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PRODUCTION OF L(+)-LACTIC ACID WITH *RHIZOPUS ORYZAE* IMMOBILIZED IN POLYURETHANE FOAM CUBES

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SUMMARY

Polyurethane foam cubes were employed as carriers to immobilize *Rhizopus oryzae* for L(+)-lactic acid production. The immobilizing capacity reached 450 g-fresh cell/l-cube. The production rate of L(+)-lactic acid could be threefold increased by using the immobilized *R. oryzae*. The immobilized cells could be steadily used in repetitive fermentations for more than 10 batches.

INTRODUCTION

Rhizopus oryzae is an important mold that metabolizes L(+)-lactic acid. It has been commercially employed for L(+)-lactic acid production (Cao and Xu, 1993). However, fermentations have been exclusively carried out using free cells (Jiang et al., 1992; Ward, 1988). Difficulties were encountered in poor oxygen supply and low fermentation efficiency because of the increase in the fermentation broth viscosity owing to the formation of large and soft mycelium aggregates. Thus several researchers have attempted the use of immobilization techniques for L(+)-lactic acid production with *R. oryzae* (Hamamci and Ryu, 1994; Hang et al., 1989; Tamada et al., 1992). Of the studies, the entrapment methods using soft gels such as Ca-alginate were mostly employed (Hamamci and Ryu, 1994; Hang et al., 1989). By the gel-entrapping methods, however, the limitation of oxygen supply by the diffusional resistance of the gel matrices might decrease the fermentation rate (Sun and Furusaki, 1990) and/or L(+)-lactic acid transformation efficiency (Cao and Xu, 1993). Hence in this work we proposed a natural attachment method using polyurethane foam cubes as the carrier for *R. oryzae* immobilization. Polyurethane foam had macropores larger than hundreds of microns and the pore volume fraction was greater than 0.9. Thus it would contribute less diffusional resistance to substrate transfers. Spores would enter the loose matrices and grow inside the cubes. Then the mycelia were embraced by the matrices after growing up. In this paper, *R. oryzae* immobilization behaviour and the production of L(+)-lactic acid with the immobilized cells in polyurethane foam cubes were studied by shaking cultures.

MATERIALS AND METHODS

Rhizopus oryzae BTC115 stored in our laboratory was maintained on potato-dextrose agar slants. The medium for cell immobilization consisted of 50g glucose, 2.5g $(NH_4)_2SO_4$, 0.13g $MgSO_4.7H_2O$, 0.045g $ZnSO_4.7H_2O$, 0.3g K_2HPO_4 and 15g $CaCO_3$ in one litre. Seventy millilitres of the medium in a 500-ml flask was autoclaved together with 14 to 20 ml of 6-mm polyurethane foam cubes. Then the spores ($6x10^6$) were inoculated and incubated in an incubation shaker for cell immobilization. After this procedure, the cell-immobilizing cubes were thoroughly washed with deionized water and a sterilized fermentation medium containing 50g glucose, 1.9g $(NH_4)_2SO_4$, 0.094g $MgSO_4.7H_2O$, 0.03g $ZnSO_4.7H_2O$, 0.23g K_2HPO_4 and 20g $CaCO_3$ in one litre, and transferred to the fermentation medium (70 ml) for L(+)-lactic acid production.. All cultures were performed at 34 °C and the shaking speed was set at 210 rpm. To detect the progress of cell growth during cell immobilization, 12-flask incubations were simultaneously carried out. One or two of them were periodically sampled and the cubes were dried after thoroughly washing with deionized water to determine the cell weights.

After the removal of calcium ions by a strong cation exchanger column, lactic acid was determined by titration with 0.05N NaOH using 0.25% phenolphthalein solution as indicator. It was checked by HPLC that the analysis error by titration was less than 5%. Glucose was analyzed by the rapid Fehling's method (Zhang, 1980).

