Immobilization of Yeast Cells by Radiation-Induced Polymerization

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Radiation-induced polymerization method was applied to the immobilization of yeast cells. The effects of irradiation, cooling and monomer, which are neccessary for polymerization, were recovered completely by subsequent aerobical incubation of yest cells. The ethanol productive in immobilized yeast cells increased with the increase of aerobical incubation period. The growth of yeast cells in immobilized yeast cells was indicated. The maximum ethanol productivity in immobilized yeast cell system was around three times as much as that in free yeast cell system.

Introduction

Immobilized microbial cells have proved their validity to produce useful compounds [1]. Many studies [2, 3] have been carried out about immobilization of yeast cells in order to obtain ethanol. Recently, Chibata *et al.* [4] reported immobilized growing yeast cells using carrageenan as carrier matrix. They entrapped very small number of yeast cells in carrier matrix and immobilized yeast cells cultivated in some complete medium grew densely than those in liquid medium.

The radiation polymerization technique has been applied to the immobilization of chloroplasts [5–8]. O_2 evolution activity in photosystem II, one of most labile activity in whole chloroplast system, was maintained in immobilized system for about ten times as long as that in intact one. This radiation polymerization technique was also applied to the fixation of microbial cells [9] and enzymes [10].

Yeast cells could be immobilized by this radiation polymerization method. The property, for instance elasticity, and structure of carrier matrix could be changed relatively freely by this technique. This method could be used as a clue to clarify the relation between the structure of carrier matrix and best condition for growth of yeast cells. Irradiation would damage a part of yeast cells, survived yeast cells could grow in carrier matrix.

In this work, the effect of irradiation on yeast cells and one carrier matrix for immobilization were investigated as the first step of study for fixation of yeast cells by radiation-induced polymerization method.

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Materials and Methods

Microorganisms

Sacchromyces formosensis was used in this study. The yeast cells were precultured under aerobic conditions for 24 h at 30 °C in a medium 1% glucose, 0.1% molasses, 0.5% pepton, 0.3% yeast extract and 0.3% malt extract (pH 5.0).

Homogeneously immobilized yeast cells by conventional method

A number of rolled gauze were sterilized and immersed in broth of precultured yeast cells. Rolled gauze with yeast cells was immersed in methoxypolyethyleneglycol methacrylate (M-23 G) or polyethyleneglycol dimethacrylate (14 G) sterilized by filtration. The sample was irradiated with γ-rays from a ^{60}Co source for 1 h with a dose rate of 1×10^6 rad/h under sterile condition.

Immobilized yeast cells were washed well with a complete medium containing 10% glucose, 1% molasses, 0.15% yeast extract, 0.25% NH_4Cl, 0.55% K_2HPO_4, 0.025% $MgSO_4 \cdot 7H_2O$, 0.1% NaCl, 0.001% $CaCl_2$ and 0.3% lactic acid (pH 5.0).

The immobilized yeast cells were then incubated using the complete medium.

Immobilized growing yeast cells

The small number of yeast cells was immobilized on rolled gauze with M-23 G or 14 G by radiation-

induced polymerization at 0 °C or 25 °C by same method as that in homogeneously immobilized yeast cell method. Carrier matrix which trap small number of yeast cells was washed well with complete medium to remove residual monomer. The entrapped yeast cells were transfered into a sterilized Erlenmeyer flask containing the same volume of complete medium and incubated with rapid shaking at 30 °C.

Analytical method

Ethanol produced was determined using alcohol dehydrogenase [11]. The number of free cells was counted by the drop-plate method and was expressed as number of free cells per ml of liquid medium.

