Impact of Dilute Sulfuric Acid Pretreatment on Fermentable Sugars and Structure of Bamboo for Bioethanol Production

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Moso bamboo (*Phyllostachys edulis*) is an important source of lignocellulosic materials because of its fast growth, its vegetative propagation, and its easy harvesting. The pretreatment of bamboo with dilute sulfuric acid and the effects on its chemical components and enzymatic hydrolysis were studied, in addition to the fibrous structural properties of pretreated residues by scanning electron microscopy (SEM), X-ray diffraction (XRD), and Fourier transform infrared (FTIR) spectroscopy. The results showed that dilute sulfuric acid pretreatment primarily hydrolyzed hemicelluloses and resulted in enhanced cellulose and lignin content in the pretreated solids. The maximum yield of hemicellulose recovery was 81.42% when pretreated with 1.00% sulfuric acid at 150 °C for 30 min, and the enzymatic hydrolysis yield was 79.45% when hydrolyzed for 72 h with an enzyme loading of cellulase 40 FPU/g of cellulose. Under these conditions, the overall sugar yield was 83.36% (cellulose and hemicellulose), with a total of 67.11 g fermentable sugars from 100 g dry bamboo. The results indicated that Moso bamboo underwent considerable changes in its chemical composition and physical properties after acid pretreatment, such as the removal of hemicellulose and lignin, an increase in specific surface area and pore volume, and exposure of internal structure, which enhances the enzymatic hydrolysis of Moso bamboo.

Keywords: Fuel ethanol; Dilute acid pretreatment; Enzymatic hydrolysis; Fermentable sugars; Structural characteristics; Bamboo

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INTRODUCTION

Because of social and industrial development over the last two decades, more and more fossil fuels have been consumed worldwide. Growing concerns about energy security and environmental impact require the large-scale replacement of petroleum-based fuels. Renewable fuels are becoming increasingly important as a consequence of heightened concern for the greenhouse effect, depleting oil reserves, and rising oil prices. Biofuel produced from renewable lignocellulosic biomass is one of the most important renewable fuels and is widely used as a partial gasoline replacement in the world. It helps to solve energy and environmental problems that many countries are facing, especially densely populated countries with high fuel consumption like China, Brazil, India, and Egypt (Ibrahim 2012).

Ethanol (bioethanol) is the most employed liquid biofuel, either as a fuel or as a gasoline enhancer. Bioconversion of renewable lignocellulosic biomass to biofuel has many advantages from both economic and environmental points of view (Gray *et al*. 2006; Xu *et al*. 2010; Li *et al*. 2012; Sarkar *et al*. 2012). Low-cost production materials, such as agricultural and forest residues, energy crops, and municipal solid wastes, make bioethanol an affordable solution (Menon and Rao 2012; Haghighi Mood *et al*. 2013). Bamboo stands out as an ideal feedstock for fuel ethanol production because of its advantages, such as biodegradability, low cost, and abundant utilization (Li *et al*. 2012; Kuttiraja *et al*. 2013). Moreover, carbohydrates (cellulose and hemicellulose) account for nearly 75% of the whole plant, indicating that bamboo is a potentially useful biomass resource for the production of bioethanol. The biological conversion of ethanol from cellulose and hemicellulose can be achieved by pretreatment, enzymatic hydrolysis, and fermentation. The effective utilization of these components may play a significant role in the economic viability of cellulosic ethanol (Sánchez and Cardona 2008; Sassner *et al*. 2008).

In bamboo-based ethanol production, enzyme-catalyzed conversion of cellulose to glucose is time-consuming because the sites available for enzymatic attacking are limited. Because native cellulose in biomass possesses a highly resistant crystalline structure and is well protected by a matrix of hemicellulose and lignin, enzymatic access is restricted by the lignin and hemicellulose interference. As a result, pretreatment of biomass is necessary. An ideal pretreatment should accomplish a reduction in lignin content, concomitant with a reduction in crystallinity and an increase in surface area (Eggeman and Elander 2005; Del Campo *et al*. 2006; Chen *et al*. 2012; Mao *et al*. 2013).

Several different pretreatment methods have been used. They are categorized into physical pretreatment, chemical pretreatment, physicochemical pretreatment, biological pretreatment, and a combination of several pretreatments, depending on the mode of their action in the pretreatment process (Xiao *et al*. 2011; Dagnino *et al*. 2013; Gu *et al*. 2013; Jin *et al*. 2013; Lee *et al*. 2013b; Sindhu *et al*. 2013; Cotana *et al*. 2014; De Bari *et al*. 2014; Hong *et al*. 2014; Maryana *et al*. 2014). Among them, dilute acid pretreatment has demonstrated to be an effective pretreatment in enhancing enzymatic hydrolysis of lignocellulosic biomass. It is performed by soaking materials in dilute acid solution and then heating to a temperature between 120 and 220 °C for a certain period of time. The dilute acid pretreatment serves to hydrolyze glycosidic bonds in hemicellulose, lignin-hemicellulose bonds, and lignin bonds (Dien *et al*. 2011; Kristiani *et al*. 2013; Perez-Cantu *et al*. 2013). This leads to the dissolution of the sugars of hemicelluloses and to an increased porosity of the plant cell walls, which makes cellulose fibers more accessible to acid or cellulose enzymes.

