Ethanosolv with NaOH Pretreatment of Moso Bamboo for Efficient Enzymatic Saccharification

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Bamboo is a potential lignocellulosic biomass for the production of bioethanol because of its high cellulose and hemicellulose contents. An acid-free ethanosolv process was proposed to overcome the problems caused by the acid catalysts commonly used in organosolv processes. In this research, ethanosolv pretreatment catalyzed by NaOH was used to enhance the enzymatic saccharification of moso bamboo. The addition of 10% (w/w on bamboo) NaOH in 75% (v/v) ethanol was demonstrated to be effective in the pretreatment and fractionation of bamboo. The pretreatment yielded a solid fraction with 60.1% cellulose. The cellulose-to-glucose conversion yield was 28.9% to 45.1%, depending on pretreatment conditions, after enzymatic hydrolysis of the solid fraction at 50 °C for 48 h using enzyme loading (15 filter paper units of cellulase/g cellulose and 30 IU β-glucosidase/g cellulose). The concentrations of fermentation inhibitors such as 5-hydroxy-2-methyl furfural (HMF) and furfural were negligible in the spent liquor after the ethanosolv pretreatment and were much lower than those in the spent liquor from H_2SO_4-water or ethanosolv only treatment.

Keywords: Bamboo; Bioethanol; Ethanosolv pretreatment

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INTRODUCTION

Bamboo is a perennial woody grass that is widely distributed in the world. Approximately 1500 commercial applications of bamboo have been identified (Scurlock *et al.* 2000). They may be divided up into the following broad categories: construction and reinforcing fibers; paper, textiles, and board; food; and combustion and other bioenergy applications (Scurlock *et al.* 2000). Due to its fast growth, short renewal time, and easy propagation, more and more attention has been paid to its applications in the bio-based energy field, including bioethanol production (Garcí-Aparicio *et al.* 2011; Leenakul and Tippayawong 2010; Shimokawa *et al*. 2009).

Many pretreatment methods have been proposed to increase fermentable sugar yield in the cellulosic bioethanol production process (Wyman *et al.* 2005). However, most research has focused on low-lignin lignocellulosic biomass such as corn stover and switch grass, and the development of effective and customized pretreatment is still needed for high-lignin lignocellulosic biomass, including bamboo. As a lignocellulosic material, bamboo mainly consists of cellulose, hemicelluloses, and lignin, which chemically and physically associate with each other to form a complex structure. Because

of its tough matrix structure, raw bamboo is recalcitrant to cellulase systems for enzymatic saccharification. Pretreatment of bamboo is necessary to make bamboo substrates accessible to enzymes.

Organosolv pretreatment has been evaluated as a potentially effective pretreatment method for high-lignin lignocellulosic biomass (Chum *et al*. 1990; Pye and Lora 1991; Koo *et al*. 2011). It is usually carried out using a strong inorganic acid catalyst, which hydrolyzes the lignin-lignin and lignin-carbohydrate bond in the biomass (Holtzapple and Humphrey 1984), and hydrochloric acid and sulfuric acid have been commonly used as catalysts (Sarkanen 1980; Pan *et al*. 2005; Zhao *et al*. 2009; Mesa *et al*. 2011). However, the spent strong acid should be recovered due to its toxic and hazardous effects, which can cause a number of environmental problems. The cellulose and hemicellulose removed by organosolv pretreatments employing dilute acid have been partially further degraded to potential fermentation inhibitors, such as formic acid, levulinic acid, furfural, and hydroxymethylfurfural (HMF). The recovery of the spent acid complicates the downstream processing steps (Teramoto *et al*. 2008). In addition, the use of sulfuric acid corrodes the reactor that is used for the pretreatment.

Alkaline catalysts such as sodium hydroxide, ammonia, and lime have been used for the pretreatment of lignocellulosic biomass because they are effective for lignin removal (Li *et al*. 2012b; Rabelo *et al*. 2008; Yamashita *et al*. 2010). To our knowledge, the organosolv pretreatment of bamboo with NaOH has not been investigated. The objective of this work was to preliminarily assess the effect of an organosolv-enhanced NaOH pretreatment process on the chemical composition and enzymatic hydrolysis of bamboo. In this study, to evaluate the feasibility of an organosolv pretreatment using alkaline catalyst, the ground moso bamboo samples were subjected to ethanosolv NaOH pretreatment at three temperatures and compared with water-NaOH pretreatment. The chemical changes involved in the process were characterized with saccharides analysis in the substrates and the spent liquor; the fermentation inhibitors in the spent liquors were investigated. The enzymatic hydrolysis of the pretreated bamboo was studied as well.