RESULTS AND DISCUSSION

During cell immobilization cultures, there were few cells observed in free suspensions and the cells were completely attached to the polyurethane foam cubes. Figure 1 shows the time course of cell immobilization. The cell concentration reached its maximum value at 24 h when glucose had been exhausted. During the process, less lactic acid was formed because most of the sugar was consumed for cell growth. We transferred the cell-immobilizing cubes to a fresh medium and repeated the immobilization. Then the cell concentration reached 67.5 g-DCW/l-cube (DCW: dry cell weight). This value was 1.3 times larger than that obtained by using Ca-alginate gels as carrier (Hamamci and Ryu, 1994). Moreover, the value corresponded to 450 g-fresh cell/l-cube if we assumed that the water content of *R. oryzae* was 85%. This result indicated that the polyurethane foam had a very large immobilizing capacity.

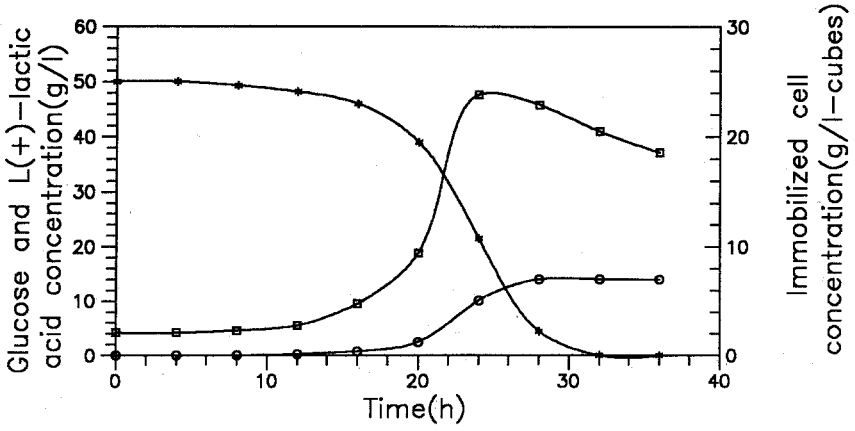

Figure 1 Time course of cell immobilization.
(※) glucose; (○) L(+)-lactic acid; (□) cell

The immobilized cells were used for L(+)-lactic acid production by transferring them to a fresh fermentation medium. The results were compared to those of a free cell fermentation where the free cells had been obtained by cultivation in the cell immobilization mediums without polyurethane foam cubes. As indicated in Figure 2, with the immobilized cells L(+)-lactic acid concentration rapidly increased corresponding to the rapid decrease in glucose concentration. L(+)-lactic acid concentration reached 37.3 g/l after 8 h when glucose was nearly exhausted. The yield of L(+)-lactic acid based upon the consumed glucose was calculated to be 77.7%, nearly the same as the theoretical yield (75%, Cao and Xu, 1993). With the free cells, however, a long initial lag period (ca. 10 h) was observed. After that, L(+)-lactic acid slowly increased corresponding to the slow decrease in glucose concentration. Glucose was nearly exhausted after 24 h when L(+)-lactic acid concentration reached 36.5 g/l. The results showed that the production rate of L(+)-

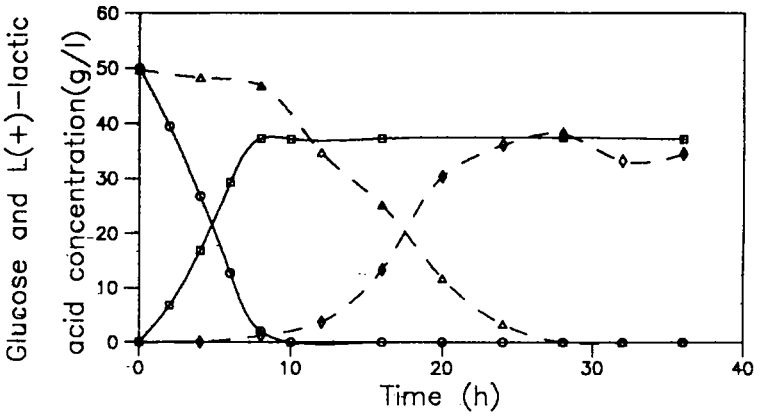

Figure 2 Comparison between immobilized (—) and free cell fermentations (- - -). (○ , △) glucose; (□ , ◇) L(+)-lactic acid

