Some Factors Affecting Production of Itaconic Acid by Aspergillus terreus Cells Immobilized in Polyacrylamide Gels

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Some Factors Affecting Production of Itaconic Acid by *Aspergillus terreus* Cells Immobilized in Polyacrylamide Gels

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SUMMARY

With repeated batch bioreactor using living *Aspergillus terreus* cells entrapped in polyacrylamide gels, production of itaconic acid from glucoce was increased 1.5 times with the addition of ferrous tartarate (10 mg/liter), 3 times with the addition of cupric sulfate (10 mg/liter), or 3.3 times with the co-addition of ferrous tartarate (10 mg/liter) and cupric sulfate (10 mg/liter) comparing with non addition. On the other hand, production of itaconic acid increased 1.4 times with 1.4 liter/min of aeration volume as large as that of 0.7 liter/min of aeration volume, or 2.4 times with 0.7 liter/min of oxygen alone as large as that with the equivalent volume of air.

With the continuous column bioreactor, the maximum itaconic acid productivity was 83.4 mg/hr/30 g gels.

INTRODUCTION

Itaconic acid is used to make polyester resins or plastics since it is a substituted acrylic acid. Itaconic acid is known to be produced from sugar by filamentous fungi, such as *Aspergillus itaconicus*[1], *Aspergillus terreus*[2], *Ustilago zeae*[3], and *Helicobasidium mompa*[4].

Recently, it is also known that *Candida sp.* produces itaconic acid from cane molasses[5]. Itaconic acid is now produced commercially by submerged culture using *Aspergillus terreus.*

In the previous report, the effects of the time of shaking culture on biocatalytic activity of immobilized cells, the types of substrate, pH and some kinds of salts, on production of itaconic acid were described[6].

In continuation of the previous work, the effects of ferrous tartarate, cupric sulfate, aeration volume, oxygen tension and size of gel blocks on itaconic acid production using *Aspergillus terreus* cells immobilized in polyacrylamide are described.

MATERIALS AND METHODS

Microorganism. *Aspergillus terreus* G-026 was used in this investigation, and was maintained on potato-dextrose agar.

Culture medium. Culture medium was composed of 10% glucose, 0.3% NH_4NO_3, 0.2% $MgSO_4 \cdot 7H_2O$, 0.27% $NH_4H_2PO_4$ and 0.1% corn steep liquor, pH 5.0.

Bioreactor substrate. Bioreactor substrate was composed of 6% glucose, 0.3% NH_4NO_3 and 0.05% $MgSO_4 \cdot 7H_2O$, pH 2.2.

Cultivation. Spores ($5x10^7$) of *Aspergillus terreus* were inoculated into 100 ml of the culture medium in a 500-ml shaking flask and cultivated on a shaker (7 cm stroke, 120 reciprocations/min)

at 30℃. Growing cells were harvested at 72 hr.

Immobilization of living cells. *Aspergillus terreus* cells were immobilzed by the same method described in the previous study[6].

Analytical method. Itaconic acid was determined by Shimadzu isotacophoretic analyzer IP-1B using 0.01 M β-alanine-0.2% Triton X-100, pH 3.1, as a leading electrolyte, and 0.01M n-caproic acid as a terminal electrolyte.

Itaconic acid production with immobilized cells.

1) **Repeated batch method.** About 20 g of gel cubes were suspended in a glass bioreactor of 400 cm^3 (5 cm in diameter, 20 cm in height) containing 100 ml of the bioreactor substrate. Incubation was carried out under aeration at a rate of 0.7 liter/min at 35℃. The medium was replaced at a 24-hr interval.

2) **Continuous method.** About 30 g of immobilized cells were incubated in the same reactor used for the repeated batch method. Incubation was carried out at 33℃ or 35℃ with an aeration volume of 1.4 liter/min and substrate at a flow rate of 4ml/hr.

RESULTS AND DISCUSSION

Repeated batch method

Effect of salts. Although it is known that the addition of zine sulfate and magnesium sulfate increases the yield itaconic acid production by fermentation[7], the effect of these two salts was only slight to judge from our experiments. However, the productivity of itaconic acid was increased 1.5 times with the addition of ferrous tartarate (10 mg/liter) alone, 3 times with the addition of cupric sulfate(10 mg/liter) and 3.3 times with the co-addition of these two salts (10 mg/liter, each) comparing with the non addition (Fig. 1).

Effect of gel block size. To observe the effect of gel block size of immobilized fungal cells on itaconic acid, both gels of 2 cubic mm and 4 cubic mm were tested. Itaconic acid productivity by gels of 2 cubic mm was 1.6 times that by gels of 4 cubic mm (Fig. 2).