EXPERIMENTAL

Materials

Moso bamboo (*Phyllostachys heterocycla*) was acquired from central Florida in the USA in the fall of 2009. Air-dried bamboo was milled using a hammer mill with a screen opening size of 2.0 mm before chemical pretreatment. The average moisture content of the ground air-dried bamboo was 6.93% (wt).

Commercial enzymes, Celluclast 1.5 L (cellulase) and Novozyme 188 (*β*-glucosidase) produced by Novozymes, were purchased from Sigma-Aldrich (St. Louis, Missouri). Celluclast 1.5 L contained 70 FPU/g of total cellulase and *β*-glucosidase activity of Novozyme-188 was 250 unit/g. One FPU is defined as the enzyme amount that releases 1 μmol of glucose equivalents from Whatman No. 1 filter paper in 1 min. One unit of *β*-glucosidase activity is defined as the enzyme amount that converts 1 μmol of cellubiose to 2 μmol of glucose in 1 min. All the chemical reagents used in this study were purchased from Fisher Scientific (Pittsburgh, Pennsylvania).

Pretreatments

Bamboo samples were pretreated in a microwave accelerated reaction system manufactured by CEM (Model MARS, CEM Corporation, Matthews, North Carolina, USA). This apparatus provided microwave radiation at three variable power levels ranging from 400 to 1600 W. A bamboo sample of 8 g on an oven-dry (OD) basis was used for each pretreatment experiment. The samples were immersed in 50 mL 75% (v/v) aqueous ethanol with 0.8 g of sodium hydroxide as ethanosolv with NaOH pretreatment. And as water-NaOH pretreatment, 8 g of bamboo sample was immersed in 50 mL of water with 0.8 g of NaOH. The mixture was placed in a 100-mL vessel and positioned at the center of a rotating circular ceramic plate in the microwave oven for treatment at the power level of 400 W. The temperature was raised to 120, 140, or 180 °C in 10 min and maintained for an additional 30 min. After the pretreatment, there was a wait of a few minutes to allow the temperature to drop down below 80 °C, and then the substrate and liquor were separated by filtration. The liquor was stored at 4 °C for the sugar and fermentation inhibitors analysis by high-performance liquid chromatography (HPLC). The solid substrate was washed with water until the pH was near neutral and then stored at 4 °C for composition analysis and enzymatic hydrolysis. Each pretreatment was carried out in duplicate; the average result is reported here, as is the ethanosolv control (without the sodium hydroxide addition).

Enzymatic Hydrolysis

Enzymatic hydrolysis was carried out in 150-mL flasks at 50 °C on a shaking incubator (Thermo Fisher Scientific, Model 4450, Waltham, MA) at 220 rev/min. Bamboo substrate equivalent to 0.8 g glucan was loaded into 40 mL of the 0.05 M sodium acetate buffer (pH 4.8). Approximately 1.5 mg of tetracycline chloride was added to control the growth of microorganisms and prevent consumption of liberated sugars. Two enzymes, cellulase (15 FPU/g glucan) and β-glucosidase (30 IU/g glucan), were loaded into the flask. The hydrolysate was sampled at 1, 3, 6, 12, 24, and 48 h to analyze glucose concentration. The same procedure was also applied to untreated bamboo for the control plot. The cellulose-to-glucose conversion yield was determined in duplicate and was defined as the percentage of obtained glucose by enzymatic hydrolysis based on structural sugar, such as glucose, in the pretreated bamboo; averages are reported. The cellulose-to-glucose conversion yield after hydrolysis was calculated by the equation:

$$\text{CGCY (\%)} = \frac{\text{Glucose in enzyme hydrolysate (g)} * 0.9}{\text{Glucan in substrate sample (g)}} * 100 \quad (1)$$

