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Recovery of 2,3-Butanediol by Vacuum Membrane Distillation*

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ABSTRACT

A vacuum membrane distillation process using a microporous polytetrafluoroethylene (PTFE) membrane was developed to concentrate 2,3-butanediol from model 2,3-butanediol solutions and fermentation broths. A 0.22-μm PTFE membrane passed water vapor while retaining 2,3-butanediol. Water flux through the membrane was 4 kg/m^2·h at 35°C and 12–14 kg/m^2·h at 55°C. Medium components, especially yeast extract and fermentation broth, reduced flux as compared to model solutions. Using this process, butanediol was concentrated from 40 g/L to about 650 g/L in the model solution, and when integrated to a fed-batch reactor, 2,3-butanediol was concentrated to over 430 g/L.

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Key Words. 2,3-Butanediol; Vacuum membrane distillation; Fermentation; Recovery

INTRODUCTION

2,3-Butanediol is an industrially important chemical with numerous applications as an ingredient in high octane aviation fuel, printing inks, lacquers, synthetic perfumes, synthetic rubber, drugs and pharmaceuticals, fumigants, emulsifying agents, and softening and moistening agents (1–4). Dehydration of 2,3-butanediol gives 1,3-butadiene which is used to make synthetic rubber. Dimerization of butandiene by the Diels–Alder reaction produces styrene, an important aromatic intermediate (5).

2,3-Butanediol is produced by *Klebsiella oxytoca* at concentrations of 100 g/L (6). Because 2,3-butanediol has a boiling point of 180°C, it is recovered from the bottoms of the distillation columns along with the other nonvolitile compounds. Recently, 2,3-butanediol was recovered from the *Klebsiella oxytaca* fermentation by salting-out using dehydrated K_2CO_3 to form a 2,3-butanediol phase and a salt phase (1). It was possible to separate 94% to 96% (w/w) 2,3-butanediol using 53–56% (w/w) K_2CO_3.

The recovery of fermentation products has also been accomplished by pervaporation, membrane distillation (MD), and vacuum membrane distillation (VMD). Pervaporation is the selective diffusion of a component through a nonporous membrane. The species of interest solubilizes into the membrane and then diffuses through the membrane due to a concentration gradient which is generated by either a vacuum or sweep gas (7). MD is a process of evaporation through a porous hydrophobic membrane which is not wetted by the aqueous mixture. The membrane is strictly a support for the liquid–vapor interface which is located at the entrance of the pore. The transport of vapor is accomplished by a temperature gradient between the membrane and the condensing surface. e.g., 30 and 15°C, respectively. The pressure on both sides of the membrane is maintained at the equilibrium pressure, and transport of vapor through the membrane is by diffusion. MD has been used for the removal of ethanol from a fermentation broth (8).

VMD is a combination of membrane distillation and pervaporation. VMD uses a microporous hydrophobic membrane to separate an aqueous phase from the permeate which is kept under vacuum. The operational unit for VMD is identical to a pervaporation system, though the mode of transport is significantly different. In VMD the aqueous phase is in direct contact with the membrane at a feed pressure that is less than that required to wet the pores. The membrane is able to retain the liquid at the surface

of the membrane, and a vapor–liquid interface develops at the pore entrance (9). Because of the pressure drop across the liquid–vapor interface and the low pressure in the vapor phase, the primary mechanism for transport through the pores is Knudsen diffusion. The molecular mean free path of the gas-phase molecules is larger than the pore size of the membrane. In the case of water at 35°C at 66 mbar, the mean free path is 2.06 μm, which is nearly an order of magnitude greater than the membrane pore diameter of 0.22 μm.

VMD has been investigated for the removal of ethanol from fermentation broths (10–12) and treatment of wastewater to remove organic contaminants (13). The objectives of this work were to develop a vacuum membrane distillation process to recover and concentrate 2,3-butanediol from model solutions, to investigate the effect of medium components and fermentation broth on membrane performance, and to integrate the membrane process to a fed-batch reactor to concentrated 2,3-butanediol.

