Bioresource Technology 151 (2014) 91–99

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Comparison of bamboo green, timber and yellow in sulfite, sulfuric acid and sodium hydroxide pretreatments for enzymatic saccharification

Zhiqiang Li [a], Zehui Jiang [a,*], Benhua Fei [a], Zhiyong Cai [b], Xuejun Pan [c,*]

[a] International Centre for Bamboo and Rattan, Beijing 100102, China
[b] USDA Forest Service, Forest Products Laboratory, 1 Gifford Pinchot Dr., Madison, WI 53726, United States
[c] Biological Systems Engineering, University of Wisconsin, 460 Henry Mall, Madison, WI 53706, United States

HIGHLIGHTS

- Bamboo fractions were compared in SPORL, acid and alkali pretreatments.
- Bamboo green, timber and yellow had different composition.
- Bamboo timber was a better feedstock than bamboo green and yellow.
- Hemicellulose removal was critical to enzymatic digestibility of bamboo substrate.
- SPORL pretreatment needs sufficient acid to remove hemicellulose.

ARTICLE INFO

Article history:
Received 4 August 2013
Received in revised form 15 October 2013
Accepted 17 October 2013
Available online 24 October 2013

Keywords:
SPORL pretreatment
Dilute acid pretreatment
Alkali pretreatment
Enzymatic saccharification
Bamboo

ABSTRACT

The response and behavior of bamboo green, timber, and yellow of moso bamboo (*Phyllostachys heterocycla*) to three pretreatments, sulfite (SPORL), dilute acid (DA), and alkali (NaOH), were investigated and compared with varied chemical loadings at 180 °C for 30 min with a 6.25:1 (v/w) liquor-to-bamboo ratio. All the pretreatments improved the enzymatic digestibility of bamboo substrates. Under the investigated conditions, the DA pretreatment achieved better enzymatic digestibility, but had lower sugar recovery yield, and formed more fermentation inhibitors. The results suggested that the SPORL pretreatment be able to generate more readily digestible bamboo substrate with higher sugar yield and fewer fermentation inhibitors than the corresponding DA pretreatment if hemicelluloses are sufficiently removed by adding more acid to bring down the pretreatment pH. Bamboo timber had higher sugar content and better enzymatic digestibility and therefore was a better feedstock for bioconversion than bamboo green and yellow.

1. Introduction

Bamboo is an abundant natural resource in Asia and South America. It has been used traditionally as a structural material for construction, flooring, and boards and as raw material for pulping and papermaking. With the increasing concerns on global warming and fossil energy use, bamboo has been considered as a potential feedstock for biofuel and biochemical production because of its fast growth, short renovation, and easy propagation (Scurlocka et al., 2000). Some studies have been conducted on the pretreatment of bamboo for bioethanol production (Garcia-Aparicio et al., 2011; Leenakul and Tippayawong, 2010; Sathitsuksanoh et al., 2010). Compared to other lignocellulosic biomass, bamboo has some unique characteristics in chemical composition and anatomical structure. It was found that bamboo was more difficult to pretreat than agricultural residues and some woods. For example, wheat straw could be easily pretreated through low-pressure steam explosion without addition of chemicals, and the cellulose-to-glucose conversion yield in enzymatic hydrolysis was higher than 90% (Chen and Liu, 2007). Contrarily, the cellulose-to-glucose conversion yield of pretreated moso bamboo by severe steam explosion at 35 atm (243 °C) was only 42.6% (Yamashita et al., 2010). Even with SO_2-enhanced steam explosion, the glucose yield was merely elevated to 62.7% (Garcia-Aparicio et al., 2011).

Bamboo stem is composed of three parts: bamboo skin, bamboo timber, and pith. Bamboo skin is the outermost thin layer of the cross section of stem wall, where no vascular bundles are present. Pith is the part of stem wall next to bamboo cavity; it also does not have vascular bundles. Bamboo timber is the part between skin

Abbreviations: SPORL, Sulfite Pretreatment to Overcome Recalcitrance of Lignocellulose; DA, Dilute acid.

* Corresponding authors. Tel.: +86 10 84789861 (Z. Jiang). Tel.: +1 608 262 4951; fax: +608 262 1228 (X. Pan).
E-mail addresses: jiangzehui@icbr.ac.cn (Z. Jiang), xpan@wisc.edu (X. Pan).

0960-8524/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biortech.2013.10.060

and pith. Vascular bundles are visible cross the timber section. The density of vascular bundles decreases from outer side of stem wall to inner side. The bamboo timber can be further divided into three layers. The outer part where vascular bundles are dense is called bamboo green, while the inner part where vascular bundles are rare is called bamboo yellow (Chand et al., 2006). The layer between bamboo green and yellow is called bamboo timber. If not indicated otherwise, the term "bamboo timber" in this study means the layer between bamboo green and bamboo yellow. The three layers (bamboo green, timber, and yellow) have different characteristics, such as density, hardness, ash and extractives, and even chemical composition. These differences may influence their behavior during pretreatment for ethanol production. Usually, bamboo green and yellow are removed from timber as wastes in bamboo processing industry, such as in bamboo board production, so they are available as feedstock for bioconversion. To our knowledge, the behaviors of green, timber and yellow in pretreatment have not been studied.

In our previous study, three pretreatment methods of sulfite based SPORL (Sulfite Pretreatment to Overcome Recalcitrance of Lignocellulose), dilute sulfuric acid (DA), and NaOH were compared for whole moso bamboo (Li et al., 2012). The SPORL pretreatment did show excellent performances than the DA pretreatment on cellulose-to-glucose conversion yield. In this study, the response and behavior of bamboo green, bamboo timber, and bamboo yellow in SPORL, DA and NaOH pretreatments were investigated and compared, respectively. The chemical changes of cell-wall components were investigated before and after each pretreatment of each fraction of moso bamboo, respectively. The enzymatic digestibility of the pretreated substrates was evaluated. Mass balance of each pretreatment for each bamboo fraction was established. The formation of inhibitors of each fraction during the pretreatments was also compared.

2. Methods

2.1. Materials

Moso bamboo (*Phyllostachys heterocycla*) culms were obtained from the central area of Louisiana, USA in the fall of 2009. After being air-dried, the culms of mature bamboo (at least 4 years old) were fractionated manually with a knife to three parts: bamboo green, bamboo timber, and bamboo yellow. The three fractions of the bamboo were milled using a grinder mill with a screen opening size of 2.0 mm before pretreatment, respectively. All of the materials samples were stored in plastic bags at room temperature until being further processed. Their chemical composition is summarized in Table 1. Commercial enzymes, cellulase and β-glucosidase, were generously provided by Novozymes (Franklinton, NC). All the chemical reagents used in this study were purchased from Fisher Scientific (Pittsburgh, PA) and used as received.

2.2. Pretreatments

Bamboo samples were pretreated in a microwave accelerated reaction system manufactured by CEM Corporation (Model MARS, CEM Corporation, Matthews, North Carolina, USA). This reactor provided microwave radiation at 3 variable power levels of 400, 800, and 1600 W. Each pretreatment was carried out in duplicate; the average of the two runs was reported. In general, bamboo sample of 8 g on an oven-dry (OD) basis was used for each pretreatment experiment. After the sample was loaded into a 100-mL vessel, 50 mL prepared pretreatment liquor was then poured into the vessel. The vessel was positioned on the rotating circular plate in the microwave oven for treatment at the power level of 400 W. The vessel with the content was heated up to 180 °C in 10 min and then maintained at the temperature for 30 min. After the pretreatment, wait a few minutes for the temperature to drop down below 80 °C by a build-in cooling fan before opening the reaction vessels. The pretreated bamboo was separated from the liquor by vacuum filtration. The liquor was stored in 4 °C for the sugars and fermentation inhibitors analysis by high performance liquid chromatography (HPLC). The solid substrate was washed with water until the pH of the washing near neutral and then stored at 4 °C for component analysis and enzymatic hydrolysis.

