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Comparison of aqueous ammonia and dilute acid pretreatment of bamboo fractions: Structure properties and enzymatic hydrolysis

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HIGHLIGHTS

- Structure and hydrolysability of SAA and DA pretreated bamboo were compared.
- Different bamboo fractions had different structure properties and hydrolysabilities.
- SAA was more efficient than DA pretreatment in the hydrolysis of bamboo.
- Supplementation of xylanase was more effective than increase of cellulases loading.
- SAA pretreatment with XYL addition was promising method for bamboo bioconversion.

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ABSTRACT

The effect of two pretreatments methods, aqueous ammonia (SAA) and dilute acid (DA), on the chemical compositions, cellulose crystallinity, morphologic change, and enzymatic hydrolysis of bamboo fractions (bamboo yellow, timber, green, and knot) was compared. Bamboo fractions with SAA pretreatment had better hydrolysability than those with DA pretreatment. High crystallinity index resulted in low hydrolysis yield in the conversion of SAA pretreated bamboo fractions, not DA pretreated fractions. The increase of cellulase loading had modestly positive effect in the hydrolysis of both SAA and DA pretreated bamboo fractions, while supplement of xylanase significantly increased the hydrolysis of the pretreated bamboo fractions, especially after SAA pretreatment. The results indicated that SAA pretreatment was more effective than DA pretreatment in conversion of bamboo fractions, and supplementation of xylanase was necessary in effective conversion of the SAA pretreated fractions into fermentable sugars.

1. Introduction

With the increasing in global energy demand and global warming concerns caused by traditional fossil fuels, the use of diverse biomass to produce renewable energy, such as biofuels is of particular interest (Berndes et al., 2003; Klass, 1998). Biomass, such as agricultural residue, grasses, and forestry wastes, are considered to be a viable and sustainable energy source for biofuels production because of its renewability, wide distribution, and abundance (Kuhad and Singh, 1993; Rubin, 2008). Bamboo, a perennial woody grass in East Asia and South East, has been widely used as a raw material for paper, textiles, food, construction and reinforcing fibers. Recently, bamboo is found to be a promising material for bioethanol production due to its extraordinary growth rate and high contents of carbohydrates that could be converted to fermentable sugars (Kobayashi et al., 2004; Shimokawa et al., 2009; Tsuda et al., 1998). In China, there are more than 200,000 ha of bamboo, and the yields per hectare may reach up to 30 tonnes a year.

According to Chand et al. (2006), bamboo stem is composed of bamboo skin, timber, and pith. There no vascular bundles exist in bamboo skin (the outer part) and pith (the inner part). Bamboo timber is the part between skin and pith, where vascular bundles are present. The bamboo timber can be further divided into three parts: bamboo green, timber, and yellow based on the density of vascular bundles. The vascular bundles in bamboo green (outer layer) are dense, while in bamboo yellow (inner layer) are rare. Bamboo timber is the part between bamboo green and yellow. In

Abbreviations: CEL, cellulases; CI, crystallinity index; DA, dilute acid pretreatment; DM, dry matter; HPLC, high-performance liquid chromatograph; SEM, scanning electron microscopy; SAA, aqueous ammonia pretreatment process; XRD, X-ray diffraction; XYL, xylanase.

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this work, the term "bamboo timber" is not the meaning of the layer between bamboo skin and pith, but the layer between bamboo green and yellow. In bamboo processing industry, bamboo timber is fully utilized while bamboo green and yellow are removed and wasted. However, the wasted bamboo fractions contain high amount of cellulose and hemicelluloses, the bioresources of fermentable sugars production. Therefore, the wasted bamboo fractions are potential materials for biofuels production. Previously, the behaviors of bamboo green, timber, and yellow in sulfite (2%, 4%, and 8% Na_2SO_3, 180 °C for 30 min), dilute sulfuric acid (2% H_2SO_4, 180 °C for 30 min), and NaOH (6% and 12% NaOH, 180 °C for 30 min) pretreatments were investigated and the pretreated bamboo fractions were hydrolyzed by cellulases (15 FPU/g cellulose cellulase and 30 IU/g cellulose β-glucosidase) (Li et al., 2014). It was observed that bamboo timber exhibited higher sugars content and better enzymatic digestibility, while the highest glucose yield of the pretreated bamboo timber was merely 60%. The relatively low hydrolysis yield is mainly due to the high content of lignin, high density and hardness of bamboo. As in other lignocellulosic materials, high degree of lignifications and density of the vascular bundles, heterogeneous and complex structure of cell–well constituents make it difficult for enzymes to access the surface of polysaccharides fibers during enzymatic hydrolysis process (Berlin et al., 2005; Himmel et al., 2007). In order to efficiently convert bamboo to fermentable sugars, it is necessary to remove part of lignin and destroy the complex structure.