Analytical Methods

Acid-insoluble lignin in the original bamboo and pretreated bamboo substrates was determined according to the National Renewable Energy Laboratory (NREL) Analytical Procedure: Determination of Structural Carbohydrates and Lignin in Biomass (with modifications) (Sluiter *et al*. 2008). In brief, monomeric sugar and lignin content of the ethanol-extracted material was determined after a two-step hydrolysis with sulfuric acid (75% for 2 h at room temperature; 3% for 1 h at 121 °C). The hydrolysate from the Klason lignin determination was retained for analysis of monosaccharides and acid-soluble lignin. Acid-soluble lignin was determined using a UV spectrometer at 205 nm according to the procedure described by Dence (1992).

Carbohydrate compositions of the original bamboo, pretreated bamboo substrates,

and spent liquors were investigated using improved high-performance anion exchange chromatography (Dionex HPLC system ICS-3000) equipped with an integrated amperemetric detector and a Carbopac™ PA1 guard and analytical columns at 20 °C.

Fermentation inhibitors including acetic acid, formic acid, furfural, levulinic acid, and 5-hydroxylmethylfurural (HMF) were analyzed using the Dionex ICS-3000 equipped with a Supelcogel C-610H column at 30 °C and a UV detector at 210 nm.

RESULTS AND DISCUSSION

Changes in Chemical Composition of Pretreated Bamboo

Changes in chemical composition through pretreatment were investigated. Chemical components of untreated original bamboo and pretreated bamboo substrates are listed in Table 1. The initial chemical composition of the moso bamboo was determined to be 41.3% glucose, 22.0% xylose, 1.1% arabinose, 0.6% mannose, 0.3% galactose, 22.8% Klason lignin, 1.5% acid soluble lignin, and 1.4% ash. The solid recovery of original bamboo was 89.6% after water/ethanol extraction. Ethanosolv pretreatment with sodium hydroxide partly degraded lignin and removed extractives; thus, the glucan content was relatively increased. Dissolution of lignin by sodium hydroxide was expected, but biomass delignification was insignificant. The ethanosolv pretreatment without the sodium hydroxide catalyst slightly changed the chemical composition of bamboo. The ethanosolv pre-treatments with sodium hydroxide were effective at removing hemicellulose and lignin, especially at 180 °C. As a result, cellulose was enriched in the pretreated substrates to as high as 60.1% (180 °C), 50.0% (140 °C), and 49.4% (120 °C). Addition of alkali to the liquid mixture played a very important role in catalyzing the removal of hemicellulose and lignin. Increasing pretreatment temperature increased lignin and hemicellulose removal.

Compared with dilute acid-water and ethanosolv pretreatment, the sodium hydroxide-water and ethanosolv pretreatments were found to minimize the loss of sugars, which serve as the main resource in bioethanol production. The content of glucan and xylan, which are major structural sugars, and lignin of moso bamboo were determined (Table 1). The content of other sugars, arabinose and galactose, was also determined. The glucose content increased with increasing pretreatment temperature. However, the content of xylose did not remain the same. With ethanosolv pretreatment, there was a slight difference in xylose content between 120 °C, 140 °C, and 180 °C. For water pretreatment, when the temperature increased, the xylose content declined. The relative lignin content decreased for all conditions except the ethanosolv control (180 °C) pretreatment. It was speculated that the residual lignin in the pretreated biomass inhibited the enzymatic hydrolysis. The relative residual lignin in pretreated biomass substrates is an important recalcitrance factor for the pretreatment process (Hundt *et al*. 2013; Wong *et al*. 1988).

For ethanosolv pretreatment with NaOH, the spent liquor and ethanol washes were combined and mixed with three volumes of water to precipitate the dissolved lignin. In contrast to organosolv pretreatment with dilute acid, there was no dissolved lignin precipitated. The sugars and soluble lignin in the combined liquor of the filtrate and water washes are listed in Table 2. Concentrations were calculated on original spent liquors. Increasing pretreatment temperature increased hemicellulose removal. The concentrations of sugars

in spent liquors showed a similar trend. More sugars were found in 180 °C ethanosolv pretreatment spent liquors than in 120 °C and 140 °C spent liquors.

