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COMMUNICATION

Simultaneous Bioreaction and Separation by an Immobilized Yeast Membrane Reactor

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INTRODUCTION

The need for immobilized whole cell reactor systems is widely recognized. Many reactor configurations have been tried using one or more semipermeable membranes to perform bioreaction and separation simultaneously (*1-3*). The major problems faced by such systems are high diffusional resistances, substrate depletion, and product inhibition. Over the past few years many membrane reactors have been proposed which address these problems individually (*4, 5*).

We have approached the problem with a twin objective 1) to overcome substrate and product diffusional resistances by forcing the nutrient solution through the cell mass and 2) to simultaneously separate product from the reaction mixture to reduce product inhibition.

MEMBRANE "SANDWICH" REACTOR CONCEPT

The biocatalyst is "sandwiched" between an ultrafiltration (UF) membrane and a reverse osmosis (RO) membrane as shown in Fig. 1. The UF membrane provides free passage for all nutrients to the cell mass

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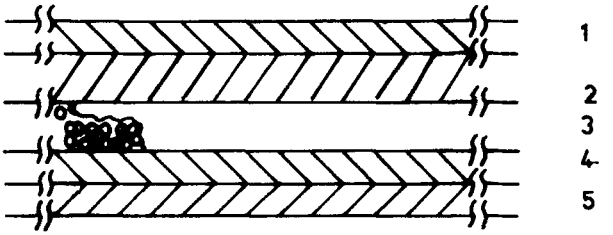

FIG. 1. Membrane "sandwich." (1) UF membrane. (2) Coarse filter paper (>10 μm). (3) Cell mass. (4) Fine (microporous) filter. (5) RO membrane.

below. The RO membrane, while immobilizing the cells, also helps in separating the product from the reaction mixture. The feed is forced through under pressure, from above, which overcomes the diffusional resistance present in previous configurations. The RO membrane chosen allows product to pass through, preferentially, which improves product purity and concentration (*6*). An additional advantage is that substrate uptake becomes more efficient. The cell layer thickness can be minimized in this configuration to prevent diffusion gradients in the cell mass due to substrate depletion. The permeate disengages at the bottom surface of the RO membrane and is collected below.

MATERIALS AND METHODS

The model cell system chosen is *Saccharomyces cerevisiae* for which extensive immobilization studies have been done (*7*).

A *Saccharomyces cerevisiae* ATCC 4126 culture was maintained on YM Agar (DIFCO 0712) slants at 4°C. Inoculum was grown aerobically in a shake flask by transferring a loopful of cells to 35 mL of YM Broth (DIFCO 0711) of pH 5.0 at 30°C. The mixture was agitated in a rotary shaker at 200 rpm for 18–24 h. A measured volume of inoculum (from 35 to 60 mL) of known cell concentration (in the exponential phase, around 10^7 cells per mL) was filtered through a 0.2-μm microfilter (MSI Magna Nylon 66 Membrane filters) to present a compact cell mass of known concentration for immobilization. The feed solution contained glucose (8–12 wt %), yeast extract (0.15 g), NH_4Cl (0.25 g), K_2HPO_4 (0.55 g), citric acid (0.30 g), NaCl (0.10 g), $MgSO_4 \cdot 7H_2O$ (0.025 g), and $CaCl_2 \cdot 2H_2O$ (0.15 g). Glucose was added from a 20% (w/v) stock solution and the amount of water added was based on the glucose concentration required in the feed solution. Nitrogen (from Matheson, extra dry, 99.9% purity)

was used to apply pressure and provide an anaerobic atmosphere in the reactor.

The cellulose acetate membranes used (UF and RO membranes) were prepared in the laboratory according to a previously described procedure in which the composition of the casting dope used was coded as Batch 316 (*8a*). Pore sizes in the UF membranes were controlled by the ethanol content in the gelation media while those of the RO membrane were controlled by the shrinkage temperature.

Glucose concentrations were measured using a glucose analyzer (YSI Model 27 Industrial Analyzer). Ethanol concentrations were measured using a gas chromatograph (Hewlett Packard 5880A series) equipped with a 1.82-m length glass column filled with 5% Carbowax 20 M and 80/120 Carbopak B-AW.