Bamboo green, bamboo timber, and bamboo yellow were pretreated with three pretreatment methods (SPORL, DA, and NaOH), as defined in Section 1, respectively. The pretreatment conditions are listed in and underneath Table 2. For SPORL pretreatment, three sulfite loadings (2%, 4%, and 8%, respectively) were investigated, and 2% H_2SO_4 was added in all SPORL pretreatment in addition to sulfite. For DA pretreatment, chemical loading was 2% H_2SO_4. For NaOH pretreatment, chemical loadings were 6% and 12% NaOH, respectively. All the chemical loadings are based on dry biomass. All pretreatments were conducted at the same temperature (180 °C), pretreatment time (10 min to 180 °C and 30 min at the temperature), and the ratio of pretreatment liquor to bamboo (6.25:1, v/w). These conditions were determined according to our previous studies (Li et al., 2012; Zhang et al., 2013; Zhu et al., 2009).

2.3. Enzymatic hydrolysis

Enzymatic hydrolysis of the pretreated and unpretreated bamboo samples was conducted as described previously (Pan et al., 2008). In brief, the enzymatic hydrolysis was carried out in 150-mL plastic jars at 50 °C on a shaking incubator (Thermo Fisher Scientific, Model 4450, Waltham, MA) at 220 rpm. Bamboo substrate equivalent to 0.8 g glucan was added into 40 mL of 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 filter paper units per gram glucan) and β-glucosidase (30 international units per gram glucan), were loaded into the plastic jars. Hydrolysates were sampled periodically at 1, 3, 6, 12, 24, and 48 h to analyze glucose concentration. The hydrolysis was conducted in duplicate for each substrate; the average is reported here.

2.4. Analytical methods

The moisture content of the bamboo samples was measured by drying in an oven for 24 h at 105 ± 2 °C. The amount of water and ethanol extractives in bamboo green, bamboo timber, and bamboo

Table 1
Chemical composition of original bamboo green, bamboo timber and bamboo yellow.

%	Arabinose	Galactose	Glucose	Xylose	Mannose	Lignin	Extractives	Ash
Bamboo green	0.7 ± 0.0	0.2 ± 0.0	43.6 ± 1.4	21.6 ± 1.0	0.6 ± 0.1	30.9 ± 0.1	6.8 ± 0.6	1.5 ± 0.1
Bamboo timber	1.0 ± 0.0	0.2 ± 0.0	46.5 ± 2.0	22.4 ± 0.9	0.4 ± 0.1	23.3 ± 0.0	10.0 ± 0.5	1.0 ± 0.1
Bamboo yellow	1.2 ± 0.0	0.2 ± 0.0	42.1 ± 1.6	23.3 ± 0.8	0.4 ± 0.1	23.8 ± 0.1	12.8 ± 0.5	1.2 ± 0.0

Note: ND-not detected.

Table 2
Chemical composition of pretreated bamboo substrates.

		Arabinose (%)	Galactose (%)	Glucose (%)	Xylose (%)	Acid-insoluble lignin (%)	Acid-soluble lignin (%)	Substrate yield (%)
8% SPORL	Bamboo green	0.3 ± 0.0	0.1 ± 0.0	58.5 ± 1.6	14.6 ± 0.5	26.4 ± 1.1	2.7 ± 0.0	72.8
	Bamboo timber	0.3 ± 0.0	0.1 ± 0.0	61.9 ± 1.9	15.4 ± 0.3	22.3 ± 1.0	3.6 ± 0.0	71.3
	Bamboo yellow	0.4 ± 0.0	0.1 ± 0.0	57.8 ± 1.9	17.2 ± 0.8	24.4 ± 1.1	4.2 ± 0.1	63.5
4% SPORL	Bamboo green	0.2 ± 0.0	ND	60.5 ± 1.1	9.2 ± 0.9	30.1 ± 1.3	2.3 ± 0.1	69.6
	Bamboo timber	0.2 ± 0.0	ND	64.3 ± 0.9	10.0 ± 0.5	25.5 ± 1.2	3.6 ± 0.0	63.9
	Bamboo yellow	0.2 ± 0.0	ND	58.1 ± 1.2	12.7 ± 0.2	29.0 ± 1.2	2.9 ± 0.0	62.4
2% SPORL	Bamboo green	0.1 ± 0.0	ND	60.7 ± 1.8	5.0 ± 0.2	34.1 ± 1.1	2.7 ± 0.0	67.4
	Bamboo timber	0.1 ± 0.0	ND	65.4 ± 1.9	4.1 ± 0.3	30.4 ± 0.8	4.4 ± 0.1	64.7
	Bamboo yellow	0.1 ± 0.0	ND	59.4 ± 1.9	7.7 ± 0.6	32.8 ± 1.2	5.8 ± 0.1	59.8
2% DA	Bamboo green	0.1 ± 0.0	ND	61.3 ± 3.2	0.4 ± 0.4	40.2 ± 1.2	2.5 ± 0.3	64.1
	Bamboo timber	0.1 ± 0.0	ND	63.9 ± 2.9	0.5 ± 0. 2	37.4 ± 1.9	1.7 ± 0.2	57.5
	Bamboo yellow	0.1 ± 0.0	ND	60.3 ± 2.8	0.7 ± 0.5	40.9 ± 1.7	2.1 ± 0.1	56.7
12% NaOH	Bamboo green	0.6 ± 0.1	0.1 ± 0.0	62.2 ± 0.4	18.9 ± 0.8	20.3 ± 1.7	1.2 ± 0.0	60.4
	Bamboo timber	0.5 ± 0.0	0.1 ± 0.0	64.5 ± 0.8	17.3 ± 0.8	19.7 ± 1.5	2.3 ± 0.0	59.2
	Bamboo yellow	0.8 ± 0.1	0.2 ± 0.0	58.8 ± 0.3	24.9 ± 0.7	17.4 ± 1.8	1.2 ± 0.0	54.4
6% NaOH	Bamboo green	1.2 ± 0.0	0.2 ± 0.0	53.6 ± 0.8	19.9 ± 0.2	27.2 ± 1.6	1.7 ± 0.5	75.9
	Bamboo timber	0.9 ± 0.0	0.2 ± 0.0	55.7 ± 0.7	21.0 ± 0.3	24.2 ± 1.6	2.2 ± 0.6	74.3
	Bamboo yellow	1.0 ± 0.0	0.2 ± 0.0	49.5 ± 0.7	25.0 ± 0.5	26.3 ± 1. 2	2.5 ± 0.0	71.4

Note: ND-not detected. 8% SPORL-SPORL pretreatment with 8% Na_2SO_3 and 2% H_2SO_4 on bamboo. 4% SPORL-SPORL pretreatment with 4% Na_2SO_3 and 2% H_2SO_4 on bamboo. 2% SPORL-SPORL pretreatment with 2% Na_2SO_3 and 2% H_2SO_4 on bamboo. 2% DA-Dilute acid pretreatment with 2% H_2SO_4 on bamboo. 12% NaOH-Alkali pretreatment with 12% NaOH on bamboo. 6% NaOH-Alkali pretreatment with 6% NaOH on bamboo. Substrate yield-weight percentage of substrate obtained after pretreatment to original unpretreated biomass, both in dry matter.

yellow were determined using Soxhlet extractor. Electric muffle furnace (programmable ramping) was used to determine the ash in samples of extractive-free bamboo green, bamboo timber, and bamboo yellow.

Acid-insoluble lignin of unpretreated and pretreated bamboo was determined according to National Energy Laboratory (NREL) Analytical Procedure: Determination of Structural Carbohydrates and Lignin in Biomass (with modifications). Acid-soluble lignin was measured at 205 nm on a UV–Visible spectrophotometer using an extinction coefficient of 110 L/g·cm (Dence, 1992).

