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방사선중합체로 고정화된 *Rhizopus oryzae*의 유산생성

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Production of L(+)-Lactic Acid by *Rhizopus oryzae* after Immobilization in Polymer Supports prepared with Gamma-ray Induced Polymerization

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ABSTRACT

Lactic acid production yield was examined for commercial use by immobilizing *Rhizopus oryzae* with radiation induced polymer. The supporting material was synthesized by the low temperature radiation polymerization method, in which the microorganism was entrapped physically by contacting together in culture broth shaking for 24 hours. Support polymers with 5~10 vol-% monomers were able to increase their catalytic activities, consuming 65 g / l of glucose, producing 56 g / l of lactic acid, thus yielding 88% of product in general. But in free culture, the microorganism consumed almost all carbon source and produced lactic acid only 60% even after 96 hours. The yield of the experiment under discussion was significantly high compared with conventional immobilization procedure.

INTRODUCTION

The research group studied and developed the immobilization technique for physically entrapping materials by means of radiation induced polymerization(1). This technique was applied to various kinds of biofunctional materials such as drugs, proteins and cells for biotechnical uses. Applications of this technique to cell cultures have also been studied(2).

Aspergillus and *Rhizopus* genus capable of producing organic acid, immobilized in calcium alginate gels, k-carrageenan and some polymers were used efficiently(3).

Lactic acid is produced commercially by the fermentation of carbohydrate with homofermentative lactic acid bacteria. Lockwood et al.(4) reported that *Rhizopus oryzae* in surface culture converted glucose to a large amount of L(+)-lactic acid in the presence of calcium carbonate. Prescott and Dunn(5) reviewed the production of L(+)-lactic acid by molds. *Rhizopus oryzae* immobilized in calcium alginate gels, k-carrageenan and some polymers have also recently been used to produce lactic acid(6).

The radiation polymerization techniques, however, have not been used until now. Our tests applied radiation polymerized support to immobilize *Rhizopus oryzae* and

obtained 88.4% yield of lactic acid from glucose. Specifically, this report details the effects on immobilized *Rhizopus oryzae* by means of radiation polymerization and demonstrates the resultant lactic acid production from glucose in a chemically defined medium.

MATERIALS AND METHODS

A lactic acid producing strain of *Rhizopus oryzae* obtained by Taki company, Japan, was maintained on potato dextrose agar slant. For the study, the microorganism was inoculated in dextrose agar slant and incubated at 25−30℃ for 4−7 days, while stored in a refrigerated environment.

The fermentation medium used in this study was composed of 7% glucose, 0.05% urea, 0.03% KH_2PO_4, 0.01% $MgSO_4 \cdot 7H_2O$, 0.0044% $ZnSO_4 \cdot 7H_2O$, 0.002% Span 80 and 0.01% yeast extract in 100-ml Erlenmeyer flask. Each flask contained 30ml of medium and 5㎤ of support material and was autoclaved.

Five monomers, such as 2-hydroxy ethyl acrylate(HEA), 2-hydroxy ethyl methacrylate(HEMA), polyethyleneglycol #400 dimethacrylate(9G), polyethyleneglycol #400 diacrylate(A-400), 2,2-bis 4-methacroxy polyethoxy phenylpropane(BPE-1300) were purchased from Shinnakamura chemical Co. Ltd., Japan. The mixture of these monomers with water was cooled to −78℃ and then irradiated with Co-60(10 kGy*) in a nitrogen gas atmosphere. The concentration range of used monomers was 2−50%. Polymers obtained were soaked distilled water for 3−4 days to wash out unreacted monomers. The water was changed regularly. The polymers for support were cut into small cubic pieces(size: 125㎟), which were then freeze-dried.

Each 100ml Erlenmeyer flask contained 30ml of media and 5㎤ of support material. One million spores in 0.5ml water were inoculated directly. These spores were cultured in a shaking incubator at 30℃ with an oscillation speed of 120 rpm for 24 hours. After 24 hours a culture of calcium carbonate 1.5 g was added to each flask for pH adjustment. The fermentation was conducted at 35℃ with the oscillation speed of 80 rpm for 1 to 3 days.

Lactic acid was analyzed by the Barker-Summerson method(7) after the removal of protein, and the glucose concentration was measured with a glucose analyzer (Mitsubishi Chemical Industry Co. Ltd., Model GL-101).

