Bioresource Technology 127 (2013) 132–137

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

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

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

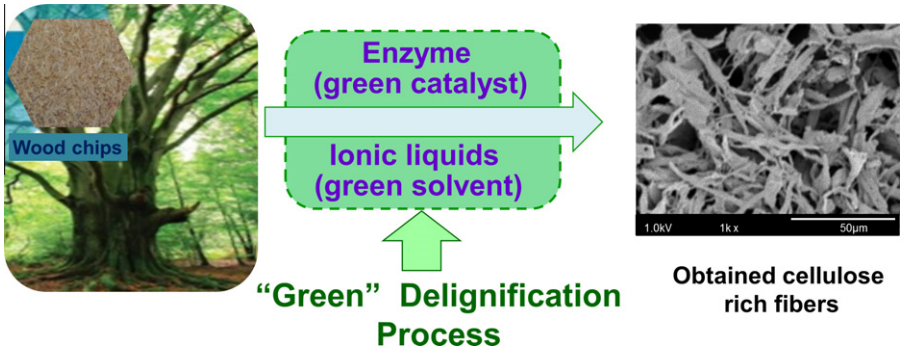
Muhammad Moniruzzaman *,†, Tsutomu Ono *

*Department of Applied Chemistry, Graduate School of Natural Science and Technology, Okayama University, 3-1-1 Tsushima-Naka, Kita-Ku, Okayama 700-8530, Japan*

## 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




## ARTICLE INFO

*Article history:*
Received 7 June 2012
Received in revised form 10 August 2012
Accepted 28 September 2012
Available online 8 October 2012

*Keywords:*
Wood biomass
Ionic liquids
Cellulose fibers
Laccase
Enzymatic delignification

## 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

* Corresponding authors. Tel./fax: +81 86 251 8072 (M. Moniruzzaman), +81 86 251 8083 (T. Ono).
*E-mail addresses:* moniru-m@cc.okayama-u.ac.jp (M. Moniruzzaman), tono@cc.okayama-u.ac.jp (T. Ono).
† Present address: Department of Chemical Engineering, Universiti Teknologi PETRONAS, 31750 Tronoh, Perak, Malaysia.

0960-8524/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biortech.2012.09.113

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

as a percentage of the original activity (considered 100%), obtained at $t = 0$ min incubation.

### 2.4. Characterization of untreated and treated materials

#### 2.4.1. Chemical characterization of the materials

The α-cellulose and hemicellulose contents of the untreated wood, IL treated wood and CRFs samples were analyzed as described by Wise et al. (1946). Briefly, the holocellulose content (α-cellulose + hemicelluloses) of the materials was determined through treatment with an acidified sodium chlorite solution at 70 °C for 1 h, with the process repeated until the product became white. Then, the α-cellulose content of the materials was determined by treatment with 6 wt.% sodium hydroxide at 80 °C overnight to leach hemicelluloses. The difference between the values of holocellulose and α-cellulose gave the hemicellulose content of the materials. The lignin content of the materials was determined using TAPPI methods (TAPPI, 1991; TAPPI, 1998) using a scaled-down process.

#### 2.4.2. Morphology of treated and untreated wood materials

The morphology of the fibers was characterized using a scanning electron microscope (SEM) (S-4700, Hitachi Ltd., Tokyo, Japan). Samples were mounted on metal stubs with double-faced tape, and coated with gold–palladium in a sputter coater (E1030 Ion Sputter, Hitachi Ltd.).

#### 2.4.3. Fourier transform infrared spectroscopy (FTIR)

The FTIR spectra of the samples were recorded from a KBr disk containing 1% finely ground sample on an IRPrestise-21 FTIR spectrophotometer (Shimadzu, Japan) in the range 4000–400 $cm^{-1}$ with a resolution of 4 $cm^{-1}$. Spectral outputs were recorded in transmittance mode as a function of wave number.

#### 2.4.4. Powder X-ray diffraction (PXRD)

The crystallinity of the untreated and treated wood materials was investigated by powder X-ray diffractometry (PXRD), using a XRD-6100 Diffraction System (Shimadzu, Japan). The diffraction patterns were measured from $2\theta = 8–40°$ with a scan speed of 0.1° $min^{-1}$ using Cu Kα radiation at 40 kV and 30 mA.

