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Evaluation of PTMSP Membranes in Achieving Enhanced Ethanol Removal from Fermentations by Pervaporation

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ABSTRACT

The use of membrane processes for the recovery of fermentation products has been gaining increased acceptance in recent years. Pervaporation has been studied in the past as a process for simultaneous fermentation and recovery of volatile products such as ethanol and butanol. However, membrane fouling and low permeate fluxes have imposed limitations on the effectiveness of the process. In this study, we characterize the performance of a substituted polyacetylene membrane, poly[(1-trimethylsilyl)-1-propyne] (PTMSP), in the recovery of ethanol from aqueous mixtures and fermentation broths. Pervaporation using PTMSP membranes shows a distinct advantage over conventional poly(dimethyl siloxane) (PDMS) membranes in ethanol removal. The flux with PTMSP is about threefold higher and the concentration factor is about twofold higher than the corresponding performance achieved with PDMS under similar conditions. The performance of PTMSP with fermentation broths shows a reduction in both flux and concentration factor relative to ethanol–water mixtures. However, the PTMSP membranes indicate initial promise of increased fouling resistance in operation with cell-containing fermentation broths.

Index Entries: Pervaporation; PTMSP; PDMS; ethanol recovery; membrane separations.

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INTRODUCTION

Importance of *In Situ* Product Removal

Fermentations often exhibit strong product inhibition, especially fermentations for the production of alcohols and organic acids *(1–3)*. In batch or fed-batch fermentations, product inhibition limits the amount of substrate that can be efficiently fermented. If high substrate loadings are used, the product accumulates to sufficiently high levels so that the process becomes inhibited. Product inhibition is typically manifested by incomplete substrate utilization, reduced fermentor productivity and low process yield. In continuous fermentations, the impact of product inhibition is more severe than in batch processes because the fermentative micro-organisms are constantly exposed to the final (effluent) product levels. In cases where fermentation processes are limited by product inhibition, simultaneous product removal to maintain product concentrations at lower, less inhibitory conditions can improve the process. For example, continuous product removal has been shown to increase fermentation productivity in ethanol and butanol fermentations *(3–10)* and in lactic acid fermentations *(3,11)*.

Simultaneous product removal is increasingly being pursued as a promising technology in cases where product inhibition is manifest *(12–14)*. In a fermentation process, *in situ* product removal involves combining the fermentation step with a compatible product recovery method such as solvent extraction, membrane separation, or pervaporation *(8)*. Simultaneous fermentation and product removal also offers the opportunity to concentrate the product stream prior to downstream purification *(14)* and to remove other components such as diacetyl *(15)* or inhibitory fermentation by-products like acetaldehyde, ethyl acetate, and acetic acid *(7)*. Recovery of alcohols and organic acids from fermentation broths is one promising application of this technology, since conventional recovery techniques such as distillation are generally not cost-effective when carried out at low feed concentrations. The potential application of simultaneous fermentation and product recovery to a commodity chemical such as ethanol would require not only enhancing fermentation yield and productivity, but also developing a recovery process with low operating and capital costs.

There are a variety of simultaneous fermentation and product recovery schemes that have been investigated for recovering alcohols *(8,12,13)*. The Biostill process combines biomass retention by centrifugation with *in situ* product recovery by stripping *(16)*. The vacuferm process similarly operates a fermentation under vacuum conditions to recover ethanol by evaporation *(17–19)*. These methods suffer from low product selectivity that results in a high downstream product purification cost. *In situ* liquid extraction is another technique that has been demonstrated for a number of

fermentation products (20–22); however, the cost of product recovery from the extract can be energy-consuming (21), or carryover of the solvent into the fermentation can cause the micro-organism to be inhibited (23,24).

