Applied Energy xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Applied Energy

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

Consolidated bioprocess for bioethanol production with alkali-pretreated sugarcane bagasse

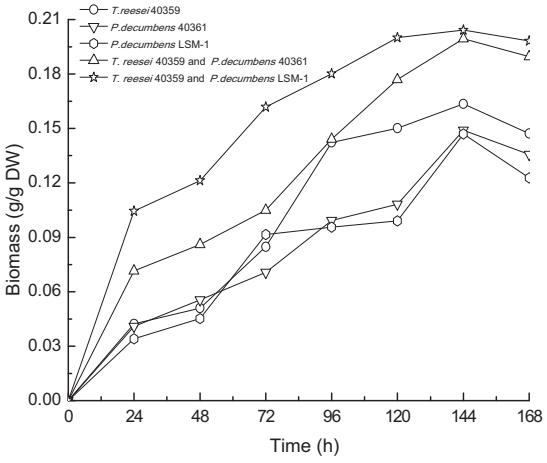
Yunyun Liu [a,b], Yu Zhang [a], Jingliang Xu [a], Yongming Sun [a], Zhenhong Yuan [a,*], Jun Xie [b]

[a] Key Laboratory of Renewable Energy, Guangzhou Institute of Energy Conversion, Chinese Academy of Sciences, Guangzhou 510640, PR China
[b] Institute of New Energy and New Materials, South China Agricultural University, Guangzhou 510642, PR China

HIGHLIGHTS

- A consolidated bioprocess for ethanol production was applied.
- Solid enzyme production and saccharification labored water and energy consumption.
- Appreciable levels of sugars and enzymes were produced in a variety of conditions.
- Enzyme preparation on site can improve the economics of ethanol production.
- Assessment of ethanol production from delignified SCB by consolidated bioprocess.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 4 February 2015
Received in revised form 15 April 2015
Accepted 2 May 2015
Available online xxxx

Keywords:
Mixed culture
Solid state fermentation
Sugars
Enzymes
Simultaneous saccharification and fermentation
Bioethanol

ABSTRACT

A consolidated bioprocess integrating enzyme production, saccharification and fermentation was applied for bioethanol production. Mixed solid-state fermentation of alkali-pretreated sugarcane bagasse (SCB) supplemented with wheat bran (WB) to produce a compound enzyme for saccharification by *Trichoderma* and *Penicillium* was carried out, and at 72 h, the obtained sugars concentration was 20.190 g/L. Then the mixture was further converted to ethanol by inoculating *Saccharomyces cerevisiae*. After 24 h, the maximum ethanol concentration 5.825 g/L (40.84% of theoretical yield) was achieved. At 144 h, the produced β-glucosidase activity and fungal biomass were 0.483 IU/mL and 0.204 g/g, respectively. The consolidated bioprocess avoided enzyme preparation, saved much water/energy consumption and reduced equipment investment, which indicated that it might be an effective approach for the economic production of bioethanol.

1. Introduction

Conversion of lignocellulose to ethanol has been considered as a potential solution to solve energy crisis and environmental pollution problems. However, the high conversion cost seriously

* Corresponding author. Tel.: +86 20 87057735.
E-mail address: yuanzh@ms.giec.ac.cn (Z. Yuan).

http://dx.doi.org/10.1016/j.apenergy.2015.05.004
0306-2619/

hindered its industrial application. Generally, the bioprocess for cellulosic ethanol production consists of pretreatment, saccharification and fermentation, and saccharification always requires the preparation of enzymes. In order to reduce the process cost, suitable process combination was necessary. Simultaneous saccharification and fermentation was proposed in the past decades [1–4], which integrated enzymatic saccharification and ethanol fermentation in a system, saved both process and time cost.

Cellulolytic enzymes play a critical role in lignocellulose saccharification. Bioconversion requires multiple enzyme activities. However, the high cost of these commercial enzymes presents a significant barrier to efficient utilization of biomass [2,4]. Toward this problem, we tried to integrate the cellulase production into saccharafication, where cellulase production and utilization were carried out on the same system. Compared to simultaneous saccharification and fermentation, the process was consolidated further, and it avoided cellulase separation and purification, which could lower the saccharification cost significantly.

Cellulase is produced by growing cellulolytic fungi either in solid state fermentation (SSF) or in submerged fermentation. Compared to submerged fermentation, SSF is a much simpler process, with higher sugars generation, less energy consumption and lower risk of contamination [5–9]. Enzymes production with SSF process, both bacteria and fungi can use cellulose as primary carbon source. The cellulosic substrate acts as carbon source and inducer for enzymes generation [10]. Fungal strains that produce cellulases mainly includes *Trichoderma*, *Penicillium*, *Aspergillus*, and *Fusarium*. *Trichoderma reesei* (*T. reesei*), which is well known for its potential for the hyper-production of cellulase, has been widely exploited for their inherent ability of cellulolytic enzymes production [11,12], but it usually has too low activity in β-glucosidase to efficiently hydrolyze cellulose into glucose [13]. The β-glucosidase hydrolyzes cellobiose, which accumulates otherwise and inhibits the activity of cellulases, subsequently results in overall low substrate conversion rate. This problem can be overcame either by supplementing β-glucosidase or by using co-culture systems [14,15]. The strains of *Penicillium* and *Aspergillus* are able to produce β-glucosidase with high activity [16,17]. It has been reported that the co-fermentation of filamentous fungi such as *T. reesei* and *Penicillium decumbens* (*P. decumbens*), or *T. reesei* and *Aspergillus niger* [18–20] can produce high activity of cellulase with harmonious composition.

