Structural Control of Core/Shell Polystyrene Microcapsule-Immobilized Microbial Cells and Their Application to Polymeric Microbioreactors

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ABSTRACT: Polystyrene microcapsules possessing a large single core and highly microporous wall were prepared as immobilization supports for microbial cells by a new method based on phase separation of polystyrene within a mixed organic solvent system in an oil-in-water (o/w) emulsion. The structures of core and micropore were controlled by changing the concentration of isooctane in the organic phase and the temperature of solvent evaporation. The immobilization of baker's yeast into the polystyrene microcapsules was carried out by entrapping the yeast into calcium alginate beads before encapsulating in the microcapsules and followed by removing the beads with HCl solution. The morphology of the microcapsules was observed by means of SEM, and the activity of the immobilized yeast was evaluated by using the microcapsules in ethanol fermentation. It was found that the formation of the core and wall pore was remarkably influenced by the isooctane concentration, and the diameter of the core was affected by the temperature of solvent evaporation. The yeast was successfully immobilized into the polystyrene microcapsules at a high density and a high catalyst activity by the proposed immobilization method. Furthermore, the polystyrene microcapsules exhibited a high operational stability in the repeated batchwise fermentation test. © 2003 Wiley Periodicals, Inc. J Appl Polym Sci 89: 1966–1975, 2003

Key words: porous polystyrene microcapsule; controlled single core; microencapsulated cell; polymeric microbioreactor; yeast cell

INTRODUCTION

Immobilization of microbial cells is increasingly applied to biotechnological processes. Recently, immobilized cells have been extensively used for the production of useful chemicals such as alcohols,[1] amino acids,[2] organic acids,[3] antibiotics,[4] or steroids,[5] and for the degradation of wastewater pollutants.[6]

To date, numerous techniques of cell immobilization have been developed. One of the promising techniques is the encapsulation of microbial cells in a polymeric support. In this technique, the hydrogel microcapsules such as polyvinyl alchohol, calcium alginate, κ-carrageenan, or chitosan are widely used as the immobilization support.[7,8] However, during the application of the immobilized cells in long-term reactions, the hydrogel microcapsules have only limited stability, in which problems such as bead disruption or cell leakage are well known.[9] Recently, the development of microcapsules with good stability have been extensively performed for the application of encapsulated cells in industrial processes.[9–11]

Solid microcapsules such as polystyrene microcapsules are known to have favorable mechanical strength and have been studied as the encapsulation support of polyelectrolyte solutions[12,13] and organic pigments,[14] for example. However, there were no reports regarding the preparation and application of polystyrene microcapsules as a highly efficient immobilization support suitable for microbial cells.

Therefore, the main objective in this present study was to develop a solid polystyrene microcapsule, which would have appropriate characteristics as an immobilization support of microbial cells. There are two important factors that must be considered for developing the immobilization support of microbial cells: (1) a high encapsulation efficiency of cells and (2) a high diffusion rate of substances. Thus, the polystyrene microcapsules are designed to possess a large single core and a highly microporous wall. In this present study, we proposed a new approach for preparing the polystyrene microcapsules with a large single core and a microporous wall by controlling the

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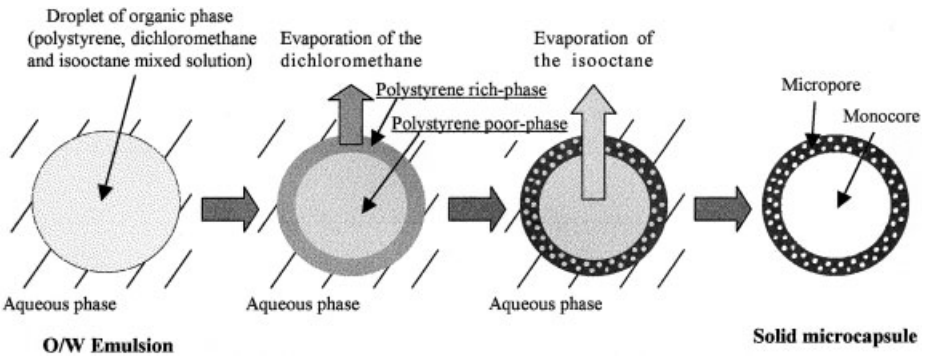

Figure 1 Schematic illustration for the preparation of the polystyrene microcapsules with a large single core and a microporous wall.

phase separation of polystyrene within the droplets of oil-in-water (o/w) emulsions. The principle of the preparation is schematically shown in Figure 1. An organic phase, which consists of polystyrene, dichloromethane as the polymer-rich solvent, and isooctane as the polymer-poor solvent, is dispersed in an aqueous phase to form the emulsions. The emulsions are then evaporated by controlling the temperature in the reactor. In this step, the phase separation of the polymer within the emulsion droplets occurs between the droplet interface and the droplet center by increasing the temperature. The evaporation of dichloromethane at a low temperature leads to the coagulation of the emulsions to form the microcapsules, and the subsequent evaporation of isooctane at a high temperature leads to the formation of a hollow core and micropores.

In this study microcapsule morphology was first evaluated by investigating in detail the effect of preparative conditions (concentration of isooctane and temperature of solvent evaporation). The immobilization of microbial cells into polystyrene microcapsules was then attempted. Baker's yeast cell was chosen as a model microbial cell. The fermentation of glucose to ethanol was carried out by evaluating the activity of yeast incorporated into the polystyrene microcapsule possessing the controlled hollow core and microporous wall.

EXPERIMENTAL

Microorganisms

A commercial dry yeast of *Saccharomyces cerevisiae* (Baker's Yeast Type I) purchased from Sigma Chemical Co. (St. Louis, MO) was used as the microorganism to be immobilized. The yeast was used without cultivating because the fermentation ability of the yeast without cultivating was quite similar to that of the yeast cultivated in the medium containing 1 wt % yeast extract, 2 wt % tryptone, and 2 wt % glucose for 24 h.