Table 1. Chemical Composition of Moso Bamboo Pretreated by Ethanosolv and by Water at a Sodium Hydroxide Charge of 10% on OD Bamboo

Pretreatments	Component Weight (%)							Solid Recovery (%)
	Arabinose	Galactose	Glucose	Xylose	Mannose	Acid-insoluble Lignin	Acid-soluble Lignin	
Original bamboo	1.1[b]	0.3	41.3	22.0	0.6±0.0	22.8	1.5	89.6
180 °C, ethanosolv control[a]	1.0	0.3	43.7	21.8	ND	24.9	1.5	88.7
180 °C, ethanosolv	1.4	0.2	60.1	25.3	ND	9.6	3.0	61.9
140 °C, ethanosolv	1.5	0.2	50.0	26.4	ND	19.4	1.8	74.3
120 °C, ethanosolv	1.2	0.3	49.4	25.9	ND	19.6	1.7	74.4
120 °C, water	1.1	0.1	51.9	24.5	ND	17.1	1.6	73.7
180 °C, water	1.0	0.1	57.8	19.7	ND	19.9	1.6	67.3

[a] without NaOH addition; ND, not detected.
[b] Mean values of the 2 tested samples. The absolute differences between pairs of replicate tests were 0.0-0.1 (arabinose), 0.0 (galactose), 0.4-2.1 (glucose), 0.7-1.8 (xylose), 0.0 (mannose), 0.1-0.4 (acid-insoluble lignin), 0.0 (acid-soluble lignin), and 0.9-2.0 (solid recovery).

Table 2. Sugars and Lignin Concentrations in Spent Liquors from Ethanosolv Pretreatment with NaOH

Pretreatments	Component in Spent Liquors (g/L)						
	Arabinose	Galactose	Glucose	Xylose	Mannose	Acid-insoluble Lignin	Acid-soluble Lignin
180 °C, ethanosolv control	0.04[a]	ND	0.80	ND	1.61	NA	5.45
180 °C, ethanosolv	0.40	0.36	6.62	0.97	1.00	NA	6.83
140 °C, ethanosolv	0.02	0.02	0.76	0.03	0.20	NA	3.07
120 °C, ethanosolv	0.05	ND	0.90	0.04	0.10	NA	4.26
120 °C, water	0.40	0.17	1.31	1.76	0.23	NA	7.57
180 °C, water	0.76	0.26	1.24	4.80	0.08	NA	13.19

ND, not detected; NA, not applicable
[a] Mean values of the 2 tested samples. The absolute differences between pairs of replicate tests were 0.0-0.1 (arabinose), 0.0 (galactose), 0.0-0.8 (glucose), 0.0-0.9 (xylose), 0.0-0.7 (mannose), and 0.5-1.0 (acid-soluble lignin).

Enzymatic Hydrolyzability of Pretreated Bamboo Substrates

There are many factors that affect the enzymatic hydrolyzability of substrates, such as hemicellulose content; lignin structure, distribution, and content; cellulose crystallinity; and degree of polymerization (Mansfield *et al*. 1999; Alvira *et al*. 2010). All these known and unknown factors make bamboo a unique biomass for efficient pretreatment, unlike agricultural wastes and wood. One of the structural differences is that bamboo has high density and hardness, even on its outer part (bamboo green) (Chand *et al*. 2006).

The enzymatic hydrolyzability of organosolv-pretreated bamboo substrates is shown in Fig. 1. The enzyme loadings were 15 FPU (filter paper units) cellulase and 30 IU (international units) β-glucosidase per gram cellulose for all enzymatic hydrolyses. The cellulose-to-glucose conversion yield of untreated raw bamboo after a 48-h hydrolysis was only 2.4%. The cellulose-to-glucose conversion yield of ethanosolv pretreatment without sodium hydroxide addition was still 2.4%, which was not a significant increase. Otherwise, the ethanosolv with sodium hydroxide pretreatment significantly improved the enzymatic digestibility of bamboo. Meanwhile, with increasing pretreatment temperature, the cellulose-to-glucose conversion yield increased. When the temperature was 180 °C and the duration time was 30 min, the glucose yield reached 45.1%. In the enzymatic hydrolysis of pretreated lignocellulosic biomass with the organosolv process using acid catalyst, increasing the temperature significantly enhanced the enzymatic conversion (Zhao *et al*. 2009). In this study, however, enzymatic conversion was not affected by the temperature conditions at 120 °C and 140 °C in the ethanosolv pretreatment. It is suggested that the ethanosolv pretreatment with sodium hydroxide should be performed at a high temperature of 180 °C. Actually, xylose and lignin contents were present in different proportions in each bamboo substrate obtained from ethanosolv with NaOH pretreatment. Considering that after ethanosolv with NaOH pretreatment, a residual fraction of the lignin and xylose is very difficult to be removed from the bamboo structure because part of these fractions is strongly bound to the cellulose, being thus very resistant to hydrolysis, more efforts need to be done in the future.

