Available online at www.sciencedirect.com
SCIENCE DIRECT
Enzyme and Microbial Technology 39 (2006) 1–5
ENZYME AND MICROBIAL TECHNOLOGY
www.elsevier.com/locate/emt

Immobilization of *Rhodococcus sp.* AJ270 in alginate capsules and its application in enantioselective biotransformation of *trans*-2-methyl-3-phenyl-oxiranecarbonitrile and amide

Xing-Lin Guo [a], Gang Deng [b], Jian Xu [a,**], Mei-Xiang Wang [b,*]

[a] *State Key Laboratory of Polymer Physics and Chemistry, Chinese Academy of Science, Beijing 100080, China*
[b] *Laboratory of Chemical Biology, Institute of Chemistry, Chinese Academy of Science, Beijing 100080, China*

Received 5 June 2004; accepted 18 January 2005

Abstract

Rhodococcus sp. AJ270, a nitrile hydratase/amidase-containing microorganism, were immobilized in alginate capsules. The influences of the bead size and the crosslinking agent on morphology and the biocatalytic activity of the cells were examined. In the presence of an organic co-solvent, such as methanol or acetone (5%), the encapsulated *Rhodococcus sp.* AJ270 has been found to catalyze efficiently the biotransformation of racemic *trans*-2-methyl-3-phenyl-oxiranecarbonitrile and its amide to yield highly enantiopure 2*R*,3*S*-(−)-2-methyl-3-phenyl-oxiranecarboxamide in at least seven runs.
© 2005 Elsevier Inc. All rights reserved.

Keywords: Encapsulation; *Rhodococcus sp.* AJ270; Enantioselective biotransformation; Nitrile hydratase; Amidase

1. Introduction

Biotransformations of nitrile [1,2], through either a direct nitrilase-catalyzed conversion of nitrile to a carboxylic acid [3,4], or a nitrile hydratase-catalyzed hydration of a nitrile followed by the hydrolysis of amide to acid by the action of the amidase [5–7], have been demonstrated as being unique and environmentally benign methods for the synthesis of chiral carboxylic acid and their amide derivatives because of the excellent enantioselectivity and very mild reaction conditions. *Rhodococcus sp.* AJ270, a robust nitrile hydratase/amidase-containing microorganism [8], has been used to transform a number of racemic α-substituted phenylacetonitriles [9–14] and cyclopropanecarbonitriles [15–17] to produce the corresponding enantiopure carboxylic acids and amides in high yields. Very recently, we have studied *Rhodococcus sp.* AJ270-catalyzed hydrolysis of oxiranecarbonitrile to synthesize optically active 2*R*,3*S*-3-aryloxiranecarboxamides [18] and 2*R*,3*S*-3-aryl-2-methyloxiranecarboxamides [19]. Although the microbial cell-catalyzed biotransformations of nitriles proceed effectively in suspension culture in phosphate buffer, the reuse or the recycle of the biocatalyst is very difficult.

Encapsulation is one of the most important techniques used to immobilize cells. It helps in segregating the cell from the adverse environment. Among many capsules derived from various materials, the one formed by alginate is probably the most frequently utilized one due to its ease of handling, its non-toxic nature and low cost. Alginate is a naturally derived linear polysaccharide comprised of (1–4)-linked β-D-mannuronic acid (M unit) and α-L-guluronic acid (G units). M and G units can vary in proportion and sequential distribution along the polymer chain [20]. Under mild conditions, alginate can form gels very rapidly in the presence of calcium or barium ions via ionic interaction between the backbone and the chelating cation [21]. A wide variety of cells or enzymes had been encapsulated in alginate polymer hydrogel beads for different applications [22–34].

As a prelude of large-scale production of chiral chemicals from biotransformations of nitriles, encapsulation of *Rhodococcus sp.* AJ270 cells in the calcium/barium alginate beads via one-step method was studied. The viability or the enzymatic activity of immobilized cells was evaluated using enantioselective biohydrolyses of racemic *trans*-2-methyl-3-

* Corresponding author. Tel.: +86 10 6254628; fax: +86 10 62569564.
** Corresponding author.
E-mail addresses: jxu@iccas.ac.cn (J. Xu), mxwang@iccas.ac.cn (M.-X. Wang).