Carbohydrate composition of the original bamboo, pretreated bamboo and spent pretreatment liquors was analyzed using an improved high performance ion chromatography (HPIC, Dionex ICS-3000 system) equipped with integrated amperometric detector and Carbopac™ PA1 guard and analytical columns at 20 °C (Shuai et al., 2010).

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 temperature 30 °C and a UV detector at 210 nm (Shuai et al., 2010).

According to the filter paper assay recommended by the International Union of Pure and Applied Chemists, the cellulase activity was determined and expressed as filter paper units (FPUs) (Ghose, 1987). β-Glucosidase activity was determined using *p*-nitrophenyl-β-D-glucoside as the substrate and expressed as international units (IUs) (Wood and Bhat, 1988).

3. Results and discussion

3.1. Composition of bamboo green, timber, and yellow

The chemical composition of the original bamboo green, bamboo timber and bamboo yellow is listed in Table 1. For all the three fractions, glucan was the dominant component, followed by lignin and xylan. The content of glucose in bamboo timber (46.5%) was slightly higher than that in bamboo green (43.6%) and bamboo yellow (42.1%). The content of lignin in bamboo green (30.9%) was much higher than that in bamboo timber (23.3%) and bamboo yellow (23.8%). Mannose was not detected in bamboo timber and bamboo yellow. More extractives were found in bamboo yellow (12.8%), followed by bamboo timber (10.0%) and bamboo green (6.8%). The ash contents of the bamboo green, bamboo timber and bamboo yellow were similar, 1.5%, 1.0% and 1.2%, respectively. Slightly higher ash content in bamboo green is probably from the epidermis.

3.2. Changes in cell-wall components of bamboo green, timber, and yellow during the pretreatments

As described in the Section 2, bamboo green, bamboo timber and bamboo yellow were pretreated with three pretreatment methods (SPORL, DA, and NaOH) at varied chemical loadings but the same temperature, pretreatment time, and the ratio of pretreatment liquor to bamboo to compare the response of bamboo fractions to different pretreatment methods. Chemical composition of the pretreated bamboo fractions is presented in Table 2. The results indicated that cellulose was enriched in all pretreated bamboo fractions because of the removal of hemicellulose and/or lignin. The order of cellulose content was bamboo timber > bamboo green > bamboo yellow, consistent with unpretreated bamboo fractions. The solid substrate yield decreased in the order of bamboo green, bamboo timber, and bamboo yellow for each pretreatment method. This might be related to the difference of the three bamboo fractions in density and lignin content. It was observed that bamboo green had higher density and hardness than bamboo timber and bamboo yellow. The high density limited the mass transfer during the pretreatment and thereby resulted in reduced removal/dissolution of hemicellulose and lignin at the same pretreatment conditions. Bamboo green had more lignin (Table 1), and the pretreated bamboo green also had more acid-insoluble lignin than pretreated bamboo timber and bamboo yellow (Table 2). In terms of hemicelluloses, no mannose was detected in any pretreated bamboo substrate, suggesting that all mannose was dissolved during the pretreatments; the majority of the hemicellulosic sugars was xylose with a small quantity of arabinose and galactose. Both unpretreated and pretreated bamboo yellow had more xylose than unpretreated and pretreated bamboo green and bamboo yellow, respectively.

The SPORL pretreatment retained more hemicellulose but less lignin in the substrate than the DA pretreatment. With the decrease of sodium sulfite loading at the same sulfuric acid loading (2% on bamboo), xylose in the substrate decreased but lignin increased. The reason is that with the addition of sodium sulfite, the pH value of the SPORL pretreatment liquor was elevated from 1.15 of DA to 2.79 of 2% SPORL, 2.98 of 4% SPORL and 6.98 of 8% SPORL, respectively. The high pH value protected cellulose and hemicelluloses from excessive hydrolysis and further decomposition to fermentation inhibitors at elevated temperature. Meanwhile, with increased sulfite loading, more lignin was removed through sulfonation. In addition, the high pH value prevented lignin from extensive condensation that occurs at strong acidic condition. The DA pretreatment apparently dissolved all hemicellulose from the bamboo fractions, leaving only cellulose and lignin in the DA substrates. The DA pretreatment had no significant delignification on bamboo, and the acid-insoluble lignin in the substrates was actually enriched after the pretreatment to 37.4% (bamboo timber), 40.2% (bamboo green), and 40.9% (bamboo yellow), respectively, with the removal of hemicellulose. It is well known that alkaline pretreatment is more effective in delignification and swelling of cellulosic fibers. It turned out that more lignin was removed at higher loading of NaOH. For example, the lignin content of the bamboo substrates pretreated with 12% NaOH was 17.4–20.3%, compared to 24.2–27.2% with 6% NaOH. In addition, the NaOH pretreatment retained more hemicellulose in the substrates than the DA and the SPORL pretreatments.

The concentrations of sugars and soluble lignin in the spent pretreatment liquors are listed in Table 3. These results provided insights into the effects of pretreatment on the changes of carbohydrates and lignin. At low sodium sulfite loading, the SPORL liquors contained more sugars but less soluble lignin. This could be attributed to that the lower pH value at low sodium sulfite loading of the SPORL liquors promoted the hydrolysis of hemicellulose and even cellulose but limited delignification through sulfonation. The higher concentration of glucose in the DA spent liquors was due to the enhanced acidic hydrolysis of cellulose during the DA pretreatment. The xylose content in the DA spent liquors was approximately 2% lower than that in the SPORL liquors, since more xylose was further degraded at lower pH during the DA pretreatment. This observation is supported by the level of inhibitors derived from the degradation of sugars, as listed in Table 3. The NaOH pretreatment dissolved less cellulose than the DA and the SPORL pretreatments. More soluble lignin was detected in NaOH spent liquors than in the DA liquors.

Table 3
Concentration of monomeric sugars, soluble lignin and fermentation inhibitors in pretreated spent liquors.

