

EFFECT OF STRUCTURAL CHANGES ON ENZYMATIC HYDROLYSIS OF EUCALYPTUS, SWEET SORGHUM BAGASSE, AND SUGARCANE BAGASSE AFTER LIQUID HOT WATER PRETREATMENT

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A woody (eucalyptus (Eu)) and two herbaceous materials (sweet sorghum bagasse (SSB) and sugarcane bagasse (SCB)) were used to evaluate the effect of liquid hot water (LHW) pretreatment on enzymatic hydrolysis of various lignocelluloses. The results showed that enzymatic hydrolysis efficiency of pretreated materials was SCB>SSB>Eu at 5% solids loading, while SSB>SCB>Eu was at 10% and 20% solids loadings. This indicated the enzymatic hydrolysis efficiency of SCB could be influenced by solids concentration. The differences in surface morphology, crystalline structure, and functional groups of pretreated samples were also examined by scanning electron microscopy (SEM), X-ray diffraction (XRD), and Fourier transform infrared (FTIR) spectroscopy. SEM photographs and crystallinity indexes (CrI) showed that the structural compactness was Eu>SSB>SCB for both of untreated and treated materials. FTIR spectra showed that the conspicuousness of physicochemical changes was SCB>SSB>Eu. The differences in enzymatic hydrolysis efficiency of pretreated materials can be ascribed mainly to the structural differences.

Keywords: *Liquid hot water; Eucalyptus; Sweet sorghum bagasse; Sugarcane bagasse; Enzymatic hydrolysis*

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INTRODUCTION

Lignocellulosic materials including agricultural residues, woody materials, and paper wastes, etc., have been identified as promising feedstocks for ethanol production (Keshwani *et al.* 2007). The conversion of lignocellulosic biomass to ethanol comprises the following main steps: pretreatment of feedstock, enzymatic hydrolysis, fermentation of monosaccharides, and purification of ethanol (Jeya *et al.* 2009; Taherzadeh and Karimi 2008). Because the porosity of lignocellulosic biomass, fiber crystallinity of cellulose, and the presence of lignin and hemicellulose can hinder the access of cellulase to cellulose, pretreatment is an essential step for the effective conversion of cellulose to ethanol (Zabihi *et al.* 2010). As one of the pretreatments, liquid hot water (LHW) treatment uses pressure above the saturation point to maintain the water in a liquid state at elevated temperatures, which provides an environmentally friendly and sustainable

way to utilize lignocellulosic material (Yu *et al.* 2010; Boussarsar *et al.* 2009; Sarkar *et al.* 2012). In comparison with acid or alkali treatment, LHW uses no chemicals except water so that no problems derived from equipment corrosion are expected, and no worries about sludge handling happen (Garrote *et al.* 1999).

LHW treatment is usually carried out at temperatures of 150 to 230 °C, which will generate hydronium ions as catalysts to depolymerize polysaccharides into oligosaccharides, especially to depolymerize hemicellulose into xylooligosaccharides (Garrote *et al.* 1999; Yáñez *et al.* 2009). Several polymerization degrees of xylooligosaccharides are contained in the liquid phase, which can be used as food, pharmaceutical, feed, and agricultural additives (Yáñez *et al.* 2009; Gullón *et al.* 2010). The residues remaining in solid phase are mainly composed of cellulose and lignin, which can produce fermentable sugars from enzymatic hydrolysis of cellulose (Yáñez *et al.* 2009). Due to the diversity of lignocellulosic material properties, different substrates subjected to LHW pretreatment may have different structural changes, and thus affect the hydrolysis of cellulose. In this study, three kinds of biomass, namely Eu, SSB, and SCB were used to evaluate the effect of LHW pretreatment on their structural changes and enzymatic hydrolysis efficiencies. Eu, as a woody biomass, has some advantages over other trees for fuel production. It has potential for high productivity over short rotations, tolerance of a wide range of soils, and denser wood than many other species (Leslie *et al.* 2011). Sweet sorghum, an herbaceous biomass, has great potential as an energy crop (Sipos *et al.* 2009). It can be adapted to almost all temperate and tropical climates as an annual or short perennial crop that is tolerant to high salinity and drought conditions, which allows it to grow in marginal areas (Kim and Day 2011; Vasilakoglou *et al.* 2011). The juice of its stalks is rich in sugars, mainly sucrose, fructose, and glucose, which can be used for production of liquid fuels such as ethanol, biodiesel, and hydrogen (Wu *et al.* 2010; Laopaiboon *et al.* 2009; Gao *et al.* 2010; Antonopoulou *et al.* 2011). The leftover stalks, after the juice extraction, can be used for ethanol production (Sipos *et al.* 2009; Li *et al.* 2010). As the same category of a potential source for fuel production as sweet sorghum, sugarcane also has high sugar content in the juice of its stalks. It is a tropical perennial grass and harvested mainly for its sucrose content (Kim and Day 2011). After pressing juice from stalks, SCB can be also utilized for ethanol production like SSB.

