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Separation and characterization of cellulose fibers from cypress wood treated with ionic liquid prior to laccase treatment

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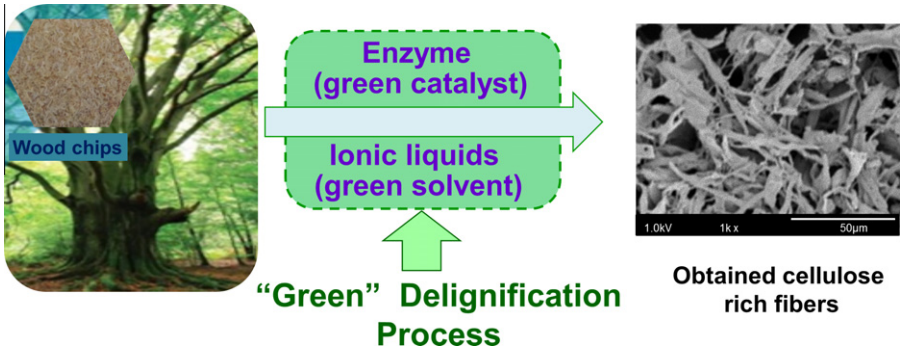
HIGHLIGHTS

- Wood biomass is treated with an IL and IL is recovered prior to enzymatic delignification.
- IL pretreatment improved the delignification efficiency significantly.
- The α-cellulose content of produced fibers is as high as 73.1%.
- Obtained cellulose fibers had a higher crystallinity and thermal stability than untreated wood fibers.

GRAPHICAL ABSTRACT



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ABSTRACT

Separation of cellulose fibers (CFs) from woody biomass with minimal structural alteration using a "green" and efficient method was achieved by treatment with the ionic liquid (IL), [emim][OAc] (1-ethyl-3-methylimidazolium acetate) at 80 °C for 1 h. The IL was recovered by rinsing with water–acetone mixture prior to treatment of the wood with *Trametes* sp. laccase in the presence of 1-hydroxybenzotriazole as a mediator. IL pretreatment did not significantly change the chemical composition of the wood, but did alter its structure and rendered its surface more accessible to the enzyme. Treated and untreated samples were characterized by SEM, FTIR, XRD, TGA, and chemical methods. The cellulose content of the produced fibers was approximately 73.1% and the lignin content was 9.8%, much lower than the lignin content of 29.3% of the untreated wood. The cellulose fibers exhibited higher cellulose crystallinity and better thermal stability compared to untreated wood materials.

1. Introduction

Wood represents a carbon-neutral renewable resource for bioenergy and biomaterials production. Wood is mainly composed of the rigid semi-crystalline polysaccharide cellulose, the amorphous multicomponent polysaccharide hemicellulose and the amorphous aromatic polymer lignin. These three biopolymers are the primary constituents of plant cell walls, in which cellulose fibers are embedded in a cross-linked matrix of lignin and hemicellulose, forming a tight and compact structure. Among these biopolymers, cellulose has been used extensively as source of raw materials for the production of biocompatible and biodegradable materials/biocomposites; however, structural heterogeneity and complexity of the cell-wall microfibrils in wood are the biggest challenges to the clean separation of celluloses with minimal polymer degradation.

Presently, physical (*e.g.*, pyrolysis and mechanical disruption) (Mosier et al., 2005), physico-chemical (*e.g.*, steam explosion and ammonia fiber explosion) (Hendricks and Zeerman, 2009; Gabriele

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et al., 2010), chemical (*e.g.*, acid hydrolysis, alkaline hydrolysis and oxidative delignification) (Zhao et al., 2008), and biological methods (Bak et al., 2009) have been investigated for extraction of cellulose at laboratory and pilot-plant scales. Most of these methods require high temperatures and pressures as well as highly concentrated chemicals for the cooking process. Sulfates and sulfite pulping processes pose serious environmental hazards. Moreover, high temperature-based cooking processes result in the production of inhibitory chemicals and degradation products. Besides, although biological treatment with enzymes can be performed under mild reaction conditions, this approach is very slow in aqueous systems, mainly due to the difficulties in enzyme accessibility to the solid substrate and the poor solubility of lignin (Sousa et al., 2009). It is therefore desirable to develop a wood pretreatment process that is not only environmentally friendly but also efficient and cost effective for wood conversion to cellulose with minimal structural alteration.

Ionic liquids (ILs) represent nonvolatile, thermally stable, nonflammable, and tunable designer solvents that can replace highly volatile organic solvents (VOSs) in a wide range of applications (MacFarlane and Seddon, 2007). Many ILs have been used to dissolve wood and other lignocellulosic biomass at high temperatures, and cellulose rich materials and lignin can readily be separated by the addition of a variety of precipitating solvents (Kilpelainen et al., 2007; Mora-pale et al., 2011; Sun et al., 2009, 2011a; Wang et al., 2011; Zavrel et al., 2009); however, significant losses of cellulose and other carbohydrates and only partial delignification have been observed (Sun et al., 2009). To address such limitations, various approaches, including addition of ammonia or oxygen during cooking (Rodriguez et al., 2011), using polyoxometalate (POMs) catalysts (Sun et al., 2011b), and designing new types of ILs (Wang et al., 2011) have been taken to enhance the delignification of biomass. IL pretreatments reduce the degree of polymerization of the recovered cellulose-rich materials (Lee et al., 2009), leading to enhanced enzymatic cellulose hydrolysis (Nguyen et al., 2010; Shill et al., 2011); however, cellulose fibers with a high degree of crystallinity are desirable as reinforcement fibers in biocomposite applications to enhance stiffness, dimensional stability, and fire, moisture diffusion, and thermal resistance. Singh et al. (2009) reported that a short IL pretreatment of switchgrass easily weaken the network of the cell wall components. Thus, selective delignification of wood via biological pretreatment of IL-swollen wood materials may be effective in isolating cellulose fibers with a minimum of modification to their structure.