Concentration (g/L)	Bamboo fraction	Arabinose	Galactose	Glucose	Xylose	Mannose	Acid-soluble lignin	Formic acid	Acetic acid	Furfural	HMF	Levulinic acid	Total inhibitor
8% SPROL	Bamboo green	0.7 ± 0.0	0.1 ± 0.0	0.4 ± 0.0	1.7 ± 0.0	0.2 ± 0.0	18.0 ± 0.4	0.1 ± 0.0	2.5 ± 0.5	1.9 ± 0.1	0.0 ± 0.0	0.2 ± 0.0	4.7
	Bamboo timber	0.8 ± 0.0	0.1 ± 0.0	0.6 ± 0.0	1.9 ± 0.0	0.3 ± 0.0	18.0 ± 0.2	0.1 ± 0.0	3.1 ± 0.3	0.9 ± 0.0	0.1 ± 0.0	0.4 ± 0.0	4.5
	Bamboo yellow	0.5 ± 0.0	0.0 ± 0.0	0.5 ± 0.0	1.3 ± 0.0	0.2 ± 0.0	15.7 ± 0.4	0.1 ± 0.0	3.7 ± 0.4	1.8 ± 0.1	0.1 ± 0.0	0.4 ± 0.0	6.0
4% SPROL	Bamboo green	1.0 ± 0.0	0.4 ± 0.0	1.2 ± 0.1	8.2 ± 0.2	0.1 ± 0.0	13.4 ± 0.3	1.0 ± 0.1	2.2 ± 0.3	0.7 ± 0.1	0.3 ± 0.0	1.1 ± 0.5	5.3
	Bamboo timber	1.0 ± 0.0	0.3 ± 0.0	3.3 ± 0. 2	7.8 ± 0.4	0.4 ± 0.0	14.7 ± 0.4	0.7 ± 0.0	2.3 ± 0.3	0.0 ± 0.0	0.8 ± 0.0	1.4 ± 0.3	5.3
	Bamboo yellow	0.8 ± 0.0	0.2 ± 0.0	3.1 ± 0.2	4.9 ± 0.3	0.4 ± 0.0	13.8 ± 0.6	0.2 ± 0.0	2.3 ± 0.4	0.3 ± 0.1	1.0 ± 0.1	1.4 ± 0.4	5.2
2% SPROL	Bamboo green	1.2 ± 0.0	0.6 ± 0.0	2.0 ± 0.2	15.5 ± 0.5	0.5 ± 0.0	8.9 ± 0.3	2.9 ± 0.2	4.5 ± 0.6	1.1 ± 0.1	0.6 ± 0.0	3.0 ± 0.4	12.1
	Bamboo timber	0.9 ± 0.0	0.6 ± 0.0	4.1 ± 0.2	14.3 ± 0.5	0.5 ± 0.0	8.5 ± 0.2	2.6 ± 0.1	5.6 ± 0.3	1.4 ± 0.3	1.5 ± 0.0	4.8 ± 0.7	15.9
	Bamboo yellow	0.9 ± 0.0	0.5 ± 0.0	5.6 ± 0.4	10.2 ± 0.3	0.4 ± 0.0	8.8 ± 0.1	0.7 ± 0.0	3.3 ± 0.5	0.2 ± 0.0	1.7 ± 0.1	2.9 ± 0.4	8.9
2% DA	Bamboo green	1.1 ± 0.0	0.5 ± 0.0	5.7 ± 0.2	12.1 ± 0.2	0.5 ± 0.0	5.6 ± 0.2	6.1 ± 0.5	5.7 ± 0.5	0.3 ± 0.0	0.5 ± 0.1	6.9 ± 0.9	19.5
	Bamboo timber	0.9 ± 0.0	0.4 ± 0.0	7.7 ± 0.4	11.7 ± 0.1	0.3 ± 0.0	6.6 ± 0.0	6.1 ± 0.4	7.1 ± 0.7	0.6 ± 0.0	1.3 ± 0.1	6.3 ± 0.4	21.3
	Bamboo yellow	0.5 ± 0.0	0.4 ± 0.0	7.8 ± 0.1	7.8 ± 0.2	0.2 ± 0.0	4.9 ± 0.1	1.4 ± 0.4	4.4 ± 0.2	0.6 ± 0.0	1.8 ± 0.1	4.3 ± 0.8	12.5
12% NaOH	Bamboo green	0.7 ± 0.0	ND	0.7 ± 0.0	3.7 ± 0.0	0.0 ± 0.0	12.7 ± 0.3	ND	1.4 ± 0.2	ND	ND	ND	1.4
	Bamboo timber	0.7 ± 0.0	ND	0.7 ± 0.0	6.9 ± 0.2	0.1 ± 0.0	18.9 ± 0.2	ND	4.6 ± 0.5	ND	ND	ND	4.6
	Bamboo yellow	1.0 ± 0.1	ND	1.6 ± 0.0	12.3 ± 0.3	0.1 ± 0.0	15.2 ± 0.2	ND	5.3 ± 0.7	ND	ND	ND	5.3
6% NaOH	Bamboo green	0.8 ± 0.0	ND	0.9 ± 0.0	5.7 ± 0.3	0.1 ± 0.0	8.1 ± 0.1	ND	4.2 ± 0.3	ND	ND	ND	4.2
	Bamboo timber	0.6 ± 0.0	ND	1.0 ± 0.0	3.2 ± 0.1	0.1 ± 0.0	6.8 ± 0.2	ND	5.4 ± 0.5	ND	ND	ND	5.4
	Bamboo yellow	0.8 ± 0.0	ND	1.8 ± 0.0	7.9 ± 0.2	0.2 ± 0.0	7.0 ± 0.1	ND	5.6 ± 0.6	ND	HMF	ND	5.6

Note: ND-not detected. 8% SPORL-SPORL pretreatment with 8% Na_2SO_3 and 2% H_2SO_4 on bamboo. 4% SPORL-SPORL pretreatment with 4% Na_2SO_3 and 2% H_2SO_4 on bamboo. 2% SPORL-SPORL pretreatment with 2% Na_2SO_3 and 2% H_2SO_4 on bamboo. 2% DA-Dilute acid pretreatment with 2% H_2SO_4 on bamboo. 12% NaOH-Alkali pretreatment with 12% NaOH on bamboo. 6% NaOH-Alkali pretreatment with 6% NaOH on bamboo.

3.3. Fermentation inhibitors formed during the pretreatments

As discussed above, certain amount of sugars were degraded in all the pretreatments, but the degradation was more severe in the DA pretreatment. For example, 77.3% of xylose in bamboo yellow was degraded during the DA pretreatment. Compounds derived from the sugar degradation were complicated. Some of them were potential inhibitors for subsequent ethanol fermentation, including formic acid, furfural, hydroxymethylfurfural (HMF), and levulinic acid. The soluble lignin (smaller lignin fragments from lignin depolymerization) was also one of fermentation inhibitors. Acetic acid was released from acetyl groups on hemicelluloses, which occurred in both acidic and basic pretreatments. Furfural was derived from pentoses, HMF from hexoses, and formic and levulinic acid from successive decomposition of HMF, all of which were usually generated in acidic pretreatments (Nevolge, 2008).

The data in Table 3 clearly indicated that fewer inhibitors were formed from degradation of sugars during the SPORL pretreatment than the DA pretreatment. The total of inhibitors (formic, acetic and levulinic acid, furfural, and HMF) formed in the SPORL pretreatment were increasing with decreased sodium sulfite loading. When no sodium sulfite was loaded (the DA pretreatment), the detected inhibitors reached the highest concentration, 19.5 g/L for bamboo green, 21.3 g/L for bamboo timber, and 12.5 g/L for bamboo yellow, respectively. As discussed above, the addition of sodium sulfite in the SPORL pretreatment slightly elevated the pH value of the pretreatment liquor, which limited the excessive degradation of sugars. Soluble lignin in the SPORL liquors decreased with reduced sodium sulfite. Soluble lignin in the SPORL spent liquors was primarily from the formation of water-soluble lignosulfonate through lignin sulfonation. Bamboo yellow had the highest total inhibitors in 8% SPORL pretreatment, while bamboo timber had the highest total inhibitors in 4% and 2% SPORL and DA pretreatments, which may be related to the differences of the bamboo fractions in structure and composition. In summary, the formation of the inhibitors was dependent on the composition and pH value of the pretreatment liquor. Acidic pretreatment at low pH generally resulted in more sugar-derived inhibitors; addition of sulfite suppressed the formation of sugar-derived inhibitors but generated more soluble lignin through lignin sulfonation; and basic pretreatment dissolved more lignin in spent pretreatment liquor.

3.4. Mass balance of sugars during pretreatments

An ideal pretreatment is not only able to produce a readily digestible substrate, but also to maximally recover available sugars in feedstock in fermentable form with limited formation of fermentation inhibitors. The mass balance of bamboo green, bamboo timber and bamboo yellow during different pretreatments is summarized in Tables 4–6, respectively.

The mass balance of the bamboo green during the pretreatments is shown in Table 4. There were 43.6 g glucose, 21.6 g xylose, 0.7 g arabinose, 0.2 g galactose, 0.6 g mannose and 30.9 g lignin from 100 g oven-dry unpretreated bamboo green (Table 1). Almost all the lignin in the bamboo green was recovered during the SPORL and the DA pretreatments, but 12.3% and 32.0% of the lignin was not detected for 6% and 12% NaOH pretreatments, respectively. The results indicated that total sugar recovery (the percentage of all sugars, including glucose, xylose, mannose, galactose, and arabinose, recovered in both solid substrate and spent pretreatment liquor after pretreatment to all available sugars in original biomass before pretreatment) was 83.2% for 8% SPORL, 83.1% for 4% SPORL, 85.3% for 2% SPORL, 77.9% for DA, and 78.7–92.2% for NaOH pretreatments, respectively. The glucose recovery was 96.8–98.2% for the SPORL, 98.4% for the DA, and 87.2–94.7% for NaOH pretreatment, respectively. The xylose recovery was quite low, compared to glucose. The xylose recovery was only 35.6% for the DA, 53.2–60.6% for the SPORL, and 63.4–86.1% for NaOH pretreatment, respectively. The recovery increased with the pretreatment pH, suggesting that more xylose underwent degradation at lower pH value.

