Bioresource Technology 102 (2011) 10548–10552

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

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

Evaluation of *Saccharomyces cerevisiae* Y5 for ethanol production from enzymatic hydrolysate of non-detoxified steam-exploded corn stover

Yun Li [1], Kai Gao [1], Shen Tian [1], Sijin Zhang, Xiushan Yang *

College of Life Science, Capital Normal University, 100048 Beijing, China

ARTICLE INFO

Article history:
Received 3 June 2011
Received in revised form 9 August 2011
Accepted 10 August 2011
Available online 19 August 2011

Keywords:
Saccharomyces cerevisiae Y5
Enzymatic hydrolysate
Non-detoxified steam-exploded corn stover
Nitrogen source
Ethanol production

ABSTRACT

Saccharomyces cerevisiae Y5 was used to produce ethanol from enzymatic hydrolysate of non-detoxified steam-exploded corn stover, with and without a nitrogen source, and decreasing inoculum size. The results indicated that the ethanol concentration of 44.55 g/L, corresponding to 94.5% of the theoretical yield was obtained after 24 h, with an inoculum size of 10% (v/v) and nitrogen source (corn steep liquor, CSL) of 40 mL/L. With the same inoculum size, and without CSL, the ethanol concentration was 43.21 g/L, corresponding to 91.7% of the theoretical value after 60 h. With a decreased inoculum size of 5% (v/v), and without CSL, the ethanol concentration was 40.00 g/L, corresponding to 85.8% of the theoretical value after 72 h. The strain offers the potential to improve the economy of cellulosic ethanol production by simplifying the production process and reducing the costs associated with the process such as water, capital equipment and nutrient supplementation.

1. Introduction

In the face of sharply rising oil prices, and environmental problems, such as the greenhouse effect caused by the burning of fossil fuels, it is extremely urgent that a new alternative energy source is identified (Balat, 2011). Because of the economic, environmental and social contributions of cellulosic ethanol to sustainable development, it is widely considered a promising alternative to oil (García-Aparicio et al., 2011; Hao et al., 2010). However, methods of producing fermentable sugar from lignocelluloses and converting the sugars into ethanol at low-cost are core challenges currently plaguing the commercial production of cellulosic ethanol (Ojeda et al., 2011).

Before lignocellulose is enzymatically hydrolyzed to produce fermentable sugar, it must be pretreated to break the tight structures of cellulose, hemicellulose and lignin. Steam explosion pretreatment is an efficient, low-cost pretreatment technology developed in recent years, which can break the protective effect of lignin and hemicellulose on cellulose, thus achieving effective separation of lignocellulose components; this method offers potential for lower capital investment, significantly lower environmental impact, less hazardous chemicals and conditions and complete sugar recovery (Alvira et al., 2010; Balat, 2011; Chen et al., 2008; Tomás-Pejó et al., 2008; Cara et al., 2008). Additionally, steam explosion has become prevalent in China for the pretreatment of corn stover in recent years. However, the materials obtained after steam explosion pretreatment contain fermentation inhibitors such as weak acids (mainly acetic acid), furfural, 5-HMF and phenols, which strongly inhibit yeast fermentation (Zhu et al., 2006). Prior to enzymatic hydrolysis and fermentation, therefore, the materials obtained after steam explosion must be detoxified using physical such as washing, chemical and biological methods. Nevertheless, detoxification causes excess sugar loss and washing water consumption, and increases in equipment cost and process complexity. Yu et al. (2011) conducted biological detoxification enzymatic hydrolysate from non-detoxified steam-exploded corn stover using *Aspergillus nidulans* (FLZ10), while simultaneous saccharification and fermentation was carried out using *Saccharomyces cerevisiae*; the ethanol concentration reached 34 g/L, but the biological detoxification also increased equipment investment and cost. Therefore, in terms of simplifying the production process, reducing equipment investment and process water consumption, and decreasing production cost, it is of great significance to develop a strain that can tolerate inhibitors such as high-concentrations of acetic acid and furfural. Development of a strain such as this will allow researchers to efficiently carry out ethanol fermentation on the enzymatic hydrolysate of non-detoxified steam-exploded corn stover.

Separate saccharification and fermentation have performed without detoxification on ammonia fiber expansion (AFEX)-pretreated corn stover using recombinant *S. cerevisiae* 424A (LNH-ST) to produce ethanol; the results demonstrated that after pretreatment of corn stover by AFEX, the original nutrients in the corn stover were sufficient for the growth of microorganisms

* Corresponding author. Tel.: +86 10 68902330; fax: +86 10 68982291.
E-mail address: cnu_xsyang@263.net (X. Yang).
[1] These authors contributed equally to this work.

0960-8524/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biortech.2011.08.039

(Lau and Dale, 2009). The temperature of the steam explosion of corn stover is much higher than that of AFEX, and concentrations of the resulting toxic compounds are higher, if microbial nutrients are not added to the enzymatic hydrolysate of steam-exploded corn stover, the production cost of cellulosic ethanol can be further reduced.

Previous studies have reported that strain *S. cerevisiae* Y5 could metabolize/tolerate furfural, hydroxymethylfurfural and other fermentation inhibitors (Tian et al., 2011). It has been reported that the strain of *S. cerevisiae* Y5 could produce ethanol from SPORL (sulfite pretreatment to overcome recalcitrance of lignocellulose) pretreated-lodgepole pine with excellent ethanol productivities of 2.0 and 0.8 g/L/h, averaged over a period of 4 and 24 h, respectively, with furfural metabolism in the non-detoxified run (Tian et al., 2010).

