Bioresource Technology 155 (2014) 8–14

Contents lists available at ScienceDirect

Bioresource Technology

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

Enhanced poly(γ-glutamic acid) fermentation by *Bacillus subtilis* NX-2 immobilized in an aerobic plant fibrous-bed bioreactor

Zongqi Xu [a,b], Xiaohai Feng [a,b,*], Dan Zhang [a,b,c], Bao Tang [a,b], Peng Lei [a,b], Jinfeng Liang [a,b], Hong Xu [a,b]

[a] State Key Laboratory of Materials-Oriented Chemical Engineering, Nanjing University of Technology, Nanjing 210009, PR China
[b] College of Food Science and Light Industry, Nanjing University of Technology, Nanjing 211816, PR China
[c] School of Agriculture and Biology, Shanghai Jiaotong University, Shanghai 200240, PR China

HIGHLIGHTS

- Aerobic fibrous-bed bioreactor using bagasse as immobilized carrier was designed.
- The time of immobilization fermentation is 48 h while free-cell 72 h.
- PGA yield of 71.21 g/L was achieved by repeated fed-batch fermentation in APFB.
- We evaluated the stability of the APFB system.
- The changes in the cells of free and immobilized cell fermentation were compared.

ARTICLE INFO

Article history:
Received 16 October 2013
Received in revised form 16 December 2013
Accepted 18 December 2013
Available online 27 December 2013

Keywords:
Bacillus subtilis NX-2
Aerobic plant fibrous-bed bioreactor
Poly(γ-glutamic acid)
Repeated fed-batch fermentation
Cell membrane permeability

ABSTRACT

To enhance poly(γ-glutamic acid) (PGA) production, a novel aerobic plant fibrous-bed bioreactor (APFB) was constructed for immobilized fermentation. Based on the analysis of the kinetics of immobilized-cell fermentation using the APFB and conventional free-cell fermentation, immobilized-cell fermentation exhibited more efficient PGA production. Furthermore, repeated fed-batch cultures for PGA production were conducted to evaluate the stability of the APFB system. Average final PGA concentration and productivity of 71.21 ± 0.83 g/L and 1.246 ± 0.008 g/L/h were respectively achieved by cells immobilized in bagasse during APFB, which was reused eight times over a period of 457 ± 18 h. Analysis of the membrane phospholipids and the key enzyme activities indicated that APFB-adapted cells had better productivity than original cells. Thus, this study demonstrated the significant potential of the APFB culture system in future industrial applications.

1. Introduction

Poly(γ-glutamic acid) (PGA) is a homopolymer that consists of D- and L-glutamic acid units connected by γ-amide linkages produced by microbes (Shih and Van, 2001). PGA has been widely utilized in a broad range of industrial fields, such as cosmetics, food, medicine, agriculture, and water treatment, for its nontoxic, biodegradable, and excellent environmental characteristics (Ashiuchi, 2010). PGA has recently been successfully used as fertilizer synergists, biodegradable fibers, drug carriers, and biological adhesives (Sonaje et al., 2010; Tajima et al., 2011; Zeng et al., 2013).

The increasing applications of PGA has caused extensive research to be conducted with a focus on increasing PGA fermentative production using a variety of approaches, such as optimization of the fermentation process (Jeong et al., 2010), agitation speed-shift control strategies (Zhang et al., 2011a), and the addition of oxygen vectors or organic acid to free cell fermentation (Zhang et al., 2012a,b). However, PGA fermentation processes still encounter problems, such as low final PGA concentration, low productivity, and low reactor productivity (Bajaj and Singhal, 2010, 2011). Thus, a more effective fermentation method is essential. The immobilization method, which has a number of excellent characteristics, such as biomass and productivity improvement as well as prevention of cell loss, is recognized as a practical tool for the enhancement of process stability and activity of microorganisms involved in fermentation systems (Zhang and Yang, 2009). The production process of a series of chemicals, including xanthan gum (Yang et al., 1996), enzymes (Bai and Yang, 2005), and organic acids (Zhang and Yang, 2009), could be effectively improved by the immobilized cell technique. Moreover, this method can also enhance the endurance capability of strain to substrates as well as the quality products (Tay and Yang, 2002). Notably, sugarcane bagasse, which is an abundant,

* Corresponding author at: College of Food Science and Light Industry, Nanjing University of Technology, Nanjing 211816, PR China. Tel./fax: +86 25 58139433.
E-mail address: fengxiaohai@njut.edu.cn (X. Feng).

0960-8524/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biortech.2013.12.080

inexpensive, and renewable lignocellulosic biomass, is an acceptable support material for cell immobilization (Plangklang et al., 2012). Therefore, sugarcane bagasse immobilization might be a suitable method to improve PGA fermentation.

In this study, a novel aerobic plant fibrous-bed bioreactor (APFB) was constructed for the production of high-viscosity PGA. This research aims to improve PGA production from glucose further by using *Bacillus subtilis* NX-2 immobilized in the APFB. Moreover, a fed-batch strategy was applied to improve PGA production base on the analysis of the kinetics of immobilized-cell fermentation, and the stability of the APFB system was evaluated. In addition, the membrane fatty acid composition and enzyme activity of cells in both free and immobilized cell fermentation were also investigated.