#### 2.4.5. Thermal characterization

Thermogravimetric analysis was performed to compare the degradation characteristics of the treated and untreated wood materials. The thermal stability of each sample was determined using a Pyris 1 thermogravimetric analyzer by heating 10 mg of sample in a platinum pan at a rate of 10 °C/min in a nitrogen atmosphere.

## 3. Results and discussion

### 3.1. Chemical composition of untreated and IL treated wood materials

Pretreatment of the wood with IL was carried out under moderate conditions (80 °C for 1 h) so that losses of major components of the wood, particularly, cellulose, were minimized. Another aim was to obtain IL treated wood containing cellulose with minimal alteration of its crystallinity. It has been reported that the crystallinity of IL treated wood highly depends on time and temperature of the IL pretreatment. Generally, elevated temperatures (100 °C or higher) and longer pretreatment times lead to complete dissolution of wood biomass, and decreased crystallinity of the regenerated cellulose-rich materials (Labbe et al., 2012; Lucas et al., 2011; Wang et al., 2011). The chemical compositions of untreated wood materials (UWMs) and IL treated wood are compared in Table 1. The wood biomass used in the present study had a composition comparable to that of typical soft-wood biomass. The yield of wood after IL treatment was about 87% of the untreated wood biomass weight. During IL treatment, swelling of wood cell walls occurs due to breaking of some of the bonds between major biopolymers in the wood matrix (Doherty et al., 2010; Lee et al., 2009). Consequently, a small portion of the hemicellulose and lignin is solubilized, resulting in the decrease of their content in the wood material after IL treatment. Thus, compared with the untreated sample, wood fibers after IL treatment had somewhat higher cellulose content due to the removal of hemicellulose, lignin, and water/IL/acetone soluble extractives during the pretreatment/recovery processes.

### 3.2. Enzymatic delignification of recovered wood after IL treatment

To examine the effects of IL pretreatment on the subsequent enzymatic delignification, the treated wood was subjected to biological pretreatment (at 50 °C for 24 h) using laccase. The reaction was carried out using acetate buffer containing 2.5 wt.% IL [emim][OAc]. This amount of IL was used during delignification because it was found that, although the initial activity of laccase decreased slightly in the presence of such an IL concentration, the stability of the enzyme at 50 °C was enhanced significantly (Fig. 1). As shown in Fig. 1B, laccase in aqueous medium retained 12.5% of its initial activity after 24 h, whereas about 52% of its original activity was retained in aqueous medium containing 2.5 wt.% IL over the same time. The IL [emim][OAc] is composed of the kosmotropic anion ($CH_3COO^-$) and the chaotropic cation ($[emim]^+$). It has been reported that the presence of such an IL at low concentration enhances the stability of many enzymes in aqueous media (Moniruzzaman et al., 2010a, 2010b; Shipovskove et al., 2008). Another possible advantage of including the IL is promotion of the dissolution of substrates and products (Pu, 2007), resulting in better process efficiency. To confirm these phenomena, the delignification efficiency of treated wood in aqueous media without IL was investigated. The results indicated that the cellulose fibers contained 16.5 wt.% lignin, whereas only 9.8 wt.% lignin remained in the fibers obtained from aqueous medium containing 2.5% IL. As shown in Table 1, after the enzymatic delignification of treated wood, the α-cellulose content of the obtained cellulose rich materials was increased from 41.2% to 73.1%, while hemicellulose and lignin contents were decreased to 8.5% and 9.8%, respectively. This outcome was as expected because IL pretreatment swells the wood structure, allowing penetration of the enzyme deep into the wood biomass for improved delignification. As shown in the process flow chart, after the end of the enzymatic delignification, the sample was diluted with 0.1 M NaOH to extract lignin. A portion of the hemicellulose was also leached in this step as reflected in the chemical composition of the finally produced CRFs. This chemical compositional change in the wood fiber may result in a higher degree of cellulose crystallinity and hence improved thermal properties and higher strength of the fibers.