Reagents

Polystyrene microcapsules. Polystyrene (n = 1000–1400) was purchased from Nacalai Tesque, Inc. Dichloromethane, a low boiling point (40°C) solvent for polystyrene, and isooctane, a high boiling point (99°C) solvent for polystyrene (both from Wako Pure Chemicals, Osaka, Japan), were used as wall materials. Sorbitan monooleate (Span 80; Nacalai Tesque, Inc.) was used as an emulsion stabilizer, and both polyvinyl alcohol (PVA, n = 500, completely hydrolyzed, Wako Pure Chemicals) and calcium triphosphate microparticles (TCP-10U; Taihei Chemical Co., Ltd.) were used as dispersion stabilizers.

Calcium alginate beads. Sodium alginate (100–150 cP), calcium chloride (anhydrous), and isooctane were purchased from Wako Pure Chemicals. The surfactant, sorbitan monooleate (Span 80), was obtained from Nacalai Tesque, Inc. Tris (hydroxymethyl) aminomethane (Merck, Darmstadt, Germany) and hydrochloric acid (Wako Pure Chemicals) were employed for the preparation of Tris–HCl buffer solution (0.1*M*, pH 7.2).

Ethanol fermentation solution. D-(+)-Glucose (Nacalai Tesque, Inc.) was used as a substrate, and yeast carbon base and casamino acid (Difco Laboratories, Detroit, MI) were used as nutrient substances.

Analytical reagent. 1-Propanol (Wako Pure Chemicals) was used as an internal standard compound for gas chromatography (GC-9A; Shimadzu, Kyoto, Japan). Iatro-chrom GLU-Lq (Iatron Laboratories, Inc.) was used for the enzymatic determination of glucose. Other reagents were commercially available special grades.

Preparation of polystyrene microcapsules

Polystyrene microcapsules were prepared by the novel o/w emulsion-solvent evaporation method. The preparation apparatus was an 800-mL glass-jacketed vessel equipped with a mechanical stirrer. A dichloromethane solution (60 g) containing 10 wt % polysty-

rene, 3 wt % Span 80, and various concentrations of isooctane was dispersed in 500 g of aqueous solution containing 1 wt % PVA and 50 wt % TCP-10U at 200 rpm for 5 min by a mechanical stirrer. Agitation was maintained at a low temperature for 12 h to remove dichloromethane and was subsequently kept at a high temperature for 18 h to eliminate isooctane. The obtained microcapsules were washed with 1*M* HCl solution, which was added to the vessel to dissolve TCP-10U for 5 min, and were then filtered off by a suction filter.

To prepare polystyrene microcapsules possessing a large single core and a microporous wall, the effects of various formulation factors on the microcapsule morphology were investigated in detail. The isooctane concentration in the organic phase was varied from 0 to 7 wt %; the solvent evaporation for the removal of dichloromethane was carried out at 30 or 35°C; and the solvent evaporation for the removal of isooctane was carried out at 45, 50, or 55°C.

Yeast immobilization in polystyrene microcapsules

In the preparation of polystyrene microcapsules, the dichloromethane, a toxic solvent, was abundantly used, and the solvent evaporation temperature of high boiling point solvent for the polystyrene was quite high toward the yeast cells. Therefore, it was necessary to protect the yeast cells against the harsh conditions of preparation. In this study, immobilization of the yeast cells in the polystyrene microcapsules was attempted by the following steps.

Step 1: Entrapment of yeast in calcium alginate beads

Calcium alginate beads (micron size) were prepared by using an emulsification method described by Wan et al.[15] Sodium alginate (1 wt %) was dissolved in 10 g of 0.1*M* Tris–HCl buffer solution (pH 7.2), with slight heating. After cooling to 25°C, 2.0 g of baker's yeast was added under agitation using a magnetic stirrer. The mixture was then dispersed in 50 g of isooctane solution containing 3 wt % Span 80 by use of a homogenizer (Polytron PT10–35; Kinematica AG, Germany) at 3000 or 4000 rpm for 10 min. During the homogenization, the flask was cooled by immersion in ice water. This was followed by the addition of 50 g of 10 wt % calcium chloride solution, and the agitation was continuously performed for 10 min. The solution was continuously agitated by using a magnetic stirrer for 2 h to further harden the formed beads. The beads were recovered by filtration and were dried at 25°C for 1 h.

Step 2: Encapsulation into the polystyrene microcapsules of yeast-entrapped calcium alginate beads

Yeast-entrapped calcium alginate beads (0.25 g) were added to 60 g of dichloromethane solution containing 10 wt % polystyrene, 3 wt % Span 80, and 5 wt % isooctane. This mixture was then dispersed in 500 g of an aqueous solution containing 1 wt % PVA and 5 wt % TCP-10U at 200 rpm for 5 min by a mechanical stirrer. Agitation was maintained at 35°C for 12 h to remove dichloromethane and was subsequently kept at 50°C for 18 h to remove isooctane.

Step 3: Removal of calcium alginate beads incorporated into the polystyrene microcapsules

The polystyrene microcapsules obtained as particles were washed with HCl solution in various concentrations (0.25, 0.5, 0.75, or 1*M*) for 6 h, by use of a magnetic stirrer, and were then filtered off. The washed microcapsules were preserved in 0.1*M* Tris–HCl buffer solution (pH 7.2).

Morphology observation

The morphology of the calcium alginate beads and the polystyrene microcapsules was observed by means of SEM (Topcon model SM-300; Topcon Co., Ltd.). The samples were coated with gold at about 300-Å thickness by using an ion coater (IB-2; Eiko Engineering Co., Ltd.) and examined by SEM.

Ethanol fermentation

The activity of the polystyrene microcapsule-immobilized yeast was evaluated by employing the microcapsules in ethanol fermentation by the batchwise method. Polystyrene microcapsule-immobilized yeast (0.1 g) was placed into a lidded vial containing 5 mL of fermentation medium (composition: glucose 10 wt %, yeast carbon base 1.17 wt %, and casamino acid 0.5 wt %). The tubes were then incubated in a shaking bath (Model BW 200; Yamato) at 30°C and 120 rpm. At the designated time intervals, the vials were removed from the shaker, and the microcapsules were filtered off with a membrane filter (pore diameter 0.45 μm; Millipore, Milford, MA). As a control experiment, the ethanol fermentation using free yeast cells and calcium alginate–entrapped yeast was carried out with the same procedures.

