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Short Communication

Evaluation of nonionic adsorbent resins for removal of inhibitory compounds from corncob hydrolysate for ethanol fermentation

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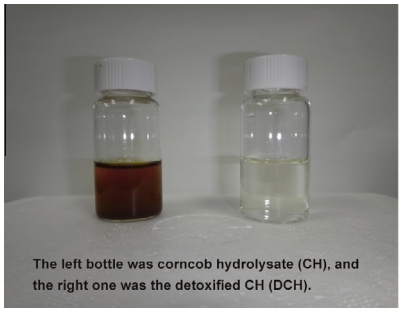
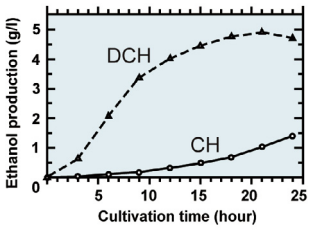
GRAPHICAL ABSTRACT





Fermentation profile regarding the production of ethanol by *Saccharomyces cerevisiae*.

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ABSTRACT

The aim of this study was to investigate the effect of XAD4-column treatment on removal of several fermentation inhibitors from corncob hydrolysate (CH). From analysis using a model hydrolysate, more than 99% of 5-hydroxy-methyl furfural, furfural and vanillin were removed by this treatment, and more than 97% of the total xylose, glucose and arabinose remained in the detoxified CH (DCH). The resulting DCH was tested as a substrate for ethanol production by *Saccharomyces cerevisiae* and *Pichia stipitis*. The highest ethanol levels for *S. cerevisiae* were 1.40 and 4.92 g l^{-1} in CH and DCH, respectively. For *P. stipitis*, the levels were 0 and 4.73 g l^{-1} in the CH and DCH media, respectively. The levels of alcohol volumetric productivity in the DCH medium were 0.374 and 0.200 g l^{-1} h^{-1} for *S. cerevisiae* and *P. stipitis*, respectively.

1. Introduction

The increasing interest in the production of fuels and chemicals by fermentation has led to investigations into the use of a compound made of wood and agro-industrial lignocellulosic-residue hydrolysates as a fermentation substrate. The hemicellulosic fraction of these hydrolysates contains xylose as a major sugar component, and the xylose can be recovered from the hydrolysates in good yields (Dominguez et al., 1997). These hydrolysates are suitable feedstocks for the production of value-added products, such as xylitol (Roberto et al., 1991), ethanol (Gulati et al., 1996), *n*-butanol (Maddox and Murray, 1983) and 2,3-butanediol (Frazer and McCaskey, 1989). The biomass hydrolysis can be performed by many pretreatments, such as concentrated acid hydrolysis, dilute acid hydrolysis, alkali treatment, sodium sulfite treatment, sodium chlorite treatment, steam explosion, ammonia fiber explosion, lime treatment and organic solvent treatment. Among these methods, dilute acid treatment and enzymatic hydrolysis have been the most popular (Cara et al., 2008).

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Dilute acid hydrolysis is a fast and convenient method to perform, but it leads to the accumulation of fermentation inhibitory compounds, such as 5-hydroxy-methyl furfural (HMF), urfural and phenolic compounds. These compounds can inhibit microbial cell growth and affect the specific growth rate and cell-mass yield depending on their concentration in the fermentation media (Modig et al., 2002). In order to improve the fermentability of acid hydrolysates into ethanol, several treatments have been reported for the detoxification of hydrolysates, including ion exchange (Mancilha and Karim, 2003; Chandel et al., 2007), over-liming (Chandel et al., 2007), activated charcoal adsorption (Chandel et al., 2007) and laccase oxidation treatment (Chandel et al., 2007). The chemical effects of these respective detoxification methods on the hydrolysates were analyzed by Nilvebrant et al. (2001), and they concluded that the anion-exchange resins affected all the types of inhibitors measured (phenolics, furan aldehydes and aliphatic acids), as well as the concentration of fermentable sugars in the hydrolysates. On the other hand, Mancilha and Karim (2003) evaluated the performance of ion-exchange resins on the detoxification of corn stover hydrolysate as a substrate for xylitol production. However, they revealed that most resins can remove only half of the HMF and that further inhibitory compounds need to be separated to achieve higher product yield.

In the present work, the nonionic adsorbent resin of Amberlite XAD4 was shown to be highly effective for the detoxification of corncob hydrolysate (CH), indicating the high removal efficiency of the fermentation inhibitors and the low loss of sugars in the detoxifying process. Furthermore, the detoxified CH (DCH) showed better efficiency than CH as a substrate in the ethanol fermentation.

2. Methods

2.1. Raw materials and chemicals

Amberlite XAD4 and XAD7-HP resins were purchased from Acros Organics (Morris Plains, NJ), were pretreated with 50% methanol for 1 h and were repetitively decanted with deionized water. *Saccharomyces cerevisiae* Meyen ex Hansen was purchased from Oriental Yeast Co., Ltd. (Tokyo, Japan). *Pichia stipitis* CBS 5773 was purchased from the Centraalbureau voor Schimmelcultures (CBS) fungal biodiversity center (Utrecht, the Netherlands). Corn fruit was purchased from a local market, and all corn seeds were thoroughly removed from the corncob. The resulting corncob was sufficiently washed by deionized water, was dried at 80 °C and was comminuted by an electrical blender to attain a particle size of 1–2 mm. Other chemicals and media components were of the highest purity grade and were purchased locally.

