Introduction

In response to the current shortage of oil resource, much attention has been paid to lignocelluloses for the production of energy, chemicals and materials [1]. Bamboo is a feedstock used widely for paper, construction, furniture, as well as bio-ethanol, etc., mainly because it does not compete with food production, and it is fastest-growing and has a high bulk density. The value-added utilization of bamboo is associated with the complex structure of bamboo, which is resistant to processing thus efficient pretreatment method is required.

Organosolv treatment is a promising processing method implementing the concept of biorefinery through fractionation, which is based on the affinity of the components of lignocellulose towards different solvents. In general, organic solvents (alcohol, organic acid, ketone or ester) are utilized to dissolve lignin, accompanying cellulose in the solid residue. In addition, hemicelluloses can also be degraded and dissolved in liquid phase if water is added in the process. Many alternatives of the organosolv processes, such as AlCELL® (ethanol for lignin solvent), Lignol (ethanol for lignin solvent), Milox (formic acid for lignin solvent), Acetosolv (acetic acid for lignin solvent), Formacell (formic and acetic acids for lignin solvent), Organocell (methanol for lignin solvent), have been developed [2-5]. Among them, the process using formic acid as solvent can achieve an ideal delignification under mild conditions. The formic acid based processes have been utilized for many lignocelluloses including birch [6], sugarcane bagasse [7], corn (Zea mays L.) cob [8], etc. These treatments, which were conducted under atmosphere as compared to higher pressure in most fractionation process, resulted in the release of most of the lignin and hemicelluloses from lignocelluloses. Our major concern was to fractionate the main components from bamboo in an environmentally friendly and mild way. Bamboo has been subjected to formic acid process to release lignin to some extent in an earlier report [9]. Since there was some lignin in the solid residue after the formic acid treatment, a subsequent treatment was required. Therefore, the aim of the present study was to fractionate bamboo by a combination way and to investigate the structural modification of lignin during the fractionation process, since the behavior of lignin is of vital importance for the separation and utilization of lignocelluloses.

In this study, bamboo was delignified with formic acid followed by alkaline hydrogen peroxide, and the lignin in the treated samples was extracted and structurally characterized as compared to...
that from the original bamboo. The comprehensive characterization, including molecular weight, Fourier-Transform Infrared (FT-IR) spectroscopy, Pyrolysis gas chromatography mass spectrometry (Py-GC/MS), etc. is important not only for providing insight into the mechanism of lignin degradation but also for the processing of bamboo for pulp or biofuel, since the structure of lignin has a great effect on the processing technology.

**Materials and Methods**

Fractionation of bamboo by formic acid and alkaline hydrogen peroxide: Bamboo (B1) was subjected to formic acid delignification and alkaline hydrogen peroxide treatment to release lignin and hemicelluloses to obtain cellulosic pulp. Bamboo (300 g) was delignified with formic acid (3000 mL, 88 wt %) at boiling point under atmosphere pressure for 2 h. After delignification, the mixture was filtrated to obtain spent liquor and cellulosic pulp. The pulp was washed with formic acid and water followed by drying to obtain formic acid pulp (B2). After this, formic acid pulp was treated with alkaline hydrogen peroxide solution containing 1% NaOH and 1% H₂O₂ at 80 ºC with a liquor to solid ratio of 20 (mL /g) for 1 h. The treated pulp was washed with water and dried before use (B3). The experiments in the present study were performed in triplicates and the errors were reported.

Milled Wood Lignin (MWL) isolation: MWL was isolated from bamboo according to a previous report. Twenty grams of dewaxed bamboo was ball-milled with a planetary mill (FRITSCH pulverisette, Germany). Then the bamboo powder was extracted with1, 4-dioxane/water (82/18, v/v) solution. The filtrate was containing 0.1 M HCl for 2 h under nitrogen. Then the solid fraction was washed with dioxane/water (82/18, v/v) solution by precipitation in 100 mL of water. The lignin fraction was dried and dissolved in 10 mL of 1,2-dichloroethane/ethanol, and precipitated in ether (100 mL). The precipitation was washed with ether and petroleum ether and dried. Then the lignin was subjected to acid hydrolysis as described in the part "Extraction of residual lignin" and named as L1.