Ethanol Removal by Pervaporation

Membrane systems have several advantages over conventional separation processes such as distillation, adsorption, and extraction (7). They often offer low operating temperatures, simplicity of design, and favorable economics, thus complementing the attributes of biotechnological processes. Membrane separations such as microfiltration and pervaporation are gaining attention for *in situ* recovery of products like ethanol (4,25). The drawbacks of microfiltration are severe membrane fouling, low product selectivity in the permeate, large membrane area requirement, and relatively complicated operation. Pervaporation offers a higher product selectivity and simpler operation; however, the permeate flux can be an order of magnitude lower than that in microfiltration. Groot et al. (4,25) compared the integration of ethanol production with recovery by either microfiltration or pervaporation. The use of pervaporation resulted in a sixfold increase in volumetric productivity over conventional continuous operation, whereas a combination of microfiltration and pervaporation yielded a 16-fold higher productivity. Although a detailed cost estimate is not available, a process coupling microfiltration to the fermentation and pervaporation to the cell-free broth merits further investigation.

In pervaporation membrane systems, a liquid feed mixture, which can be a recycle loop with the fermentor, is contacted with the membrane, and the membrane permeate is removed from the other side of the membrane as a vapor (6,13). A low concentration (vapor pressure) of the product is maintained on the permeate side to provide the driving force for diffusion through the membrane (8,13). Thus, pervaporation can be particularly effective in the separation of a volatile product such as ethanol. In the case of ethanol, three distinct approaches can be used, individually or in combination, to control the permeation rate and selectivity of a particular membrane:

1. Decrease the vapor pressure on the permeate side;
2. Introduce a sweep gas stream to carry away the vapor; and
3. Use a temperature differential to increase the driving force for permeation (6,26).

Previous research has reported low flux rates (membrane flux is a measure of the amount of material that passes through the membrane [g m^{-2} hr^{-1}]), low membrane selectivity (membrane selectivity is a measure of the separation efficiency of the molecule isolated by the process), and membrane fouling in fermentations (7,25–27).

The development of new membranes based on poly[(1-trimethylsilyl)-1-propyne] (PTMSP) with improved permeability and/or selectivity relative to conventional systems of poly(dimethyl siloxane) (PDMS) will increase the potential of realizing efficient product removal *(6,28,29)*. Specifically, the selectivity of PTMSP for ethanol over water is more than four times higher than that of PDMS, whereas the permeability is 30 times higher *(28,29)*. This higher selectivity allows ethanol to be concentrated from 5 to over 50% in process streams with the PTMSP membrane. A traditional PDMS membrane would yield only 30% ethanol as the concentrate, and it would require 30 times more membrane area. Substituted polyacetylenes, such as PTMSP, are rigid rod polymers with bulky substituents that restrict rotational mobility and limit the polymer ability to pack together *(30,31)* (*see* Fig. 1). This limited molecular packing results in an unusually high free volume for these polymers, yielding the highest air permeability of any organic polymer and a high organic vapor permeability. PTMSP also has high ethanol permeability and selectivity over water, which makes it an ideal candidate for recovery of ethanol from fermentation broths *(28,29)*. The selectivity of PTMSP can be further increased by modifying its structure, for example by incorporating copolymers or blends *(32–34)*. Additional advantages of glassy PTMSP over rubbery PDMS include higher possible transmembrane operating pressures (the modulus of PTMSP is more than three orders of magnitude higher than that of PDMS), greater chemical stability, limited swelling of the glassy polymer resulting in greater durability, and limited fouling of the membrane surface by using copolymers or surface fluorination *(30)*.

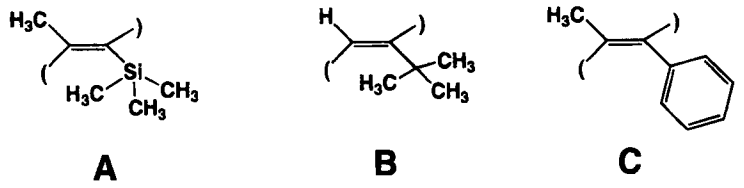
National Renewable Energy Laboratory (NREL) researchers have recently developed the ability to tailor the permselective properties and molecular morphology of PTMSP through the preparation of copolymers and blends. It is reported here on studies carried out to evaluate the characteristics of NREL-produced PTMSP membranes for achieving enhanced pervaporative ethanol removal from fermentation broths.

MATERIALS AND METHODS

Membranes

PDMS was a standard membrane obtained from Membrane Technology and Research, Inc. (Menlo Park, CA). It is a rubbery dense film (20 μ) on a microporous support. It was used as received.