Results and Discussion

The effect of irradiation on yeast cells up to various dosage at some temperatures

In order to confirm the possibility for the immobilization of yeast cells by radiation-induced polymerization, the effect of irradiation on yeast cells at some temperatures was investigated and shown in Fig. 1. Yeast cells in preculture medium was irradiated with up to 1×10^6 rad at some temperatures and incubated at 30 °C in preculture medium for 50 h, then in complete medium fro 46 h. The number of yeast cells are plotted against incubation time in Fig. 1. Just after the irradiation, the number of yeast cells in the sample irradiated at 25 °C, 0 °C and − 24 °C was much smaller than that in unirradiated control sample. However, the number of yeast cells in irradiated sample increased with incubation time. After incubation for 96 h, the number became almost same as that in control sample.

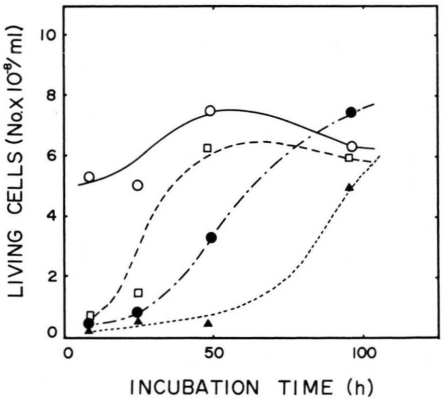

Fig. 1. Effect of irradiation at various temperatures on yeast cells. The precultured yeast cells was irradiated up to 1 Mrad at various temperatures. Irradiated yeast cells were incubated at 30 °C in preculture medium for 50 h and then in complete medium for 46 h. (○) control, (□) −24 °C irradiation (●) 0 °C irradiation, (▲) 25 °C irradiation.

The change in the shape of irradiated yeast cells was observed continuously by optical microscope. After irradiation, the shape of some irradiated yeast cells was abnormal and that of the others was normal. The percentage of normal yeast cells increased rapidly when the sample was incubated at 30 °C. After 96 h incubation, the shape of all yeast cells was completely normal.

Recovery in the number and shape of irradiated yeast cells by incubation was made clear. However, the number of yeast cells just after irradiation was one tenth of that of control yeast cells without irradiation. In order to increase the number of yeast cells just after irradiation, the effect of smaller dosage irradiation on yeast cells was investigated and results are shown in Table I. For the dosage between 10^6 rad and 10^5 rad, the number of yeast cells irradiated was less than one tenth of that in yeast cells without irradiation. For the dosage below 5×10^4 rad, the number of yeast cells increased to almost same number as that in yeast cells without irradiation after 24 h incubation at 30 °C, instead of 96 h incubation as results shown in Fig. 1. Recovery in the number of yeast cells is significant at the dosage below 5×10^4 rad. These results open the possibility for the immobilization of yeast cells by irradiation.

The effect of cooling on yeast cells

The effect of cooling on yeast cells and recovery during incubation at 30 °C are shown in Table II. Yeast cells in preculture medium were cooled down to 0 °C for 1 h and incubated at 30 °C. After 18 h-incubation, the number of yeast cells cooled was around half of that of control yeast cells without cooling. After 24 h-incubation, however, the number of cooled yeast cells increased to almost same as that of control yeast cells. These results show that the damage of yeast cell by cooling down to 0 °C can be recovered by incubation at 30 °C.

Table I. Effect of irradiation on yeast cells at lower dosage.

Irradiation condition		Living cells
dosage [rad]	temperature [°C]	[No × 10^{-7}/ml]
control (without irradiation)		125
10^6	−24	5
	0	2.5
10^5	−24	10
	0	12.5
	25	10
5×10^4	0	50
	25	100
10^4	0	65
	25	150
5×10^3	0	87.5
	25	60

One ml of precultured yeast cells were irradiated and incubated with 10 ml of preculture medium at 30 °C for 24 h.

Table II. Effect of cooling on yeast cells.

Cooling temperature	Number of yeast cells [No × 10^{-7}/ml]		
	original number	incubation time	
		18 h	24 h
control (30 °C)		150	150
0 °C		75	140
−24 °C	12	150	150
−78 °C		50	150

One ml of precultured yeast cells were cooled down to various temperatures for 1 h and warmed up to 30 °C rapidly and incubated at 30 °C with 10 ml of complete medium.

