Journal of Biotechnology 164 (2012) 202–210

Contents lists available at SciVerse ScienceDirect

Journal of Biotechnology

journal homepage: www.elsevier.com/locate/jbiotec

Propionic acid production in a plant fibrous-bed bioreactor with immobilized *Propionibacterium freudenreichii* CCTCC M207015

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A R T I C L E I N F O

Article history:
Received 21 May 2012
Received in revised form 29 August 2012
Accepted 31 August 2012
Available online 12 September 2012

Keywords:
Propionibacterium freudenreichii CCTCC M207015
Propionic acid
Sugar cane bagasse
Plant fibrous-bed bioreactor
Cell physiological change
Metabolic flux analysis

A B S T R A C T

A plant fibrous-bed bioreactor (PFB) was constructed for propionic acid production. Sugar cane bagasse was applied to the PFB as immobilizing material. Starting at a concentration of 80 g/L of glucose, *Propionibacterium freudenreichii* CCTCC M207015 produced 41.20 ± 2.03 g/L of propionic acid at 108 h in the PFB. The value was 21.07% higher than that produced by free cell fermentation. Intermittent and constant fed-batch fermentations were performed in the PFB to optimize the fermentation results. The highest propionic acid concentration obtained from constant fed-batch fermentation was 136.23 ± 6.77 g/L, which is 1.40 times higher than the highest concentration (97.00 g/L) previously reported. Scanning electron microscopy analysis showed that cells exhibited striking changes in morphology after PFB domestication. Compared with free cell fermentation, the fluxes of propionic acid synthesis and the pentose phosphate pathway in PFB fermentation increased by 84.65% and 227.62%, respectively. On the other hand, a decrease in succinic and acetic acid fluxes was also observed. The metabolic flux distributions of the two PFB fed-batch fermentation strategies also demonstrated that constant fed-batch fermentation is a more beneficial method for the immobilized production of propionic acid. The relevant key enzyme activities and metabolic flux variations of the batch cultures showed good consistency. These results suggest that the PFB was effective in high-concentration propionic acid production.

1. Introduction

Propionic acid is a bulk chemical frequently used in the synthesis of cellulose fibers, herbicides, perfumes, and pharmaceuticals (Woskow and Glatz, 1991; Quesada et al., 1994; Kerjean et al., 2003). The calcium, sodium, and potassium salts of propionic acid are good mold inhibitors that are added as preservatives to animal feed and human food worldwide (Quesada et al., 1994). Despite its broad application, the massive production of propionic acid relies solely on the petrochemical process. Considering the cost, environmental impact, and growing preference for bio-based materials, an alternative based on the fermentation of biomass with propionibacteria has become a research focus. However, a high-concentration propionic acid production is hard to achieve, and productivity is relatively poor, mainly due to end-product inhibition (Woskow and Glatz, 1991; Gu et al., 1998).

Immobilized cell technology was applied in the current study to overcome the barrier. The productivity of propionic acid was improved through the enhanced resistance of propionibacteria toward organic acids (Paik and Glatz, 1994; Rickert et al., 1998; Coronado et al., 2001; Suwannakham and Yang, 2005; Feng et al., 2010). Embedding calcium alginate or calcium polygalacturonate is a common method reported for propionibacteria immobilization and propionic acid fermentation (Paik and Glatz, 1994; Rickert et al., 1998; Coronado et al., 2001). However, self-renewable embedding of propionibacteria was difficult to achieve in gel. Moreover, the cost of calcium alginate and calcium polygalacturonate minimizes their potential for industrial use.

Cotton fiber is a good immobilizing material for propionic acid production by fibrous-bed bioreactor (FBB) (Suwannakham and Yang, 2005) and multi-point fibrous-bed bioreactor (MFB) (Feng et al., 2010). Despite the high propionic acid concentration obtained, the fermentation was extremely time-consuming

Abbreviations: GLC, glucose; G6P, glucose-6-phosphate; F6P, fructose-6-phosphate; DHAP, dihydroxyacetone phosphate; GAP, glyceraldehyde-3-phosphate; G3P, 3-phosphoglycerate; RU5P, ribulose-5-phosphate; CO_2, carbon dioxide; XU5P, xylulose-5-phosphate; R5P, ribose-5-phosphate; S7P, sedoheptulose-7-phosphate; E4P, erythrose-4-phosphate; PEP, phosphoenolpyruvate; PYR, pyruvate; AACoA, acetyl CoA; OACE, oxaloacetate; SUC, succinic acid; MMCoA, methylmalonyl CoA; PPCoA, propionyl CoA; SUCCoA, succinyl CoA; PA, propionic acid; LA, lactic acid; AC, acetic acid; PTA, phosphotransacetylase; ACK, acetate kinase; OTC, oxaloacetate transcarboxylase; CoAT, propionyl CoA:succinyl CoA transferase; PPC, phosphoenolpyruvate carboxylase; G6PDH, glucose-6-phosphate dehydrogenase.

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http://dx.doi.org/10.1016/j.jbiotec.2012.08.025

(97.00 g/L in FBB at 2000 h and 67.05 g/L in MFB at 496 h of fermentation) (Zhang and Yang, 2009; Feng et al., 2010) and power-consuming, which makes cotton fiber inefficient for the industrial production of propionic acid. In addition, cotton fiber has a low porosity and a small specific surface area. This not only reduces the number of immobilized cells on the fiber but also decreases its update rate. Sorghum bagasse was used for ethanol fermentation as immobilizing material because of its high fiber content and good water retention capacity (Yu et al., 2007, 2010). This prompted us to explore a material with a similar structure using sugar cane bagasse (the by-product from sugar refineries) for the immobilization of propionibacterium, which may be more effective.