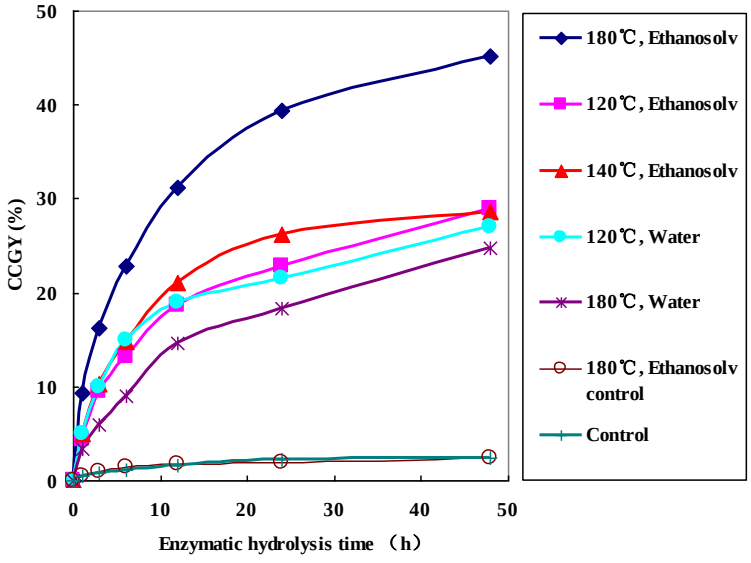

Fig. 1. Comparisons of glucose yield on time-dependent enzymatic hydrolyzability of pretreated bamboo substrates with an enzyme loading of 15 FPU cellulase and 30 IU β-glucosidase per gram of cellulose, 50 °C, pH 4.8, and on a 220-rpm shaker. CGCY: cellulose-to-glucose conversion yield

Comparing the ethanosolv pretreatments, the cellulose-to-glucose conversion yields increased when the content of xylose and lignin decreased. The removal of hemicellulose and lignin increased cellulose susceptibility to enzymes. The results indicated that in addition to the content of hemicellulose and lignin affected the enzymatic digestibility of the substrates. Meanwhile, other factors such as cellulose crystallinity and degree of polymerization maybe also main factors affected the enzymatic digestibility of the substrates. These factors will be investigated in future research.

Although the cellulose-to-glucose conversion yield of pretreated bamboo increased significantly compared with that of untreated bamboo, the feasible monomeric sugars were released in small amounts within 48 h, and the yield was much lower than the result from organosolv pretreatment with acid catalyst (Pan *et al*. 2005, 2006; Li *et al*. 2012a). In comparison with alkali pretreatment without organic solvent, the higher sugar yield was obtained at a lower temperature (Zhao *et al.* 2007). Although the maximum sugar yield was obtained at 180 °C for the organosolv pretreatment, sugar yields of the pretreated biomass at 120 °C and 140 °C were likewise high, at approximately 28%. The enzymatic conversion was 27.0% (120 °C water) and similar to the results obtained from the bamboo pretreated at 180 °C water. Therefore, the applied temperatures (120 °C and 180 °C) in this study did not affect the enzymatic hydrolysis of the pretreated biomass by a water-based system, and a proper temperature could be applied to the process in the future.

Mass Balance of Sugars During Pretreatments

An ideal pretreatment should readily provide enzymatic digestible substrates. That is not enough, however; it should also maximally recover all the components of the original biomass. As shown in Table 3, sugars and lignin in two fractions, solid substrates and spent liquors, were calculated based on 100 g of untreated raw bamboo.

