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Comparison of citric acid production by *Aspergillus niger* immobilized in gels and cryogels of polyacrylamide

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***Aspergillus niger* was immobilized in cryogels and in conventional gels of polyacrylamide. The growth of cells entrapped in two kinds of gels and the production of citric acid by the immobilized cells were investigated and compared. Cells immobilized in cryogels were more suitable for citric acid production.**

Keywords: citric acid; *Aspergillus niger*; immobilization

Introduction

Citric acid, an intermediate of the tricarboxylic acid cycle, is produced almost exclusively by fermentation with *Aspergillus niger*, and widely used in the food, beverage and pharmaceutical fields. Considerable amounts of citric acid are required by large-scale industrial processes. The demand is steadily increasing and the industry is seeking cheap, economic, and newer process technology.

In the past few years, immobilization of microbial cells has received increasing interest. The successful application of immobilized microorganisms as living biocatalysts, involving more careful handling and often having higher production rates than free microorganisms, has prompted a rapid development of this technique.

The use of immobilized cells as a novel fermentation technology has been investigated for organic acids. Citric acid production has also been reported on a laboratory scale with *Aspergillus niger* immobilized in calcium alginate gel [1,8,9,11], polyacrylamide gel [2,3,6,10] and on polyurethane foam [4,7]. In this study, acrylamide was cryopolymerized for the immobilization of *Aspergillus niger* for citric acid production, and compared with a conventional polymerization method.

Materials and methods

Microorganism: *Aspergillus niger* W1–2, was isolated in our laboratory. It was maintained on malt extract agar slants and stored at 4°C and renewed every other month [12].

Medium: The medium for citric acid production contained the following composition (g L^{-1}): sucrose, 100; NH_4Cl, 4.0; KH_2PO_4, 1.0; $MgSO_4 \cdot 7H_2O$, 0.25; and (mg L^{-1}): $FeSO_4$, 0.3; $ZnSO_4$, 0.4; $MnSO_4$, 0.15; $CuSO_4$, 0.4.

Analytical methods: Citric acid was determined by the colorimetric method of Marier and Boulet [5]. Reducing sugar was analyzed by the DNS (3,5-dinitrosalicyclic acid) method [9]. The dry weight of immobilized cells was estimated by subtracting the dry gel weight of a spore-free gel used as control [6].

Immobilization techniques

Immobilization by polyacrylamide gel: Thirty millilitres of spore suspension were added to 20 ml sterile physiological saline containing 5 g of acrylamide (ACAM) monomer, 0.75 g of N,N′-methylenebis-acrylamide (BIS). The polymerization was initiated with 0.1 g of ammonium persulfate and 1.0 ml of 25% N,N,N′,N′-tetramethylethlenediamine (TEMED). The mixture was shaken, then poured into a glass tray (25 × 15 × 5 cm) to give a thickness of 3 mm, allowed to polymerize at 20°C for 30 min. After gel formation, 3 × 3 × 3-mm blocks were cut and washed with saline.

Immobilization by cryo-polyacrylamide gel: The cell immobilization process using cryo-polyacrylamide gel is illustrated in Figure 1. Cryogel immobilization of cells in polyacrylamide was carried out by freezing a solution of polymerizing agent containing spores at −10°C for 24 h.

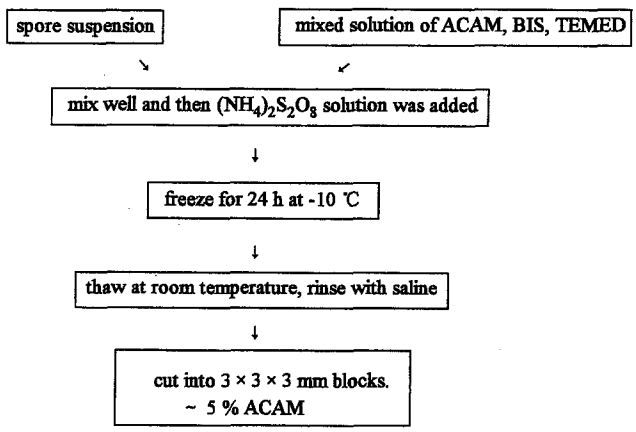

Figure 1 The cryo-polyacrylamide immobilization process.

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The gels formed were then thawed at room temperature, rinsed with physiological saline, and cut into 3 × 3 × 3-mm blocks. The final concentration of acrylamide in cryogels was about 5%.

