Immobilization of Bacteria and *Saccharomyces cerevisiae* in Poly(Tetrafluoroethylene) Membranes

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A novel method for immobilization of bacteria and *Saccharomyces cerevisiae* cells is described. Microorganisms may be entrapped in a matrix of poly(tetrafluoroethylene) (PTFE) fibrils. Cells are blended with an aqueous emulsion of PTFE stabilized with Triton X-100 surfactant to form a thick paste. The paste is calendered biaxially in a standard rubber mill. This process causes fibrillation of the PTFE and formation of the fibrill matrix, which serves only to impart physical integrity to the composite microporous membrane. The cells trapped in the membrane were shown to be viable by incubation of the membrane on solid media and in broth culture. This bioactive membrane represents a new means of immobilization of cells for bioprocessing.

Immobilization of living microorganisms has been described by several investigators (1) as being useful in the production of specialty chemicals for industrial use. Fermentations which are currently performed in large vessels have problems with complete mixing of nutrients and biomass. Problems exist also with the purification of chemicals generated by microorganisms in fermentation vessels.

Immobilization of bacteria, yeast cells, and fungi has been done in a variety of ways. Matrices for entrapment include calcium alginate, carageenan, agar, cellulose, polyacrylate, and polyamide (1). These methods have their own problems associated with them, such as dispersion of cells, flow of nutrients into and wastes away from the cells (largely inhibited by the viscosity of the immobilization preparation), and purification of the desired cell product from the immobilization matrix.

In this report, we describe a novel means of immobilizing microbial cells in a fibril matrix of poly(tetrafluoroethylene) (PTFE) (Teflon 30B; Dupont, Wilmington, Del.). The process involves working a mixture of wet cells and PTFE on a standard rubber mill to generate a tough, leathery membrane which is permeable to growth substrates but not to foreign cells or colloidal materials which have diameters equal to or greater than those of the cells trapped in the membrane (4). Despite the mechanically harsh procedure of preparing the microporous membrane, there is little, if any, detrimental effect on the viability of the cells entrapped therein. We have demonstrated the viability of cells entrapped in the matrix by chemical means, such as production of ethanol by yeast cells, and by cultivation, such as growth of a membrane composed of 85% *Serratia marcescens* cells.

**MATERIALS AND METHODS**

**Construction of biomembranes.** PTFE membranes containing microorganisms were constructed by mixing viable cells with a PTFE emulsion and calendering the mixture on a standard rubber mill. Bacterial cells (*S. marcescens* or *Pseudomonas aeruginosa*) were grown in tryptic soy broth (Difco Laboratories, Detroit, Mich.) to mid-log phase in a 6-liter Erlenmeyer flask. The cells were harvested after 24 h of growth at 37°C by centrifugation in a Sorvall RC5C high-speed centrifuge. The yeast was commercially available baker’s yeast (*Saccharomyces cerevisiae*) purchased from a local grocery outlet. Bacterial cell pellets were washed once with phosphate-buffered saline (PBS) (10 mM sodium phosphate [pH 7.3] with 15 mM NaCl). The wet weight of the cells was approximately 12 g. A slurry of PTFE particles in emulsion form (Teflon 30B [59.3% solids by weight; DuPont]) was added dropwise to the slurry of cells to a final concentration of Teflon of 15% by weight for the *S. marcescens* mixture and 10% for the *P. aeruginosa* mixture. Each mixture was centrifuged at 5,000 rpm for 10 min to concentrate the PTFE and cells. The supernatant was removed, and the pellet was resuspended in PBS to remove the surfactant. After a second centrifugation, the mixture was removed from the centrifuge bottle, leaving a tough dough which was milled in multiple directions on a standard rubber mill. The resulting membrane was washed overnight in nutrient broth.

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**FIG. 1.** *S. marcescens* membrane. The dark area, bacterial growth, appears red (not evident in this black and white photograph). When incubated for several more days at 30°C, the red growth spread to the entire surface of the membrane.
which had been diluted 1:10 with water. The membrane was cut into the appropriate size and shape for the experiments on cell viability.

