Immobilization of *Saccharomyces cerevisiae* Cells by Adhesion to Polymeric Matrices Obtained by Radiation-induced Polymerization[a]

M. CARENZA,[b] E. DE ALTERIIS,[c] S. LORA,[b]
P. PARASCANDOLA,[d] AND V. SCARDI[c,e]

[b]*Istituto di Fotochimica e Radiazioni di Alta Energia*
C.N.R.
Sezione di Legnaro
I-35020 Legnaro (PD), Italy

[c]*Dipartimento di Fisiologia Generale e Ambientale*
Università di Napoli
I-80134 Napoli, Italy
and
Stazione Zoologica "A. Dohrn"
I-80121 Napoli, Italy

[d]*Istituto di Ingegneria Chimico-Alimentare*
Facoltà di Ingegneria
Università di Salerno
I-84081 Baronissi (SA), Italy

INTRODUCTION

An immobilization method based on the spontaneous adhesion of invertase-active cells of yeast (*Saccharomyces cerevisiae*) to tuff granules was described by Parascandola, Scardi, and Tartaglione.[1] Compared with gel entrapment, immobilization by adhesion is much more simple and free from diffusional limitations. However, adhesion is a rather complicated process involving surface interactions between microbial cells and the so-called substratum, that is, the solid support to which they attach. Because there are still many unanswered questions about the mechanism of adhesion, the selection of suitable substrata for a given microbial species can be made only empirically. Thus, to find substrata better than tuff or insolubilized gelatin,[2] polymeric hydrogels that were obtained by radiation-induced polymerization below 0 °C and that were employed successfully for immobilizing enzymes, cells, and antibodies[3] were considered. A dozen of such polymer matrices with different hydrophilicities were synthesized and assayed as possible substrata for *S. cerevisiae* cells used in continuous ethanol production.

[a]This work was financially supported in part by a grant from Consiglio Nazionale delle Ricerche (Rome, Italy).
[e]To whom all correspondence should be addressed.

MATERIALS AND METHODS

Microorganism

A strain of *S. cerevisiae* was isolated from commercial baker's yeast (Eridania, Genoa, Italy) and was precultured under aerobic conditions for 24 h at 30 °C in a medium containing 10 g/L glucose, 5 g/L bactopeptone, and 10 g/L yeast extract (pH adjusted to 5.0). The cells were harvested by centrifugation (4 °C, 10 min, 1600*g*) and then were washed with sterile saline (9 g/L NaCl) and stored in a refrigerator until use.

Synthesis of Polymeric Matrices

The following monomers were used: 2-hydroxyethyl acrylate (HEA), 2-hydroxyethyl methacrylate (HEMA), *N*,*N*-dimethylacrylamide (DMAA), and *N*-vinyl-2-pyrrolidone (NVP) from Aldrich (Germany); the sodium salt of styrene sulfonic acid (StS) from Fluka (Switzerland); and methoxypolyethyleneglycol methacrylate (M-3G) from Rohm (Germany). Monomer/water mixtures in a 1:2 volume ratio were placed in Pyrex tubes and were degassed under vacuum. The sealed tubes were frozen at −73 °C and were exposed to the γ-rays of a 2000-Ci ^{60}Co at a dose rate of 0.55 Gy/s for 5 h. After irradiation, the tubes were opened and the contents were sliced into thin disks; they were then thoroughly washed with deionized water.

Evaluation of Water Content

The polymeric matrix was allowed to swell for a week in a large volume of water, which was changed every day; then, the swollen matrix was dried at 60 °C in a vacuum oven. The equilibrium water content (in %) was given by $100(W_s - W_d)/W_s$, where W_s and W_d are the weight of the matrix after swelling and drying, respectively.

Immobilization of Yeast Cells

A sample of precultured yeast was incubated at 30 °C under agitation in 200 mL of culture medium (2.5 mg cells/mL) with a suspension of regularly shaped fragments of the polymeric matrix under examination, previously swollen in the culture medium and autoclaved. Yeast growth was allowed to occur for five days, with the medium being changed every 6 h. The fragments were decanted and washed with sterile saline.

