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Xylitol production from D-xylose and horticultural waste hemicellulosic hydrolysate by a new isolate of *Candida athensensis* SB18

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ABSTRACT

This paper describes the production of xylitol from D-xylose and horticultural waste hemicellulosic hydrolysate by a new strain of *Candida athensensis* SB18. Strain SB18 completely consumed 250 and 300 g L^{-1} D-xylose and successful converted it to xylitol in the respective yield of 0.83 and 0.87 g g^{-1}, resulting in 207.8 and 256.5 g L^{-1} of xylitol, respectively. The respective volumetric productivity were 1.15 and 0.97 g L^{-1} h^{-1}. Approximately 100.1 g L^{-1} of xylitol was obtained from the bioconversion of detoxified horticultural waste hemicellulosic hydrolysate using strain SB18. The yield and productivity were 0.81 g g^{-1} xylose and 0.98 g L^{-1} h^{-1}, respectively. Strain *C. athensensis* SB18 was able to completely utilize glucose, mannose, xylose and partially arabinose. This work demonstrates that stain *C. athensensis* SB18 is a promising strain for high-titer and high-yield xylitol production and it has great potential in bioconversion of hemicellulosic hydrolysate.

1. Introduction

As a natural occurring five carbon alcohol sugar, xylitol is a high-value polyalcohol produced by the reduction of D-xylose derived from hemicellulose fraction of lignocellulose. As an alternative sugar, owning the properties of low energy and inhibition against the metabolism of dental plaque formation, xylitol was widely used in oral hygiene and pharmaceutical products to reduce tooth decay and ear infection (Makinen, 2000). Currently, xylitol is widely used in chewing gums as a sweetener due to its high sweetening power.

Xylitol can be produced by catalytic hydrogenation of D-xylose from hemicellulosic hydrolysates (Aminoff et al., 1978) or be produced by some xylose-utilizing microorganisms as a natural metabolic intermediate (Chang and Knight, 1960; Antti et al., 2005). Microbial xylitol production is more favorable for industrial applications because it can be conducted under mild conditions such as atmospheric pressure and ambient temperature. In addition, microorganisms can be metabolically engineered to convert most of the reducing sugars in hemicellulosic hydrolysates to xylitol in a single step. Recently, considerable attention has been drawn to the bioconversion of xylose into xylitol (Rao et al., 2006; Rodrigues et al., 2006; Canilha et al., 2008; West, 2009). As far as the commercial application and industrial scale-up are concerned, high substrate concentration is very critical for a cost-effective xylitol production process. It is essential that the microorganisms are tolerant to the high osmotic pressure of the substrates and are resistant to product inhibition. Many efforts have therefore been made in strain improvement for xylitol-producing microorganisms (Bae et al., 2004; Antti et al., 2005; Ko et al., 2006). While genetic engineering is an effective method for strain improvement, it is time-consuming, requiring high level of techniques and complicated operating procedures. In addition, the engineered strains may probably have defective metabolism and characteristics due to the change of intracellular structure of the strains. The optimization of the fermentation conditions is therefore very restricting in the improvement of product yield due to the limitations of the natural potential of the strains. On the other hand, screening of natural occurring microorganisms with high xylitol yield is a promising approach to obtain a potential xylitol-producing strain for industrial applications.

Horticultural waste (HW) refers to tree trunks and branches, plant parts, and trimmings generated during the maintenance and pruning of trees and plants. It is the most common type of woody waste available in Singapore and more than 229,300 tonnes of horticultural waste was generated in 2008, while the recycling rate was only 42.4% (www.zerowastesg.com). The reutilization of HW could not only help solve pollution problems arisen from

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incineration and land filling but also could create their added value. HW is a potential raw material for cellulosic ethanol production. However, in order to enhance its competitiveness, one possibility is the utilization of xylose-rich hemicellulosic hydrolysate for production of value-added chemicals. Approximately, HWs are stored in the form of 34.5% cellulose, 28.6% hemicellulose and 36.0% lignin (Geng et al., 2011). The hemicellulose hydrolysate of HW can be obtained through dilute acid hydrolysis or organosolv fractionation. Such HW hemicellulosic hydrolysate can be further utilized through bioconversion to produce value-added chemicals, such as xylitol.

Strain SB18 was isolated from Singapore soil samples and it was identified through 18S rDNA sequence information as *Candida athensensis* (GenBank Accession No. JN683655). This study explores the potential of this newly isolated strain of *C. athensensis* SB18 for xylitol production from D-xylose and organosolv fractionated HW hemicellulosic hydrolysate. To our knowledge, it is the first report on a *C. athensensis* strain for xylitol production. Effects of the key fermentation factors were investigated to optimize the cultivation conditions. Both batch fermentation and fed-batch fermentation were conducted to enhance the xylitol production efficiency. Furthermore, strain SB18 was evaluated in the bioconversion of HW hemicellulosic hydrolysate to xylitol.

