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Effect of the support matrix on biotransformation of benzaldehyde to benzyl alcohol by yeast cells in aqueous and aqueous-organic two phase systems

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SUMMARY

Biotransformation of benzaldehyde to benzyl alcohol by *Saccharomyces cerevisiae* immobilized in different support matrices was investigated. Polymers with intrinsic hydrophobic and/or hydrophilic nature as well as mixed hydrophobic and hydrophilic supports were examined both in aqueous and bisphasic aqueous-organic systems. The hydrophobic support material ENTP-2000 or mixed silicone:alginate (50–25:50–75) proved to be most suitable not only for nonconventional media but also for conventional aqueous media for production of benzyl alcohol. With ENTP-2000, catalytic activity and maximum yield were 159 μmol h^{-1} g^{-1} dry weight catalyst and 0.89 mM, respectively, in hexane containing 2% moisture. Corresponding values in aqueous media were 246 μmol h^{-1} g^{-1} dry weight catalyst and 1.53 mM. With 50:50 silicone:alginate, catalytic activity and maximum yield were 177 μmol h^{-1} g^{-1} dry weight catalyst and 1.18 mM, respectively, in hexane containing 2% moisture. Corresponding values in aqueous media were 192 μmol h^{-1} g^{-1} dry weight catalyst and 0.8 mM.

INTRODUCTION

Implementation of biocatalysis in organic media offers potential to facilitate the solubilization of poorly water-soluble organic compounds, thereby extending the range of possible substrates for biocatalytic reactions. In nonconventional media, dispersion of biocatalyst is easily achieved by use of immobilization methods which also facilitate continuous operation procedures, biocatalyst recovery and often improve enzyme stability. Nonpolar organic solvents are more suitable than polar solvents in nonconventional biocatalysis [7,8] because the latter solvents tend to strip essential water from the enzyme micro-environment [2,14]. While most biotransformation reactions using organic reaction media have utilized isolated enzymes, relatively little emphasis has been placed on whole cell systems [13]. Whole cell biocatalysts have advantages over isolated enzymes in that enzyme extraction and purification costs are avoided, enzyme-containing cells may be easily reused, essential cofactors can be retained or recycled within the cell, and the enzyme may be more stable within the cell environment.

Hydrophilic immobilization support materials such as calcium alginate have been widely used in conventional biotransformation reactions but diffusion of water-insoluble substrates into these support matrices is often the rate limiting step [9,12]. Recently, interest has developed in evaluation of hydrophobic support material and mixed hydrophilic/hydrophobic polymeric matrices for cellular biotransformation reactions in nonconventional media [1,5].

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MATERIALS AND METHODS

Microorganisms used as biocatalyst

Fresh pressed commercial baker's yeast (30 g dry weight) was obtained from Fleischmann's Inc., Kitchener, Ontario, Canada. Yeast cake, 50 g, was suspended in 50 ml of 50 mM sodium succinate buffer, pH 5.0 (Buffer A), and lyophilized. The lyophilized biocatalyst was stored at 4 °C for use in biotransformations.

Immobilization of biocatalyst

The silicone and alginate polymer system used was sylgard 184^R obtained from Dow Corning Co. (Midland, MI, USA). The system consisted of a dimethyl-siloxane prepolymer called sylgard 184^R (30 g) and curing agent sylgard 184^R (3 g). Previously lyophilized yeast cells (0.3 g) were suspended in 3 ml of Buffer A and added to the above mixture by gentle stirring with a spatula. The resulting emulsion was allowed to stand overnight at 37 °C to give a rubber-like solid matrix, which was cut in small particles in the range 3–5 mm. The procedure was adapted from those described by Kawakami et al. [4] and Oriel [10]. In the case of the mixed matrix consisting of silicone prepolymer and calcium alginate, different proportions were mixed to obtain the desired ratio between the two polymers. Above 50% calcium alginate,

bead formation was easily achieved by dropwise addition of the emulsion (consisting of cells, silicone prepolymer and 2.5% (w/v in water) sodium alginate) into 0.2 M $CaCl_2$ solution. The calcium alginate beads were prepared according to the procedure of Kierstan and Coughlan [6]. All alginate-containing beads were 3–5 mm in diameter.

