digital Instrument Co. Ltd., Shanghai). For each sample, five spots were measured and the results were averaged. Three samples were employed for each treatment.

Jute fiber membranes were cut into dimensions of 150 mm (length) × 10 mm (width). The wetting time was defined as the time required for water to rise to 100 mm in height, from the bottom of the membrane. The average wetting time is the ratio of the

wetting time above to the thickness of the jute fiber membrane. For each treatment, three samples were measured and then the results were averaged.

FT-IR Analysis

Fourier transform infrared spectroscopy (FT-IR) analysis of the jute fiber membranes was performed on a Nicolet iS10 FT-IR spectrometer (Thermo Fisher Scientific, USA) with the attenuated total reflectance (ATR) technique. The spectra were recorded in the range 4000 to 650 cm^{-1} at 4 cm^{-1} resolution and 16 scans per sample.

SEM Analysis

The surface of the jute fiber membranes was scanned using a SU1510 scanning electron microscope (SEM, Hitachi, Japan) at an accelerating voltage of 5.00 kV.

RESULTS AND DISCUSSION

Effect of Laccase Treatment on the Mechanical Properties of Jute Fiber Membranes

Table 1 shows the mechanical properties of jute fiber membranes prepared after several biological treatments of jute fragments. Tensile strength, tear strength, and burst strength were chosen as indicators to reveal the physical characteristics of the membranes. Inactive laccase treatment only led to a slight variation in the three indicators, probably because of the residue of laccase proteins in the fiber membranes. Laccase treatment induced cross-coupling and polymerization of lignins on the different surfaces of jute fragments, which resulted in stronger combination between the jute fragments and higher mechanical performance of the jute fiber membranes. The tensile strength, tear strength, and burst strength increased by 30.2%, 21.4%, and 1.9%, respectively, from control to laccase treatment. The defibrillation process can enhance the coupling effect of the laccase treatment, represented by increases in the tensile strength, tear strength, and burst strength of 39.7%, 28.6%, and 5.8%, respectively, from control to defibrillation/laccase treatment. This can be attributed to the fact that more lignins appeared on the jute surface through the defibrillation procedure, enabling the macromolecular laccase to contact and oxidize them (Schroeder *et al.* 2007).

Table 1. Mechanical Properties of Jute Fiber Membranes Processed by Various Biological Treatments

Performance indicators	Biological treatments before defibrillation			Laccase treatment after defibrillation
	Control	Inactive laccase	Laccase	
Tensile strength (N·m/g)	6.3±0.2	7.5±0.2	8.2±0.1	8.8±0.1
Tear strength (mN·m ² /g)	1.4±0.3	1.6±0.4	1.7±0.2	1.8±0.2
Burst strength (kPa·m ² /g)	1.038±0.003	1.040±0.004	1.058±0.003	1.098±0.003

Effect of Laccase Treatment on the Surface Hydrophobicity of Jute Fiber Membranes

The surface hydrophobicity of jute fiber membranes was evaluated in terms of contact angle and wetting time. As presented in Fig. 1, the contact angle and the wetting time per thickness of the fiber membranes both increased after the laccase treatments. The

contact angle and wetting time reached 95.48° and 3058 s, respectively, when the defibrillation and laccase treatment of jute fragments were conducted in sequence. The surface hydrophobic results show a similar trend with the mechanical properties of jute fiber membranes above and indicate that the polymerization of lignin mediated by laccase could partly hydrophobize the fiber membrane surface. This may have occurred because of the cross-linking of laccase-generated phenoxyl radicals and the decrease in phenolic hydroxyl groups in lignin structures (Kim *et al.* 2009; Zhou *et al.* 2013b).