As lignocellulosic material, Eu, SSB, and SCB must be pretreated prior to enzymatic hydrolysis and fermentation. Several pretreatments such as LHW, acid, alkali, and steam explosion, *etc.*, can be employed. LHW was applied in this work. Although there have been several studies evaluating the effect of LHW on enzymatic hydrolysis of Eu, SSB, or SCB (Yu *et al.* 2010, 2011; Romaní *et al.* 2010; Boussarsar *et al.* 2009; Sasaki *et al.* 2003), their focuses were not on the influence of structural changes of pretreated residues on enzymatic hydrolysis of lignocellulosic materials, but on the effect of LHW pretreatment conditions on enzymatic hydrolysis of pretreated residues. No research has explored the different structural changes in different materials after LHW pretreatment. The aim of this research was to find differences in the enzymatic hydrolysis efficiency of LHW-pretreated Eu, SSB, and SCB to explore the reasons leading to the different hydrolysis efficiency and to determine which kind of lignocellulosic material is suitable for being pretreated by LHW to reach considerable enzymatic hydrolysis.

EXPERIMENTAL

Raw Materials

Eucalyptus residues, which had been milled, were obtained from a wood processing plant in Jiangmen, Guangdong Province, China. Sweet sorghum bagasse, the solid residues left after solid-state ethanol fermentation, was provided by Beijing Tai Tian Di Energy Technology Development Co. Ltd. Sugarcane bagasse, leftover after juice extraction, was provided by the National Engineering Research Center for Non-food Biorefinery, Guangxi Academy of Sciences, China. The three kinds of materials were milled and screened. The 40 to 60 mesh particles were selected, then washed and dried at 105 °C until a constant weight was achieved.

Enzyme

Cellulase, mixed with small quantities of other enzymes such as xylanase, was produced from *Penicillium* sp. and bought from Imperial Jade Bio-technology Co. Ltd. (China). The filter paper activity (FPA) is 740 FPU/g protein, as assayed according to Ghose (1987).

LHW Pretreatment

LHW pretreatment was carried out with a laboratory hydrolysis facility set up in the Bio-Chemistry Lab of Guangzhou Institute of Energy Conversion (Zhuang *et al.* 2009). Thirty grams of substrates mixed with 600 mL deionized water were transferred into the reactor which was heated to 180 °C and kept at this temperature for 20 min with a magnetic agitator operating at 500 rpm. After 20 min, the reactor was rapidly cooled down to less than 140 °C. The pretreated residues were not discharged from the reactor until it reached room temperature. Then the residues were dried at 60 °C until a constant weight was reached and stored in a desiccator at room temperature as the substrate for the downstream enzymatic hydrolysis. The “severity factor” R_0 of this pretreatment is 4534.5 ($\log R_0=3.7$), as calculated by the equation provided by Overend and Chornet (1987).

Enzymatic Hydrolysis

The hydrolysis process was performed with 5%, 10%, and 20% (weight/volume) pretreated materials in 50 mmol/L acetate buffer (pH 4.8) with an enzyme loading of 20 FPU/g biomass. The mixtures were incubated at 50 °C in a rotator shaker at the speed of 150 rpm. After 72-h hydrolysis, the hydrolyzates were collected in 1.5-mL Eppendorf tubes and centrifuged at 12,000 rpm for 2 min (5417R, Eppendorf, Germany). Component contents of supernatants were determined using a Waters 2698 HPLC system (Milford, Massachusetts, USA) equipped with a Shodex sugar SH-1011 column at 50 °C. 5 mmol/L H₂SO₄ was used as an eluent at a flow rate of 0.5 mL/min. All of the experiments were duplicated.

Analytical Methods

Composition analysis and calculation

Composition analyses of the untreated, treated, and enzymatic-hydrolyzed materials were carried out following a National Renewable Energy Laboratory (NREL)

analytical procedure (Sluiter *et al.* 2008). The enzymatic hydrolysis efficiency was assessed by the glycan conversion (ξ) which is defined as the ratio of cellobiose, glucose, and xylose in the hydrolyzate to cellulose and hemicellulose remaining in the pretreated residue and calculated as following:

$$\xi = \frac{r_{Gg}c_gv + r_{Gcb}c_{cb}v + r_{Xx}c_xv}{m_P(\eta_G + \eta_X)} \times 100 \quad (1)$$

In this equation r_{Gg} is the molecular weight (MW) ratio of a glucan monomer to glucose [162.16/180.18]. The term r_{Gcb} is the MW ratio of two glucan monomers to cellobiose [324.32/342.34], while r_{Xx} is the MW ratio of a xylan monomer to xylose [132.11/150.13] (Roche *et al.* 2009). The quantities c_g , c_{cb} , and c_x (g/L) refer to the concentration of glucose, cellobiose, and xylose in the liquid phase. The symbol v (L) stands for the volume of solution. η_G and η_X are the ratios of glucan and xylan to pretreated material, and m_P (g) represents the mass of pretreated material.