RESULTS AND DISCUSSION

Typical cultures of *R. oryzae* were conducted in a free state and in an immobilized state, as shown in Figure 1. In the free state, the growing speed was very slow as indicated by glucose consumption being nullified at 96 hours and lactic acid production reaching 43 g as a final amount. In the immobilized state, lactic acid was maximized after 48 hours of fermentation reaching 54 g. In the fermentation using immobilized *R. oryzae* the incubation period for the first 24 hours was without considerable changes in glucose consumption or lactic acid production. Following this initial period, the fermentation rate increased consumption and lactic acid production yield 66 g and 58 g, respectively. After that time production yield decreased slightly so that no additional time was necessary

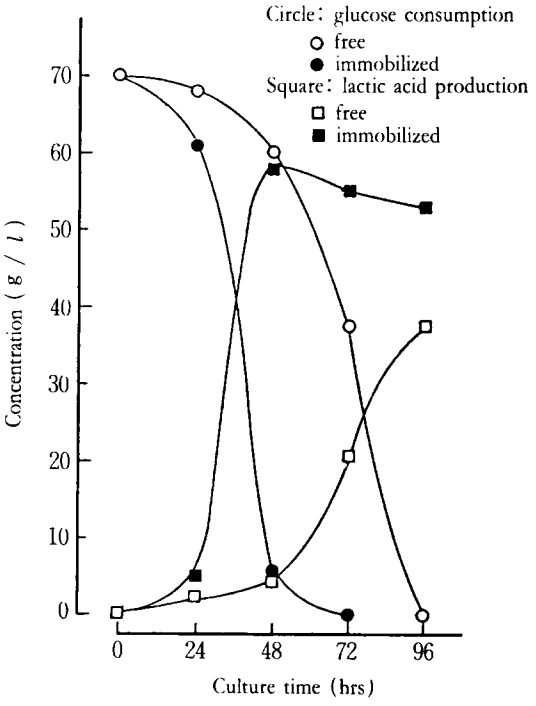

Fig. 1. Time required for glucose consumption and lactic acid production during fermentation of free and immobilized *Rhizopus oryzae*.

Table 1. The immobilized effects of microorganism *Rhizopus oryzae* after 24 hours of fermentation

Polymer description	Monomer / water (V / V %)	Glucose consumption (g / l)	Lactic acid production (g / l)	Yield of lactic acid (%)
2 Hydroxyethylacrylate (HEMA) copolymer	50	23.2	16.0	69.0
	20	64.1	56.0	87.0
	10	70.0	60.0	86.0
	5	70.0	59.0	84.0
	2	70.0	60.0	86.0
2 Hydroxyethylmethacrylate (HEMA) copolymer	50	35.0	20.0	57.1
	20	60.0	46.0	76.6
	10	67.5	60.0	88.9
	5	61.0	50.0	82.0
Polyethyleneglycol #400 dimethacrylate (9G) copolymer	50	26.5	17.0	64.0
	20	45.0	36.0	80.0
	10	62.2	55.0	88.4
	5	69.8	61.0	87.4
	2	64.0	52.0	81.1
Polyethyleneglycol #400 diacrylate (A−400) copolymer	50	30.0	20.0	76.7
	20	46.0	32.0	69.6
	10	61.6	50.0	81.0
	5	62.8	53.0	84.4
	2	63.0	50.0	79.4
2,2-Bis 4-methacroxy polyethoxyphenylpropane (BPE-1300) copolymer	50	22.0	10.0	45.5
	20	42.0	32.0	76.2
	10	67.0	60.0	89.5
	5	70.0	62.0	88.6

to increase the product.

Immobilization of fungi with polymer support occurred during the shaking of the culture. Polymers made from 2 to 50% of monomer concentration by radiation polymerization technique were added for fermentation(Table 1). Support prepared with 50% monomer could not immobilize the fungi. But polymers prepared with 5−10% monomers were able to increase their catalytic activities and facilitate the immobilization after 24 hours, producing 65 g / l of lactic acid production, 88% of product yield in 5% monomer on the average. Especially in case of BPE-1300, lactic acid production and yield were 60 g / l and 89.5% in 10% monomer, and 62 g / l and 88.6% in 5% monomer respectively. It was concluded that polymers constituted with 5 to 10% monomer offer the optimal conditions for the fungi.

Lockwood et al.(4) reported that free cells of *R. oryzae* usually yielded about 43% of lactic acid yield and required a fermentation time of 17 to 21 days, and Hang et al.(8) reported that the immobilized *R. oryzae* cells which were obtained by dropping Ca-alginate produced large amountes of L(+)−lactic acid up to 72% of yield. Compared with these, the yield of the experiment under discussion was significantly high.

* kGy(kilogray): when a kilogram of matter absorbs the energy of one joule, this matter is said to have received a dose of one gray.

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요 약

방사선 중합체로 고정화한 *Rhizopus oryzae*의 유산생성

방사선 공중합 고분자로 *Rhizopus oryzae*를 흡착 고정화하였다. 이 고정화 균체는 고정화하지 않은 생균체보다 반응시간도 짧았으며 제품수율도 아주 높았다. 즉 글루코스를 주 탄소원으로 한 경우 고정화 하지 않은 균체 배양은 반응후 96시간에 탄소원을 거의 소비하고 유산생성 수율이 약 60%에 다다랐으나 고정화 균체는 반응후 48시간 안에 수율이 약 88% 이상으로 되었다. 또 중합체 중 모노머의 비율이 5−10%의 것이 고정화에 좋은 결과를 보였다.

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