Many researchers have focused on bamboo pretreatment for bioethanol production (Cheng *et al*. 2014; Li *et al*. 2012). Pretreatment with acid or alkali is used to enhance the digestibility of bamboo. Leenakul and Tippayawong (2010) employed dilute acid pretreatment of bamboo for fermentable sugar production. Dilute phosphoric acid retreatment of moso bamboo was studied, which showed that the optimum dilute acid pretreatment conditions were 170 °C and 45 minutes (Hong *et al*. 2012). Pretreatment was carried out by immersing the bamboo in KOH (12% and 8% w/w bamboo) solutions and exposing the slurry to microwave radiation power of 400 W for 30 min. The results showed that the pretreated substrate with microwave assisted KOH had a significantly high sugar yield. The fermentation inhibitors (formic acid, furfural, HMF and levulinic acid) were much lower than those with acid pretreatment. Ethanosolv with NaOH

pretreatment of moso bamboo for efficient enzymatic saccharification was reported by Li *et al*. (2012). It was shown that the addition of 10% (w/w on bamboo) NaOH in 75% (v/v) ethanol could be effective in the pretreatment and fractionation of bamboo. Ethanol organosolv pretreatment with dilute sulfuric acid as catalyst was studied in order to enhance enzymatic saccharification of bamboo (Li *et al*. 2012). The work showed that organosolv pretreatment with sulfuric acid as a catalyst significantly accelerated hemicellulose and lignin removal and increased the enzymatic digestibility of bamboo substrates. The comparison of dilute organic and sulfuric acid in pretreating bamboo was reported (Li *et al*. 2014). It was demonstrated that the pretreatment with dilute formic acid at 180 °C and 30 min could be an acceptable alternative to dilute sulfuric acid pretreatment. Bioconversion of bamboo to bioethanol using the two-stage organosolv and alkali pretreatment was reported by Li *et al*. (2012). This treatment resulted in about 96.5% lignin removal after organosolv pretreatment (2% H_2SO_4 in 75% ethanol w/w) followed by sodium hydroxide pretreatment (10% w/w).

Some researchers have found that the cellulose conversion to glucose yield of bamboo was lower than that of both wood and agricultural waste. In this study, we endeavored to investigate how dilute acid pretreatment affects the solubilization of cellulose and hemicellulose in bamboo and the subsequent enzymatic hydrolysis. We also analyzed the changes in the chemical composition and physical characteristics caused by the pretreatment and study how those factors affect enzymatic digestibility. The physical features of fibers, as well as the structure and properties of pretreated residues, were examined using scanning electron microscopy (SEM), X-ray diffraction (XRD), and Fourier transform infrared (FTIR) spectroscopy.

EXPERIMENTAL

Materials

Moso bamboo (*Phyllostachys edulis*) from Jiangxi, having an age of six years, was comminuted by the combination of chipping and milling to attain a powder with particle sizes of approximately 1 mm. After air-drying at room temperature to an equilibrium moisture content of about 10%, the ground bamboo was homogenized and stored in sealed plastic bags at 4 °C until use.

Methods

Pretreatment

The bamboo samples were presoaked at room temperature in the corresponding concentration of dilute sulfuric acid for at least 4 h. The presoaked slurry was then transferred to the reactor for treatment. Agitation was set at 500 rpm, and the average heating rate was 3 °C/min. Dilute acid pretreatment was performed in a laboratory-scale stirred autoclave, which has a total volume of 1 L, with an electric heater and magnetic agitation. Bamboo at a solid-to-liquid ratio of 1:5 to 1:15 (g:mL) was mixed with dilute sulfuric acid (acid concentrations: 0.75 to 1.50% w/w) and reacted at four temperatures in the range of 130 to 160 °C for a residence time of 30 to 60 min. When the reaction ended, the autoclave reactor was transferred to room-temperature water to complete the reaction. The contents of the autoclave were washed with deionized water into a 500-mL volumetric flask, followed by filtration to separate the slurry into the solid residue and liquid prehydrolyzate. The solid residue was analyzed for hemicellulose sugar, glucose,

and acid-insoluble lignin contents, and used as the substrate in enzymatic hydrolysis tests. The liquid prehydrolyzate was analyzed for sugars and inhibitors. Monomeric sugars and degradation products in the prehydrolyzate were identified using a modified NREL laboratory analytical procedure (Sluiter *et al*. 2008).

Enzymatic hydrolysis

The solid residues recovered after dilute acid pretreatment were hydrolyzed by cellulase at 50 °C and 80 rpm for 72 h in a water bath shaker. A sodium citrate buffer was used in the mixture to maintain the pH at 4.8 while sodium azide (0.3% w/v) was added to inhibit microbial growth. Cellulase enzyme loading was 40 filter paper units (FPU)/g cellulose. Fungal *β*-glucosidase, at an enzyme loading of 20 international units (IU)/g cellulose, was used as a supplement to *β*-glucosidase activity in the cellulase. Sugars were analyzed after enzymatic hydrolysis. All enzymatic hydrolysis experiments were performed in duplicate, and the average results are given.

Enzymatic digestibility (*Y*) was determined in Eq. 1,

$$Y\,\% = (C_{HPLC} \times 0.02) \div (G \times W \times 1.11) \times 100 \quad (1)$$

where C_{HPLC} is the concentration of sugar as determined by HPLC (g/L); 0.02 is the total volume (L); 1.11 is the conversion factor for glucan to equivalent glucose; G is the mass of residue (g); and W is the content of cellulose (%).

Analytical methods

Samples of the wet biomass were weighed with a four-digit analytical balance and dried to a constant weight in an oven at 105 °C. The typical composition of the untreated and all pretreated bamboo substrates were determined according to the National Renewable Energy Laboratory (NREL) standard analytical procedure for biomass (Sluiter *et al*. 2012). Prior to determination, the raw material was extracted consecutively with water and ethanol in a two-step extraction procedure.