Cell concentration was measured using a hemocytometer (grid volume 0.001 mL) by counting under a microscope. Before measurement, samples were diluted to keep the concentration around 2.5×10^6 cells per mL. After mounting, samples were stained with methylene blue and observed at 400× magnification. Cells which appeared blue were considered inactive. At the end of each experiment the "sandwich" was transferred to a 200-mL physiological saline solution and agitated at 30°C and 200 rpm in a rotary shaker for 30 min to disperse the cell mass. Cells were then counted by the same procedure described above. Viable cell numbers were counted using a viable plate count. After diluting the saline mixture (1000 times), 0.3–1 mL of the solution was mixed with 40–50 mL growth agar (at 30–37°C) and spread over Petri dishes. After incubation at room temperature for 24–48 h, the cell colonies were counted.

All sterilization was done in a steam sterilizer (Castle 3120 from Sybron Medical Products Division) at conditions recommended by the manufacturer.

REACTOR DESCRIPTION AND OPERATION

A schematic of the bioreactor system is given in Fig. 2. The reactor consists of a stainless steel 316 pipe with a threaded flange at one end. This flange is bolted to another flange which has a radial bore to withdraw permeate. The microporous filter with *Saccharomyces cerevisiae* cells is sandwiched between the UF and RO membranes and then clamped between the two flanges. Two neoprene rubber O-rings seal the feed from the permeate and prevent external leaks. A port is provided at the top as an inlet for the inert gas which is used to maintain feed

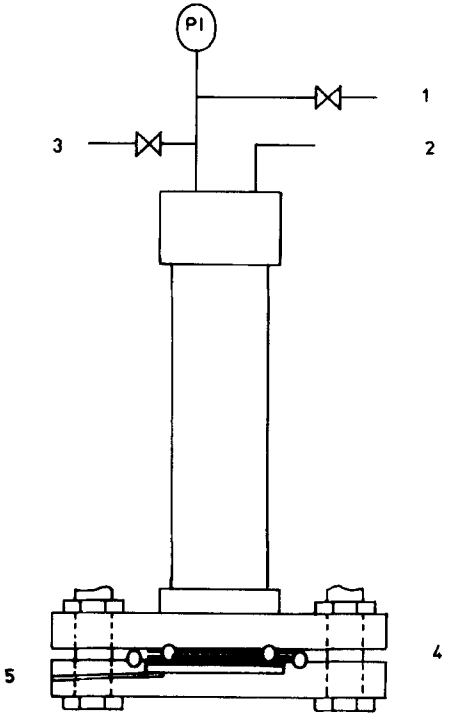

FIG. 2. Membrane reactor schematic. (1) Inert gas inlet. (2) Feed port. (3) Bleed port. (4) Membrane "sandwich" (see Fig. 1). (5) Permeate collection port.

pressure and an anaerobic atmosphere. A pressure gauge and bleed valve are also provided for pressure measurement and an initial purge, respectively.

After the "sandwich" has been placed between the flanges, the reactor is assembled and glucose solution is filled in the feed chamber. The reactor is purged from the top port with nitrogen for 15 min to remove all air and is then pressurized to the required operating pressure. Samples are collected periodically and analyzed for glucose and ethanol concentrations. Temperature of operation is room temperature and all samples are stored at 4°C before analysis. At the end of each run, feed volume is measured and a sample is removed for analysis. The reactor is opened and cell concentration is measured including the number of dead, viable, and nonviable cells.

RESULTS

In this experiment, which is representative of other runs, the UF membrane was prepared from casting dope with an evaporation time of 60 s and a gelation time of 60 min in 50% (v/v) ethanol solution in water at 0°C. The RO membrane was prepared with the same evaporation time of 60 s, and gelation was in pure water at 0°C for 60 min following which it was shrunk in water for 10 min at 72°C. The latter membrane is known to be effective for the fractionation of ethanol and glucose solutes in aqueous solution at an operating pressure of 6900 kPag (*6*).

The results of a typical experimental run are shown in Fig. 3. The conditions of this experiment were 825 kPag operating pressure, 125 g feed solution (glucose concentration 12.27% by wt) and initial cell concentration of 1.8×10^8 cells in the "sandwich." There is a sharp maximum observed in the permeate glucose concentration. The sharp drop is normally followed by a much slower but steady decline in concentration until the end of an experiment when a slight rise is sometimes observed. The ethanol concentration in the permeate always begins at modest values and increases steadily, as can be seen in the same figure. Concentrations as high as 7.5% (by wt) have been achieved. At the end of the run (which is about 160 h), cell numbers rise by a small factor of only 2–3. The observed permeation rate is reasonably constant. At lower permeation rates, oscillatory trends in both concentrations have been observed.