To evaluate the stability of the prepared polystyrene microcapsules, the repeated fermentation by the batch operation was carried out three times. Polystyrene microcapsule-immobilized yeast (2 g), washed with 0.5*M* HCl solution, was put into a 200-mL Erlenmeyer flask containing 100 mL of fermentation medium (the composition of solution was the same as that men-

tioned above), and then incubated at 30°C under continuous shaking at 120 rpm. After the first fermentation (F_1) was completed, the microcapsules were collected by a suction filtration, and redispersed in the second batch. The same procedure was repeated twice, hereafter called F_2 and F_3, respectively.

The fermentation results were shown as glucose consumption yield and ethanol formation yield. The concentration of glucose remaining in the reaction solution was determined enzymatically by using an Iatro-chrom Glu-Lq reagent (Iatron Laboratories, Ltd.). The concentration of formed ethanol was determined by a gas chromatograph, equipped with a G-205 capillary column (GL Sciences Inc.). The injection or column temperature was maintained at 150 or 80°C, respectively. The flow rate of carrier gas (helium) was controlled at 85 mL/min. 1-Propanol (1.0 wt %) was used as an internal standard solution.

RESULTS AND DISCUSSION

Structural control of single-core polystyrene microcapsules

The effects of preparation conditions such as isooctane concentration and solvent evaporation temperature were first researched to develop polystyrene microcapsules possessing a large single core and a highly porous wall.

In the method of microcapsule preparation, isooctane may play an important role in the formation of the core and micropores of the microcapsule wall. Therefore, the influence of isooctane concentration in the organic phase was evaluated with eight different concentrations (0–7 wt %). The solvent evaporation temperature was performed at 35°C for the removal of dichloromethane and then at 50°C for the removal of isooctane. SEM photographs of the obtained microcapsules are shown in Figure 2. The microcapsules prepared with the isooctane concentration up to 5 wt % were obtained as spherical polymeric particles, about 25–400 μm in diameter. On the other hand, when the isooctane concentration was 6 wt %, a high degree of aggregation on the microcapsules was observed, and at an isooctane concentration of 7 wt %, the microcapsules could not be obtained at all because of the aggregation of the emulsion during the solvent evaporation process.

In the absence of isooctane in the organic phase, the obtained microcapsules had a smooth surface without apparent wall pores. The cross-sectional photographs revealed that a hollow core could not be confirmed in the microcapsules, and the inner structures were slightly rough. These rough structures are probably formed during the evaporation of the organic solvent. For the preparation at an isooctane concentration of 1 wt %, the surface of the microcapsules became slightly rough, and a single core with a diameter of about 50 μm was formed in the internal structure. In addition, a number of pores with diameters of 3–5 μm were observed in the inner wall. Furthermore, in the microcapsules prepared at an isooctane concentration of 5 wt %, it was found that the diameter of a single core and the number of wall pores increased with increasing isooctane content in the organic phase. From these results, it is clear that the isooctane concentration plays an important role in the formation of both the core and the wall pores. The addition of the polymer-poor solvent in the organic phase led to the phase separation within the o/w emulsion droplets, which finally resulted in the controlled formation of a single core and wall pores. These results also suggest that the microcapsules with a controlled single core can be easily prepared by the o/w emulsion technique. In these experiments, it was demonstrated that the addition of 5 wt % isooctane is required for producing a polystyrene microcapsule possessing a large single core and a highly porous wall.

Because the organic solvent in the emulsion droplets is removed by the encapsulation technique, it is predicted that the evaporation temperature influences the morphology of the microcapsules. To study this effect, six different temperature combinations in the solvent evaporation were adopted, as shown in Figure 3. The evaporation temperature for the removal of dichloromethane was varied at 30 or 35°C, and the subsequent evaporation temperature for the removal of isooctane, whose concentration was 5 wt %, was at 45, 50, or 55°C. The core size of the microcapsules prepared at 35°C, which is the temperature of dichloromethane removal, was larger than that of microcapsules prepared at 30°C. The core size also increased with increasing temperature of isooctane removal. These results suggest that solvent evaporation at a higher temperature effectively transferred the polymer-rich phase to the interfaces of the emulsion droplets, resulting in the formation of a large core (about 150 μm in diameter). Furthermore, it was found that the wall of the microcapsules prepared at 50°C, which is the temperature of isooctane removal, had a well-defined and compact structure, whereas the microcapsules prepared at 45 or 55°C had a large crack wall. From these results, it is noteworthy that the core size of the microcapsules can be easily controlled by changing the solvent evaporation temperature. We demonstrated that the most suitable temperature of solvent evaporation for preparing polystyrene microcapsules with the best characteristics for cell immobilization is 35°C for dichloromethane removal and subsequently 50°C for isooctane removal.

Yeast immobilization into polystyrene microcapsules

Because the core size of the obtained polystyrene microcapsules was about 150 μm, the size of yeast-en-

