Bioresource Technology 192 (2015) 471–477

Contents lists available at ScienceDirect

Bioresource Technology

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

Facilitating the enzymatic saccharification of pulped bamboo residues by degrading the remained xylan and lignin–carbohydrates complexes

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HIGHLIGHTS

- Effect of kraft pulping on bamboo saccharification was evaluated.
- Hemicellulase could degrade the linkage of LCCs.
- Xylan and LCCs degradation improved enzymatic saccharification.

ARTICLE INFO

Article history:
Received 27 April 2015
Received in revised form 1 June 2015
Accepted 2 June 2015
Available online 9 June 2015

Keywords:
Moso bamboo residues
Kraft pulping
Lignin carbohydrate complexes
Hemicellulase
Enzymatic saccharification

ABSTRACT

Kraft pulping was performed on bamboo residues and its impact on the chemical compositions and the enzymatic digestibility of the samples were investigated. To improve the digestibility of sample by degrading the xylan and lignin–carbohydrates complexes (LCCs), xylanase and α-L-arabinofuranosidase (AF) were supplemented with cellulase. The results showed more carbohydrates were remained in the samples pulped with low effective alkali (EA) charge, compared to conventional kraft pulping. When 120 IU/g xylanase and 15 IU/g AF were supplemented with 20 FPU/g cellulase, the xylan degradation yield of the sample pulped with 12% EA charge increased from 68.20% to 88.35%, resulting in an increased enzymatic saccharification efficiency from 58.98% to 83.23%. The amount of LCCs in this sample decreased from 8.63/100C_9 to 2.99/100C_9 after saccharification with these enzymes. The results indicated that degrading the remained xylan and LCCs in the pulp could improve its enzymatic digestibility.

1. Introduction

Moso bamboo (*Phyllostachys pubesescens*) is a kind of polysaccharides-rich agriculture products, with the crops covering an area of 3.19 million hectares in China (Huang et al., 2015). In the course of utilization of moso bamboo stem, some fractions are not effectively utilized due to the weakness in mechanical properties. Only 30% of these residues are burned to recover energy or made into activated carbon; the remainder are discarded resulting in environment pollution (Fu, 2001; Mui et al., 2008). Therefore, efficient utilization of bamboo residues is an ideal choice for bio-based chemical production and reducing the risk of pollution.

Technically, bio-based chemical productions are based on the sugar platform, which can be created by hydrolysis of the polysaccharide fraction (Barr et al., 2012). While, the complex structure of lignocellulosic biomass makes lignocellulose recalcitrant to deconstruction and creates a barrier to convert the polysaccharides to monomeric sugars (Chen et al., 2011; Barr et al., 2012). Hence, pretreatments should be carried out to disrupt of the complex structure of biomass by removing lignin or hemicellulose (Chen et al., 2011; Gu et al., 2012). Our previous study (Huang et al., 2015) and Li et al. (2014b) both suggested that acid-based pretreatments are not effective for improving bamboo enzymatic saccharification efficiency, except for kraft pulping and other alkali-based pretreatments. Kraft pulping with low EA charge is a mild chemical pretreatment, most of the original carbohydrates (glucan and xylan) are remained in the pretreated sample. Meanwhile, the degree of delignification can reach up to 50%, which is beneficial for glucan and xylan degradation by cellulase and xylanase. Hence, it is speculated that kraft pulping with low EA charge can be an effective pretreatment for moso bamboo enzymatic saccharification and available sugars released.

During the pulping process, a certain amount of original lignin are remained in the treated samples, linking with carbohydrates by benzyl ether, phenyl glycoside and γ-ester (Martin et al., 2004).To quantificationally characterize the linkages and moieties in the lignin–carbohydrates complexes (LCCs), Balakshin et al.

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http://dx.doi.org/10.1016/j.biortech.2015.06.008
0960-8524/

(2011) proposed an approach using a combination of quantitative ^{13}C NMR and two-dimensional heteronuclear single quantum coherence NMR (2D HSQC NMR). For the biorefinery, the LCCs in biomass has been shown to inhibit the availability of the carbohydrates for digestion (Jeffries, 1990; Min et al., 2014). Jeffries (1990) investigation showed the steric hindrance of multiple cross-linkages of LCCs can limit the action of cellulase to attack the cellulose. Besides, hemicellulose has also been thought to be an obstacle for the enzymatic digestibility. Since hemicelluloses usually coat the cellulose microfibril in the plant cell wall and form a physical barrier for efficient cellulose degradation (Hu et al., 2011; Zhang et al., 2013). Meanwhile, the lignin in the LCCs usually links to the C-5 hydroxyl position of arabinofuranoside in arabinoxylan through ester-ether bridges of ferulic acid (Chesson et al., 1983). Hence, hemicellulase, such as xylanase and α-L-arabinofuranosidase (AF), have been applied to degrade xylan and disrupt the chemical bonds between lignin and carbohydrates, facilitating the enzymatic saccharification of pretreated biomass (Kumar and Wyman, 2009; Hu et al., 2011). Xylanase can increase the cellulase accessibility to cellulose by degrading the xylan coating around cellulose fibers (Kumar and Wyman, 2009; Zhang et al., 2013), and the ester-linked LCCs structures as well as γ-acetylated lignin moieties in the biomass can be degraded by the esterase activity in commercial xylanase preparation (Balakshin et al., 2014). AF can remove arabinan from the xylan backbone and help to disrupt LCCs structure, favouring xylan degradation by xylanase (Sun et al., 2005).

