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Short communication

Sugarcane bagasse hemicellulose hydrolysate for ethanol production by acid recovery process

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Abstract

In order to increase the reducing sugar concentration in the sugarcane bagasse hemicellulose acid hydrolysate and recover the acid, the acid hydrolysis was carried out in an acid recycle process and detoxification of hydrolysate was performed by electrodialysis. Two cycles of acidic treatments increased the reducing sugar concentration from 28 to 63.5 g l^{-1} and sulphuric acid consumption decreased to 0.056 g g^{-1} bagasse. After treatment by electrodialysis, 90% of acetic acid in hydrolysate was removed and the recovery ratio of sulphuric acid was 88%. The pretreated hydrolysates, supplemented with nutrient materials, were fermented to ethanol using *Pachysolen tannophilus* DW06. A batch culture with pretreated hydrolysate as substrate was developed giving 19 g ethanol l^{-1} with a yield of 0.34 g g^{-1} sugar and productivity of 0.57 g l^{-1} h^{-1}.

Keywords: Acid recovery; Ethanol; Fermentation; Hemicellulose; Hydrolysate; Sugarcane bagasse

1. Introduction

For large-scale biological production of fuel ethanol, it is desirable to use cheaper and more abundant substrates and, consequently, lower its high price which has hindered the use of fuel ethanol in the energy industries. Lignocellulose is considered as an attractive raw material for the production of fuel ethanol, because of its availability in large quantities at low cost [1,2]. The efficient utilization of the hemicellulose component of lignocellulosic feedstock offers an opportunity to reduce the cost of fuel ethanol production greatly [3].

Amongst the various agricultural crop residues, sugarcane bagasse is the most abundant agricultural material in Southern China. The cellulosic and hemicellulosic fractions can be hydrolyzed to sugars, which eventually could be fermented to ethanol. The ideal organism for the production of ethanol would be one that can utilize pentose and hexose sugars generated by lignocellulose hydrolysis. The best-known alcohol fermenting organism, *Saccharomyces cerevisiae* is capable of fermenting only hexose sugars to ethanol. However, pentose fermenting organisms are limited. Among xylose fermenting yeasts, *Pachysolen tannophilus* is promising for ethanol production using acid and enzymatic hydrolysates from lignocelluloses [4].

The hemicellulose fraction of sugarcane bagasse contains up to 35% of the total carbohydrate that can be readily hydrolyzed to monomeric sugars by dilute sulphuric acid [5]. However, the concentration of reducing sugar in hydrolysate is relatively low due to high liquid/solid ratio during the acid hydrolysis. So the hydrolysate is needed to concentrate before fermentation. During acid hydrolysis, an assortment of microbial inhibitors are produced that can be removed by treatment with lime [6]. During overliming, sulphuric acid and acetic acid (HAC) are removed from the initial hydrolysate by adding lime to adjust the pH and precipitation as gypsum. A potential drawback of overliming is that the acid cannot be reused any more because it has become to salt [7].

Electrodialysis (ED) is an electrochemical separation process in which electrically charged membranes and an electrical potential difference are applied to separate ionic species from an aqueous solution and other uncharged components. Recently, ED has been widely applied not only in the demineralization of

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natural water, desalination of saline solution and production of table salt, but also in separation of organic acids such as lactic, citric, acetic acid and their salts in bio-separation processes [8–10]. In our previous report [11], ED pretreatment was applied to remove acetate in the glycerol broth (pH 2), where acetate was a inhibitor in 1,3-propanediol fermentation with glycerol broth as a substrate. After the pretreatment, acetate in glycerol broth could not be detected.

In this paper, sugarcane bagasse hemicellulose acid hydrolysis was carried out in an acid recycle process and detoxification of hydrolysate was performed by ED. The purpose of this study was to increase the reducing sugar concentration in the hydrolysate and recover the acid. After pretreatment, the hydrolysate was used for ethanol fermentation using *P. tannophilus* DW06. This research can provide important information on the commercial utilization of sugarcane bagasse for industrial scale ethanol production.

2. Materials and methods

2.1. Hemicellulose hydrolysate preparation

Sugarcane bagasse from Guangxi province in Southern China was used as raw material. Particles in the size ranged from 0.45 to 0.9 mm (20–40 mesh) were used in the experiments. The biomass at a solid loading of 10% (w/w) was mixed with dilute sulphuric acid (1.25%, w/w) and pretreated in an autoclave at 121 °C with residence time of 2 h. The liquid fraction was separated by filtration and the unhydrolysed solid residue was washed with 20 ml warm water (60 °C). The filtrate and wash liquid were pooled together and then used in the next stage acid hydrolysis, in order to raise the reducing sugar concentration in the solution. Before every stage subsequent to the first one, the concentration of sulphuric acid was determined by sodium hydroxide titration with phenolphthalein as indicator. The sulphuric acid is reintegrated to keep the same concentration and liquid/solid ratio as those in first stage.