MATERIALS AND METHODS

Organism, Media, and Growth Conditions

Klebsiella oxytoca ATCC 8724 was obtained from the American Type Culture Collection (Rockville, Maryland). Cells were propagated by weekly transfer in Medium I. Media I and II (Table 1) were used to prepare inocula for fermentation studies and for production of 2,3-butanediol, respectively. For inocula preparation, the glucose level was 30 g/L. Inocula were prepared by transfer of 10 mL of 24 hour cells into 100 mL Medium I. Cells were incubated at 35°C for 20–24 hours in a shaker incubator (175–200 rpm), then used to inoculate the bioreactor. Medium I was sterilized at 121°C for 15 minutes, and Medium II was sterilized using a 0.45-μm sterile filter. For fouling studies, the medium was not sterilized. Fed-batch experiments were performed with Medium II minus 2,3-butanediol. All tubing and air filters were sterilized at 121°C for 15 minutes. 2,3-Butanediol model solutions with specified 2,3-butanediol concentrations were made in distilled water and adjusted to 5.4 with either 2 N NaOH or 2 N H_2SO_4. The concentration of 2,3-butanediol in the model solutions varied from 40 g/L to over 500 g/L.

Membrane and Bioreactor Operation

A 0.22-μm polytetrafluoroethylene (PTFE) membrane was used to recover 2,3-butanediol from model solutions and fermentation broth. A schematic of the process is shown in Fig. 1 with the membrane module and membrane as described previously (14). Modifications to the mem-

TABLE 1
Compositions of Media

Components	g/L	Vendor
Medium I:		
Glucose	100	Sigma Chemicals, St. Louis, MO
Yeast extract	5	Difco Laboratories, Detroit, MI
Tryptone	5	Difco Laboratories, St. Louis, MO
K_2HPO_4	1	Mallinckrodt, Paris, KY
2,3-Butanediol	40	Aldrich, Milwaukee, WI
Medium II:		
Glucose	100	Sigma Chemicals, St. Louis, MO
$K_2HPO_4 \cdot 3H_2O$	13.7	Mallinckrodt, Paris, KY
KH_2PO_4	2.0	Aldrich, Milwaukee, WI
$(NH_4)_2HPO_4$	3.3	Sigma Chemicals, St. Louis, MO
$(NH_4)_2\ SO_4$	6.6	E. M. Science, Cherry Hill, NJ
$MgSO_4 \cdot 7H_2O$	0.25	Fisher Scientific, Fairlawn, NJ
$FeSO_4 \cdot 7H_2O$	0.05	Sigma Chemicals, St. Louis, MO
$ZnSO_4 \cdot 7H_2O$	0.001	Allied Chemicals, Morristown, NJ
$MnSO_4 \cdot 7H_2O$	0.001	Sigma Chemicals, St. Louis, MO
$CaCl_2 \cdot 2H_2O$	0.01	Sigma Chemicals, St. Louis, MO
EDTA	0.05	Sigma Chemicals, St. Louis, MO
2,3-Butanediol	40	Aldrich, Milwaukee, WI

brane system included replacing the 1/8″ PEEK tubing with 19 mm stainless steel vacuum tubing (vacuum side) and using liquid nitrogen to trap the condensate. The system was recirculated at 2.8 L/min, the backside membrane pressure was 6–9 torr, and the inlet and outlet retentate pressures were 6 and 4 psig, respectively.

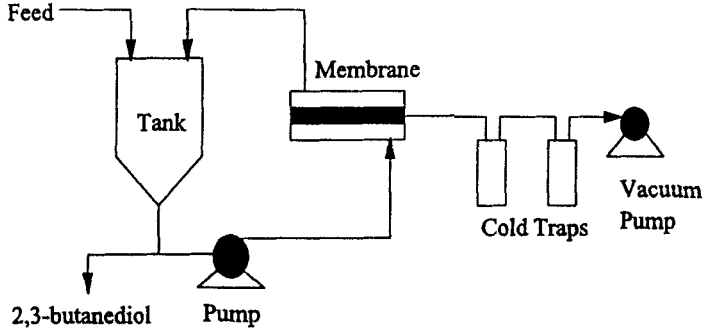

FIG. 1 A schematic diagram of the vacuum membrane distillation system for butanediol recovery.

A schematic of the integrated bioreactor and product recovery system is shown in Fig. 2. Cells were recycled to the bioreactor using a 500,000 MW cutoff hollow filber membrane (Model UFP-500-E-5A, A/G Technology, Needham, Massachusetts) and 2,3-butanediol was recovered from the permeate using the PTFE membrane. The hollow fiber module was sterilized at 121°C for 30 minutes and then cooled to 30°C before being attached to the reactor. A monostate parastaltic pump (Varistaltic Pump, New York, New York) was used to circulate the fermentation broth through the hollow fiber module.

