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Citric Acid Production with Alginate Bead Entrapped *Aspergillus niger* ATCC 9142

J. VAIJA, YU-YEN LINKO, AND P. LINKO*

Helsinki University of Technology, Kemistintie 1, SF-02150 Espoo 15, Finland

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Abstract

Aspergillus niger ATCC 9142 mycelium was entrapped in calcium alginate beads and employed in an air–lift completely stirred reactor for continuous production of citric acid. Maximum yield obtained from 10% (w/v) sucrose was 12 g dm^{-3} with about 40% fermentation efficiency. Maximum rate of production 70 mg g^{-1} h^{-1} was about five times that obtained in classical batch fermentation.

Index Entries: Citric acid production, by entrapped mycelia; alginate bead entrapped mycelia, citric acid production from; *Aspergillus niger*, citric acid production from entrapped; immobilized bacteria, citric acid production from; immobilized cells, citric acid production from.

Introduction

Few reports have been published on the applications of immobilized live cell technology for the biotechnical production of organic compounds. Organic acids (*1–6*), antibiotics and enzymes (*7*), steroids (*8*), and ethanol (*6, 9*) have been produced by heterogeneous biocatalysis on a laboratory scale. Immobilized live cell systems allow the utilization of complex native multienzyme reaction systems with cofactor regeneration *in situ*. In aerobic fermentations, one of the key problems is to maintain a sufficient dissolved oxygen concentration and transport rate, and to obtain a biocatalyst with small diffusional limitations, but long operational stability. Citric acid is one of the few organic chemicals that today is produced biotechnologically in bulk. Consequently, there is considerable interest in improv-

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ing citric acid productivity and production economy. Briffaud and Engasser (*3*) investigated the kinetics of citric acid formation with *Saccharomyces lipolytica* yeast grown on beechwood shavings. Vieth and Venkatasubramanian (*4*) observed an increased rate of citric acid production with hide collagen immobilized *Aspergillus niger*, although yield and overall productivity were considerably lower than obtainable by classical fermentation. We have investigated citric acid production as an examplary process to find how feasible immobilized live cell technology may be for oxygen-requiring multienzyme biosynthesis.

Biocatalyst Preparation

Aspergillus niger ATCC 9142 was grown at 28–30 °C, initial pH 3.5, as pellets in 250 cm^3 shake flasks containing 100 cm^3 of nutrient solution (15% w/v glucose or sucrose, 0.22% NH_4NO_3, 0.1% $K_2HPO_4 \cdot 3H_2O$, 0.02% $MgSO_4 \cdot 7H_2O$, 0.0001% $FeSO_4 \cdot 7H_2O$, 0.0001% $ZnSO_4 \cdot 7H_2O$). Potassium ferrocyanide (5.35 mg/100 cm^3) was added after 20–24 h of fermentation, and the mycelium was normally harvested after 4–7 days (0.25–0.75% w/v citric acid). An example of citric acid formation in a shake culture is shown in Fig. 1.

In a typical experiment, 25 g of mycelium (15% dm) was suspended in 75 g of 8% sodium alginate. The suspension was extruded under pressure through hollow needles (ϕ, 0.6 mm) into continuously aerated 0.5*M* $CaCl_2$ containing 10% (w/v)

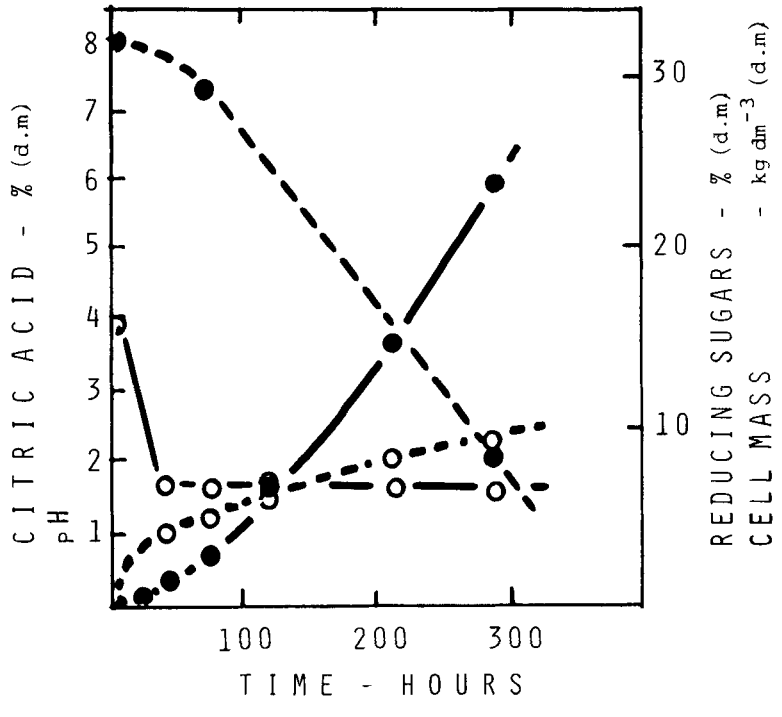

Fig. 1. Formation of citric acid from 15% (w/v) sucrose in a shake culture (details in text); (— ● —) citric acid, % (w/v); (— ● —) reducing sugars, % (w/v); (— ○ —) pH; (— ○ —) cell mass kg dm^{-3} (dm).

sucrose and 0.001 % (w/v) $K_4Fe(CN)_6 \cdot 3H_2O$ at pH 1.9. The biocatalyst beads (ϕ, 2 mm) were allowed to harden under stirring for 20 min. *A. niger* pellets remained intact during immobilization, and cell division after immobilization could be demonstrated.

