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Efficient ethanol production from inulin by two-stage aerate strategy

Jiaoqi Gao [a], Wenjie Yuan [a,*], Liang Kong [b], Ruijuan Xiang [a], Shijun Zhong [a]

[a] School of Life Science and Biotechnology, Dalian University of Technology, Dalian 116023, China
[b] College of Marine Technology and Environment, Dalian Ocean University, Dalian 116023, China

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ABSTRACT

A major concern for ethanol production from inulin-containing materials, is the higher unconverted sugar, which increases the cost of ethanol production and wastewater treatment. Some key factors, such as inulinase, biomass or aeration rates, were studied to solve the problems in the process of ethanol fermentation from inulin. It was showed that more inulinase and increasing inoculum size can shorten the fermentation time, but could not reduce residual sugars. Two-stage aerate strategy was developed to utilize the remained sugars: keep the aeration at 5 h^{-1} at the first 12 h, and drop it to 1.2 h^{-1}. Under this condition, contradiction between fermentation time and high ethanol yield was solved (60 h and 0.43 g g^{-1}), and the final residual sugar concentration decreased to about 10 g L^{-1} with 98 g L^{-1} ethanol. The ethanol productivity was up to 1.63 g L^{-1} h^{-1}, which is the highest productivity of ethanol fermentations from inulin-containing materials.

1. Introduction

Sustainable development and environmental protection requires renewable biological energies instead of non-renewable fossil energies, which may be exacerbated for countries like China with much more energy consumption. Biofuels, as the significant substitutions for fossil energy, will be the main solution for the development of energy industry [1]. Fuel ethanol is the earliest and most mature biofuel product so far and widely considered as one of the most promising biomass energies [2].

Production of ethanol from starch-based materials is not suitable for China with large population and insufficient grain, consequently, fuel ethanol from non-grain materials is going to be the solutions to energy problems considering the increasing prices of grain and other concerns on food security, or land-use [3,4]. Inulin is a linear biopolymer made up of fructose residues linked by β-2,1 bonds [5], which can be hydrolyzed into fructooligosaccharide or single-fructose by acid, or inulinase and be further transformed into other products [6]. Inulin, as the main reserve carbohydrate, can be stored in the roots and tubers of some plants, such as Jerusalem artichoke, chicory, dahlia, and yacon [5,6]. All these inulin-containing materials, especially, Jerusalem artichoke, have great potential for biofuels production because of the advantages of high yield, resistance to poor soil, drought, cold temperature and pests [7].

Consolidated bioprocessing (CBP) technology combined inulinase production, inulin hydrolysis and ethanol

* *Corresponding author*. Tel./fax: +86 41184706308.
E-mail address: ywj@dlut.edu.cn (W. Yuan).
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production, which had very outstanding advantages in the process of ethanol production [6,8]. Recently, more works focused on strains screening with inulinase activity, optimization of the fermentation conditions and modified the strains by over expression the inulinase gene (*INU1*) to improve the ethanol production from inulin-containing materials by CBP technology [9–14]. Some serious problems in the process of ethanol production, such as the long fermentation time, lower ethanol yield and the higher concentration of residual sugar at the end of the fermentation, have not been solved so far at home and abroad.

Commonly, ethanol is produced under non-aeration, but aeration can increased the cell viability, inulinase activity and cell density. Inulinase activity, the most important factor for the process of CBP, can be influenced by aeration rates and biomass concentration at the same time [15,16]. Unfortunately, it was paid little concerned during the ethanol fermentation [6,13,17,18].

Consequently, in this article, the key factors, such as, the aeration, inulinase activity, and the concentration of biomass were adopted to conduct the fermentation. Inulin, as the main carbon source for fermentation, is supposed to be convenient for detecting the growth and viability of cells. Therefore, this study might achieve the pivotal explanation for solving the problems in the ethanol production from inulin-containing materials and to promote the development of the industrialization.