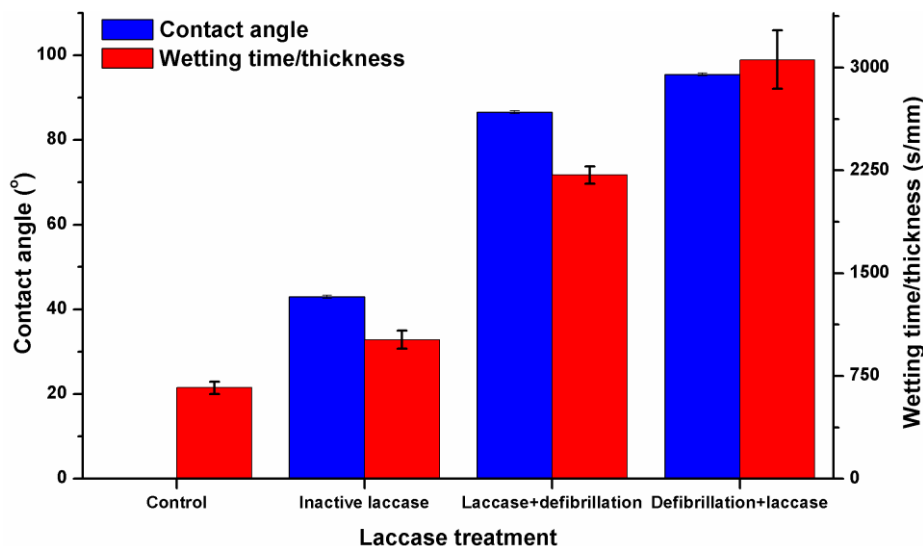


Fig. 1. Effect of laccase treatments on the surface hydrophobicity of jute fiber membranes

Optimization of the Enzymatic Process Parameters

The process parameters in the laccase treatment of jute fragments before being used to prepare jute fiber membranes include laccase concentration, pH of phosphate buffer, incubation temperature, and incubation time. These parameters were optimized as shown in Fig. 2. As a mechanical indicator of the jute fiber membranes, the tensile strength was chosen to monitor the effect of the treatments. From the curves, the optimum reaction condition was obtained when jute fragments were incubated at a pH 4.5 (phosphate buffer) with 0.92 U/mL laccase at 60 °C for 3 h. The effects of buffer pH and incubation temperature on the enzymatic coupling corresponded well with the laccase activity. The incubation time data suggest that laccase-mediated cross-coupling of lignin can reach the maximum reactivity within several hours. After that, with increasing depolymerization of lignin, the two opposite reactions tended to remain balanced (Zhou *et al.* 2013b).

Comparison of Laccase/Mediator Systems on the Mechanical Properties of Jute Fiber Membranes

The tensile strength of jute fiber membranes treated with various laccase/mediator systems (LMS) was compared, as shown in Fig. 3. Four excellent substrates for laccase, *i.e.*, ABTS, DMP, AL, and G, were applied as mediators in the LMS. The addition of ABTS in the LMS showed the largest decrease in the tensile strength of fiber membranes (17.0%) when contrasted with the laccase treatment alone. This representative synthetic mediator of laccase has been reported to facilitate the laccase-induced cleavage of lignins into phenolic pieces with lower molecular weight, rather than cross-coupling and polymerization (Bourbonnais *et al.* 1997).