Extraction of residual lignin: Extraction of residual lignin from the treated samples was conducted according to the procedure reported previously with minor modifications [10]. Cellulosic fraction (100 g) was refluxed with dioxane-water (82/18, v/v) solution (1 500 mL) containing 0.1 M HCl for 2 h under nitrogen. Then the solid fraction was washed with dioxane-water (82/18, v/v) solution. The filtrate was evaporated to 50 mL under reduced pressure (0.1 MPa) at 40 ºC, and then the lignin was precipitated by the addition of water (500 mL). The precipitated lignin was then filtrated and washed with pentane to obtain purified lignin and named as L2 and L3, corresponding to the lignin extracted from cellulosic fractions obtained by formic acid delignification and alkaline hydrogen peroxide treatment, respectively.

Analysis methods: The chemical components of the samples were determined according to the National Renewable Energy Laboratory (NREL) method [11]. Molecular weight of the lignin was measured by a Gel Permeation Chromatography (GPC) system (Agilent Technologies) after acetylation. Elemental analysis was performed using a Vario El III analyzer. FT-IR spectra were recorded on a Thermo Scientific Nicolet iN10 FT-IR Microscope (Thermo Nicolet Corporation, Madison, WI, USA) equipped with liquid nitrogen cooled MCT detector.

Py-GC/MS analysis of the lignin was conducted with a multi-shot pyrolyzer (EGA/Py-3030D, Frontier Laboratories, Japan) combined with a GC-MS system (QP2010 Ultra, Shimadzu, Japan). The volatile pyrolysis products were separated by using an Ultra ALLOY+5 (30 m × 0.25 mm × 0.25 μm) columns. The fine powder (0.25 mg) was loaded into a small platinum cup placed into a quartz tube and introduced into the pyrolysis chamber. The pyrolysis was conducted at 500 ºC for 10 s. The pyrolysis interface was kept at 320 ºC and purged with helium to transfer the pyrolysis products to the GC column. The temperature of the chromatograph oven was programmed to rise from 50 ºC (1 min) to 280 ºC at a rate of 3 ºC/min, and to 300 ºC at a rate of 30 ºC/min. The final temperature was held for 3 min. The mass spectrometer was operated by electron impact ionization at 70 eV. The temperatures of the detector and the GC/MS interface were 200 ºC and 280 ºC, respectively. Identification of the products was conducted by comparison of their mass spectra with GC/MS library (National Institute of Standards and Technology 2005) and data from literature. Relative quantification was performed on the basis of the relative area of each lignin-derived compound divided by the total area of the chromatogram [12].

**Result and Discussion**

Effect of the formic acid delignification and alkaline hydrogen peroxide extraction: Bamboo was subjected to two environmentally friendly and mild fractionation processes to fractionate bamboo to obtain cellulose-rich pulp together with degraded lignin and hemicelluloses sugars. After the formic acid treatment and alkaline hydrogen peroxide fractionation process, the lignin and hemicelluloses in bamboo were notably removed. As it can be seen from the solid residue B2 (Table 1), the hemicelluloses content was 6.84% and the lignin content was 8.17%. After a further fractionation with alkaline hydrogen peroxide, the hemicelluloses and lignin contents further decreased to 6.81% and 3.50%, respectively. It was concluded that the combination of formic acid delignification and alkaline hydrogen peroxide degradation result in an effective removal of both lignin (delignification rate 94.9%) and hemicelluloses (removal rate 87.4%) from bamboo. The extraction efficiency was higher than the delignification with alcohols, which is usually conducted at higher temperatures (above 140 ºC) the delignification rate is less than 90% [13]. In addition, the extraction of hemicelluloses was conducted by diluted alkaline solution as compared to the conventional extraction with concentrated NaOH, which was easy to treat the waste liquor after the fractionation process [14].

Table 1: Yield and chemical composition of the fractionated bamboo samples as compared to the original bamboo.

| Sample | Yield(%) | Cellulose(H%) | Hemicelluloses(H%) | Lignin(H%) | Extractives(%) |
|--------|----------|---------------|-------------------|------------|---------------|
| B1     | 46.5±1.21| 19.7±0.23     | 50.7±0.55         | 6.8±1.20   | 3.5±0.02       |
| B2     | 46.0±1.10| 18.4±0.55     | 50.7±0.56         | 6.7±1.21   | 3.5±0.06       |
| B3     | 36.5±1.02| 30.7±0.89     | 61.5±1.20         | 3.5±0.03   | 3.2±0.03       |

*B: Based on the original bamboo; *B: Based on the measured sample.