DISCUSSION

The above results indicate that a considerable amount of glucose passes through the RO membrane. The initial maximum of glucose concentration observed in the experiment was probably due to two reasons. First, a lag was present due to the sudden change in environment for the organism in terms of mechanism of metabolism, nutrient media, and cell concentration (the sudden crowding on transfer to the microporous filter) and second, the fact that the membranes are not prepressure treated before their use in the experiment and hence they will not separate effectively until an equilibrium pore size is reached (*8b*). Once the cells switch to the anaerobic growth pattern the glucose consumption increases rapidly till all cells perform the metabolic switch. The sudden changes in environment may also result in synchronous growth of cells in the initial stages of growth. The high cell density also reduces the growth rate drastically due to cell inhibition. The combined

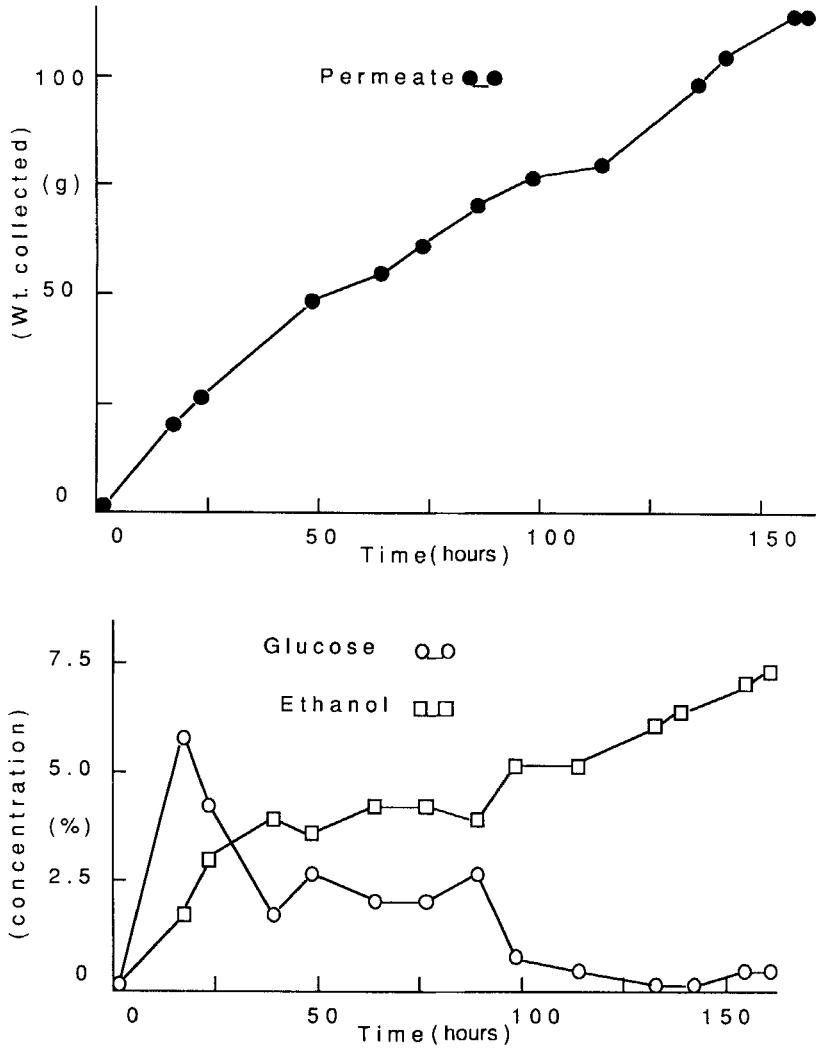

FIG. 3. Results of a typical run.

effect of all these phenomena may lead to the oscillatory pattern observed in the experimental data.

CONCLUSION

The concept of a membrane "sandwich" reactor is presented where nutrient was forced through the "sandwich" to reduce mass transfer problems. A special membrane was used to simultaneously separate product from the reaction mixture. The results from the experiment to convert glucose to ethanol using *Saccharomyces cerevisiae* as biocatalyst demonstrate that the concept is workable. At present, further studies are being conducted to have a better understanding of the interaction of membrane transport, bioreaction kinetics, and cell growth kinetics on the performance of the reactor system.

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