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Retamycin production by immobilized cells of *Streptomyces olindensis* ICB20 in repeated-batch cultures

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Abstract

Repeated-batch cultures of Ca-alginate immobilized cells of *Streptomyces olindensis* ICB20 for retamycin production were carried out in two different bioreactors: a basket-type stirred tank reactor (BSTR) and a bubble column reactor (BCR). Higher average values of retamycin content (R) and productivity (P_R) were achieved in the BSTR cultures (about 1.7 AU and 0.031 AU h^{-1}, respectively) compared to those obtained in the BCR cultures (about 0.6 AU and 0.012 AU h^{-1}, respectively). The BCR, on the other hand, presented significantly better operation stability than the BSTR, which makes the former much more promising regarding future industrial applications.

Keywords: *Streptomyces olindensis*; Immobilized cells; Retamycin; Repeated-batch; Basket-type tank reactor; Bubble column reactor

1. Introduction

Antibiotic production is usually carried out in batch and fed-batch free cell suspension cultures. Antibiotics are derived from the secondary metabolism pathways in microbial cells and the production activities of these microorganisms are often unstable [1]. Bioprocesses using immobilized cells in inert supports have been considered as an alternative to increase overall productivity and minimize production costs. Actinomycin [2], cephamycin [1], chephalosporin C [3], neomycin [4] and daunorubycin [5] production by immobilized cells has been studied in bubble column and airlift bioreactors in batch, repeated-batch and continuous modes [3,6–8]. These studies have shown that the cell entrapment leads to higher specific production rates compared to those obtained in conventional free cell suspension cultures. Productivity has also been maintained at high levels in long-term entrapped cell cultures [5].

Anthracycline antibiotics are potent antitumor drugs used for the treatment of a wide variety of neoplasias [9]. Retamycin is an anthracycline complex [10] similar in structure and activity to cosmomycin D [11]. It is produced in submerged fermentations of free cells of *Streptomyces olindensis* ICB20 in batch [12,13], fed-batch [14] and continuous modes [15].

Immobilized cell systems may be an alternative to free cell systems for the enhancement of productivity and the maintenance of production stability in long-term fermentations in several kind of bioprocesses [16–21].

In the present study, retamycin production was investigated in repeated-batch cultures of immobilized cells of *S. olindensis* ICB20 in two different bioreactors and the results were compared with those previously obtained in free cell suspension cultures in batch mode [12].

2. Materials and methods

2.1. Microorganism

S. olindensis ICB20, a mutant strain isolated from CCT (Tropical Culture Collection) 4859 [22], was stored in cryotubes as 10 ml aliquots of vegetative mycelia in glycerol 20% (p/v) at −80 °C. This stock suspension was prepared by submerged cultivation on a rotary shaker using the R5 modified culture medium (R5Mod) [12].

2.2. Culture medium

The R5Mod medium for both inoculum and bioreactor cultivations had the following composition (g l^{-1}): glucose, 10.0; K_2SO_4, 0.25; yeast extract, 5.0; casein hydrolysate, 0.10; $MgCl_2{\cdot}6H_2O$, 10.12; and tris(hydroxymethyl)-aminomethane 3.09. The pH was adjusted to 7.0. After sterilization at 120 °C for 20 min, the medium was complemented with the following separately sterilized solutions (per 250 ml): trace elements solution, 0.5 ml; KH_2PO_4 (0.5% w/v), 2.5 ml; $CaCl_2$ (5 M), 1 ml. The composition of the trace elements solution (in

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1000 ml) was: 40 mg $ZnCl_2$; 200 mg $FeCl_3{\cdot}6H_2O$; 10 mg $CuCl_2{\cdot}2H_2O$; 10 mg $MnCl_2{\cdot}4H_2O$; 10 mg $Na_2B_4O_7{\cdot}10H_2O$ and 10 mg $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$.

2.3. Inoculum and pre-immobilization cultures

Erlenmeyer flasks of 1000 ml containing 90 ml of the culture medium (R5Mod) were inoculated with 10 ml of frozen mycelium suspension. The flasks were incubated for 16 h at 30 °C and 200 rpm [12]. Afterwards, 1000 ml flasks containing 180 ml of the culture medium were inoculated with 20 ml of the inoculum and incubated for 48 h at 30 °C and 200 rpm (pre-immobilization culture).

2.4. Preparation of immobilized cells

The pre-immobilization cell suspension was centrifuged and the harvested cells were suspended in a 3% sodium alginate solution. The resulting suspension (2 g cells on dry cell weight basis) was then extruded into a 0.2 M $CaCl_2$ solution through a small orifice (1.2 in.) to form beads using a peristaltic pump. Beads of about 3 mm diameter were formed after being cured for 24 h in the $CaCl_2$ solution at 4 °C.

