# STUDY ON IMMOBILIZED YEAST CELLS WITH HRDROPHILIC POLYMER CARRIER BY RADIATION—INDUCED COPOLYMERIZATION*

Li Zhengkui (李正魁), Zhang Bosen (张伯森)

*(Institute of Application of Atomic Energy, Jiangsu Academy of Agricultural Sciences, Nanjing 210014, China)*

You Jingjing (游静静)

*(Jiangsu Food and Fermentation Institute, Nanjing 210008, China)*

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## ABSTRACT

In this paper, various kinds of monomers 2-hydroxyethyl methacrylate (HEMA), 2-hydroxyethyl acrylate (HEA), hydroxypropyl methacrylate (HPMA) and methoxy polyethylene glycol methylacrylate (M-23G) were copolymerized by radiation technique at low temperature (-78 °C) and several kinds of copolymer carriers were obtained. Yeast cells were immobilized through adhesion and multiplication of yeast cells themselves on these carriers. The ethanol productivity of immobilized yeast cells with these carriers was related to the monomer composition and water content of copolymer carriers and the optimum monomer composition was 20 %:10 % in poly (HEA-M23G). In this case, the ethanol productivity of immobilized yeast cells was 26 mg /( ml · h), which was 4 times as high as that of free cells. In this study, the effect of adding of crosslinking reagent (4G) in copolymer on activity of yeast cells immobilized with the carriers were also studied. It was found that the effect of adding crosslinking reagent (4G) in lower monomer composition of poly(HEA-M23G) on the ethanol productivity of immobilized cells was better than that in higher one in this work.

**Keywords:** Ethanol productivity Yeast cells Immobilization Radiation Copolymer

## 1 INTRODUCTION

Recently, several studies have been reported on the immobilization of yeast cells. A number of methods with natural and artificial carriers has been proposed for immobilization[1-3]. Among these the radiation polymerization method is considered to be one of the most promising. Fujimura and Kaetsu have studied on this method to immobilized yeast cells with artificial polymer carriers produced by radiation-induced polymerization[4]. The immobilized cells thus obtained exhibited activity of around

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5—10 times higher than that of cells in free system. This greater activity was due to much higher density of yeast cells trapped inside and on the surface of carriers. Radiation polymerization can produce artificial polymer carrier for immobilizing microoganism and change continously nature of carriers. In this paper, the effect of several copolymer systems and adding crosslinking reagent in polymer on activity of yeast cells immobilized with the carriers, the relationship between the activity of immobilized yeast cells and the water content of polymer carrier and the structure of polymer carriers are discussed.

## 2 MATERIALS AND METHODS

### 2.1 Microorganism

Saccharomyces formesensis was used in this work. The yeast cells were precultured under aerobic condition for 48 h at 28 °C in a medium consisting of 1 % glucose, 0.1 % molasses, 0.5 % pepton, 0.3 % yeast extract and 0.3 % malt extract (pH 4.8).

### 2.2 Preparation of swelling copolymer carriers and immobilization of yeast cells

Various kinds of glass-forming monomers, 2-Hydroxyethyl methacrylate (HEMA), 2-hydroxyethyl acrylate (HEA), hydroxypropyl methacrylate (HPMA), methoxy polyethylene glycol methylacrylate (M-23G), and crosslinking reagent polyethylene glycol dimethacrylate (4G) were used in this work, and were mixed with water to various composition. The mixtures were irradiated at -78 °C with γ-rays of $^{60}Co$ source with a dose of 10 KGy. The resultant polymer carriers were cut into small pieces, approximately 5—10 mm in diameter, and shaken with excess amount of water for one week until fully swollen. The swollen copolymer carriers were sterilized by autoclaving at 120 °C for 40 min. The sterilized carriers were immersed into the nutrient medium for 2 d to be filled with the nutrient medium. The carriers swollen and sterilized (10 $cm^3$) were added to the mixture of precultured yeast cells (1 ml) and the nutrient medium (20 ml). The resultant suspension was incubated at 30 °C under an aerobic condition in a rotary shaker with 130 rpm for 72 h. For every 24 h, the nutrient medium was changed. The composition of the nutrient medium used in this work was 12 % glucose, 1 % molasses, 0.15% yeast extract, 0.25 % $NH_4Cl$, 0.1 % NaCl, 0.001 % $CaCl_2$ and 0.3% lactic acid (pH 4.8).

### 2.3 Evaluation of water content of copolymer carriers

Fully swollen copolymer carrier were decanted and dried under vacuum at 45 °C until no changed in weight of carriers. The water content was calculated from the weight of carriers before and after vacuum drying.

### 2.4 Evaluation of activity of immobilized yeast cells

After aerobic incubation for 72 h, the copolymer carriers immobilized yeast cells

were washed well with the nutrient medium. The washed immobilized yeast cells were put into 10 ml of the nutrient medium and fermented by incubator at 30 °C under gental rotary shaker. After fermentation for 60 min, the concentration of ethanol was determined by using alcohol dehydrogenase.