Surface morphology observation

Surface morphologies of untreated, treated, and hydrolyzed samples were imaged using a scanning electron microscope (S-4800, Hitachi) with an accelerating voltage of 2.0 kV. Prior to observation, the samples were coated with a thin layer of gold.

XRD analysis

Crystallinity of different samples was analyzed by wide-angle X-ray diffraction on an X'Pert Pro MPD (PW3040/60, Philips, Holland) instrument using Cu K α radiation ($\lambda=0.154$ nm) at 40 kV and 40 mA, with a step size of 0.017° and a recorded range from 10° to 50°. In order to compare the crystalline changes among different samples, CrI (%) was calculated as follows (Segal *et al.* 1959),

$$\text{CrI} = \frac{I_{002} - I_{am}}{I_{002}} \times 100 \quad (2)$$

where I_{002} is the intensity of diffraction (002) plane at about $2\theta=22.5^\circ$ and I_{am} is the intensity of the baseline at about $2\theta=18.4^\circ$.

FTIR analysis

FTIR spectra of different samples were measured by direct transmittance using the KBr pellet technique. Discs were prepared with mixture of 2 mg of dried sample and 200 mg of KBr, which were pressed at 10 MPa for 20 s. The spectra were recorded between 400 and 4000 cm $^{-1}$ using a TENSOR 27 Fourier transform infrared spectrometer (Bruker Optics, Germany) with a spectral resolution of 2 cm $^{-1}$ and 64 scans per sample. The hydrolyzed samples containing a 5% solids loading were chosen to do the analysis of SEM, XRD, and FTIR. The reason for choosing them is that the viscosity of 5% solids loading influencing on their hydrolysis is weaker than that of 10% and 20% solids loadings, which makes it necessary to consider structural changes of the three lignocellulosic materials after LHW pretreatment for the main factors affecting enzymatic hydrolysis.

RESULTS AND DISCUSSION

Compositions of the Untreated and LHW-pretreated Eu, SSB, and SCB

The compositions of untreated and LHW-pretreated Eu, SSB, and SCB are summarized in Table 1. The glucan (representing mainly the cellulose) content of untreated Eu was 2.2% and 6.7% more than untreated SSB and SCB, and the xylan (representing the hemicellulose) content of untreated Eu was 7.4% and 6.7% less than untreated SSB and SCB. As for the untreated SSB and SCB, the glucan content of SSB was 4.5% more than that of SCB, while the xylan content is almost the same. The lignin contents of these three raw materials showed no significant difference.

Table 1. Compositions of Untreated and LHW-pretreated Eu, SSB, and SCB

Materials	Residues (%, g/100 g untreated material)	Glucan (%)	Xylan (%)	Acid-insoluble lignin and ash (%)
Untreated Eu		42.0±0.0	13.4±0.1	24.4±0.2
Untreated SSB		39.8±0.5	20.8±0.2	23.5±3.0
Untreated SCB		35.3±0.5	20.1±0.3	24.2±0.0
Pretreated Eu *	67.0	37.2±0.7 55.4±1.0	0 0	18.9±0.9 28.2±1.3
Pretreated SSB *	64.1	39.9±0.3 62.2±0.4	5.2±0.3 8.2±0.5	16.0±1.2 24.9±1.8
Pretreated SCB *	62.4	34.6±0.5 55.4±0.9	3.4±0.1 5.4±0.1	18.2±0.3 29.2±0.5

* Data on the top line are based on raw materials and on the bottom line are based on pretreated materials.

After LHW pretreatment, the xylan content decreased sharply, especially for Eu, in which the xylan was removed completely. For SSB and SCB, the xylan was removed by 75.0% and 83.1% respectively. These results imply that hemicellulose, which can hinder access of cellulase to cellulose, has little or no influence on enzymatic hydrolysis of cellulose (Mussatto *et al.* 2008). The lignin removal for SSB was 31.9%, which was more than that of Eu and SCB, 22.5% and 24.8%, respectively. In addition, most of glucan remained in pretreated residues, especially for SSB, where no loss of glucan was observed. The removal of cellulose, hemicellulose, and lignin is in agreement with that reported by others (Yáñez *et al.* 2009; Yu *et al.* 2010; Romaní *et al.* 2010).