Cellulose and hemicellulose contents of the extracted solid residues were determined based on monomer content measured after a two-step acid hydrolysis procedure. In the first step, the sample was treated with 72% (w/w) H_2SO_4 at 30 °C for 60 min. In the second step, the reaction mixture was diluted to 4% (w/w) H_2SO_4 and autoclaved at 121 °C for 60 min. The hydrolyzates were then analyzed for sugar content by high-performance liquid chromatography (HPLC) with an Agilent 1100 liquid chromatograph (USA) with a refractive index detector. An AMINEX HPX-87P carbohydrate analysis column (Bio-Rad, Hercules, CA) operating at 85 °C with ultrapure water as the mobile phase (0.6 mL/min) was used. The sugar content (cellobiose, glucose, xylose, and arabinose) of the liquid fraction after pretreatment was determined by HPLC. Formic acid, acetic acid, levulinic acid, furfural, and hydroxymethlfurfural were analyzed by HPLC in an Agilent 1100 liquid chromatograph with a Bio-Rad HPX-87H column at 65 °C. The mobile phase was 5 mM H_2SO_4 at a flow rate of 0.6 mL/min. All analytical determinations were performed in duplicate, and the average results are shown.

Scanning electron microscopy

Scanning electron microscopy of the untreated and pretreated residues was carried out with a Philips Quanta D8594 (The Netherlands) operating at an acceleration voltage

of 20 kV after gold coating. Images were obtained at magnifications ranging from 300× to 2500×, depending on which feature was to be traced.

X-ray diffraction

X-ray diffraction (XRD) measurements were performed on Rigaku D/max-2500/PC (Rigaku Corporation, Japan). The crystallinity of the bamboo samples was determined in a 2θ range between 3° and 40° while the instrument was operated at 40 kV and 200 mA.

Fourier transform infrared spectroscopy

Fourier transform infrared (FTIR) spectroscopy was performed using a Nicolet IR 6700 FTIR spectrometer (Thermo Scientific, USA) with a diffusive reflection accessory and the KBr disc technique. The spectra were obtained using 64 scans of the sample with no dilution, triangular apodization, a resolution of 4 cm^{-1}, and an interval of 1 cm^{-1}.

RESULTS AND DISCUSSION

Effect of Pretreatment Conditions on Chemical Composition of Bamboo

The solid yield was calculated in relation to the raw material. The total solid mass, which is shown in Table 1, was reduced by about 40%, and the remaining solid was in the range of 58 to 68%. Chemical compositions of untreated and pretreated bamboo substrates are shown in Table 1. Compared to the untreated sample, the chemical composition of the pretreated bamboo solids was changed. The pretreatment hydrolyzed most of the hemicelluloses and a small amount of lignin, but hardly solubilized any of the cellulose. As a result, 65 to 90% of the total hemicellulose of the bamboo feedstock was solubilized, and 15 to 30% of the total lignin was removed. Therefore, the solid material recovered after acid pretreatment mostly consisted of cellulose and lignin, whereas hemicellulose comprised a relatively smaller portion of the carbohydrates in the treated material as compared to the untreated material (Xiao *et al*. 2011). Under the acidic pretreatment conditions, the hydronium ion initially caused hemicellulose depolymerization and cleavages of the acetyl group. The primary effect of acid pretreatment on the composition of the biomass was the partial and substantial hydrolysis of hemicellulose (Canilha *et al*. 2012). Increasing the acid concentration, temperature, and residence time produced higher hydrolysis of hemicellulose to a minimum amount of 3.12% of raw bamboo with 1.5% acid at 160 °C for 40 min. Consequently, with the content of hemicellulose solubilized into prehydrolyzate, a cellulose-enriched solid residue fraction was obtained. The total cellulose content range increased from 62% to 68%, compared with the initial percentage of 49%. Overall, the amount of solubilized hemicellulose increased obviously with increasing temperature and residence time.

The recovered solid fractions were found to have a much higher level of lignin content compared to the untreated material. Concerning the lignin content, the maximum value of pretreated material reached nearly 20% when pretreated with 1.00% dilute acid at 130 °C for 30 min.

Ideally, the sulfuric acid pretreatment should fully utilize cellulose and hemicelluloses as ethanol or other high-value products (Shi *et al*. 2013). From Table 1, the cellulose was well preserved, which is crucial to the pretreatment process. Acid pretreatment of lignocellulosic materials resulted in a decrease in the amounts of

hemicelluloses and lignin in the residues and an increase in the cellulose content. Eventually, such changes can increase the efficiency of enzymatic hydrolysis and the yield of the reducing sugar (Sindhu *et al*. 2011).