PTMSP was synthesized by the method of Masuda et al. *(35)*. $TaCl_5$ (1.20 g, 3.35 mmol) was dissolved in 150 mL toluene by heating at 80°C for 15 min. A solution of 22.44g (0.202 mol) 1-(trimethylsilyl)-1-propyne in 50 mL of toluene was cannulated into the $TaCl_5$ solution and heated at 80°C for 24 h. After cooling to room temperature, the solid was stirred with 400 mL methanol and filtered to give a brown solid. The polymer was redis-



A B C

Fig. 1. Chemical structures of substituted polyacetylenes of interest for this project; **(A)** poly[(1-trimethylsilyl)-1-propyne] (PTMSP), **(B)** poly(t-butylacetylene) (PTBA), and **(C)** poly(1-phenyl-1-propyene) (PPP).

solved in 500 mL THF, precipitated by slow addition of methanol (500 mL) and filtered to give 22.20g (99%) white powder. This sample had a number average molecular weight of 300,000 Dalton and a glass transition temperature of greater than 150°C. PTMSP is a rigid polymer with good chemical stability and very good mechanical strength. The PTMSP polymer was dissolved to form a 5% w/v solution in toluene, and filtered to 7 μ. Films were hand-cast using a six-inch BYK Gardner casting knife, at 750 microns onto Teflon-taped glass. PTMSP dense films were 20–70 μ in thickness.

Experimental Set-Up

A Minitan S Ultrafiltration unit (Millipore) was used as the pervaporation cell. Membranes were installed between the two acrylic plates of the Minitan S using two silicone rubber gaskets (one above and one below the membrane) that sealed the perimeter of the filtration surface of the Minitan S. The effective membrane surface area in the unit was 0.0055 m^2. The inlet and retentate lines were connected to the feed vessel by silicone tubing. Feed was circulated at 100 mL/min by means of a Cole-Parmer pump. The filtrate lines of the unit were piped with stainless steel tubing to a simple on/off valve. Downstream of the valve, the collection vessel, a vacuum flask, was connected by stainless steel tubing inserted through a rubber stopper in the top of the flask. The side-arm of the collection vessel was then connected via vacuum tubing and stainless steel tubing through a rubber stopper into the top of the trap (a larger vacuum flask). Vacuum was applied to the system with a Welch 1400 Duoseal vacuum pump. A McCleod gauge in-line between the vacuum and trap monitored the vacuum pressure and a second on/off valve between the vacuum and the gauge controlled when vacuum was applied to the system. Vacuum was between 1 and 3 mm Hg. Both the collection vessel and trap were cooled by a dry ice/ethanol bath (about −40°C). Samples from the collection vessel were taken by closing the valve connected to the filtrate lines and then closing the valve controlling the vacuum. The stainless steel tubing between the filtrate lines and the collection vessel was then disconnected, and the vacuum tubing on the collection flask removed, and the openings covered with Parafilm to prevent evaporation while the flask was warmed to thaw the frozen collected sample. The collection vessel was weighed, and the increase in weight recorded as the weight

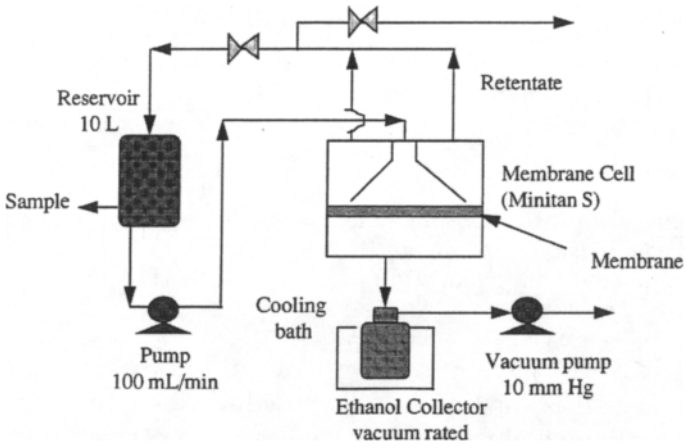


Fig. 2. Pervaporation system schematic.

of sample collected for that time period. Feed samples were also collected at each sampling time. To resume collection, the collection vessel was reconnected to the filtrate tubing, the vacuum valve opened, and then the filtrate valve opened. At the end of a run, the trap was weighed in the same manner as the collection vessel. The pervaporation experiments were carried out at an ambient temperature of about 25°C. Figure 2 shows a schematic of the experimental set-up. The concentration of the feed in the pervaporation runs showed only a small change (about 4%) during these experiments.

