Journal of Molecular Catalysis B: Enzymatic 122 (2015) 289–295

Contents lists available at ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

journal homepage: www.elsevier.com/locate/molcatb

Laccase-mediated biografting of *p*-coumaric acid for development of antibacterial and hydrophobic properties in coconut fibers

Kamini Thakur[a], Susheel Kalia[b,*], Neha Sharma[c], Deepak Pathania[a]

[a] School of Chemistry, Shoolini University, Solan, 173212 HP India
[b] Department of Chemistry, Army Cadet College Wing, Indian Military Academy, Dehradun,248007 UK India
[c] Department of Microbiology, Shoolini University, Solan, 173212 HP India

ARTICLE INFO

Article history:
Received 10 May 2015
Received in revised form
14 September 2015
Accepted 6 October 2015
Available online 20 October 2015

Key words:
Coconut fibers
Lignin
Biografting
Antibacterial property
Hydrophobicity

ABSTRACT

Laccase-mediated biografting of *p*-coumaric acid was carried out on coconut fruit fibers to develop the new properties. Optimization of reaction parameters was carried out in order to get maximum biografting of *p*-coumaric acid. Maximum percentage biografting was observed at 4.5% (w/w) of *p*-coumaric acid, 40 U/g of laccase and 24 h incubation time. Grafted fibers were characterized by FTIR, SEM, XRD and TGA techniques in order to check the biografting, change in morphology, crystallinity and thermal stability, respectively. Moisture retention studies were carried out at 55% and 75% RH and biografting of *p*-coumaric acid has developed the hydrophobic nature in coconut fibers. Colony forming unit method was used to study the antibacterial behavior of fibers against the gram negative (*Escherichia coli*) and gram positive (*Staphylococcus aureus*) bacteria. Laccase-mediated biografting of *p*-coumaric acid has developed antibacterial property in the coconut fibers.

1. Introduction

Enzymatic biografting is a green approach to modify the lignocellulosic materials with the development of hydrophobic and antibacterial functional groups. Laccase treatment improves the physical, chemical and mechanical properties of lignocellulosic material [1,2]. Enzymatic biografting of natural antimicrobial and hydrophobic organic molecules to lignocellulosic fibers is a valid solution to meet the growing demands of consumers regarding the safer products, hygiene standards and environment protection [3]. Lignocellulosic fibers have poor microbial resistance [3] and high hydrophilicity [4]. Laccase-assisted surface functionalization of flax [5,6], kraft pulp [7], wood surface [8,9] and beech wood [10] was carried out to impart new properties such as antimicrobial and hydrophobicity. Laccase catalyzes the oxidation of various phenolic substrates with simultaneous reduction of molecular oxygen to water and results into grafting, coupling and cross coupling processes by free radical mechanism [11]. Biografting of phenolic compounds takes place in the active sites generated by laccase. Acero et al. [12] have studied the two-step mechanism for the grafting of ferulic acid on the surface of polyamide by laccase (from *Trametes hirsuta*).

Lignin is a three dimensional complex structure containing structural units of guaiacyl, syringyl and p-hydroxyphenyl [13]. Lignin has been considered as a suitable substrate for laccase as phenolic sites of lignin could be oxidized to phenoxyl radicals. As a result, a radical-rich surface was generated, where oxidized phenolic molecules can be grafted by laccase simultaneously. Various phenolic compounds extracted from natural sources are found to exhibit antimicrobial activities because of phenolic acids present in the extracts [14–17]. Antimicrobial raw materials have gained interest in a wide range of fields such as food packaging, sanitary materials, households, military and medical items [18]. The hydrophilic nature of lignocellulosic materials was reduced by the laccase assisted treatment with different hydrophobic moieties [19,20].

Laccase-mediated biografting is mainly targeted on lignin and coconut fibers have 40–45% lignin content [21]. In the present work, lignin-rich coconut fibers were used as substrate for laccase-mediated biografting of *p*-coumaric acid (PCA). Antibacterial and hydrophobic properties of biografted coconut fibers were analyzed by colony forming unit (CFU) and moisture retention methods, respectively.

* Corresponding author.
E-mail address: susheel.kalia@gmail.com (S. Kalia).

http://dx.doi.org/10.1016/j.molcatb.2015.10.002
1381-1177/

2. Materials and methods

2.1. Fiber sample, enzyme and natural phenol

Coconut fibers were collected from the local market (H.P.) India. Fibers were washed with mild detergent followed by distilled water to remove the water soluble impurities. Clean fibers were then Soxhlet extracted with acetone for 24 h for further purification. Laccase from *Trametes versicolor*) and *p*-coumaric acid were purchased from Sigma–Aldrich. Citric acid (Himedia), sodium citrate (Himedia), nutrient broth (Himedia) and nutrient agar (Himedia) were used as received.