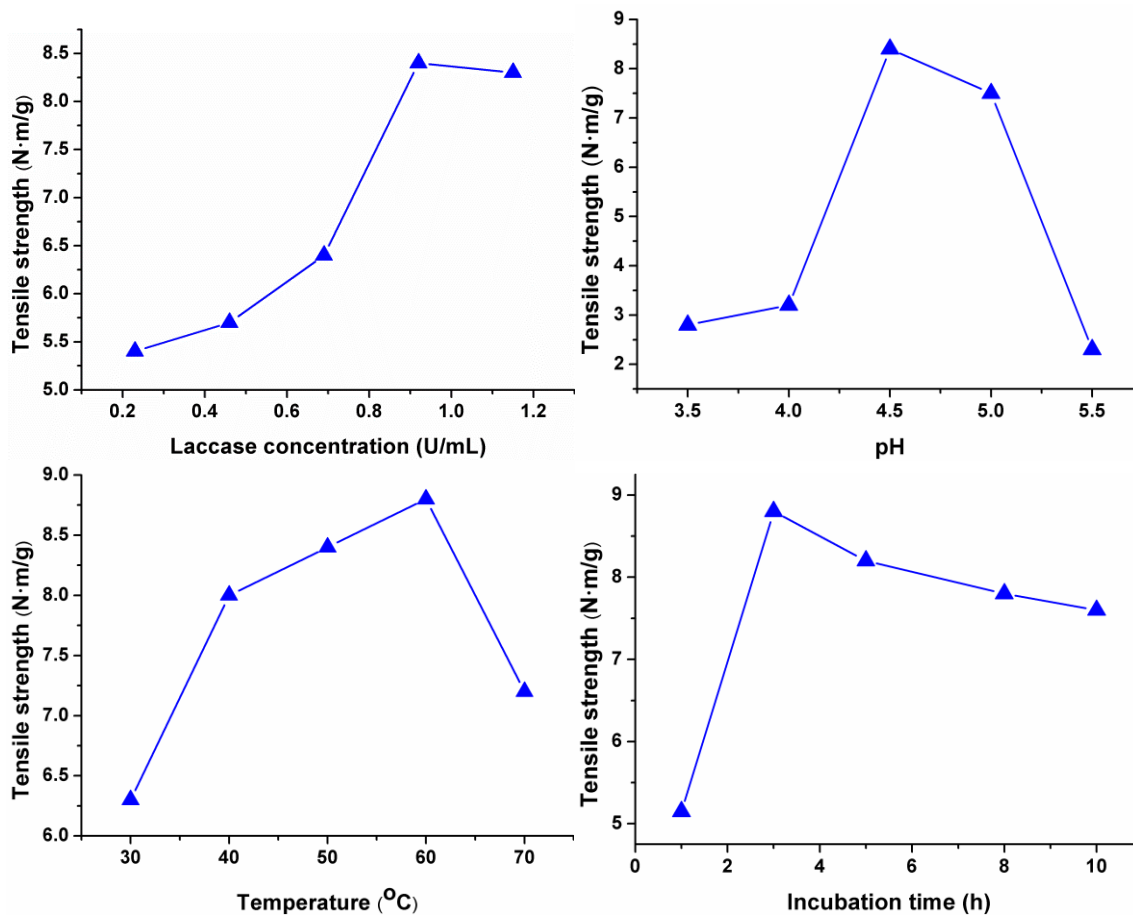


Fig. 2. Optimization of process parameters in the laccase treatment: laccase concentration, pH of phosphate buffer, incubation temperature, and incubation time

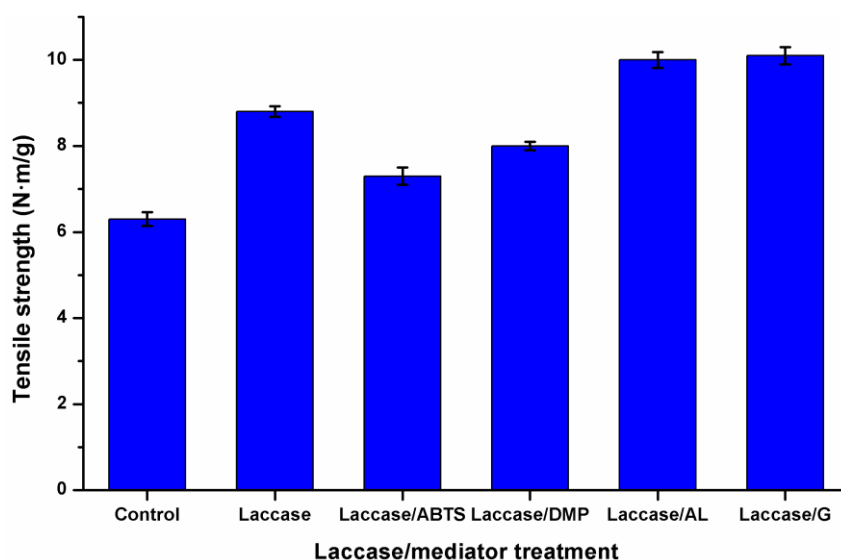


Fig. 3. Tensile strength of jute fiber membranes treated by laccase with various mediators

DMP exhibited the same trend as ABTS, with a smaller decrease (9.1%). However, AL and G accelerated the laccase-mediated oxidation and coupling of lignin or even were activated to radicals by laccase and acted as linking agents between the lignin sections

(Aracri *et al.* 2009). The tensile strengths of jute fiber membranes treated by the laccase/AL system and laccase/G system increased by 13.6% and 14.8%, respectively, compared with the laccase treatment alone.

The comprehensive mechanical indicators of the jute fiber membranes after laccase/AL treatment and laccase/G treatment are listed in Table 2. All physical properties of jute fiber membranes treated by the laccase/AL system were similar to those *via* the laccase/G treatment, which showed excellent ability to replace low-molecular weight phenols with AL as the mediator of the laccase oxidation.