Enzymatic Hydrolysis of Pretreated Eu, SSB, and SCB

Hemicellulose and lignin have been demonstrated to block enzymatic hydrolysis of cellulose. The lower the proportions of hemicellulose and lignin that are contained in

the biomass, the higher will be the enzymatic hydrolysis efficiency of cellulose (Mussatto *et al.* 2008; Zhu *et al.* 2008). According to the xylan contents of pretreated materials, it might be inferred that the enzymatic hydrolysis efficiency should be Eu>SCB>SSB, while the lignin contents of pretreated materials imply that the enzymatic hydrolysis efficiency should be SSB>Eu>SCB. The enzymatic hydrolysis efficiency of pretreated materials deduced from lignin content is inconsistent with that inferred from xylan content. Further research on enzymatic hydrolysis of pretreated materials is necessary. The glucan contents of pretreated materials are different from each other, which will lead to the production of different sugar concentrations. So the glycan conversion is more suitable for evaluating the enzymatic hydrolysis efficiency of pretreated Eu, SSB, and SCB than sugar concentration.

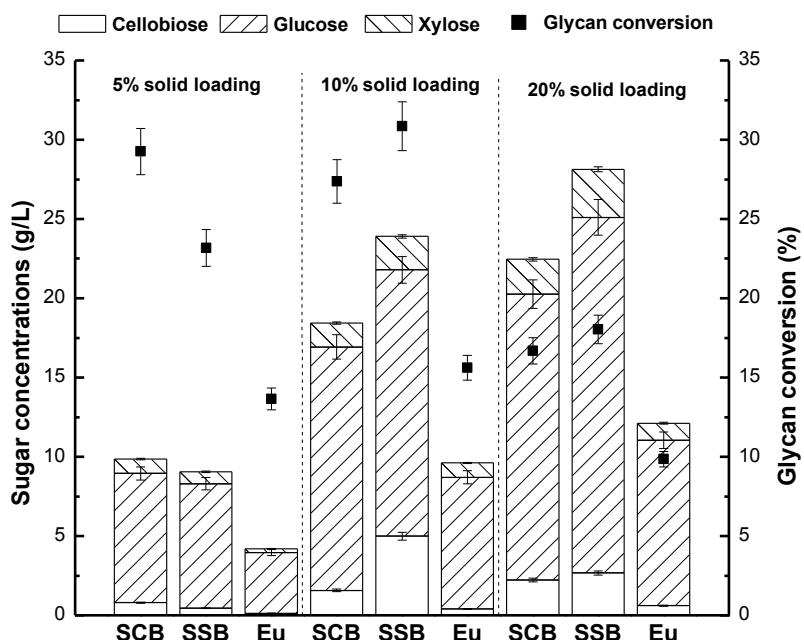


Fig. 1. Comparison of enzymatic hydrolyzing effectiveness of Eu, SSB, and SCB at different solids loadings

According to the xylan and lignin contents of pretreated Eu and SCB in Table 1, it might be deduced that enzymatic hydrolysis of pretreated Eu would be more convenient than pretreated SCB. From Fig. 1, it can be seen that the enzymatic saccharification of pretreated SCB is more effective than pretreated Eu, which is contrary to the foregoing interpretation. This indicates that the enzymatic hydrolysis efficiency of biomass depends on not only the lignin and xylan contents, but also other factors, such as the structural characteristics of biomass. Figure 1 also shows that increasing biomass loadings can lead to higher sugar concentrations after enzymatic hydrolysis for 72 h. At 10% and 20% solids loadings, after enzymatic hydrolysis for 72 h, pretreated SSB attained the highest sugar concentrations and glycan conversion, while pretreated Eu got the lowest. However, at 5% solids loading, pretreated SCB reached the highest sugar concentrations and glycan conversion. These results might be caused by the viscosity of solid-liquid mixture, which increases with rising solids loading. After adding the dry biomass into a

liquid, the biomass is first saturated by a certain quantity of water to reach absorption equilibrium. With the loading of biomass increasing, the need of water to saturate biomass increases as well. This will result in the reaction system becoming more viscous for a certain volume of solution, thus hindering the mass transfer to perform enzymatic hydrolysis. It was found that the pretreated SCB could absorb larger quantities of buffer than the pretreated SSB to reach its absorption equilibrium. Comparing 10% and 20% solids loadings, the free water left after saturating pretreated SCB at 5% solids loading was enough for mass transfer in the process of enzymatic hydrolysis. But at the solids loadings of 10% and 20%, after saturation, the quantity of free water left for the pretreated SSB was more than the pretreated SCB, and thus the pretreated SSB achieved higher enzymatic hydrolysis efficiency. The water absorption is suitable for interpreting the difference in enzymatic hydrolysis of pretreated SSB and SCB, which belong to herbaceous biomass, but not appropriate to explain the different results between those two herbaceous lignocelluloses and the pretreated Eu, of which water absorption is the lowest. The viscosity of the reaction system is not the only reason to affect the effectiveness of enzymatic hydrolysis. The results obtained from enzymatic hydrolysis show discordance with inferences only deduced from composition analyses. Further research is necessary to assess the causes. In addition, xylose is produced in the process of enzymatic hydrolysis of pretreated Eu, which is contrary to the result of the component analysis. This might have resulted from the degradation of xylose during the component analysis. The degradation efficiency of xylose was proven to be 15.9% in this study.

