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Functional Modification of Jute Fiber by Enzymatic Grafting of Gallate Esters

Chunxiao Zhou[a,b], Qiang Wang[a], Yuanyuan Yu[a], Xuerong Fan*[a], Yuanlin Cao*[c], Tiejun Li[b]

[a] Key Laboratory of Eco-Textiles, Ministry of Education, Jiangnan University, Wuxi 214122, China
[b] Nantong Vocational University, Nantong 226007, China
[c] Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China
wxfxr@163.com

To improve the interfacial compatibility between the hydrophobic polymer matrix and the jute fiber as a composite reinforcement, laccase-mediated hydrophobic modification of jute fiber by grafting gallate monomers was performed. The influence of pH, temperature, reaction time, laccase, monomer concentrations, and Cu^{2+} concentration on grafting percentage of jute was investigated to optimize the grafting process. The grafting products were characterized by infrared spectroscopy and scanning electron microscopy. The Hydrophobicity of jute before and after grafting were determined. Gallate monomers were successfully grafted onto jute fiber. Moreover, an ideal grafting percentage can be obtained for jute through process optimization. Jute-dodecyl gallate graft shows the optimum hydrophobicity (water contact angle = 127.8; wetting time > 30 min).

1. Introduction

Jute has attracted great attention in the field of composite materials due to their excellent mechanical properties, easy biodegradability, wide raw source, low price and many other advantages (Rafiquzzaman et al. 2016). However, enzymatic biochemical modification of laccase catalysis jute has become a research hotspot according to its poor chemical composition, low performance uniformity and weak interfacial bonding with hydrophobic substrates, which greatly limit the expansion of their applications (Román-Aguirre et al. 2004).
Laccase can catalyze the oxidation of jute lignin to form free radicals and then lead to hydrophobic monomers forms a hydrophobic polymer structure on jute surface, which effectively improves its interfacial compatibility with the hydrophobic resin, thereby enhancing the mechanical property of the plant-fiber-reinforced composite (Terzopoulou et al. 2015). The proposed enzymatic pathway is as shown in Figure 1.

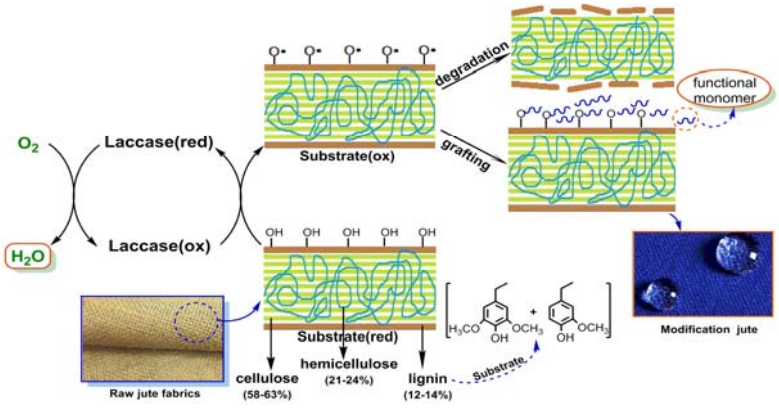

Figure 1: Grafting process of jute fiber through its activation and formation of radicals by laccase.

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In our previous work, reactive oxygen species free radicals generated by laccase-activated jute fiber were determined (Zhou et al. 2014). Here, we further compared the effects of free radical formation during enzymatic grafting of the jute fiber using gallate esters. The grafting parameters were optimized by saponification and back-titration method, and the structure and property changes of the jute fiber before and after grafting were also discussed.

2. Experimental

2.1 Materials

Jute fibers were obtained from Yingjie Textile Co., Ltd (Shandong, China). Laccase (DeniLite II) from *Aspergillus* was provided by Novozymes Biotechnology Co., Ltd. (Shanghai, China). N-tert-butyl-phenylnitrone (PBN) was purchased from Sigma-Aldrich Inc. (Beijing, China). Propyl gallate (PG), octyl gallate (OG), and dodecyl gallate (DG) were purchased from Shanghai Shifeng Biological Technology Co., Ltd. (Shanghai, China).

