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Short Communication

Ethanol production from xylan-removed sugarcane bagasse using low loading of commercial cellulase

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HIGHLIGHTS

- Xylan-removed residue was used to produce bioethanol.
- Enzymatic digestibility was 82% by using cellulase of 7.5 FPU/g cellulose.
- Increasing cellulase loading from 7.5 FPU/g cellulose showed no improvement.
- Ethanol concentration reached 40.59 g/L by using fed-batch mode.

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ABSTRACT

Xylan was always extracted as the feedstock for xylooligosaccharides production. The xylan-removed residue may contain high content of cellulose and thus had a possibility to be converted into ethanol. After soaked in 12% of NaOH at room temperature overnight, solubilization of cellulose, xylan, and lignin was 4.64%, 72.06%, and 81.87% respectively. The xylan-removed sugarcane bagasse (XRSB) was enzymatically hydrolyzed by using decreased cellulase loadings. The results showed that 7.5 FPU/g cellulose could obtain a cellulose conversion yield of 82%. Increasing the cellulase loading did not result in higher yield. Based on this, bioethanol production was performed using 7.5 FPU/g cellulose by employing fed-batch fermentation mode. The final ethanol concentration reached 40.59 g/L corresponding to 74.2% of the theoretical maximum. The high titer ethanol and low cellulase loading may reduce the overall cost.

1. Introduction

Sugarcane bagasse (SCB) is a waste from sugar production (Li et al., 2013). Its high yield and high cellulose and xylan content makes it a competitive candidate for biorefinery (Li et al., 2014c). The cellulose part is always converted into hexose which could be fermented easily by wild-type *Saccharomyces cerevisiae*. However, pentose could not be metabolized by wild-type *S. cerevisiae*. The xylan can be separated and further converted into high-value xylooligosaccharides (Jayapal et al., 2013).

The commonly used method for extracting xylan from lignocellulose is the alkali extraction which includes the concentrated solutions of NaOH or KOH, and alkaline hydrogen peroxide solution (Jayapal et al., 2013). NaOH was found to be better than KOH when considering the recovery yield of xylan. Higher concentration of NaOH or KOH gave rise to higher xylan recovery and steam cooking improved the recovery further (Jayapal et al., 2013). Although many reports were focused on xylan extraction and xylooligosaccharides production, the utilization of the xylan-extracted residue was rare.

The cost of cellulase is still one of the dominant factors of the whole process even though significant advances in reduction of enzyme costs have been made through enzyme production process optimization and cellulase engineering (Klein-Marcuschamer et al., 2012). A lot of literatures underestimated the cost of cellulase. It was found that the cost of enzyme was $0.68/gal if the sugars in the corn stover could be converted at maximum theoretical yields. To lower the cost of enzyme still needs significant efforts for biofuel production economically (Klein-Marcuschamer et al., 2012). Based on this, Sathitsuksanoh et al. (2009) focused on decreasing the overall cellulase loading to reduce the capital investment.

The use of high solid loading ($\geqslant$15%) for bioethanol production potentially offers many advantages such as increased concentration of sugar and ethanol and decreased capital costs (Modenbach and

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Nokes, 2012). In the present study, xylan-extracted residue was used as feedstock. Its digestibility was investigated at different cellulase loadings and ethanol was produced by employing fed-batch simultaneous saccharification and fermentation.

2. Methods

2.1. Preparation of xylan-removed SCB (XRSB)

Raw SCB was provided by the Qianwu sugar refinery plant in Zhuhai, Guangdong, China. It was air-dried and placed at room temperature in woven bags. The raw SCB was milled by a hammer milling machine to get the particles between 0.15–0.27 mm. Xylan was extracted by 12% (w/v) NaOH solution at a solid loading of 5% under room temperature overnight (Jayapal et al., 2013). The liquid fraction was separated by filtering through a 500-mesh filter cloth. The solid fraction was washed by using tap water until pH 6–7. The XRSB with moisture content of 74% was stored at 4 °C till utilization.

2.2. Microorganism strain, media, and inocula preparation

The *S. cerevisiae* strain, CICC 1445, obtained from China Center of Industrial Culture Collection, was used in the present study. The strain was cultured aerobically at 30 °C in an incubator at 200 rpm in a yeast extract peptone dextrose medium (YPD) containing 20 g/L of glucose, 10 g/L of yeast extract, and 5 g/L of peptone. The medium was sterilized by steam autoclaving at 121 °C for 20 min. The seed culture for the fermentation inocula was prepared from the culture of YPD medium in sterile test tubes and incubated at 30 °C at 200 rpm for 24 h in a shaker. Then, 20 mL of activated cells was aseptically transferred to 200 mL of sterile YPD medium in a 500-mL Erlenmeyer flask. The flask was incubated at 30 °C at 200 rpm for another 48 h. The cells were harvested by centrifugation in 50-mL sterilized centrifuge tubes for 10 min at 5000 rpm at 4 °C using a centrifuge (Shanghai Anke, Shanghai, China). The cell pellets were washed twice with sterile deionized water. The cells were then resuspended by sterile deionized water and used to initiate fermentation. The entire process was completed within 2 h to ensure the activity of the cells.

