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Evaluation of aqueous ammonia pretreatment for enzymatic hydrolysis of different fractions of bamboo shoot and mature bamboo

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HIGHLIGHTS

- Hydrolyzabilities of different fractions of bamboos were compared.
- High hydrolysis yields were obtained from non-pretreated bamboo shoots.
- Pretreatment was not necessary for the enzymatic hydrolysis of bamboo shoots.
- Aqueous ammonia pretreatment was effective for mature bamboos.
- Different mature bamboo fractions exhibited different hydrolyzabilities.

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ABSTRACT

The production of fermentable sugars from different fractions of bamboo shoots and mature bamboos (*Phyllostachys heterocycla* var. *pubescens*) by cellulase and/or xylanase was investigated. Aqueous ammonia pretreatment exhibited high but different delignification capacities for different bamboo fractions. Supplementation of cellulases with xylanase synergistically improved the glucose and xylose yields of mature bamboo fractions. High hydrolyzability was observed in the hydrolysis of both non-pretreated and pretreated bamboo shoot fractions, suggesting pretreatment was not necessary for the hydrolysis of bamboo shoots. High hydrolyzability together with the advantages of low lignin content, fast growth, and widely distribution demonstrated that bamboo shoots were excellent lignocellulosic materials for the production of bioethanol and other biochemicals.

1. Introduction

Bamboo, a perennial and rapidly growing woody grasses, has been distributed widely in Asia. China is the main bamboo-producing country, approximately occupies 1/4 of total bamboo forest area in the world and the annual production reaches 1356 billion culms (Li et al., 2012). Bamboo has been used as materials in many fields, such as construction, pulp and paper, food, furniture, and combustion (Scurlock et al., 2000).

The aboveground portions of mature bamboo mainly include bamboo culm and culm branches with their leaves. Bamboo culm can be further divided into three layers: bamboo green (the outer part where vascular bundles are dense), bamboo yellow (the inner part where vascular bundles are rare) and bamboo timber (the part between bamboo green and bamboo yellow) (Chand et al., 2006). Some fractions of bamboo, such as bamboo green, bamboo yellow, bamboo node, and bamboo branches, were not effectively utilized during the manufacturing of bamboo floor, furniture, and mats, etc. (Sun and Sun, 2012). Besides, immature bamboo shoots gradually become inedible due to the increase of rough fiber (Nirmala et al., 2007); meanwhile, it could not be used as raw materials for the furniture, construction, and pulp and paper before maturation due to the weakness in mechanical properties. So these underused bamboo residues and bamboo shoots are available as feedstocks for bioconversion into bioethanol and other biochemicals.

Bamboo, like other lignocellulosic materials, is mainly composed of three polymeric components: cellulose, hemicelluloses and lignin. Cellulose is the main component of lignocelluloses, and the major source of fermentable sugars. However, hemicelluloses and

Abbreviations: CEL, mixture of Celluclast 1.5 L and Novozyme 188; CrI, crystalline index; DM, dry matter; FPU, filter paper unit; SEM, scanning electron microscope; TAC, theoretical amount of cellulose in substrates; TAX, theoretical amount of xylose in substrates; XRD, X-ray diffraction; XYL, xylanase.

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lignin in lignocelluloses are closely associated with cellulose and form complex structure, which block the accessibility of enzymes to cellulose (Liese, 1987). Thus, a pretreatment step is crucial to removing part of lignin and hemicelluloses, breaking the complex structure of lignocelluloses, and improving the accessibility of enzymes to cellulose fibers. The common pretreatment methods mainly included physical methods (Bonn et al., 1983); chemical methods such as acid pretreatment (Esteghlalian et al., 1997) and alkaline pretreatment (Millet et al., 1976); physical–chemical methods such as steam explosion (Mackie et al., 1985); and biological methods (Akin et al., 1995). Among these, alkaline pretreatment has been found to be a promising pretreatment method because of its low cost, and importantly, highly effective in delignification, cellulose swelling, which can significantly improve the hydrolysis rate and yield of lignocelluloses. So far, many researches on the hydrolysis of bamboo after alkaline pretreatment have been reported. Li et al. (2012) explored the effect of microwave coupled with KOH pretreatment (12%, 180 °C, 30 min) on enzymatic hydrolysis of moso bamboo, and the glucose and xylose yields of pretreated bamboo reached 20.87% and 63.06%, respectively, compared with non-pretreated bamboo (2.4% of glucose yield and 2.9% of xylose yield). Subsequently, they studied the feasibility of ethanosolv with NaOH pretreatment to enhance enzymatic hydrolysis of moso bamboo, and the cellulose-to-glucose conversion yield increased from 2.4% (before pretreatment) to 45.1% after NaOH pretreatment at 180 °C for 30 min (Li et al., 2013). As an easily recycled alkaline pretreatment method, soaking in aqueous ammonia pretreatment process has been proved effective in the lignin removal of wheat straw and corn stover (Rémond et al., 2010; Sun et al., 2014), but researches about the effect of soaking in aqueous ammonia pretreatment on the hydrolysis of bamboo have not been reported yet.