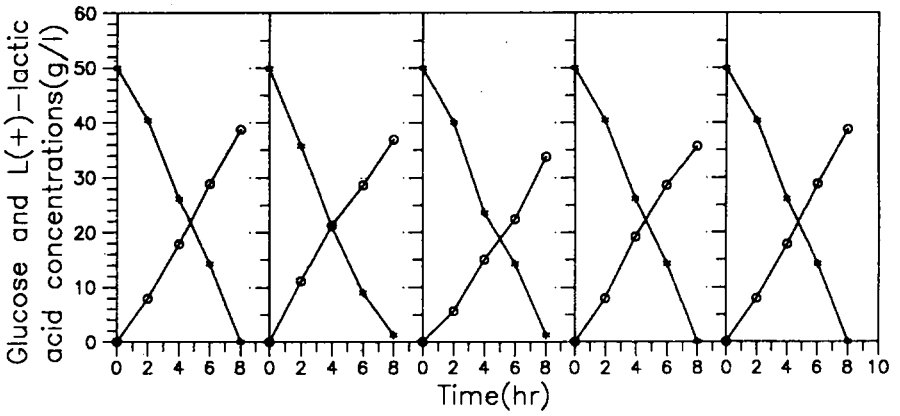

Figure 3 Time courses of the repetitive batch fermentation (※) glucose; (○) L(+)-lactic acid

lactic acid with the immobilized cells was three times greater than that with the free cells. Moreover, since 20 ml polyurethane foam cubes (containing ca. 15 ml medium in the cubes) in 70 ml medium were used in the fermentation, the productivity could be calculated to be $37.3\times(0.07+0.015)/(8\times0.02) = 19.8$ g/h.l-cube. This value was approximately 1.8 times as large as that obtained with *R. oryzae* immobilized in Ca-alginate gel beads (Hamamci and Ryu, 1994). Free cells were not observed in the fermentation using immobilized cells.

Repetitive batch fermentations were carried out as well to demonstrate the stability of the immobilized cells. Ten batches were performed. Each was continued for 8 h. Figure 3 shows the results of the first five batches. For the later five, only the final glucose and L(+)-lactic acid concentrations were determined. Similar behaviour was observed between the first five batches. L(+)-lactic acid yield, represented in Figure 4, kept over 72% in the repetitive fermentations.

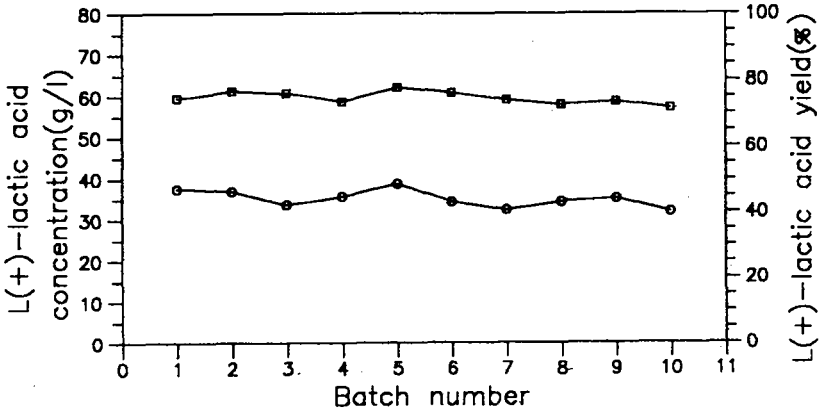

Figure 4 L(+)-lactic acid concentrations (○) and yields (□) in the repetitive batch fermentations.

In conclusion, polyurethane foam was a good carrier for immobilizing *R. oryzae*. The immobilizing capacity reached 450 g-fresh cell/l-cube. The production rate of L(+)-lactic acid could be threefold increased by using the immobilized *R. oryzae*. The immobilized cells could be steadily used in repetitive fermentations for more than 10 batches.

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