Efficient separation and utilization of structural components in lignocellulosic waste

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Abstract

Lignocellulosic waste, i.e. wood, straw, and bamboo, represents an abundant carbon-neutral renewable resource, which is used for the production of biofuels and biomaterials, and their enhanced use would lower the environmental impact such as the emission of greenhouse gas, i.e. carbon-dioxide, and fossil fuel depletion, helping to create the sustainable environment. With advances in technologies such as genetics, biotechnology, process chemistry, and engineering are leading to the concept of biorefinery. In this work, for the development of total biorefinery process of lignocellulosic waste, the efficient separation and utilization of woody structural components in the white poplar chopsticks waste was carried out using steam explosion as a pretreatment followed by water and acetone extractions. Not only cellulose component was converted into cellulose nanofiber (CNF) but also lignin component was used as a raw material for the synthesis of epoxy resin. The components of steam-exploded product was extracted and separated into water extract, acetone extract, and holocellulose. Water extract had a high catechin equivalent and the cured epoxy resin was synthesized from acetone extract as a raw material. Furthermore, the significant reinforcement effect of CNF obtained from holocellulose on polylactic acid was confirmed. The steam explosion, extraction and separation method, and various conversion process proposed in this work seems to be one of the most efficient and environmentally friendly conversion methods of lignocellulosic waste into eco-materials, i.e. CNF, cured lignin epoxy resin, etc., with generating little pollutants.

Keywords lignocellulosic waste, steam explosion, cellulose, lignin.

Introduction

Recently, for a breakaway from the fossil resources-dependent society, the development of energy and material production process using not edible biomass, i.e. sugar and starch material, but non-edible biomass, lignocellulosic material, as a raw material has attracted
increasing interest in the world because the use of edible biomass competes with food and feed supplies [1-3]. Ethanol production from lignocellulosic material, i.e. wood, straw, oil palm, bamboo, etc., has been studied domestically and abroad [4]. However, since the enzyme (cellulase) necessary for hydrolysis of cellulose into glucose (a substrate for alcohol fermentation) is very expensive and its activity is very low compared with the enzyme (amylase) necessary for hydrolysis of starch into glucose, the manufacturing cost of ethanol from lignocellulosic material is too high for practical use [5-7]. Therefore, it is desirable to develop a new profit-generating type refinery process by effectively utilizing the lignocellulosic biomass components, i.e. not only cellulose but also lignin, as a raw material for useful chemicals without using a high cost cellulase instead of lowering biomass component into low molecular weight product, i.e. ethanol (a raw material for energy).

Lignocellulosic waste represents an abundant carbon-neutral renewable resource, which is used for the production of biofuels and biomaterials, and their enhanced use would lower the environmental impact such as the emission of greenhouse gas, i.e. carbon-dioxide, and fossil fuel depletion, helping to create the sustainable environment. With advances in technologies such as genetics, biotechnology, process chemistry, and engineering are leading to the concept of biorefinery [8]. Biorefinery is a new manufacturing concept for converting renewable biomass to valuable fuels and products. In East Asia, disposable wooden chopsticks are used in restaurants, school cafeterias, and homes and are generally made of white poplar or white birch wood and bamboo. The annual average amount of waste wooden chopsticks is about 90,000 t in Japan [9]. Therefore, disposable wooden chopsticks can be used as a raw material for useful chemicals production.

In this work, for the development of total biorefinary process of lignocellulosic waste, the efficient separation and utilization of woody structural components in the white poplar chopsticks waste was carried out using steam explosion as a pretreatment followed by water and acetone extractions. Not only cellulose component was converted into cellulose nanofiber (CNF) but also lignin component was used as a raw material for the synthesis of epoxy resin. Furthermore, the mechanical and thermal properties of CNF/PLA composite and cured epoxy resin were evaluated.

