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A facile and efficient pretreatment of corncob for bioproduction of butanol

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HIGHLIGHTS

- An efficient and convenient method was proposed for corncob pretreatment.
- Simultaneous CBMAS pretreatment and hydrolysis could reduce the processing time.
- The hydrolysates from CBMAS-pretreated corncob could be utilized without detoxication.
- Fermentable sugars were completely consumed with a yield of 9.52 g/L butanol.

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ABSTRACT

The present study developed a combined ball milling-aqueous swelling (CBMAS) pretreatment to accelerate the hydrolysis of corncob. The enzymatic hydrolysis of microcrystalline cellulose carried out in the plates and flasks indicated that the response of enzymatic hydrolysis to CBMAS was quite evident. The fermentable reducing sugars of hydrolysates from CBMAS-pretreated corncob was 59.8 g/L, which was 1.3 and 1.7 folds higher than those from diluted acid and alkaline pretreated corncob hydrolysates, respectively. Simultaneous CBMAS pretreatment and enzymatic hydrolysis was also conducted, reducing the processing time from 66 h to 28 h. The enzymatic hydrolysates from CBMAS-pretreated corncob could be directly utilized as the substrate for butanol fermentation without detoxication. Under the optimal conditions, fermentable sugars in the corncob hydrolysate were completely consumed to generate 9.52 g/L butanol.

1. Introduction

Corncob is a major waste from corn (maize) production, which contains 30–35% hemicellulose, 40–45% cellulose and 10–20% lignin (Sheng and Marquis, 2006). As a low-cost lignocellulosic biomass, corncob is a reliable and renewable source for the production of various value-added products, such as biofuel. However, the highly ordered structure of lignocellulose constitutes the major obstacle for corncob saccharification to generate fermentable sugars (Demiral et al., 2012). Although various pretreatment methods have been developed to improve the hydrolysis efficiency of lignocellulosic complex, the main challenges still exist in the matter of saccharification performance and environmental pollution, etc. (Kumar et al., 2009). Furthermore, by-products generating during the hydrolysis process are often toxic to the fermenting microorganisms and affects the subsequent fermentation process (Palmqvist and Hahn-Hägerdal, 2000).

To aim at above problems, the present work has developed a facile and efficient method called combined ball milling-aqueous swelling (CBMAS) for corncob pretreatment. The shaker-driving ball milling was of low energy consumption, but notably improved the enzymatic hydrolysis of microcrystalline cellulose combined with the aqueous swelling effect. This method was then adopted for corncob pretreatment, where the processing performance was compared with that of chemical pretreatment. Thereafter, the pretreatment and saccharification of corncob was optimized to shorten the processing time. Finally, the generated corncob hydrolysates were directly used as the substrate for biobutanol production.

2. Methods

2.1. Materials and reagents

Corncob was collected from local market and air dried. After chipping, corncob was sieved by a 40 mesh screen and stored for future use. Cellulase (150 FPU/g) and xylanase (1.67×10^5 U/g) were supplied by Sino Enzymes Co. Ltd. (China).

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2.2. Pretreatment and saccharification of microcrystalline cellulose and corncob

The weighed sample (20 g) of microcrystalline cellulose or chipped corncob was prepared in a flask with the supplement of 200 mL water and 50 glass balls (1 mm diameter). The sample was stirred at 200 rpm in a shaker at room temperature for adequate time (~24 h). In addition, chipped corncob was pretreated with diluted acid or alkaline, as described by previous reports (Chang et al., 2012; Torre et al., 2008).

To evaluate the effectiveness of CBMAS method, 1.0% (w/v) CBMAS- and non-pretreated microcrystalline cellulose was paved in the plates supplemented with 2% agar, respectively. One microlitre cellulase solution of 5 FPU/mL was distributed in the centre of plates and placed in the 50 °C incubator for 24 h. For liquid hydrolysis, samples of 20 g pretreated microcrystalline cellulose or corncob were thrown in the 200 mL solution of 50 mM sodium citrate buffer (pH 5.0) in all experimental cases. Enzymatic hydrolysis was performed in the shake flasks agitated at 200 rpm and 50 °C for 28 h, initiated by an enzyme cocktail constituting 10 FPU cellulase and 2500 U xylanase per gram dry substrate, except only 10 FPU cellulase per gram substrate for microcrystalline cellulose.

2.3. Butanol fermentation

Clostridium beijerinckii G-23 was isolated from forest soil and used as the host for butanol fermentation. Tryptone-yeast extract-acetate (TYA) medium was used for the preparation of inoculum (Tanaka et al., 2012). Batch fermentation for butanol production was conducted for 88 h at 37 °C within the P2 medium (Lu et al., 2011) containing corncob hydrolysate as the carbon source. The samples were periodically withdrawn from the broth and centrifuged at 15,000 rpm for 2 min. The remaining supernatants were subjected for subsequent analysis.