2.2 Methods

Laccase-activated grafting of jute with gallate esters:
Jute sample of 4 g was incubated in 200 mL 0.2 M acetate acetic acid-sodium acetate buffer/ethanol solutions (4:1, V:V) among a shaking bath with the optimum enzyme catalyzed grafting conditions such as laccase and gallate ester dosage, Cu^{2+} concentration, pH value, temperature and reaction time. After the reaction is over, the sample was removed and washed first with cold water and then with warm water (80 °C) for 10min and dried. Lastly, the jute fiber was extracted at 75 °C for 12 h and dried to obtain the grafted product.
Grafting percentage measured by saponification-back titration:
The grafting percentage (Gp) was calculated by determining the amount of ester groups introduced into the jute-gallate ester graft copolymer. Here, 50 mL of sodium hydroxide solution (0.1 mM) was added into a flask containing about 1 g of the dried grafted jute powder. The mixture was then heated under reflux at 90 °C degrees for about 2 h to fully saponify the ester group. The reaction mixture was cooled and the excess sodium hydroxide was titrated with 0.0997 mol/L standard hydrochloric acid solution. The volume of HCl used at the titration endpoint was recorded. Three independent tests were performed and the average volume of HCl, V_G, was taken. The same titration process was carried out on a control jute sample (blank sample), and the volume of HCl used was denoted as V_0. The grafting percentage (Gp) was calculated as Eq(1) (Ren and zhu 2002):

$$Gp\ (\%) = \frac{(V_0 - V_G) \cdot C_{HCl} \cdot M_G}{3} \cdot 100 \quad (1)$$

where C_{HCl} is the concentration of HCl (mol/L) used in the titration and M_G is the molar mass of monomers to be grafted (g/mol).
Optimization of enzyme-catalyzed grafting:
The grafting process was optimized by varying each individual factor. First, the grafting of the jute samples was carried out for different time (1, 2, 3, 4, and 5 h) under the following conditions: laccase=1.5 U/mL; pH=4; temperature=50 °C; and gallate ester=10 mM, and the Gp was determined to obtain the optimal reaction time. Using the optimal grafting time, and by keeping the other conditions unchanged, the pH of the buffer was altered (2.5, 3, 3.5, 4, 4.5, and 5) to find the optimum pH. Adopting both the optimal pH and reaction time and keeping the amount of laccase and gallate ester monomers same as above, grafting was carried out to select the optimum temperature. Then grafting was carried out to optimize the concentration of laccase by adopting the best grafting pH, temperature, and time, and keeping the monomer concentration of 10 mM. Under the optimized conditions of laccase concentration, pH, temperature, and reaction time, the concentration of gallate ester was changed (4, 6, 8, 10, 12, and 14 mM) to optimize the monomer concentration. Lastly, the concentration of external Cu^{2+} was optimized by changing the amount of $Cu2^{+}$ (0, 5, 10, 15, 20, and 40 mmol/L) in reaction system under the optimum enzyme catalyzed grafting conditions.
The capture and determination of ROS free radicals produced by Laccase catalyzsis jute lignin:
First, PBN solution (50 μL, final concentration of 10 mM) and a measurable amount of laccases (0-0.72U/mL) and $CuSO_4$ (only added when the effect of the concentration of Cu^{2+} was being determined) were added into 2.5-mL centrifuge tubes (containing 0.045 g of jute powder per tube). Then, buffer solutions with various pH values were added into these centrifuged tubes ensuring each reaction system totals 500 μL. The suspensions were mixed by shaking and incubated under various conditions same as Zhou et al (2014). Finally, the reactions were stopped by placing the tubes in an ice bath at the end of the reaction. Ethyl acetate

(300 µL) was added into each tube to extract the PBN–ROS adduct. The ROS levels were measured by using a 200D-SRC Spectrometer (Bruker, Germany) at room temperature. The mean height of the three characteristic peaks in each EPR signal was taken as the relative intensity of the ROS free radical. Every sample was measured in triplicate (Zhou et al. 2014).