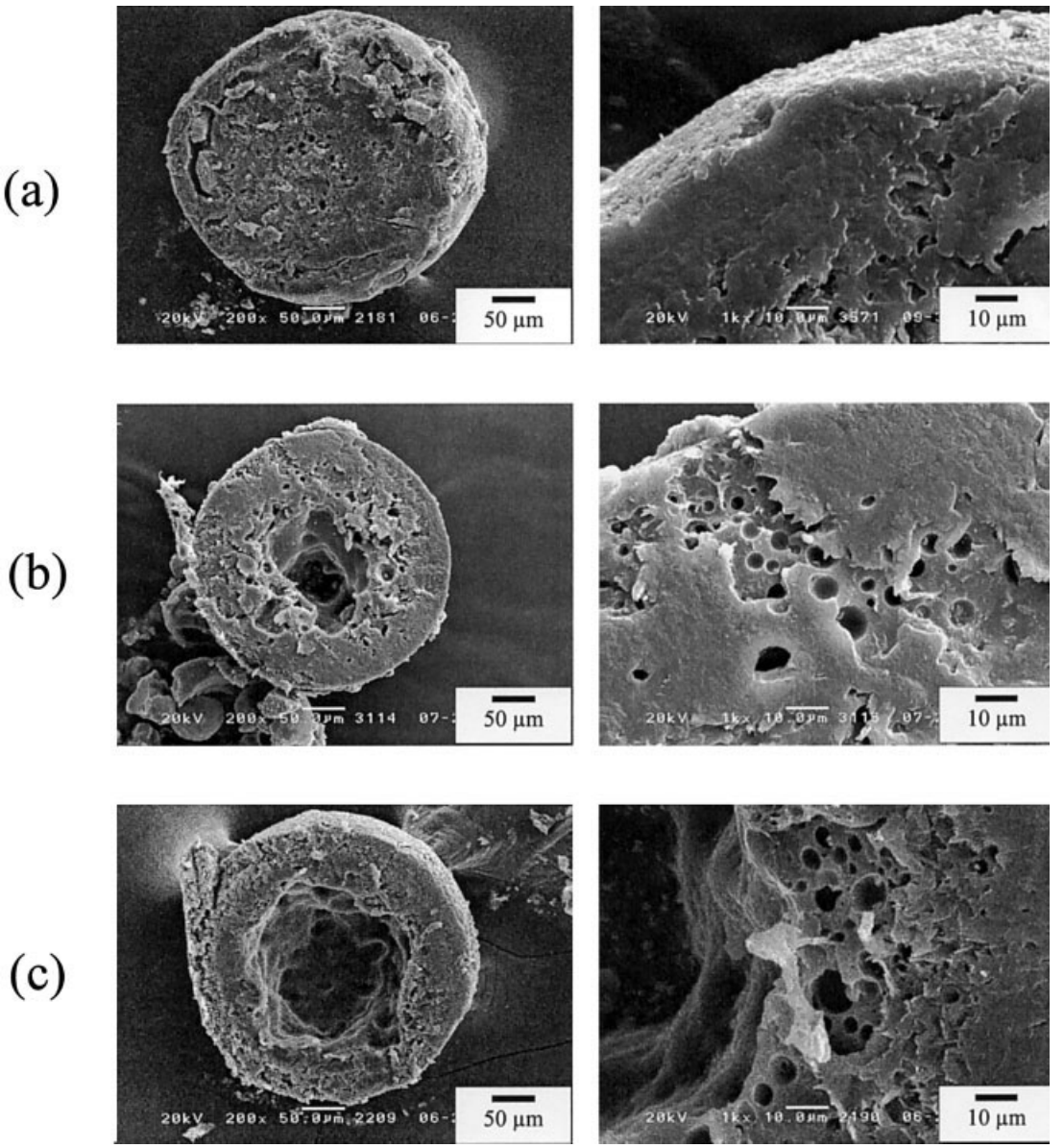

Figure 2 SEM photographs of cross-sectional wall structure of polystyrene microcapsules prepared with various concentrations of isooctane: (a) 0 wt %, (b) 1 wt %, and (c) 5 wt %. Right-hand pictures show the photographs taken at higher magnification. Evaporation temperature of dichloromethane was 35°C, and evaporation temperature of isooctane was 50°C.

trapped calcium alginate beads that will be encapsulated into the polystyrene microcapsules must be less than 150 μm. In this study, micron-size calcium alginate beads were prepared by using an emulsification method described by Wan et al.[15] To study the effect of agitation rate on the bead size, the homogenization was performed at 3000 or 4000 rpm. SEM photographs of the prepared calcium alginate beads are shown in Figure 4. The morphology of the calcium alginate beads was spherical and, as expected, the bead size decreased with increasing agitation rate. Calcium alginate beads, prepared at an agitation rate of 3000 rpm, had a diameter ranging from 20 to 200 μm, whereas calcium alginate beads prepared at 4000 rpm were smaller, with diameters ranging from 20 to 150 μm. It can be seen that the most suitable agitation rate for preparing yeast-entrapped calcium alginate beads is 4000 rpm.

The immobilization of yeast in the polystyrene microcapsules was performed by employing yeast-entrapped calcium alginate beads as the core material. The SEM observation of the prepared microcapsules is shown in Figure 5(a). As shown in the figure, the calcium alginate beads are successfully immobilized in the polystyrene microcapsules at a high density. To obtain polystyrene microcapsule-immobilized free yeast cells effectively, the microcapsules were washed with HCl solution for dissolving the calcium alginate beads included in the polystyrene microcapsules. The concentrations of HCl were varied from 0 to 1.0*M*.