2.2. Hydrolysate treatment

Dilute acid hydrolysis was undertaken in batch mode in an S-90N autoclave (Tomy Digital Biology, Tokyo, Japan), employing 2% (w/v) sulfuric acid at 121 °C for 1 h. Prior to hydrolysis, corncobs were impregnated in acid solution at a solid–liquid ratio of 1:10. After autoclaving, the hydrolyzed corncob was filtered using an ADVANTEC paper filter No. 2 (Tokyo, Japan). Excessive calcium carbonate was added to the filtrate, and the resulting supernatant was again filtered to remove calcium sulfate and was kept at 4 °C until further experiments.

2.3. Adsorption chromatography

The concentrations of HMF, furfural and vanillin were respectively set to 0.20, 0.35 and 0.80 mg ml^{-1} by referring to the hydrolysate composition of wood scrap (Japanese patent publication, 2005-270056). The HMF solution was applied to an XAD4 or XAD7-HP column (1.4 × 14 cm; stands vertically) in upflow mode, in order to avoid the solution/water convection inside the column (Hatano et al., 2009), and the column was rinsed using 40% methanol in downflow mode. For furfural and vanillin solutions, each solution was applied to the column in upflow mode, the column was rinsed using deionized water in downflow mode, and the furfural and vanillin adsorbed on the resin were eluted using 70% methanol in the downflow direction. In order to examine whether these inhibitors could be removed simultaneously, a loading solution including these inhibitors was prepared here (a model hydrolysate). For the model hydrolysate, glucose, fructose, galactose and xylose were added as well as the inhibitors, and their concentrations were respectively set to 1.0, 0.33, 0.33 and 0.33 mg ml^{-1} (Japanese patent publication, 2005-270056). The model hydrolysate solution was applied in upflow mode, and the column was then rinsed in the downflow direction by using 70% methanol or 70% isopropanol when the absorbance at 280 nm (A_{280}) of the eluate increased to more than 0.2. For CH, the solution was applied in upflow mode, and the column tube was then flipped upside-down to avoid the sugar/water convection inside the column. The column was rinsed in the downflow direction by using deionized water until the sugar content of the column eluate fell to zero degrees Brix (°Brix), and then the column was rinsed in downflow mode by using 70% isopropanol. The sugar fractions were pooled and designated the detoxified CH (DCH).

The above-mentioned operations were performed at room temperature, and the flow rate was set to 0.8 ml min^{-1} by using a Gilson Minipuls 3 peristaltic pump (M&S Instruments, Osaka, Japan). HMF, furfural and vanillin in the column eluate were monitored by the absorbance at 284 nm (A_{284}), the absorbance at 277 nm (A_{277}) and the absorbance at 230 nm (A_{230}), respectively. For instance, the efficiency of the HMF removal was calculated by the following equation:

$$\text{removal efficiency}(\%) = 100(A_{\text{total}} - A_{\text{through}})/A_{\text{total}} \quad (1)$$

where A_{adsorb} is the sum of the products (ml × A_{284}) of the fraction volume (2.4 ml) and the value of the absorbance at 420 nm (A_{420}) in each flow-through fraction from the column, and A_{total} is the product of the loading dose (400 ml) and its A_{284} value (25.3).

2.4. Fermentation

P. stipitis was maintained on an agar slant containing the following: xylose, 40.0 g l^{-1}; Bacto yeast extract (Becton, Dickinson and Co., Sparks, MD), 4.0 g l^{-1}; Bacto Tryptone (Becton, Dickinson and Co.), 5.0 g l^{-1}; agar, 15.0 g l^{-1}. The preculture medium for *S. cerevisiae* was composed of the following: glucose, 20.0 g l^{-1}; Bacto yeast extract, 20.0 g l^{-1}; Bacto Tryptone, 10.0 g l^{-1}; on the other hand, that for *P. stipitis* was composed of Bacto yeast extract (3.0 g l^{-1}), Bacto Tryptone (5.0 g l^{-1}) and KH_2PO_4 (2.5 g l^{-1}) with xylose (30.0 g l^{-1}) as an additional carbon source. All media were adjusted to pH 4.5 by 1 M NaOH or HCl, and yeasts were grown in a 100-ml Erlenmeyer flask that contained 50 ml of the sterilized medium and was plugged by cotton. The preculture was conducted in an SB-20 Shaking Bath with a Thermal Robo TR-1A (AS ONE, Osaka, Japan) at 140 rpm and 30 °C. After the preculture, fungus cells were collected by centrifugation at 1500*g* and were stored at 4 °C. The correlation between the cell number and optical density at 660 nm (OD_{660}) was examined using a OneCell Counter (OneCell, Hiroshima, Japan), and the cell concentration in the CH or DCH medium was standardized using OD_{660} to 4.9×10^5 cells ml^{-1} (OD_{660} = 0.320) for *S. cerevisiae* and 1.7×10^7 cells ml^{-1} (OD_{660} = 0.337) for *P. stipitis*. The main cultures for *S. cerevisiae* and *P. stipitis* were respectively performed in the shaking incubator