Among a considerable amount of pretreatment technologies, alkaline pretreatment, such as aqueous ammonia exhibits strong selectivity in lignin removal by degrading the ester bonds those between *p*-coumaric acid and lignin or between ferulic acid and hemicelluloses and has an ability to swell biomass solids by converting cellulose I to cellulose III (Kim and Lee, 2005; Wada et al., 2004). Removing lignin and swelling cellulose with aqueous ammonia could effectively increase the accessible surface of carbohydrates (cellulose and hemicelluloses) with enzymes, and hence enhance the conversion of carbohydrates to fermentable sugars. It was reported that 73.5% of the lignin in corn stover was removed by 29.5 wt.% aqueous ammonia at room temperature and 1:12 of solid-to-liquid ratio for 60 d, and high glucose yield (92%) and xylose yield (85%) were obtained (Kim and Lee, 2005). However, to our knowledge, less attention has been focused on the aqueous ammonia pretreatment of bamboo.

Therefore, in this work, bamboo green, timber, yellow, and another wasted bamboo fractions in bamboo processing industry, bamboo knot, were pretreated by aqueous ammonia. Additionally, dilute acid pretreatment, the most efficient pretreatment method in the work of Li et al. (2014), was performed on the four bamboo fractions as a comparative pretreatment method. The structural characteristics of raw and pretreated bamboo fractions were assessed by X-ray diffraction (XRD) and scanning electron microscopy (SEM). The enzymatic hydrolysis of the pretreated bamboo fractions by different dosages of cellulase was evaluated. Synergistic action of cellulase and xylanase in the hydrolysis of pretreated bamboo fractions was carried out.

2. Methods

2.1. Materials

Bamboo (4 years old) was collected from Hangzhou city, Zhejiang Province, China. Air-dried bamboo was fractionated to four parts: bamboo yellow, bamboo timber, bamboo green, and bamboo knot. The four fractions of the bamboo were milled and sieved through a 60 mesh screen scale. The milled fractions (⩽0.3 mm) were then pretreated by aqueous ammonia and dilute acid, as described below.

2.2. Aqueous ammonia and dilute acid pretreatments

The bamboo fractions were pretreated with 25 wt.% of aqueous ammonia at 70 °C for 72 h (SAA) and 1 wt.% of H_2SO_4 at 121 °C for 1 h (DA) in screw-capped bottles at a solid:liquid ratio of 1:10. The pretreated bamboo fractions were washed with distilled water until the pH of the washing to neutral. After that, the solids were air-dried and stored at −20 °C for composition analysis and enzymatic hydrolysis.

2.3. Enzymes

The commercial enzyme preparations Celluclast 1.5L and Novozyme 188 (Novo Nordisk A/S, Bagsværd, Denmark) were used as cellulase preparation. Pentopan Mono BG (Novo Nordisk A/S, Bagsværd, Denmark) was used as xylanase (XYL) preparation. Celluclast 1.5L had an activity of 74.7 FPU/ml measured according to IUPAC standard assay (Ghose, 1987). The activity of Novozyme 188 was determined to be 8451 nkat/ml of βG as described by Bailey and Nevalainen (1981). Protein was quantified by the Lowry method, using bovine serum albumin (Sigma Chemical Co., St. Louis, MO, USA) as standard (Lowry et al., 1951).

2.4. Enzymatic hydrolysis

The hydrolysis of the SAA and DA pretreated bamboo fractions by cellulase preparation was performed in tubes with a working volume of 3 ml in 50 mM sodium citrate buffer (pH 5.0) at 50 °C. The hydrolysis was conducted in a shaking incubator, and the shaking speed in the incubator was 200 rpm. The dry matter (DM) content of substrate was 2%. 0.02% NaN_3 was added to the hydrolysis broth to prevent bacterial infection. The cellulase preparation contained both Celluclast 1.5L and Novozyme 188 preparations, which were dosed in the range from 10 to 50 FPU/g DM and 500 nkat/g DM, respectively. Samples were withdrawn at 6, 24, and 48 h and boiled for 10 min to stop the enzymatic hydrolysis. After cooling, the samples were centrifuged at 10,000*g* for 10 min and the supernatants were analyzed for glucose and xylose with a high-performance liquid chromatography (HPLC).

The synergy between CEL and XYL in the hydrolysis of the SAA and DA pretreated bamboos was investigated as described above. CEL was dosed at 20 FPU Celluclast 1.5L per gram DM, and 500 nkat Novozyme 188 per gram DM. The dosage of XYL was 0.5 and 2 mg protein per gram DM. Samples were withdrawn at 48 h and boiled for 10 min to stop the enzymatic hydrolysis. After cooling, the samples were centrifuged and the supernatants were analyzed for glucose and xylose by HPLC. Two replicate tests were carried out in all hydrolysis experiments and average values were presented.

2.5. XRD analysis

The cellulose crystallinity index (CI) of nonpretreated and pretreated samples was measured by XRD using a Rigaku D/max-3C generator (Rigaku Corporation, Japan). The dried samples were scanned in 2θ range from 5° to 50° using the steps of 0.02° in width, and using Cu/Kα radiation (1.54 Å) generated at 35 kV and 35 mA. The CI of cellulose was calculated from the XRD spectra according to the method of Segal et al. (1954):

$$\mathrm{CrI} = \frac{I_{002} - I_{\mathrm{am}}}{I_{002}} \times 100$$

In which I_{002} is the maximum intensity of the (002) lattice diffraction, and I_{am} is the peak of the amorphous portion evaluated as the minimum intensity between the (101) and (002) lattice planes.