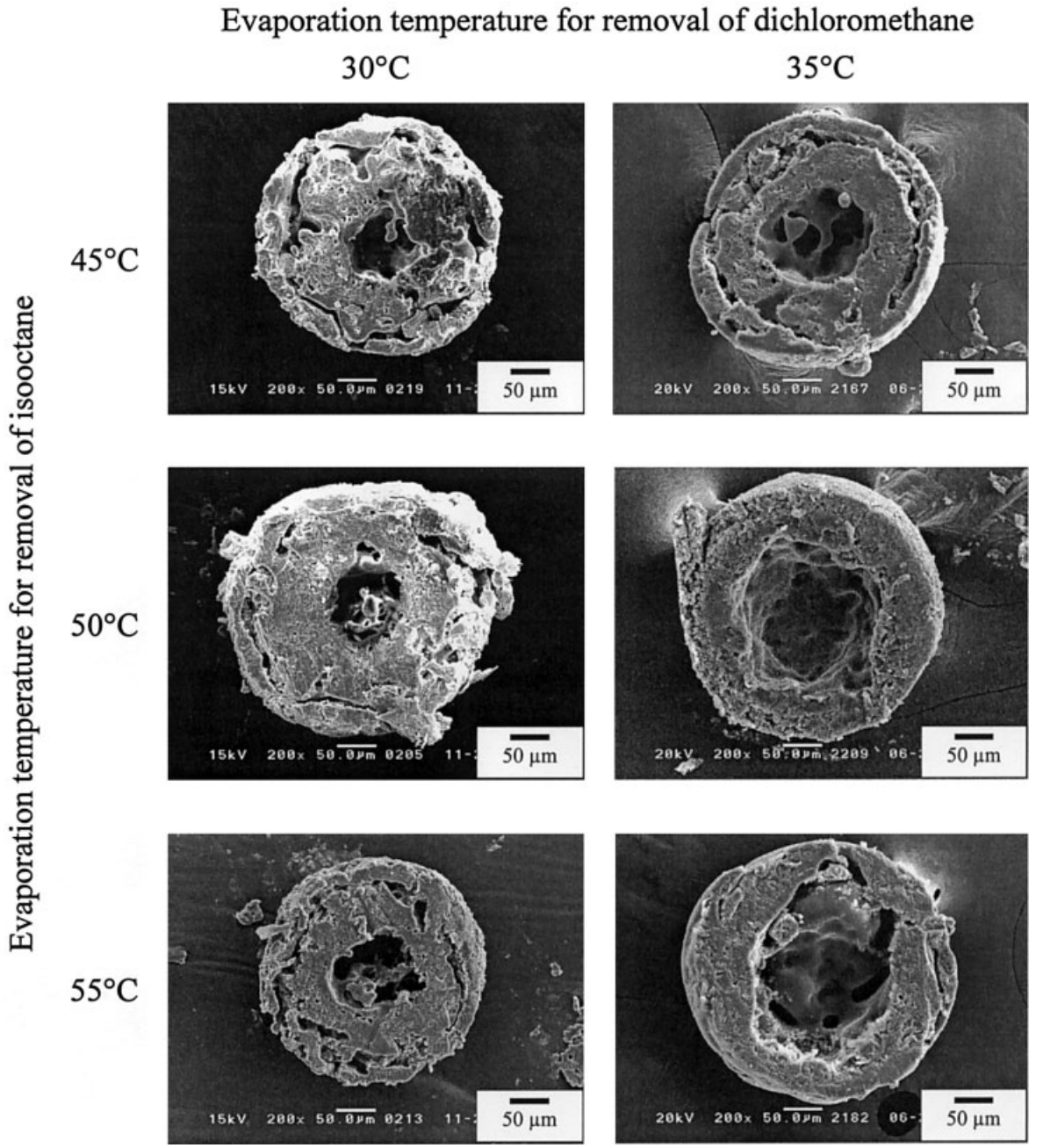

Figure 3 SEM photographs of cross-sectional wall structure of polystyrene microcapsules prepared at various temperatures of solvent evaporation. Isooctane concentration was 5 wt %.

SEM photographs of the microcapsules washed with 0.5 or 1.0*M* HCl solution are shown in Figure 5(b) and (c), respectively. It is apparent that the degree of dissolution of calcium alginate beads increases with increasing the concentration of HCl solution. We demonstrated that the polystyrene microcapsule-immobilized free yeast cells at high density can be prepared by encapsulating yeast cells entrapped in the calcium alginate beads and by washing the microcapsules with HCl solution to remove calcium alginate, in which yeast cells are incorporated.

Ethanol fermentation of polystyrene microcapsule-immobilized yeast

As mentioned above, the calcium alginate beads used as a protection support of yeast cells were removed by washing the polystyrene microcapsules with HCl solution. However, it is considered that the toxicity of HCl solution used to carry out the washing treatment will affect the activity of yeast cells. The ethanol fermentation ability of the yeast-immobilized polystyrene microcapsules, washed with different concentra-

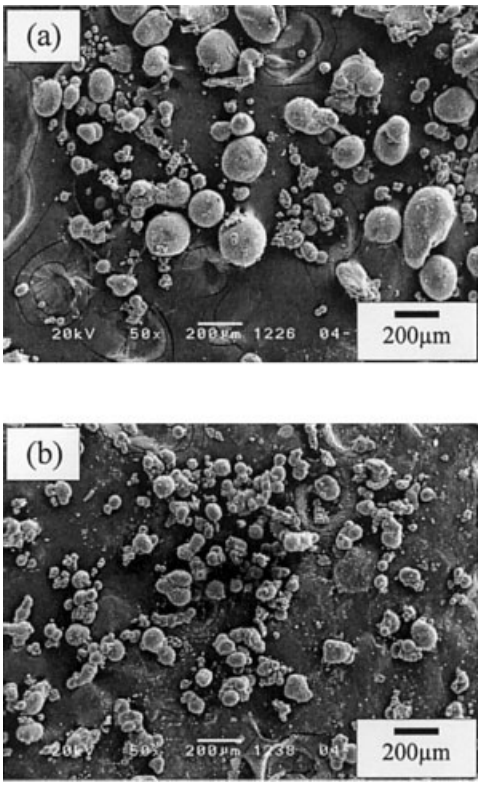

Figure 4 SEM photographs of yeast-entrapped calcium alginate beads prepared at various speeds of homogenization: (a) 3000 rpm, (b) 4000 rpm.

tions of HCl solution, was studied to find the most suitable concentration of HCl solution for dissolving the calcium alginate beads, as shown in Figure 6. In the case of microcapsules unwashed with HCl solution, the conversion of glucose to ethanol did not occur. This is probably caused by the high limitation of mass diffusion through the duplicated walls (the wall of the polystyrene microcapsule and the calcium alginate bead), so that glucose cannot permeate the duplicated walls and contact with the yeast inside the capsule. The microcapsules washed with 0.5*M* HCl solution exhibited a higher conversion rate compared to that of microcapsules washed with 0.25*M* HCl solution. This result can be attributed to an efficient dissolution of calcium alginate beads at a higher concentration of HCl solution (Fig. 5), and the conversion to alcohol was accelerated by the enhancement of a high mass diffusion through the microcapsule wall. Furthermore, the fermentation did not occur when the microcapsules were washed with either 0.75 or 1.0*M* HCl solution, caused by the extinction of the yeast at the high concentration of HCl. From these results, it was demonstrated that the most suitable concentration of HCl solution used for washing treatment is 0.5*M*.