Fermentations

Saccharomyces cerevisiae D_5A was used in the yeast fermentations. Inoculum was grown for 12 h at 37°C, 150 rpm, in a baffled shake flask with Morton closure using 2% w/v yeast extract, 1% w/v peptone, 5% w/v glucose It was then used 10% v/v to inoculate a baffled shake flask with Morton closure containing 2% w/v yeast extract, 1% w/v peptone, 12% w/v glucose. The flask was incubated at 37°C, 150 rpm overnight. For cell-free broth, the material was then centrifuged at 4000 rpm for 10 min (1800 *g*), and the supernatant passed through a 0.2 mm filter.

Zymomonas mobilis 39676 (pZB4L) (NREL's proprietary strain) was used for the bacterial fermentations. Inoculum was grown in a baffled shake flask with Morton closure at 30°C, 150 rpm for 12 h using RM medium (1% w/v yeast extract, 0.02% w/v KH_2PO_4, 2.5% w/v glucose, 2.5% w/v xylose, plus 12.5 mg/L tetracycline). The inoculum was concentrated by centrifugation at 4000 rpm for 10 min (1800 *g*), and then used to inoculate a 2.5 L Bioflo fermentor (New Brunswick, NJ) containing RM medium with 5% w/v glucose, 3% w/v xylose, and 12.5 mg/L tetracycline. Temperature was controlled at 37°C, and pH was controlled at 6.0 with 3*M* KOH. The broth was harvested at 48 h. For cell-free broth, the same centrifugation/filtration procedure was used as was used for the yeast fermentations.

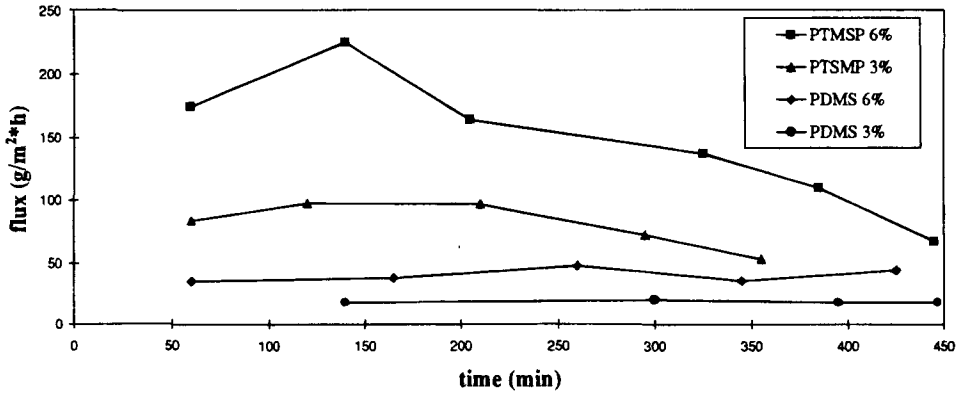


Fig. 3. Effect of feed concentration on membrane flux.

