BIOMASS AND BIOENERGY 77 (2015) 53–63

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Efficient enzymatic hydrolysis and simultaneous saccharification and fermentation of sugarcane bagasse pulp for ethanol production by cellulase from *Penicillium oxalicum* EU2106 and thermotolerant *Saccharomyces cerevisiae* ZM1-5

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ARTICLE INFO

Article history:
Received 23 April 2014
Received in revised form
16 December 2014
Accepted 20 March 2015
Available online 6 April 2015

Keywords:
Sugarcane bagasse pulp
Enzymatic hydrolysis
Cellulase
Simultaneous saccharification and fermentation
Bioethanol production

ABSTRACT

Sugarcane bagasse (SB) is a promising alternative feedstock for second-generation fuel ethanol production. Chemical SB pulp has high cellulose content (73.89%) and low lignin content (0.49%). SB pulp and pretreated SB were subjected to digestion by cellulase produced by *Penicillium oxalicum* EU2106. The results showed that SB pulp released the highest amount of reducing sugars, when compared with other pretreated SB. The glucan conversion reached 93.36% after 96 h hydrolysis of SB pulp at an enzyme loading of 20 FPU/g solid and a solid loading of 60 g/L. Thermotolerant *Saccharomyces cerevisiae* ZM1-5 was employed in simultaneous saccharification and fermentation of SB pulp to ethanol, which was performed in multiple parallel fermentation tanks (500 mL working volume). A considerable amount of ethanol (18.79 g/L at 0.42 g ethanol/g cellulose) was produced when the solid loading was 60 g/L. All data indicated that SB pulp could be employed as an alternative material for bioethanol production.

1. Introduction

The inevitable fossil oil depletion, energy security concerns, and increasing problem of CO_2 emissions have strengthened the interest in the search for alternative, non-petroleum-based sources of energy. Sugarcane bagasse (SB) is one of the most important and widely available lignocellulosic biomass in tropic and subtropic climates, which is obtained

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http://dx.doi.org/10.1016/j.biombioe.2015.03.020
0961-9534/

after extracting the juice from sugarcane in the sugar production process. It has been estimated that about 5.4×10^8 tons of sugarcane are processed annually throughout the world [1].

Pretreated lignocellulose can be degraded into fermentable sugars by using acids or enzymes. Enzymatic hydrolysis of cellulosic materials by cellulase is the most promising approach to obtain relatively high ethanol yields [2]. Cellulose, the major fraction of lignocellulosic biomass, can be hydrolyzed to glucose by cellulase enzymes [3]. However, the complicated deconstruction of lignocellulosic materials accounts for the major challenges in degrading them into monosaccharides. Furthermore, both lignin and hemicellulose act as physical barriers to the cellulase enzymes, hindering the degradation of cellulose [4,5]. Besides, the enzymes might be non-productive and may irreversibly bind to the lignin components, resulting in the loss of enzyme activities [6]. In addition, cellulose is highly crystalline and has a high degree of polymerization, making it more resistant to cellulase decomposition. Thus, considering all these obstacles, efficient pretreatment of lignocellulose is necessary before the hydrolysis step [7]. Several pretreatment processes, including use of enzymes, dilute acid [8], steam explosion [9], and alkaline agents [10], have been employed to remove lignin and hemicellulose.

For the conversion of pretreated lignocellulose into fuel ethanol, one of the following four processes can be employed. The first process is the separate hydrolysis and fermentation (SHF), in which hydrolysis of lignocellulosic materials and fermentation of sugars are completely separate. One of the advantages of the SHF process is that both hydrolysis and fermentation steps can be performed under their optimal conditions, mainly pH and temperature. However, undesirable features have also been noted, such as end-product inhibition of cellulase in the hydrolysis step, leading to incomplete hydrolysis of cellulose, or the presence of toxic inhibitors of microbial growth in the lignocellulose hydrolysates, affecting the performance of fermentation microorganism such as yeast. To address these problems, attempts had been made to develop simultaneous saccharification and fermentation (SSF) processes or methods to detoxify the hydrolysates [1,11].