Figure 7 shows the comparison of fermentation characteristics of the unimmobilized yeast, the calcium alginate immobilized yeast, and the polystyrene microcapsule-immobilized yeast washed with 0.5*M* HCl solution. The yeast immobilized into the polystyrene microcapsule or the calcium alginate exhibited a longer initial reaction time compared to that of the unimmobilized yeast. The long initial reaction time is supposed to be caused by the mass transfer limitation in the support matrices. In addition, the initial reaction time of polystyrene microcapsule-immobilized yeast was longer than the calcium alginate–entrapped yeast. This is probably attributable to the hydropho-

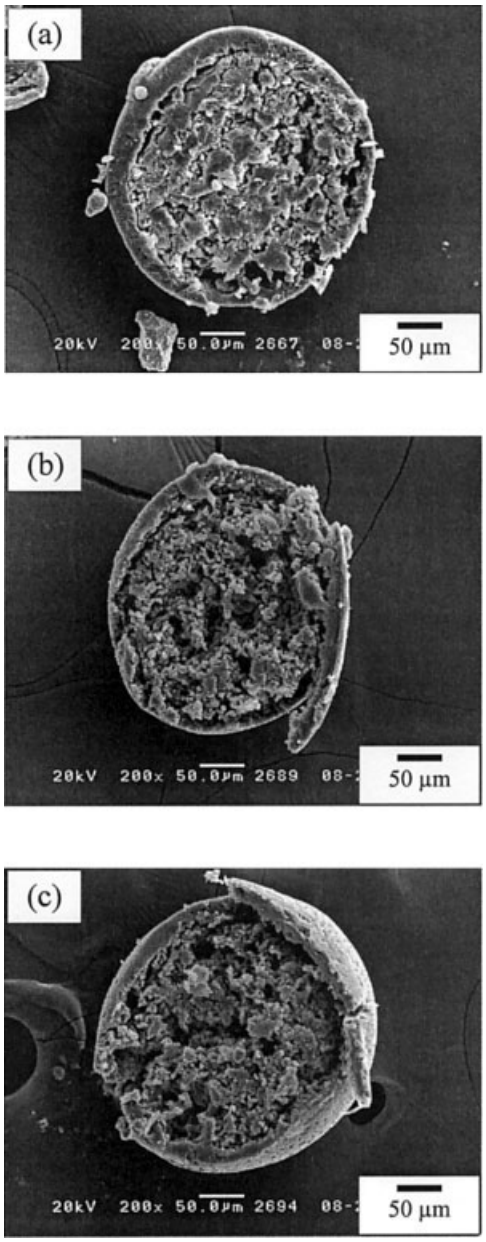

Figure 5 SEM photographs of cross-sectional structure of yeast-immobilized polystyrene microcapsules washed with different concentrations of HCl solution: (a) unwashed microcapsule, (b) microcapsule washed with 0.5*M* HCl, and (c) microcapsule washed with 1.0*M* HCl.

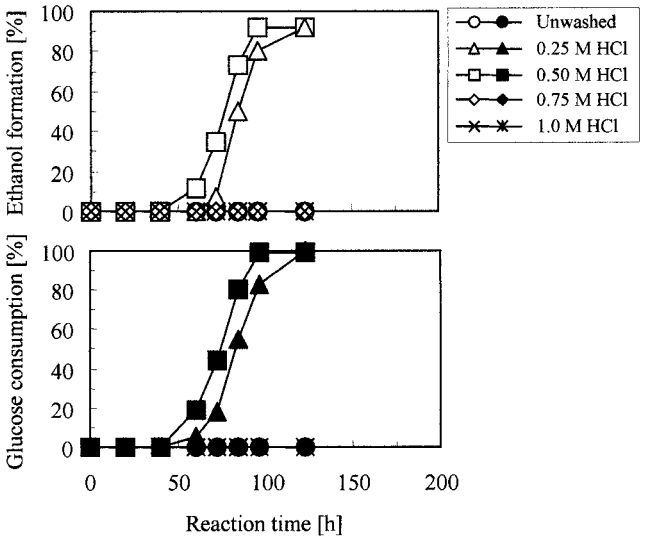

Figure 6 Ethanol fermentation result of yeast-immobilized polystyrene microcapsules washed with different concentrations of HCl solution.

bicity of polystyrene walls, so that the substrates of hydrophilic fermentation medium find it difficult to permeate the walls of polystyrene microcapsules.

Stability of polystyrene microcapsule-immobilized yeast

One of the significant points on the development of cell immobilization supports is the stability of supports during their use in a long-term reaction. To evaluate the stability of the prepared polystyrene microcapsule-immobilized yeast, the repeated ethanol fermentation in a batchwise process was carried out three times, as shown in Figure 8. This result indicates that the prepared polystyrene microcapsules are quite stable and can be reutilized. The first-batch fermentation (F_1) exhibited a long initial reaction time; however, the initial reaction times of the second- and third-batch fermentations (F_2 and F_3) were substantially shorter, similar to the case of unimmobilized yeast (Fig. 7). These results reveal that the repeated use of polystyrene microcapsules can be increased by the catalytic activity of the immobilized yeast inside the microcapsules.