2.2. Laccase-mediated biografting

Optimum incubation conditions were determined for enzymatic biografting by using an experimental design with three parameters, i.e. laccase concentration, PCA concentration and incubation time. Biografting process was carried out in a 250 mL Erlenmeyer flask containing 200 mg of coconut fibers, 40 mM citrate buffer (pH 4), 3.5% (w/w) *p*-coumaric acid (relative to dry fiber weight), and 40 U laccase/g fiber. The reaction mixture was incubated with 30 rpm shaking at 50 °C for 24 h. Control reaction was also carried out under identical conditions in the absence of PCA. After biografting, treated fibers were washed thoroughly with distilled water and then Soxhlet extracted with acetone for 12 h to remove the fraction of unreacted PCA. Fiber samples were then dried in a vacuum oven to a constant weight [6].

2.3. Quantitative analysis of biografting

Quantitative analysis of biografting was done by weighing method. The percentage of biografting was determined by using the following equation:

$$\text{Biografting}\,(\%) = \frac{w_2 - w_1}{w_1} \times 100$$

where w_2 is weight of the biografted fibers and w_1 is the final weight of control sample [22].

2.4. Characterization of raw and biografted fibers

FTIR spectra of original and grafted coconut fibers were taken with KBr pellets on PerkinElmer RXI Spectrophotometer over a range of 400–4000 cm^{-1}. This technique is useful to identify the chemical groups or unknown composition and the intensity of absorption spectra was associated with the molecular composition of the chemical group. The surface morphology of raw and biografted coconut fibers was examined by scanning electron microscope (Jeol JSM-6610LV). Thermogravimetric analysis (TGA) of raw and biografted fibers was done at a heating rate of 10 °C/min in an inert atmosphere using a Perkin-Elmer TGA. XRD studies were done on a Brucker D_8 Advance X-ray diffractometer under ambient conditions. Percentage crystallinity (%Cr) was calculated as follows:

$$\%\text{Cr} = \frac{I_{22}}{I_{22} + I_{18}} \times 100$$

where I_{22} and I_{18} are the crystalline and amorphous intensities at 2θ scale close to 22° and 18° respectively [23].

2.5. Antibacterial property

Antibacterial property was studied by Colony Forming Unit (CFU) method. This method decides whether the sample is able to inhibit the growth of bacteria or not by comparing colony count with the control. 0.05 g of raw and biografted coconut fibers were added to 10 mL of nutrient broth followed by the addition of inoculated culture of *Staphylococcus aureus* or *Escherichia coli*. (1 mL) and incubated at 37 °C. Colonies were counted after 24–48 h by seeding the aliquot of incubated sample on nutrient agar plates. The number of microorganisms growing on the plate was multiplied by dilution factor and divided by the volume used to seed the plate in order to determine the CFU mL^{-1} [24].

$$\frac{\text{CFU}}{\text{mL}} = \frac{\text{colony count on plate} \times \text{dilution factor}}{\text{sample poured on plate}}$$

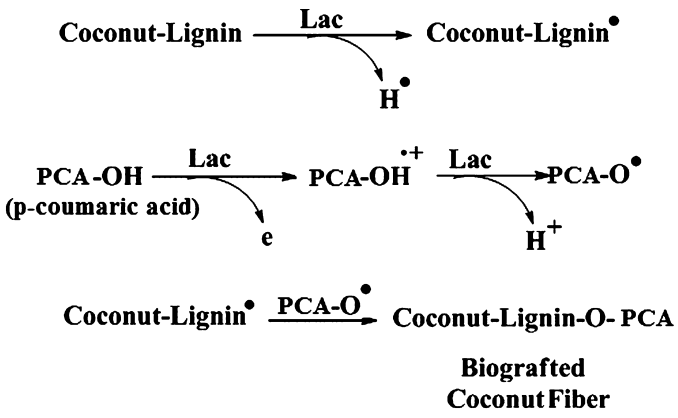

Fig. 1. Mechanism of laccase catalyzed biografting of PCA on coconut fibers.

2.6. Hydrophobicity of raw and biografted fibers

Hydrophobic studies of raw and biografted fibers was done in a humidity chamber. Raw and biografted coconut fibers were dried at 80 °C for 4 h to attain the equal moisture level. Fiber samples were then exposed to 55% and 75% relative humidity (RH) at 23 °C in a humidity chamber. Fibers were removed quickly from the chamber after every hour and weighed till the constant weight was obtained. The percentage weight gain was calculated by following formula [25].

$$\text{Weightgain\%} = \frac{w_f - w_i}{w_i} \times 100$$

where w_i and w_f are the initial and final weight of fibers, respectively.

3. Results and discussion

Laccase-mediated biografting of PCA on coconut fibers was proceeded in three steps. Lignin free radicals were generated by laccase in the first step. In the second step, radical cation was formed due to the electron transfer process, which was followed by a quick removal of hydrogen ion (deprotonation) and this result in the generation of phenoxy radical. The formation of phenoxy radical is a rate determining step in the laccase catalyzed oxidation of phenolic compounds. Phenoxy radical of *p*-coumaric acid formed in the second step has very less stability. In the last step, phenoxy radicals coupled with the lignin radicals to get stabilized and results in biografting (Fig. 1) [26].

3.1. Optimization of reaction parameters

The amount of reactants affects the enzymatic reactions to a great extent. So, PCA concentration, laccase concentration and the incubation period were optimized in laccase-mediated biografting. Different concentrations of phenol i.e. 2.5%, 3.5%, 4.5% and