0141-0229/$ – see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.enzmictec.2005.01.044

phenyl-oxiranecarbonitrile (t-MPON) and *trans*-2-methyl-3-phenyl-oxiranecarboxamide (t-MPOA) as the model reactions. In order to optimize the parameters for the bioreactor, the effect of metal cations such as Ca^{2+} and Ba^{2+}, bead size and morphology, and the organic solvent on the catalytic activity of encapsulated *Rhodococcus sp.* AJ270 were examined, respectively.

2. Materials and methods

2.1. Bacteria and growth conditions

Rhodococcus sp. AJ270, a powerful and robust nitrile hydratase/amidase containing microorganism, was isolated from a soil sample [8]. They were readily cultivated from fermentation [14].

2.2. Materials

Sodium alginate (from macrocystis pyrifera, medium viscosity) was purchased from Sigma Chemical Co. Sodium bicarbonate buffer solution was prepared by dissolving 2 g of sodium bicarbonate in 1 l of distilled water and its pH value was adjusted to 7.5 with 1 M HCl and/or NaOH solution. Calcium chloride and sodium bicarbonate were purchased from Beijing Yili Chemical Inc. Barium chloride was purchased from Beijing Hongxing Chemical Inc.

2.3. Entrapment of Rhodococcus sp. AJ270

Four grams (wet weight) of *Rhodococcus sp.* AJ270 cell were thoroughly suspended in 20 ml of sodium bicarbonate buffered solution. Then it was mixed with 40 ml of sodium alginate solution (1.5%). Fifteen milliliters of the mixture (containing 1 g wet weight of the cell) was taken for the preparation of beads. The device was shown in Fig. 1. Droplets of the suspension, formed by a syringe pump with 26G syringe at a rate of 60 ml/h, were gelled in a 0.2 M, 0.1 M $CaCl_2$ and 0.1 M, 0.05 $BaCl_2$ aqueous solution to give spherical entrapment beads. The beads were then washed two times with buffer solution, and were stored in 4 °C for viability evaluation. The bead radius (ca. 0.5, 1.0 and 2.5 mm, respectively) was controlled by airflow.

2.4. Experimental

Racemic t-MPON and t-MPOA were synthesized following the literature methods [35,36].

General procedure for the biotransformation of racemic t-MPON and t-MPOA: To an Erlenmeyer flask (150 ml) with a screw cap were added entrapment beads which contain 1 g wet weight cells, sodium bicarbonate buffer solution (47.5 ml, 2 g/L). The entrapment beads were then activated at 30 °C for 0.5 h with orbital shaking. The mixture of racemic t-MPON or t-MPOA (1 mmol) with acetone or methanol (2.5 ml) were added in one portion to the flask and the mixture was incubated at 30 °C with use of an orbital shaker (200 rpm). After a specified period of time the reaction was quenched by filtration and beads were washed with 150 ml of distilled water. The entrapment beads was marinated in buffer and placed in a refrigerator for reuse. The resulting aqueous solution was extracted with ethyl acetate (3× 60 ml). After the solution was dried ($MgSO_4$) and the solvent removed under vacuum, the residue was chromatographed on a silica gel column with a mixture of petroleum ether and ethyl acetate as the mobile phase to give optically active 2*R*,3*S*-(−)-2-methyl-3-phenyl-oxiranecarboxamide: mp 141–142 °C; ^{1}H NMR (300 MHz, $CDCl_3$, TMS) δ 7.28–7.41 (m, 5H), 6.45 (s, br, 1H), 5.39 (s, br, 1H), 4.13 (s, 1H), 1.33 (s, 3H); ^{13}C NMR($CDCl_3$): δ 169.9, 140.5, 128.9, 128.5, 125.6, 63.7, 63.2, 17.9; IR(KBr) ν 3391, 3196 ($CONH_2$), 1637 (C=O); EI (*m/z*): 177 (M^+,10.5), 107 (69), 106 (100), 105 (21), 91 (23); Anal. Calcd for $C_{10}H_{11}NO_2$: C, 67.78; H,6.26; N,7.90. Found: C, 67.87; H,6.17; N,7.81.

