Bioresource Technology 100 (2009) 6651–6654

Contents lists available at ScienceDirect

Bioresource Technology

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

Short Communication

Effects of growth stage on enzymatic saccharification and simultaneous saccharification and fermentation of bamboo shoots for bioethanol production

Tomoko Shimokawa [a,*], Mutumi Ishida [b], Shigeki Yoshida [b], Masanobu Nojiri [a]

[a] Department of Applied Microbiology, Forestry and Forest Products Research Institute, 1 Matsunosato, Tsukuba, Ibaraki 305-8687, Japan
[b] Graduate School of Life and Environmental Sciences, University of Tsukuba, Tennodai 1-1-1, Tsukuba, Ibaraki 305-8572, Japan

ARTICLE INFO

Article history:
Received 26 March 2009
Received in revised form 26 June 2009
Accepted 27 June 2009
Available online 6 August 2009

Keywords:
Bamboo
Bioethanol
Enzymatic saccharification
Simultaneous saccharification and fermentation

ABSTRACT

Bamboo is a fast-growing renewable biomass that is widely distributed in Asia. Although bamboo is recognised as a useful resource, its utilization is limited and further development is required. Immature bamboo shoots harvested before branch spread were found to be a good biomass resource to achieve a high saccharification yield. The saccharification yield of the shoots increased (up to 98% for immature *Phyllostachys bambusoides*) when xylanase was used in addition to cellulase. Simultaneous saccharification and fermentation (SSF) processing converted immature shoots of *P. bambusoides* and *Phyllostachys pubescens* to ethanol with an ethanol yield of 169 and 139 g kg^{-1}, respectively (98% and 81%, respectively, of the theoretical yields based on hexose conversion) when 12 FPU g^{-1} enzyme and the yeast *Saccharomyces cerevisiae* were used.

1. Introduction

Bamboo plants grow rapidly, reaching their maximum size within a few months. In Japan, the above-ground biomass of *Phyllostachys pubescens* has been estimated to be 116.5 t DM (dry matter) ha^{-1} for culms and the gross production was reported to be 52.3 t DM ha^{-1} yr^{-1} (Isagi et al., 1997). In 2007, the area of bamboo fields (mainly *Phyllostachys bambusoides* and *P. pubescens*) was 36,294 ha (National Statistic Center, 2009). Although bamboo is recognised as a useful resource, its utilization is limited and further development is required. There have been reports on improved pre-treatment processes that increase the enzymatic saccharification rates of bamboo for bioethanol production (Demenezes et al., 1983; Ram and Seenayya, 1991). Mature bamboo requires physical, chemical or biological pre-treatments to destroy the cell wall structure of lignocelluloses (Asada et al., 2005; Zhang et al., 2007).

In general, the lignin content of biomass material is strongly related to its enzymatic saccharification yield (Kumar and Wyman, 2009; Ohgren et al., 2007; Chang and Holtzapple, 2000). Since the lignin content of immature bamboo shoots is lower than that of mature bamboo (Fujii et al., 1993), bamboo materials that are more easily hydrolyzed by enzymes could be obtained by optimizing the harvest time. In this study, immature bamboo shoots were converted to ethanol with good ethanol yields.

2. Methods

2.1. Raw material

Mature culms and immature shoots of *P. bambusoides* were harvested from the arboretum of the Forestry and Forest Products Research Institute (Tsukuba, Ibaraki, Japan) from June to July 2007. The culms of mature (at least 1 year old) *P. bambusoides* were cut into sections 100 cm in length from the lower part. Immature shoots of *P. bambusoides* were also cut into sections after removing the sheath; the sections were then cut into small pieces. Immature shoots of *P. pubescens* were harvested from the same arboretum from April to May 2007 and cut into sections. The middle 30 cm of each 100 cm section of *P. pubescens* was removed and cut into pieces for analysis. The pieces were dried at 45 °C to moisture content <10%, then pulverized with a rotor mill equipped with a 0.5 mm sieve ring (Pulverisette 14; Fritsch, Germany). Three shoots of *P. bambusoides* and two shoots of *P. pubescens* before branch spread were harvested for SSF experiments. For those experiments, the whole shoots were cut into pieces and milled as described above.

The cellulase preparation, Meicellase (Lot No. CEPB-5081, Meiji Seika, Japan) from *Trichoderma viride*, was provided by Meiji Seika

* Corresponding author. Tel.: +81 29 829 8281; fax: +81 29 873 3797.
E-mail address: tshimo@ffpri.affrc.go.jp (T. Shimokawa).

0960-8524/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biortech.2009.06.100

Co., Ltd. The xylanase preparation, Cellulosin TP 25 from *T. viride*, was supplied by HBI Co., Ltd.

2.2. Analytical methods

2.2.1. Analyses of bamboo components

Acid-insoluble lignin content was determined according to Effland (Effland, 1977). The ash estimate was subtracted from the acid-insoluble measurement. The amount of total sugars in the hydrolysate was measured using the Somogyi–Nelson method (Somogyi, 1952) after neutralization with diluted NaOH. A mixed sugar solution with equal concentrations of glucose and xylose was used as standard. To determine the relative sugar composition, the corresponding neutral monosaccharides in the acid hydrolysates were analyzed as described previously (Shimokawa et al., 2008).

2.2.2. Enzymes and enzyme assays

The enzyme preparations used were Meicellase (Meiji Seika Co., Ltd., *T. viride*, 332 FPU g^{-1}; β-glucosidase activity, 1050 U g^{-1}) and Cellulosin TP 25 (HBI Co., Ltd., *T. viride*, 157 FPU g^{-1}; β-glucosidase activity, 344 U g^{-1}; xylanase activity, 17,200 U g^{-1}). Cellulase activity was measured as FPU using IUPAC guidelines (Ghose, 1987). β-Glucosidase and xylanase activities were determined as described previously (Shimokawa et al., 2007).

