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Enhanced butanol production by solvent tolerance *Clostridium acetobutylicum* SE25 from cassava flour in a fibrous bed bioreactor

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HIGHLIGHTS

- AquaMats AO was used as the immobilization carrier in ABE fermentation.
- The butanol-producing ability of the producing strain SE25 was fairly stable.
- The FBB system is a promising approach for enhancing the butanol productivity.
- The correlation between the biofilm and solvent tolerance was investigated.

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ABSTRACT

To enhance the butanol productivity and reduce the material cost, acetone, butanol, and ethanol fermentation by *Clostridium acetobutylicum* SE25 was investigated using batch, repeated-batch and continuous cultures in a fibrous bed bioreactor, where cassava flour was used as the substrate. With periodical nutrient supplementation, stable butanol production was maintained for about 360 h in a 6-cycle repeated-batch fermentation with an average butanol productivity of 0.28 g/L/h and butanol yield of 0.32 g/g-starch. In addition, the highest butanol productivity of 0.63 g/L/h and butanol yield of 0.36 g/g-starch were achieved when the dilution rate were investigated in continuous production of acetone, butanol, and ethanol using a fibrous bed bioreactor, which were 231.6% and 28.6% higher than those of the free-cell fermentation. On the other hand, this study also successfully comfirmed that the biofilm can provide an effective protection for the microbial cells which are growing in stressful environment.

1. Introduction

Due to the increasing concerns over the environmental issues associated with the impact of petroleum fuel emissions and the decreasing of fossil fuel reserves, production of alternative fuels such as butanol and ethanol by fermentation has drawn worldwide attention (Zheng et al., 2013). Amongst these biofuels, butanol has many advantages compared to ethanol, such as higher boiling point, higher energy content, less corrosive and a reduced need to modify current combustion energies. Therefore, butanol is regarded as one of the most appropriate candidates for biofuels. In addition, butanol is a multipurpose chemical feedstock which has also been extensively used in plastic and food industries. Commercial butanol fermentation processes have triggered increasing attention by some companies (such as DuPont and BP) (Lepiz-Aguilar et al., 2013; Li et al., 2016). Some solventogenic *Clostridium,* including *Clostridium saccharoperacetobutylicum*, *Clostridium saccharobutylicum*, *Clostridium beijerinckii* and *Clostridium acetobutylicum*, are used as butanol producing strains during acetone, butanol, and ethanol (ABE) fermentation. These four species can be roughly divided into two classes such as starch and sugar species according to the utilization of carbon sources. Furthermore, it was believed *C. acetobutylicum* and *C. beijerinckii* are the major butanol producing strains in present ABE fermentation industries (Keis et al., 2001; Patakova et al., 2013). Butanol production in ABE fermentation processes can be divided into two phases, i.e., acidogenic phase and solventogenic phase. Moreover, some studies showed that the phase shift (from acidogenesis to solventogenesis) can be trigged if the pH value is below 5 and the concentration of butyric acid is over 2 g/L (Cheng et al., 2012; Li et al., 2014b). However, practical ABE fermentation processes are often extremely complicated and difficult to being controlled. Consequently, fur-

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ther system modifications for these processes are inevitably required (Chauvatcharin et al., 1998).

Presently, the major obstacles associated with ABE fermentation include conversion efficiency of substrate to product, product toxicity (especially butanol) to the producing strains, the potential for culture degeneration, and the ability to utilize cheaper raw materials or renewable agricultural wastes as the substrates, which resulted in the production of butanol from ABE fermentation less competitive when compared with the petroleum-based butanol production (Qureshi et al., 2014). Furthermore, economic analysis of ABE fermentation processes showed that the separation cost would be reduced and led to an economically viable process if the final butanol concentration has been slightly increased (Ting et al., 2012). Therefore, in order to enhance the economic competitiveness of biobutanol production, the improvement of substrates, microbial strains and processes are the key issues of priority research. Diverse methods such as solvent tolerant microorganisms screening, genetic engineering, metabolic engineering, modern fermentation techniques and downstream processing have been constantly used to surmount the above hampers (Ezeji et al., 2010). Amongst those methods, fed-batch fermentation coupling with an immobilization technology, is a relatively simple and an economic bioprocess, and worth to maximize the butanol production as exhibited herein this study.

In traditional ABE fermentation, corn starch or glucose were mostly used as the major feedstocks. As the case stands, the fermentation substrate cost, which account for 60–70% of the total production cost, is the most influential factor in ABE fermentation (Qureshi and Blaschek, 2000). Therefore, it is necessary to explore inexpensive raw materials for making ABE fermentation more economically attractive. Cassava, a non-cereal starchy crop, can widely grow in harsh climates and poor soils. In 2010, the worldwide yield of cassava was 242 million tons, especially in China, the cassava output was about 7.3 million tons which accounted for about 3.0% of the current global cassava production (Li et al., 2015). In addition, cassava is regarded as a nonfood crop in China, and the concerns for food security would thus be minimized. Therefore, cassava represents an alternative cheap substrate for ABE fermentation, which is attractive and promising in both geographical and economic considerations.