SEM Analysis

Based on the SEM images of untreated, treated, and hydrolyzed Eu, SSB, and SCB in Fig. 2, it is found that the surface of untreated Eu is smooth and flat, and no fiber bundles can be observed, while the surfaces of untreated SSB and SCB consist of compact fiber bundles. This suggests that the structure of untreated Eu is more compact than untreated SSB and SCB. After LHW pretreatment, the surface morphologies of Eu, SSB, and SCB are changed significantly. The fiber bundles of treated Eu emerge from the inside and are arranged with many deep longitudinal cracks and tighter than the treated SSB and SCB, which pose an obstacle for the access of cellulase to cellulose. This may be one of the reasons that the enzymatic hydrolysis of the treated Eu is less effective than that of the treated SSB and SCB. The compact fiber bundles of SSB and SCB are split and fractured, contributing to the increase of rough and fresh surfaces, which can increase the accessibility of the cellulase. In addition, the surface of pretreated SCB is more porous than the two others, which makes it more hydrophilic and thus tending to absorb more water. From Fig. 2, it can be also observed that after treating Eu, SSB, and SCB with LHW, the conformations of their single fiber bundle are not changed obviously except some intact fractures. Combined with the sample composition in Table 1, it can be inferred that the removal of lignin and hemicellulose causes the changes of external and internal structure of the materials, and LHW pretreatment under the condition of this research has little impact on cellulose destruction. After enzymatic hydrolysis, little debris can be found except gap expansion of fiber bundles.

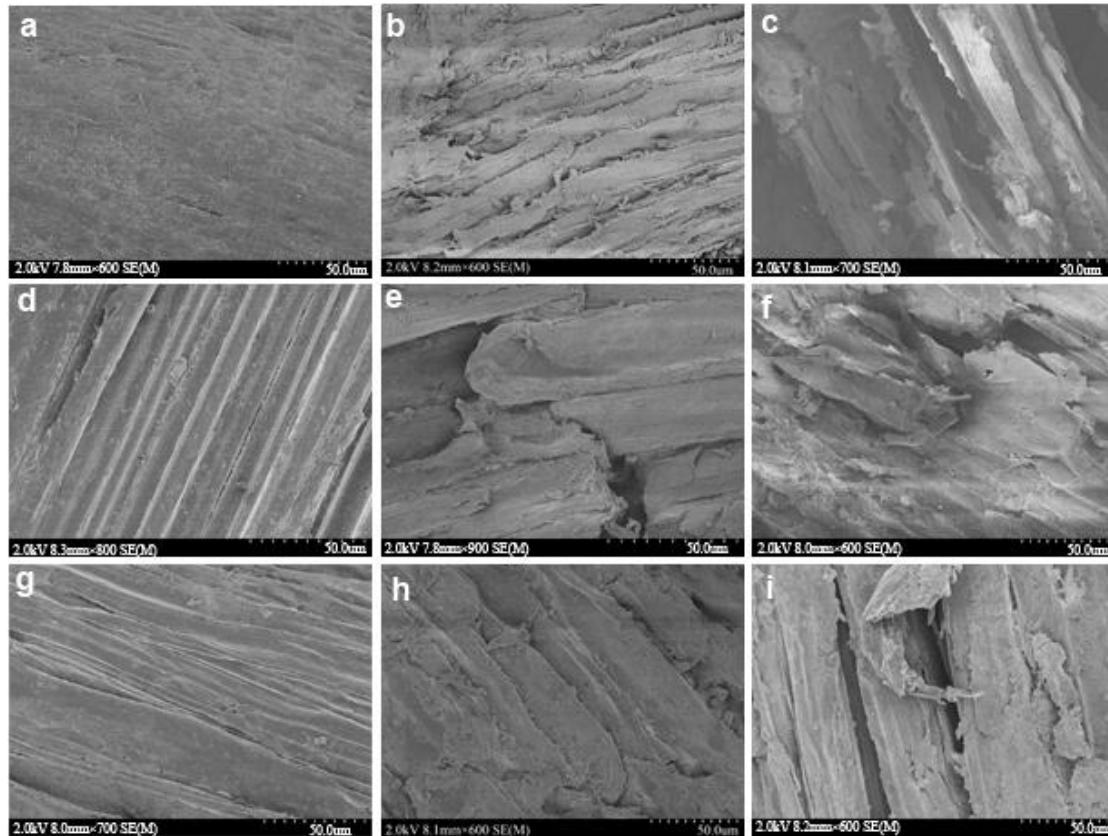


Fig. 2. SEM micrographs of samples, (a) untreated Eu; (b) pretreated Eu; (c) hydrolyzed Eu; (d) untreated SSB; (e) pretreated SSB; (f) hydrolyzed SSB; (g) untreated SCB; (h) pretreated SCB; (i) hydrolyzed SCB

XRD Analysis

X-ray diffraction curves of untreated, pretreated, and hydrolyzed substrates are shown in Fig. 3 and the CrI are given in Table 2.