2.3. Enzymatic hydrolysis

The enzyme activity of Celluclast 1.5 L and Novozym 188 (both kindly provided by Novozymes (China) Investment Co. Ltd.) was 83.43 FPU/mL and 748 pNPGU/mL, respectively (Li et al., 2014b). The ratio of Celluclast 1.5 L to Novozym 188 was fixed as 1 FPU:2 pNPGU. Enzymatic hydrolysis of samples was conducted at the solid loading of 2% (wet weight/v, equivalent to 1.87% of dry matter (DM)) with different cellulase loadings (1.5, 3.7, 7.5, 11.2, 14.9, 22.4, and 29.9 FPU/cellulose) in 10 mL of 0.1 M citrate buffer (pH 4.8) supplemented with 80 μg/mL tetracycline and 60 μg/mL nystatin (dissolved in DMSO) to prevent microbial contamination. The mixture was incubated at 50 °C in a rotary shaker at 200 rpm. Samples were collected from the reaction mixture after hydrolysis for 48 h. Each sample from the hydrolysate was centrifuged for 2 min at 12,000 rpm. The supernatant was used to determine the products by HPLC.

2.4. Fed-batch simultaneous saccharification and fermentation (SSF)

Fed-batch SSF was conducted with initial solid loading of approximately 7.78% (38.5 g wet XRSB mixed with 100 mL of in 0.1 M citrate buffer (pH 4.8)) without prehydrolysis. 19.2 g of wet substrate and the corresponding fresh enzymes were fed into the mixture at 24 h, 60 h, and 96 h. The final solid loading was 14.6% (dry weight/v). Yeast extract (1%, w/v) and peptone (0.5%, w/v) were supplemented as nutritious sources. The media were sterilized in an autoclave at 121 °C for 30 min. Celluclast 1.5 L and Novozym 188 were filtered through 0.22 μm sterilized membranes (Millipore Corp. Carrigtwohill, Co. Cork, Ireland) before being added to the media. Celluclast 1.5 L and Novozym 188 loadings were 7.5 FPU and 14.9 pNPGU/g cellulose, respectively. The yeast cell suspension was added at concentration of 1 g dry yeast cells/L (DCW/L) to initiate fermentation. Subsequently, the bottles were sealed with rubber stoppers equipped with a water trap, which permitted CO_2 removal without air injection. The bottles were placed on a shaker at 200 rpm at 30 °C. Samples were periodically collected under aseptic conditions for determining the products.

2.5. Analytical methods

Chemical composition of SCB and the XRSB was analyzed following the NREL method (Sluiter et al., 2008). Products were determined using a HPLC equipped with an Aminex HPX-87H column with a Cation H+ Cartridge Micro-Guard column (Bio-Rad, Hercules, CA, United States) as previously described (Li et al., 2014a).

2.6. Statistical analysis

All experiments were conducted in duplicate, and the data are presented as the mean value ± standard deviation (SD). Statistical analyses were done using the PASW statistics 18 software using one-way ANOVA and Duncan's multiple range tests. The results were considered statistically significant at a 95% confidence interval ($p < 0.05$).

3. Results and discussion

3.1. Chemical composition

The chemical composition of the SCB and XRSB is shown in Table 1. Compared with the raw SCB, the cellulose content was increased by almost 2-fold while the xylan content was decreased by almost 2-fold and the lignin content was decreased by 2.78-fold after the extraction. However, when pretreatment of SCB by using 2% of NaOH with steaming under 121 °C for 1 h, the xylan content was increased by 29.7%, and the lignin content was reduced by 6.47-fold (Li et al., 2014c). It suggested that the pretreatment conditions affected the role of NaOH significantly. NaOH pretreatment coupled with steam cooking may be a better way to extract acid insoluble lignin part while higher concentration of NaOH pretreatment followed by overnight incubation may be the suitable for extracting both lignin and xylan (Table 1). Solubilization of cellulose was very low compared with other components (Table 1), suggesting that cellulosic part of SCB could be refined by the employed extraction method without much loss. Silverstein et al. (2007) studied different concentrations of NaOH under different temperatures with different residence times on solubilization of cellulose, xylan, and lignin of cotton stalks. The results showed that glucan solubilization was ranged from 10% to 30% and that for xylan was ranged from 20% to 40% and that for lignin was ranged from 35% to 65%. Compared with that, cellulose solubilization in the present study was rather low while xylan and lignin solubilization were very high (Table 1). The main reason may be the high concentration of NaOH and longer soaking time which were used in this work. The employed pretreatment procedure might be a