Batch Fermentation

A 2-L Multigen fermentor (New Brunswick Scientific Co., Edison, New Jersey) was inoculated with 7–10% of the original volume, maintained at 37°C, and aeration was set at 0.5 vvm. The total volume in the reactor was 1,400 mL. The initial glucose concentration was 100 g/L, and the pH was maintained at 5.4 with 2 N NaOH using a NBS Model 4000 pH controller. A condensor was attached to the top of the reactor to condense any water/solvent vapors and fitted with a 0.45-μm sterile vent filter. Silicone-based antifoam (Sigma Chemical Co, St. Louis, Missouri) was used to control foaming. Two milliliter samples were removed at intervals, cooled to 2°C, centrifuged to remove cells, and frozen at 20°C for later analysis.

Fed-Batch Fermentation

The fermentation was initiated in a batch mode using Medium II. After 39 hours the glucose level reached 1.6 g/L, and a 500k ultrafiltration membrane was used to reduce the reactor volume to 450 mL and recycle the cells. The reactor was operated in a fed-batch mode until the reactor volume increased to 1180 mL (86 g/L of 2,3-butanediol), at which time the volume was reduced to 520 mL. The fed-batch fermentation continued to a final volume of 830 mL (98 g/L of 2,3-butanediol) and was then terminated. The feed contained concentrated glucose (267 g/L) and minerals (Medium II), and the flow rate was varied to maintain a glucose concentration of approximately 3 g/L. The permeate from each step was pooled and used for the 2,3-butanediol membrane recovery step.

Analyses

The dry weight concentration of cells was determined by correlating the optical density at 600 nm (Beckman DU-70; Beckman Instrument Inc, Fullerton, California) with cells dried overnight at 105°C. Glucose was measured using a glucose analyzer (YSI Model 27, Yellow Springs Inc.,

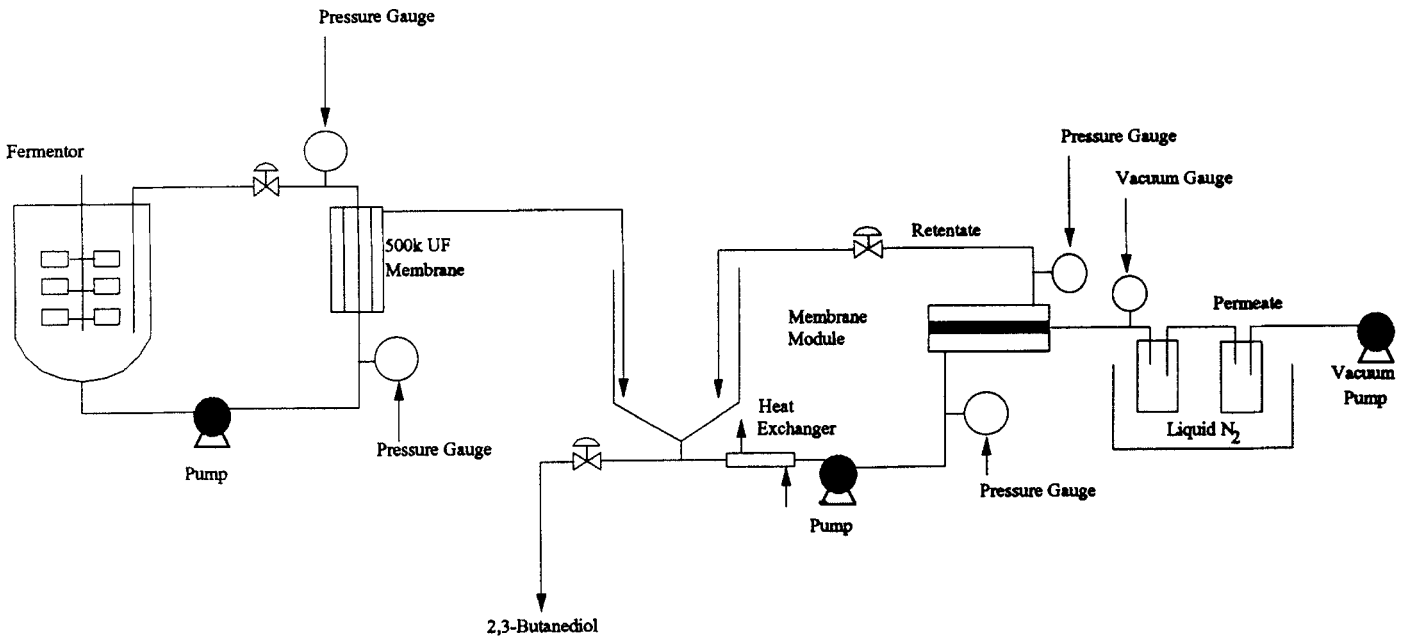

FIG. 2 An integrated process for production of 2,3-butanediol in a fed-batch reactor and recovery by vacuum membrane distillation.