Materials and Methods

Raw Material

White poplar (Populus Tremuloides) chopsticks waste were cut into the half, i.e. about 10 cm in length, and steam-exploded under various operating conditions. Polylactic acid (Landy PL-2000, Miyoshi Oil & Fat Co., Ltd., Japan) was used as filler. All chemicals were purchased from Nacalai Tesque Co., Ltd., Japan and were of analytical grade.
Steam Explosion Pretreatment

Figure 1 shows the flow chart of steam explosion process from white poplar chopsticks waste for cellulose nanofiber and cured epoxy resin co-product. The chopsticks were steam-exploded in a batch system with a 2-L reactor (steam explosion apparatus NK-2L; Japan Chemical Engineering and Machinery Co. Ltd., Osaka, Japan) [10]. The reactor was charged with 150 g of chopsticks per batch and heated to a pressure of 2.5 MPa (225 °C), 3.0 MPa (234 °C), and 3.5 MPa (243 °C) for a steaming time of 1 min and 5 min. The prescribed temperature was reached in a few seconds. After exposure to the saturated steam, a ball valve at the bottom of the reactor was opened suddenly to bring the reactor rapidly to atmospheric pressure, thereby obtaining the product of liquid and solid materials, i.e., the steam-exploded product. The steam-exploded product was extracted with water followed by acetone and then converted into cellulose nanofiber and cured epoxy resin co-product.

![Flow chart of steam explosion followed by water and acetone extractions from white poplar chopsticks for cellulose nanofiber and cured epoxy resin co-product](image)

**Fig. 1** Flow chart of steam explosion followed by water and acetone extractions from white poplar chopsticks for cellulose nanofiber and cured epoxy resin co-product
Component Analysis

The components, i.e. water extract, acetone extract (a low molecular weight lignin), bleaching extract (a high molecular weight lignin), and residue after bleaching (holocellulose), in the steam-exploded product were separated and measured by the following procedure, with modifications according to Wayman’s extraction method [11]. 5 g of dry steam-exploded product was added to 100 mL of distilled water and extracted for 24 h at room temperature. The solid and liquid materials were separated by filtration, and the filtrate, i.e. water extract, was recovered from the liquid. The residue after water extraction was extracted with 150 mL acetone for 24 h at room temperature to dissolve an extract, i.e. acetone extract. After concentration and drying of the extract, the acetone extract was weighed. The residue after acetone extraction consisted holocellulose (cellulose and hemicellulose) and residual lignin. 150 mL of 1 g NaClO₂ was added to this residue (2.5 g) with gentle mixing and then 0.2 mL acetic acid was subsequently added. The suspended mixture was reacted at 80°C for 1 h. Next, the addition of 1 g NaClO₂ and 0.2 mL acetic acid was repeated 4 times in 1 h intervals for removing the NaClO₂ extract. After the solid fraction was rinsed 5 times with distilled water and dried, the residue after bleaching, i.e. holocellulose, which was defined as a steam-exploded pulp in this work, was weighted. Furthermore, 1 g this residue was soaked with 25 mL of 17.5 wt% NaOHaq at room temperature for 30 min and then added to 25 mL distilled water with stirring for 6 min. The solid fraction was rinsed 5 times with distilled water and neutralized with 10% acetic acid. After drying, the residue, i.e. α-cellulose, was weighed.

Measurement of Catechin Equivalent of Water Extract

Amount of phenolic compounds in the water extract was measured according to the Folin–Ciocalteu method [12]. The extract (200 μL) was added to the test tube containing 4 mL of distilled water, followed by addition of 1 mL phenol reagent. The mixture was thoroughly stirred. In addition, 1 mL of 10%(w/v) sodium carbonate was added to this solution. The absorbance of reaction was measured at 760 nm after 1 h of reaction. Estimations were carried out in triplicate and calculated from a calibration curve obtained with catechin. The amount of phenolic compounds was expressed as catechin equivalent (mg-catechin equiv./g-dry steam-exploded product).

Synthesis of Cured Epoxy Resin from Acetone Extract

Acetone extract (a low molecular weight lignin) was used as a sample for not only epoxidized lignin, i.e. lignin epoxy resin, but also a curing reagent of epoxidized lignin. The cured epoxy
resin was synthesized from the acetone extract according to the method reported by Asada et al. [13].

**Preparation of Cellulose Nanofiber from Residue after Bleaching**

10 g residue after bleaching (holocellulose) was suspended with 1 L distilled water and passed twice through a grinder (MKCCA6-2, Masuko Sangyo Co., Ltd., Saitama, Japan) at 1500 rpm.