Evaluation of Cell Loading

Fragments of the polymeric matrix under examination were disintegrated mechanically by an Ultraturrax, and an aliquot of the resulting fine suspension was assayed for protein according to Stickland.[4] Because protein represents 46% of the yeast cell on a dry weight basis, cell loading was expressed as mg dry cells/cm^2.

Continuous Fermentation

In a shake flask containing the fermentation medium formulated by Williams and Munnecke,[5] carrier fragments with immobilized yeast cells were suspended. The shake flask was continuously fed with fresh medium from a reservoir by means of a peristaltic pump. The fermented medium was withdrawn at the same flow rate by another peristaltic pump and was assayed for ethanol by using a Boehringer test kit.

RESULTS AND DISCUSSION

Hydrogels by definition do not dissolve in water, but merely swell up and retain a significant fraction of water in their structure. This peculiarity depends on both the monomer nature and the monomer-to-water ratio in the reaction mixture. In other words, the more hydrophilic the monomer employed, the higher the polymer water content of the resulting polymer, whereas the higher the monomer dilution in water, the more porous the matrix. In the present work, a dozen polymers and copolymers were prepared from six monomers differing from one another in hydrophilicity and/or acidity/basicity, thus providing various potential substrata for *S. cerevisiae*.

TABLE I. Hydrophilicity and Cell Loading of Synthetic Polymeric Hydrogels Assayed as Possible Substrata for *S. cerevisiae* Cells

No.	Monomers Used for Preparation of Polymers[a]	Water Content (%)	Cell Loading (mg/cm^2)
1	HEA[b]	69.9	null
2	HEA	86.3	3.1
3	HEA + M-3G	87.4	3.1
4	HEA + NVP	90.5	6.0
5	HEA + DMAA	91.6	6.0
6	HEA + StS	88.4	11.8
7	DMAA	94.8	7.4
8	M-3G	88.3	10.6
9	HEMA[b]	38.9	null
10	HEMA	66.4	15.0
11	HEMA + DMAA	91.0	8.5
12	HEMA + NVP	82.1	7.6

[a]Monomer mixtures were 1:1 (v/v).
[b]Used without addition of water.

From the data reported in TABLE 1, it appears that all the polymeric hydrogels, except poly-HEA and poly-HEMA when prepared without addition of water, are better substrata for *S. cerevisiae* cells than tuff and insolubilized gelatin, whose maximum cell loadings were 0.5 and 0.3 mg dry cells/cm^2, respectively.[1,2]

At first sight, the data of TABLE 1 might suggest that no relationship exists between water content and cell loading. This is not in agreement with the conclusion drawn by several authors[6–8] using *S. formosensis* cells immobilized with polymeric hydrogels.

They found that the ethanol production in batch fermentation experiments was higher when the matrix water content was higher. However, it should be kept in mind that the polymeric matrices used by these authors[6-8] were obtained either from monomers belonging to an homologous series or from graded mixtures of two different monomers. Therefore, an appropriate comparison of the data of TABLE 1 [e.g., between poly-HEA and poly-HEMA prepared either with or without water; poly-(HEA + DMAA) and poly-HEA or poly-DMAA; and so on] would confirm the existence of a relationship between water content and cell loading.

Hydrophilicity and porosity of the polymeric matrices are considered by the authors mentioned earlier[6-8] as playing important roles in cell immobilization in that they not only facilitate the diffusion of nutrients, but also the adsorption of cells into the cavities of the carrier. However, the results of the following experiments seem to reduce the importance of both hydrophilicity and porosity.

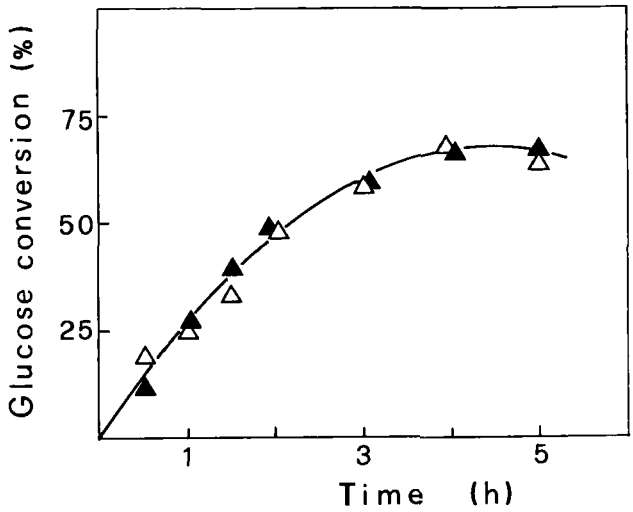

FIGURE 1. Glucose conversion by poly-HEMA-immobilized yeast cells (△) and the same amount of free cells (▲) in batch fermentation experiments.