The larger the surface of immobilized gels was, the more itaconic acid was produced.

Effect of aeration volume. Effect of aeration volume on the productivity was investigated by comparing rates of 0.7 liter/min, 1.0 liter/min and 1.4 liter/min of air. The higher the aeration was, the higher was the productivity (Fig. 3).

Effect of oxygen tension. Since the productivity of itaconic acid increased by increasing

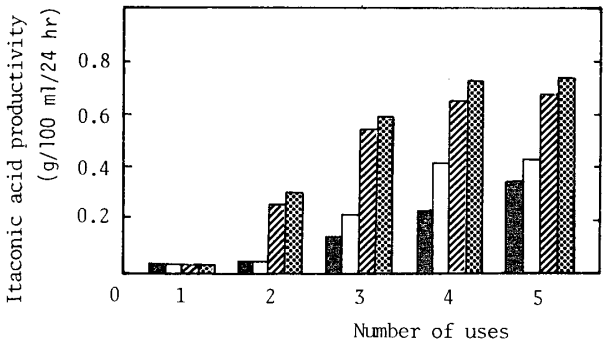

Fig. 1. Effect of ferrous tartarate, $CuSO_4 \cdot 5H_2O$ and mixture of ferrous tartarate and $CuSO_4 \cdot 5H_2O$ on itaconic a cid productivity.
■, non addition: □, ferrous tartarate, 10 mg/liter; ▨, $CuSO_4 \cdot 5H_2O$, 10 mg/liter ; ⁙, mixture of ferrous tartarate, 10 mg/liter and $CuSO_4 \cdot 5H_2O$, 10 mg/liter.

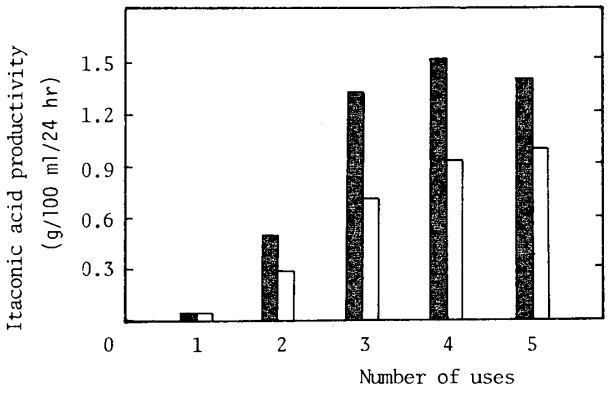

Fig. 2. Effect of size of immobilized cells on itaconic acid productivity.
Size of immobilized cells :■, 2cubic mm ; □, 4cubic mm.

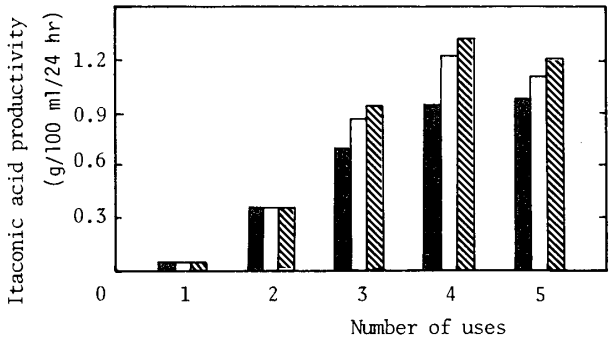

Fig. 3. Effect of aeration volume on itaconic acid productivity.
Aeration volume : ■, 0.7 liter/min ; □, 1.0 liter/min ; ▨, 1.4 liter/min.

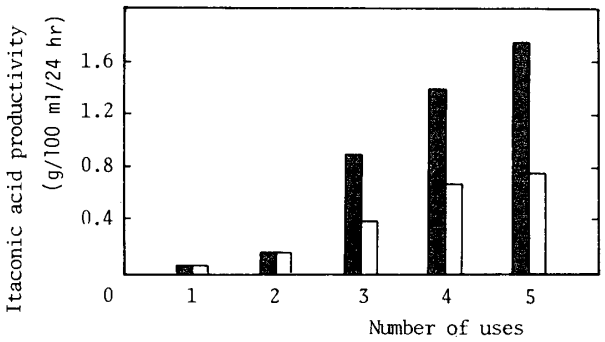

Fig. 4. Effect of air or oxygen on itaconic acid productivity. ■, oxygen ; □, air.

the aeration volume, comparison of oxygen tension was improved. The productivity using oxygen alone (0.7 liter/min) was twice that with only air (0.7 liter/min) (Fig. 4). This indicates that immobilized cells require more oxygen tension for itaconic acid production.

Effect of glucose concentration. Effect of glucose concentration on the productivity was investigated by varying glucose concentration. Among concentrations tested, 6% glucose gave the highest productivity (Fig. 5).