Photo-crosslinkable resin prepolymers ENT and ENTP

The prepolymers were obtained as a gift from A. Tanaka, Kyoto University, Japan. Methods for their preparation are described elsewhere [1,9]. ENT-4000 or ENTP-2000, 1 g, was mixed with 10 mg of the photosensitive ethyl ether (Aldrich Chemical Co. Inc., Milwaukee, USA) and melted at 60 °C. One milliliter of Buffer A was added to the molten mass and the mixture was cooled to 4 °C. The yeast cells previously lyophilized (0.3 g) were suspended in 2 ml of Buffer A in the case of ENT-4000 and in 2 ml of water-saturated *n*-hexane in the case of ENTP-2000. The cell suspension was added to the molten mixture to give the prepolymer biocatalyst mixture, which was layered in glass plates (thickness ~ 0.5 mm). The layer was covered with transparent thin film to eliminate air and was illuminated with UV light (365 nm) for several minutes. The polymer gel formed was cut into small pieces, ~3–5 mm, and used for biotransformations.

Urethane prepolymers

One gram of PU-3 or PU-6 (provided by A. Tanaka) was melted at 50 °C and cooled to room temperature (not to solidify). Lyophilized cells, 0.3 g, were suspended in 2 ml of Buffer A and mixed quickly with the prepolymer solution. The mixture was stirred until gelation started and was left at 4 °C for 1 h to complete gelation. The resulting porous gel was cut into small pieces and used for the biotransformation.

Biotransformation conditions

In the case of biotransformation of benzaldehyde to benzyl alcohol, two systems were tested: a two-phase system of hexane containing 2% (v/v) moisture (Buffer A) and an aqueous phase system consisting of Buffer A. The benzaldehyde concentration was 2.5 g L^{-1}, unless otherwise stated. For both systems a 30-ml reaction volume was used in 250-ml Erlenmeyer flasks incubated at 28 °C on an orbital shaker at 225 r.p.m. In all cases the amount of biocatalyst was the same, 0.3 (dry weight) yeast cells entrapped in the different polymers.

Gas chromatography (GC) analysis

Samples of the organic solvent system (hexane with 2% moisture) were subjected to GC analyses. Aqueous phase samples were extracted twice with an equal volume of diethyl ether. Benzaldehyde and benzyl alcohol concentrations were determined by GC analysis using a Shimadzu GC 14A gas chromatograph equipped with FID and connected to a Shimadzu Chromatopac CR6A integrator. The column was a fused silica megabore 30 m long and 0.52-mm internal diameter coated with 1-μm thickness of 25% cyanopropyl, 25% phenyl, 50% methyl polysiloxane (Durabon 225; Chromatographic Specialities, Brockville, Ontario, Canada). Analytical conditions were: injection and column temperature 150 °C and detector temperature 200 °C.

Scanning electron microscopy (SEM)

The immobilized cells were gold coated (200–300 Å) with SEM Coating Unit PS3. An accelerating voltage of 15 kV was used in a Hitachi S-570 Scanning Electron Microscope.

RESULTS

Conversion of benzaldehyde to benzyl alcohol by whole yeast cells entrapped in the different polymeric matrices was investigated in hexane containing 2% (v/v) moisture and in aqueous media (Table 1). Best overall performance in both reaction media was observed with cells entrapped in ENTP-2000 in comparison with the rest of the polymers. Poorest performance was observed in cells entrapped in hydrophilic and hydrophobic urethane polymers, PU-3 and PU-6, respectively.

As gel hydrophobicity may be an important parameter for biocatalysis, particularly for reactions involving poorly water-soluble compounds, the effect of hydrophobicity–hydrophilicity balance on reaction performance was investigated in the aqueous and aqueous organic reaction media (Table 2). In hexane containing 2% (v/v) moisture the reaction was only carried out in polymers consisting of silicone:alginate ratios of 50:50; 25:75, and 0:100. Due to the swelling behavior of polymers containing silicone:alginate ratios of 100:0 and 75:25 the reaction was not investigated in these matrices. This swelling phenomenon was also observed when hexane was replaced by other hydrophobic solvents such as decane and dodecane. However, when the same biotransformation reaction was carried out in aqueous media, higher silicone:alginate ratios (100:0; 75:25) were compatible with the reaction medium and no swelling was observed. In hexane containing 2% moisture, altering the silicone:alginate ratio from 0:100 to 50:50 resulted in an increase in catalytic activity and yield of biotransformation product. Maximum yield in aqueous media was noted with a silicone:alginate ratio of 25:75.