**Preparation of Cellulose Nanofiber (CNF) and Polylactic Acid (PLA) Composite**

CNF water slurry containing 5 wt% fibers was added to the melted PLA (Landy PL-2000, Miyoshi Oil & Fat Co., Ltd., Japan), and CNF/PLA mixture was kneaded and mixed under vacuum condition. The composite was preheated in a die (100 mm × 100 mm) at 105°C for 1 h and then compressed at 180°C and 1 MPa for 10 min. The resulting sheet was 1.4 mm. Specimens with 80 mm long and 10 mm width were prepared from the sheet.

**Analyses**

*Fourier Transform Infrared (FTIR) Spectroscopy*

Changes in the functional groups of the steam-exploded product after various extraction and separation treatments were recorded by FTIR spectrometry (FT/IR-670 Plus; JASCO, Tokyo, Japan). First, the samples were ground and dried at 105°C. The sample (1.5 mg) was mixed with 200 mg potassium bromide (KBr). The role of KBr was to hold the fiber flour during the test. Transparent pellets were prepared from the blend and analyzed from 400 to 4000 cm⁻¹.

*Molecular Weight Measurement*

To measure the molecular weight of the sample (α-cellulose in the steam-exploded product), 250 mg of the sample was soaked with 25 mL distilled water and stirred at room temperature for 1 h. Next, 25 mL of copper ethylene diamine solution (CEDS) was added and stirred for 30 min. The temperature of the water bath was maintained at 25 ± 0.1°C. According to JIS P8215 [14], the molecular weight of the sample was measured.

*FE-SEM (Field-Emission Scanning Electron Microscope)*

Steam-exploded product after various extraction followed by separation treatments and CNF
were observed using a FE-SEM (6400F, Hitachi, Tokyo, Japan), operating at 1.6 kV and a working distance of 8 mm. A small piece of the CNF mat was fixed on carbon tape and then sputtered with Pt.

**Thermal Property of Cured Epoxy Resin and CNF/PLA Composite**

The thermogravimetric (TG) curve was measured using a TG analyzer (TG/DTA SII EXSTAR 6300; Seiko Instruments Inc., Chiba, Japan) under an atmosphere of nitrogen (heating rate of 5°C/min) using alumina as a primary standard. The thermal tests were performed in triplicate and average values are shown.

**Mechanical Property of CNF/PLA Composite**

The tensile modulus and strength of the samples was measured using a tensile tester (Dual Column Series for Mechanical Testing 5667, INSTRON Japan Co. Ltd, Kamasaki, Japan) at room temperature. The dimensions of the samples were 80 mm×10 mm×1.4 mm. The gauge length was 30 mm and a testing speed of 2 mm/min was applied for the test. For each sample, five repetitions were performed and the average of five tests was reported.

**Result and Discussion**

**Chemical Composition of Steam-Exploded White Poplar Chopsticks**

Figure 2 shows the ratio of component to dry weight of steam-exploded white poplar chopsticks. Three treatment conditions, i.e. a steam pressure of 2.5, 3.0, and 3.5 MPa for a steaming time of 5 min, were evaluated. The highest ratio, i.e. 56.6%, of component of the residue after bleaching, i.e. holocellulose (hemicellulose plus cellulose), was obtained at a steam pressure of 2.5 MPa for a steaming time of 5 min. This component could be used as a raw material for cellulose nanofiber. Similar values of the residue after bleaching, i.e. 53.1% and 51.1% were observed with a steam pressure of 3.0 and 3.5 MPa for a steaming time of 5 min, respectively. In general, hemicellulose and cellulose decompose to be sugars and more decomposed materials such as furan derivatives and organic acids by severe treatment condition, i.e. long time, high temperature, and high concentration of acidic catalyst [15]. These compounds were detected in water extract with phenolic compounds derived from high molecular weight lignin (data not shown). Kurosumi et al. [16] reported that water extract from steam-exploded bamboo plant (Sasa palmate (Bean) Nakai, a steaming pressure of 3.9 MPa for a steaming time of 1 min) contained phenolic compounds derived from high molecular weight lignin and indicated an antioxidant activity. In this study, the highest ratio of
component of the water extract, i.e. 16.7%, was observed at a steam pressure of 2.5 MPa and
the amount of phenolic compounds in the water extract corresponded to 76 mg-catechin
equiv/g-dry steam-exploded product (data not shown). Furthermore, acetone extract mainly
contains a low molecular weight lignin derived from a high molecular weight lignin. Asada et
al. [13] mentioned that a low molecular weight lignin extracted from steam-exploded plant
biomass could be useful resource to synthesize epoxy resin. The highest ratio of component of
acetone soluble material, i.e. 36.7%, was observed at a steam pressure of 3.5 MPa, and this
means that the depolymerization reaction is promoted by the increasing treatment pressure
(severity). As a result, it seems that each component of steam-exploded product, i.e., residue
after bleaching, water extract, and acetone extract, can be a promising resource for various
useful chemicals.