2. Methods

2.1. Microorganism

B. subtilis NX-2, a PGA production strain, was originally isolated from a soil sample in previous study (Xu et al., 2005). The strain was deposited in the China General Microbiological Culture Collection Center with the accession number CGMCC No. 0833.

2.2. Culture and medium

B. subtilis NX-2 was first inoculated into a 500 mL flask with 80 mL of seed medium, which was composed of 20 g/L of glucose, 15 g/L of glutamate, 5 g/L of yeast extract, 2 g/L of $K_2HPO_4 \cdot 3H_2O$, and 0.1 g/L of $MgSO_4$. The strain was then aerobically incubated at 32 °C for 16 h with shaking at 220 rpm. The fermentation medium contained 60 g/L of glucose, 50 g/L of glutamate, 5 g/L of $(NH_4)_2SO_4$, 2 g/L of $K_2HPO_4 \cdot 3H_2O$, 0.1 g/L of $MgSO_4$, and 0.03 g/L of $MnSO_4$.

2.3. Free-cell fermentation

Free-cell fermentation was performed in a 7.5 L bioreactor (BioFlo 110, New Brunswick Scientific, USA). The seed culture (2%, v/v) was then transferred to the bioreactor containing 4.5 L of basal medium and was incubated at 32 °C with an agitation speed controlled at 400 rpm. The pH was automatically controlled at 7.0 ± 0.1 by adding 2 N NaOH or 2 N HCl, and the aeration rate was maintained at 1.2 vvm.

2.4. APFB construction

To improve the final concentration of PGA, APFB was employed to immobilize the cells of *B. subtilis* NX-2. The APFB system consisted of a 7.5 L bioreactor (BioFlo 110, New Brunswick Scientific, USA) and immobilized cells in jacketed glass column (height 60 cm and tubing ID 4 cm) connected through a recirculation loop with a peristaltic pump (Fig. 1). The temperature of the glass cell-immobilized column was controlled at 32 °C by a super thermostatic water bath, and the consistency was retained with the use of a stirred-tank fermenter. The bagasse was chopped into small pieces (1–10 mm) and then dried. Thereafter, 30 g of bagasse was loaded onto the glass cell-immobilized column. Stainless steel wire mesh was placed on top and at the bottom of the column to keep the bagasse in place and to avoid leakage. Air pipes were installed at the bottom of the stirred-tank fermenter and glass column.

The empty bioreactor system was sterilized before use by autoclaving at 121 °C for 20 min and then cooled. The bioreactor system was then filled with 4.5 L of fresh fermentation medium and autoclaved for another 20 min. The APFB was then ready to be used for batch, fed-batch, and repeated fed-batch fermentation.

2.5. Immobilized-cell fermentation

For batch fermentation, PGA was produced in the APFB with an initial glucose concentration of 60 g/L. Culture conditions were the same as those for free-cell fermentation. Fed-batch fermentation was also conducted in the APFB with a total glucose concentration of 90 g/L. Once the residual glucose in both culture media was exhausted (below 10 g/L), the feeding solution (500 g/L glucose) was added to the bioreactor using a peristaltic pump to maintain the glucose level at approximately 10 g/L. For the repeated fed-batch fermentation experiment in the APFB, eight repeated fed-batch fermentations were applied to investigate the stability of PGA production by the APFB. The total glucose concentration of each batch was 90 g/L. Cells immobilized on the surface and the apertures of the fibrous bagasse were used as the inoculums for the second batch.

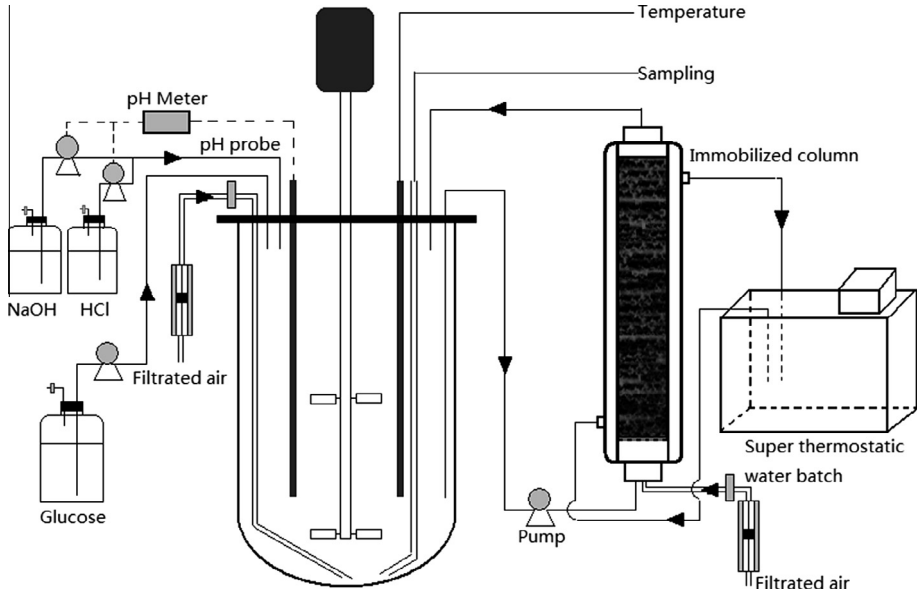

Fig. 1. Structure of the APFB system.