In this study, in order to enhance the butanol productivity and reduce the production cost, continuous butanol production using a high butanol tolerant strain *C. acetobutylicum* SE25 with cassava flour as the substrate was carried out in a fibrous bed bioreactor (FBB) system. AquaMats AO was firstly used as an immobilized material for the ABE fermentation processes. And the effect of various dilution rates on solvent production in continuous ABE fermentation was then investigated. In addition, the correlation between biofilm and butanol tolerance in *C. acetobutylicum* was also evaluated. To the best of the authors' knowledge, little literatures has been reported on the correlation between biofilm and butanol tolerance in *C. acetobutylicum*. Consequently, the results presented herein also would provide a valuable reference for further elucidating the solvent tolerance mechanisms of other microorganisms.

2. Materials and methods

2.1. Microorganism and medium

The solvent tolerance strain *C. acetobutylicum* SE25, derived from *C. acetobutylicum* PW12, was isolated from the waste water of Daqing Oilfield Company in North China and used as the producing strain. The cell suspension was routinely maintained in 20% (v/v) of steriled glycerol at −80 °C in screw-capped bottles. SE25 cell suspension in a glycerol tube was heat-shocked for 90 s at 80 °C and then cooled to room temperature on ice. The heat shocked cell suspension was transferred into an anoxic sterilized tryptone-yeast extract-acetate medium (TYA medium) at 37 °C for 16–18 h. Following growth, 10–15 mL of activated cultures was inoculated into 250 mL screw-capped bottles containing 100 mL of fermentation medium (Li et al., 2014a).

The developed P2 medium contained the following substances in per liter of distilled water: 60 g glucose, 3 g yeast extract, 1 mL of filter-sterilized stock solutions (vitamin: 0.1 g thiamin, 0.001 g biotin, 0.1 g para-aminobenzoic acid, mineral: 1 g $MnSO_4{\cdot}H_2O$, 1 g NaCl, 20 g $MgSO_4{\cdot}7H_2O$, 1 g $FeSO_4{\cdot}7H_2O$, and buffer: 220 g ammonium acetate, 50 g K_2HPO_4, 50 g KH_2PO_4) was added into 1 L of P2 medium. Minerals and vitamins were filter-sterilized through 0.22 μm sterilized membrane filters. Buffer, nitrogen and carbon sources were separately sterilized by autoclaving at 121 °C for 15 min (Li et al., 2013).

The developed TYA medium comprised of the following substances in per liter of distilled water: 40 g glucose, 6.0 g tryptone, 2.0 g yeast extract, 0.2 g $MgSO_4{\cdot}H_2O$, 0.01 g $FeSO_4$, 3.0 g CH_3COONH_4, 0.75 g K_2HPO_4 and 0.75 g KH_2PO_4. The medium was sterilized by autoclaving at 121 °C for 15 min (Loyarkat et al., 2013).

2.2. Cassava flour pretreatment and hydrolysis

The cassava flour was pretreated and hydrolyzed according to the method reported by Yang et al. (2015). Required amounts of cassava flour were immersed in distilled water to obtain a suspension of 80 g/L cassava flour, which was used for subsequent enzymatic hydrolysis. Enzymatic hydrolysis process consisted of two steps: liquefaction and saccharification. $CaCl_2$ (1 g/L), previous to liquefaction, was added into cassava flour suspensions to obtain a final concentration of 40 mg-Ca^{2+}/L. At the same time, the pH was adjusted to 6.5 using 2 M HCl or 2 M NaOH. For liquefaction, the processes were carried out in flasks which were placed in a bath shaker (150 rpm), and α-amylase was added at a dosage of 8 U/g-cassava. The enzymatic reaction was carried out for 45 min at 100 °C, followed by cooling down to 60 °C on ice and a decrease in pH to 4.5 with 2 M HCl. Subsequently, the process of saccharification was performed by adding β-glucoamylase (120 U/g-cassava) for 4 h at 60 °C, and the enzyme was then inactivated by heating at 80 °C for 5 min after saccharification. The hydrolysis liquid was sterilized at 121 °C for 15 min at the final pretreatment and hydrolysis procedure. The sterilized hydrolysis liquefied was then used as the medium for ABE fermentation. α-amylase (20,000 U/mL) and β-glucoamylase (100,000 U/mL), which were used as the enzymatic treatment, were obtained from Boli Biological Products Co. Ltd, Taizhou, China.

2.3. Preparation of fibrous bed bioreactor (FBB) and operation

2.3.1. The construction of FBB

The FBB reactor was prepared as reported by (Silva and Yang, 1995) and the schematic drawing of FBB system was shown in Fig. 1. AquaMats AO, which was purchased from Zhongyu water ecological technology Co., Ltd in South China, was used as the immobilization carrier. AquaMats AO (300 mm × 260 mm) and stainless steel wire were coiled into a spiral cylindrical. At the same time, the clearance of each ring of stainless steel wire mesh was 3 mm. The glass column reactor (Φ 60 mm × 300 mm) was filled till to reach the height of 25–30 mm by ceramic tube glass. Afterwards, the stainless steel wire mesh was put into the glass column reactor. This reactor system was designated as FBB. The FBB and the bioreactor were separately autoclaved for 30 min, and then aseptically connected with the fermentation tank by a hosepipe after sterilization.