The mass balance of the bamboo timber during the pretreatments is shown in Table 5. From Table 1, 100 g oven-dry bamboo timber contained 46.5 g glucose, 22.4 g xylose, 1.0 g arabinose, 0.2 g galactose, and 0.4 g mannose. The total recovery of all components of bamboo timber was slightly lower than that of bamboo green for the pretreatments except for 12% NaOH pretreatment. The results indicated that total sugar recovery was 78.9–82.0% for the SPORL, 71.5% for the DA, and 76.7–86.4% for NaOH pretreatment, respectively. The glucose recovery was 93.1–96.6% for the SPORL, 89.5% for the DA, and 83.2–90.3% for NaOH pretreatment, respectively. The xylose recovery was relatively low, and it was 51.3–54.5% for the SPORL, 33.9% for the DA, and 65.2–78.6% for NaOH pretreatment, respectively.

The mass balance of the bamboo yellow during the pretreatments is shown in Table 6. The data in Table 1 indicated that 100 g oven-dry bamboo yellow contained 42.1 g glucose, 23.3 g xylose, 1.2 g arabinose, 0.2 g galactose, and 0.4 g mannose. The recovery of bamboo yellow components was slightly lower than that of bamboo green and bamboo timber for corresponding SPORL and DA pretreatments. The results indicated that total sugar recovery was 73.7–76.5% for the SPORL, 67.0% for the DA, and 82.6–90.5% for NaOH pretreatment, respectively. The glucose recovery was 87.9–92.9% for the SPORL, 92.9% for the DA, and 78.4–86.9% for NaOH pretreatment, respectively. The xylose recovery was the lowest, compared to the results of bamboo green and timber, and it was only 46.8–50.2% for the SPORL, 22.7% for the DA, and 91.4–97.4% for NaOH pretreatment, respectively.

In summary, since more xylose underwent degradation at low pH, the DA pretreatment had lowest xylose recovery for all bamboo fractions, compared to the SPORL and NaOH pretreatments. The SPORL and the DA pretreatments had the same acid loading, pretreatment temperature, and pretreatment time. The only difference is that sodium sulfite was added in the SPORL pretreatment (2, 4, and 8%, respectively). The results indicated that total sugar recovery of the SPORL pretreatments was higher than the DA pretreatment. The reason is that the addition of sodium sulfite raised the pH value of the pretreatment liquor, as indicated above, which reduced the sugar degradation. For the SPORL pretreatment, bamboo green had the highest total sugar, glucose, and xylose recovery, followed by bamboo timber and bamboo yellow. For the DA pretreatment, bamboo green also had the highest total sugar, glucose, and xylose recovery. Slightly more glucose was degraded in bamboo timber during the DA pretreatment than bamboo yellow. For NaOH pretreatment, the results were dependent on the loading of NaOH. When NaOH loading was 12%, the bamboo yellow had the highest total sugar and xylose recovery, but more glucose was degraded than the bamboo green and the bamboo timber. When NaOH loading was 6%, the bamboo green had the highest total sugar and glucose recovery, but the bamboo yellow got the highest xylose recovery.

3.5. Enzymatic hydrolysability of pretreated bamboo substrates

There are many factors that affect enzymatic hydrolysability of a substrate, such as hemicellulose content, lignin structure, distribution and content, cellulose crystallinity and degree of polymerization, and so on (Mansfield et al., 1999; Alvira et al., 2010). Bamboo is a unique biomass, different from agricultural residues and woods. For example, bamboo has high density and hardness, in particular the outer part of bamboo stem (bamboo green)

Table 4
Mass balance of 100 g bamboo green during SPORL, DA and NaOH pretreatment.

Mass balance (g)		Arabinose	Galactose	Glucose	Xylose	Mannose	Insoluble lignin	Soluble lignin	Total	Recovery (%)
8% SPORL	Substrate	0.2 ± 0.0	0.1 ± 0.0	42.6 ± 1.6	10.7 ± 0.5	ND	19.2 ± 1.1	2.0 ± 0.0	74.8	87.9
	Spent liquor	0.4 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	1.1 ± 0.0	0.1 ± 0.0	NA	11.2 ± 0.4	13.1	
4% SPORL	Substrate	0.1 ± 0.0	0.0 ± 0.0	42.1 ± 1.1	6.4 ± 0.9	ND	20.9 ± 1.3	1.6 ± 0.1	71.1	86.4
	Spent liquor	0.7 ± 0.0	0.2 ± 0.0	0.7 ± 0.1	5.1 ± 0.2	0.1 ± 0.0	NA	8.4 ± 0.3	15.2	
2% SPORL	Substrate	0.1 ± 0.0	ND	40.9 ± 1.8	3.4 ± 0.2	ND	23.0 ± 1.1	1.8 ± 0.0	69.2	87.2
	Spent liquor	0.8 ± 0.0	0.4 ± 0.0	1.3 ± 0.2	9.7 ± 0.5	0.3 ± 0.0	NA	5.6 ± 0.3	18.0	
2% DA	Substrate	0.1 ± 0.0	ND	39.3 ± 3.2	0.2 ± 0.4	ND	25.7 ± 1.2	1.6 ± 0.3	66.9	82.8
	Spent liquor	0.7 ± 0.0	0.3 ± 0.0	3.6 ± 0.2	7.5 ± 0.2	0.3 ± 0.0	NA	3.5 ± 0.2	15.9	
12% NaOH	Substrate	0.3 ± 0.1	0.1 ± 0.0	37.6 ± 0.4	11.4 ± 0.8	ND	12.3 ± 1.7	0.8 ± 0.0	62.4	73.5
	Spent liquor	0.4 ± 0.0	0.0 ± 0.0	0.4 ± 0.0	2.3 ± 0.0	0.0 ± 0.0	NA	7.9 ± 0.3	11.1	
6% NaOH	Substrate	0.9 ± 0.0	0.1 ± 0.0	40.7 ± 0.8	15.1 ± 0.2	ND	20.7 ± 1.6	1.3 ± 0.5	78.7	88.4
	Spent liquor	0.5 ± 0.0	0.0 ± 0.0	0.6 ± 0.0	3.5 ± 0.3	0.1 ± 0.0	NA	5.1 ± 0.1	9.7	

Note: NA-Not applicable. ND-not detected. 8% SPORL-SPORL pretreatment with 8% Na_2SO_3 and 2% H_2SO_4 on bamboo. 4% SPORL-SPORL pretreatment with 4% Na_2SO_3 and 2% H_2SO_4 on bamboo. 2% SPORL-SPORL pretreatment with 2% Na_2SO_3 and 2% H_2SO_4 on bamboo. 2% DA-Dilute acid pretreatment with 2% H_2SO_4 on bamboo. 12% NaOH-Alkali pretreatment with 12% NaOH on bamboo. 6% NaOH-Alkali pretreatment with 6% NaOH on bamboo. Recovery-the sum of all sugars and lignin recovered in substrate and spent liquor to that in original unpretreated biomass.

Table 5
Mass balance of 100 g bamboo timber during SPORL, DA and NaOH pretreatment.