2. Methods

2.1. 1. Materials

Horticultural waste (HW) was collected from a horticultural waste treatment plant, ecoWise Solution Pte Ltd. The collected HW was firstly washed using tap water to remove the dirt and soil and then immediately dried at 80 °C overnight. They were then cooled down to room temperature and stored in sealed plastic bags until use. Before organosolv fractionation, the dried HW was mechanically milled using a lab mill (Ultra Centrifugal Mill ZM 200, Retsch GmbH, Germany) and sieved through standard mesh sieves using a Cisa® Sieve Shaker model RP09 (Barcelona, España) to obtain the powder of 200–500 μm particle sizes. All chemicals were of analytical grade and obtained from Sigma–Aldrich (St. Louis, MO, USA) unless otherwise stated. Distilled water was used throughout, except for HPLC analysis where Millipore water was used.

2.2. Microorganisms and cultivation media

C. athensensis SB18 was maintained in yeast extract–peptone–xylose (YPX) agar slants containing (g L^{-1}) yeast extract (Acumudia, USA), 10; peptone, 20; xylose 20; and agar–agar (MP Biomedicals, France), 20 at 4 °C. Yeast nitrogen base–yeast extract (YNB–YE) medium containing (g L^{-1}) yeast nitrogen base (YNB, USBiologicals), 6.76; yeast extract (YE), 1.0 and xylose, 20, was used for seed culture preparation. Yeast nitrogen base–yeast extract–urea (YNB–YE–urea) medium containing (g L^{-1}) yeast nitrogen base (YNB, USBiologicals), 6.76; yeast extract (YE), 1.0 and urea, 2.0, supplemented with predefined amount of xylose was used for xylitol production.

2.3. Shake-flask xylitol production

Shake-flask xylitol production was carried out in 100 mL Erlenmeyer flasks containing 30 mL cultivation medium. Seed culture was prepared by inoculating loopfuls of yeast cells stored from the YPX slants into 100 mL Erlenmeyer flasks containing 30 mL of YPX–YE medium incubating at 30 °C and 250 rpm for 24 h. Cells were then collected and centrifuged at 12,000×*g* for 10 min at 4 °C. Cell pellets were washed twice with sterile water and stored at 4 °C for the use as inoculums. Flasks were inoculated with an inoculum size of 0.5 g L^{-1} and incubated at 30 °C for 4–7 days. Fermentation experiments were conducted at varied nitrogen source, shaking speed, and initial xylose concentration. Stages of fermentation were also studied. Samples were withdrawn periodically to determine the cell density at 600 nm (OD_{600}) and the concentration of residual substrates and products.

Typical batch fermentation was conducted in 500 mL Erlenmeyer flasks with an overall 100 mL YNB–YE–urea medium containing 250 g L^{-1} xylose with an inoculum size of 0.5 g L^{-1}. Flasks were incubated at 30 °C and 200 rpm for 36 h and then at 100 rpm for the remaining time. Samples were withdrawn periodically to measure xylose and xylitol concentration and the cell biomass density.

Xylitol yield was calculated by examining the residual xylose concentration and the final xylitol concentration. Fermentative parameters were calculated and compared. All experiments were performed in duplicate and the average data are reported below along with the corresponding standard deviations (SD).

2.4. Batch bioreactor xylitol production

Batch xylitol production were performed in a BIOSAT® 2-L Microbial Bioreactor (Sartorius Stedim Biotech, France) containing 1.5 L YNB medium supplemented with 250 g L^{-1} xylose and the chosen nitrogen sources. Seed culture from the 24-h culture of *C. athensensis* SB18 was inoculated to the fermentation medium with an inoculum size of 0.5 g L^{-1}. The fermentation temperature was maintained at 30 °C. The airflow was maintained at 2.0 L min^{-1} with an agitation speed of 250 rpm for the first 24 h for cell growth. The agitation speed was then switched to 200 rpm and the air flow rate was adjusted to 0.7 L min^{-1} for the remaining time for xylitol accumulation. Foaming of the culture medium was minimized by addition of BioSpumex 36 K antifoam (Cognis, Cincinnati, OH, USA). Samples were withdrawn periodically to measure xylose, xylitol and cell biomass concentration.

2.5. Fed-batch bioreactor xylitol production

Fed-batch xylitol production was performed in the BIOSAT® 2-L Microbial Bioreactor (Sartorius Stedim Biotech, France) initially containing 1.6 L YNB medium supplemented with 187.5 g L^{-1} xylose. The preparation of seed culture and the culture conditions such as nitrogen sources, agitation speed, inoculum size, pH, temperature, and aeration rate, were the same as those of batch fermentation except that the cell growth phase was controlled to 48 h. At 96 and 168 h, 200 mL of concentrated xylose solution (750 g L^{-1}) was successively added to the bioreactor under aseptic conditions. The total xylose concentration for fed-batch fermentation was 300 g L^{-1} and the overall fermentation volume was 2 L. Samples were withdrawn periodically to measure xylose, xylitol and cell biomass concentration.

2.6. Organosolv fractionation of horticultural waste and detoxification of hemicellulosic hydrolysate

Horticultural waste was fractionated using a mild-condition organosolv pretreatment method with slight modifications (Geng et al., 2011). Horticultural waste powder (200–500 μm) was firstly soaked in water at room temperature overnight and then fractionated using 70% ethanol in the presence of 1% H_2SO_4 (solid–liquid was 1:8 (w/v)) at 65 °C for 4 h. The slurry was filtered through filter paper (Whatman No. 1 filter paper) and the black liquor was collected. Ethanol was removed and the black liquor was concentrated by vacuum evaporation. After removal of the precipitate, the pH of the raw hydrolysate was increased to pH 10 using