Yellow Springs, Ohio). Fermentation end products were measured using a Hewlett Packard 5880A gas chromatograph equipped with a flame ionization detector and a 2 m by 2 mm glass column packed with Chromosorb W [(AW: 80/100)/10% carbowax 20M-TPA + 0.1% H_3PO_4] (Supelco, Inc., Bellefonte, Pennsylvania). Injection and column temperatures were 220 and 210°C, respectively. The FID detector temperature was 230°C and the nitrogen carrier gas flow rate was 25–30 mL/min.

2,3-Butanediol selectivity was defined as $x = [y/(1 - y)]/[x/(1 - x)]$, where x and y are weight fractions of the 2,3-butanediol in the reactor and condensate, respectively. Selectivity is a measure of the preferential removal of 2,3-butanediol. Flux was defined as the weight of the desired component/m^2 membrane area·h.

RESULTS AND DISCUSSION

The focus of this work was to investigate the recovery of 2,3-butanediol by VMD using a 0.22-μm PTFE membrane. The hydrophobicity of the membrane allows vapors to move through the pores while the liquid is unable to penetrate the pores. 2,3-Butanediol has a boiling point of 180°C and is primarily retained behind the membrane, while in this case the more volitile components, i.e., water, ethanol, and acetic acid, are transported across the membrane as a vapor. The flux of the 0.22-μm PTFE membrane was characterized with water, model solutions of 2,3-butanediol/water, 2,3-butanediol/fermentation media, and fermentation broth.

Factors Affecting Water Flux

The water flux of the 0.22-μm PTFE membrane at 35°C was constant at 4 kg/m^2·h over a 4-hour period. When a 40-g/L 2,3-butanediol solution was recirculated under the same conditions, the 2,3-butanediol concentration in the retentate increased from 40 to 72 g/L, while the flux was constant at 4 kg/m^2·h (Fig. 3a). The flux of 2,3-butanediol through the membrane was approximately 5–8 g/m^2·h and the concentration of butanediol in the permeate was 2–3 g/L, indicating a 2,3-butanediol selectivity of 0.05.

The effect of temperature on water flux and 2,3-butanediol flux and selectivity at 40 and 55°C was investigated at an initial 2,3-butanediol concentration of 40 g/L. At 40°C the flux was constant at 5.8 kg/m^2·h, while the 2,3-butanediol flux was 10 to 12 g/m^2·h over a 2-fold increase in 2,3-butanediol concentration (Fig. 3b). The concentration of 2,3-butanediol in the permeate varied from 2 to 3 g/L. At 55°C the water flux increased to 13–14 kg/m^2·h and the 2,3-butanediol flux fluctuated between

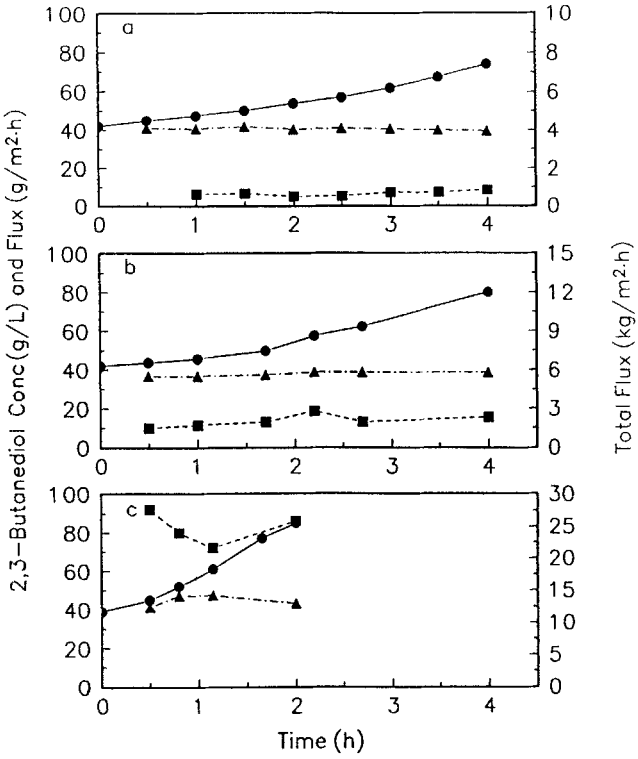

FIG. 3 Concentration of 2,3-butanediol from a model solution using a PTFE membrane at (a) 35°C, (b) 40°C, and (c) 55°C. 2,3-Butanediol retentate concentration (●), 2,3-butanediol flux (■), and flux (▲).