In order to better understand the nature of the immobilization matrices, each support, including cells, was examined using SEM (Figure 1(A–G)). In the case of PU-3 (A) and PU-6 (B), the cells appeared to be embedded and covered with a thick film of polymer. When ENT-4000 was examined (C), the cells appeared to be protruding more into the spaces. This phenomenon was even more pronounced in the ENTP-2000 matrix (D) which manifested highest activity in organic solvents. A 100% silicon matrix (E) exhibited pore-like structure while a 100% alginate matrix (F) had leaf-like structure. The 50:50 silicon alginate mixed matrix manifested a partly porous partly leaf-like structure (G).

DISCUSSION

Conversion of benzaldehyde to benzyl alcohol by non-growing yeast cells in aqueous media was most efficient with

TABLE 1

Effect of polymers on reduction of benzaldehyde to benzyl alcohol by non-growing yeast cells

Polymer	Properties	Catalytic activity[a] (μmol h^{-1} g^{-1} dry wt catalyst)		Maximum yield[b] (mM)	
		Hexane[c]	Aqueous phase	Hexane[c]	Aqueous phase
ENTP-2000	Hydrophobic	159	246	0.89	1.53
ENT-4000	Hydrophilic	53	153	0.51	1.30
Alginate	Hydrophilic	81	132	0.56	0.73
PU-3	Hydrophobic	23	12	0.14	0.13
PU-6	Hydrophilic	14	27	0.14	0.12

[a] Initial reaction rates; [b] 115 h incubation period; [c] Hexane containing 2% moisture.

TABLE 2

Effect of the hydrophobicity–hydrophilicity of the silicone-alginate mixed matrix on reduction of benzaldehyde to benzyl alcohol by non-growing yeast cells

Mixed matrix (%)		Catalytic activity[a] (μmol h^{-1} g^{-1} dry wt catalyst)		Maximum yield[b] (mM)	
Silicone	Alginate	Hexane[c]	Aqueous phase	Hexane[c]	Aqueous phase
100	0	NA	116	NA	0.36
75	25	NA	103	NA	0.38
50	50	177	192	1.18	0.80
25	75	145	178	1.13	1.10
0	100	81	132	0.56	0.73

[a,b,c] As for Table 1; NA = Not analyzed.

cells entrapped in ENTP-2000 as compared with the other polymers. In hexane containing 2% (v/v) moisture the biotransformation was most efficient with the mixed 50:50 silicone:alginate polymer. Kawakami et al. [4] also compared the performances of several different polymers in aqueous-organic two-liquid phase systems using non-growing cells of *Nocardia* for the production of epoxide. Entrapment in PU-3 and PU-6 resulted in no biocatalytic activity. Differences in biocatalyst performance in various polymers could also be ascribed to differences in the physical properties of the polymers (size, porosity, surface) and orientation of the cells in the matrix [5]. Park and Hoffman [11] observed that, in aqueous media, *Arthrobacter* cells exhibited higher steroid-converting activity in a hydrophobic gel matrix than in a hydrophilic matrix. Our results also confirmed that, in the aqueous phase, hydrophobic matrices such as ENTP-2000 performed better than the corresponding hydrophilic matrix (ENT-4000).

When compared with other matrices, the two urethane polymers exhibited poor biotransformation performance. Similarly, lipase entrapped with the urethane polymers PU-3, PU-6 and PU-9 exhibited poor biocatalytic activity in water-saturated hexane [15]. The observation that micrographs of cells encapsulated in PU-3 and PU-6 are covered with a thick film suggests that these matrices may retard substrate/product diffusion.

Increasing the silicone to alginate ratio increased conversion of benzaldehyde to benzyl alcohol in hexane containing media. Efficiency of conversion of liquid alkenes to epoxides in the presence of hexadecane as organic solvent varied with the silicone:alginate ratio. Ratios of 80–90%:20–10% were optimal for 1,2-epoxy tetradecane production; 40–50%:60–50% were maximum for 1,2-epoxy octane formation and almost 100% alginate was optimal for styrene oxide formation [3,4].