### 3.3. Morphology of treated and untreated wood fibers

SEM images were taken to investigate the structure and morphology of the untreated wood, IL treated wood, and CRF samples, and are shown in Supplemental Figure. The surface of the untreated wood fibers was irregular due to the coating of the cellulose fibers with lignin and other non-cellulosic substances (Supplemental Fig. A). However, IL treated wood fibers (Supplemental Fig. B) had a relatively homogeneous macrostructure with increased porosity, possibly due to the fusion of wood fibers during IL pretreatment (Sun et al., 2009). This observation suggests that pretreatment with IL may restructure the wood to provide a more

**Table 1**
Chemical composition of the treated and untreated wood chips.[a]

| Materials | α-cellulose (%) | Hemicelluloses (%) | Total lignin (%)[b] |
|---|---|---|---|
| Untreated wood materials | 41.2 ± 1.3 | 25.2 ± 1.1 | 29.3 ± 1.7 |
| IL treated wood materials | 46.3 ± 2.1 | 15.3 ± 1.2 | 25.2 ± 3.2 |
| Cellulose rich fibers (CRFs) (IL + enzymatic treatment) | 73.1 ± 3.5 | 8.5 ± 1.0 | 9.8 ± 1.4 |

[a] The data represent the average of three experiments with standard deviation.
[b] Total lignin = Klasion lignin + acid soluble lignin.

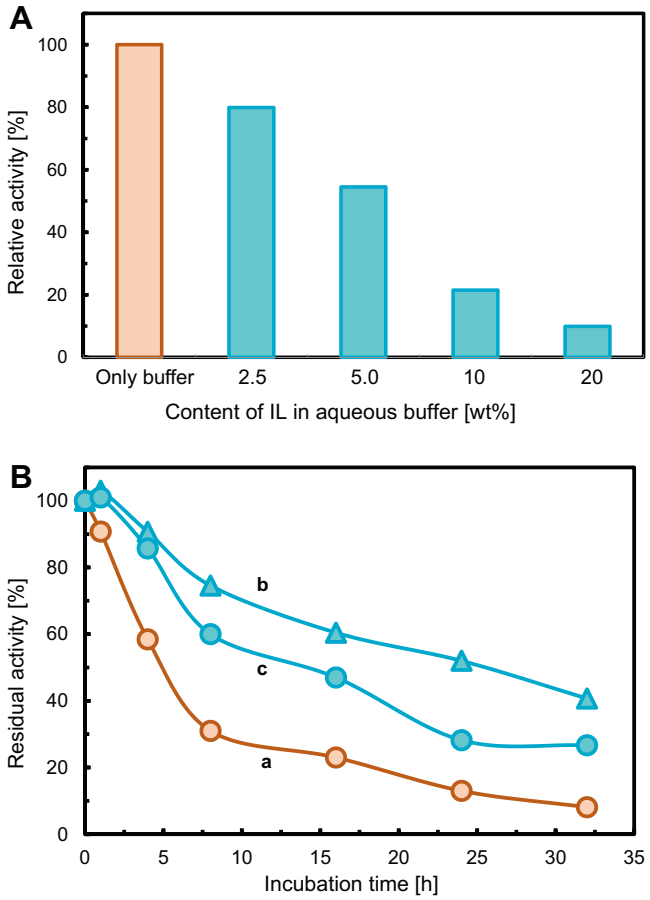



**Fig. 1.** Activity and stability of laccase in aqueous buffer solution containing various amounts of IL at 50 °C. (A) Relative percent activity of laccase in 100 mM sodium acetate pH 4.5 as a function of IL concentration. The activity of enzyme in pure buffer was taken as 100% activity. Activities plotted are averages of three measurements. (B) Influence of IL on laccase stability: (a) laccase in aqueous buffer, (b) laccase in buffer containing 2.5 wt.% IL, and (c) laccase in buffer containing 5 wt.% IL. Data plotted here are averages of three measurements.

accessible surface area, leading to enhanced enzymatic delignification. Interestingly, CRFs obtained from enzymatic delignification have smooth and clean surfaces (data not shown) because most of the non-cellulosic materials (*e.g.*, lignin) were removed during the IL and biological treatments. The wood cell networks composed of cellulose, hemicellulose, and lignin were broken down and cellulose fibers were partially separated into individual microsized fibers (Supplemental Fig. D), as previously observed for cellulose fibers obtained from wood by chemical pretreatment (Chen et al., 2011).