Table 2. Mechanical Properties of Jute Fiber Membranes after Various Laccase/Mediator Treatments

Performance indicators	Control	Laccase	Laccase/AL	Laccase/G
Elongation percentage (%)	0.40±0.04	0.60±0.04	0.60±0.02	0.60±0.02
Tensile strength (N·m/g)	6.3±0.2	8.8±0.1	10.0±0.2	10.1±0.2
Absorption strength (mJ/g)	15.7±0.9	30.8±1.5	35.9±2.2	36.6±2.3
Tear strength (mN·m ² /g)	1.4±0.3	1.8±0.2	2.0±0.2	2.0±0.1
Burst strength (kPa·m ² /g)	1.038±0.003	1.098±0.003	1.194±0.005	1.202±0.003

Comparison of Laccase/AL Treatment and Laccase/G Treatment on the Surface Hydrophobicity of Jute Fiber Membranes

The contact angle and wetting time of jute fiber membranes *via* laccase/AL and laccase/G treatments are illustrated in Fig. 4. Jute fiber membranes after the laccase/AL treatment displayed lower surface hydrophobicity than those after the laccase treatment alone, with a contact angle of only 68.69° and wetting time of 1438 s. The high solubility in water of AL should be the main reason for this shortcoming of the jute fiber membranes. In contrast, the laccase/G treatment can give rise to surface hydrophobicity enhancement of the fiber membranes, with a contact angle of 106.26° and wetting time of up to 4199 s.

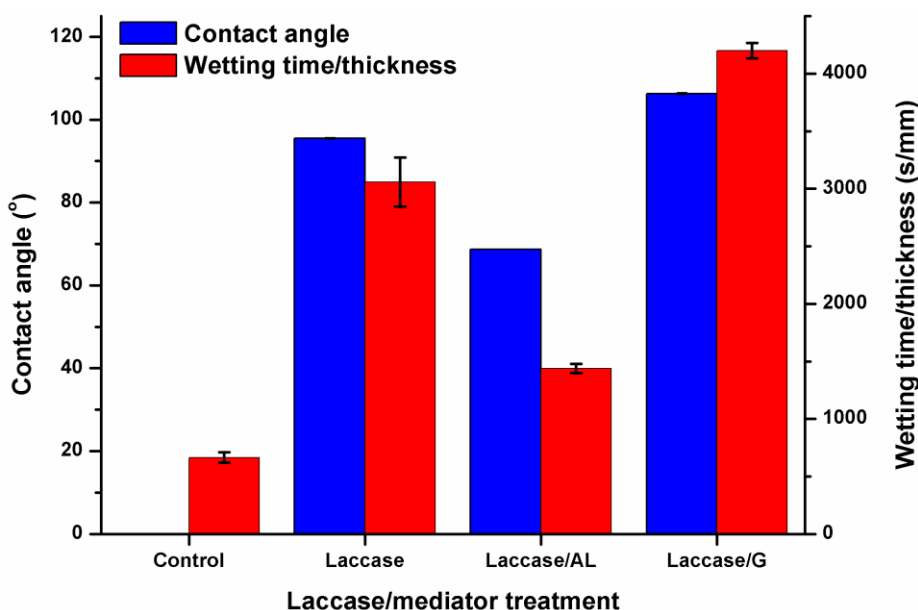


Fig. 4. Surface hydrophobicity of jute fiber membranes treated by laccase/G and laccase/AL systems

Influence of Xylanase or Cellulase Pretreatment on the Mechanical Properties and Surface Hydrophobicity of Jute Fiber Membranes

Pretreatment of jute fragments by xylanase or cellulase before the laccase treatment was conducted to utilize the multi-enzyme synergism to expand the effect of the laccase-mediated cross-linking of lignin. The mechanical properties and surface hydrophobicity of the jute fiber membranes *via* multi-enzyme processes (xylanase/laccase treatment and cellulase/laccase treatment) are given in Table 3. After the enzymatic multi-step treatments, the physical indicators of the jute fiber membranes increased remarkably. Xylanase/laccase treatment and cellulase/laccase treatment showed increases in tensile strength of 10.2% and 26.1%, respectively, compared with the laccase treatment and of 54.0% and 76.2%, respectively, compared with the control. Xylanase can remove hemicellulose from jute fibers, and cellulase, with a short incubation time, can remove cellulose on the jute surface. As a result, more lignins were exposed on the surface and the oxidative coupling of lignin by laccase proceeded to larger scales. However, the surface hydrophobic indicators of jute fiber membranes only displayed limited increases after the combined enzymatic processes. Compared with the cellulase or xylanase pretreatments, the laccase-mediated cross-linking was the main factor to affect the surface hydrophobicity of the jute fiber membranes.