Mass balance (g)		Arabinose	Galactose	Glucose	Xylose	Mannose	Insoluble lignin	Soluble lignin	Total	Recovery (%)
8% SPORL	Substrate	0.2 ± 0.0	0.1 ± 0.0	44.1 ± 1.9	11.0 ± 0.3	ND	15.9 ± 1.0	2.5 ± 0.0	73.8	87.3
	Spent liquor	0.5 ± 0.0	0.1 ± 0.0	0.4 ± 0.0	1.2 ± 0.0	0.2 ± 0.0	NA	11.3 ± 0.2	13.5	
4% SPORL	Substrate	0.1 ± 0.0	0.0 ± 0.0	41.1 ± 0.9	6.4 ± 0.5	ND	16.3 ± 1.2	2.3 ± 0.0	66.2	83.4
	Spent liquor	0.6 ± 0.0	0.2 ± 0.0	2.1 ± 0.2	4.8 ± 0.4	0.3 ± 0.0	NA	9.2 ± 0.4	17.2	
2% SPORL	Substrate	0.1 ± 0.0	ND	42.3 ± 1.9	2.6 ± 0.3	ND	19.7 ± 0.8	2.9 ± 0.1	67.6	85.7
	Spent liquor	0.6 ± 0.0	0.4 ± 0.0	2.6 ± 0.2	8.9 ± 0.5	0.3 ± 0.0	NA	5.3 ± 0.2	18.1	
2% DA	Substrate	0.1 ± 0.0	ND	36.8 ± 2.9	0.3 ± 0.2	ND	21.5 ± 1.9	1.0 ± 0.2	59.6	76.9
	Spent liquor	0.6 ± 0.0	0.3 ± 0.0	4.8 ± 0.4	7.3 ± 0.1	0.2 ± 0.0	NA	4.1 ± 0.0	17.3	
12% NaOH	Substrate	0.3 ± 0.0	0.0 ± 0.0	38.2 ± 0.8	10.3 ± 0.8	ND	11.7 ± 1.5	1.4 ± 0.0	61.8	78.8
	Spent liquor	0.5 ± 0.0	0.0 ± 0.0	0.5 ± 0.0	4.3 ± 0.2	0.0 ± 0.0	NA	11.8 ± 0.2	17.1	
6% NaOH	Substrate	0.7 ± 0.0	0.1 ± 0.0	41.4 ± 0.7	15.6 ± 0.3	ND	18.0 ± 1.6	1.6 ± 0.6	77.4	84.8
	Spent liquor	0.4 ± 0.0	0.0 ± 0.0	0.6 ± 0.0	2.0 ± 0.1	0.1 ± 0.0	NA	4.3 ± 0.2	7.3	

Note: NA-Not applicable. ND-not detected. 8% SPORL-SPORL pretreatment with 8% Na_2SO_3 and 2% H_2SO_4 on bamboo. 4% SPORL-SPORL pretreatment with 4% Na_2SO_3 and 2% H_2SO_4 on bamboo. 2% SPORL-SPORL pretreatment with 2% Na_2SO_3 and 2% H_2SO_4 on bamboo. 2% DA-Dilute acid pretreatment with 2% H_2SO_4 on bamboo. 12% NaOH-Alkali pretreatment with 12% NaOH on bamboo. 6% NaOH Alkali pretreatment with 6% NaOH on bamboo. Recovery-the sum of all sugars and lignin recovered in substrate and spent liquor to that in original unpretreated biomass.

Table 6
Mass balance of 100 g bamboo yellow during SPORL, DA and NaOH pretreatment.

Mass balance (g)		Arabinose	Galactose	Glucose	Xylose	Mannose	Insoluble lignin	Soluble lignin	Total	Recovery (%)
8% SPORL	Substrate	0.3 ± 0.0	0.1 ± 0.0	36.7 ± 1.9	10.9 ± 0.8	ND	15.5 ± 1.1	2.7 ± 0.1	66.2	77.6
	Spent liquor	0.3 ± 0.0	0.0 ± 0.0	0.3 ± 0.0	0.8 ± 0.0	0.1 ± 0.0	NA	9.8 ± 0.4	11.4	
4% SPORL	Substrate	0.2 ± 0.0	0.0 ± 0.0	36.2 ± 1.2	7.9 ± 0.2	ND	18.1 ± 1.2	1.8 ± 0.0	64.2	78.7
	Spent liquor	0.5 ± 0.0	0.2 ± 0.0	1.9 ± 0.2	3.0 ± 0.3	0.3 ± 0.0	NA	8.6 ± 0.6	14.5	
2% SPORL	Substrate	0.1 ± 0.0	ND	35.6 ± 1.9	4.6 ± 0.6	ND	19.6 ± 1.2	3.5 ± 0.1	63.3	79.7
	Spent liquor	0.6 ± 0.0	0.3 ± 0.0	3.5 ± 0.4	6.4 ± 0.3	0.3 ± 0.0	NA	5.5 ± 0.1	16.4	
2% DA	Substrate	0.0 ± 0.0	ND	34.2 ± 2.8	0.4 ± 0.5	ND	23.2 ± 1.7	1.2 ± 0.1	59.0	72.5
	Spent liquor	0.3 ± 0.0	0.2 ± 0.0	4.9 ± 0.1	4.9 ± 0.2	0.1 ± 0.0	NA	3.1 ± 0.1	13.5	
12% NaOH	Substrate	0.4 ± 0.1	0.1 ± 0.0	32.0 ± 0.3	13.6 ± 0.7	ND	9.5 ± 1.8	0.7 ± 0.0	56.2	75.1
	Spent liquor	0.6 ± 0.1	0.0 ± 0.0	1.0 ± 0.0	7.7 ± 0.3	0.1 ± 0.0	NA	9.5 ± 0.2	18.9	
6% NaOH	Substrate	0.7 ± 0.0	0.2 ± 0.0	35.4 ± 0.7	17.8 ± 0.5	ND	18.7 ± 1.2	1.7 ± 0.0	74.6	85.6
	Spent liquor	0.5 ± 0.0	0.0 ± 0.0	1.2 ± 0.0	4.9 ± 0.2	0.1 ± 0.0	NA	4.4 ± 0.1	11.0	

Note: NA-Not applicable. ND-not detected. 8% SPORL-SPORL pretreatment with 8% Na_2SO_3 and 2% H_2SO_4 on bamboo. 4% SPORL-SPORL pretreatment with 4% Na_2SO_3 and 2% H_2SO_4 on bamboo. 2% SPORL-SPORL pretreatment with 2% Na_2SO_3 and 2% H_2SO_4 on bamboo. 2% DA-Dilute acid pretreatment with 2% H_2SO_4 on bamboo. 12% NaOH-Alkali pretreatment with 12% NaOH on bamboo. 6% NaOH-Alkali pretreatment with 6% NaOH on bamboo. Recovery-the sum of all sugars and lignin recovered in substrate and spent liquor to that in original unpretreated biomass.

(Chand et al., 2006), which limits mass and heat transfer during pretreatment. In addition, the wax in the bamboo green may be another factor affecting pretreatment and enzymatic digestibility.

The enzymatic hydrolysability of unpretreated and the SPORL, the DA, and NaOH pretreated bamboo green, timber, and yellow was compared. The enzyme loadings were 15 FPU cellulase and

30 IU β-glucosidase per gram cellulose for all enzymatic hydrolysis experiments. The unpretreated bamboo green, timber, and yellow were almost indigestible, and their cellulose-to-glucose conversion yields after a 48-h hydrolysis were only 1.3%, 4.3%, and 3.2%, respectively (data not included in Fig. 1). As expected, all the pretreatments did improve the digestibility of all the bamboo fractions, as shown in Fig. 1, but the digestibility was dependent on the pretreatment methods, pretreatment conditions, and bamboo fractions.

In general, regardless pretreatment methods and conditions, the enzymatic digestibility of pretreated bamboo fractions decreased in the order of bamboo timber, bamboo yellow, and bamboo green. As mentioned above, higher density and wax content of bamboo green retarded the penetration of pretreatment chemicals, and thereby the recalcitrance of the bamboo green was not sufficiently removed during the pretreatments. In addition, the high lignin content (Table 1) of bamboo green was probably another important cause of the worst digestibility of pretreated bamboo green.

