Journal of Solid-Phase Biochemistry, Vol. 3, No. 3, 1978

IMMOBILIZATION OF MICROBIAL CELLS BY RADIATION-INDUCED POLYMERIZATION OF GLASS-FORMING MONOMERS AND IMMOBILIZATION OF *Streptomyces phaeochromogenes* CELLS BY POLYMERIZATION OF VARIOUS HYDROPHOBIC MONOMERS

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Accepted September 14, 1978

The immobilization of *Streptomyces phaeochromogenes* cells was studied by the radiation-induced polymerization of various hydrophobic glass-forming monomers at low temperatures. The glucose isomerase activity of cells immobilized in hydrophobic polymers showed no decrease in activity with repeated use (batch enzyme reaction). Activity increased with increasing monomer concentration in contrast to results with the immobilized enzyme. The hydrophobic polymer composite was microspheric in form. The particle diameter of the composite increased with the increasing monomer concentration. K_m values of the immobilized cells were close to that of intact cells. It was deduced that the cells were trapped on the surface part of the hydrophobic polymer ready to react with the substrate, and not within the matrix where diffusion would play an important role in reaction rates.

INTRODUCTION

We studied the radiation-induced polymerization at low temperatures using glass-forming monomers that take a stable super-cooled state and have remarkable polymerizability at low temperatures (1). We found that the low-temperature polymerization of glass-forming monomers could be applicable to the entrapment of biofunctional substances within the polymerized matrix. In a previous paper, we showed that microbial cells and enzymes could be immobilized effectively by low-temperature polymerization (2). Previously immobilization of enzymes by entrapment has generally been carried out using hydrophilic monomers and polymers (3,4) in which enzymes were entrapped into the polymer matrix.

In this report, *Steptomyces phaeochromogenes* cells containing active glucose isomerase were immobilized by radiation-induced polymerization

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of hydrophobic glass-forming monomers at low temperatures. The characteristic of the immobilized cells was studied. In the present immobilization method, the immobilized cell–polymer composite had a microspheric form in which the cells were trapped on the microsphere surface. Unlike other entrapment methods the diffusion of substrate into the matrix was not necessary.

MATERIALS AND METHODS

Streptomyces phaeochromogenes cells were obtained from Nagase Sangyo Co., Ltd. Butanediol monomethacrylate(BDMM), 1,3-butyleneglycole dimethacrylate(BG), neopentylglycole dimethacrylate(NPG), and trimethyrolpropane triacrylate(A-TMPT) were obtained from Shin-Nakamura Chemical Co., Ltd.

Method of Immobilization

The cells (50 mg) were suspended in 0.05 M phosphate buffer solution (0.01 M $MgSO_4 7H_2O$, pH 7.2) containing the hydrophobic monomer. The final volume was 2.0 ml. This solution was charged in a glass ampoule and quickly shaken. Immediately after shaking, the ampoule was frozen at a low temperature (−78°C). The immobilized cell–polymer composite of microspheric form was obtained by γ-ray irradiation (1×10^6 R) at −78°C for 1 h at a dose rate of 1×10^6 R/h.

Examination for Leakage of the Cells from the Polymer Matrix

Examination for leakage of the cells from the polymer matrix was carried out by the repeated use (repeating of batch enzyme reaction, 1.0 h at 65°C). Ten milliliters of 1.0% D-glucose solution (0.05 M) phosphate buffer solution containing 0.01 M $MgSO_4 7H_2O$, pH 7.2) were used as substrate. After each reaction, the D-fructose formed was determined by the cysteine-carbazole method (5). The enzymatic (glucose isomerase) activity(%) was obtained from the D-fructose formation ratio in immobilized and intact cells with each batch enzyme reaction.