**Cell viability in PTFE membranes.** To determine that microorganisms survived the milling-calendering process, experiments were performed to evaluate growth and biochemical activity in membranes. To determine that microorganisms were indeed viable, a small piece of the bacterial or yeast membrane was excised from the master sheet and placed onto the surface of tryptic soy agar (Difco) plates. The plates were placed into a 30°C incubator for 72 h to evaluate growth of the organisms. The cut membrane would allow any viable microorganisms to escape from the PTFE matrix and grow out onto the solid medium. The bacterial membranes were placed at 30°C because *Serratia* spp. produce a bright red pigment at this temperature.

**Biological activity of microorganism-filled PTFE membranes.** Experiments were performed to determine the biochemical characteristics of the yeast membrane on the basis of conversion of glucose to ethanol by the entrapped yeast cells. Membranes were cut into a circular shape of 44.2 cm² and placed into the flow cell of an Amicon (Danvers, Mass.) 402 model 3155 ultrafiltration device. A glucose solution (1% [wt/vol] in water) was passed through the yeast membrane at various flow rates. The ethanol present in the filtrate was detected by gas chromatography with a Hewlett Packard 5880 gas chromatograph. The membranes evaluated for biological functionality were all of the same size. *P. aeruginosa* was evaluated for its ability to remove a pesticide, 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), from water. The membrane was permeated with tryptic soy broth for 72 h and then with 420 ml of distilled water. The reservoir above the membrane was charged with a solution of 2,4,5-T (21 μg/ml in water). The pesticide was passed through the membrane at a flow rate of 8 ml/h. The amount of 2,4,5-T remaining in the water sample was determined by gas chromatography.

**Visualization of microorganisms in PTFE membranes.** Electron microscopy of the PTFE membrane containing *S. cerevisiae* was performed with a freeze-fractured membrane. The membrane was frozen in liquid nitrogen and broken. The membrane was sputter coated with uranyl acetate and

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**FIG. 2.** Scanning electron micrographs of freshly constructed microporous yeast membranes. (A) Cut yeast membrane (original magnification, ×2,000). (B) Oblique view of yeast membrane (original magnification, ×2,000). (C) Micrograph of edge of yeast membrane showing Teflon fibrils (original magnification, ×2,000). (D) Fivefold magnification of panel C.
placed into the electron path of a Jeol 840 scanning electron microscope. Images at a magnification of ×500 were exposed on Polaroid type 55 film.

RESULTS

The PTFE-microorganism matrix formed easily on a standard rubber mill when a work-intensive calendering procedure was used. The permeability of the membrane was determined by the expression \( F = 1.52 - 0.280V \), where \( F \) is the flow rate in milliliters per minute and \( V \) is the total volume of substrate solution that has passed through the membrane (2, 3). This relationship was established by using water to permeate the membrane.

When the water was replaced by a nutrient solution such as tryptic soy broth, the flow rate decreased logarithmically according to the expression \( F = 0.176 - 0.213(V - 1.2) \), because of the faster rate of accumulation of particulate material (now in gel form) on the proximal side of the membrane. When this gel was removed from the membrane, the flow rate returned to that exhibited at \( V = 1.2 \) liters (i.e., just prior to the change from water to nutrient broth).

Membranes containing 85% (wt/wt) S. marcescens placed on the surface of a tryptic soy agar plate were incubated at 30°C. No color change was noted after 36 h at this temperature. A spreading red area was observed on the membrane after 48 h at 30°C (Fig. 1). The color spread until the entire membrane was red (approximately 6 days). The slow growth of the organism is likely attributable to the recovery period necessary for the organisms after the milling process and to the slow diffusion of nutrients from the agar into the membrane. Several red colonies were visible on the surface of the membrane after 48 h, the progeny of organisms situated at the surface of the membrane.

Membranes containing 85% (wt/wt) S. cerevisiae cells were placed onto tryptic soy agar plates and incubated at 30°C. Series of electron micrographs of the membrane immediately after construction (Fig. 2) and after 1 month of use (Fig. 3) are shown. The fibrils noted in the photographs are the PTFE fibrils, and intact yeast cells are abundant in the area. After 24 h at this temperature, yeast cells were detected spreading away from the cut edge of the membrane, and colonylike growth (small areas of butter-textured growth) was observed on the surface of the membrane. This indicated that the yeast cells had survived the milling process, as had the bacteria.

