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Ethanol production from steam-pretreated sweet sorghum bagasse with high substrate consistency enzymatic hydrolysis

Fei Shen [a,*], *Jinguang Hu* [b], *Yuehua Zhong* [c], *Michael L.Y. Liu* [b], *Jack N. Saddler* [b], *Ronghou Liu* [d]

[a] *Provincial Key Laboratory of Agricultural Environmental Engineering, Sichuan Agricultural University, Chengdu Campus, 211 Huimin Road, Chengdu, Sichuan 611130, PR China*

[b] *Forest Products Biotechnology, Department of Wood Science, University of British Columbia, 2424 Main Mall, Vancouver, BC V6T 1Z4, Canada*

[c] *School of Chemical Engineering, Sichuan University, 24 South Section 1,Yihuan Road, Chengdu, Sichuan 610065, PR China*

[d] *Biomass Energy Engineering Research Centre, School of Agriculture and Biology, Shanghai Jiao Tong University, Shanghai 201101, PR China*

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ABSTRACT

In this work, separate hydrolysis and fermentation (SHF) and simultaneous saccharification fermentation (SSF) with high substrate consistency (the mass fraction: 12%) were performed with the steam-pretreated sweet sorghum bagasse (SSB). Fermentation ability of four yeast strains and influences of residual solids after hydrolysis on fermentation were investigated in SHF. Meanwhile, influences of inorganic salts on fermentation were assessed to determine their suitable supplementations for ethanol production. Additionally, influences of initial yeast inoculation on SHF and SSF were further investigated. The results showed the adopted yeast strain, Tembec 1, displayed the best fermentation performance on the hydrolysate of pretreated SSB, and the residual solids in hydrolysate had negligible influences on ethanol fermentation. Although the deficiency or overdose of $(NH_4)_2HPO_4$ or $MgSO_4 \cdot 7H_2O$ could reduce ethanol yield in SHF, the suitable supplementation of $(NH_4)_2HPO_4$ (0.5 g L^{-1}) and $MgSO_4 \cdot 7H_2O$ (1.0 g L^{-1}) could increase ethanol yield by 5.2% and 8.3%, respectively. The initial yeast inoculation of 3 g L^{-1} could satisfy both SSF and SHF, which achieved 63.8% and 57.9% ethanol yield of theoretical one with final ethanol concentration of 23.3 g L^{-1} and 21.2 g L^{-1}, respectively. In addition, ethanol yield kept almost constant as yeast was inoculated from 3 to 5 g L^{-1} in SHF, whereas it decreased significantly in SSF.

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1. Introduction

Concerns about the supply of oil and the environmental consequences of large-scale fossil fuel consumption are driving the search for more sustainable alternatives [1]. Ethanol, as an alternative fuel energy resource, has been a subject of great interest since the oil crisis in the 1970s [2]. Obviously, the production of first generation ethanol from food-based biomass has induced a competition between fuel and food, and consequently fluctuated world food price.

* *Corresponding author*. Tel./fax: +86 28 8629 1390.
E-mail addresses: fishensjtu@gmail.com, fishen@sicau.edu.cn (F. Shen).

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Therefore, the second generation, by utilizing inedible biomass, is gradually attracting wide attention. In recent decades, sweet sorghum has been regarded to be one of the most promising crops for ethanol production [3], because it is a high-yielding sugar crop with characteristics of wide climate adaptability and high tolerance to abiotic stresses such as drought, water logging, salinity, and alkalinity [4]. Additionally, both the soluble carbohydrates (glucose, fructose and sucrose, nearly 9.5% mass fraction) and insoluble carbohydrates (cellulose and hemicellulose, nearly 10.0% mass fraction) in sweet sorghum stalk could be potentially utilized for ethanol production [5–7]. Nowadays, the relatively high cost is a key issue to hinder the industrialization of ethanol production from sweet sorghum. It is estimated that the logistic cost, including the harvesting, collecting, preprocessing, transporting and handing of the raw materials, has occupied a relatively high part in the whole cost of ethanol production [8]. Thus, if the sweet sorghum bagasse (SSB) could be effectively utilized for ethanol production integrating with juice fermentation, the overall cost of refining ethanol from sweet sorghum would be reduced by sharing the co-logistic cost. Thus, this work focused on improving ethanol production from the SSB by enzymatic hydrolysis and fermentation.