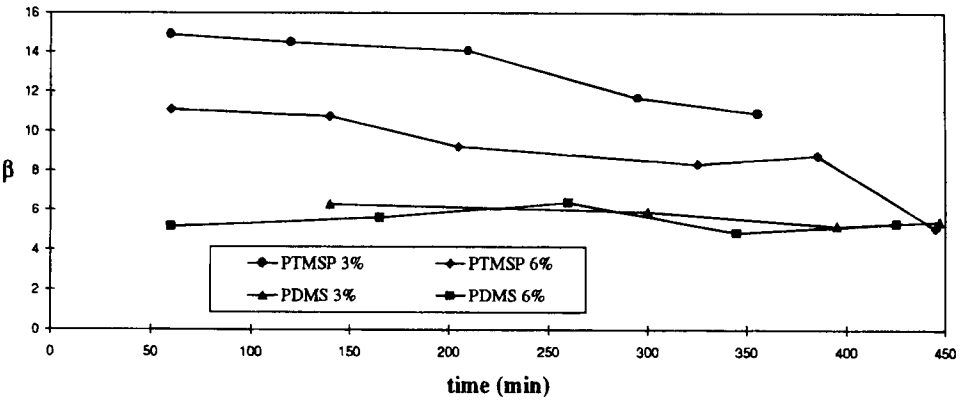


Fig. 4. Effect of feed concentration on β.

Analytical Techniques

Samples from the feed, collection vessel and trap were analyzed by HPLC using a Hewlett Packard Series II 1090, with an Aminex 87H organic acid column from Biorad installed and mobile phase of 0.01N sulfuric acid. The temperature of the column was 65°C.

RESULTS AND DISCUSSION

Comparison of PDMS and PTMSP in Ethanol–Water Mixtures

Effect of Concentration on Membrane Performance

Ethanol mixtures with water were tested as feed to the pervaporation cell in concentrations of 3% w/v and 6% w/v to represent the ethanol range achieved in fermentations. Figures 3 and 4 describe the performance of the two membranes by the ethanol flux and the concentration factor, β, respectively. Flux is expressed as rate of ethanol removal in g ethanol per square meter of membrane surface per hour. The factor, β, is the ratio of

ethanol concentration in the permeate to that in the feed. With PDMS, the ethanol in the permeate was concentrated by a factor of about 5.5 for both feed concentrations. The flux of ethanol showed a marked dependence on the feed concentration of ethanol; the flux at 6% w/v concentration of 39 g/m^2-h was twofold higher than that at 3% w/v ethanol feed. The feed concentration provides the key driving force for ethanol permeation through the membrane.

In contrast to PDMS, PTMSP showed a dependence of both β and flux on the feed concentration. The flux at 6% w/v feed concentration was 149 g/m^2-h compared to 79 g/m^2-h at 3% w/v. The concentration factor showed the reverse trend; at 6% w/v feed, the permeate was about 10-fold more concentrated than the feed whereas, at 3% w/v feed, the β was about 13. Previous work with PDMS and PTMSP has been carried out at operating conditions different from those in our experiments. However, the flux and concentration factors in our studies of PTMSP carried out at ambient conditions correspond well with previous reports by Nagase et al. *(32,33)*. Nagase et al. report a membrane permeability of 1.91×10^{-2} g-m/m^2-h compared to about 6.0×10^{-3} g-m/m^2-h in our experiments. The concentration factor is about 6.8 from earlier reports compared to 8.0 in the current study.

Comparison of Membranes in Ethanol Removal

PTMSP showed a distinct improvement over PDMS in both flux and β of ethanol. At both feed concentrations, the flux with PTMSP was at least fourfold higher than that obtained with PDMS. The concentration factor achieved with PTMSP at 6% w/v ethanol feed was about twofold higher and that at 3% w/v, feed was threefold higher than the corresponding values shown by PDMS membranes. The PTMSP performance seemed to indicate a gradual declining trend with time against the steady results obtained with PDMS. The PTMSP membranes that were prepared in-house were used unsupported in the pervaporation cell. The slight deterioration of performance may be a result of gradual physical degradation of the membrane during the experiment. In a commercial set-up, use of supported PTMSP membranes is not likely to show the same deterioration in performance.