2.3 Characterization

Hydrophobicity analysis:
Water contact angle: The jute sample was equilibrated at 21 °C and a constant relative humidity of 65% for over 4 h. And then was measured on an SL200B Contact Angle Goniometer (Kino Industry Co., USA). During testing, a droplet of deionized water was dispensed from 10 mm above the fabric surface and photographed continuously, and the contact angle between the water droplet and fabric surface was measured. Each sample was measured five times and the average was taken.
Wetting time: The equilibrated jute sample was laid out on a platform. A droplet of deionized water was dropped from 5 mm above the fabric. The time for the droplet to completely wet the fabric from their first contact was recorded. Each sample was measured five times and the average was taken.
FT-IR analysis:
Nicolet iS10 Fourier Transform Infrared Spectrometer (Thermo Fisher Scientific, USA) was used to perform infrared spectroscopy of the jute fabric. The scanning range was 4000–650 cm^{-1}, the resolution was 4 cm^{-1}, and 16 scans per sample.
Scanning electron microscopy imaging:
The surface of control and grafted jute fiber samples was scanned with the SU1510 scanning electron microscope (Hitachi, Japan). The voltage used was 10.0 kV, and magnification was 1.00 k.

3. Optimization of the enzymatic grafting process

The technical parameters of reaction time, pH, reaction temperature, laccase and monomer concentrations, and concentration of external Cu^{2+} ion were optimized. Furthermore, the effects of free radical formation during enzymatic grafting of the jute fiber using gallate esters were compared. The results of process optimization are shown in Figure 2.

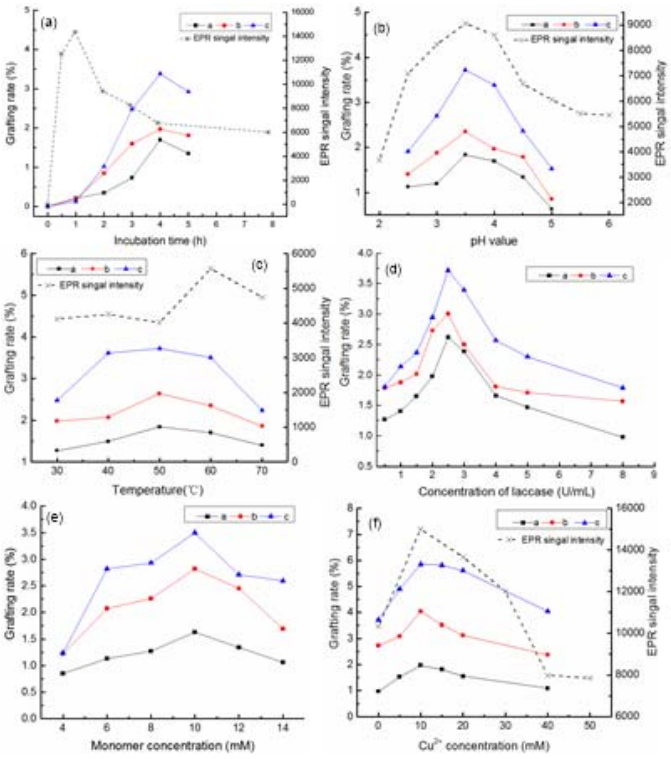

Figure 2: Optimization of laccase-catalyzed grafting of jute (a: grafting rate with PG; b: grafting rate with OG; and c: grafting rate with DG).

From Figure 2 (a), it can be seen that time significantly affects laccase-catalyzed free radical formation of jute and the subsequent grafting of gallate ester monomers. The concentration of free radicals rapidly reaches the peak after 1 h, while the optimum reaction time for the grafting of gallate esters was extended to 4 h. The reason could be the high reactivity of free radicals produced by laccase catalysis in jute, which, over time, become prone to reduction or coupling reaction between laccase and the substrate (Zhou et al. 2014). In the enzymatic grafting of jute, the free radicals formed in jute by laccase catalysis are rapidly consumed in the reaction with high concentration of grafting monomers, which reduces the probability of reduction and other side reactions on free radicals. When the grafting rate also exceeds the degradation rate of lignin, the grafting percentage reaches its peak (4 h) long after the enzyme-catalyzed free radical formation does (1 h). The monomers are consumed continuously with time. The increase in the grafting percentage slows down as the grafting rate is taken over by the degradation rate of lignin by laccase. The grafting percentage thus shows an overall decrease. Therefore, the optimum reaction time of laccase-activated grafting of jute fiber is 4 h.