The aim of this research project is to test and optimize the efficiency of propionic acid production by *Propionibacterium freudenreichii* CCTCC M207015 in a plant fibrous-bed (PFB) bioreactor embedded with sugar cane bagasse through intermittent fed-batch and constant fed-batch fermentations. A comparison between the free cell and immobilized cell in terms of physiological changes, metabolic fluxes, and related enzyme activities was conducted to elucidate the PFB fermentation mechanism. These analyses are expected to further the current understanding of the effect of using a PFB on the improvement of propionic acid fermentation.

2. Materials and methods

2.1. Strain, medium and cultivation

P. freudenreichii CCTCC M207015 was used in this study. This strain was originally isolated from cheese in our laboratory (Xu et al., 2007).

Peptone and yeast extract were purchased from Sinopharm Chemical Reagent Co., Ltd. (China). Nitrogen gas was obtained from the Nanjing Sanle Group Co., Ltd. (China). All other chemicals were of analytical grade. The seed medium was composed of 20 g/L glucose, 5 g/L peptone, 5 g/L yeast extract, and 5 g/L NaCl. The cultures were transferred from deep agar into serum bottles containing 100 mL of fresh seed medium, and were incubated at 35 °C for 24 h. The seed cultures were then transferred to a 1000 mL serum bottle containing 400 mL of seed medium, and were incubated at 35 °C for 48 h before use. Anerobiosis was established by sparging the medium in the serum bottle with nitrogen gas for 10 min. The fermentation medium contained 5 g/L peptone, 10 g/L yeast extract, 3 g/L NaCl, 5 g/L $(NH_4)_2SO_4$, 5 g/L KH_2PO_4, and glucose.

2.2. PFB bioreactor construction

Fig. 1 shows the structure of the PFB. The PFB was primarily composed of a 7.5 L stirred-tank fermentor (BioFlo 110, New Brunswick Scientific, USA) and an immobilized glass column (ID: 4 cm, height: 60 cm). The column and stirred-tank fermentor were connected with a peristaltic pump. A super thermostatic water bath was applied to maintain the temperature of the immobilized glass column and ensure consistency with in the stirred-tank fermentor (35 °C). The bagasse was chopped into small pieces (1–10 mm) using a crushing mill. The chopped bagasse was dried to achieve a constant weight and loaded onto the glass cell-immobilized column with an estimated weight of approximately 30 g. The bagasse was kept in place using a stainless steel wire mesh on top and at the bottom of the column to avoid leakage. The stirred-tank fermentor with fresh seed medium and glass cell-immobilized column containing bagasse were sterilized by autoclaving at 121 °C for 20 min, and then connected with each other for propionic acid production.

2.3. Propionic acid production

Free cell fermentation was performed in a 7.5 L stirred-tank fermentor. A 450 mL aliquot of the seed culture was used to inoculate 4.0 L of bioreactor culture medium. The seed was cultured at 35 °C with stirring rate of 100 rpm. A nitrogen flow of 0.1 L/min was applied to ensure that the fermentation proceeded under anaerobic conditions. The pH was automatically controlled at 6.0 by adding 6 mol/L of NaOH during fermentation.

The culture conditions of PFB fermentation were similar with free cell fermentation. The fermentation broth in the stirred-tank fermentor and the immobilized cells in the glass column were circulated with a peristaltic pump at the flow rate of 600 mL/h. Subsequently, most of the cells were immobilized onto the bagasse after 48 h of adsorption. The fresh fermentation medium was poured into the fermentor instead of the seed medium for the propionic acid batch or fed-batch fermentation.

In PFB fed-batch fermentation, two feeding approaches were performed. In the intermittent fed-batch culture, the initial glucose concentration was 40 g/L. Each time the glucose was completely

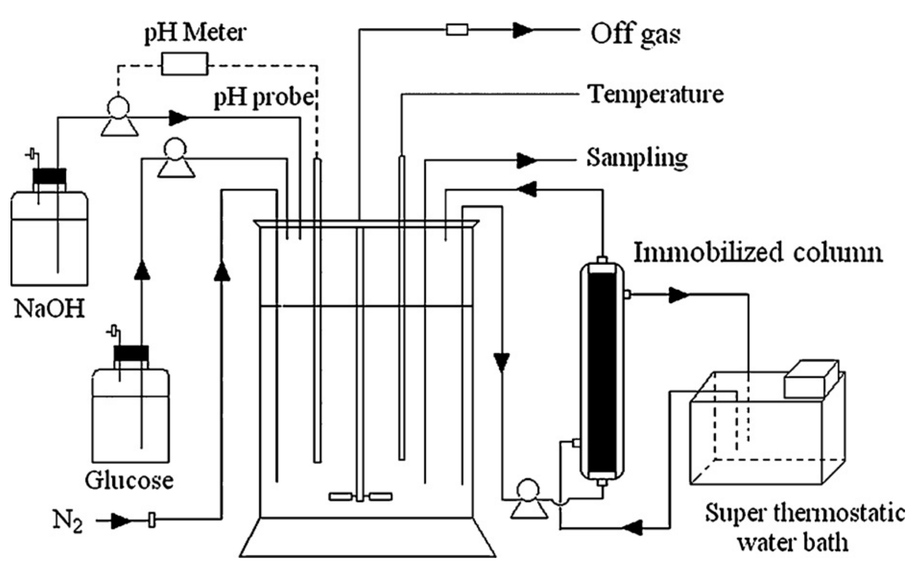

Fig. 1. Diagram of the PFB structure.