Comparing the three pretreatment methods investigated in the present study, it seems that under the pretreatment conditions investigated, the DA pretreatment performed the best, and the NaOH pretreatment with high NaOH dosage gave similar results. The SPORL pretreatment did not perform as well as expected. The DA pretreatment did not delignify the bamboo, and the lignin was actually enriched in the DA pretreated bamboo fractions with the removal of hemicelluloses (Table 2). Therefore, the digestibility enhancement of the DA pretreated bamboo was primarily from the removal of hemicellulose. The NaOH pretreatment was conducted at two levels of NaOH dosage. As seen in Fig. 1, at 6% NaOH dosage, the NaOH pretreatment did not improve the digestibility substantially, simply because the alkali loading was not enough to remove significant amount of either lignin or hemicellulose (Table 2). Increasing NaOH dosage to 12% significantly improved the digestibility of all bamboo fractions, in particular the bamboo timer. This should be attributed to the removal of more hemicellulose and lignin (Table 2). The swelling/softening of cell walls by the

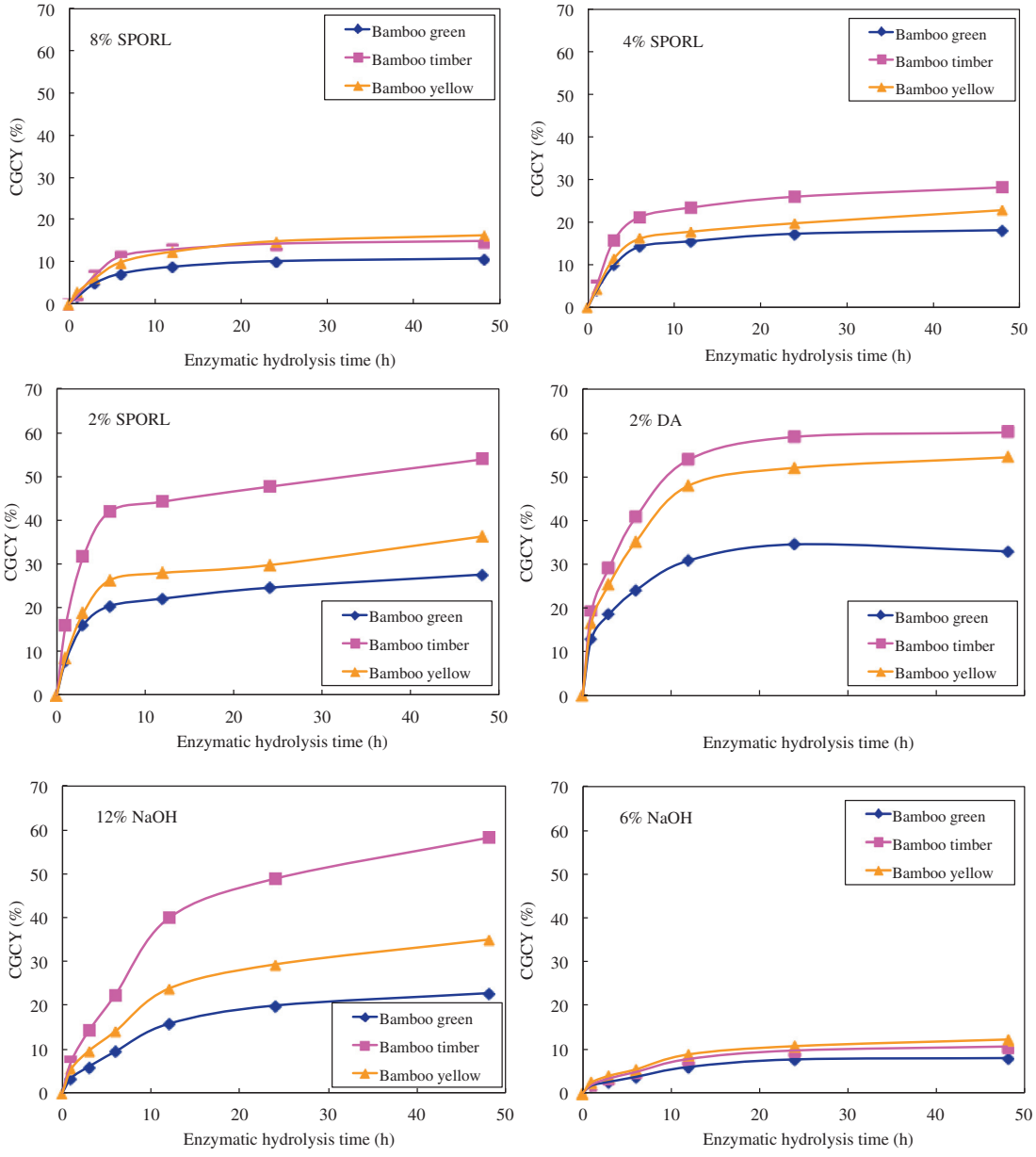

Fig. 1. Comparison of time-dependent enzymatic hydrolysis profiles of the SPORL, DA, and NaOH pretreated bamboo fractions. (CGCY – cellulose to glucose conversion yield).

high-concentration NaOH presumably was another contributor to the better digestibility, which made cellulose more accessible to the enzymes.

It was expected that the SPORL should have performed better in pretreating the bamboo, compared to the DA and NaOH pretreatments, because of its ability to dissolve hemicellulose and lignin and to sulfonated lignin (Wang et al., 2009; Shuai et al., 2010). The sulfonation of lignin does not only result in partial delignification (dissolution of lignin) but also makes the residual lignin in the pretreated substrate more hydrophilic, which reduces the hydrophobic impact of the lignin on cellulases (Wang et al., 2013). However, the performance of the SPORL was surprisingly worse than that of the DA pretreatment and 12% NaOH pretreatment in the present study, as shown in Fig. 1. Compared to the DA pretreatment with 2% H_2SO_4, the SPORL pretreatment included sodium sulfite as a supplementary pretreatment chemical. The addition of sodium sulfite, as a buffer, elevated pH value of the SPORL pretreatment liquor, as discussed above. The elevated pH reduced the ability of the liquor to dissolve/hydrolyze hemicelluloses. The composition of pretreated bamboo substrates in Table 2 clearly shows that all SPORL substrates had higher hemicellulose content than the DA ones. The higher the sulfite dosage was, the more the hemicelluloses retained in the SPORL substrates. This was the primary reason why the SPORL substrates in this study had worse digestibility than the DA substrates in this study. It was found in our previous studies (Li et al., 2012; Wang et al., 2012; Yang and Pan, 2012; Zhang et al., 2013) that for the feedstock that has high hemicellulose content such as hardwood, corn stover, and switchgrass, the removal of hemicellulose is more crucial to improve enzymatic digestibility than the removal of lignin when the delignification is not extensive. The removal of hemicelluloses created pores, exposed cellulose, and thereby increased cellulose susceptibility to enzymes. That is the reason why in spite of lower lignin content, the SPORL substrates had poorer digestibility than the DA substrates as the former had higher hemicellulose content than the later, as shown in Table 2. Based on the discussion above, the enzymatic digestibility of the SPORL pretreated bamboo substrates could be improved by removing more hemicellulose through increasing acid loading slightly to lower the pH of the SPORL pretreatment liquor. The results in Fig. 1 have somehow verified the hypothesis. Lowering the pH of SPORL pretreatment from 6.98 of 8% SPORL, 2.98 of 4% SPORL, to 2.79 of 2% SPORL significantly enhanced the cellulose-to-glucose conversion yield (CGCY) of bamboo timber at 48 h from 13%, 27%, to 55%, respectively. The optimization of SPORL pretreatment for bamboo needs further investigation. For example, taking the 2% SPORL pretreatment as an example, if the acid loading was increased from the current 2% to around 3%, the pH of the pretreatment liquor would be brought down to about 2 from the current 2.79, which would therefore be able to dissolve more hemicellulose and generate more readily digestible substrate than the corresponding DA pretreatment (H_2SO_4 alone). In general, the higher the sulfite is loaded, the more the acid should be added to maintain an appropriate pH ($\sim$2) to sufficiently remove hemicellulose (Wang et al., 2012; Zhang et al., 2013).

