IMMOBILIZATION OF YEAST CELLS ON POLYPHENYLEDIHYDROXIDE

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Summary: The potential of a polymeric product of 2,6-dimethylphenol as a support for immobilized intact yeast cells was investigated. The procedure used is based on modification of the polymeric adsorbent by adsorption of glutaraldehyde, and the immobilization of cells is probably accomplished by their adsorption and covalent linkage.

INTRODUCTION

The use of immobilized microorganisms as an enzyme source generally eliminates the high cost of enzyme purification. Maintenance of the enzyme in its natural environment prolongs its activity, and the application of immobilized cells makes it possible to carry out multistep reactions (Abbot, 1978; Broun et al., 1978; Chibata, 1978; Fukui and Thomas, 1978).

MATERIAL AND METHODS

Strain and cell preparation. Kluyveromyces lactis 35-3-1 was obtained from the collection of the Institute of Chemistry of the Slovak Academy of Sciences. Cells were grown in 0.7% (w/v) Yeast Nitrogen Base (Difco) with 0.7% lactose at 30°C (rotary water bath shaker). The yeast cells in the exponential or stationary growth phase were taken for permeabilization and immobilization procedure.

Permeabilization and enzyme assay. Cells were made permeable by drying in a flow of warm air as described previously (Jirka et al., 1979).
β-Galactosidase was assayed according to Lederberg (1950).

**Support.** Polyphenyleneoxide (PFO) was prepared as a polymeric product of 2, 6-dimethylphenol according to Kubánek et al (1978). The porous polymer obtained had a specific surface area of \(600 \text{ m}^2\cdot\text{g}^{-1}\) and a 250 \(\mu\text{m}\) particle size.

**Cell immobilization.** The time course of whole cell immobilization was followed by the decrease of β-galactosidase activity in the stirred mixture containing \(10^6\) permeabilized cells per 100 mg PFO in 50 mM phosphate buffer (pH 7.2). The amount of bound dry weight per gram of carrier was determined from the content of nitrogen measured by Kjeldahl's method.

**Scanning electron microscopy.** Specimens of the carrier were, without any previous fixation procedure, coated with gold and observed on a JEOL SEM.

**RESULTS AND DISCUSSION**

The preliminary experiments revealed a fast and significant uptake of permeabilized cells by particles of PFO. However, the observation of complete release of β-galactosidase activity from a carrier after one washing indicated that the uptake of cells was caused by weak adsorption only. We therefore investigated the possibility of immobilizing yeast cells via adsorbed glutaraldehyde, taking advantage of the fact that PFO is noted for a specific adsorption of aldehydes (Kubánek et al, 1978). PFO (0.5 g) was suspended in 5 ml of 1% - 10% aqueous solutions of glutaraldehyde. After 24 h stirring the adsorbent was transferred into a column and washed with distilled water until the reaction of the eluate with 2, 4-dinitrophenylhydrazine was negative. Afterwards the adsorbent was washed to equilibrium with 200 ml 50 mM phosphate buffer (pH 7.2). The bound aldehyde was determined by an indirect procedure based on the degradation of bound L-leucine-4-nitroanilide (Turková, unpublished method). The results obtained revealed that maximum adsorption of aldehyde was obtained in 5% glutaraldehyde.

In the experiment shown in Figure 1, the time course of the fixation of