**Fig. 2** Ratio of component to dry weight of steam-exploded white poplar chopsticks waste.
(A) 2.5 MPa and 5 min, (B) 3.0 MPa and 5 min, (C) 3.5 MPa and 5 min

**FTIR Analysis of Steam-Exploded Product after Various Extraction and Separation
Treatments**

Figure 3 shows changes in functional groups of steam-exploded product at 2.5 MPa for 5 min
by water extraction, acetone extraction, bleaching, and NaOH treatment using FTIR analysis.
The assignments of FTIR absorption bands are shown in this figure caption [17-21]. Though
the peaks at (4) and (12) ascribed to lignin structure were observed in the steam exploded
product, the residue after water extraction, and the residue after acetone extraction, they
became weak in the residue after bleaching, i.e. holocellulose, and disappeared in the residue
after NaOH treatment, i.e. α-cellulose. This means that the acetone extraction cannot remove
high molecular weight lignin, but the NaClO₂ and NaOH treatments can degrade and remove
high molecular weight lignin. Since the peaks of (4) and (12) are completely eliminated, it
seems to be that lignin was completely removed by alkali treatment. Also, trace amounts of lignin or lignin-derived compounds were present in holocellulose. The intensity of the peaks at (6), (9), (10), and (11), which relate to COO’ stretching, stretching of C-O in ring or bending of C-OH, C-O-C stretching, and O-H bending, respectively, in the holocellulose (cellulose and hemicellulose), increased due to the removal of lignin that covered the cellulose and hemicellulose strongly.

**Fig. 3** FTIR of (A) steam-exploded product, (B) residue after water extraction, (C) residue after acetone extraction, (D) residue after bleaching, and (E) α-cellulose (E) obtained from white poplar waste treated at 2.5 MPa for 5 min. Assignments of FTIR absorption bands: (1) O-H stretching, (2) C-H stretching, (3) CO₂, (4) C=O band from ester groups, (5) O-H stretching, (6) COO’ stretching, (7) CH₂ bending, (8) O-H in plane bending or C-H bending, (9) stretching of C-O in ring or bending of C-OH, (10) C-O-C stretching, (11) O-H bending, (12) C-H bending or CH₂ stretching amorphous, (13) O-H out-of-plane bending.
Synthesis of Cured Epoxy Resin from Acetone Extract

Epoxy resins are one of the most important and highly valuable thermosetting resins, and are known to have good electrical characteristics, chemical resistance, mechanical strength, and low absorption of moisture. Strong mechanical strength with high thermal resistance properties of epoxy resins render them versatile and applicable in various fields, such as in electronics, aerospace, and automotive applications. Therefore, the synthesis of epoxy resin from acetone extract was attempted.