Thus, the nature of the substrates and products as well as the hydrophilic or hydrophobic nature of the reaction medium influences the relative efficiencies of different cell immobilization matrices. Varying the ratios of the polymers to alter hydrophobicity/hydrophilicity balance can lead to changes in the physical structure of the support and alters reaction performance.

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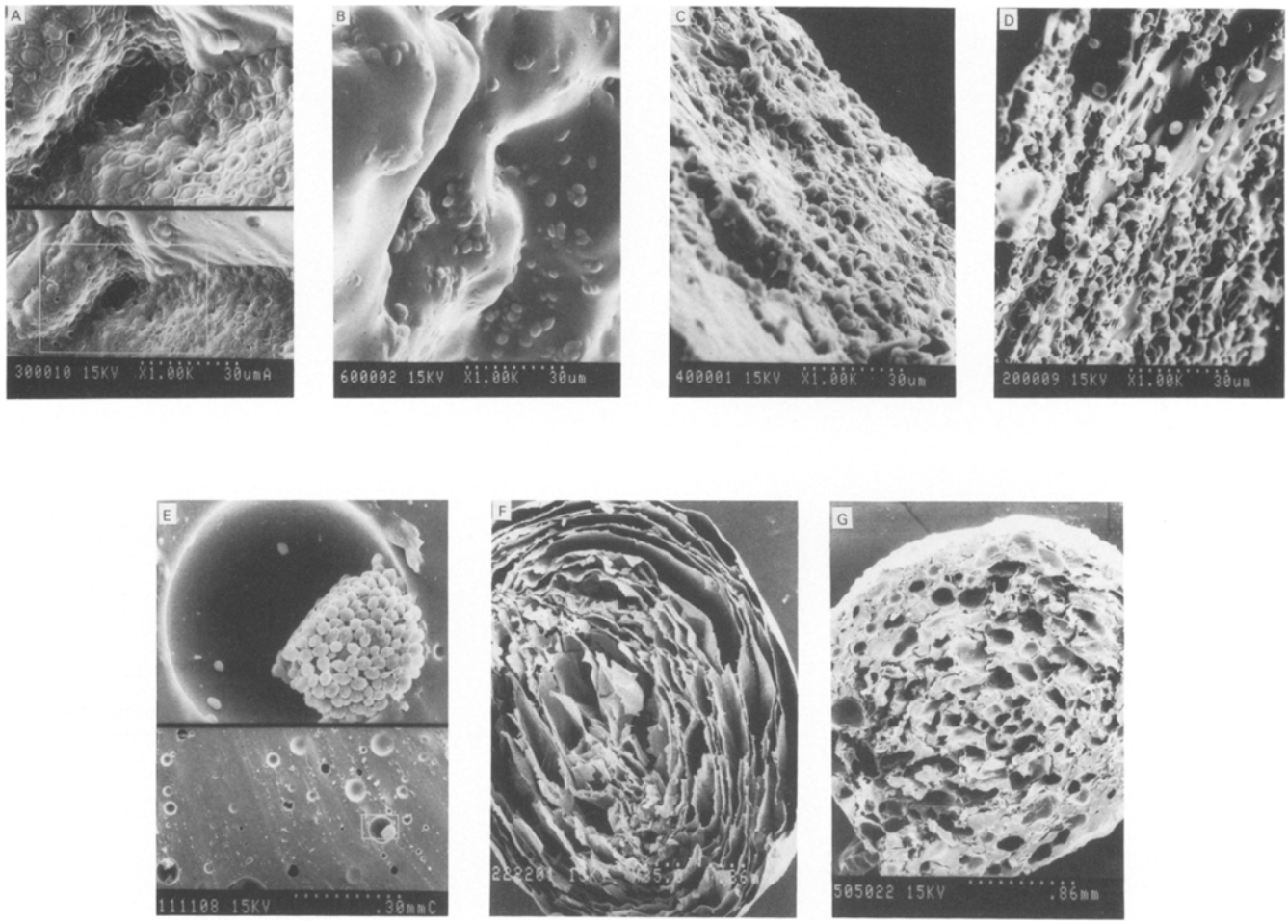

Fig. 1. Scanning electron micrographs of *Saccharomyces cerevisiae* entrapped in: (A) PU-3; (B) PU-6; (C) ENT-4000; (D) ENTP-2000; (E) 100% silicone; (F) 100% alginate; and (G) 50% silicone–50% alginate.

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