The present study investigated whether the strain *S. cerevisiae* Y5 could ferment the enzymatic hydrolysate of non-detoxified steam-exploded corn stover to produce ethanol, whether it could ferment the enzymatic hydrolysate with a decreased inoculum size and in the absence of nutrients, and whether the final benchmark concentration for commercial cellulosic ethanol production could achieved.

2. Methods

2.1. Strain

Strain *S. cerevisiae* Y5 is a newly developed strain in the lab (Patent No. ZL200810222897.7, CGMCC2660, China General Microbiological Culture Collection Center). The strain has the ability to metabolize furfural/tolerate fermentation inhibitors and efficiently metabolize glucose to produce ethanol.

2.2. Enzymatic hydrolysate of steam exploded – pretreated corn stover

The enzymatic hydrolysate used in this study was provided by Henan Tianguan Group Co., Ltd., in NanYang city Henan province, China. The natural corn stover was chopped to 2 cm size. The steam explosion pretreatment conditions of the corn stover raw materials were 205 °C, 2.0 Mpa and 5 min. After pretreatment, the corn stover without detoxification was hydrolyzed enzymatically and the water-insoluble solid (WIS) concentration was 25% (w/v) with the enzyme loading of 20 FPU/g WIS using Celluclast 1.5 L complemented with 20 IU/g WIS of Novozym 188 under the condition of citrate buffer (pH 4.8). The hydrolysis lasted for 96 h. The main components and contents of the hydrolysate were determined to be as follows: glucose 102.90 g/L, xylose 4.81 g/L, acetic acid 12.91 g/L, propionic acid 0.15 g/L, butyric acid 0.61 g/L, furfural 0.41 g/L and a pH of 4.5. The hydrolysate not only contained a high concentration of glucose, but also 12.91 g/L acetic acid, which was much higher than the previously reported acetic acid content in hydrolysates or enzymatic hydrolysates (Cunha-Pereira et al., 2010; Huang et al., 2009).

2.3. Medium

2.3.1. YPD medium

Yeast extract 10 g/L, peptone 20 g/L, glucose 20 g/L.

2.3.2. Fermentation medium

Enzymatic hydrolysate; pH 5.4, sterilized for 20 min at 121 °C.

2.4. Cultivation of strain

A loop of Y5 colonies was inoculated into 100 mL of YPD medium, and incubated to log phase at 30 °C 150 rpm. Then, the culture medium was transferred to the YPD medium again until log phase under the same conditions prior to use; the cell dry content weight was 6 g/L.

2.5. Ethanol fermentation characteristics

2.5.1. Nitrogen source

Two nitrogen sources were selected: one was YP (yeast extract 10 g/L and peptone 20 g/L), a nitrogen source commonly used in the laboratory, and the other was CSL (corn steep liquor 40, 20 and 0 mL/L), a nitrogen source commonly used in industrial production. Seed liquid was inoculated to 100 mL of the fermentation medium, with YP or CSL as the nitrogen source, and incubated at 30 °C, 150 rpm; samples were taken every 12 h to measure the residual sugar and ethanol content.

2.5.2. Nitrogen source concentration

After CSL was determined to be the optimum nitrogen source (according to the methods in the above Section 2.5.1), CSL nitrogen source concentrations were set to 40, 20 and 0 mL/L. The seed liquid was inoculated to the fermentation media (100 mL) with three CSL nitrogen source concentrations and incubated at 30 °C, 150 rpm; samples were taken every 12 h to measure residual sugar and ethanol content.

2.5.3. Inoculum size

With CSL as the nitrogen source, the seed liquid was inoculated to the fermentation media (100 mL) with three CSL nitrogen source concentrations and incubated with inoculum sizes of 10% (v/v) and 5% (v/v) at 30 °C and 150 rpm; samples were taken every 12 h to measure the residual sugar and ethanol content.

Three parallel tests were carried out for each group, and the results were averaged.

2.6. Analysis methods

Determination of ethanol content and furfural content was performed using a gas chromatograph (Agilent 7890A GC). Determination conditions for ethanol were as follows: the chromatographic column was HJ-PEG-20M, column temperature was 120 °C, injection temperature was 120 °C, detector temperature was 200 °C, N_2 was used as the carrier gas, and the flow rate was 4 mL/min. Determination conditions for furfural were as follows: the column temperature was 120 °C, injection temperature was 200 °C, detector temperature was 50 °C, and the sample size was 0.5 μL.

Determination of weak acid content was performed using a gas chromatograph (Shimadzu GCZO01). The chromatographic column was a glass column, N_2 was used as the carrier gas, the column pressure was 100 kPa, the column temperature was 170 °C, inlet temperature was 200 °C, the detector was FID and the detector temperature was 200 °C.

Determination of sugar content was performed using a high performance liquid chromatograph (Waters 2690). An ammonia column (200 × 4.6 mm) and Waters 410 differential detector were used; the column temperature was 40 °C, acetonitrile in mobile phase: water = 75:25, flow rate was1 mL/min, and sample size was 0.4 μL.

3. Results and discussion

3.1. Enzymatic hydrolysate of non-detoxified steam-exploded corn stover

In general, under the conditions of low pH and high acetic acid concentration, the acetic acid exists in a binding state; acetic acid