Continuous Citric Acid Fermentation

A typical jacketed glass reactor of 78 cm^3 contained 20 g (26 cm^3) of biocatalyst beads. Substrate (10 % w/v sucrose, 0.03% NH_4NO_3, 0.005% $MgSO_4 \cdot 7H_2O$, 0.00001% $ZnSO_4 \cdot 7H_2O$, 0.37% $CaCl_2 \cdot 2H_2O$, 0.001% $K_4Fe(CN)_6 \cdot 3H_2O$) and sterile air were fed to the bottom of the reactor through a glass sinter No. 2. Temperature was maintained at 30°C, and the substrate pH was adjusted to 1.9 with HCl.

A small quantity of ammonium nitrate appeared to be necessary for optimum yield and productivity. On the other hand, the addition of phosphate (0.015% w/v $K_2HPO_4 \cdot 3H_2O$) resulted in a relatively rapid destruction of the biocatalyst beads and in excessive cell growth with a decreased citric acid yield. Figure 2 illustrates results from continuous citric acid production experiment. The maximum yield obtained was 12 g dm^{-3} (13 % of theory), and the rate of citric acid production 70 mg g^{-1} h^{-1} was up to five times that obtained in a classical batch fermentation with free mycelium. Overall fermentation efficiency was about 40%. Isocitric acid

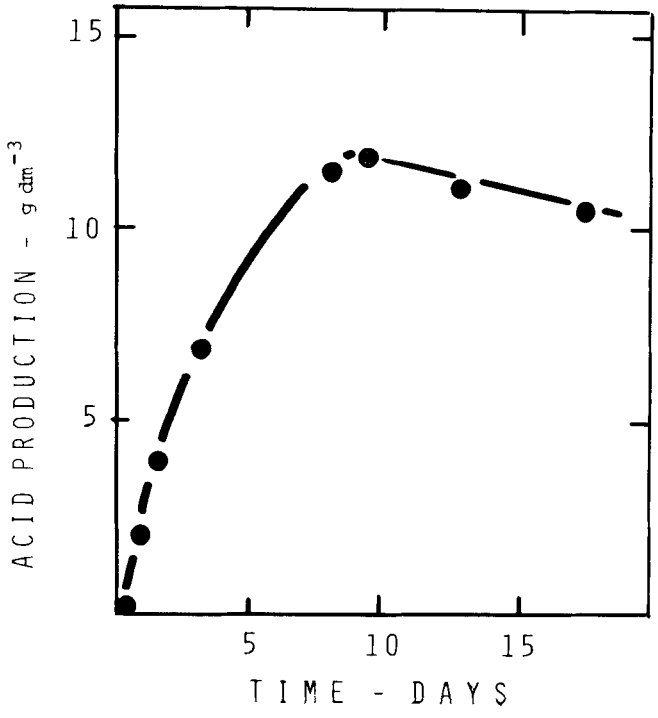

Fig. 2. Continuous production of citric acid by calcium alginate immobilized *A. niger* from 10% (w/v) sucrose (details in text).

was formed as main side product in accordance with the observation of Vieth and Venkatasubramanian (*4*) with collagen-immobilized *A. niger*. After reaching a maximum in about a week, production declined slowly with a half-life of about a month.

References

1. Slovinski, E., and Charm, S. W., *Biotechnol. Bioeng.* **15,** 973 (1973).
2. Griffith, W. L., and Compare, A. L., *Dev. Ind. Microbiol.* **18,** 733 (1977).
3. Briffaud, J., and Engasser, J. M., *Preprints, First European Congress on Biotechnology,* Interlaken, Part 2, 1978, p. 133.
4. Vieth, W. R., and Venkatasubramanian, K., in *Immobilized Microbial Cells,* Venkatasubramanian, K., ed., ACS Symp. Ser. 106, ACS, Washington, DC, 1979, p. 1.
5. Kennedy, J. F., in *Enzyme Engineering,* Broun, G. B., Manecke, G., and Wingard, L. B., Jr. eds., Vol. 4, Plenum, New York, 1978, p. 323.
6. Linko, P. in *Food Process Engineering,* Vol. 2, *Enzyme Engineering in Food Processing,* Linko, P., Malkki, Y., Olkku, J., and Larinkari, J., eds., Applied Science Publishers, London, 1980, p. 27.
7. Suzuki, S., and Karube, I., in *Immobilized Microbial Cells,* Venkatasubramanian, K., ed., ACS Symp. Ser. 106, ACS, Washington, DC, 1979, p. 59.
8. Larsson, P. O., Ohlsson, S., and Mosbach, K., *Appl. Biochem. Bioeng.* **2,** 290 (1979).
9. Chibata, I., in *Food Process Engineering,* Vol. 2, *Enzyme Engineering in Food Processing,* Linko, P., Malkki, Y., Olkku, J., and Larinkari, J., eds. Applied Science Publishers, London, 1980, p. 1.