In this work, aqueous ammonia pretreatment were used for the pretreatment of different fractions of bamboo shoots and mature bamboos (*Phyllostachys heterocycla* var. *pubescens*), and the enzymatic digestibility of different fractions (bamboo green, timber, yellow, node, and branch) of bamboo shoots (4- and 6-meter-height) and mature bamboos (2- and 10-year-old) were investigated and compared. The 4- and 6-meter-height here refers to the height of bamboo shoots as they cut down. The structure characteristics of non-pretreated and aqueous ammonia pretreated bamboo fractions were analyzed by scanning electron microscope (SEM) and X-ray diffraction (XRD). The role of xylanases in the hydrolysis of different bamboo fractions by celluloses was also evaluated.

2. Methods

2.1. Substrate

Moso bamboo (*P. heterocycla* var. *pubescens*) was collected from Hangzhou, Zhejiang Province, China. The types of materials included 2- and 10-year-old mature bamboos and 4- and 6-meter-height bamboo shoots. The 2- and 10-year-old mature bamboos are about 11 and 15 m height, respectively. For 4- and 6-meter-height bamboo shoots, they are about one month old. The culms of mature bamboos and bamboo shoots were fractionated manually with a knife to four parts: bamboo green, timber, yellow, and node. The branches of mature bamboos were also used as the materials of experiment. All the bamboo fractions were milled and passed through 60 mesh screen sieve, and then air dried to less than 10% moisture content. All of samples were stored in sealed plastic bags at −18 °C for further use. Chemical compositions were determined based on the standard Laboratory Analytical Procedures from National Renewable Energy Laboratory (Sluiter et al., 2008).

2.2. Enzymes

Celluclast 1.5 L and Novozyme 188 (Novo Nordisk A/S, Bagsværd, Denmark) were used as cellulase preparations. Pentopan Mono BG (Novo Nordisk A/S, Bagsværd, Denmark) was used as xylanase preparations. Celluclast 1.5 L had an activity of 74.7 filter paper units (FPU)/mL measured according to International Union of Pure and Applied Chemistry 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).

2.3. Aqueous ammonia pretreatment

About 20 g of bamboo power and 200 mL 26% ammonia (solid to liquid ratio of 1:10) were mixed in a vial and capped. These vessels were placed in water bath at 70 °C for 72 h. After incubating, the supernatant and solid were separated by centrifugation (5000*g* for 10 min), and the solid was washed and centrifuged repeatedly using distilled water until the pH of supernatant was neutral. Then, the obtained solids was air-dried and stored for structure analysis and enzymatic hydrolysis.

2.4. Non-structural carbohydrate

Non-pretreated 4- and 6-meter-height bamboo shoot fractions were incubated in tubes with a working volume of 2 mL in 50 mM sodium citrate buffer (pH 5.0) at 50 °C. The hydrolysis was conducted in a shaking incubator with a shaking speed of 200 rpm. The dry matter (DM) consistency of substrate was 2%. A solution of 0.02% NaN_3 was added to the hydrolysis broth to prevent bacterial infection. Samples were withdrawn at 48 h and boiled for 10 min. After cooling, the samples were centrifuged at 10,000*g* for 10 min and the supernatants were analyzed for glucose and xylose. All experiments were performed in duplicate and the average values are shown.

2.5. Enzymatic hydrolysis

Enzymatic hydrolysis of the non-pretreated and aqueous ammonia pretreated bamboo fractions was performed in the same system as the release of non-structural carbohydrate in non-pretreated bamboo shoots (Selig and Weiss, 2008). The cellulase preparations contained both Celluclast 1.5 L and Novozyme 188 preparations, which were dosed at 20 FPU/g DM and 500 nkat/g DM, respectively. The dosage of XYL was 2 mg protein/g DM.

2.6. X-ray diffractometry

The crystalline structure of non-pretreated and pretreated 4-meter-height bamboo shoot and 2-year-old mature bamboo fractions were analyzed by means of an X-ray diffractometer (Rigaku D/max 3C generator, Rigaku Corporation, Japan) with Cu Kα radiation generated at 35 kV and 35 mA. The Cu radiation was of Kα (λ = 1.5405 Å) and the scattering angle (2θ) ranged from 5° to 50° with a scan step of 0.02°. The background intensity without bamboo powder was subtracted from the sample intensity. The crystalline index (CrI) was determined from the XRD data according to the XRD spectra's peak height (Segal et al., 1959) using the following formula:

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