Prior to the enzymatic hydrolysis and fermentation, the lignocellulosic materials should be pretreated to open up the integrity cell wall structure by disrupting the lignin/hemicellulose matrix and exposing the cellulose to the cellulase enzymes [9]. Steam pretreatment is one of the most thoroughly investigated methods applied for the bioconversion [10,11]. It has been shown to be an inexpensive and efficient process for pretreatment of various lignocellulosic substrates, especially for agricultural residues [12]. Hydrolysis of lignocellulose is typically carried out at low substrate consistency (2–5% mass fraction), which can get no more than 2% mass fraction of final ethanol content. Previous techno-economic assessment has suggested that an increase in substrate consistency from 5 to 8% could reduce the total ethanol production cost by nearly 20% [13,14]. Thus, the enzymatic hydrolysis of steam-pretreated SSB was employed with a relatively high substrate consistency in this study. In addition, no matter in the separate hydrolysis and fermentation (SHF) process or in the simultaneous saccharification fermentation (SSF) process, residual solids always exist in the hydrolysate [15], However, the influence of those residuals on ethanol fermentation is still unclear. Therefore, the comparison fermentation was carried out to investigate the necessity of removing the residual solids in SHF. Meanwhile, various types of yeast were compared for selecting the most robust yeast strain for ethanol production from steam-pretreated SSB. As some nutrients (inorganic salts, trace metals, etc.), which are essential for supporting yeast metabolism, could probably be extracted and flow into the water soluble fraction (WSF) during the steam pretreatment process [16]. Thus, there might be not enough nutrients left in the water insoluble fraction (WIF) for the downstream yeast fermentation process. For example, the pretreated wheat straw contains only 0.4% mass fraction of total nitrogen on the basis of dry weight, while the mashes used in the traditional fuel ethanol production contains 10 times more [17,18]. Thus, the effects of main inorganic nutrient ions, such as NH_4^+ and Mg^{2+} sources, were assessed on fermentation process, and their suitable supplementations were also evaluated. Some of previous studies showed that a high initial yeast inoculation (6 g L^{-1}, 10 g L^{-1}, even to 23.6 g L^{-1}) were employed to obtain high ethanol yield and productivity [19–21].However, as an excessive high yeast inoculation could also increase the entire ethanol production cost, the reasonable initial yeast density for efficient ethanol production during the SHF and SSF processes were also determined.

2. Material and methods

2.1. Raw material and enzymes

Sweet sorghum {*Sorghum biocolor (L.) Moench*} cultivar of Liaotian 1 was employed for this work. It was cultivated in the farm of Shanghai Jiao Tong University-Qibao Campus (N 31°08′49″, E 121°21′31″) on May 28, 2008. The fresh stalks were harvested on Sep. 30, 2008. The wet bagasse was collected after the juice extraction from stalks with a three-roller mill (130 , Debao Machinery Plant, Guangxi, China). Bagasse was sun-dried for 2–4 days till its moisture decreased to less than 10% mass fraction. The dried SSB was stored in the plastic zipper bags. It was ground to less than 4 mm in length using a small scale industry rubbing machine (9PR-1.6, Forestry and Agricultural Machinery Plant, Liaoning, China) before the steam pretreatment.

The commercial cellulase (Spezyme-CP, Genencor Danisco, Palo Alto, CA) with bata-glucosidase (Novzymes188, Bagsværd, Denmark) was used in enzymatic hydrolysis process. Cellulase activity was 48.6 filter paper units (FPU) mL^{-1} with protein content of 133.9 g L^{-1}. Bata-glucosidase activity was 458.4 cellobiose units (CBU) mL^{-1} with 233.4 g L^{-1} protein content.

2.2. Steam pretreatment

The steam pretreatment for the ground SSB was carried out in a 2 L steam gun (Stake Tech II, Stake Technology, Norval, Ontario, Canada) at the Department of Wood Sciences in the University of British Columbia, Canada. The detailed protocol was totally same with the description in reference [22]. After pretreatment, the WIF was separated by vacuum filtration, and washed 10 times with tap water. The washed WIF was stored in a −20 °C freezer for further analysis and hydrolysis.

2.3. Yeast strains and their preparation

Tembec 1 and Tembec 2 strains of *Saccharomyces cerevisiae* were provided by Tembec Limited (Témiscaming, Québec, Canada). Y 1528 and Baker's yeast (BY 4742) strain of *S. cerevisiae* respectively obtained from the Agricultural Research Service, U.S. Department of Agriculture, Peoria, Illinois, U.S.A. and the Wine Research Centre in the University of British Columbia, Canada. The employed strains were stored in the libratory of Forest Products Biotechnology/Bioenergy Group, the University of British Columbia, Canada. Yeasts were

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