The effect of monomer on yeast cells

In order to find out best monomer for immobilization of yeast cells, the effect of monomer on yeast cells was investigated and shown in Table III. After monomer was added to yeast cells in preculture medium, yeast cells were incubated at 30 °C for 24 h. After 24 h-incubation, the number of yeast cells with M-23 G or 2-hydroxyethyl methacrylate (HEMA) was almost same as that of yeast cells without monomer.

Homogeneoulsy immobilized yeast cells by conventional method

In order to search best immobilization condition for immobilized growing yeast cells, yeast cells were homogeneously immobilized by conventional method at first. Homogeneously immobilized yeast cells were transfered into a sterilized Erlenmeyer flask containing the complete medium and incubated at 30 °C with gentle shaking. As a control, the same number of free cells as that of immobilized cells was also incubated. The results after 24 h-incubation with gentle shaking are shown in Table IV. Amount of ethanol produced after 24 h-incubation in homogeneously immobilized yeast cells was more than half of that in free cell system.

Immobilized growing yeast cells

The carrier matrix trapped a small number of yeast cells (6×10^5/ ml carrier matrix) was aerobically incubated in the complete medium containing nutrients for growth.

After various periods of aerobical incubation time (A), a part of immobilized yeast cells was

Table III. Effect of monomer on yeast cells.

Monomer	Living cell [No × 10^{-7}/ml]
control (without monomer)	80
HEMA	62
HEA	30
M-23 G	78

Ten ml of precultured yeast cells were incubated with 0.1 ml of various monomers at 30 °C for 24 h.

Table IV. Ethanol production by homogeneously immobilized yeast cells.

Immobilization condition				Ethanol concentration
irradiation dose [rad]	irradiation temperature [°C]	monomer	monomer concentration [%]	[%]
5×10^4	25	M-23 G	10	2.7
1×10^5	0	14 G	10	4.53
		Free cells [a]		5.19

The batch production of ethanol was carried out by homogeneously immobilized yeast cells using complete medium with shaking at 30 °C for 24 h.

[a] Same number of free yeast cells as that of yeast cells homogeneously immobilized were suspended in the complete medium. The ethanol production of free cells was determined using this suspension under the same condition of batch ethanol production as described in the text except for the use of immobilized yeast cells.

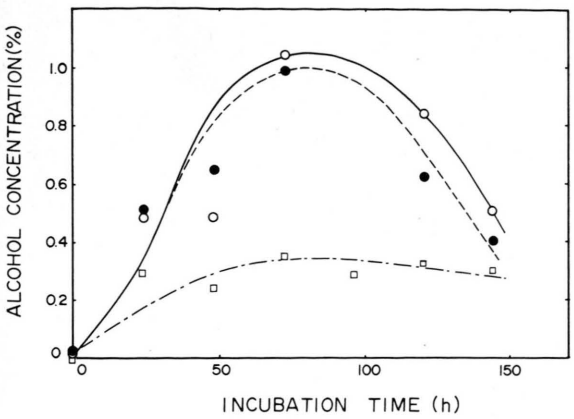

Fig. 2. The change of ethanol concentration for 1 h batch production of ethanol in immobilized growing yeast cells system. Carrier matrix trapped a small number of yeast cells was incubated aerobically at 30 °C for various periods (A). Immobilized yeast cells were washed well with complete medium. One hour batch ethanol production was carried out by using these immobilized yeast cells, and ethanol concentration (B) was measured. The relation between A and B is shown in this figure. As a control, free cells were incubated aerobically for various periods (A'). Alcohol concentration (B') after 1-h ethanol production was measured under the same conditions as immobilized yeast cells as described in text precisely. Irradiation conditions and monomer concentration; (○) 5×10^4 rad, 25 °C, 10% M-23G; (●) 5×10^4 rad, 25 °C, 10% 14G; (□) free yeast cells.

