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

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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.

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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.

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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.

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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.

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