Effect of Acetic Acid on Ethanol Separation

Organic acids such as acetic, lactic, and succinic acids are common by-products in an ethanol fermentation. Acetic acid at 1.5% w/v concentration in the feed was selected as a representative by-product. Mixtures of ethanol with acetic acid were used as feed to study the effect of an acidic fermentation by-product on ethanol recovery with PTMSP membranes. Figure 5 describes the performance of PTMSP on a 6% w/v ethanol, 1.5% w/v acetic acid mixture. Both the flux and ethanol concentration factor declined in comparison with the 6% w/v ethanol feed. In the acetic acid runs, the flux of 96 g/m^2-h was a 33% decrease and the β of 8.4 represented a 17% decrease over the corresponding parameters in runs with no feed acetic

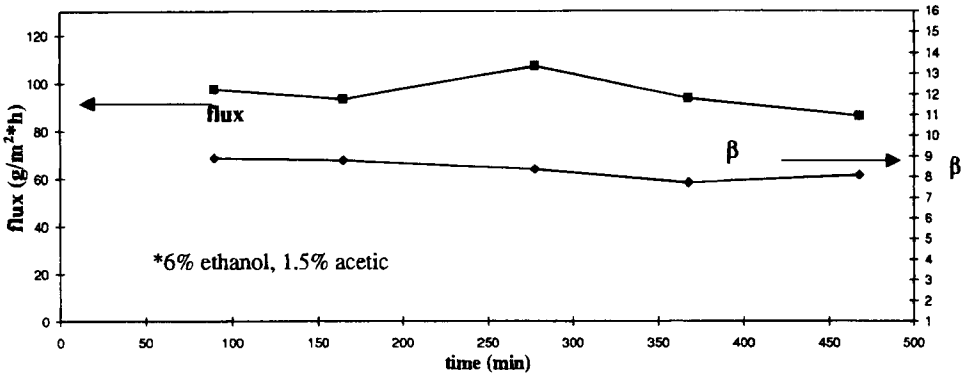


Fig. 5. Effect of acetic acid on ethanol pervaporation.

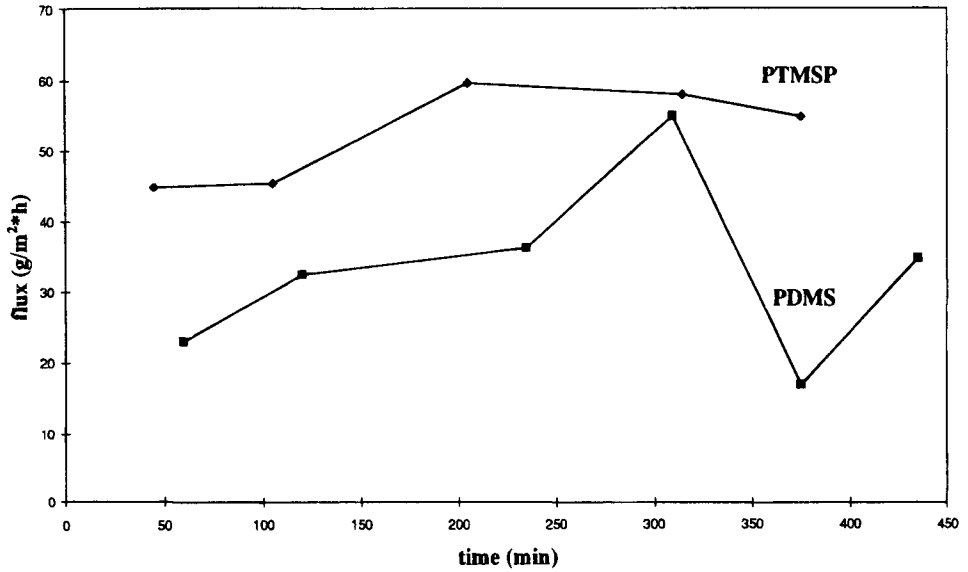


Fig. 6. Pervaporation of cell-free yeast fermentation broth.

acid. Acetic acid was detected in the permeate at concentrations of about 4.5 g/L, a threefold decrease compared to the feed concentration. The flux of acetic acid was two orders of magnitude lower than that of ethanol. These results indicate that the PTMSP selectivity for ethanol is much greater than that for acetic acid. Thus, PTMSP can be used effectively for ethanol recovery in the presence of an organic acid; however, the presence of the impurity does impact the performance to some extent.