This finding indicates that the osmotic pressure due to 6% glucose was the most favorable among concentrations tested.

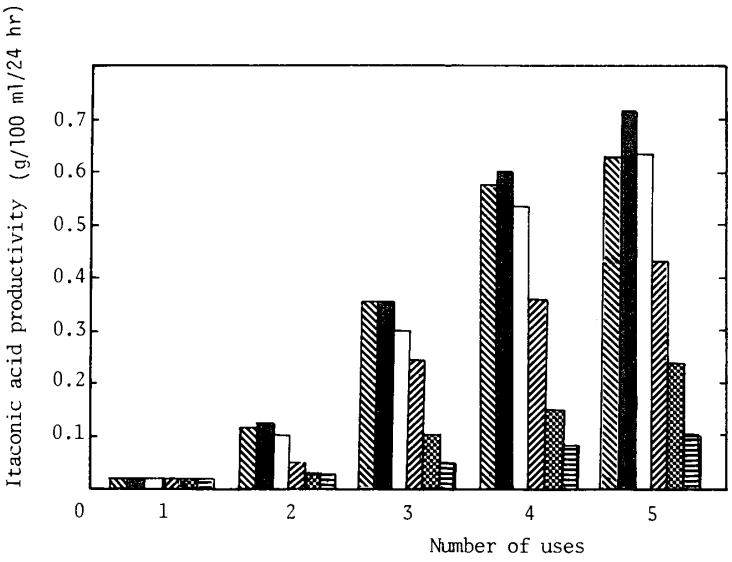

Fig. 5. Effect of glucose concentration on itaconic acid productivity.
Glucose concentration : ▧, 4% ; ■, 6% ; □, 8% ; ▨, 10% ; ▩, 15% ; ▤, 20%.

Continuous method.

Since the addition of ferrous tartarate (10 mg/liter), cupric sulfate (10 mg/liter) or a mixture of these two salts increased the productivity by the repeated batch method, the effect of these two salts on the productivity by the continuous method was also tested. In the case of non addition. the production of itaconic acid reached 38 mg/hr/30 g gels after 13 days (Fig. 6).

However, the productivity reached 52 mg/hr/30 g gels after 11 days with addition of ferrous tartarate (10 mg/liter). By the co-addition of ferrous tartarate (1 mg/liter) and cupric sulfate (10 mg/liter), the productivity reached 58 mg/hr/30 g gels after 9 days.

Since it was found that the productivity was increased by decreasing gel size of immobilized cell by the repeated batch method, the effect of gel size of immobilized cell on continuous reaction was investigated. The productivity reached 83.4 mg/hr/30 g gels, and the rate of glucose consumed was 82.3%, the yield of itaconic acid from glucose, was 59.1% using 2 cubic mm gel of immobilized cell (Fig. 7).

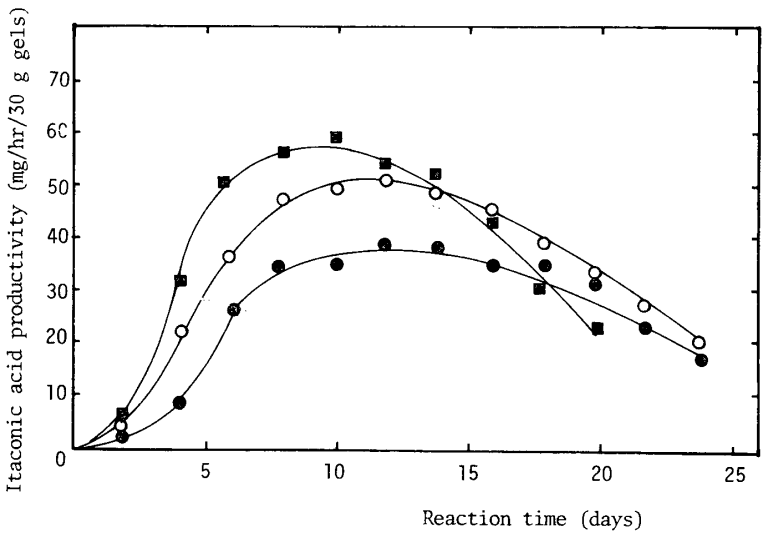

Fig. 6. Effect of ferrous tartarate, and mixture of ferrous tartarate and $CuSO_4 \cdot 5H_2O$ on continuous production of itaconic acid.
●, non addition ; ○, ferrous tartarate, 10 mg/liter ; ■, mixture of ferrous tartarate, 10 mg/liter and $CuSO_4 \cdot 5H_2O$, 10 mg/liter.