Fermentation condition: Fermentations were carried out in 250-ml Erlenmeyer flasks containing 20 g wet weight of immobilized cells in 50 ml fermentation medium, incubated at 30°C and 200 rpm in an orbital shaker.

Results and discussion

Growth of Aspergillus niger in gels

The development of *Aspergillus niger* cells immobilized in polyacrylamide and cryopolyacrylamide gels was investigated by measuring the biomass weight. The results are shown in Figure 2.

The growth profile of immobilized *Aspergillus niger* cells may be tentatively divided into three stages: (1) lag phase, from swelling of the spore to mycelium formation, which was characterized by a low biomass growth rate. The lag phase lasted 48 h in polyacrylamide gel and 24 h in cryogel; (2) phase of biomass growth, which can be further subdivided into initial growth and rapid growth phases. During this phase, the biomass content increased from 0.3 and 0.5 g L^{-1} to 6.8 and 8.3 g L^{-1} for cells immobilized in cryogels and conventional gels of polyacrylamide, respectively; (3) stationary phase, in which no marked increase in biomass was observed.

Citric acid production by immobilized Aspergillus niger

The time course of citric acid production using *Aspergillus niger* cells immobilized in polyacrylamide gels and cryogels is shown in Figure 3.

In the case of polyacrylamide-immobilized cells, 20 days were required for apparently complete fermentation with a maximum yield of 14.8 g L^{-1} citric acid. On the other hand, using cryopolyacrylamide-immobilized cells, it took 14 days to reach the maximum concentration of citric acid, *ca* 21.6 g L^{-1}.

A probable explanation for these differences could be

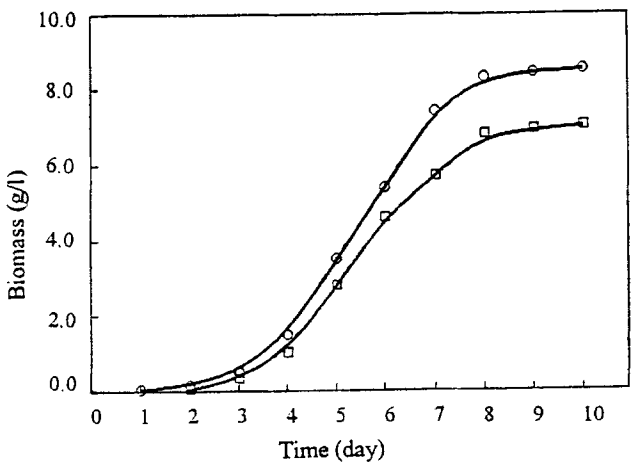

Figure 2 The growth curve of cells immobilized in (○) cryogel; (□) conventional gel.

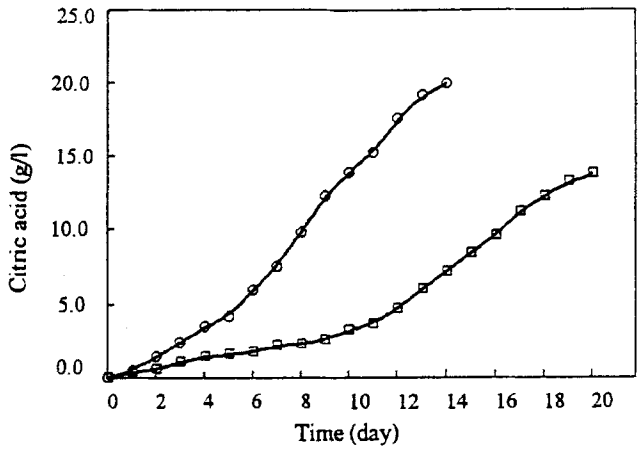

Figure 3 Time course of citric acid production by immobilized cells (○) cryogel; (□) conventional gel.

that: (1) The toxic effect of acrylamide monomer on *Aspergillus niger* cells entrapped in polyacrylamide cryogels was less than that in conventional gels, because the concentration of acrylamide in gels and cryogels were 10% and 5%, respectively. (2) Because cryopolymerization was carried out at a low temperature, loss of cell activity caused by polymerizing heat was small, the cryopolymerization was mild and maintained a higher viability of cells. (3) The biomass content in cryogels is higher than that in conventional gels, therefore the productivity of the former is higher. (4) The sponge-like porous structure of cryogels is more beneficial to diffusion of oxygen and substrate to the cells and of product away from the cells.

These preliminary experimental results indicate that cryopolymerization is more suitable for immobilization of *Aspergillus niger* for citric acid production. Further investigations are underway.

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