72 to 92 g/m^2·h with no apparent trend (Fig. 3c). The concentration of 2,3-butanediol in the permeate was initially 3.7 g/L and increased to 6.6 g/L at the end of the experiment.

Effect of Media and Medium Components on Flux and Selectivity

The media components yeast extract (5 g/L), glucose (100 g/L), and 2,3-butanediol (40 g/L) caused the flux to drop by 25% from 4 to 3 kg/m^2·h, while 2,3-butanediol flux remained constant at 6 g/m^2·h (Fig. 4a). Medium I components had a similar effect (Fig. 4b). Permeate concentrations and selectivities of 2,3-butanediol for the two fermentation media ranged from 1.1 to 2 g/L and 0.02 to 0.05, respectively.

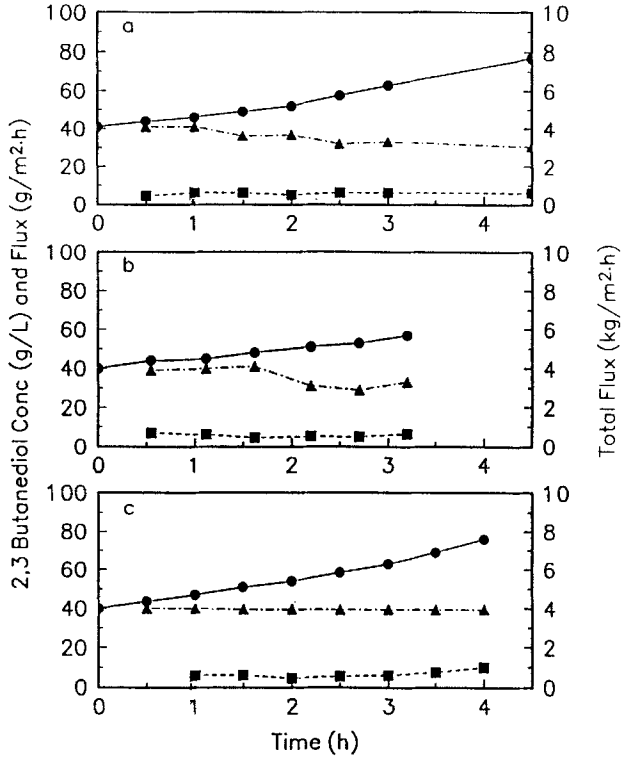

FIG. 4 Concentration of 2,3-butanediol from fermentation broths (a) 5 g/L yeast extract, 100 g/L glucose, and 40 g/L 2,3-butanediol; (b) Medium I; and (c) Medium II at 35°C. Symbols as Fig. 3.

A defined medium (II) based on Jansen et al. (15) was investigated. The flux was constant throughout the run at 3.8 kg/m^2·h, and 2,3-butanediol flux, concentration, and selectivity were similar to the 2,3-butanediol/water system (Fig. 4c). The results from these experiments indicated that the yeast extract and tryptone affected flux. Included in the defined media was glucose (100 g/L). A high final glucose concentration would not be typical of a completed 2,3-butanediol fermentation, but it was included in the media as a worst-case scenario. It is interesting to note that the glucose concentration was the same for all three media, but the synthetic media did not effect flux, indicating that a high glucose concentration was not effecting flux. Because of these results, the synthetic media was used during the fermentation studies.

A series of experiments were performed to determine the maximum 2,3-butanediol concentration that could be achieved with a 2,3-butanediol/water solution. Over six experiments, using the same membrane, the butanediol concentration was increased to 651 g/L (Fig. 5). The experiment was performed in steps because of the large concentration factor, the small membrane surface area of the system, and the minimum system hold-up volume (300 mL). The beginning and end of each experiment is indicated in Fig. 5. At the completion of a step the 2,3-butanediol concentration in the retentate was measured. The retentate reservoir was then filled with 2,3-butanediol to the concentration at the end of the previous experiment, and the experiment was resumed.

The flux decreased as the concentration of 2,3-butanediol increased in the retentate and fluctuated significantly above a 2,3-butanediol retentate concentration of 250 g/L. As the 2,3-butanediol concentration in the retentate increased to 500 g/L, the flux of 2,3-butanediol increased from 10–20 to 200–400 g/m^2·h, which corresponded to 170–227 g/L of 2,3-butanediol in the permeate. At this point the average pressure on the retentate side increased from 5 to 20 psig.