2.5. Basket-type stirred tank reactor (BSTR) culture

The cultures in BSTR (Fig. 1) were performed in a 3 l New Brunswick Bioflo fermentor equipped with a stainless steel basket (2 mm mesh). The operating conditions were: reactor working volume, 2.4 l; agitation rate, 500 rpm; air flow rate, 2.4 l min^{-1} and temperature, 30 °C. The reactor containing 1.8 l of culture medium (V_M) was inoculated with 0.6 l of Ca-alginate gel beads (V_{gel}).

2.6. Bubble column reactor (BCR) culture

The schematic diagram of the bubble column reactor used in this study is shown in Fig. 1. The design details and operating conditions were: internal diameter, 8 cm; height, 56 cm; working volume, 1.6 l; air flow rate, 3.2 l min^{-1} and temperature, 30 °C. The bubble column reactor containing 1.2 l of culture medium (V_M) was inoculated with 0.4 l of Ca-alginate gel beads (V_{gel}).

2.7. Repeated-batch operations

Repeated-batch cultures in both the BSTR and the BCR were performed by replacing the exhausted medium with 1.8 l or 1.2 l of fresh, sterile culture medium, respectively, right after glucose exhaustion.

2.8. Analytical methods

Samples were collected periodically in order to estimate the following variables: free and immobilized cell concentrations, residual glucose concentration and retamycin content. The immobilized cell concentration was determined only at the end of each batch in order to reduce contamination risk, since this procedure required opening one screw-cap of the bioreactor and introducing a sterile pipette inside the gel bed in order to withdraw a sample.

The free cell concentration in the culture medium (X_M) was evaluated in the following way: cells were harvested by vacuum filtration and dried in a microwave oven (180 W) for 15 min. The immobilized cell concentration (X_I) was determined as follows: about 2 g of beads were washed with distilled water, dissolved in a 2% (w/v) sodium hexametaphosphate solution [17], previously heated to about 40 °C. The cells were then collected by centrifugation (7300 × g, 20 min, 5 °C) and the dry weight was determined. The free cell concentration in the culture medium (X_M) and the immobilized cell concentration in the gel beads (X_I) are related to the reactor working volume (V_R) by the following equations:

$$X_{M,R} = X_M \frac{V_M}{V_R} \quad (1)$$

$$X_{I,R} = X_I \frac{V_{gel}}{V_R} \quad (2)$$

$$X_T = X_{I,R} + X_{M,R} \quad (3)$$

where $X_{M,R}$ is the free cell concentration in the reactor, X_M is the free cell concentration in the culture medium, V_M is the volume of culture medium in the reactor, V_R is the reactor working volume, $X_{I,R}$ is the immobilized cell concentration in the reactor, X_I is the immobilized cell concentration in the gel beads, V_{gel} is the volume of gel beads in the reactor and X_T is the total cell concentration in the reactor.

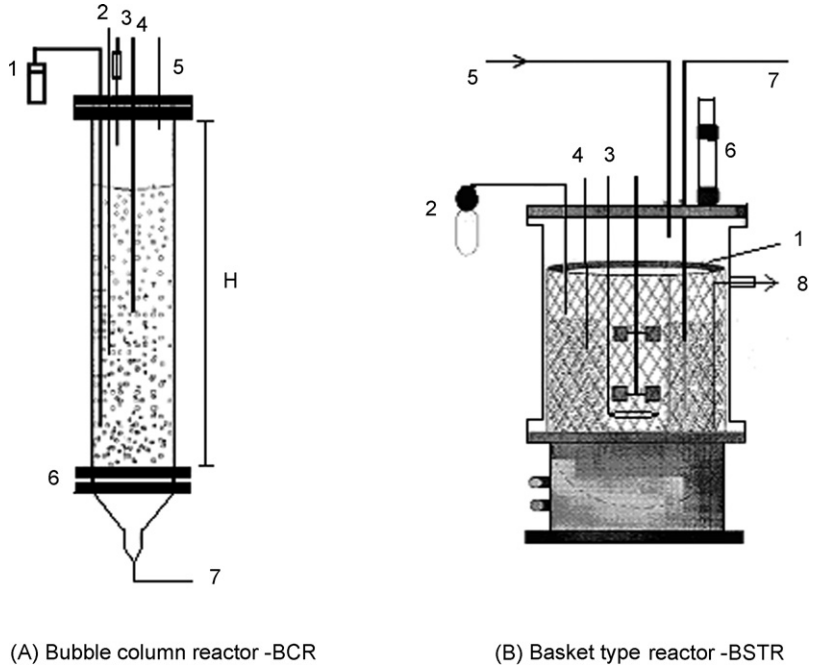

Fig. 1. Schematic diagram of the bubble column reactor (A) 1: sampling and effluent outlet; 2 and 4: sensors (T, OD); 3: condenser and air outlet; 5: medium inlet; H = 0.56 m; $\phi_{(int.)}$ = 0.08 m. Schematic diagram of the basket-type tank reactor (B) 1: steel basket; 2: sampling; 3: air inlet; 4 and 7 sensors (T, OD); 5: medium inlet; 6: condenser and air outlet; 7: effluent outlet.