### 3.4. Spectroscopic analysis of the untreated and treated wood fibers

To determine any chemical changes in the wood materials occurring during IL pretreatment or enzymatic delignification, FTIR spectra of the untreated, IL-treated, and CRF samples were obtained and are compared in Fig. 2. The dominant peaks at 3346 $cm^{-1}$ (O–H stretch) and 2892 $cm^{-1}$ (C–H stretch) represent aliphatic moieties. The prominent peak at 1731 $cm^{-1}$ in the untreated wood is attributed to a C=O stretching vibration in acetyl groups of the hemicelluloses (Labbe et al., 2005). After IL pretreatment (Fig. 2, b), this peak disappeared, indicating that some of the hemicellulose was removed in the recovery step. The characteristic peaks of lignin at 1592/1503 (C=C stretching vibration), 1256 $cm^{-1}$ (asymmetric bending in $CH_3$), and 1251 $cm^{-1}$ (C–O vibration in the syringyl ring) (Labbe et al., 2005) remained in the IL treated sample but with lower intensity when compared with those from the untreated sample. This decrease in intensity is due to partial removal of lignin during IL treatment. The peaks disappeared after enzymatic delignification due to the removal of most of the lignin. The absorbance bands at 1150, 1052 and 896 $cm^{-1}$, corresponding to C–O–C asymmetric bridge stretching vibration in cellulose/hemicellulose, C–O stretching vibration in cellulose/hemicellulose, and C–H deformation vibration in cellulose, respectively, (Labbe et al., 2005), were more resolved in the CRF sample, indicating that the produced cellulose-rich wood fibers are richer in carbohydrates, consistent with the chemical composition study.

### 3.5. Crystallinity of untreated and treated wood fibers

Powder X-ray diffraction (PXRD) studies of the untreated wood materials, IL treated wood fibers, and CRFs were conducted to investigate the crystalline behavior of the fibers as shown in Fig. 3. All three samples showed major intensity peaks related to their crystalline structure at 2$\theta$ values of around 15.5° and 22.2°, indicating that IL pretreatment did not significantly change the crystallinity of the wood fibers. The peak intensities of the IL treated sample were slightly increased compared to those of the untreated sample. This outcome is in contrast to that reported by others who say a decrease in crystallinity upon IL pretreatment

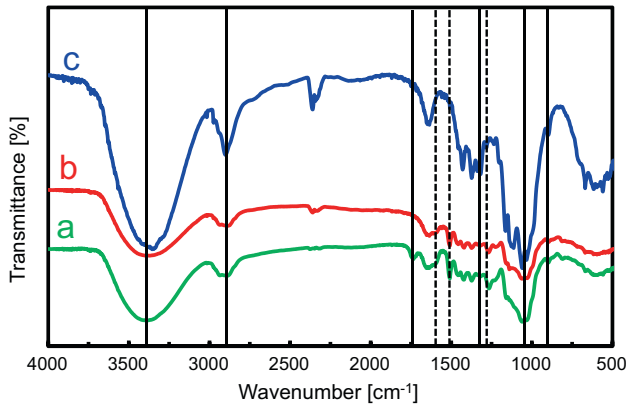



**Fig. 2.** FTIR spectra for (a) untreated wood materials, (b) IL treated wood materials and (c) CRFs after enzymatic delignification of IL treated wood. Vertical solid lines represent characteristic peaks of cellulose and hemicelluloses and vertical dashed lines represent lignin.