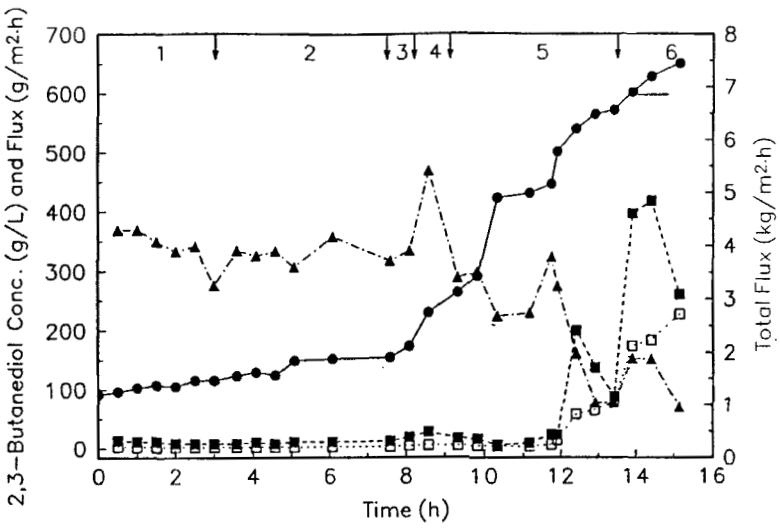

FIG. 5 Concentration of 2,3-butanediol in water at 35°C. 2,3-Butanediol permeate concentration (□) and other symbols as in Fig. 3. The arrows indicate the beginning and end of an experiment.

2,3-Butanediol Fermentations with Medium II

Batch fermentation were run for 45 hours; fermentation results are listed in Table 2. Acetoin and ethanol were also produced in addition to 2,3-butanediol. The initial glucose concentration was 100 g/L, which was completely consumed during the fermentation, resulting in production of 46 g/L of total solvents with a productivity of 1 g/L·h. The fermentation broth, including cells, was concentrated using the PTFE membrane (Fig. 6). The 2,3-butanediol concentration increased from 41 to 88 g/L and the total flux decreased from 4.2 to 3.6 kg/m^2·h in 4.5 hours. As expected, ethanol rapidly passed through the membrane; however, the flux of 2,3-butanediol and acetoin was very low.

A fed-batch system was used to increase the 2,3-butanediol concentration in the fermentor to 100 g/L; results are summarized in Table 3. Liquid level in the reactor, feed rate, and cell concentration versus time are presented in Fig. 7 (top). Fluctuations in the cell concentration were due to the dilution effect of the feed medium. A small bleed was maintained in the later part of the run to maintain a cell concentration of about 14 g/L. Product concentration in the fermentor increased with time and, except for 2,3-butanediol, the end products stayed below 10 g/L (Fig. 7, bottom).

At the completion of the fed-batch fermentation, the 2,3-butanediol was concentrated to 430 g/L. The concentration was performed in two stages. The first stage lasted 16 hours with an initial flux of 4 kg/m^2·h and a final flux of 1 kg/m^2·h. The 2,3-butanediol concentration increased from 40 to 200 g/L in the retentate and 2 to 7 g/L in the permeate (Fig. 8a). The

TABLE 2
Fermentation Parameters of 2,3-Butanediol Production in a Batch Reactor

Parameter	Yield
2,3-Butanediol [g/L]	42.6
Ethanol [g/L]	1.2
Acetoin [g/L]	2.0
Acetic acid [g/L]	Trace
Lactic acid [g/L]	0.0
Cells [g/L]	3.0
Total solvents [g/L]	45.7
Solvent yield [g/g]	0.46
Solvent productivity [g/L·h]	1.02
Glucose utilized [g/L]	100

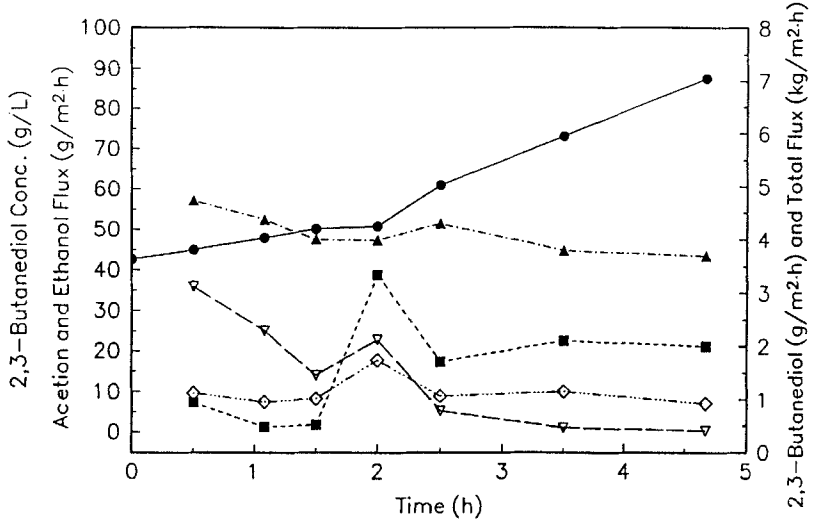

FIG. 6 Concentration of 2,3-butanediol from a batch fermentation. Acetoin flux (◇), ethanol flux (▽), and other symbols as in Fig. 3.