Table 1 shows the characteristics of acetone extract from steam-exploded product at 2.5 MPa and 5 min. The purity of lignin contained in the extract was 99%, which implies that high-purity lignin was obtained in this work. The number-average molecular weight, the weight-average molecular weight, and the hydroxyl equivalent of the extract were 1200, 5100, and 130, respectively. Asada et al. [13] reported that the weight-average molecular weight and the hydroxyl equivalent of methanol extract from various steam-exploded plant biomass were 1330-1600 and 115-118, respectively. The reason why the weight-average molecular weight and the hydroxyl equivalent of acetone extract were higher than those of methanol extract seems to be that acetone can extract lignin more than methanol. This means that not only a small molecular weight lignin but also a comparatively large molecular weight lignin were extracted by acetone extraction.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Purity (%)</th>
<th>Number-average molecular weight (g/mol)</th>
<th>Weight-average molecular weight (g/mol)</th>
<th>Hydroxyl equivalent (g/equivalent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetone extract</td>
<td>99</td>
<td>1200</td>
<td>5100</td>
<td>130</td>
</tr>
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</table>

The resinification of acetone extract was carried out with epichlorohydrin. Figure 4 shows the $^1$H NMR spectra of acetone extract and epoxidized lignin synthesized from acetone extract. Both spectrum varied significantly. Hydroxyl signals were observed at 8-9 ppm in the acetone extract but they were not observed in the epoxidized lignin. Furthermore, in the epoxidized lignin the epoxide signals appeared at 2.7-2.9 ppm. These results suggests the incorporation of epoxy group into the acetone extract, i.e. a low molecular weight lignin.

Epoxidized lignin, i.e. epoxy resin synthesized from the acetone extract, was cross-linked with the acetone extract as a curing agent. The thermal properties (i.e., thermal stability and thermal decomposition) of cured epoxy resin were investigated by using TG/DTA analysis. Figure 5 shows the TG/DTA profiles of cured epoxy resin under a nitrogen atmosphere. The
thermal decomposition temperature at 5% weight loss (Td₅), 10% weight loss (Td₁₀), and 30% weight loss (Td₃₀) were 260, 294, and 358°C, respectively. Benyaha et al. [22] reported that the thermal decomposition temperature at 30% weight loss of cured bio-based epoxy resin using a green tea extract, i.e. catechin with isophorone diamine, was 299°C. Since, this value was much lower than that of the cured epoxy resin obtained in this work, the low molecular weight lignin is a more suitable biopolymer than catechin for the synthesis of heat-resistant bio-based epoxy resin. Furthermore, since Td₅ exceeded the temperature of heat-stability property for solder-dip resistance, i.e. beyond 250°C [23], it can be used in the electronic board material field.

Fig. 4 "H NMR of (A) acetone extract and (B) epoxidized lignin synthesized from acetone extract

Fig. 5 TG/DTA profiles of cured epoxy resin made from acetone extract, i.e. low molecular weight lignin
Synthesis of CNF from Residue after Bleaching

Degree of polymerization of α-cellulose in the residue after bleaching, i.e. holocellulose, obtained from steam-exploded cedar white poplar chopsticks waste was compared to that of BiNFi-s WMa-10002 (a commercial cellulose nanofiber, Sugino Machine Ltd., Japan). With increasing the treatment condition severity, the degree of polymerization decreased. Molecular weight of cellulose can be calculated by degree of polymerization × 162 [24], therefore, the lowest molecular weight, i.e. approximately 17,000, in this work was obtained with a steam pressure of 3.5 MPa for a steaming time of 5 min. The degree of polymerization at 2.5 MPa and 5 min was a little lower than that of BiNFi-s WMa-10002. However, since a comparative high degree of polymerization, i.e. approximately 500, was obtained from the residue at 2.5 MPa and 5 min, it seems to be the most adequate for the production of CNF as a reinforcement material.

Fig. 6 Degree of polymerization of α-cellulose in residue after bleaching obtained from steam-exploded white poplar chopsticks waste and commercial cellulose nanofiber (BiNFi-s WMa-10002, Sugino Machine Ltd.). (A) 2.5 MPa and 5 min, (B) 3.0 MPa and 5 min, (C) 3.5 MPa and 5 min, (D) BiNFi-s WMa-10002