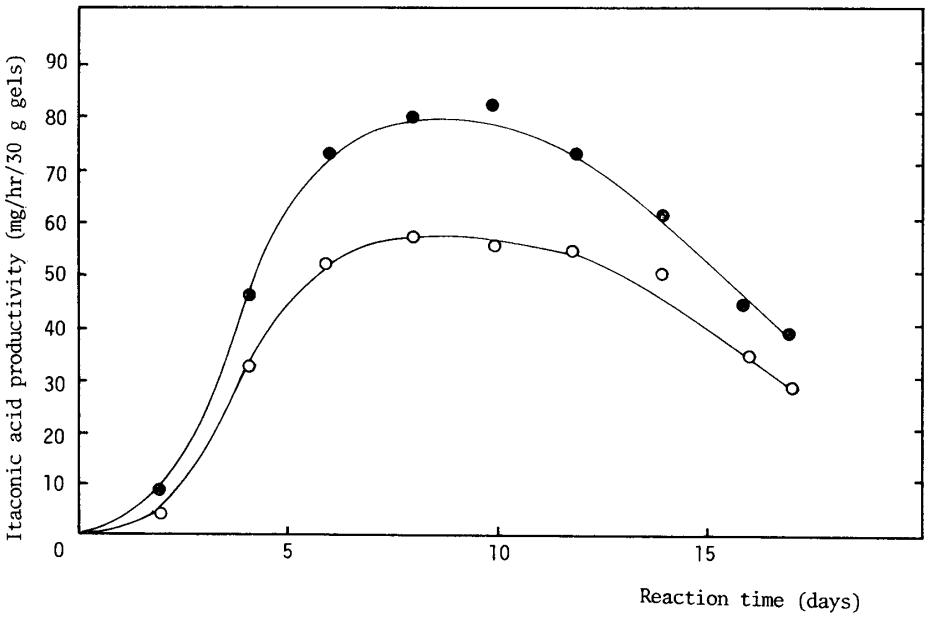

Fig. 7. Effect of size of immobilized cells on continuous production on itaconic acid.
Size of immobilized cells : ●, 2 cubic mm ; ○, 4 cubic mm.

The effect of the reaction temperature on continuous reaction was tested.

For the repeated batch method, the productivity was the highest at 35℃, but the period of half-life was shorter at 35℃. For improvement of the biocatalytic activity, it was tested at 33℃. When the reaction temperature was 33℃, the amount of itaconic acid production was about 88.6% of that at 35℃, but itaconic acid productivity did not decrease until 30 days (Fig. 8).

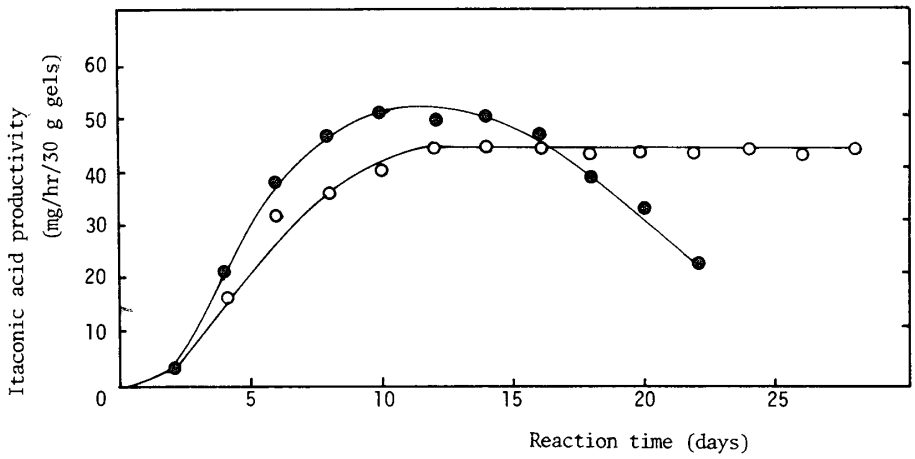

Fig. 8. Effect of temperature on continuous production of itaconic acid. Temperature: ●, 35℃; ○, 33℃.

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固定化 *Aspergillus terreus* を用いてのイタコン酸生産に対する影響因子について

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要　　約

ポリアクリルアミドゲルに固定した *Aspergillus terreus* を用い基質としてグルコースを使用，イタコン酸生産を回分法で行なった。その結果，無添加の場合に対し酒石酸鉄の添加で1.5倍，硫酸銅添加で３倍，そして両者の添加により3.3倍の生産増加をみた。又，空気の通気量を２倍にすることにより1.4倍，純酸素通気では2.4倍の生産増加をみた。連続法では最高30ｇゲル当り１時間83.4mgのイタコン酸生産をみ，その活性半減期は33℃では30日であった。

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