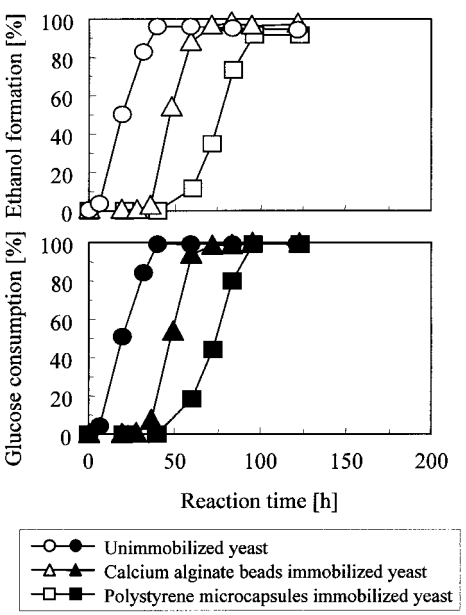

Figure 7 Ethanol fermentation result of yeast immobilized in various supports. Fermentation was carried out in batch process at 30°C and 120 rpm.

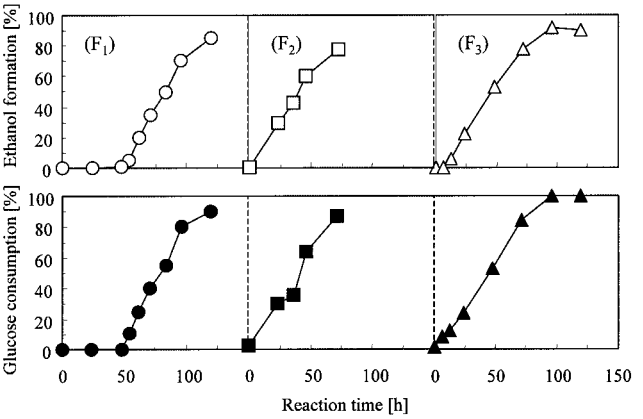

Figure 8 Ethanol fermentation result of polystyrene microcapsule-immobilized yeast in the repeated batch fermentation: (F_1) first-batch fermentation, (F_2) second-batch fermentation, and (F_3) third-batch fermentation. Fermentation was carried out in batch process at 30°C and 120 rpm.

The short initial reaction time can be attributed to three factors: (1) the disruption of polystyrene microcapsules, (2) the dissolution of calcium alginate beads, and (3) the leakage of yeast cells. To investigate the main factor causing the shortening of initial reaction time after repeated use, SEM observations of the reused microcapsules were carried out.

Figure 9 shows the internal structures of the polystyrene microcapsules used in repeated fermentation. From this figure, it is confirmed that the disruption of polystyrene microcapsule did not occur. Furthermore, the observation at higher magnification (right-hand panel of Fig. 9) indicated that there is no significant dissolution of the calcium alginate beads. The calcium alginate beads still remained inside the repeatedly used polystyrene microcapsules; that is, it can be supposed that microcapsule disruption and dissolution of alginate beads are not the main factors causing the acceleration of initial time reaction.

Figure 10 shows the SEM photographs of surface structures on the polystyrene microcapsules used in repeated fermentation. As shown in Figure 10, the yeast cells were hardly observed on the surface of the reused microcapsules. It is predicted that the immo-