Table 3. Mass Balance of 100 g Moso Bamboo Pretreated by Ethanosolv and by Water with NaOH

Pretreatments		Component Recovery (g)							
		Arabinose	Galactose	Glucose	Xylose	Mannose	Acid-insoluble Lignin	Acid-soluble Lignin	Recovery
Oven-dry bamboo		1.1	0.3	41.3	22.0	0.6	22.8	1.5	89.6
180 °C, ethanosolv control	Substrate	0.9	0.3	38.7	19.3	ND	22.1	1.7	87.9
	Liquor	0.0	0.0	0.5	0.0	1.0	NA	3.4	
180 °C, ethanosolv	Substrate	0.9	0.1	37.2	15.7	0.2	5.9	1.9	71.0
	Liquor	0.2	0.2	3.1	0.6	0.6	NA	4.3	
140 °C, ethanosolv	Substrate	1.1	0.1	37.2	19.6	0.0	14.4	1.3	76.2
	Liquor	0.0	0.0	0.5	0.0	0.0	NA	1.9	
120 °C, ethanosolv	Substrate	0.9	0.2	36.8	19.3	0.0	14.6	1.3	76.3
	Liquor	0.0	0.0	0.6	0.0	0.1	NA	2.7	
120 °C, water	Substrate	0.8	0.0	38.2	18.1	0.0	13.8	1.0	79.1
	Liquor	0.3	0.1	0.8	1.1	0.1	NA	4.7	
180 °C, water	Substrate	0.6	0.0	38.9	13.3	0.0	13.4	1.1	80.1
	Liquor	0.5	0.2	0.8	3.0	0.0	NA	8.2	
ND, not detected; NA, not applicable									

As discussed above, this amount of oven-dried bamboo contained 41.3 g glucose, 22.0 g xylose, 1.1 g arabinose, 0.3 g galactose, and 0.6 g mannose. The total recovery of sugars and lignin from the ethanosolv pretreatment without sodium hydroxide was 87.9%. Pretreatment with the sodium hydroxide addition obtained recovery values in the range of 71.0% to 76.3%. This was lower than for the water-NaOH pretreatment, which ranged from 79.1% to 80.1%. The low substrate yield from the pretreatment was attributable in part to the dissolution of more cellulose and xylose. The amount of detected sugars in the spent liquors was much lower than that found after the organosolv with dilute acid pretreatment.

Calculations using these data indicated that the glucose recovery increased with higher pretreatment temperature for both ethanosolv and water pretreatment, while the xylose recovery decreased with higher temperature. The result indicated that xylose underwent greater degradation than glucose at high temperature and long pretreatment time.

Comparisons of Fermentation Inhibitors' Formation During Pretreatments

The cellulose and hemicellulose removed by organosolv pretreatments were partially hydrolyzed to fermentable sugars and even further degraded to potential fermentation inhibitors, such as formic acid, levulinic acid, furfural, and hydroxymethyl-furfural (HMF). Furfural derived from pentoses, HMF, was a result of the degradation of hexoses, whereas levulinic and formic acids came from successive decompositions of HMF. The acid-soluble lignin was also a kind of fermentation inhibitor. Acetic acid was released from acetyl groups on hemicelluloses and was detected in both acidic and basic pretreatment liquors.

Table 4. Acetic Acid Released During Pretreatment

Pretreatments	Acid-soluble Lignin (g/L)	Acetic Acid (g/L)
180 °C, ethanosolv control	5.45[a]	0.0
180 °C, ethanosolv	6.83	7.0
140 °C, ethanosolv	3.07	2.3
120 °C, ethanosolv	4.26	1.6
120 °C, water	7.57	5.6
180 °C, water	13.19	5.7

[a] Mean values of the 2 tested samples. The absolute differences between pairs of replicate tests were 0.5-1.0 (acid-insoluble lignin), and 0.0-1.0 (acetic acid).