experiment was discontinued at this point and restarted with a new membrane. The initial flux of the new membrane was 5.5 kg/m^2·h which decreased to just over 1 kg/m^2·h in 6 hours as the 2,3-butanediol concentration increased from 200 g/L to 430 g/L. The 2,3-butanediol flux increased dramatically with the new membrane from 10 to 47 g/m^2·h. This sudden increase in 2,3-butanediol flux was attributed to the high overall flux of the new membrane. The permeate concentration of 2,3-butanediol at the completion of the first stage was 7.53 g/L. After the new membrane was installed, the permeate concentration after 15.6 hours of operation was 8.00 g/L which increased to 32 g/L after 17.1 hours.

The flux and permeate concentration of the two by-products ethanol and acetion are presented in Fig. 8(b). As expected, the ethanol flux was high, but it decreased significantly within 7 hours. The acetoin (bp 148°C) flux was initially 27 g/m^2·h and was maintained at a level of 10–15 g/m^2·h through the duration of the experiment until the new membrane was installed, at which time it increased. The permeate concentration was consistantly 4 to 6 g/L for both membranes (Fig. 8b).

Comparing the membrane performance for the water/2,3-butanediol mixture to the fermentation broth, the fermentation broth experienced a steady decline from 4 to 2 kg/m^2·h as the 2,3-butanediol retentate concentration increased from 40 to 150 g/L, while the total flux for the model

TABLE 3
Fermentation Parameters of 2,3-Butanediol Production in a Fed-Batch Reactor

Parameter	Yield
2,3-Butanediol [g]	171.2
Ethanol [g]	6.3
Acetoin [g]	18.0
Acetic acid [g]	11.6
Total solvents [g]	195.5
Total glucose utilized [g]	362.5
% Glucose utilization	99.0
Total solvent yield [g/g]	0.54
2,3-Butanediol yield [g/g]	0.47
Solvent productivity [g/L·h]	1.60
2,3-Butanediol productivity [g/L·h]	1.40

solution remained at about 3.8 kg/m^2·h over the same concentration range. As the concentration of 2,3-butanediol in the retentate increased, the overall flux for both cases decreased, though much more dramatically for the fermentation broth. When a new membrane was installed after 16 hours of the fermentation broth run, the flux rate decreased faster than with the first membrane. The membrane flux increased dramatically after a new membrane was installed, indicating that the first membrane was fouled by the fermentation broth. The actual mechanism for fouling is unknown at this time.

The threshold retentate concentration at which the 2,3-butanediol flux dramatically increased is significantly different between the model solution and the fermentation broth. For the model solution the overall flux rate was 3 to 4 kg/m^2·h and the 2,3-butanediol flux rate was 20 to 25 g/m^2·h at a 2,3-butanediol retentate concentration up to 430 g/L. Once the retentate concentration increased to 500 g/L, the 2,3-butanediol flux rate increased, i.e., 170 g/m^2·h. The 2,3-butanediol concentration in the permeate increased dramatically to 60 g/L. As the retentate concentration increased to 650 g/L, the permeate concentration increased to 227 g/L. The overall flux at the end of the run was 0.97 kg/m^2·h, and the 2,3-butanediol flux was 0.26 kg/m^2·h or 27% of the total flux. Though the 2,3-butanediol flux increased significantly, it did not appear that the membrane had wetted for several reasons. First, if the membrane wetted, a sudden increase in overall flux rate should have occurred, which was not indicated by the flux curve; and second, the concentration of 2,3-butanediol in the permeate was still less than the retentate concentration.