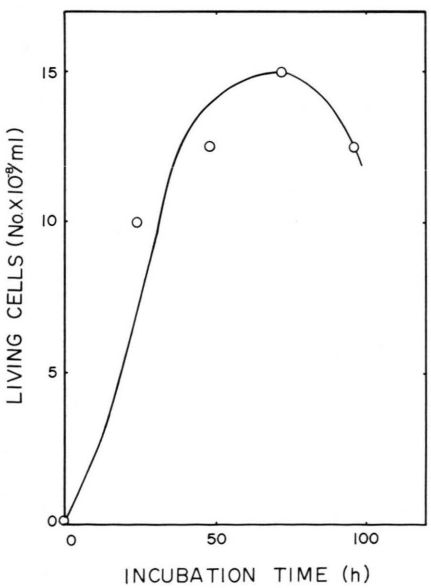

Fig. 3. The change in number of free yeast cells. The number of free cells incubated aerobically shown in Fig. 2 was plotted against incubation time.

transfered into a sterilized Erlenmeyer flask and washed well with the complete medium. The complete medium was changed three times in order to remove alcohol completely which was produced during aerobical incubation and to eliminate yeast cells attached to the surface of carrier matrix. Immobilized growing yeast cells were incubated at 30 °C with same volume of the complete medium as that of carrier matrix under gentle shaking condition for 1 h to test alcohol productivity. After 1 h-incubation, the concentration (B) of alcohol produced was measured. The relation between the ethanol concentration (B) after 1-h batch production and the period of aerobical incubation (A) was investigated.

Without aerobical incubation, ethanol concentration after 1-h batch ethanol production was very low (see the first points of Fig. 2). The ethanol concentration (B) in 1-h batch production increased rapidly with the increase of aerobical incubation period (A) as shown in Fig. 2. After 72-h incubation, the ethanol concentration after 1-h batch production reached around 1%. In this case, during 1-h batch production, a violent evolution of CO_2 gas was observed on the surface of carrier matrix.

As a control, same number of free yeast cells as that of immobilized yeast cells were transfered into a sterilized Erlenmeyer flask containing the complete medium and incubated aerobically under the same conditions. After aerobical incubation, same volume of cultured medium containing free cells as that of carrier matrix with immobilized growing yeast cells was transfered into sterilized Erlenmeyer flask containing same volume of complete medium as that of cultured medium containing free cells and incubated under same condition for 1 h. In this free cell system, the change of alcohol concentration and the change in the number of free cells were investigated. The results are shown in Fig. 2 and Fig. 3.

After 72-h aerobical incubation, the concentration of alcohol after 1-h production reached maximum, around 0.3%, as shown in Fig. 2. In this case, the curve of the change in alcolhol concentration is rather flat. The curve of change in number of yeast cells showed clear maximum 1.5×10^9 per ml of cultured medium, after 72 h aerobical incubation as shown in Fig. 3. As shown in Fig. 2, the maximum alcohol concentration after 1-h batch alcohol production in immobilized system was around 1%,

three times of that in free cell system which was around 0.3%.

Chibata *et al.* [4] obtained ethanol productivity of immobilized growing yeast cells which was around ten times higher compared to the volumetric productivity of free cells. In their immobilized growing cell system, specific productivity of alcohol (mg alcohol/10^8 of cells/h) was almost same as that in free system. Their results indicate that specific ethanol productivity of the cell in carrier matrix was fully utilized.

In our present work, the alcohol concentration in the immobilized system was around three times of that in free cell system. Ethanol production rate is proportional to the product of specific ethanol productivity and number of immobilized yeast cells. Specific ethanol productivity in immobilized system could not be more than that in free system. It is reasonable that number of immobilized yeast cell was equal to or more than three times of that of free cell system in the present work. These results indicate that yeast cells grew inside of carrier matrix in this immobilized cell system.

Improvements could be done in our present work because the structure and property of carrier matrix could be changed more freely by radiation-induced polymerization method than other methods. Further investigation will be shown in the near future.

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