Table 1. Chemical Analysis of Untreated and Pretreated Bamboo Substrates

Pretreatment test	Sulfuric acid concentration (%, w/w)	Temperature (°C)	Residence time (min)	Solid-to-liquid radio (g:mL)	Solid recovery (%)	Composition (%)		
						Cellulose	Hemicellulose	Lignin
CT	untreated				100.00	49.06±1.89	24.12±0.56	15.85±0.60
1	0.75	160	40	1:10	62.68±2.90	65.40±2.93	10.81±0.53	18.91±0.85
2	1.00	160	40	1:10	60.95±2.87	64.05±3.15	3.15±0.16	17.83±0.83
3	1.25	160	40	1:10	59.30±1.95	63.20±2.82	3.13±0.21	17.63±0.96
4	1.50	160	40	1:10	58.77±2.12	62.25±2.65	3.12±0.14	17.93±0.78
5	1.00	130	30	1:10	67.34±2.34	67.23±2.34	14.55±0.63	19.77±1.02
6	1.00	140	30	1:10	66.28±2.54	68.01±2.75	7.03±0.36	19.61±0.93
7	1.00	150	30	1:10	63.06±2.87	67.63±3.15	3.18±0.16	17.34±0.74
8	1.00	160	30	1:10	61.76±1.92	64.95±2.96	3.20±0.14	17.15±0.80
9	1.00	150	30	1:10	63.06±2.87	67.63±3.15	3.18±0.16	17.34±0.74
10	1.00	150	40	1:10	65.15±2.82	67.63±2.82	4.03±0.21	18.98±0.95
11	1.00	150	50	1:10	63.86±2.33	68.07±2.79	3.96±0.20	17.90±0.91
12	1.00	150	60	1:10	62.56±2.11	68.09±2.81	3.19±0.18	17.31±0.82
13	1.00	150	30	1:15	63.35±2.54	67.62±2.80	3.96±0.15	17.20±0.93
14	1.00	150	30	1:10	63.06±2.87	67.63±3.15	3.18±0.16	17.34±0.74
15	1.00	150	30	1:8	63.17±2.23	67.25±2.45	4.22±0.18	18.10±0.65
16	1.00	150	30	1:5	63.05±2.75	67.07±2.28	4.47±0.17	18.27±0.97
Data presented as mean ± standard deviation								

Effect of Pretreatment Conditions on the Prehydrolyzates

After pretreatment under different conditions, the prehydrolyzates were collected. Table 2 shows the composition of the filtrates obtained after pretreatment of 100 g raw material. The prehydrolyzate, a mixture of sugars, contains cellobiose, glucose, xylose, and arabinose. Under the assayed conditions, a maximum content of 23.69 g sugars was acquired from 100 g bamboo when pretreated with 1.0% acid at 150 °C for 30 min. Because hemicellulose was more easily digested by acid than cellulose, xylose was the most abundant sugar in the filtrate. The highest hemicellulose recovery was 85.89%, when pretreatment conditions were 1.00% sulfuric acid at 150 °C for 30 min. Xylose was the primary sugar among the hemicellulose-derived sugars and can be converted into ethanol by xylose-fermenting yeasts or recombinant microorganisms (Sasaki *et al*. 2013). When the pretreatment conditions were less effective, the hemicellulose was not solubilized completely and remained in the solid residues. Increased severity of the pretreatment conditions resulted in higher solubility of hemicellulose. However, the xylan in the biomass was degraded into furfural or other byproducts.

The liquid phase of pretreatment also contained variable amounts of non-sugars, such as furfural, hydroxymethylfurfural, acetic acid liberated from acetyl groups in the hemicellulose fraction of raw material, formic acid, and levulinic acid. All of these

compounds have been described as inhibitors to the fermentation process to different extents (Oliva *et al*. 2006; Chandel *et al*. 2007; Hodge *et al*. 2009; Bellido *et al*. 2011; Zhu *et al*. 2011) and hence must be taken into account for the evaluation of pretreatment. Table 2 shows the composition of non-sugar compounds in the filtrates. The lowest content of inhibitors was 2.01 g/100 g bamboo when pretreated with 1.00% acid at 130 °C for 30 min. The content of inhibitors was 4.22 g/100g bamboo when pretreated with 1.00% acid at 150 °C for 30 min. The maximum content of 8.98 g/100 g bamboo of inhibitors was determined in the prehydrolyzate pretreated with 1.50% acid at 160 °C for 40 min.

Table 2. Composition of the Prehydrolysates (g/100 g Oven-dried Bamboo) Resulting from Dilute Acid Pretreatment at Different Conditions

