Biochemical Engineering Journal 91 (2014) 92–98

Contents lists available at ScienceDirect

Biochemical Engineering Journal

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

Fermentation process for continuous production of succinic acid in a fibrous bed bioreactor

Qiang Yan[a,b], Pu Zheng[a,*], Sheng-Tao Tao[a,b], Jin-Jun Dong[a]

[a] The Key Laboratory of Industrial Biotechnology, Ministry of Education, School of Biotechnology, Jiangnan University, Wuxi 214122, Jiangsu, People's Republic of China

[b] State Key Laboratory of Food Science and Technology, Jiangnan University, Wuxi 214122, Jiangsu, People's Republic of China

ARTICLE INFO

Article history:
Received 22 May 2014
Received in revised form 26 July 2014
Accepted 2 August 2014
Available online 10 August 2014

Keywords:
Actinobacillus succinogenes
Succinic acid
Bioprocess design
Bioreactors
Fermentation
Immobilized cells

ABSTRACT

Succinic acid (SA) was produced from *Actinobacillus succinogenes* with high cell density by continuous fermentation using fibrous bed bioreactor (FBB). The effects of feeding glucose concentration, dilution rate, and pH on continuous production of SA were examined to achieve an efficient and economical bioprocess. The optimum feeding glucose concentration, dilution rate, and pH were 80 g/L, 0.05 1/h, and 6.0–6.5, respectively. A SA concentration of 55.3 ± 0.8 g/L, productivity of 2.77 ± 0.04 g/L/h, and yield of 0.8 ± 0.02 g/g were obtained, and the continuous fermentation exhibited long-term stability for as long as 18 days (440 h) with no obvious fluctuations in both SA and biomass levels. The Jerusalimsky equation for the specific rate of SA production presented the inhibition phenomenon of the product, demonstrating that 60 g/L SA might be a critical concentration in this continuous FBB system. The results obtained could be beneficial for future fermentor designs and improvements in SA production.

1. Introduction

Owing to the declining oil reserves, increasing petrochemical prices, and environmental impact of oil-based industries, the need for the development of bio-based processes for fuel and chemical production has increased [1,2]. Succinic acid is an intermediate in the Krebs cycle, and is predicted to be a C4 platform chemical that can be obtained from the fermentation of renewable resources [3]. Although succinic acid is produced petrochemically to meet the needs of the specialty chemical market, bio-based succinic acid (bio-SA) production has the environmental benefit of using carbon dioxide and meeting the global demands [4]. The predicted annual bio-SA production has been estimated to be more than 150 kton by the end of 2015, and, currently, at least five companies have been implementing pilot- or industrial-scale succinic acid production [5,6]. For the development of a more cost-effective industrial succinic acid fermentation process that can replace the oil-based industry, advancements in several aspects are required to overcome limitations such as product inhibition and slow growth of the anaerobic microorganisms during anaerobic fermentation [5,7]. In large-scale fermentation of succinic acid, efficiency of production is a crucial factor. Therefore, except for the most focused reports on microorganism improvement with batch and fed-batch fermentation, reactor design and operation of the fermentation process might possibly be more important [6].

As continuous fermentation has the advantages of higher productivity as well as lower capital expenditure and operating costs, several continuous fermentation processes have been studied by using wild succinic acid producing strains. Normal suspended cell systems ("chemostat"), membrane cell recycle systems, and biofilm bioreactors have been used to improve succinic acid productivity [8–13]. In a continuous biofilm fermentation system comprising a plastic composite support (PCS) tube reactor containing large amounts of biofilms formed by *Actinobacillus succinogenes*, a maximum productivity of 8.8 g/L/h was achieved, which was ten-fold higher than that obtained in repeated-batch biofilm fermentation [8,9]. In another study [11], by using an external membrane cell recycle reactor with a high-speed pump between the fermentor and membrane unit, the productivity of succinic acid was increased from 3.71 to 6.63 g/L/h at dilution rates from 0.2 to 0.5 1/h, which was about 2.8- to 5.0-fold higher than that obtained in batch fermentation. However, besides the distinctive improvement in succinic acid productivity, the concentration of succinic acid obtained from continuous fermentation was seldom more than 40 g/L. To improve the production of succinic acid, an electrodialysis system was integrated to an external membrane recycle system by an ultra-filtration flat module, and by continuously removing succinic acid and acetic acid from the medium, a final succinic

* Corresponding author. Tel.: +8651085918156; fax: +8651085918156.
E-mail address: zhengpu@jiangnan.edu.cn (P. Zheng).

http://dx.doi.org/10.1016/j.bej.2014.08.002
1369-703X/

Nomenclature

AA	acetic acid
bio-SA	bio-based succinic acid
CO_2	carbon dioxide
C_{suc}	succinic acid concentration produced (g/L)
D	dilution rate (1/h)
F	volumetric flow rate (L/h)
K_p	succinic acid inhibition constant (g/L)
P	succinic acid volumetric productivity (g/L/h)
q_{suc}	specific succinic acid production rate (g/g/h)
q_{max}	maximum specific succinic acid production rate (g/g/h)
q'_{suc}	succinic acid production rate (g/L/h)
q'_{max}	maximum succinic acid production rate (g/L/h)
SA	succinic acid
V	reactor volume (L)

acid concentration of 83 g/L was obtained with a productivity of 10.4 g/L/h and yield of 0.89 g/g [10]. This was an exclusive bioprocess system, which could simultaneously achieve high succinic acid productivity, concentration, and yield, irrespective of the complexity of an additional operation unit. For the extension to the biofilm bioreactor, *A. succinogenes* immobilized by perlite packing was utilized. A highest succinic acid productivity of 6.35 g/L/h was achieved at a dilution rate of 0.56 1/h, and the overall succinic acid yield varied between 0.67 and 0.71 g/g; however, the "chemostat" operation was described as impossible [12]. To improve the biofilm stability and increase the total biomass concentration in the reactor, stainless-steel wool was used as the packing bed to form a novel external recycle biofilm, and the steady-state metabolic flux variations were compared. The highest succinic acid yield and succinic acid titer obtained were 0.91 g/g and 48.5 g/L, respectively, at a dilution rate of 0.05–0.50 1/h [13].