A field-emission scanning electron microscope (FE-SEM) was used to investigate the changes of surface structure of white poplar chopsticks waste received with the steam explosion, water and acetone extractions, bleaching, and grinder treatment. Figure 7 shows FE-SEM of (A) untreated white poplar chopsticks waste, (B) steam-exploded product at 2.5 MPa for 5 min, (C) residue after water extraction, (D) residue after acetone extraction, (E) residue after bleaching, and (F) cellulose nanofiber. Though the rough and linty surface of untreated white...
Poplar chopsticks waste was observed, the woody fibers were defibrilled by the steam explosion and the fiber size became about 100 nm width as shown in Fig. 7(B). However, there are variations in the degree of disintegration, and it has not been fibrillated to a uniform thickness. The residue after water extraction had rough and spherical surface as shown in Fig. 7(C), but the residue after acetone extraction had clean and smooth surface as shown in Fig. 7(D). This means that acetone extraction removed a low molecular weight lignin from the residue after water extraction. Though compared before and after bleaching, little change of surface was observed by FE-SEM as shown in Figs. 7(D) and (E), the sample was decolorized from brown to white due to the removal of high molecular weight lignin. The CNF which was produced from the residue after bleaching, i.e. holocellulose, by a grinder treatment had comparatively smaller nanofibers (about 20 nm width) as shown in Fig. 7(F).

**Fig. 7** FE-SEM of (A) untreated white poplar chopsticks waste, (B) steam-explored product at 2.5 MPa for 5 min, (C) residue after water extraction, (D) residue after acetone extraction, (E) residue after bleaching, and (F) cellulose nanofiber
**Effect of CNF on Mechanical and Thermal Properties of CNF/PLA Composite**

The reinforcement effects of CNFs produced from holocellulose and α-cellulose on the mechanical and thermal properties of CNF/PLA composites were evaluated using CNFs obtained from steam-exploled white poplar chopsticks waste at 2.5 MPa and 5 min. Figure 8 shows the tensile strength and Young’s modulus of various composites. As can be seen, the tensile strength and Young’s modulus of PLA with 5 wt% CNF obtained from holocellulose increased to 3.7 and 27.8 times in comparison with neat PLA, respectively. Compared the tensile strength of CNF/PLA composite from holocellulose with that from α-cellulose, CNF/PLA composite from holocellulose was a little stronger than that from α-cellulose. This means that hemicellulose contained in the holocellulose fibers binds not only cellulose fibers but also PLA resin each other resulting in the strong strength of CNF/PLA. Though the tensile strength and Young’s modulus of CNF/PLA composite from holocellulose were a little lower than those of CNF/PLA composite with a commercial CNF due to lower degree of polymerization of α-cellulose as shown in Fig. 6, the significant reinforcement effect of CNF obtained from steam-exploled product on PLA resin was confirmed.

![Graph showing Tensile strength and Young’s modulus of various composites](image)

**Fig. 8** Tensile strength and Young’s modulus of various composites. (A) PLA, (B) PLA with 5% CNF obtained from holocellulose, (C) PLA with 5% wtCNF obtained from α-cellulose, and (D) PLA with 5% wtCNF (a commercial CNF, BiNFi-s WMa-10002)

The TG/DTA profiles of CNF/PLA composite show their thermal stability and degradation characteristics. Figure 9 shows TG/DTA profiles of PLA, CNF/PLA composite from holocellulose, and CNF/PLA composite with a commercial CNF. Since the thermal decomposition temperatures at 5% weight loss (Tds) were almost the same and the similar
TG/DTA profiles were observed regardless of samples, it was found that the addition of 5 wt% CNF to PLA did not affect the thermal property of neat PLA.

Fig. 9 TG/DTA profiles of PLA and CNF/PLA composite. Solid line: PLA, dashed line: PLA with 5 wt% CNF obtained from holocellulose, and dotted line: PLA with 5 wt% CNF (a commercial CNF, BiNFi-s WMa-10002)

Conclusions

This work proposed a new effective and environmentally friendly biorefinery process of lignocellulosic waste using a steam explosion followed by water and acetone extractions. The water extract, the acetone extract, and the residue after bleaching, i.e. holocellulose, obtained from steam-exploaded white poplar chopsticks waste were converted into useful eco-materials. The water extract corresponded to 76 mg-catechin equiv./g-dry steam-exploaded product and it can be used as an antioxidant. The acetone extract was converted into a cured lignin epoxy resin with high heat-resisting property. The residue after bleaching was used as a raw material of CNF and its reinforcement effect on PLA resin was clarified. This process seems to be useful for total biorefinary of not only white poplar chopsticks waste but also lignocellulosic waste.

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References