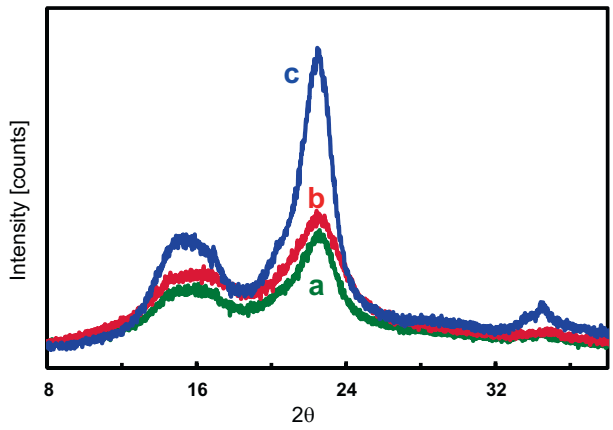



**Fig. 3.** Powder X-ray diffraction patterns for (a) untreated wood materials, (b) IL treated wood materials and (c) CRFs after enzymatic delignification of IL treated wood.

(Labbe et al., 2012; Lee et al., 2009; Lucas et al., 2011). A possible explanation for the different results could be that crystalline regions of the cellulose obtained in the present study were unaffected but amorphous parts were partially destroyed during IL pretreatment due to the mild pretreatment conditions (80 °C for 1 h). As shown in Fig. 3, the peaks around $2\theta = 15.5°$ and 22.2° are sharper for the enzymatically treated wood fibers, which is typical for the cellulose I (Alemdar and Sain, 2008). An increase in number of crystallinity regions enhances the rigidity of cellulose, and higher crystallinity in isolated cellulose fibers is associated with higher tensile strength, which is expected to be beneficial for producing high-strength composite materials (Bhatnagar and Sain, 2005).

### 3.6. Evaluation of thermal properties by TGA analysis

Investigation of the thermal properties of natural fibers is important to gauge their applicability for biocomposite processing, in which the processing temperature for thermoplastic polymers rises above 200 °C. TGA results obtained for untreated, IL treated, and CRF wood chips are shown in Fig. 4. These results illustrate that the thermal stability of the wood fibers increased after IL treatment and further increased after enzymatic delignification. After heating to 550 °C, fiber residue remained in all three samples, indicating the presence of carbonaceous materials in the wood biomass in the nitrogen atmosphere (Sain and Panthapulakkal, 2006). However, there was a difference between the amounts of fiber residues remaining after heating to 550 °C in the treated and untreated wood fibers presumably due to partial removal of hemicelluloses and lignin from the fibers and higher crystallinity of the cellulose-rich materials. These results are consistent with the results obtained from XRD studies.

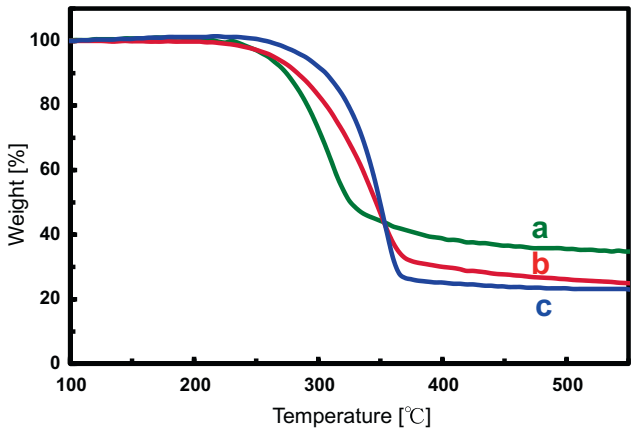



**Fig. 4.** Thermogravimetric analysis for (a) untreated wood materials, (b) IL treated wood materials and (c) CRFs after enzymatic delignification of IL treated wood.

## 4. Conclusions

A highly effective and clean pretreatment method for isolation of cellulose fibers from wood biomass is described that results in minimal structural alteration. The delignification efficiency of IL treated wood was notably improved in the presence of 2.5 wt.% IL in aqueous system. Although IL pretreatment did not significantly change the cellulose chemical composition, crystal structure, or thermostability, it provided a more accessible surface area, leading to enhanced enzymatic delignification. The cellulose fibers had a higher degree of crystallinity and thermal stability than those of the native wood fibers. The present approach may be useful for isolation of cellulose fibers for composite, textile, and other industrial applications.

## Acknowledgements

This work was supported by the Okayama Prefecture Green Project. We are grateful to Prof. H. Kanzaki for providing laccase. We would like to thank Prof. T. Namba and Prof. M. Azhar for providing access to XRD and TGA analysis facilities.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2012.09.113.