**2.5 Microscopic observation of the copolymer carriers**

The copolymer carriers were cut into slices 10—15 μm thick using a freezing type microtome (Bright). The micrograph of its membrane (polymerized composite with a porous structure) was then taken using an Olympus Model TO 41 microscope. Electroscopic photographs were taken by a scanning electron microscope (model HITACHI H6010A). The average area of a pore was read directly from the micrograph. The average diameter of a pore in the pore structure of copolymer carriers was determined from following equation:

$$\text{Average diameter of pore } (\mu m) = 2 \times (\text{Average area of a pore}/\pi)^{1/2}$$

## 3 RESULTS AND DISCUSSION

**3.1 Immobilization of yeast cells during indubation by physical adsorption**

The different copolymer carriers were immersed in the mixture of precultured yeast cells and the nutrient medium. The resulted suspension was incubated at 30 °C under aerobic condition on a rotary shaker for 24 h, the many colonies of yeast cells were observed on the surface and in the inside of different copolymer carriers through microscope, but the highest density of yeast cells was observed in the polymer carrier poly (HEA-M-23G). Fig.1 shows the situation of yeast cells in the inside of the poly (HEA-M-23G) and the process of immobilizing yeast cells. The immobilized cells multiplied with increasing the incubation time and the cells were full of the whole carrier for 72 h of incubation. The result suggested that the mechinism of immobilizing yeast cells was as following steps: first, the yeast cells adsorbed on the surface of the copolymer carrier, especially in the dent of it, or infiltrate in the interior of polymer surface, then the cells in the surface intruded or infiltrated in the interior of polymer carrier by brisk multiplication and mutiplication through the pore of polymer carrier.

**3.2 The effect of the structure of copolymer carriers on the ethanol productivity of yeast cells**

The present immobilization cells method by radiation copolymerization using different monomers at low temperature was carried out in a water-monomer mixture. Therefore, absorbing situation of the yeast cells in the process of immobilization could be affected by the hydrophilicity of monomers and structure of copolymer carriers. In this paper, the effect of the structure of copolymer carriers on the ethanol productivity of yeast cells was studied. The structure of the copolymer carriers produced from different copolymer systems (poly (HEA-M23G), poly (HEA-HEMA),

poly (HEA-HPMA)) were observed by means of optical and electron microscope. The results were shown in Fig.2. As be seen, the structure of the copolymer carrier, which was produced from high hydrophilic monomer system (HEA-M23G (10 %:10 %)) and 80 % water, had sponge like pore and mechanical strength was soft, elastic, the pore number was more and the pore diameter was about 30 μm—40 μm. At same time, the ethanol productivity of immobilized yeast cells with this kind of carrier was the highest among three kinds of copolymer systems. On the other hand, the structure of the copolymer carriers, which were produced from HEA-HEMA and HEA-HPMA monomer systems (10 %:10 %) and 80 % water was more dense and the mechanical strength was harder, the pore number was less and the pore diameter was about 5 μm — 15 μm, the ethanol productivity of immobilized cells with these carriers were lower. This difference might be due to the hydration ability of the monomers.

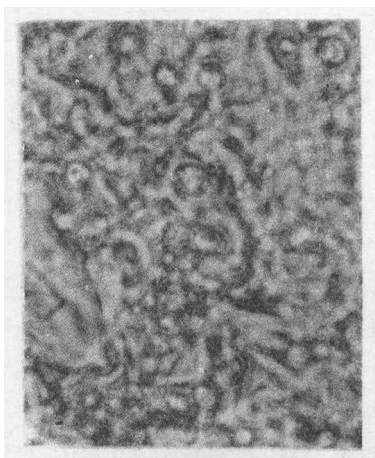

**Fig.1 Microphotograph of immobilized yeast cells**

Copolymer carrier: HEA-M23G 20 % : 10 %

**Fig.2 Electron microphotograph of pore structure of copolymer**

Carrier: Poly (HEA-M23G) 10 %:10 %

### 3.3 The effect of monomer composition and water content of different copolymer carriers on the ethanol productivity of yeast cells

The relationship between the ethanol productivity of immobilized yeast cells and the copolymer carrier with different copolymer systems as shown in Table 1. It was found that ethanol productivity of free cells with these copolymer carriers was all higher than that of free cells, but it varied with the monomer composition in the different kinds of copolymer systems. Among these carriers, the ethanol productivity of cells immobilized with poly (HEA-M23G) produced from 20 %:10 % monomer composition was highest, it was 26 mg / (ml · h), which was 4 times as high as that of free cells, However, the ethanol productivity of cells immobilized with polymer carrier (poly(HEA-M23G)) produced from low and high concentration was lower. The result indicated that the yeast cells immobilized with the copolymer carrier by radiation polymerization had high activity in the ethanol productivity and the better polymer

carrier for immobilizing yeast cells could be selected out by changing the monomer composition and the monomer kinds.