In the SSF process, hydrolysis and fermentation steps are performed in the same bioreactor. The SSF shows tremendous advantage over SHF by minimizing end-product inhibition. In addition, the SSF process is more economical, when compared with the SHF, because it can increase the ethanol concentration in the fermentation broth [12], leading to reduction in the energy costs during distillation. However, a major shortcoming of this process is that hydrolysis and fermentation cannot always be performed under optimal conditions, making it more difficult to optimize the fermentation parameters [13].

The third process that can be employed for bioethanol production is the simultaneous saccharification and co-fermentation (SSCF). The purpose of this process is to allow complete assimilation of both hexoses and pentoses released from the lignocellulosic materials by the fermentation strains. Therefore, a microorganism capable of efficient ethanol production and assimilating hexoses as well as pentoses is required [14].

The fourth alternative route for bioethanol production is consolidated bioprocessing (CBP), which is characterized by combining cellulase production, cellulose hydrolysis, and fermentation together in a single process step. The CBP is distinctively distinguished from the above-mentioned three processes because it does not comprise a separate and dedicated step. The primary microorganisms employed in CBP could be bacteria or yeasts [15]. As a result of highly integrated configurations, the CBP exhibits the potential for reducing the cost of operation and increasing the efficiency [15].

Large quantities of SB are generated by sugar industries in China [16]. Many previous studies had used pretreated SB to produce ethanol [9,17–20]. However, the conversion of glycan in SB into fermentable sugars was constrained to a limited range owing to insufficient delignification or inadequate depolymerization of cellulose or other factors, adding to the difficulties in lignocellulose degradation, although various pretreatment strategies were introduced to increase degradability [21,22]. Ding et al. [5] showed that lignin is one of the most important limiting factors in the enzymatic saccharification of plant cell walls. Accordingly, materials containing low content of lignin could be a suitable feedstock for ethanol production. It is well known that the chemical pulping process can effectively remove lignin from lignocellulose. Hence, the present study was aimed to investigate the digestibility of SB pulp by cellulase and the suitability of SB pulp as an alternative material for bioethanol production.

It was demonstrated in this study that the SB pulp could be well hydrolyzed by the cellulase produced by *Penicillium oxalicum* EU2106. A relatively high concentration of glucose of 45.43 g/L was obtained when using a solid loading of 60 g/L, corresponding to 93.36% of glucan conversion. The SSF experiments conducted in multiple parallel fermentation tanks of 500 mL working volume showed that the SB pulp at a solid loading of 60 g/L could be well converted into ethanol at 0.42 g ethanol/g cellulose.

2. Materials and methods

2.1. Microorganisms

The microorganism used to produce cellulase in the present study was *P. oxalicum* EU2106, the mutant strain of the wild-type *P. oxalicum* HP7-1. *P. oxalicum* HP7-1 was isolated from Huaping National Natural Reserve in Guilin, Guangxi, China. After three rounds of $Co^{60}\gamma$-ray irradiation and two rounds of ethylmethane sulfonate/ultraviolet combined mutagenesis, *P. oxalicum* EU2106 was eventually picked out as a cellulase hyper-producing strain. The strain EU2106 was deposited in China General Microbiological Culture Collection Center under the number of CGMCC 6471. The stock culture was maintained on potato dextrose agar (PDA) plates at 4 °C. The strains used for ethanol production were thermotolerant *Saccharomyces cerevisiae* ZM1-5 (deposited in China General Microbiological Culture Collection Center under the number of CGMCC 3761), which could grow well at 40 °C.

2.2. The SB pulp and pretreatments of SB

The methods employed for the pretreatment of SB included dilute acid (HCl) pretreatment and peracetic acid (PAA)-NaOH pretreatment. The SB pulp and all the pretreated SB were milled and sieved through an 80-mesh sieve before being used.