Table 2. CrI of Untreated, Pretreated, and Hydrolyzed Eu, SSB, and SCB

Materials	CrI (%)
Untreated Eu	83.83±4.2
Pretreated Eu	81.58±4.1
Hydrolyzed Eu	82.69±4.1
Untreated SSB	71.83±3.6
Pretreated SSB	79.16±4.0
Hydrolyzed SSB	79.72±4.0
Untreated SCB	62.28±3.1
Pretreated SCB	75.45±3.8
Hydrolyzed SCB	75.74±3.9

All of the diffraction curves are of typical cellulose I structure (Oh *et al.* 2005), which indicates that LHW pretreatment and enzymatic hydrolysis do not alter the crystal type of cellulose. Two obvious peaks at $2\theta=15.9^\circ$ and 22.5° can be visualized from Fig. 3. The presence of the peak at $2\theta=15.9^\circ$, representative of an amorphous region, is mostly due to the presence of non-cellulosic materials such as hemicellulose and lignin, while

the sharp peak at $2\theta=22.5^\circ$ can be assigned to the (002) plane of cellulose I, which is ascribed to α -cellulose (Jayaramudu *et al.* 2011).

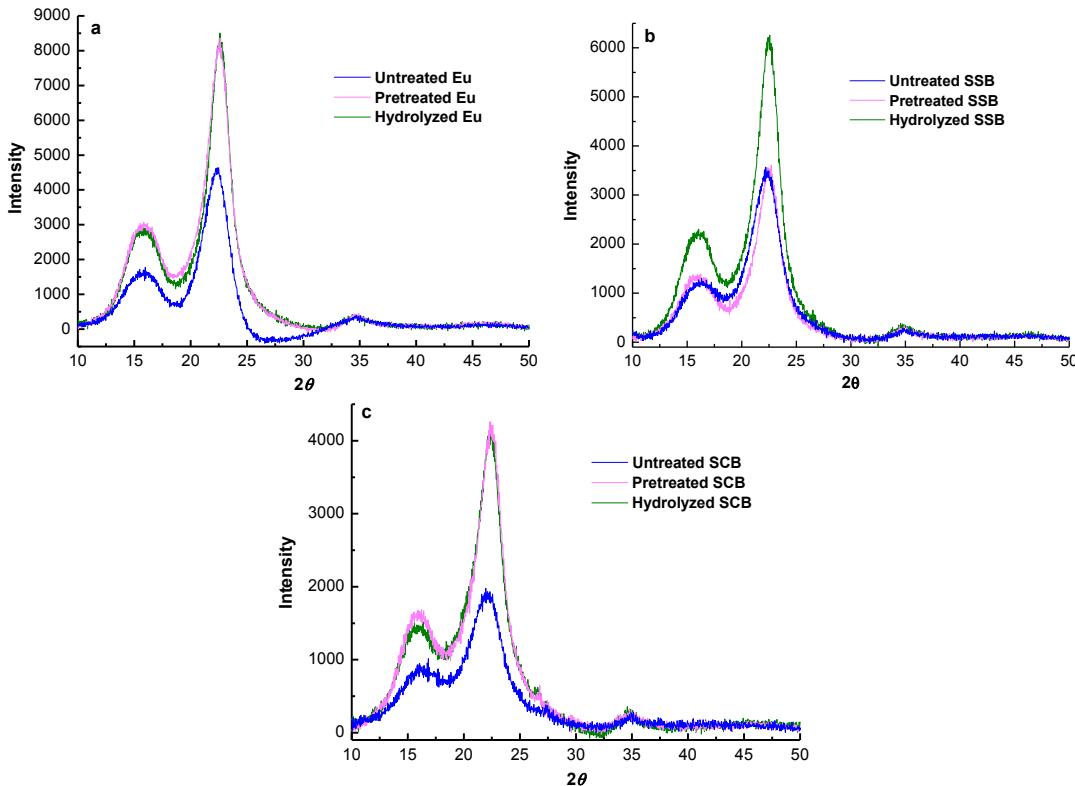


Fig. 3. XRD pattern of untreated, pretreated, and hydrolyzed materials at 5% solids loading for LHW pretreatment, (a) Eu; (b) SSB; (c) SCB