## References

Alemdar, A., Sain, M., 2008. Isolation and characterization of nanofibers from agricultural residues-wheat straw and say hulls. Bioresour. Technol. 99, 1664–1671.

Bak, J.S., Ko, J.K., Choi, I.G., Park, Y.C., Seo, J.H., Kim, K.H., 2009. Fungal pretreatment of lignocellulose by *Phanerochaete chrysosporium* to produce ethanol from rice straw. Biotechnol. Bioeng. 104, 471–482.

Bhatnagar, A., Sain, M., 2005. Processing of cellulose nanofiber reinforced composites. J. Reinf. Plast. Compos. 24, 1259–1268.

Blanchette, R., 1991. Delignification by wood decay fungi. Annu. Rev. Phytopathol. 29, 381–403.

Chen, W., Yu, H., Liu, Y., Chen, P., Zhang, M., Hai, Y., 2011. Individualization of cellulose nanofibers from wood using high-intensity ultrasonication combined with chemical pretreatments. Carbohydr. Polym. 83, 1804–1811.

Doherty, T.V., Mora-Pale, M., Foley, S.E., Linhardt, R.J., Dordick, J.S., 2010. Ionic liquid solvent properties as predictors of lignocelluloses pretreatment efficacy. Green Chem. 12, 1967–1975.

Gabriele, G., Cerchiara, T., Salerno, G., Chidichimo, G., Vetere, M.V., Alampi, C., Gallucci, M.C., Conidi, C., Cassano, A., 2010. A new physical–chemical process for the efficient production of cellulose fibers from Spanish broom (*Spartium junceum* L.). Bioresour. Technol. 101, 724–729.

Hendricks, A.T.W.M., Zeerman, G., 2009. Pretreatments to enhance the digestibility of lignocellulosic biomass. Bioresour. Technol. 100, 10–18.

Kilpelainen, I., Xie, H., King, A., Granstrom, M., Heikkinen, S., Argyropoulos, D.S., 2007. Dissolution of wood in ionic liquids. J. Agric. Food Chem. 55, 9142–9148.

Labbe, N., Rials, T.G., Kelley, S.S., Cheng, Z.M., Kim, J.Y., Li, Y., 2005. FT-IR imaging and pyrolysis-molecular beam mass spectrometry: new tools to investigate wood tissues. Wood Sci. Technol. 39, 61–77.

Labbe, N., Kline, L.M., Moens, L., Kim, K., Kim, P.C., Hayes, D.G., 2012. Activation of lignocellulosic biomass by ionic liquid for biorefinery fractionation. Bioresour. Technol. 104, 701–707.

Lee, S.H., Doherty, T.V., Linhardt, R.J., Dordick, J.S., 2009. Ionic liquid-mediated selective extraction of lignin from wood leading to enhanced enzymatic cellulose hydrolysis. Biotechnol. Bioeng. 102, 1368–1376.

Lucas, M., Wagner, G.L., Nishiyama, Y., Hanson, L., Samayam, I.P., Schall, C.A., Langan, P., Rector, K.D., 2011. Reversible swelling of the cell wall of poplar