Pretreatment Test	Sugars (g)					Inhibitors (g)				
	Cellobiose	Glucose	Xylose	Arabinose	Hemicellulose recovery (%)	Formic acid	Acetic acid	Furfural	Hydroxymethylfurfural	Levulinic acid
1	0.27±0.01	1.27±0.05	19.12±0.47	1.01±0.04	75.11±2.23	1.35±0.01	2.37±0.05	0.70±0.03	0.33±0.01	0.54±0.02
2	0.48±0.02	1.34±0.06	18.64±0.75	0.87±0.03	72.80±3.42	1.50±0.02	2.42±0.11	0.81±0.03	0.42±0.01	0.67±0.01
3	0.67±0.02	1.59±0.08	18.02±0.78	0.91±0.03	70.63±3.10	1.70±0.02	2.58±0.12	1.75±0.06	1.43±0.07	0.97±0.03
4	0.79±0.03	1.60±0.06	17.89±0.63	0.86±0.04	69.96±2.98	1.74±0.03	2.81±0.12	2.04±0.11	1.34±0.06	1.05±0.04
5	0.39±0.01	0.34±0.01	16.34±0.67	0.94±0.01	64.48±1.47	0.33±0.01	0.82±0.03	0.23±0.01	0.30±0.01	0.36±0.01
6	0.71±0.04	1.00±0.04	17.59±0.55	0.96±0.02	69.22±2.55	0.37±0.01	1.01±0.05	0.79±0.03	0.65±0.02	0.61±0.02
7	0.48±0.02	1.53±0.06	20.85±0.75	0.97±0.03	81.42±3.42	0.50±0.02	1.82±0.11	0.81±0.03	0.42±0.01	0.67±0.01
8	0.45±0.02	1.49±0.06	18.96±0.84	0.95±0.02	74.37±3.10	1.34±0.03	2.24±0.11	1.10±0.05	0.73±0.03	0.41±0.02
9	0.48±0.02	1.53±0.06	20.85±0.75	0.97±0.03	81.42±3.42	0.50±0.02	1.82±0.11	0.81±0.03	0.42±0.01	0.67±0.01
10	0.63±0.03	1.35±0.05	19.67±0.82	0.95±0.03	76.94±2.84	1.04±0.02	2.32±0.10	1.01±0.05	0.89±0.03	0.61±0.03
11	0.55±0.02	1.51±0.06	19.16±0.81	0.91±0.03	74.89±2.18	1.60±0.07	2.82±0.14	1.25±0.10	1.06±0.07	0.81±0.06
12	0.55±0.03	1.69±0.07	18.38±0.80	0.99±0.04	72.28±2.32	1.65±0.12	2.97±0.20	1.50±0.11	1.33±0.12	0.98±0.11
13	0.79±0.04	1.56±0.05	19.65±0.91	0.98±0.05	76.98±3.02	0.52±0.02	2.06±0.22	0.98±0.02	0.45±0.02	0.57±0.02
14	0.48±0.02	1.53±0.06	20.85±0.75	0.97±0.03	81.42±3.42	0.50±0.02	1.82±0.11	0.81±0.03	0.42±0.01	0.67±0.01
15	0.71±0.02	1.43±0.06	19.07±0.77	0.94±0.04	74.66±2.92	0.53±0.03	1.98±0.11	0.78±0.03	0.89±0.01	0.65±0.01
16	0.79±0.02	1.36±0.05	18.91±0.69	0.96±0.03	74.10±2.24	0.51±0.01	1.95±0.09	0.72±0.03	0.90±0.02	0.59±0.02
Data presented as mean ± standard deviation										

As a rule, the contents of acetic and formic acids increased as pretreatment temperature or acid concentration increased. Furfural and hydroxymethylfurfural contents also increased concomitantly with increasing pretreatment acid concentration and temperature (Nakashimada *et al*. 1999; Oliva *et al*. 2006; Chandel *et al*. 2007; Hodge *et al*. 2009; Alriksson *et al*. 2011; Bellido *et al*. 2011; Zhu *et al*. 2011; Lee *et al*. 2013a). Levulinic acid content in the prehydrolyzates was slight, and the acid concentration and pretreatment temperature had no significant effect on the formation of levulinic acid. These products generated in the pretreatment process strongly inhibited cell growth and negatively affected fermentative

microorganisms. The detoxification step was an inevitable step, removing those inhibitors from the pretreated lignocelluloses material (Sun 2009), but it was a significant cost factor for most of the ethanol production (Huang *et al*. 2011; Pereira *et al*. 2012). Thus, an ideal pretreatment method is needed to make the cellulosic biomass amenable to the action of cellulose enzymes, accrue more sugars, and generate no degradation products.

Effect of Pretreatment Conditions on Enzymatic Hydrolysis and Overall Sugar Content of Bamboo

The effectiveness of a pretreatment is measured by its success in increasing the susceptibility of the biomass to enzymatic hydrolysis. Enzymatic hydrolysis was performed with an enzyme loading of 40 filter paper units (FPU)/g cellulose and 20 *β*-glucosidase international units (IU)/g cellulose for 72 h on untreated samples and pretreated solid residues to assess the effect of pretreatment (Fig. 1). Regardless of the treatment conditions, the enzymatic digestibility of the pretreated sample was improved greatly compared to that of the untreated sample. The enzymatic digestibility of the untreated sample was only 24.83%. The lowest enzymatic digestibility of the pretreated sample was 63.54% when the pretreatment condition was 1.0% of sulfuric acid at 130 °C for 30 min, which was 38.71% higher than that of control test. The highest enzymatic hydrolysis yield was 82.69% after pretreatment with 1.25 % of sulfuric acid at 160 °C for 40 min, which was 3.3 times that of control test. The enzymatic hydrolysis yield was 80.45% (sugar recovery 43.42 g) after pretreatment with 1.00% sulfuric acid at 150 °C for 30 min.

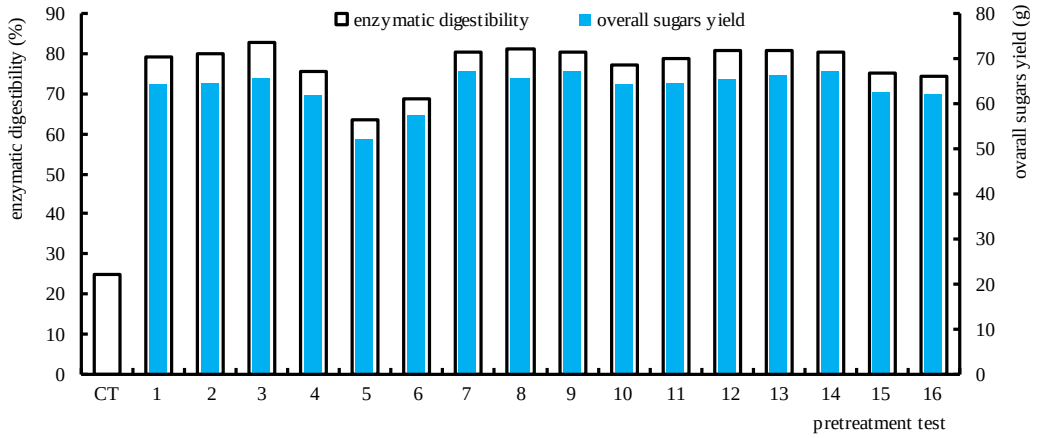

Fig. 1. Enzymatic digestibility of untreated sample and pretreated residue under various conditions (see pretreatment test CT, 1-16.)