In present study, moso bamboo residues was treated by kraft pulping with different EA charge, and the recovery yields of original carbohydrates, degree of delignification and enzymatic digestibility of the pretreated samples were investigated. The LCCs in the untreated bamboo and pulped bamboo were isolated according to Björkman (1954) and quantified according to Balakshin et al. (2011). To improve the enzymatic saccharification efficiency of this sample by degrading the remained xylan and LCCs, an enzyme cocktail composed with xylanase X2753 and α-L-arabinofuranosidase E-AFASE was associated with cellulase C2730 during enzymatic hydrolysis process.

2. Methods

2.1. Materials

The bamboo residues used in this study were from the stems of 3-year-old or older moso bamboo (*Phyllostachys heterocycla*) provided by the Shaowu Bamboo Processing Factory in Fujian, China. Air-dried moso bamboo residues without classification were collected and stored in sealed plastic bags at room temperature. The bamboo residues consist of 38.22% glucan, 19.26% xylan, 6.15% arabinan and 30.62% total lignin. The commercial cellulase (No. C2730) and xylanase (No. X2753) were purchased from Sigma–Aldrich Inc. (USA), and the α-L-arabinofuranosidase (No. E-AFASE) was purchased from Megazyme (Ireland).

2.2. Kraft pulping

Kraft pulping liquors were prepared with Na_2S and NaOH. The effective alkali (EA) charge (as Na_2O on dry material) was varied from 2% to 28%. Sulfidity, Na_2S/(Na_2S and NaOH) (on Na_2O basis) was 20%. The pretreatments were carried out in 1-L autoclave bombs that were heated with an oil bath. The liquid: solids ratio was 6:1. Dry bamboo residues (100 g) were first impregnated with the liquor at 60 °C for 30 min. After impregnation, the temperature was raised at a rate of 2 °C/min to 150 °C and maintained 60 min. The pretreated bamboo residues were washed with distilled water at a solids: liquid ratio of 1:10 to remove the spent chemicals. The resulting pulps were stored at 4 °C for subsequent experiments.

2.3. Enzymatic hydrolysis

Enzymatic saccharification of pulped bamboo residues with different EA charge were conducted at a substrate loading of 5% (w/v) with a cellulase loading of 20 FPU/g glucan. An enzymatic cocktail composed with xylanase (30, 60, 90, 120 and 150 IU/g glucan) and AF (5, 10, 15, 20 and 25 IU/g glucan) were supplemented with the cellulase (20 FPU/g glucan). All of the enzymatic saccharification experiments (30 mL) were performed in a 150-mL Erlenmeyer flask at 50 °C using 50 mM citrate buffer (pH 4.8), which were shaken at 150 rpm for 48 h. Aliquots of the enzymatic saccharification were withdrawn and centrifuged for 10 min at 4000 rpm; the supernatants were subsequently filtered through a 0.22-μm syringe filter and analyzed to determine the sugar content.

2.4. Isolation and purification of LCC

The isolation and purification of LCCs in untreated and pulped bamboo residues were accorded to the classical method proposed by Björkman (1954). A planetary ball milling (Pulverisette 7, Fritsch, Germany) was used to mill the samples before isolating the LCCs. The bamboo particles (4 g) was subjected to 5 h milling at 600 rpm using a 100 mL ZrO_2 bowls with 25 ZrO_2 balls. The ball-milled bamboo meals were extracted with 96% dioxane for three days. The extracted residues were extracted with acetic acid (50% v/v) and the solvent was then evaporated under vacuum at 35 °C. To remove traces of acetic acid, a few drops of H_2O were added to the solid matter and evaporated again. This procedure was repeated three times. Finally, the solid matter was dried in a vacuum oven at 35 °C to obtain un-purification LCCs preparations.

To purify the LCCs, the solid matter was dissolved in DMSO (20 ml/g LCCs) and supernate was dropped into dichloroethane-ethanol (2:1) mixture (10 ml/ml DMSO). The precipitated was washed by ether, freeze-dried and dissolved in 50% acetic acid (10 ml/g LCCs). The solution was precipitated drop-wise into acetone (10 ml/ml acetic acid), and the precipitate was filtered and washed with ether and petroleum ether to obtain purified LCC-Björkman preparation.

2.5. Analysis chemical constituent and enzymatic saccharification

The constituents of the bamboo residues and pretreated samples were determined based on the procedure developed by the National Renewable Energy Laboratory for analyzing biomass materials. The sugars of the constituent analysis and enzymatic saccharification were measured using a high-performance liquid chromatography (HPLC) system equipped with an Aminex HPX-87H column (300 × 7.8 mm) and a refractive index (RI) detector, and 5 mM H_2SO_4 solution was used as the eluent at a flow rate of 0.6 mL/min. The analysis of sugar composition in the present study was run in duplicate, and average values were calculated. The recovery yield of original carbohydrates, degree of delignification, and enzymatic hydrolysis efficiency were calculated as follows:

$$\text{Recovery yield}\,(\%) = \frac{\text{glucan or xylan in pretreated bamboo residue}\,(g)}{\text{glucan or xylan in the raw bamboo residue}\,(g)} \times 100\%$$

$$\text{Delignification}\,(\%) = 1 - \frac{\text{lignin in pretreated bamboo residue}\,(g)}{\text{lignin in the raw bamboo residue}\,(g)} \times 100\%$$