2.4. Analytical methods

The concentration of total reducing sugars was analyzed by DNS method (Chang et al., 2012). Monosaccharides in the liquid were determined by Dionex ion chromatography ICS-5000 (Sunnyvale, CA, USA) with a Dionex pulsed amperometric detector equipped with an Au electrode and a Dionex Carbopac PA20 column (150 mm × 3 mm). The mobile phase was composed of 250 mM NaOH, 1 M NaAc and water, with a flow rate of 0.5 mL/min. The gradient elution program was indicated in Table 1.

The fermentation products were analyzed using a gas chromatograph (Agilent 6820, America) equipped with a flame ionization detector (FID) and a 3000 × 0.32 mm capillary column. The oven temperature was set at 90 °C, and the injector and detector temperatures were set at 240 °C.

Table 1
Gradient elution program of monosaccharide analysis.

Time (min)	Water (%, v/v)	250 mM NaOH (%, v/v)	1 M NaAc (%, v/v)
0	98.2	1.8	0
21.0	98.2	1.8	0
21.1	93.2	1.8	5.0
30.0	78.2	1.8	20.0
30.1	20.0	80.0	0
50.0	20.0	80.0	0

3. Results and discussion

3.1. Evaluation of CBMAS pretreatment

A drop of cellulase solution caused the formation of a distinct transparent zone on the plate with CBMAS-treated microcrystalline cellulose, while no change was observed on the plate with non-treated microcrystalline cellulose. This result suggested that the CBMAS pretreatment could effectively disrupt the configuration of microcrystalline cellulose and increase its accessibility to cellulase. Accordingly, the resulted glucose in the hydrolysate of CBMAS-treated microcrystalline cellulose was 112.1 g/L, with a conversion rate of 100.0% (Fig. 1), which were about two folds higher than those of non-treated microcrystalline cellulose.

The enhanced performance of enzymatic hydrolysis of CBMAS-treated microcrystalline cellulose may derive from the combined effects of both mechanical attrition and aqueous swelling. The reduction of crystallinity and the crystal size caused by mechanical attrition (Abdullah and Wu, 2009) could increase the content of amorphous cellulose, which was considered as the main reason for improved digestibility of milled cellulose (Yu et al., 2010). Compared with other mechanical milling methods, glass ball milling assisted by the shaker rotation in this study was mild and consumed low energy, but giving a much satisfactory processing performance. This could be the contribution of aqueous swelling. It was found that the treatment performance of wet milling was much better than that of dry milling (Taherzadeh and Karimi, 2008). And the enzymatic hydrolysis of microcrystalline cellulose (cellulose I) was greatly accelerated via its conversion to the cellulose II hydrate form (Zhao et al., 2006). Thus, with the assistance of water, the crystalline state of cellulose was easily changed and facilitated the simultaneous mechanical milling process. In spite of literature support, the above-mentioned assumptions should be verified in the subsequent study regarding the mechanism of CBMAS pretreatment.

3.2. Comparison of different pretreatment methods for corncob saccharification

As a lignocellulosic biomass, corncob needs pretreatment for the subsequent saccharification. Although various methods have been tested (Demiral et al., 2012), acid or alkaline hydrolysis are still considered as the effective and cost-efficient alternatives. These two methods thus were adopted to compare with CBMAS

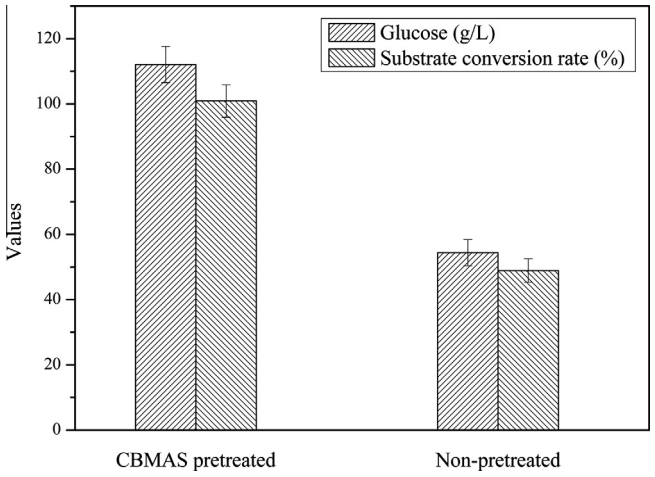

Fig. 1. Effects of CBMAS pretreatment on the enzymatic hydrolysis of microcrystalline cellulose in the flask.