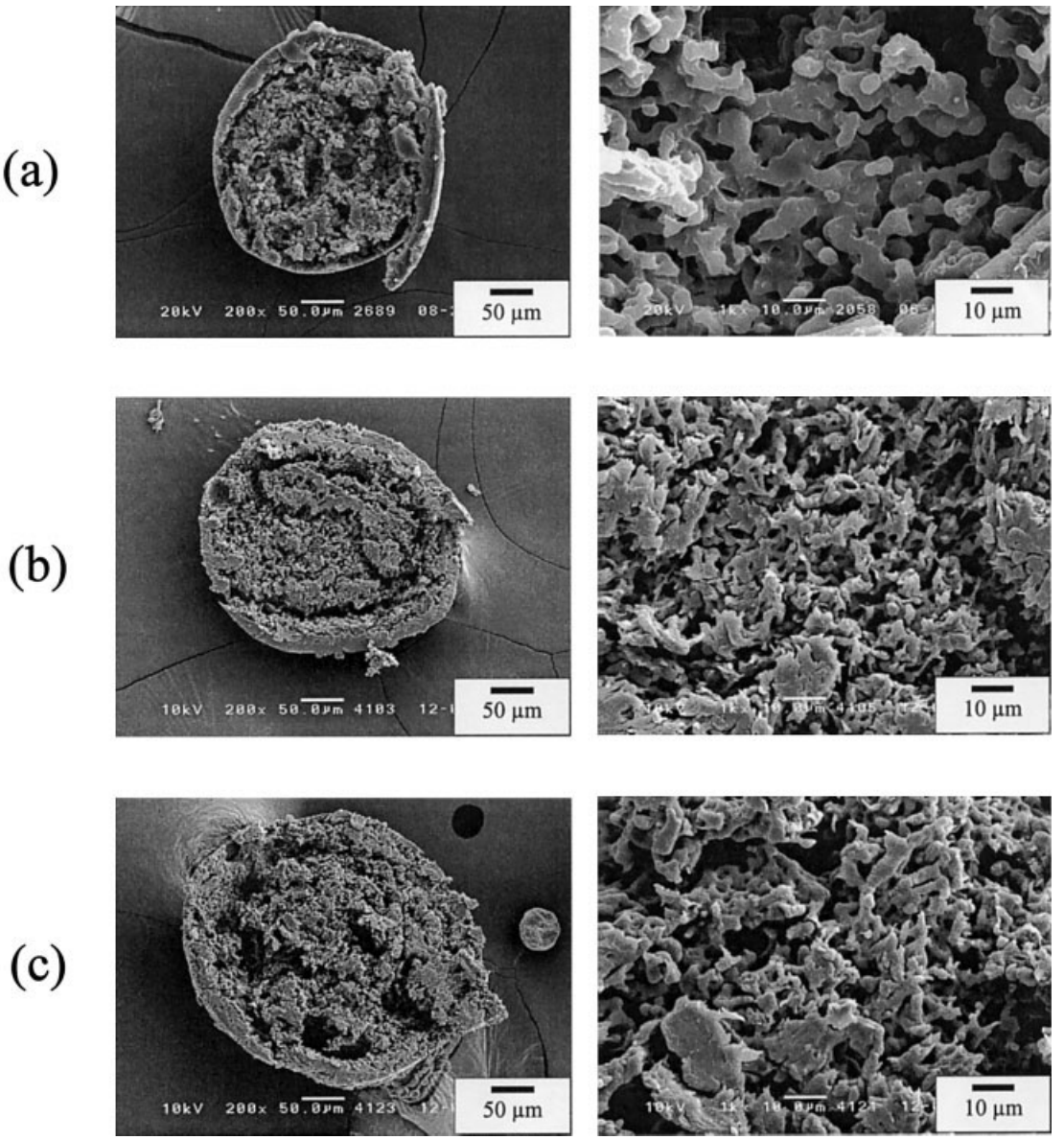

Figure 9 SEM photographs of cross-sectional wall structure of yeast-immobilized polystyrene microcapsules used in repeated batch fermentation: (a) before F_1, (b) after F_1, and (c) after F_2. Right-hand pictures show the photographs taken at higher magnification.

bilized yeast leaked out of the microcapsules during the fermentation process. The leakage yeast might grow in the fermentation medium and accelerate the initial time of reaction. To evaluate the influence of the released yeast on the fermentation result, the following experiment was carried out. After third-batch fermentation, the solution was recovered by suction filtration. Glucose (10 wt %) was added to the reaction medium, and ethanol fermentation was then performed statically at 30°C for 27 h. As a result, the ethanol was slightly formed in the reaction medium, in which ethanol formation was only 3.04% after 27 h reaction. This result reveals that leakage of the yeast is not the dominant factor accelerating the initial time reaction (Fig. 8).

We assume that the acceleration of initial time reaction in F_2 and F_3 was predominantly caused by the sufficient water available inside the reused microcapsules. The adequate aqueous solution for the fermentation is considered to penetrate the microcapsules during F_1 fermentation. Thus, when this microcapsule is reused in the subsequent batch fermentation, the immobilized yeast is already in a suitable condition for catalyzing the reaction. Therefore, the conversion of glucose to ethanol is immediately performed, resulting in elimination of the lag phase of reaction. This assumption can be attributed to the similar fermentation results of both F_2 and F_3. We demonstrated that the polystyrene microcapsule has the optimal conditions as a biocatalyst support by the repeated use of the microcapsules.

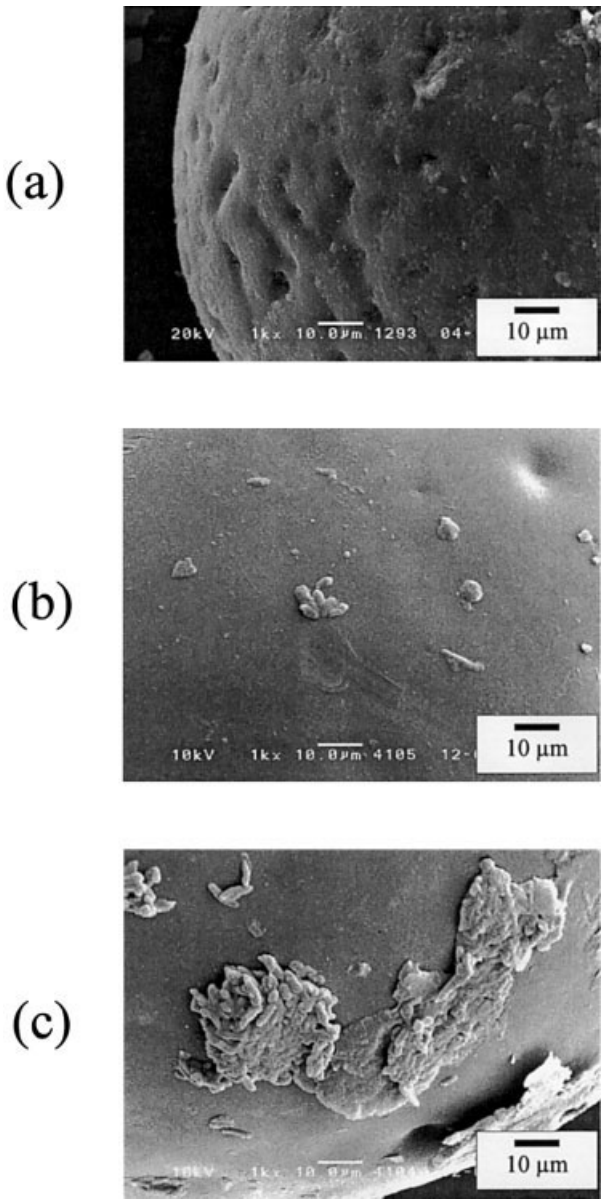

Figure 10 SEM photographs of surface structure of yeast-immobilized polystyrene microcapsules used in repeated batch fermentation: (a) before F_1, (b) after F_1, and (c) after F_2.

CONCLUSIONS

Polystyrene microcapsules possessing the single large core and highly microporous wall were obtained by a novel preparation method based on a combination of phase-separation and solvent-evaporation techniques. The formation of the core and wall pore was markedly influenced by the isooctane concentration, and the diameter of the core was affected by the temperature of solvent evaporation. It was found that the addition of 5 wt % isooctane, the evaporation temperature of dichloromethane at 35°C, and the evaporation temperature of isooctane at 50°C are the optimal conditions for the preparation of the single-core and highly porous polystyrene microcapsules.

The high baker's yeast cell density in the polystyrene microcapsules was obtained by encapsulating calcium alginate bead–entrapped yeast. The entrapped beads inside the microcapsules could be removed by washing the microcapsules with HCl solution. In the ethanol fermentation test that uses the immobilized yeast, the highest conversion of glucose to ethanol was obtained when the polystyrene microcapsules were washed with 0.5*M* HCl solution. Moreover, the prepared polystyrene microcapsules exhibited a high operational stability in the repeated batch-wise fermentation. It was demonstrated that the polystyrene microcapsule developed in this study is a promising immobilization support of microbial cells. We believe that use of the microcapsule-immobilized high-density cells will find various biological applications in the future.

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