FIG. 3. Scanning electron micrographs of the yeast-filled microporous membrane after 1 month of use. The membrane was prepared as described in the text for scanning electron microscopy. (A) Yeast cells enmeshed in the membrane. (B) Cross section of freeze-fractured membrane. The large particles are 20-μm-diameter polystyrene beads picked up from the mill, where they had been deposited by previous experiments. (C) ×10 magnification of panel B. (D) ×3.3 magnification of panel C. Scale is denoted on each plate.
To confirm the above data, biochemical testing was done to determine conversion of specific substrates to known end products. Membranes containing S. cerevisiae cells permeated with a 1% (wt/vol) glucose solution showed production of ethanol, as determined by gas chromatography. The ethanol produced was proportional to the time of residence of the solution in the membrane: the slower the flow of the glucose solution through the membrane, the greater the conversion to ethanol (Fig. 4). The conversion was found to be independent of the total volume of glucose solution passed through the membrane.

The electron micrograph of the used membrane (Fig. 3) revealed the presence of a 20-μm-diameter polystyrene bead, picked up from the mill from a previous experiment. This bead serves as a good size reference. A membrane freshly constructed (with a looser "weave") is shown in Fig. 2. The membrane in Fig. 3 shows the cells isolated in compartments which were created by the PTFE fibrillation. The freeze-fractured edge and the surface of the membranes are shown in Fig. 2 and 3. These figures illustrate the isolation of cells into their own chambers. The porosity of the membranes varies with the amount of milling used to form the membrane.

Membranes composed of 15% PTFE and 85% P. aeruginosa organisms were tested for their ability to remove the pesticide 2,4,5-T from water. The membrane was treated in the same manner as the Serratia membrane. After a wash with 420 ml of distilled water in an Amicon TM ultrafiltration cell, 420 ml of a 21-μg/ml solution of 2,4,5-T was forced through the membrane at a rate of 8 ml/h. The time of residence of the solution in the membrane was 1.1 h. Gas chromatography of the starting material and the material which permeated the membrane revealed decreased levels of the pesticide. The removal of the pesticide was dependent on the time that the membrane was exposed to the pesticide: the longer the exposure, the less efficient the membrane was in removing the 2,4,5-T (Table 1). The cells in this membrane were not preconditioned to tolerate 2,4,5-T.

In the control experiments with Sephadex alone, no pesticide removal was noted at any time for a period of 1 week (data not shown). The flow rate of the pesticide-containing broth was kept constant for the duration of the experiments. The decreasing rate of depletion suggests that the cells were killed during the time the membrane was in use.

### DISCUSSION

Process development in biotechnology has inherent purification problems because cellular debris and other particulates must be separated from the processing effluent. Currently, methods such as ultrafiltration and centrifugation are used to accomplish this task. Some investigators have experimented in the past with immobilization of microorganisms in polysaccharide matrices, such as dextran, or in other polymers, such as carageenan or calcium alginate. These methods have inherent problems associated with the ability of the solutes to permeate the cell mass, the removal of the metabolic wastes and the recovery of the desired product. Entrapment of microorganisms into PTFE eliminates these problems because the cells are trapped into a porous fibril Teflon matrix. Solutes can be easily delivered to the cells, wastes can be efficiently removed, and the desired product can be more easily purified because the step to remove cells and debris is eliminated.

We have demonstrated that bacteria and yeast cells are able to survive a harsh manufacturing process and are able to perform bioconversions while entrapped in a Teflon membrane. The viability of the S. marcescens membrane demonstrated the survivability of microorganisms in the membrane-forming process. The conversion of glucose by S. cerevisiae and the elimination of the pesticide 2,4,5-T by P. aeruginosa demonstrate but two uses of these types of membranes. The membranes may be made thicker, increasing the number of cells available for bioprocessing. It is necessary to determine empirically the optimal flow rate for conversion of substrate, since efficient conversion is dependent on the time of residence of the substrate in the membrane and on other factors, such as nutrient and oxygen concentrations.

### REFERENCES


### TABLE 1. Removal of the pesticide 2,4,5-T by PTFE membranes filled with P. aeruginosa and Sephadex R

<table>
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<th>No. of days membrane was in use</th>
<th>% Removal of 2,4,5-T</th>
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FIG. 4. Ethanol production by PTFE-entrapped S. cerevisiae cells. The experimental conditions are described in the text. As the flow rate of a buffered glucose solution through the membrane decreases, the rate of ethanol production increases linearly. The longer time of residence of the glucose solution in the membrane allows more of the sugar to be metabolized.