2. Materials and methods

2.1. Microorganisms and culture mediums

Kluyveromyces marxianus Y179 was deposited at China Center for Type Culture Collection (CCTCC) with a reference number of M202031. Cultures were maintained on YPD medium (20 g L^{-1} glucose, 20 g L^{-1} peptone, 10 g L^{-1} yeast extract) agar slants at 4 °C for routine use. For long-term preservation, cultures were stored at −80 °C in 20% glycerol. YPD medium was used for preparation of the inoculum. The high-performance inulin (HP-inulin, Orafti), as the main carbohydrate in Jerusalem artichoke tubers was utilized to conduct the fermentation experiments in this study, and the fermentative medium was composed of (g L^{-1}): inulin 235, peptone 20, yeast extract 10 and had a nature pH, then sterilized at 115 °C for 15 min.

2.2. Culture conditions

For preparation of the inoculum, the yeast was cultivated in 100 cm^3 of YPD medium in 250 cm^3 flasks at 30 °C with orbital shaking at 2.5 Hz for 16–18 h. Then the cultures were inoculated into a 3 L fermenter with a 1.0 L working volume at 30 °C and 2.5 Hz. For investigating effects of the aeration rate on the fermentation by Y179 via CBP, 0, 5 or 10 h^{-1} (space velocity) was controlled in the experiments. To examine the effect of the more inulinase, 10 U cm^{-3} of inulinase, which was kindly presented by Professor Du from a recombinant *K. marxianus* [14], was added into the initial medium (Strategy 1, S1). In the experiment of increased biomass concentration, cells grown in 1.0 L YPD medium was collected, after washing the cells with distilled water, were inoculated into the fermentation medium (Strategy 2, S2). Double two-stage aerate strategies were used in the experiments: 1) keep the aeration rate at 5 h^{-1} at the first 12 h of fermentation, and then drop it to 1.2 h^{-1} until the end (Strategy 3, S3); 2) keep the aeration rate at 5 h^{-1} at the first 12 h of fermentation, and then drop it to 0.12 h^{-1} until the end (Strategy 4, S4). (Fig. 1)

2.3. Analytical methods

Cells concentration was measured using optical density at 620 nm. Concentrations of the reducing sugar were determined by the dinitrosalicylic acid method [19], and the total sugar was firstly hydrolyzed by 0.2 mol L^{-1} H_2SO_4 at 100 °C for 1 h, then measured by methods of Miller after adding equivalent amount of 0.4 mol L^{-1}NaOH.

Inulinase activity was measured as described by Parekh & Margaritis [20]. Culture broth samples were centrifuged at 6000 g for 10 min. The supernatant was then collected and diluted appropriately with distilled water, and subjected to inulinase activity assay. Briefly, 0.5 cm^3 culture supernatant was incubated with 2% inulin prepared in 0.02 mol L^{-1} sodium acetate buffer (pH 4.6) at 55 °C for 10 min, and the reducing sugar was analyzed by the dinitrosalicylic acid method [19]. One enzyme unit was defined as the amount of fructose (μmol) hydrolyzed per min under the above conditions [20]. Fructose was used as the standard substance to plot a standard curve.

The ethanol was analyzed by gas chromatography (Agilent 6890A, USA) as previously described [21]. Ethanol lost because of evaporation was absorbed by distilled water, and then measured by gas chromatography. Therefore, concentration of ethanol in this article was the sum of ethanol in broth and ethanol dissolved in distilled water. Glycerol and acetic acid was determined by HPLC (Waters 1525) as described before [22]. Briefly, an organic acids analysis column (Aminex HPX-87H, 300 mm × 7.8 mm) and photodiode array detector were used, operating at 50 °C and room temperature respectively. The mobile phase was 0.005 mol L^{-1} H_2SO_4 with a flow rate of 0.50 cm^3 min^{-1}.

Duplicate analysis was applied to biomass, sugars, ethanol and by-products, and the mean values were given in the results.

3. Results and discussion

3.1. Impact of aeration rates on the ethanol production by Y179

Batch fermentation was carried out in the medium containing about 230 g L^{-1} inulin at the aeration rates of: 0.0, 5 and 10 h^{-1}. As is shown in Fig. 1, biomass concentration increased as the time extended and reached the maximum at about 30 h, then kept stable or decreased a little, which were similar among the three aeration rates. But as the aerate rate increased, the maximum of biomass also enhanced, 10 OD at 10 h^{-1} and only 4.03 OD at 0.0 h^{-1}. Total sugars in the medium fell down quickly, but it kept over 30 g L^{-1} and did not decrease any more under anaerobic condition; on the contrary, the total sugar