Cells of *S. cerevisiae* immobilized by adhesion to five polymeric hydrogels chosen in the order of increasing cell loading (i.e., nos. 4, 7, 8, 6, and 10 of TABLE 1) were employed for the continuous fermentation of glucose to ethanol under comparable conditions (total surface-to-void volume ratio, 1:4; flow rate, 1.3 mL/min). By measuring the specific rate of ethanol formation, instead of obtaining, within the limits of the experimental error, the same value in all the cases examined, the following values in order were obtained: 0.12, 0.14, 0.16, 0.21, and 0.26 h^{-1}. Such a difference of values roughly indicates that the immobilized yeast cells, independently of the carrier cell loading, are not equally active in the fermentation process because a fraction of them are confined in the narrow pores and/or deep cavities of the matrix. In fact, the lowest values for the specific rate of ethanol formation found in these experiments are

associated with the highest water content and porosity of the polymeric carrier. The highest value found, that is, 0.26 h^{-1}, indicates that almost all the cells (or a large fraction) are distributed evenly onto the outer surface of the matrix (poly-HEMA) so that internal diffusion limitations are negligible. FIGURE 1 shows the glucose conversion operated in batch fermentation by free cells and poly-HEMA-immobilized cells (in equal amounts); it is a further demonstration of the negligible diffusional resistance offered by the cell layer covering the matrix.

Compared with the other polymeric hydrogels assayed as substrata for *S. cerevisiae* cells, poly-HEMA—in addition to the highest cell loading and the lowest water content—exhibits the best mechanical properties. These characteristics make poly-HEMA a substratum for *S. cerevisiae* that is particularly suitable for continuous operation. In this connection, another peculiarity of the *S. cerevisiae* immobilized by adhesion to poly-HEMA deserves to be reported here. Contrary to batch fermentation with free yeast cells, which is inhibited by glucose concentrations higher than 10%, continuous fermentation with poly-HEMA-immobilized cells at a flow rate of 11 mL/min seems to be inhibited by glucose concentrations higher than 25%.

REFERENCES

1. PARASCANDOLA, P., V. SCARDI & O. TARTAGLIONE. 1987. Immobilization of yeast cells by adhesion on tuff granules. Appl. Microbiol. Biotechnol. **26:** 507–510.
2. DE ALTERIIS, E., P. PARASCANDOLA, M. A. PECORELLA & V. SCARDI. 1987. Un nuovo modello di bioreattore a membrana piana. 1° Convegno Nazionale sui Biocatalizzatori (ENEA, Roma, 28–30 Ottobre).
3. KAETSU, I. 1985. Recent progress on the immobilization of biocomponent by radiation polymerization and the application to biomedical uses. Radiat. Phys. Chem. **25:** 517–528.
4. STICKLAND, L. H. 1951. The determination of small quantities of bacteria by means of the biuret reaction. J. Gen. Microbiol. **5:** 698–703.
5. WILLIAMS, D. & D. M. MUNNECKE. 1981. The production of ethanol by immobilized yeast cells. Biotechnol. Bioeng. **23:** 1813–1825.
6. FUJIMURA, T. & I. KAETSU. 1983. Growth of yeast cells immobilized with porous swelling carriers produced by radiation polymerization. Appl. Biochem. Biotechnol. **8:** 145–151.
7. FUJIMURA, T. & I. KAETSU. 1987. Nature of yeast cells immobilized by radiation polymerization. Activity dependence on the water content of polymer carriers. Biotechnol. Bioeng. **29:** 171–175.
8. FUJIMURA, T., M. CARENZA & I. KAETSU. 1988. Activity dependence of immobilized yeast cells on the various natures of polymer carriers for immobilization produced with radiation polymerization at low temperatures. Radiat. Phys. Chem. **31:** 653–655.