SCB has a rich cellulosic composition containing 40–45% cellulose. WB is well known as a great source of minerals, carbon, vitamins, and nitrogen for the growth of microorganisms and enzyme production [21,22]. Alkali-pretreatment can efficiently dissolve lignin [23]. After delignification, SCB was mainly composed of glucan and xylan. Supplemented with WB, it could be used as a substrate for the production of enzymes and sugars in an SSF process [24–26].

Our previous studies showed that high concentration of sugars could be obtained from SSF process, which indicated that the crude unprocessed enzymes produced was well hydrolyzing the substrate. Therefore it might be a consolidated way to provide enzymes on-site for the ethanol bioconversion process after inoculated with *Saccharomyces cerevisiae*. The majority of published reports mainly focused on cellulase production or ethanol fermentation. It is still unclear whether it is feasible for cellulosic ethanol production from SSF-generated sugars employing crude unprocessed enzymes instead of commercial cellulases.

In this paper, the individual and mixed culture solid state fermentation (MSSF) of alkali-pretreated SCB supplemented with WB using *T. reesei* and *P. decumbens*, and a consolidated bioprocess of directly using the generated sugars and crude unprocessed enzymes from SSF in the ethanol conversion process were investigated.

2. Materials and methods

2.1. Materials

Substrates: SCB was kindly provided by Guangxi Feng Hao alcohol Co., Ltd. in China for ethanol fermentation; the material was ground and milled to a particle size in the range of 2–3 mm. The milled bagasse (1 g dry weight basis) was mixed with 20 mL of 0.5 M NaOH/g dry weight and incubated in a water bath at 80 °C for 2 h with agitation. After reaction, the treated bagasse was washed with tap water, until neutrality, and dried at 80 °C. For all fermentations the insoluble organic nutrients WB was added at the ratio of 3:7 dry substrate weight with alkali-treated bagasse, and thoroughly mixed.

Microorganisms: *T. reesei* CICC 40359 (ATCC 56765), CICC 40358 (ATCC 26921), and *P. decumbens* CICC 40361 were supplied by China Center of Industrial Culture Collection. The three strains and laboratory-preserved *P. decumbens* LSM-1 were used for the production of enzymes in all solid-state fermentations. They were grown and maintained on potato dextrose agar (PDA). Spore suspension of 107 spore mL^{-1} was prepared by harvesting from 4 to 7 days old cultures of each molds with 10 mL of sterile distilled water.

Commercial *S. cerevisiae* preparation (24 h of activation prior to inoculation) containing nutrients was used to fermentate the generated sugars from SSF. Yeast preparation was added at 10% (V/V) of the mixed system.

Medium: The nutrient solution was Mandel's mineral salt solution [27], with composition (g/L): urea 0.3, $(NH4)_2SO_4$ 1.4, KH_2PO_4 2.0, $CaCl_2{\cdot}2H_2O$ 0.3, $MgSO_4{\cdot}7H_2O$ 0.3, and microelement solution (added 1 mL/L). The solid state fermentation medium for sugars and enzymes production was the following (%): alkali-treated bagasse 70, wheat bran 30, and the nutrient solution was added in the ratio of 2.5 mL: 1 g dry mixed solid. The initial pH value of the medium was 4.5 after sterilization at 121 °C for 20 min, and the water content of the substrate was about 70% (w/v) for most experiments to be described.

2.2. Solid substrate tray fermentation

SSF was conducted in metal shallow trays (D10 × 2 cm), each containing 3 g dried mixed solid and 7 mL Mandel's medium. The tray was placed in a glass container with ventilation holes which was designed and manufactured by our laboratory, to form a small fermentation system. The filled trays were autoclaved at 121 °C for 20 min. Each tray was then inoculated with 8% spore suspension of *T. reesei* CICC 40359, *P. decumbens* CICC 40361, LSM-1 or mixture of both *T. reesei* and *P. decumbens*. All the small bioreactors were incubated in an environment chamber in which air humidity and temperature were kept at 92% and 30 °C, respectively, for 1 to 7 days (7 trays for single or mixed strains). The samples were taken from the trays at regular intervals of 24 h and were analyzed for different indexes. Duplicate trays were set up for each experimental condition.

After 3 days SSF, the contents of the trays were transferred into a 50 mL flask containing 30 mL of HAc–NaAc buffer (pH 5.0) and thoroughly blended, meanwhile inoculating *S. cerevisiae*. Ethanol fermentation was started at 30 °C, 150 rpm for 72 h in duplicate.

2.3. Sample processing

After the substrates of the trays for each time point (every 24 h) were thoroughly blended in 30 ml distilled water by shaking for 1 h at 150 rpm, the mixture was decanted into 100 mL tubes, centrifuged at 4000 rpm for 15 min and the supernatant was used for

Please cite this article in press as: Liu Y et al. Consolidated bioprocess for bioethanol production with alkali-pretreated sugarcane bagasse. Appl Energy (2015), http://dx.doi.org/10.1016/j.apenergy.2015.05.004