Table 3. Mechanical Properties and Surface Hydrophobicity of Jute Fiber Membranes *via* Multi-Enzyme Treatments

Performance indicators	Control	Laccase	Xylanase/Laccase	Cellulase/Laccase
Tensile strength (N·m/g)	6.3±0.2	8.8±0.1	9.7±0.2	11.1±0.1
Tear strength (mN·m ² /g)	1.4±0.3	1.8±0.2	1.9±0.2	2.2±0.2
Burst strength (kPa·m ² /g)	1.038±0.003	1.098±0.003	1.106±0.005	1.182±0.003
Contact angle (°)	0.00±0	95.48±0.28	96.17±0.12	97.53±0.29
Wetting time/thickness (s/mm)	664.0±45.5	3058.0±213.5	3454.4±58.9	3655.6±39.5

FT-IR Analysis

Figure 5 shows a comparison of the ATR-IR spectra of control jute fiber membranes and jute fiber membranes modified by cellulase/laccase combined treatment. The broad peak at 3335 cm⁻¹ corresponds to -OH stretching vibration, and the aromatic skeletal vibration of lignin appears at 1594 cm⁻¹. The C=O stretching vibration of ester groups in lignin structures is observed at 1646 cm⁻¹. The strong peak at 1031 cm⁻¹ represents the C-O stretching vibration in cellulose, hemicellulose, and lignin. Compared with control jute fiber membranes, cellulase/laccase treated fiber membranes had stronger peaks in the aromatic skeletal vibration and C=O stretching vibration of ester, which suggested more lignin was exposed on the fiber surface *via* the cellulase process. The cellulase-assisted hydrolysis of cellulose can occur on the jute fiber surface, which is mostly composed of cellulose, hemicellulose, and lignin. Additionally, the cellulase/laccase combined treatment led to an enhancement in the C-O stretching vibration at 1031 cm⁻¹. This can be attributed to the subsequent cross-linking of lignin by laccase with ether bonds. Several studies have reported an increase in the number of hydroxyl groups of lignin after laccase treatment (Kim *et al.* 2009; Zhou *et al.* 2013b). This finding was also

supported in this work by an increase in the –OH stretching vibration in the IR spectrum of cellulase/laccase-treated fiber membranes.

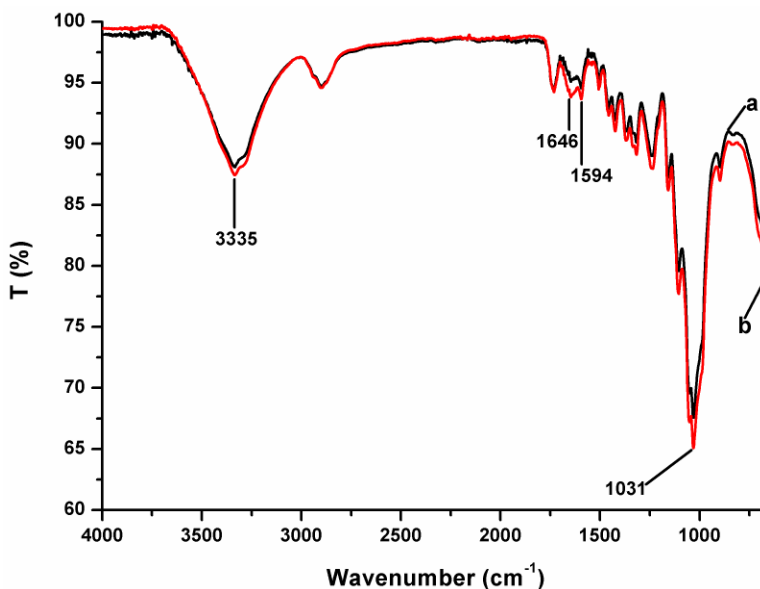


Fig. 5. FT-IR spectra of (a) control jute fiber membranes and (b) cellulase/laccase-treated jute fiber membranes