FT-IR spectra of the solid fractions were recorded for comparison (data not shown). For bamboo, the band at 3420 cm⁻¹ is due to O-H stretch and that at 2915 cm⁻¹ is attributed to methyl, methylene and methine groups. Aromatic skeletal vibrations in lignin were observed at 1596, 1506, and 1422 cm⁻¹. The peak at 834 cm⁻¹ from the C-H out of plane bending of S unit in lignin. These peaks of lignin noticeably diminished after the formic acid delignification as well as alkaline hydrogen peroxide fractionation. The peak at 1732 cm⁻¹, increased
Table 2: Weight-average (Mw) and number-average (Mn) molecular weights and polydispersity (Mw/Mn) of bamboo lignin.

| Sample | Mw (g/mol) | Mn (g/mol) | Mw/Mn |
|--------|------------|------------|--------|
| L1     | 4650±100   | 2100±150   | 2.2    |
| L2     | 8490±250   | 4390±220   | 1.9    |
| L3     | 10340±120  | 3200±220   | 3.2    |

Table 3: Elemental analysis and C9 formula of lignin.

| Sample | C % | H % | O % | OCH3 % | C9 | H | O | OCH3 | C9H9.85O2.14(OCH3)1.09 |
|--------|-----|-----|-----|--------|----|---|---|------|------------------------|
| L1     | 63.10±1.20 | 5.67±2.01 | 31.23±1.23 | 17.74±0.29 | C9H10O6(OCH3)11.06 |
| L2     | 64.58±0.06 | 6.04±1.81 | 29.38±1.54 | 17.47±1.05 | C9H9O5(OCH3)10.65 |
| L3     | 65.17±1.51 | 7.10±2.12 | 27.73±1.06 | 18.14±0.67 | C9H9O4.64(OCH3)0.90 |

The residual lignin of bamboo exhibited an increase in C content but a decrease in O content when compared to the corresponding lignin from the original bamboo. This feature was in well agreement with the dissolution of non-condensed structure of lignin during the delignification process.

Figure 1: FT-IR spectra of lignin isolated from bamboo.

Figure 2: Py-GC/MS chromatograph of lignin isolated from bamboo.
The intensities at 1127 cm⁻¹ and 1034 cm⁻¹ are due to the aromatic in-plane C-H bending and aromatic in-plane C-H bending, respectively. The similarity of the signals between 1597 and 836 cm⁻¹ suggested that the core structure of lignin did not change after the treatments. As compared to the L1, the intensity at 1719 cm⁻¹ increased slightly for L2 due to esterification, and it increased largely for L3 probably due to oxidation.

Py-GC/MS is a rapid and sensitive technique for analyzing the composition of lignin [15-17]. The chromatograms of lignin samples are illustrated in Figure 2 and the identities and relative abundances of the degraded compounds are shown in Table 4. The chromatogram of bamboo MWL indicated that this lignin was a GSH type, in accordance with the literature [18]. The main compounds released were 4-vinylphenol (H/PCA), 4-methylsyringol (S), 4-methylguaiacol (G), guaiacol (G). The Py-GC/MS data indicated the relative ratio of the S/G ratio was 1.63 for L1. The residual lignin isolated from the formic acid treated cellulose-rich fraction (L2) presented a chromatograph similar to that of bamboo MWL (L1) but had a low proportion of S units, resulting in a lower S/G ratio of 1.28. This indicated that a preferential removal of S units in bamboo during the formic acid fractionation process. Alkaline hydrogen peroxide treatment also resulted in more removal of S units, as indicated by a lower S/G ratio of 0.71 in L3. In short, formic acid and alkaline hydrogen peroxide treatments could liberate both S and G type lignin, in which more S units were removed under the conditions studied.

Conclusion

Bamboo was fractionated by formic acid delignification and alkaline hydrogen peroxide post-treatment to remove lignin and hemicelluloses, producing cellulose-rich pulp. The structural modification of lignin was investigated by extracting lignin from bamboo and cellulose-rich residue and characterized by multiply technologies. Py-GC/MS analysis indicated that the relative ratio of S/G was decreased from 1.28 in formic acid treated bamboo lignin to 0.71 in alkaline hydrogen peroxide treated bamboo lignin, as compare to 1.63 in the milled wood lignin of bamboo. This indicated that the preferential removal of S units during the formic acid fractionation process and that alkaline hydrogen peroxide treatment resulted in more removal of S units.

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