biomass by ionic liquid at room temperature. Bioresour. Technol. 102, 4518–4523.
MacFarlane, D.R., Seddon, K.R., 2007. Ionic liquids – progress on the fundamental issues. Aust. J. Chem. 60, 3–5.
Moniruzzaman, M., Kamiya, N., Goto, M., 2010a. Activation and stabilization of enzymesin ionic liquids. Org. Biomol. Chem. 8, 2887–2899.
Moniruzzaman, M., Nakashima, K., Kamiya, N., Goto, M., 2010b. Recent advances of enzymes in ionic liquids. Biochem. Eng. J. 48, 295–314.
Moniruzzaman, M., Ono, T., 2012. Ionic liquid assisted enzymatic delignification of wood biomass: a new 'green' and efficient approach for isolating of cellulose fibers. Biochem. Eng. J. 60, 156–160.
Mora-Pale, M., Meli, L., Doherty, T.V., Linhardt, R.J., Dordick, J.S., 2011. Room temperature ionic liquids as emerging solvents for the pretreatment of lignocellulosic biomass. Biotechnol. Bioeng. 108, 1405–1422.
Mosier, N., Wyman, C., Dale, B., Elander, R., Lee, Y.Y., Holtzapple, M., Ladisch, M., 2005. Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresour. Technol. 96, 673–686.
Nguyen, T.A.D., Kim, K.-R., Han, S.J., Cho, H.Y., Kim, J.W., Park, S.M., Park, J.C., Sim, S.J., 2010. Pretreatment of rice straw with ammonia and ionic liquid for lignocellulose conversion to fermentable sugars. Bioresour. Technol. 101, 7432–7438.
Pu, Y., 2007. Ionic liquids as a green solvent for lignin. J. Wood Chem. Technol. 27, 23–35.
Rodriguez, H., Padmanabhan, S., Poon, G., Prausnitz, J.M., 2011. Addition of ammonia and/or oxygen to an ionic liquid for delignification of miscanthus. Bioresour. Technol. 102, 7946–7952.
Sain, M., Panthapulakkal, S., 2006. Bioprocess preparation of wheat straw fibers and their characterization. Ind. Crops. Prod. 23, 1–8.
Shipovskove, S., Gunaratne, H.Q.N., Seddon, K.R., Stephens, G., 2008. Catalytic activity of laccases in aqueous solutions of ionic liquids. Green Chem. 10, 806–810.
Shill, K., Padmanabhan, S., Xin, Q., Prausnitz, J.M., Clark, D.S., Blanch, H.W., 2011. Ionic liquid pretreatment of cellulosic biomass: enzymatic hydrolysis and ionic liquid recycle. Biotechnol. Bioeng. 108, 511–520.
Singh, S., Simmons, B.A., Vogel, K.P., 2009. Visualization of biomass solubilization and cellulose regeneration during ionic liquid pretreatment of switch grass. Biotechnol. Bioeng. 104, 68–75.
Sousa, L.D., Chundawat, S.P.S., Balan, V., Dale, B.E., 2009. Cradle-to-grave' assessment of existing lignocellulose pretreatment technologies. Curr. Opin. Biotechnol. 20, 339–347.
Sun, N., Rahman, M., Qin, Y., Maxim, M.L., Rodriguez, H., Rogers, R.D., 2009. Complete dissolution and partial delignification of wood in the ionic liquid 1-ethyl-3-methylimidazolium acetate. Green Chem. 11, 646–655.
Sun, N., Rodriguez, H., Rahman, M., Rogers, R.D., 2011a. Where are ionic liquid strategies most suited in the pursuit of chemicals and energy from lignocellulosic biomass? Chem. Commun. 47, 1405–1421.
Sun, N., Jiang, X., Maxim, M.L., Metlen, A., Rogers, R.D., 2011b. Use of polyoxometalate catalysts in ionic liquids to enhance the dissolution and delignification of woody biomass. ChemSusChem 17, 65–73.
TAPPI Useful Methods, UM 250, 1991. Acid-soluble lignin in wood and pulp. TAPPI Press, Nocross, GA.
TAPPI T222om 98, 1998. Acid-insoluble lignin in wood and pulp. TAPPI Press, Nocross, GA.
Wang, X., Li, H., Cao, Y., Tang, Q., 2011. Cellulose extraction from wood chip in an ionic liquid 1-ally-3-methylimidazolium chloride (AmimCl). Bioresour. Technol. 102, 7959–7965.
Wise, L.E., Murphy, M., D'Addieco, A.A., 1946. Chlorite holocellulose: its fractionation and bearing on summative wood analysis and on studies on the hemicelluloses. Pap. Trade J. 122, 35–43.
Zavrel, M., Bross, D., Funke, M., Buchs, J., Spiess, A.C., 2009. High-throughput screening for ionic liquids dissolving (ligno-)cellulose. Bioresour. Technol. 100, 2580–2587.
Zhao, Y., Wang, Y., Zhu, J.Y., Ragauskas, A., Deng, Y., 2008. Enhanced enzymatic hydrolysis of spruce by alkaline pretreatment at low temperature. Biotechnol. Bioeng. 99, 1320–1328.