RESULTS AND DISCUSSION

Enzymatic Activity of Immobilized Cells by Hydrophobic Polymer Matrix

The immobilization of the cells was carried out by radiation-induced polymerization of various glass-forming hydrophobic monomers. The

observed enzymatic activity of the immobilized cells was examined by the batch method, and the results are plotted against the number of batch enzyme reactions (repeated use, see Fig. 1). No decrease in enzymatic activity was observed with each batch enzyme reaction in any of the immobilized cell preparations regardless of the source of the hydrophobic monomer. This fact was attributed to the fact that no cell leakage occurred. In this immobilization method, the enzymatic activity at the initial stage of the batch enzyme reactions was high, and the high activity was retained at even a later stage. This appears to be characteristic of the immobilization of the cells by polymerization in hydrophobic monomers. This high activity could be because of the entrapment of the cells on the microsphere surface and other factors, such as larger size of immobilized cells as opposed to pure enzyme.

The monomer concentration dependence of the enzymatic activity is shown in Figure 2. The enzymatic activity decreased slightly with the decreasing monomer concentration in most of the monomer systems. This could be due to the increase of cell loss with the decreased water content in the immobilization stage, since the hydrophobic polymer–cell composite formed separately with the water phase. The enzymatic activity increased

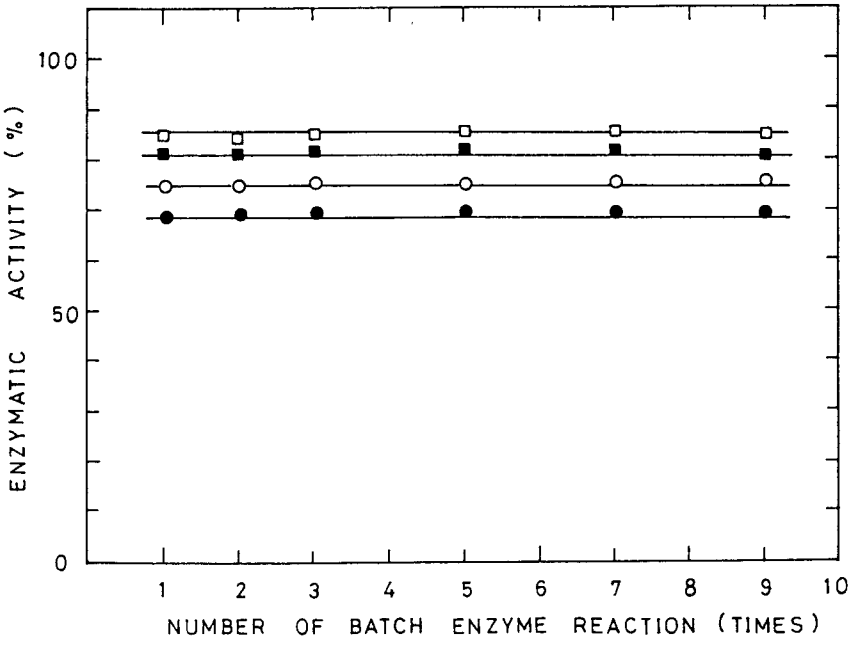


FIG. 1. The effect of repeated batch enzyme reaction on enzymatic activity at 50% monomer concentration. (□) NPG, (■) A-TMPT, (○) BG, (●) BDMM.