With increasing sulfuric acid concentration, temperature, and retention time, the yield of enzymatic hydrolysis increased gradually, showing that the enzymatic hydrolysis of pretreated materials was highly correlated with the removal of hemicelluloses and lignin. Dilute acid pretreatments through hydrolysis of the hemicellulose components produced a syrup of monomeric sugars, exposing cellulose for enzymatic digestion and removing hemicellulose and part of the lignin.

The overall sugar yield after pretreatment and enzymatic hydrolysis is the most important factor in pretreatment. Taking into account sugars present in the liquid that resulted from pretreatment and were released by enzymatic hydrolysis, the overall sugar yield increased with increasing acid concentration, temperature, and residence time. The

maximum overall sugar yield (67.11 g sugar/100 g raw material) was obtained when pretreatment with 1.00% acid at 150 °C for 30 min was followed by enzymatic hydrolysis, representing 83.36% of all carbohydrates in the bamboo biomass (73.18 g/100 g). Leenakul and Tippayawong (2010) reported that the maximum yields of total reducing sugar (85 mg/g) were obtained for the pretreatment conditions at 120 °C, 1.2% sulfuric acid concentration, and 60 min.

SEM Analysis

To investigate how the different pretreatments changed the appearance of the bamboo, SEM analysis was carried out to compare the untreated samples and samples pretreated with 1.00% acid at 130 °C and 1.00% acid at 150 °C for 30 min. Figures 2a1, 2a2, and 2a3 are images of the untreated sample under increasing magnifications. In Figs. 2b1, 2b2, and 2b3 and Figs. 2c1, 2c2, and 2c3, samples treated with 1.00% acid at 130 °C and 1.00% acid at 150 °C for 30 min are shown. The bamboo structure is clearly visible, with rigid and highly ordered fibrils. At higher magnification, SEM images (Fig. 2a3) show that the macrofibril surface of the untreated sample was smooth.

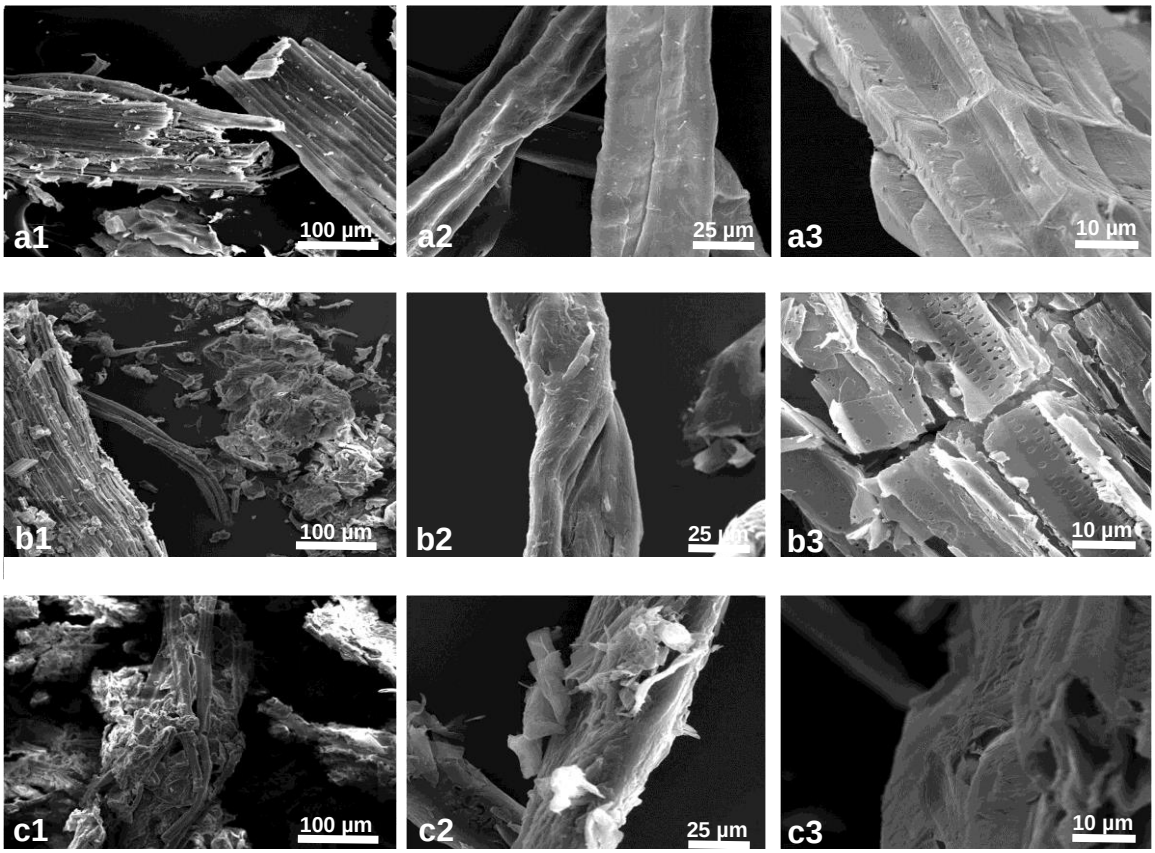

Fig. 2. Scanning electron micrographs of untreated and pretreated bamboo: (a) untreated, (b) pretreated with 1.00% acid at 130 °C for 30 min, and (c) pretreated with 1.00% acid at 150 °C for 30 min