In our previous study, an internal fibrous bed bioreactor (FBB) was first investigated for the production of succinic acid by using *A. succinogenes* through batch and fed-batch fermentation. A maximum succinic aid concentration of 98.7 g/L was achieved, with a productivity of 2.77 g/L/h and yield of 0.89 g/g through fed-batch fermentation, demonstrating that high succinic acid concentration, yield, and productivity could be simultaneously achieved using FBB [14]. In the present study, continuous fermentation in FBB was performed to further improve succinic acid productivity. The effects of pH, dilution rates, and various glucose concentrations, as well as the FBB operation stability during continuous fermentation were studied. Furthermore, a product kinetic model was proposed to describe the effect of product inhibition on succinic acid productivity.

2. Materials and methods

2.1. Strain

A. succinogenes CCTCC M2012036, obtained in our laboratory and stored at the China Center for Type Culture Collection, was used in the fermentation process for succinic acid production [15].

2.2. Media

The seed medium used in this study was a defined medium, which contained (per L): glucose, 10 g; yeast extract, 10 g; $NaH_2PO_4{\cdot}2H_2O$, 9.6 g; $K_2HPO_4{\cdot}3H_2O$, 20.3 g; and $NaHCO_3$, 10 g. The fermentation medium contained the following (per L): glucose, 50–80 g; corn steep liquor, 25 g; $K_2HPO_4{\cdot}3H_2O$, 2.5 g; $NaH_2PO_4{\cdot}2H_2O$, 2.5 g; $MgCl_2$, 0.2 g; $CaCl_2$, 0.2 g; Vitamin B_{12}, 20 μg; Vitamin B_6, 50 μg; riboflavin, 50 μg; lipoic acid, 50 μg; niacin, 50 μg; thiamine, 50 μg; folic acid, 50 μg; pantothenate, 50 μg; *p*-aminobenzoate, 50 μg; and biotin, 100 μg. The pH of the fermentation medium was adjusted to 6.5 with 2 mol/L NaOH or HCl before sterilization, and the fermentation medium was autoclaved at 121 °C for 20 min.

2.3. Inocula preparation

The inocula were prepared by cultivating 5% seed culture in 100-mL anaerobic bottles containing 50 mL of the seed medium at 38 °C for 12 h with CO_2 as the gas phase.

2.4. Setup of the fermentation system

As shown in Fig. 1, two 3-L stirred fermentors (Biotech-3BG, Shanghai, China), reactors A and B, were used in the FBB fermentation system to improve the uniformity of the entire fermentation broth, particularly the pH distribution of the fermentation broth. Reactor A was a FBB and reactor B was a general fermentor with pH controller. The procedures for the construction and autoclaving of FBB were described in our previous studies [14]. A fibrous matrix was bound to a stainless steel cylinder mounted on the agitator shaft in reactor A. The stainless steel screen cylinder (15 cm in diameter, 10 cm in height) was affixed with a sheet of 100% cotton terry cloth (100 × 16 cm^2, thickness of 1 mm, porosity >95%). The gap between each turn of the winding layers was 2–5 mm. Before use, the matrix was washed with purified water and dried at 85 °C for 12 h. Subsequently, the matrix was attached to the surface of the stainless steel cylinder mounted on the agitator, and the reactor was autoclaved at 121 °C for 30 min. The area of carrier material to liquid ratio was 2:1 (cm^2/mL, previously optimized, data not shown). Each reactor was equipped with a gas filter as well as air flow rate, pH, and temperature controllers. The two reactors were in series such that the sterilized fermentation medium was first fed continuously into reactor A for succinic acid production. At the same time, the broth was withdrawn from reactor A to reactor B by peristaltic pump (LongerPump BT100-2J, Shanghai, China), and the products were collected from reactor B and stored in a product reservoir. The working volumes of the reactors were kept constant by removing excess broth from reactor B at a rate equal to the feed rate.

2.5. Continuous fermentation in FBB

A total of 50 mL of the seed medium was inoculated into reactor A and incubated at 38 °C under constant stirring at the rate of 100 rpm. Reactor A was started by adding 2 L of the seed medium to immerse the fibrous matrix for sufficient cell attachment, whereas reactor B was started by adding 1.6 L of the fermentation medium. The volume of the fermentation medium to be added into reactor B was calculated by summing the total working volume of reactors A and B. The cells attached to the surface of the cotton cloth were ready after around 6 h of inoculation when the initial pH dropped from 7.2 to 6.0. Subsequently, the seed medium in reactor A (working volume, 1.0 L) was rapidly replaced with the fermentation medium from reactor B (working volume, 600 mL). The fermentation was allowed to proceed in batch mode for 24 h, after which the fermentation feed medium was continuously pumped into the bioreactor at different dilution rates. To achieve proper mixing in both the reactors, peristaltic pumps were used with a circulation rate of 100 mL/min. The adsorption of the cells onto the fibrous matrix reached saturation during batch growth before the start of continuous fermentation. When the residual glucose concentration during batch fermentation was less than 5 g/L, fresh medium was continuously fed into reactor A by using