Acid-soluble lignin and the fermentation inhibitor acetic acid were detected in these organosolv or water basic pretreatments, as listed in Table 4. The data clearly indicated that the acid-soluble lignin in the spent liquors was slightly different. It was about 13.19 g/L in the sodium hydroxide-water spent liquor (180 °C), which was two times as much as that (6.83 g/L) in the sodium hydroxide-ethanosolv pretreated spent liquor (180 °C). It was almost the same for 120 °C pretreated spent liquors, 7.57 g/L (sodium hydroxide-water), and 4.26 g/L (sodium hydroxide-ethanosolv). There was no inhibitor detected in the ethanosolv pretreatment liquors without the sodium hydroxide addition. The acetic acid increased after an increase in pretreatment temperature. It was

only 22.9% (120 °C, ethanosolv) and 32.9% (140 °C, ethanosolv) of those formed in the 180 °C ethanosolv pretreatment (7.0 g/L). For the sodium hydroxide–water pretreatment, the acetic acid amount was almost the same (5.6 and 5.7 g/L).

CONCLUSIONS

1. Ethanosolv pretreatment (180 °C, 30 min) without sodium hydroxide addition had no observable influence on the cell-wall change or enzymatic digestibility of bamboo.

2. Ethanosolv pretreatment with sodium hydroxide as the catalyst resulted in hemicellulose and lignin removal and an increase in the enzymatic digestibility of the bamboo substrates.

3. Based on the same sodium hydroxide loading (10% on OD bamboo), when the pretreatment temperature increased from 140 °C to 180 °C, more hemicellulose and lignin were removed and the cellulose-to-glucose conversion yield of enzymatic hydrolysis increased.

4. Ethanosolv pretreatment with sodium hydroxide enhanced the cellulose-to-glucose conversion yield significantly from 2.4% to 45.1%, demonstrating its applicability as a potential effective pretreatment for enhancing enzymatic digestibility of moso bamboo.

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REFERENCES CITED

Alvira, P., Tomás-Pejó, E., Ballesteros, M., and Negro, M. J. (2010). "Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: A review," *Bioresource Technology* 101(13), 4851-4861.

Chand, N., Jain, D., and Nigrawal, A. (2006). "Investigation on gradient dielectric characteristics of bamboo (*Dentroclamus strictus*)," *Journal of Applied Polymer Science* 102(1), 380-386.

Chum, H. L., Johnson, D. K., and Black, S. K. (1990). "Organosolv pretreatment for enzymatic hydrolysis of poplars. 2. Catalyst effects and the combined severity parameter," *Ind. Eng. Chem. Res*. 29(2), 156-162.

Dence, C. W. (1992). "The determination of lignin," in: *Methods in Lignin Chemistry*, C. W. Dence (ed.), Springer-Verlag, Berlin.

García-Aparicio, M., Parawira, W., Rensburg, E. V., Diedericks, D., Galbe, M., Rosslander, C., Zacchi, G., and Gorgens, J. (2011). "Evaluation of steam-treated giant bamboo for production of fermentable sugars," *Biotechnol. Prog.* 27(3), 641-649.

Holtzapple, M. T., and Humphrey, A. E. (1984). "The effect of organosolv pretreatment on the enzymatic hydrolysis of poplar," *Biotechnology and Bioengineering* 26(7), 670-676.

Hundt, M., Schnitzlein, K., and Schnitzlein, M. G. (2013). "Alkaline polyol pulping and enzymatic hydrolysis of softwood: Effect of pulping severity and pulp properties on cellulase activity and overall sugar yield," *Bioresource Technology* 134, 307-315.

Koo, B. W., Kim, H. Y., Park, N., Lee, S. M., Yeo, H., and Choi, I. G. (2011). "Organosolv pretreatment of *Liriodendron tulipifera* and simultaneous saccharification and fermentation for bioethanol production," *Biomass and Bioenergy* 35(5), 1833-1840.

Leenakul, W., and Tippayawong, N. (2010). "Dilute acid pretreatment of bamboo for fermentable sugar production," *Journal of Sustainable Energy & Environment* 1(3), 117-120.

Li, Z., Jiang, Z., Fei, B., Pan, X., Cai, Z., Liu, X., and Yu, Y. (2012a). "Ethanol organosolv pretreatment of bamboo for efficient enzymatic saccharification," *BioResources* 7(3), 3452-3462.