SEM Analysis

The surface morphologies of control jute fiber membranes and cellulase/laccase-treated fiber membranes were investigated by SEM and are shown in Fig. 6. The control surface in Fig. 6a was rough and covered with some natural impurities. After the cellulase/laccase combined treatment, the jute fiber became neat and smooth (Fig. 6b), probably because of the removal of cellulose by cellulase, as well as the enzymatic dislodgement and redistribution of bulky lignins on the surface. The lignin removed from the jute surface at the beginning of the laccase treatment can polymerize and covalently reattach to the surface in the later stages of the reaction (Zhou *et al.* 2013b; Dong *et al.* 2015). In addition, cellulase/laccase-treated jute fiber membranes showed more combinations between jute short fibers, which indicated higher mechanical properties for the fiber membranes.

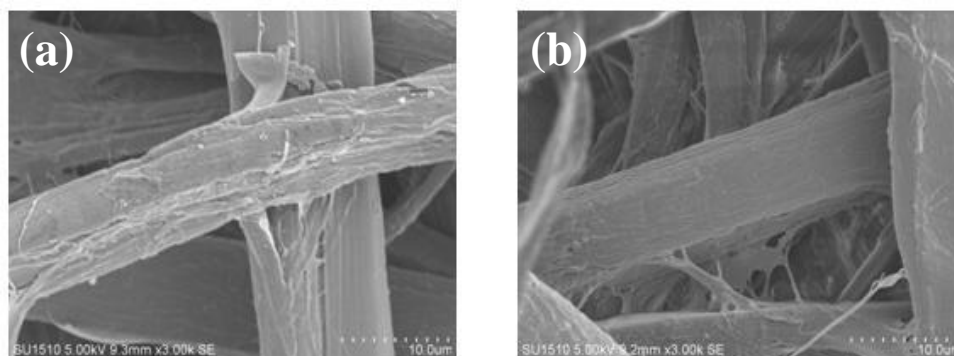


Fig. 6. SEM images of jute fiber membranes after (a) control treatment and (b) cellulase/laccase combined treatment

CONCLUSIONS

1. Fiber membranes were successfully prepared from jute fragments, a rich lignocellulosic bioresource. To enhance the properties of the jute fiber membranes without adhesives, green chemistry, using enzymes as biotechnological catalysts, was employed.
2. After the laccase treatment of jute fragments, the mechanical properties and surface hydrophobicity of the produced fiber membranes both increased because of the cross-coupling of lignin mediated by laccase with ether bonds. The optimum reaction conditions were a buffer pH of 4.5 and an incubation temperature of 60 °C with 0.92 U/mL laccase for 3 h.
3. Laccase/mediator systems had various effects on the jute fiber membranes. Laccase/guaiacol and laccase/alkali lignin treatments resulted in increases in the mechanical properties of the fiber membranes. In contrast, laccase/ABTS and laccase/DMP treatments led to a decrease in the mechanical properties. The laccase/guaiacol system increased the surface hydrophobicity of jute fiber membranes. However, the laccase/alkali lignin system had the opposite effect.
4. Xylanase/laccase and cellulase/laccase combined treatments can enhance both the mechanical properties and the surface hydrophobicity of the jute fiber membranes. Among these, cellulase/laccase treatment was the best; compared to mechanical properties, the surface hydrophobicity of the jute fiber membranes only showed a slight increase after the enzymatic multi-step processes.
5. This eco-friendly enzymatic process provides an attractive alternative to the current methods for improving the mechanical and surface hydrophobic properties of jute or other lignocellulosic fiber membranes. Enzyme-modified jute fiber membranes or other natural fiber membranes containing lignins could fulfill the mechanical and hydrophobic requirements of their corresponding products with better performance.

ACKNOWLEDGMENTS

This work was financially supported by the National Natural Science Foundation of China (51173071, 21274055), Program for New Century Excellent Talents in University (NCET-12-0883), Program for Changjiang Scholars, Innovative Research Team in University (IRT_15R26), and Fundamental Research Funds for the Central Universities (JUSRP51312B, JUSRP51505).

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Article submitted: December 6, 2015; Peer review completed: January 23, 2016; Revised version received and accepted: February 6, 2016; Published: February 12, 2016.

DOI: 10.15376/biores.11.2.3289-3302