Enzymatic delignification efficiency can be improved by IL pretreatment of the wood biomass (Moniruzzaman and Ono, 2012). In this one-step process, 10 wt.% wood chips in IL were cooked and an aqueous solution containing laccase from *Trametes sp.* was added directly to start delignification. Preliminary results indicated that the enzymatic delignification efficiency of IL-swollen wood biomass was higher than that of untreated materials. At the optimum IL concentration, 50% delignified wood fibers were obtained. More significantly, the cellulose fibers showed a higher degree of crystallinity than untreated wood fibers. This result provided the impetus to design a process whereby wood was first treated with the IL, [emim][OAc] (1-ethyl-3-methylimidazolium acetate), the IL was recovered from the treated wood, and the wood was subjected to enzymatic treatment. Chemical characterization of treated and untreated wood materials was performed to determine their cellulose, hemicellulose, and lignin contents. Physical characteristics including cellulose crystallinity, thermal stability, and wood cell wall morphology were investigated using X-ray diffraction (XRD), scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), and thermogravimetric analysis (TGA). Commercial laccase, a copper-containing oxidase enzyme obtained from white rot fungi, was selected as a biocatalyst because it can degrade biomass lignin while leaving other components (*e.g.*, cellulose) virtually untouched (Blanchette, 1991).

2. Methods

2.1. Materials and reagents

Wood chips from hinoki cypress (*Chamaecyparis obtusa*) from the Okayama Biomass Center, Japan were dried in an oven at 110 °C and atmospheric pressure. The IL, [emim][OAc] (1-ethyl-3-methylimidazolium acetate) (⩾95%), was obtained from Ionic Liquids Technologies GmbH (Heilbronn,Germany) and used as received. Commercial laccase Y120 (EC.1.10.3.2, 1000 U/g) from *Trametes sp.* was kindly supplied by Amano Enzyme Inc. (Nagoya, Japan). 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt (ABTS) (98%) and 1-hydroxybenzotriazole (HBT) were obtained from Sigma (St. Louis, MO, USA). All other reagents used in the experiments were analytical grade.

2.2. Ionic liquid pretreatment and delignification

In a typical experiment, 0.4 g wood chips (110–550 μM) and 4.0 g IL were placed in a flask and heated at 80 °C in an oil bath with magnetic stirring for 1 h. After cooling the wood–IL mixture to RT, water–acetone (1:1 v/v) was added as an anti-solvent and stirred at RT for 20 min to separate the solid materials from the dissolved lignin and IL. After allowing the mixture to settle, the treated wood, lignin, and IL were recovered as reported by Sun et al. (2009). The treated wood was washed several times with distilled water to remove residual IL. Dried treated wood and sodium acetate buffer (100 mM, pH 4.5) (ca. 5 wt.% biomass) were placed in a three-neck flask and homogenized by ultrasonic homogenizer (Sonic & Materials Inc.). Laccase (200 U/g biomass) was added to the flask while 1-hydroxybenzotriazole (HBT) (1.5 wt.% of biomass) was added as a mediator. Reactions were carried out at 50 °C with O_2 bubbling and stirring, with or without IL. After 24 h, 0.1 M NaOH was added and the mixture was stirred for 1 h to extract lignin from the enzymatically delignified IL treated wood. The mixture was filtered under mild vacuum and cellulose-rich wood fibers (CRFs) were collected. To remove traces of NaOH, the CRFs were washed with distilled water until the wash water became neutral. The CRFs were oven dried at 70 °C and 0.1 MPa for 24 h to constant weight.

2.3. Measurement of laccase activity and stability

Laccase activity was determined by oxidation of 2–2′-azino-bis-(3-ethylbenzthiazoline-6-sulfonate) (ABTS). Stock laccase and ABTS solutions were prepared in 0.1 M sodium acetate buffer at pH 4.5. For enzymatic reactions, 20 μL laccase solution (2 mg mL^{-1}) was added to 1.96 mL buffer (with or without IL) and the contents were gently shaken at 50 °C so that they were combined with the reaction mixture at ambient temperature. Finally, 20 μL of 50 mM ABTS buffer solution was added to initiate the reaction. The change in absorbance at 420 nm ($\varepsilon_{420} = 3.6 \times 10^4\ M^{-1}\ cm^{-1}$) at 50 °C was recorded for 30 s and catalytic activity was determined from the slope of the resulting kinetic curve. The activity was expressed in relative units (%) where the activity value in aqueous buffer solution was set as 100%.

To determine the stability, buffer solutions with 0, 2.5, or 5 wt.% IL containing laccase were incubated in the absence of substrates at 50 °C. After incubation, the samples were withdrawn at predetermined time intervals in order to measure the remaining enzyme activity by the addition of substrate (ABTS). The stability of the enzyme was expressed as the residual activity, which was calculated