Li, Z., Jiang, Z., Fei, B., Yu, Y., and Cai, Z. (2012b). "Effective of microwave-KOH pretreatment on enzymatic hydrolysis of bamboo," *Journal of Sustainable Bioenergy System* 2, 104-107.

Mansfield, S. D., Mooney, C., and Saddler, J. N. (1999). "Substrate and enzyme characteristics that limit cellulose hydrolysis," *Biotechnology Progress* 15(5), 804-816.

Mesa, L., González, E., Cara, C., González, M., Castro, E., and Mussatto, S., I. (2011). "The effect of organosolv pretreatment variables on enzymatic hydrolysis of sugarcane bagasse," *Chemical Engineering Journal* 168, 1157-1162.

Pan, X., Arato, C., Gilkes, N., Gregg, D., Mabee, W., Pye, K., Xiao, Z., Zhang, X., and Saddler, J. (2005). "Biorefining of softwoods using ethanol organosolv pulping: Preliminary evaluation of process streams for manufacture of fuel-grade ethanol and co-products," *Biotechnology and Bioengineering* 90(4), 473-481.

Pan, X., Gilkes, N., Kadla, J., Pye, K., Saka, S., Gregg, D., Ehara, K., Xie, D., Lam, D., and Saddler, J. (2006). "Bioconversion of hybrid poplar to ethanol and co-products using an organosolv fractionation process: Optimization of process yields," *Biotechnology and Bioengineering* 94(5), 851-861.

Pye, E. K., and Lora, J. H. (1991). "The Alcell process: A proven alternative to kraft pulping," *Tappi J*. 74(3), 113-118.

Rabelo, S. C., Maciel, R., and Costa, A. C. (2008). "A comparison between lime and alkaline hydrogen peroxide pretreatments of sugarcane bagasse for ethanol production," *Applied Biochemistry and Biotechnology* 148(1-3), 45-58.

Sarkanen, K. V. (1980). "Acid-catalyzed delignification of lignocellulosics in organic solvents," *Prog. Biomass Convers.* 2, 127-144.

Scurlock, J. M. O., Dayton, D. C., and Hames, B. (2000). "Bamboo: An overlooked biomass resource?," *Biomass and Bioenergy* 19, 229-244.

Shimokawa, T., Ishida, M., Yoshida, S., and Nojiri, M. (2009). "Effects of growth stage on enzymatic saccharification and simultaneous saccharification and fermentation of

bamboo shoots for bioethanol production," *Bioresource Technology* 100(24), 6651-6654.

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., and Crocker, D. (2008). "Determination of structural carbohydrates and lignin in biomass," *National Renewable Energy Laboratory Analytical Procedure*: *Determination of Structural Carbohydrates and Lignin in Biomass (with Modifications).*

Teramoto, Y., Tanaka, N., Lee, S. H., and Endo, T. (2008). "Pretreatment of eucalyptus wood chips for enzymatic saccharification using combined sulfuric acid-free ethanol cooking and ball milling," *Biotechnology and Bioengineering* 99, 75-85.

Wong, K., Deverell, K., Mackie, K., Clark, T., and Donaldson, L. (1988). "The relationship between fiber porosity and cellulose digestibility in steam-exploded *Pinus radiata*," *Biotechnology and Bioengineering* 31, 447-456.

Wyman, C. E., Dale, B. E., Elander, R. T., Holtzapple, M., Ladisch, M. R., and Lee, Y. Y. (2005). "Coordinated development of leading biomass pretreatment technologies," *Bioresource Technology* 96(18), 1959-1966.

Yamashita, Y., Shono, M., Sasaki, C., and Nakamura, Y. (2010). "Alkaline peroxide pretreatment for efficient enzymatic saccharification of bamboo," *Carbohydrate Polymers* 79, 914-920.

Zhao, Y., Wang, Y., Zhu, J. Y., Ragauskas, A., and Deng, Y. (2007). "Enhanced enzymatic hydrolysis of spruce by alkaline pretreatment at low temperature," *Biotechnology and Bioengineering* 99(6), 1320-1328.

Zhao, X., Cheng, K., and Liu, D. (2009). "Organosolv pretreatment of lignocellulosic biomass for enzymatic hydrolysis," *Appl. Microbiol. Biotechnol.* 82(5), 815-827.

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