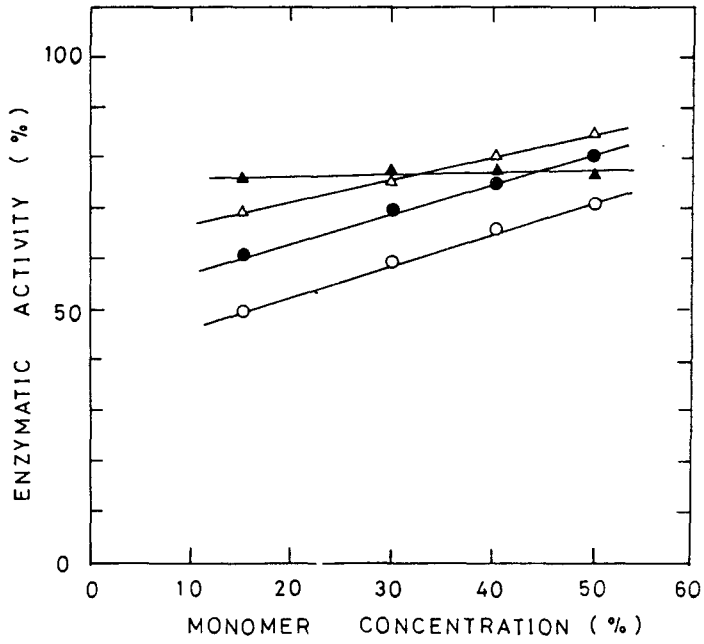


FIG. 2. The effect of monomer concentration on enzymatic activity. (▲) BG, (△) NPG, (●) A-TMPT, (○) BDMM.

with increasing monomer concentration. However, it tended to decrease again at very high monomer concentrations perhaps due to the increase in cells occluded in the matrix which did not contribute to the total enzyme activity. Such monomer concentration dependency on the enzymatic activity shows a remarkable contrast with that observed with enzymes immobilized by the same method. The monomer concentration dependence of the enzymatic activity showed a clear concentration dependence of the enzymatic activity showed a clear optimum at 30–50% monomer concentration. The difference perhaps could be attributed to the lower leakable rate from the matrix and occlusive property of matrix with the cells, owing to their large size. It could be concluded that various hydrophobic polymers were also effectively utilizable as polymer carriers. The formed polymer–cell composite took a microspheric form with a range of between 50 and 200 μ diameter, having a large surface area as a polymerized state (except in a very high-monomer concentration condition, in which the polymer–cell composite does not take a microspheric form).

The order of the enzymatic activity of the immobilized cells for various hydrophobic monomers at a 50% monomer concentration was NPG > A-

TMPT > BG > BDMM (Fig. 2). The enzymatic activity in the BG monomer did not change with increasing monomer concentration.

The microscopic photograph of polymer–cell composite is shown in Figure 3. The composites in dimethacrylate and trimethacrylate monomers such as NPG and A-TMPT took a spherical form (Fig. 3a,b), and that in the monomethacrylate monomer such as BDMM took a quasi-spherical form (Fig. 3c). The composite of the NPG monomer has a dent on its surface in which the cells are conveniently trapped. The polymer–cell composite obtained in a hydrophobic monomer was a very hard particle. Therefore, such a dent gives a larger surface area for the composite. In fact, the enzymatic activity of the composite in the NPG monomer had the highest value of various hydrophobic monomers examined. The enzymatic activity of the BDMM monomer composite was lower than that of the A-TMPT monomer (Fig. 2). Accordingly, it is believed that the composite, such as the quasispherical form, is not advantageous for the enzyme activity. In addition, in the composite formed by polymerization of the BDMM monomer, it is assumed that small amounts of the cells are included in the polymer matrix. On the other hand, in the composites made from A-TMPT, NPG, and BG monomers, it is probable that the cells are trapped on the surface of microspheres and that the enzyme reaction can be carried out on the surface part of hydrophobic polymer without the necessity of substrate diffusion into the polymer matrix.