Figures 2b1, 2b2, and 2b3 show that the porous nature of the fiber surface was exposed and there were obvious boundary edges in different regions after pretreatment with 1.00% acid at 130 °C for 30 min. In Figs. 2c1, 2c2, and 2c3, SEM images show that

the sample surfaces were rougher with 1.0% acid at 150 °C for 30 min. As shown in Figs. 2b1 and 2b2, the fibers of the pretreated samples were visibly separated from the initially connected structure. Figures 2c1 and 2c2 showed that some macrofibrils remained separated and unorderly. For both pretreatments, the bamboo became more degraded with stronger acid and higher temperature. Figure 2b3 shows that many terraces, steps, and kinks formed on the macrofibril surface. The terraces, steps, and kinks disappeared in Fig. 2c3, while numerous holes became apparent. These changes may have resulted from the removal of reactive amorphous cellulose on the surface. It is likely that the surface area and the porosity of the pretreated samples also increased. There, the pretreatment altered the bamboo structure significantly (Li *et al*. 2010).

XRD Analysis

Cellulose is composed of crystalline and amorphous components. The X-ray diffraction patterns of both untreated and pretreated biomass were measured. The powdered samples were dispersed onto a stub and placed within the chamber of an analytical X-ray powder diffractometer (Danilkin *et al.* 1998). The relative crystallinity indices were calculated according to Eq. 2,

$$[(I(002)-I_{am})/I(002)]\times 100 \quad (2)$$

where $I(002)$ is the maximum intensity of the (002) lattice diffraction (2θ = 22.5°) attributed to the crystalline region of the sample, and I_{am} is the intensity of diffraction at 2θ = 18.5° attributed to the amorphous region of the sample. The peak patterns of untreated samples were the typical crystalline structures of native cellulose, and there was no discernible difference between the peak patterns of the three samples (Fig. 3). Although dramatic morphology changes involving cellulose macrofibrils were observed in the pretreated samples, no changes in the XRD patterns were detected, as shown in Figs. 3a, 3b, and 3c. The crystallinity indices of the cellulose pretreated with 1.00% sulfuric acid at 130 °C for 30 min and 1.00% acid at 150 °C for 30 min were 48.5% and 46.5%, respectively, which was lower than that of the untreated sample, *i.e.*, 46.3%. The crystallinity index increased slightly with the severity of pretreatment.

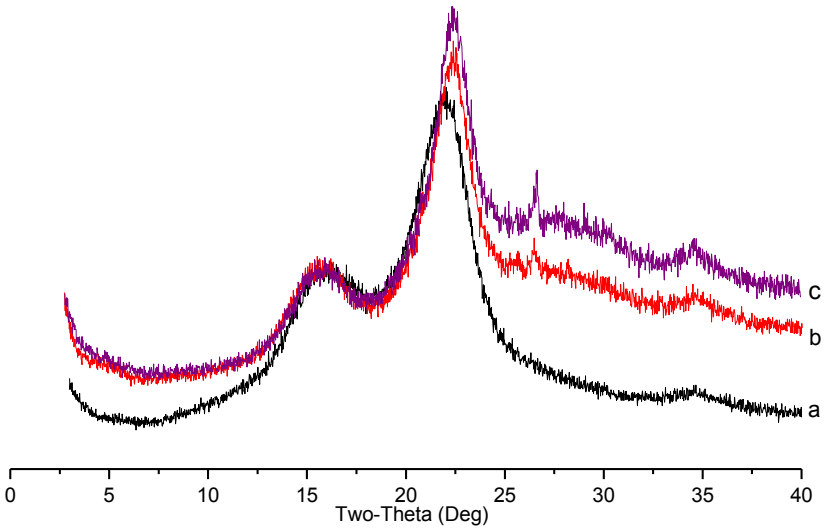

Fig. 3. X-ray diffraction spectra of untreated and pretreated bamboo: (a) untreated, (b) pretreated with 1.00% acid at 130 °C for 30 min, and (c) pretreated with 1.00% acid at 150 °C for 30 min

For lignocellulosic biomass, the crystallinity index measures the relative amount of crystalline cellulose in the total solid. Dilute acid pretreatment removed most hemicelluloses and some lignin, so the crystallinity index increased after pretreatment (Zhao *et al*. 2007; Xu *et al*. 2012). This was caused by the removal of amorphous lignocellulose near the macrofibrils surface, leading to the exposure of microfibril bundles but leaving the crystalline cellulose fraction intact in the pretreated solid residues. Some slight crystallization was found after pretreatment. This indicated that some of the crystalline components were broken, and cellulose underwent changes in crystallinity upon chemical and physical treatment, which can enhance the yields of enzymatic hydrolysis.

FTIR Analysis

The FTIR analysis was performed on the original and pretreated bamboo. Figure 4 shows the FTIR spectra of untreated samples and samples pretreated with 1.00% acid at 130 °C for 30 min and 1.0% acid at 150 °C for 30 min in the fingerprint region of 3500 to 500 cm^{-1}. The IR spectra showed a strong band associated with hydrogen bonded O-H stretching absorption around 3390 cm^{-1} and a prominent C-H stretching absorption around 2920 cm^{-1}. In the fingerprint region, 1790 to 500 cm^{-1}, many absorption bands associated with various contributions from vibrations can be seen. Modes in carbohydrates and lignin were also present in bamboo (Xu *et al*. 2013).