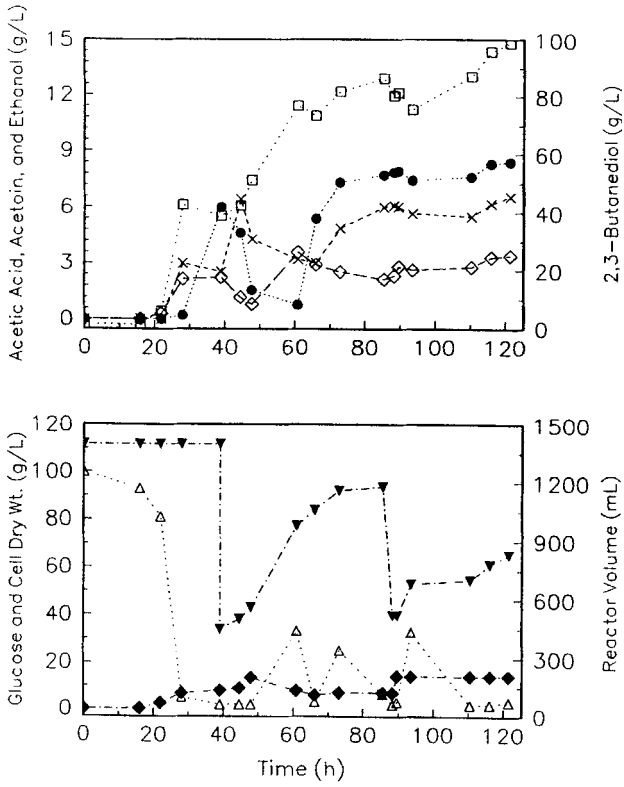

FIG. 7 Fed-batch fermentation of *Klebsiella oxytoca* ATCC 8724 using Medium III. Acetic acid (×), acetoin (●), ethanol (◇), 2,3-butanediol (□), reactor volume (▼), glucose (△), and cell dry weight (◆).

At this time it is difficult to explain why 2,3-butanediol "breaks through" the membrane at a lower retentate concentration for the fermentation broth as compared to the water/2,3-butanediol system. Investigations are ongoing to determine what is causing this phenomenon.

CONCLUSIONS

A 0.22-μm PTFE membrane was used to concentrate 2,3-butanediol from model solutions and fermentation broth. 2,3-Butanediol has a boiling point of 180°C and was primarily retained by the membrane when volatile compounds, such as ethanol and water, were passed through. 2,3-Butanediol was concentrated to a level of 650 g/L from a water/2,3-butanediol

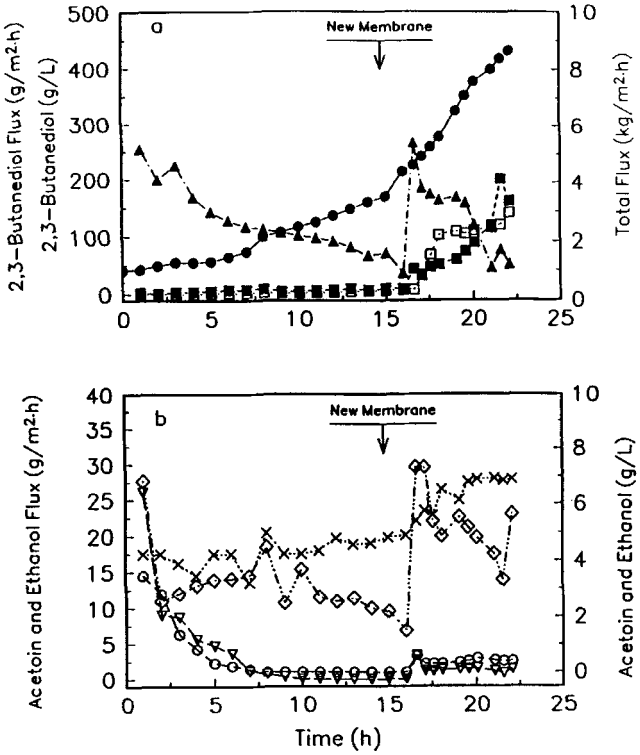

FIG. 8 Concentration of 2,3-butanediol from the fed-batch fermentation. Plot (a): 2,3-Butanediol concentration (●), 2,3-butanediol flux (■), flux (▲), and 2,3-butanediol in the permeate (□). Plot (b): Acetoin flux (◇), ethanol flux (▽), acetoin (×), and ethanol (○).

solution, and to 430 g/L from a fermentation broth; however, water flux decreased rapidly at these higher 2,3-butanediol concentrations. The 2,3-butanediol flux rate increased from 25 to 170 g/m^2·h at a retentate concentration of 500 g/L for the water/2,3-butanediol system, while the selectivities of 2,3-butanediol were very low. The fermentation broth apparently fouled the membrane although this phenomenon was not investigated further and is a subject for further study.

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