After LHW pretreatment, the CrI of SSB and SCB increased 7.33% and 13.17%, respectively, while the CrI of Eu decreased 2.25%. This is because the removal of hemicellulose and partial lignin leads to rearrangement of crystalline regions, and the removal of cellulose is little or even none for SCB and SSB, but relatively more for Eu. The CrI intensity of untreated, pretreated, and hydrolyzed substrates was Eu>SSB>SCB, indicating that the structural compaction was Eu>SSB>SCB, which is the reason for the lowest enzymatic hydrolysis efficiency of Eu. Moreover, it can be observed from Fig. 3 that the peak intensity at $2\theta=22.5^\circ$ of the pretreated SSB is lower than that of the pretreated SCB, whereas the opposite result is obtained for the peak intensity at $2\theta=15.9^\circ$. It can be deduced that the smaller crystalline region makes pretreated SSB easier to be hydrolyzed at high solids loading, and the bigger amorphous region renders the pretreated SCB easier to be hydrolyzed at low solids loading.

FTIR Analysis

FTIR spectra with baseline correction of Eu, SSB, and SCB from untreated, pretreated, and hydrolyzed phases are presented in Fig. 4. The assignments of main bands are summarized in Table 3 (Schwanninger *et al.* 2004; Abidi *et al.* 2007; Abidi *et al.* 2010; Liang *et al.* 2011).

The absorption band between 3600 cm^{-1} and 3000 cm^{-1} is attributed to O-H stretching vibration. It is comprised of valence vibrations of H-bonded OH groups at 3570 to 3450 cm^{-1} , O(2)H \cdots O(6) intramolecular hydrogen bonds at 3455 to 3410 cm^{-1} , O(3)H \cdots O(5) intramolecular hydrogen bonds at 3375 to 3340 cm^{-1} , and O(6)H \cdots O(3) intermolecular hydrogen bonds at 3310 to 3230 cm^{-1} (Schwanninger *et al.* 2004; Oh *et al.* 2005). From Fig. 4, it can be observed that the width of bands between 3600 cm^{-1} and 3000 cm^{-1} for all samples became narrower after LHW-pretreatment. It is noted that all the changes happened to the band at 3310 to 3230 cm^{-1} , which suggests that LHW pretreatment mainly destroys the intermolecular hydrogen bonds in cellulose. After enzymatic hydrolysis, the intensities of the peaks in this area for hydrolyzed SSB and SCB were increased, and the band for hydrolyzed SCB became narrower than untreated and pretreated SCB, which suggests that the enzymatic hydrolysis of pretreated SCB is more effective than that of SSB. This corresponds to the result of enzymatic hydrolysis of samples at low solids loading shown in Fig. 1. But the band of hydrolyzed Eu is broader than the untreated and pretreated materials. This might be ascribed to the fact that hydrolysis of pretreated Eu produces more hydroxyl groups, which enable the formation of new intermolecular hydrogen bonds.