The relation between the water content of copolymer carriers and the ethanol productivity of immobilized cells was also studied and the result was shown in Table 1 as a function of the water content. The ethanol productivity of immobilized yeast cells with poly (HEA-M23G) increased with the increase of the water content of the copolymer carriers, reached the maximum value at 94.85 % of water content, then sharply decreased with a little increase of the water content of the copolymer carriers. The tendency was the similar in the rest of the copolymer systems. It was found that the less the water in the monomer solution was, the smaller the pore diameter was and the less the pore number in the copolymer carriers. In this case, it was difficult for yeast cells to enter and for the nutrient and $O_2$ to diffuse into the carriers. So the density of yeast cells immobilized was smaller. On the other hand, the more the water in the monomer solution was, the bigger the pore diameter and the more the pore number. When the water content was came up to a certain value, in which both pore diamter and pore number were the most suitable for the immobilization of yeast cells and multiplication. Therefore, the density of the yeast cells immobilized large in the carrier and the ethanol productivity increased. However, it was found that the water content of copolymer carriers reaching the highest ethanol productivity was different in the different copolymer systems. This result might be due to the different hydrophilicity of the copolymer carriers.

**Table 1**

**Effect of monomer composition and water content of different copolymer carriers on the ethanol productivity of yeast cells**

| Monomer | Concentration /% | | Water content /% | Ethanol prod. /mg(h · ml)$^{-1}$ |
|---|---|---|---|---|
| | HEA | M23G | | |
| | 30 | 30 | 87.54 | 13.5 |
| | 25 | 25 | 89.36 | 16.0 |
| HEA-M23G | 15 | 15 | 94.22 | 22.0 |
| | 20 | 10 | 94.85 | 26.0 |
| | 10 | 10 | 95.72 | 18.5 |
| | 6 | 6 | 97.86 | 15.0 |
| | 4 | 4 | 97.98 | 12.5 |
| | HEA | HEMA | | |
| HEA-HEMA | 20 | 20 | 86.67 | 12.5 |
| | 15 | 15 | 92.41 | 18.5 |
| | 10 | 10 | 96.77 | 16.0 |
| | 7 | 7 | 97.25 | 13.0 |
| | HEA | HPMA | | |
| HEA-HPMA | 20 | 20 | 85.25 | 11.0 |
| | 15 | 15 | 87.47 | 13.0 |
| | 10 | 10 | 95.25 | 14.5 |
| | 7 | 7 | 97.87 | 12.0 |
| Free cells | | | | 6.5 |

### 3.4 The effect of crosslinking reagent (4G) on ethanol productivity of immobilized yeast cells

The effect of monomer concentration on water content of carrier and ethanol productivity of yeast cells was discussed more easily as shown in Table 1. Although the copolymer carrier poly(HEA-M23G) had higher water content produced from

4 %:4 %, and 6 %:6 % than that from 20 %:10 %, but the ethanol productivity of immobilized yeast cell with these carriers were lower than that of immobilized yeast cells with carrier poly(HEA-M23G) 20 %:10 %. It was found that the pore of carriers (monomer concentration below 6 %:6 %) was too big like tube, the yeast cells could not immobilized effectively, owing leaking out of the carrier. A decrease of monomer concentration also resulted in a decrease of the mechanical strength of the prepared polymer carrier. In this case, the polymer carrier could break into small pieces when shaken in a rotary shaker for initial aerobic incubation of the immobilized cells and the yeast cells cound liberated. In order to improving this situation, bifunctional monomer 4G was added in HEA-M23G monomer system as a crosslinking reagent. The composition of HEA-M23G monomer had three kinds: 10 %:10 %, 6 %:6 % and 4 %:4 %. The concentration of the crosslinking reagent 4G added were 1.5 %, 2.0 %, 2.5 % out of total monomer volume in each composition respectively. The dependence of the ethanol productivity of immobilized cells on the concentration of the crossliking reagent was plotted as Fig.3. The ethanol productivity of yeast cells immobilized with the crosslinked carrier all increased, and increased with the increase of 4G concentration, reached the highest value, then decreased. It was found that the effect of adding 4G in lower monomer concentration such as 4 %:4 % on the ethanol productivity of immobilized cell was better than in higher one in this work. The effect of adding biofunctional monomer 4G in HEA-M23G monomer systems not only made the copolymer carrier mechanically stronger, but also changed the pore diameter and pore number of the carriers. The growth state of immobilized yeast cells with the crosslinked carrier incubating for 72 h has been observed through microscope, the yeast cells inside this kind of carriers were filled with the field of vision. The result indicated that the effect of immobilization of yeast cells with the carriers was better, the density of cells was high. Therefore, the ethanol productivity reached the highest value.

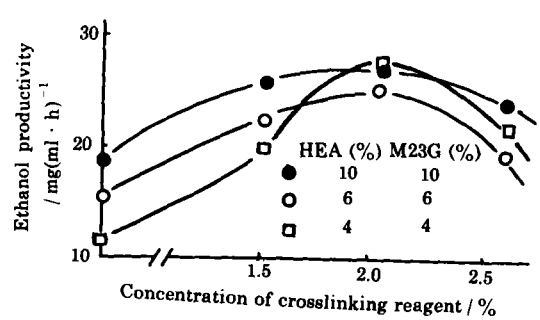


**Fig.3 The effect of crosslinking reagent on ethanol productivity of immobilized yeast cells**

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