2.2.3. Determination of ethanol concentration

Ethanol concentration was determined by HPLC using two SUGAR KS-802 columns (300 × 7.5 mm, Shodex) connected in series and equilibrated with deionized water at 80 °C at 1 ml min^{-1} flow rate. The elution patterns were monitored using a differential refractometer.

2.3. Enzymatic hydrolysis

The reaction mixture consisted of 20 mg milled sample and enzyme solutions (2–83 FPU g^{-1}) in 1 ml 50 mM sodium citrate buffer, pH 4.8. The mixture was incubated at 40 °C with continuous shaking, and the reaction was stopped by heating the mixture at 100 °C for 5 min. The amounts of liberated reducing sugars were measured using the Somogyi–Nelson method with mixed glucose and xylose solution (1:1) as standard. The saccharification yield was calculated from the weight of polysaccharides in the substrate. Results are expressed as the means of two independent experiments.

2.4. Simultaneous saccharification and fermentation

The yeast (*Saccharomyces cerevisiae* NBRC 2347) was pre-cultured for 24 h in 100 ml YM broth (Difco) in a 300 ml flask at 30 °C on an orbital shaker at 200 rpm. The culture broth was centrifuged and the yeast pellet was washed with deionized autoclaved water. The SSF experiments were conducted in 50 ml flasks with a working volume of 25 ml. The mixture contained 5% milled bamboo, 25 mM Na-citrate buffer (pH 4.8), enzyme preparation (Meicellase:Cellulosin TP 25 = 1:1, 2–12 FPU g^{-1}), and 0.25 g (wet weight) of the washed yeast pellet. The reaction mixture was stirred at 30 °C. Milled bamboo and buffer solution in the flasks were autoclaved at 121 °C for 20 min before addition of enzyme and yeast. All experiments were carried out in triplicate.

3. Results

3.1. Characteristics of mature and immature P. bambusoides

P. bambusoides reaches the branch spread stage approximately 60 days after budding from the ground, and each plant grows to a height of 7–10 m at the arboretum. The acid-insoluble lignin content of immature shoot was only 4.7%, while that of mature culm was 25.2% of DM. The polysaccharide contents of immature shoot and mature culm were 50.9% and 61.9%, respectively. Hydrolysis of the immature shoot with Meicellase (83 FPU g^{-1}) gave a saccharification yield of 69%, while that of the mature culm was less than 15%. The immature sample was much more readily hydrolyzed by the enzyme than was the mature sample. Since glucose and xylose residues were detected as the major sugar ingredients in the acid hydrolysate from both mature and immature *P. bambusoides*, we added a xylanase preparation (Cellulosin TP 25) in addition to cellulase to improve the saccharification yield. The highest saccharification yield (up to 98%) was obtained when immature shoots were hydrolyzed with Meicellase and Cellulosin TP 25 (61 FPU g^{-1}).

3.2. Effects of growth stage on enzymatic hydrolysis

Shoots of *P. bambusoides* were harvested at various growth stages, and their lignin contents analyzed to clarify the relationship between growth stage and saccharification yield. The data (Table 1) shows that the lignin contents of each shoot before branch spread were higher at the bottom portions and lower at the top portions. Lignin became more evenly distributed along the length of the culm after branch spread (when plants reached approximately 7 m height). Fig. 1 shows the saccharification yields of sections taken from shoots of *P. bambusoides* from 1 to 7 m. Saccharification yields were higher in the upper sections, and lower in the lower sections of the shoot. Almost all of the polysaccharides in the upper sections of the shoots were hydrolyzed by enzyme preparations containing both Meicellase and Cellulosin TP 25 (61 FPU g^{-1}). After branch spread, the enzymatic saccharification yields of the sections of *P. bambusoides* were low, and did

Table 1
Acid-insoluble lignin contents of *P. bambusoides* cut into sections.

Height from ground (m)	Acid-insoluble lignin (%)					
	0–1	1–2	2–3	3–4	4–5	5–6
1 m (86.8 cm)	2.9					
2 m (218.7 cm)	4.0	2.4				
3 m (298.3 cm)	9.0	5.9	4.7			
4 m (415.8 cm)	13.4	6.4	3.7	5.2		
5 m (554.8 cm)	13.3	10.8	9.3	6.7	4.9	
7 m[a] (696.5 cm)	17.2	16.3	16.3	15.5	15.0	15.9

[a] The culm after branch spread. Results are expressed as the means of two independent experiments.

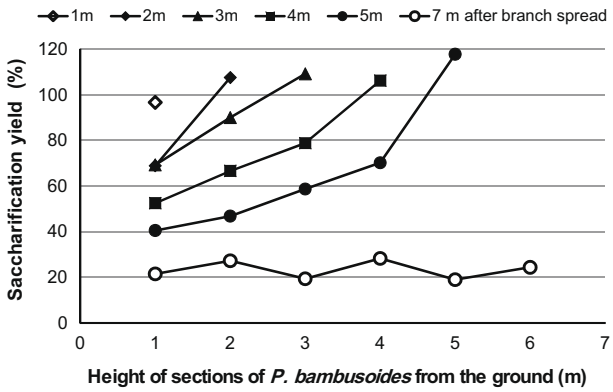

Fig. 1. Relationships between saccharification yields and heights of sections from bottom to top of *P. bambusoides*. Each section was hydrolyzed by enzyme preparations containing both Meicellase and Cellulosin TP 25 (61 FPU g^{-1}).