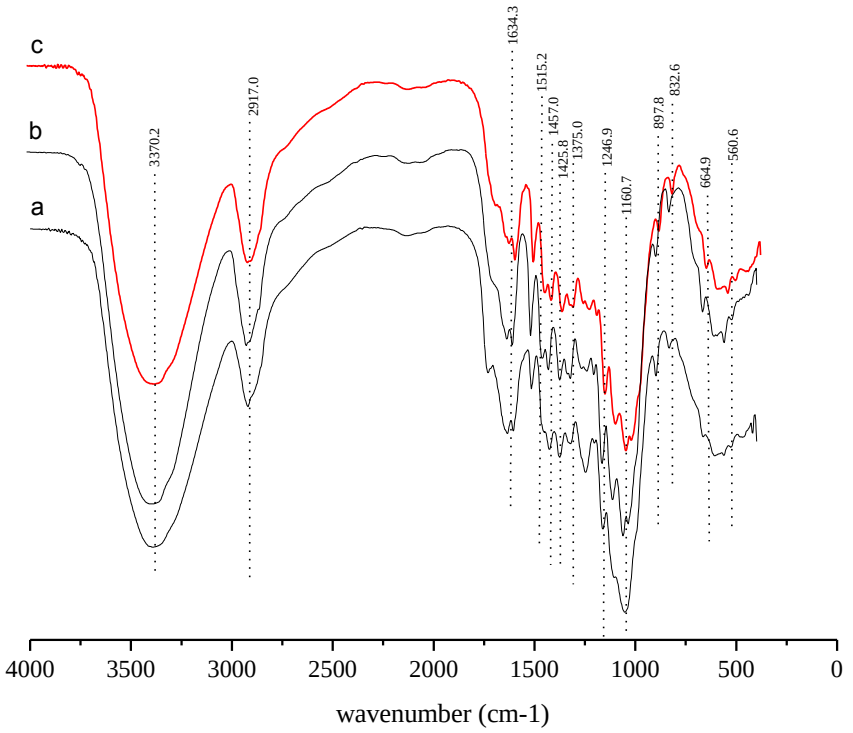

Fig. 4. FTIR of untreated and pretreated bamboo: (a) untreated, (b) pretreated with 1.00% acid at 130 °C for 30 min, and (c) pretreated with 1.00% acid at 150 °C for 30 min

As shown in Fig. 4, cellulose characteristic peaks were observed at 3395 cm^{-1} (-OH), 1425 cm^{-1} ($-CH_2$), and 1375 cm^{-1} (δCH). The IR spectra of each sample were very similar. There were negligible changes in the cellulose characteristic peaks.

The characteristic hemicellulose band appeared at 1730 cm^{-1} for the original sample. This IR absorbance band was not discernible after treatment, which indicated that hemicellulose was almost entirely removed by the pretreatment applied. Reduction in the peak intensity found at around 1631 to 1633 cm^{-1} in treated samples indicated the partial reaction of the C=O bonds of hemicellulose. The intensity of the peak of acetyl and methyl ester group at 1590 and 1240 cm^{-1} was sharply weakened after the pretreatment, confirming the removal of hemicellulose.

An aromatic stretching band that was typical of the unconjugated guaiacyl nucleus was present at 1605 and 1515 cm^{-1}, and the band intensity was strongly influenced by the structures bordering the aromatic nuclei. Figure 4 showed that the band intensities at lignin peaks (1605 to 1515 cm^{-1}) of the untreated sample were higher than those of dilute sulfuric pretreated samples, and a broad peak in the untreated sample faded after treatment, suggesting that the dilute acid pretreatment removed some lignin.

Pretreatment with 1.00% acid at 150 °C for 30 min, as compared to that with 1.00% acid at 130 °C for 30 min, showed an increase in amorphous cellulose and O-H peaks. This implied that an increase in concentration and temperature led to breakage into smaller molecules and decrystallization. When compared with the untreated sample, pretreatment of 1.0% acid at 150 °C showed an increase in aldehyde and O-H bonds and a decrease in ester carbonyl, indicating depolymerization and hydrolysis of the hemicellulose.

CONCLUSIONS

1. Moso bamboo is a potential material for fuel ethanol production. Dilute acid pretreatment followed with enzymatic hydrolysis is a suitable process to produce sugars from bamboo for further processing to ethanol. The results indicated that the pretreated solid residues changed markedly from the original bamboo. The dilute acid pretreatment hydrolyzed hemicellulose to sugars (xylose, L-arabinose, and others) that are water soluble. The maximum yield of bamboo's hemicellulose was 81.42% in the prehydrolyzate when pretreated with 1.00% sulfuric acid at 150 °C for 30 min, and the enzymatic hydrolysis yield of the treated residues was 79.45% after a 72-h enzymatic reaction. Taking into account sugars present in the liquid issued from pretreatment and released by enzymatic hydrolysis, 83.36% of all carbohydrates present in bamboo biomass (67.11 g sugar/100 g raw material) were available.
2. Pretreatment effectively altered the fine structure of bamboo cellulose and opened up the lignin-carbohydrate complex. The SEM micrographs confirmed that the dilute acid pretreatment both increased pore volume and produced more reachable surface area for cellulose enzymes to react with cellulose. The XRD analysis showed that some of the crystalline component was broken and the crystallinity index of bamboo increased after dilute acid pretreatment. The FTIR analysis showed that the peculiar peaks of hemicellulose disappeared after dilute acid pretreatment, primarily due to the removal of hemicellulose. Therefore, the dilute sulfuric acid pretreatment technique enhanced enzymatic hydrolysis of bamboo by destructing chemical composition and changing structural features.

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