However, for the 2% SPORL and the DA pretreatments, the content of hemicellulose in bamboo green substrates was lower than that in bamboo timber or bamboo yellow, but the cellulose-to-glucose conversion yield of the pretreated bamboo green was significantly lower than that of the pretreated bamboo timber or bamboo yellow. This suggested that besides the content of hemicellulose, other factors such as cellulose crystallinity and degree of polymerization, lignin content and distribution, and physical properties of the substrates (particle size, density, and so on) affect the enzymatic digestibility of the substrates as well. These should be further investigated in the following study.

In addition, the results of the present study suggested that bamboo, in particular bamboo green and bamboo yellow, is a difficult feedstock to pretreat, compared to switchgrass, corn stover, and even woody biomass. For example, corn stover pretreated with 1% sulfuric acid at 170 °C by dilute acid flowthrough method could be hydrolyzed almost completely within 48 h with enzyme loading of 15 FPU/g cellulose (Zhu et al., 2005). Switchgrass pretreated with 10% NaOH at 180 °C for 30 min could be hydrolyzed by 73% within 48 h with enzyme loading of 15 FPU/g cellulose (Zhang et al., 2013), while the bamboo timber pretreated with 12% NaOH was hydrolyzed less than 60% at 48 h with the same enzyme loading (Fig. 1). Softwood spruce and red pine pretreated with SPORL under similar conditions had much better enzymatic hydrolysability at the same enzyme loading (Zhu et al., 2009), although the softwoods had higher lignin content than the bamboo.

4. Conclusion

Bamboo green, timber, and yellow had different composition, in particular in cellulose, lignin, and extractives. The three pretreatments (SPORL, DA, and NaOH) did improve the enzymatic digestibility of bamboo substrates. Under the investigated conditions, DA pretreatment achieved better digestibility, but had lower sugar recovery yield and generated more fermentation inhibitors; SPORL pretreatments had higher sugar recovery and less inhibitors but poor digestibility. The results suggested that appropriately lowering pH of SPORL pretreatment should be investigated to improve the digestibility of SPORL substrate by removing more hemicellulose. Bamboo timber was a better feedstock for bioconversion than bamboo green and yellow.

Acknowledgements

The authors acknowledge Drs. Qiang Yang and Li Shuai for their assistance in HPLC analyses. This work was supported by "the Fundamental Research Funds for the International Centre for Bamboo and Rattan" (Grant No 1632012001) and partially by the NSF Career Award to Dr. Xuejun Pan (CBET-0847049).

References

Alvira, P., Tomás-Pejó, E., Ballesteros, M., Negro, M.J., 2010. Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: A review. Bioresour. Technol. 101, 4851–4861.

Chand, N., Jain, D., Nigrawal, A., 2006. Investigation on gradient dielectric characteristics of bamboo (*Dentroclamus strictus*). J. Appl. Polym. Sci. 102, 380–386.

Chen, H.Z., Liu, L.Y., 2007. Unpolluted fractionation of wheat straw by steam explosion and ethanol extraction. Bioresour. Technol. 98, 666–676.

Dence, C.W., 1992. The determination of lignin. In: Dence, C.W. (Ed.), Methods in Lignin Chemistry. Springer-Verlag, Berlin, pp. 33–61.

Garcia-Aparicio, M., Parawira, W., van Rensburg, E., Diedericks, D., Galbe, M., Rosslander, C., Zacchi, G., Gorgens, J., 2011. Evaluation of steam-treated giant bamboo for production of fermentable sugars. Biotechnol. Prog. 27, 641–649.

Ghose, T.K., 1987. Measurement of cellulase activities. Pure Appl. Chem. 59, 257–268.

Leenakul, W., Tippayawong, N., 2010. Dilute acid pretreatment of bamboo for fermentable sugar production. J. Sustain. Ener. Environ. 1, 117–120.

Li, Z.Q., Yang, Q., Jiang, Z.H., Fei, B.H., Cai, Z.Y., Pan, X.J., 2012. Comparative study of sulfite (SPORL), dilute acid and NaOH pretreatments of bamboo for enzymatic saccharification. J. Biobased Mater. Bio. 6, 544–551.

Mansfield, S.D., Mooney, C., Saddler, J.N., 1999. Substrate and enzyme characteristics that limit cellulose hydrolysis. Biotechnol. prog. 15, 804–816.

Nevolge, E., 2008. Progress in metabolic engineering of *Saccharomyces cerevisiae*. Microbiol. Mol. Biol. Rev. 72, 379–412.

Pan, X.J., Xie, D., Yu, R.W., Saddler, J.N., 2008. The bioconversion of mountain pine beetle-killed lodgepole pine to fuel ethanol using the organosolv process. Biotechnol. Bioeng. 101, 39–48.

Sathitsuksanoh, N., Zhu, Z.G., Hoc, T.J., Bai, M.D., Zhang, Y.H.P., 2010. Bamboo saccharification through cellulose solvent-based biomass pretreatment followed by enzymatic hydrolysis at ultra-low cellulase loadings. Bioresour. Technol. 101, 4926–4929.

Scurlocka, J.M.O., Daytonb, D.C., Hamesb, B., 2000. Bamboo: an overlooked biomass resource? Biomass Bioenergy 19, 229–244.
Shuai, L., Yang, Q., Zhu, J.Y., Lu, F.C., Weimer, P.J., Ralph, J., Pan, X.J., 2010. Comparative study of SPORL and dilute-acid pretreatments of spruce for cellulosic ethanol production. Bioresour. Technol. 101, 3106–3114.
Wang, G.S., Pan, X.J., Zhu, J.Y., Gleisner, R., Rockwood, D., 2009. Sulfite pretreatment to overcome recalcitrance of lignocellulose (SPORL) for robust enzymatic saccharification of hardwoods. Biotechnol. Prog. 25, 1086–1093.
Wang, Z.J., Zhu, J.Y., Zalesny, R.S., Chen, K.F., 2012. Ethanol production from poplar wood through enzymatic saccharification and fermentation by dilute acid and SPORL pretreatments. Fuel 95, 606–614.
Wang, Z.J., Lan, T.Q., Zhu, J.Y., 2013. Lignosulfonate and elevated pH can enhance enzymatic saccharification of lignocelluloses. Biotechnol. Biofuels 6, 9.
Wood, T.M., Bhat, M., 1988. Methods for measuring cellulase activities. In: Wood, T.M., Bhat, M. (Eds.), Methods in Enzymology, Biomass (Part a, Cellulose and Hemicellulose), vol. 160. Academic Press Inc., New York, pp. 87–112.
Yamashita, Y., Shono, M., Sasaki, C., Nakamura, Y., 2010. Alkaline peroxide pretreatment for efficient enzymatic saccharification of bamboo. Carbohydr. Polym. 79, 914–920.
Yang, Q., Pan, X.J., 2012. Pretreatment of Agave americana stalk for enzymatic saccharification. Bioresour. Technol. 126, 336–340.
Zhang, D.S., Yang, Q., Zhu, J.Y., Pan, X.J., 2013. Sulfite (SPORL) pretreatment of switchgrass for enzymatic saccharification. Bioresour. Technol. 129, 127–134.
Zhu, Y.M., Lee, Y.Y., Elander, R.T., 2005. Optimization of dilute-acid pretreatment of corn stover using a high-solids percolation reactor. Appl. Biochem. Biotechnol. 121, 1045–1054.
Zhu, J.Y., Pan, X.J., Wang, G.S., Gleisner, R., 2009. Sulfite pretreatment (SPORL) for robust enzymatic saccharification of spruce and red pine. Bioresour. Technol. 100, 2411–2418.