Biorefining of Bamboo Processing Residues

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Abstract

Bamboo is known as China's second forest laudatory. Bamboo plants grow quickly, have the ability to propagate and are easy to update. Bamboo-cutting would result in a lot of leaves and sawdust residues. Comprehensive utilization of bamboo processing residues would improve resource utilization and economic benefits of bamboo. Due to the bamboo's nature and recalcitrant for fractionation, the bamboo processing residues were firstly pretreated by NaOH to remove hemicellulose fraction, which would be used for production of xylooligosaccharides. The left residues were enzymatically hydrolyzed to monosaccharides and further fermented to L-lactic acid by Rhizopus oryzae. In this article, hemicellulose fraction was extracted by NaOH varying concentrations from 1% (w/v) to 10%(w/v) at 80°C,100°C,120°C, 7% (w/v) NaOH at 100°C was proved to be suitable for extraction of hemicellulosic fraction from bamboo processing residues and a higher xylan yield of 57.81% was achieved. The alkali-treated residues were rich in cellulose, which could be hydrolyzed to glucose by cellulase. The enzymatic hydrolysis yield at 7% (w/v) NaOH was 62.15%. Furthermore, glucose would be fermented to produce optical pure L-lactic acid by Rhizopus oryzae. The optimal lactic acid production condition was determined by the cellulose hydrolysis yield. Biorefining of bamboo processing residues is a promising alternative for the comprehensive utilization of bamboo processing residues.

Key words: Bamboo processing residues; Pretreatment; NaOH; xylooligosaccharides; L-lactic acid
1 Introduction

Bamboo is known as China's second forest laudatory. Bamboo plants grow quickly, have the ability to propagate and are easy to update. Bamboo-cutting would result in a lot of leaves and sawdust residues. Comprehensive utilization of bamboo processing residues would improve resource utilization and economic benefits of bamboo.

The chemical compositions of bamboo are similar to wood, including of cellulose, hemicellulose, lignin and a small amount of extract and ash. Physical, chemical or physico-chemical pretreatment is used to break down the hemicellulose and lignin structure in order to improve the enzymatic hydrolysis rate (Zhang et al. 2007). The pretreatment approaches: steam explosion, dilute acid, and lime were discussed for enzymatic hydrolysis (Zhang et al. 2011). Binod et al. reported that sodium hydroxide pretreatment of cotton stalk was effective for deriving fermentable sugars (Binod et al. 2012). Lin Y.S. studied that production of xylooligosaccharides could use immobilized endo-xylanase of Bacillus halodurans (Lin Y.S. et al. 2011). The enzymatic production of xylooligosaccharides from alkali solubilized xylan of Sehima nervosum was economically feasible (Samanta et al. 2012). Production of xylooligosaccharides from the steam explosion liquor of corncobs coupled with enzymatic hydrolysis using a thermostable xylanase has been reported (Teng et al. 2010).

Raw materials, starchy and cellulosic materials are currently receiving a great deal of attention to produce L-lactic acid, because they are cheap, abundant, and renewable (Hofvendahl et al. 2000). The utilization of waste office paper, fishmeal water has been reported as well (Park et al. 2004, Huang et al. 2007). In this paper, the bamboo processing residues were firstly pretreated by NaOH to remove hemicellulosic fraction, which would be used for production of xylooligosaccharides. The left residues were enzymatically hydrolyzed to monosaccharides and further fermented to L-lactic acid by Rhizopus oryzae (R. oryzae). Biorefining of bamboo processing residues is a promising alternative for the comprehensive utilization of bamboo processing residues.

2 Materials and Methods

2.1 Pretreatment of bamboo processing residues

Bamboo processing residues were collected from Fujian province, which were the residues of bamboo processing. The bamboo processing residues were firstly pretreated by NaOH to remove hemicellulosic fraction at 80 °C, 100 °C, 120 °C. The NaOH concentrations varied from 1 % to 10 %.
2.2 Enzymatic hydrolysis
The alkali-treated filtrate was hydrolysised by xylanase after ultrafiltration to obtain xylooligosaccharides. The alkali-treated residues was initiated by the addition of cellulose with 25 FPIU/g cellulose and cellobiase with 4 IU/g cellulose. Hydrolysis was carried out in a 250 ml Erlenmeyer flask, and cellulase and cellobiase were purchased from Sigma-Aldrich and used in the hydrolysis experiments. The reaction was performed at 50 °C for 48 h with pH adjusted to 4.5-5.0 by addition of 72 % (w/w) sulfuric acid. All the flask level experiments were carried out in duplicate and presented as an average.

2.3 Strain and spore culture medium
The microorganism was R. oryzae NLX-M-11 (Nanjing Forestry University). The fungus was first grown on potato-dextrose agar slants at 30 °C for 3-5 d. A spore solution of 10^7 spores/ml was precultured into 250 ml Erlenmeyer flasks containing 50 ml of the culture broth in flask cultivation with CaCO₃. The composition (g/l) of medium used in the preculture (g/l) consisted of 50 glucose, 3 (NH₄)₂SO₄, 0.75 MgSO₄·7H₂O, 0.2 ZnSO₄·7H₂O, and 0.30 KH₂PO₄. Preculture flasks were incubated at 30 °C with 170 r/min shake speed for 12 h.