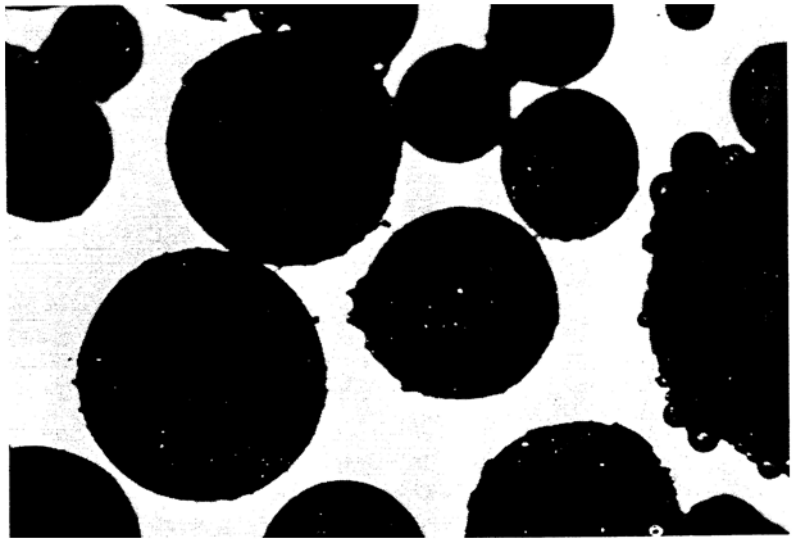
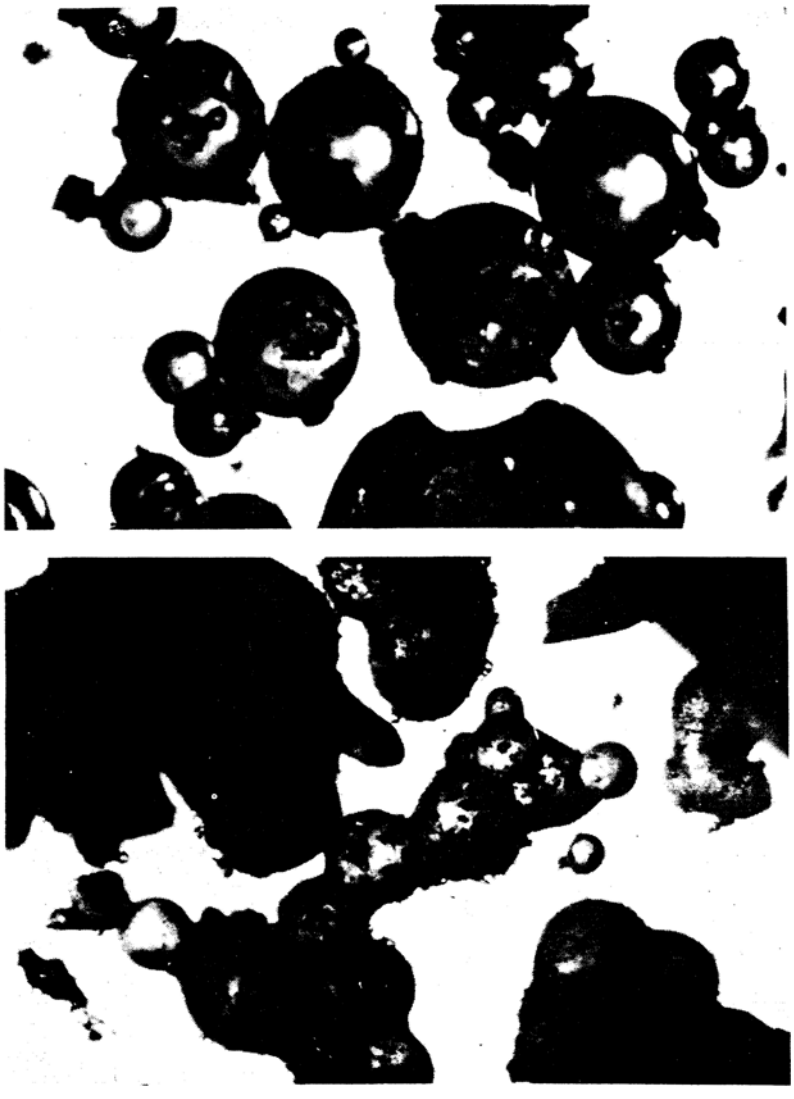


FIG. 3. Optical micrographs of polymer–cells composite (×80). (a: above) A-TMPT, (b: following page, above) NPG, (c: following page, below) BDMM.