As can be seen from Figure 2 (b), both the peak for laccase-catalyzed free radical formation in jute and the highest grafting percentage in gallate ester grafting appeared near the pH of 3.5–4.0. This indicates the high catalytic activity of laccase under such conditions. The optimum temperature for laccase-catalyzed free radical formation is around 60 °C, whereas that for the enzymatic grafting of jute is lower (at around 50 °C) (Figure 2 (c)). This could be due to the increased homopolymerization of monomers at the higher temperature of 60 °C. And the homopolymer easy adhesion on the surface of jute hindered the reaction of the monomer and jute lignin which resulted in the decrease of grafting rate. Therefore, the optimum grafting temperature should be around 50 °C. As shown in Figure 2 (d) and (e), laccase exerts a great influence upon the enzymatic grafting reaction and the highest grafting percentage occurs at 2.5 U/mL of laccase and 10 mmol/L of the monomer.

The copper ion added to the system facilitates the laccase-mediated reaction. Thus, the effects of Cu^{2+} concentration on the laccase-catalyzed free radical formation in the jute fiber and the subsequent enzymatic grafting were explored (Figure 2 (f)). The activation of Cu^{2+} on the enzymatic grafting reaction is remarkable . When 10–15 mM of Cu^{2+} is used, the grafting percentage of jute increases by more than 50%, reaching its highest value. Therefore, an appropriate amount of Cu^{2+} (10 mM) could enhance the catalytic activity of laccase.

4. Surface characterization of jute fabrics

4.1 FT-IR analysis

FT-IR spectroscopy was used to analyze the control jute and the jute grafted with propyl gallate, octyl gallate, and dodecyl gallate. The results are shown in Figure 3.

As shown in Figure 3, the control jute fiber displays strong characteristic absorption peaks at 3335 cm^{-1}, 2901 cm^{-1}, 1732 cm^{-1}, and 1650 cm^{-1}, corresponding to the stretching vibration of phenolic hydroxyl, saturated C-H, and C=O groups and C=O with absorbed water (Wen et al. 2013; Dong et al. 2014). The jute fiber is preprocessed to remove its wax and pectin, and mainly cellulose, hemicellulose, and lignin are left on the surface. Hydroxyl and saturated C-H bonds are present in all the three components of jute fiber, while the C=O group is derived from lignin (Faix 2009).

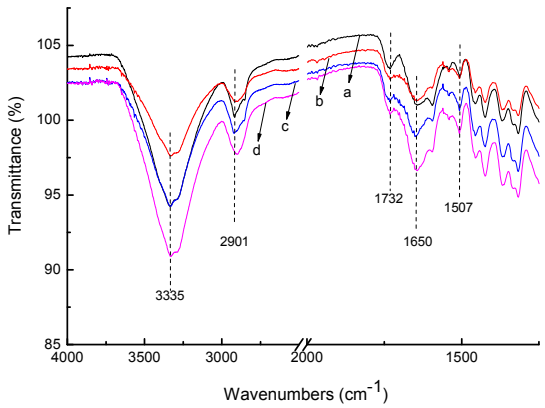

Figure 3: IR spectra for laccase-catalyzed grafting of jute with gallate ester monomers (a: control jute, b: jute grafted with PG (Gp = 1.97%), c: jute grafted with OG (Gp = 4.04%), and d: jute grafted with DG (Gp= 5.85), The symbols in the following figures and tables are the same as those in this figure).

Compared with the control jute fiber, the intensity of the absorption peak at 3200-3500 cm^{-1} for jute changes after gallate ester grafting. This could be due to the change in the content of phenolic hydroxyl groups at the jute surface after the grafting of monomers. In addition, the grafted jute fiber shows enhanced C=O stretching vibration at 1650 cm^{-1}. This confirms the sharp increase in the C=O content on the surface of the jute fiber following laccase-catalyzed grafting (Gadhe *et al*. 2006; Zhang et al. 2013). Moreover, the vibration absorption peak of the benzene ring at 1507 cm^{-1} also shows a significant increase (Dong et al. 2014). All these observations indicate the successful grafting of gallate esters onto the jute fiber by laccase-catalyzed reaction.

4.2 SEM analysis

Scanning electron microscopy was used to observe the change on the grafting jute surface. The results are shown in Figure 4.