2.4 Fermentation for lactic acid production
For the production of lactic acid, preculture was inoculated into 250 ml Erlenmeyer flasks containing 100 ml of fresh medium. The medium (g/l) consisted of enzymatic hydrolysate from bamboo processing residues, 2 (NH₄)₂SO₄, 0.75 MgSO₄·7H₂O, 0.2 ZnSO₄·7H₂O, and 0.3 KH₂PO₄. The culture temperature was maintained at 35 °C throughout the experiments. After the culture time of 12 h, 30 g/l CaCO₃ powder was added to avoid a decrease of pH. Culture was carried out 2-3d containing the bamboo processing residues hydrolysate. Lactic acid yield expressed as g lactic acid produced/g sugars consumed. Each experiment was done in duplicate and presented as an average.

2.5 Analytical methods
After sampling, the samples were capped tightly in the test tube and immersed in boiling water for 3 min to deactivate the hydrolytic enzyme, then the slurry was centrifuged and the supernatant kept for sugar assay. To determine the amounts of glucose, cellobiose and lactic acid produced, liquid fractions were analyzed by high performance liquid chromatography (HPLC) using an Aminex BioRad HPX-87H column, a mobile phase of H₂SO₄ (5 mmol/l) at a flow rate of 0.6 ml/min, and a column temperature of 55 °C.

3 Results and Discussion

3.1 Xylan extraction
Hemicellulose fraction was extracted by NaOH varying concentrations from 1 % (w/v) to 10 % (w/v) at 80 °C, 100 °C and 120 °C, 100 °C, 30 % (w/v) NaOH at 100 °C was proved to be suitable for extraction of hemicellulosic fraction from bamboo processing residues. The results of glucan yield of alkali-treated residues and xylan concentration of alkali-treated filtrate at different temperatures were shown in figure 1,2,3,4.
Fig. 1 The effect of different NaOH concentrations on glucan yield of bamboo processing residues at 80 °C.

Fig. 2 The effect of different NaOH concentrations on glucan yield of bamboo processing residues 100 °C.
Fig. 3 The effect of different NaOH concentrations on glucan yield of bamboo processing residues 120 °C

As shown in Figures 1, 2, 3, with the increase of NaOH concentration, the glucan yield declined. The temperature has little effect on glucan yield. According to Figure 4, xylan concentration in alkali-treated filtrate was the highest at 100 °C. About 10% of material was removed as black liquor, a major portion of which include hemicellulosic sugars (Binod et al. 2012). The water-soluble materials contained various monosaccharides and oligosaccharides (Asada et al. 2005). The xylan concentration increased along with the increase of NaOH concentration from 1% to 7%, not much have changed from 7% to 10%.

3.2 Production of Xylooligosaccharides
The bamboo processing residues were pretreated by NaOH to remove hemicellulosic fraction, which would be used for production of xylooligosaccharides.

Fig. 5 Ion Chromatography analysis of the hydrolysate of alkali-treated filtrate.
Different degree of polymerization of the xylooligosaccharides could be detected by Ion Chromatography analysis.

### 3.3 Enzymatic hydrolysis

Enzymatic hydrolysis studies were carried out to investigate the effect of lignin and hemicellulose removal on hydrolysis yield (Öhgren et al. 2007). The alkali-treated residues were rich in cellulose, which could be hydrolyzed to glucose by cellulase. To investigate the influences of different NaOH concentrations on enzymatic hydrolysis, the results were as follows in figure 6.

![Enzymatic hydrolysis yield of alkali-treated residues with different NaOH concentrations.](image)

The enzymatic hydrolysis yield were increasing sharply from 1% to 4% of NaOH concentrations, then the enzymatic hydrolysis yield were steady. The enzymatic hydrolysis yield at 7% NaOH was 62.15%, which could be promoted by different measures.

### 3.4 Production of L-lactic acid

Glucose would be fermented to produce optical pure L-lactic acid by *R. oryzae*. The optimal lactic acid production condition was determined by the cellulose hydrolysis yield.

![HPLC analysis of the fermentation broth of alkali-treated residues hydrolysate.](image)
L-lactic acid was detected by HPLC analysis. Lactic acid is currently considered as the most potential feedstock monomer for chemical conversions (Gao et al. 2011). Interest in L-lactic acid production has increased recently also due to its ability to serve as raw material for the manufacture of green solvent, such poly-L-lactic acid (PLLA), which are biodegradable and environmental friendly (Yu et al, 2007).

4 Conclusion

In the present study, the hemicellulose and cellulose of bamboo processing residues were utilized efficiently. Xylooligosaccharides and L-lactic acid were obtained from the two fractions respectively. In this artical, 7% (w/v) NaOH at 100°C was proved to be suitable for extraction of hemicellulosic fraction from bamboo processing residues and a higher xylan yield of 57.81% was achieved. The alkali-treated residues were rich in cellulose, which could be hydrolyzed to glucose by cellulase. Furthermore, glucose would be fermented to produce optical pure L-lactic acid by R. oryzae. Comprehensive utilization of bamboo processing residues would improve resource utilization and economic benefits of bamboo.

References


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