Michaelis Constant (K_m) of Immobilized Cells

The Linewaver–Burk plots of the immobilized cells are shown in Figure 4. K_m values for the immobilized cells formed by polymerization of the A-TMPT and NPG monomers were observed to be 0.32 and 0.34 M. These values agreed approximately with that of intact cells (0.33 M). This fact supported the mechanism that the cells are trapped mainly on the surface of

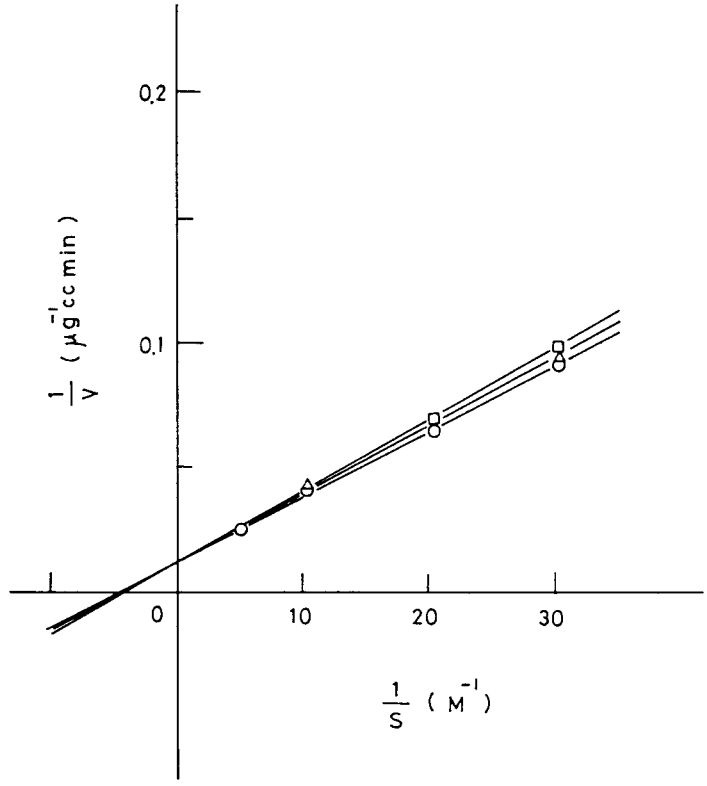


FIG. 4. Lineweaver–Burk plots in immobilized cells. (△) Intact cells, (○) A-TMPT, (□) NPG.

the polymer matrix ready to contact and react with the substrate without the inner diffusion of the substrate into the polymer matrix.

Particle Size

The relationship between particle diameter and monomer concentration in various hydrophilic monomers is shown in Figure 5. In the A-TMPT and NPG monomers, the particle diameter of the formed polymer–cell composite increased with increasing monomer concentration, while the particle diameter of the composite made from the BG monomer did not change with increasing monomer concentration. It is suggested that the effect of monomer concentration on the particle diameter is dependent on the properties of the hydrophilic monomer. Figure 6 shows the relationship between the particle diameter and the enzymatic activity. Figure 7

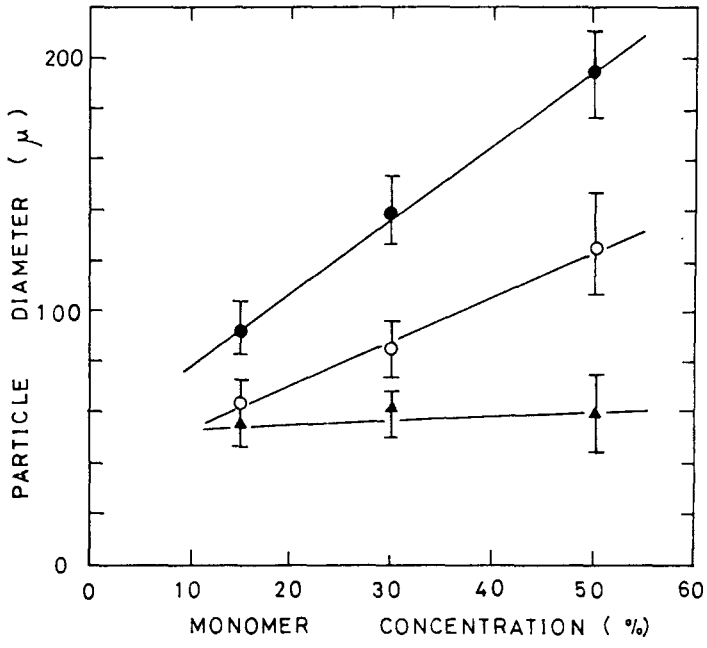


FIG. 5. The relationship between particle diameter and monomer concentration in various hydrophobic monomers. (○) A-TMPT, (○) NPG, (▲) BG.