The enantiomeric excesses (Ee%) of compounds were obtained with a Shimadzu LC-10AVP HPLC system, using a Chiracel OJ column. A mixture of hexane:2-propanol [9:1] as the mobile phase at a flow rate of 0.8 ml/min was employed.

$$\mathrm{Ee}\,(\%) = \frac{[2R, 3S] - [2S, 3R]}{[2R, 3S] + [2S, 3R]} \times 100\%$$

Chemical yield Y (%) = amide obtained experimentally/theoretical amount of amide based on nitrile used × 100%.

2.5. Morphologies of entrapped Rhodococcus sp. AJ270

The morphology of *Rhodococcus sp.* AJ270 within the alginate beads was observed as a function of culture time using a Nikon polarizing optical microscope (ECLIPSE E600W POL).

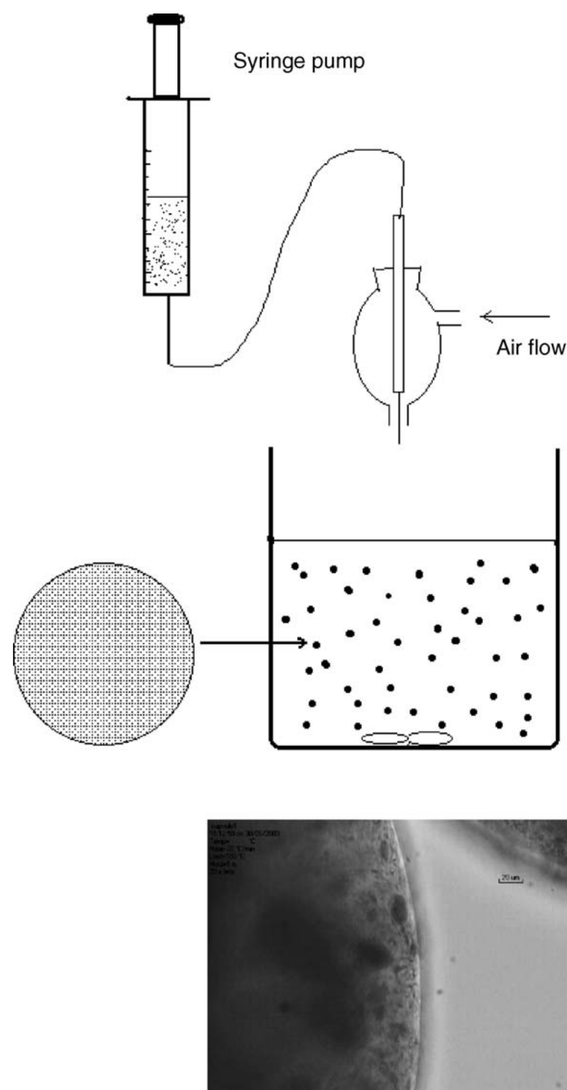

Fig. 1. Schematic diagram showing the preparation of entrapment beads. The flux of the syringe pump was 60 ml/h. The bead size that can be adjusted between 0.4 and 2.5 mm was controlled by airflow rate. Three kinds of bead size, 0.5, 1.0 and 2.5 mm, were used in this work.

3. Results and discussion

3.1. Morphologies of entrapped Rhodococcus sp. AJ270

The morphologies of entrapped *Rhodococcus sp.* AJ270 after a period of time [day 1 (a), day 4 (b) and day 7 (c)] were shown

Download English Version:

https://daneshyari.com/en/article/18742

Download Persian Version:

https://daneshyari.com/article/18742

Daneshyari.com