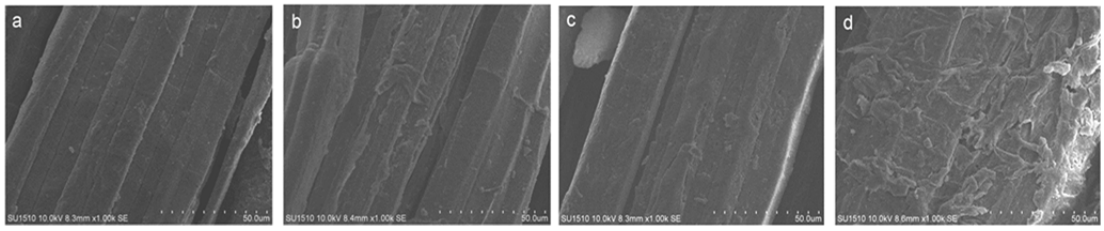

Figure 4: Side-way SEM images of control jute and laccase-catalyzed grafting of jute with gallate ester monomers.

As shown in Figure 4 (a), the fibers of the control jute are elongated and cylindrical, arranged in parallel with a rough surface and a gelatinous substance binding them together. The layers of jute fibers are separated by a soft membrane, which is destroyed during chemical digestion, but the gelatinous substance between the fibers is not completely decomposed (Mukherjee et al. 1981). Compared with the control jute, the grafted fibers show no mutual adhesion, except for the case shown in Figure 4 (d). This supports the notion that the laccase-catalyzed grafting occurs primarily between the monomers and jute lignin, and is not in the form of a physical attachment. A large amount of fibrous material is present on the jute surface grafted with DG, which is due to the long carbon chain of DG covering the jute surface.

5. Effects of grafting on the hydrophobicity of jute fiber

5.1 Measurement of water contact angle

To better illustrate the change in the hydrophobicity of the jute fiber after grating with gallate ester monomers, the static water contact angle of jute was measured. The results are shown in Table 1.

Table 1: Changes in the water contact angle of control jute and the jute grafted with PG, OG, and DG.

Sample	Water contact angle (°)			
	3 s	10 s	40 s	600 s
a	68.32 ± 0.95	0	0	0
b	109.59 ± 0.51	49.52 ± 0.33	0	0
c	114.12 ± 0.63	113.04 ± 0.35	111.44 ± 0.40	0
d	127.78 ± 0.42	126.96 ± 0.35	126.50 ± 0.24	118.19 ± 0.28

Table 1: shows that the surface contact angle of the jute fiber grafted with gallate ester monomers is greater than 90° at the beginning of the measurement in all cases, exhibiting water repelling/hydrophobic property (zhu et al. 2007). The contact angle increases gradually with the increase in the length of the hydrophobic carbon chain, i.e., the hydrophobic property stabilizes and increases. Among them, the jute grafted with DG shows the most persistent, stable, and best hydrophobic property, followed by that shown by jute fibers grafted with OG. The jute grafted with PG only obtained transient hydrophobicity.

5.2 Measurement of wetting time

To assess the change in the wetting time of the control and grafted jute, a water drop test is carried out on the fabric following the measurement of water contact angle. The results are shown in Table 2.

Table 2: Wetting time of control and grafted jute

Sample	a	b	c	d
Wetting time (s)	4.7± 0.5	12.3 ± 0.5	245 ± 2.0	>1800

From Table 2, it can be seen clearly that jute grafted with DG shows wetting time of more than 30 min, which reveals the best hydrophobicity. In contrast, the wetting time of jute grafted with OG is slightly shorter, which is consistent with the water contact angle measurements. Thus, grafting prolongs the wetting time of jute.

6. Conclusions

Laccase is capable of catalyzing the genesis of free radicals in jute fiber lignin, which triggers the hydrophobic modification of jute by grafting gallate ester monomers. The hydrophobic property increases gradually with the increase in the length of hydrophobic carbon chain. At conditions of pH of 3.5; temperature of 50 °C; laccase concentration of 2.5 U/mL; gallate ester concentration of 10 mM; external Cu^{2+} concentration of 10 mM and reaction time of 4 h, the ideal grafting percentage can be realized on jute. This eco-friendly approach for surface functionalization of lignocellulosic fiber meets the community's environmental requirements. Furthermore, given jute excellent hydrophobic properties, which greatly expanding its application in the field of composite materials.

Acknowledgments

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