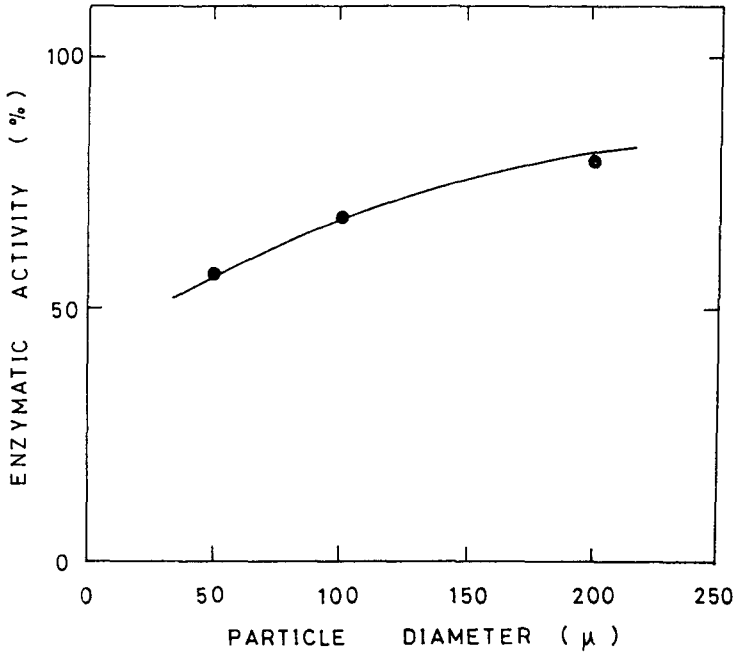


FIG. 6 The relationship between particle diameter and enzymatic activity at A-TMPT monomer.

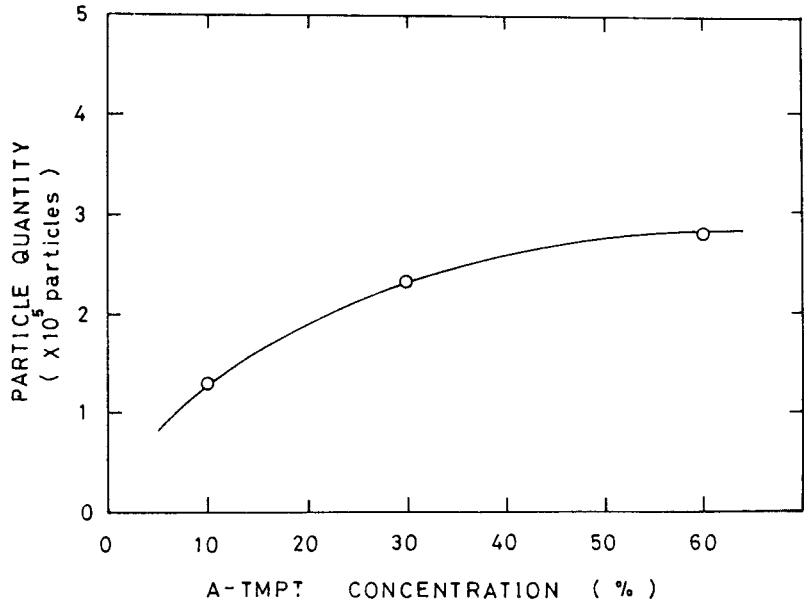


FIG. 7. The relationship between particle quantity and A-TMPT concentration.

shows the relationship between the formed particle quantity and A-TMPT monomer concentration. The formed particle quantity increased with increasing monomer concentration. From these results it is found that the increase of the enzymatic activity with increasing monomer concentration is attributable to the increase of the particle size and quantity.

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