2.2. Detoxification by electrodialysis

Hemicellulose acid hydrolysate was heated to 100 °C, and then maintained for 15 min to remove or reduce the volatile components. The hydrolysate was filtered to remove insolubles. The filtrate was treated using an electrodialysis device that was described in our previous report [11]. The electrodialysis operation was carried out at 20 V. The flow rates in the dilute and concentrated compartments were kept at 50 l h^{-1}.

2.3. Microorganism and culture medium

P. tannophilus DW06 was grown on the preculture medium containing 5 g KH_2PO_4 l^{-1}, 2 g $(NH_4)_2SO_4$ l^{-1}, 1 g peptone l^{-1}, 3 g yeast extract l^{-1}, 0.2 g $MgSO_4{\cdot}7H_2O$ l^{-1}, 20 g xylose l^{-1}. The pretreated sugarcane bagasse hemicellulose hydrolysate, adjusted to pH 5 with 3 M NaOH and supplemented with 5 g KH_2PO_4 l^{-1}, 2 g $(NH_4)_2SO_4$ l^{-1}, 0.2 g $MgSO_4{\cdot}7H_2O$ l^{-1}, 1 g peptone l^{-1}, 5 g yeast extract l^{-1}, was used as the fermentation medium. The seed cells for the bioreactor were prepared in 500 ml flasks containing 100 ml preculture medium. The flasks were incubated at 30 °C for 14 h and subsequently inoculated into the bioreactor at 5% (v/v). The batch cultivations were conducted in a 1000 ml stirred-vessel bioreactor (Biostat Q1000, B. Braun, Germany) containing 750 ml fermentation medium under 0.1 vvm air flow. The pH was controlled at 5 by automatic addition of 3 M NaOH and all fermentation experiments were carried out at 30 °C and 150 rpm.

2.4. Analytical methods

The liquid samples were analyzed by HPLC, equipped with UV and RI detectors. The concentrations of glucose, xylose, galactose, mannose and arabinose were determined using refractive index detector and Aminex HPX-87P column at 85 °C with H_2O as mobile phase at 1 ml min^{-1}. Cellobiose, and acetic acid and ethanol were analyzed using refractive index detector and Aminex HPX-87H column at 65 °C with 5 mM H_2SO_4 as mobile phase at 0.8 ml min^{-1}. Furfural was detected on UV chromatograms at 250 nm. Cell growth was monitored at 600 nm and converted to cell dry weight (CDW) by an appropriate calibration curve.

3. Results

3.1. Sugarcane bagasse acid hydrolysis and detoxification

The action of sulphuric acid on bagasse hydrolysis is as a catalyst, so in principle no sulphuric acid should be consumed during this process. However, the acid had to be reintegrated in an acid recycle owing to unavoidable losses (absorption by bagasse, salification of inorganic cations and dilution with washings). In this experiment, six stages of acidic recycle treatments were carried out. The composition of the hemicellulose acid hydrolysate in the first stage was 17.1 g xylose l^{-1}, 7.2 g glucose l^{-1}, 2 g arabinose l^{-1}, 0.5 g cellobiose l^{-1}, 0.9 g galactose l^{-1}, 0.3 g mannose l^{-1}, 4 g acetic acid l^{-1}, 1.4 g furfural l^{-1}. With the increase of hydrolysis stage, the concentration of reducing sugar increased. However, a significant decrease in hydrolysis efficiency was observed at the fourth stage treatment and only 4.3 g xylose l^{-1} was increased in the hydrolysate. So two cycles of acidic treatments could be reasonable. Compared with sulphuric acid consumption of 0.125 g g^{-1} bagasse in the single stage acid hydrolysis, the consumption of sulphuric acid in two cycles of acidic treatments decreased to 0.056 g g^{-1} bagasse, saved by 55%. In the third stage, the composition of the hemicellulose acid hydrolysate was 45 g xylose l^{-1}, 9.3 g glucose l^{-1}, 4.8 g arabinose l^{-1}, 1.3 g cellobiose l^{-1}, 2.4 g galactose l^{-1}, 0.7 g mannose l^{-1}, 8.4 g acetic acid l^{-1}, 2 g furfural l^{-1}. The overall xylose yield was about 52% of the theoretical xylose content of the original bagasse.

The main problem encountered when treating the lignocellulose with acids is the formation of furan derivatives and other unidentified toxic products. This is particularly true in the case