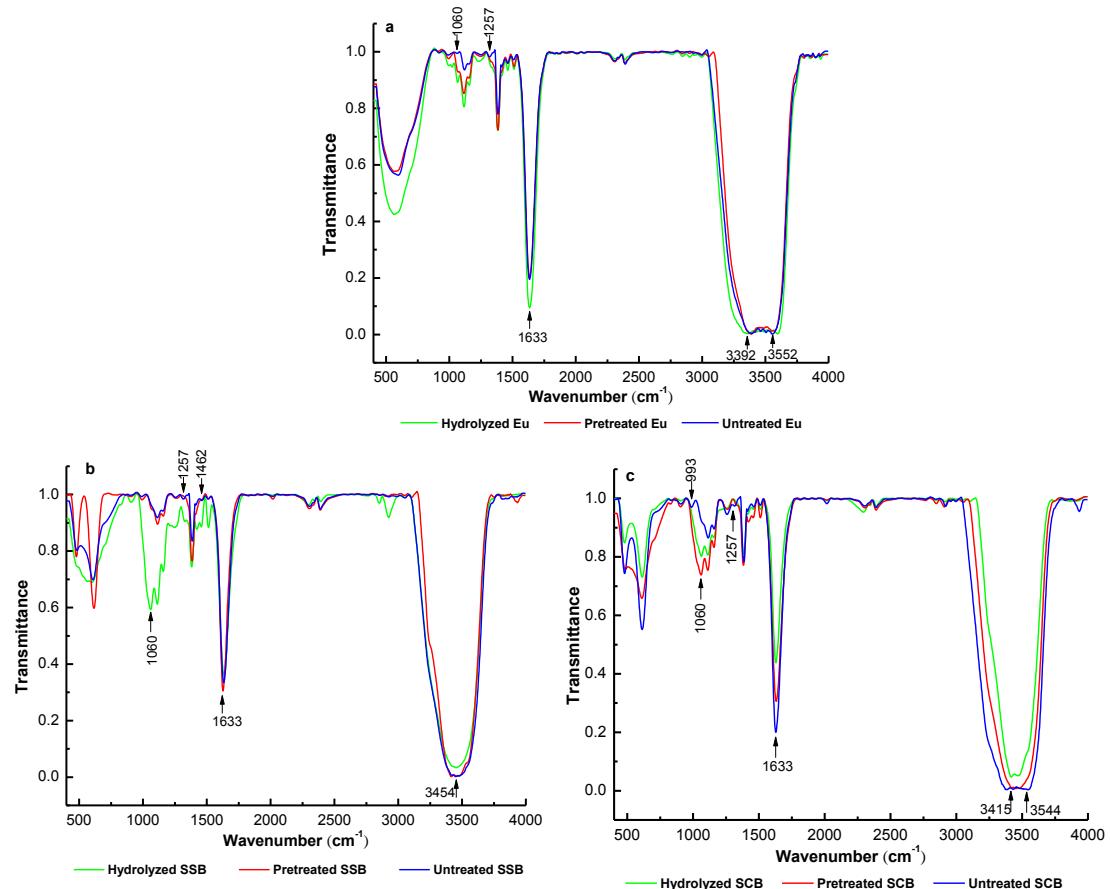


Fig. 4. FTIR spectra of untreated, pretreated, and hydrolyzed materials at 5% solids loading for LHW pretreatment, (a) Eu; (b) SSB; (c) SCB

It can also be observed that the most prominent difference was the band at 1635 to 904 cm⁻¹. The band at ~1633 cm⁻¹ originates from absorbed water molecules via hydrogen bonding in the amorphous regions of the cellulose macromolecules (Abidi *et al.* 2007). The increase in the intensity at 1633 cm⁻¹ for pretreated and hydrolyzed SCB indicates that LHW pretreatment and enzymatic hydrolysis lead to physicochemical changes in the amorphous regions, which reduces reaction with water via hydrogen bonding. The intensity of peak at 1633 cm⁻¹ for hydrolyzed Eu is decreased due to the appearance of new groups, which can form hydrogen bond with water.

Table 3. Peak Wave Numbers of Bands and Their Assignments

Wavenumber (cm ⁻¹)	Assignment
3600-3000	O-H stretching
~2850	-CH ₂ - asymmetric stretching
~1633	O-H bending of adsorbed water
1514-1512	Aromatic skeletal vibration
1462	C-H deforming in methyl and methylene
1384	C-H deforming in methyl
1257	C-O stretching, C=O vibration, Amide III
1159	C-O-C stretching in pyranose rings, C=O stretching in aliphatic groups
1112	Ring asymmetric valence vibration
1060	C-O deforming in secondary alcohols and aliphatic ethers
993-987	C-O valence vibration
908-904	C-H stretching out of plane of aromatic rings

Compared with raw materials, the peaks of pretreated Eu at 1060 cm⁻¹ and 1257 cm⁻¹ disappear, the peaks of pretreated SSB at 1257 cm⁻¹ and 1462 cm⁻¹ disappear, and the peaks of pretreated SCB at 993 cm⁻¹ and 1257 cm⁻¹ disappear. These changes might be caused by the removal of protein and some groups originated from hemicellulose and lignin (Table 3). In addition, the peak at 1060 cm⁻¹ appears in these three hydrolyzed residues, which might have originated from residual cellulose or lignin (Table 3). For all of the pretreated residues, the increase in the intensity at other bands between 1635 cm⁻¹ and 904 cm⁻¹ might be caused by the rising content of lignin and cellulose (Table 3).

CONCLUSIONS

1. The effect of LHW pretreatment on enzymatic hydrolysis of different biomass was evaluated by SEM, XRD, and FTIR in this study. SEM images showed that the fiber bundles of treated Eu were arranged more tightly than the treated SSB and SCB. XRD tests indicated that the crystallinity value of Eu was scarcely affected by LHW pretreatment and was higher than the corresponding values for SSB and SCB. FTIR tests implied that the chemical bond changes of Eu were gentler than SSB and SCB after LHW pretreatment and enzymatic hydrolysis. These structural differences in Eu, SSB, and SCB make the enzymatic hydrolysis of LHW-pretreated Eu less effective

than that of SSB and SCB in all tests. LHW is more suitable for treating SSB and SCB than Eu.

2. For pretreated SSB and SCB, the structural characteristic of SCB is looser and more porous than SSB, and thus the hydroscopicity of SCB is stronger than SSB. The enzymatic hydrolysis efficiency of LHW-pretreated SCB is higher than SSB at low solids loading, while lower at high solids loading.
3. Lignocellulosic materials having moderate structural compactness should be suitable for LHW pretreatment to obtain high sugar concentration and enzymatic hydrolysis efficiency at high solids loading.

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