Membrane Performance with Fermentation Broths

Figures 6 through 8 show a comparison of flux and β for ethanol-containing broths obtained from glucose fermentations by two organisms: *Saccharomyces cerevisiae* and *Zymomonas mobilis*. The final ethanol concen-

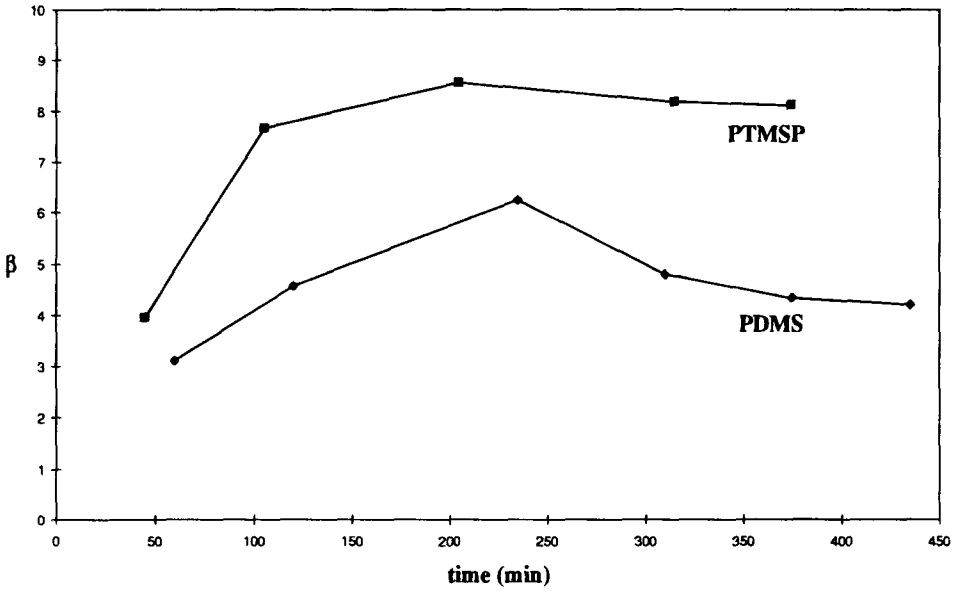


Fig. 7. Pervaporation with cell-free yeast fermentation broth.

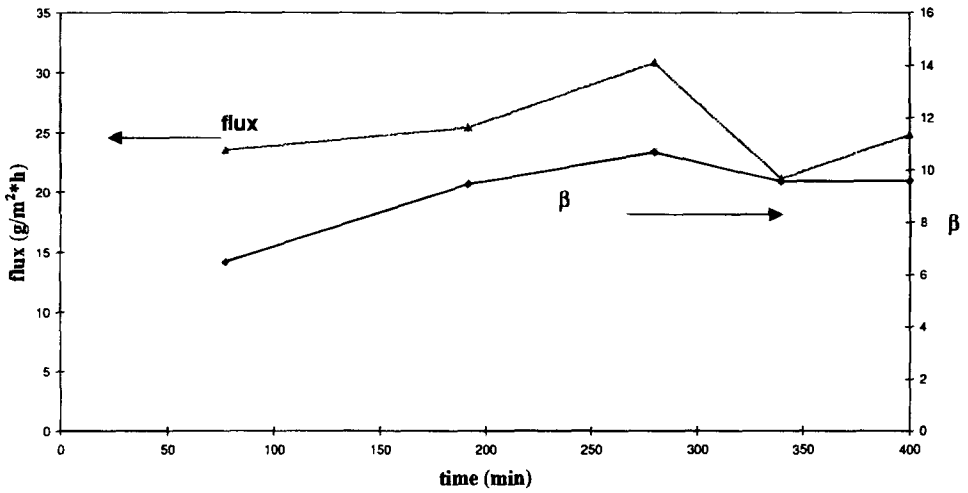


Fig. 8. Pervaporation of cell-free bacterial fermentation broth.

trations for the yeast and bacterial fermentations were about 6% w/v and 3% w/v, respectively, and were, therefore, compared with previous results from corresponding ethanol feed concentrations.

Pervaporation with Cell-Free Broths

The flux of ethanol from fermentation broths achieved with PTMSP were 25 and 52 g/m^2 for *Zymomonas* and *Saccharomyces*, respectively. These were about threefold lower than those achieved previously with corresponding ethanol–water mixtures. The concentration factor declined by

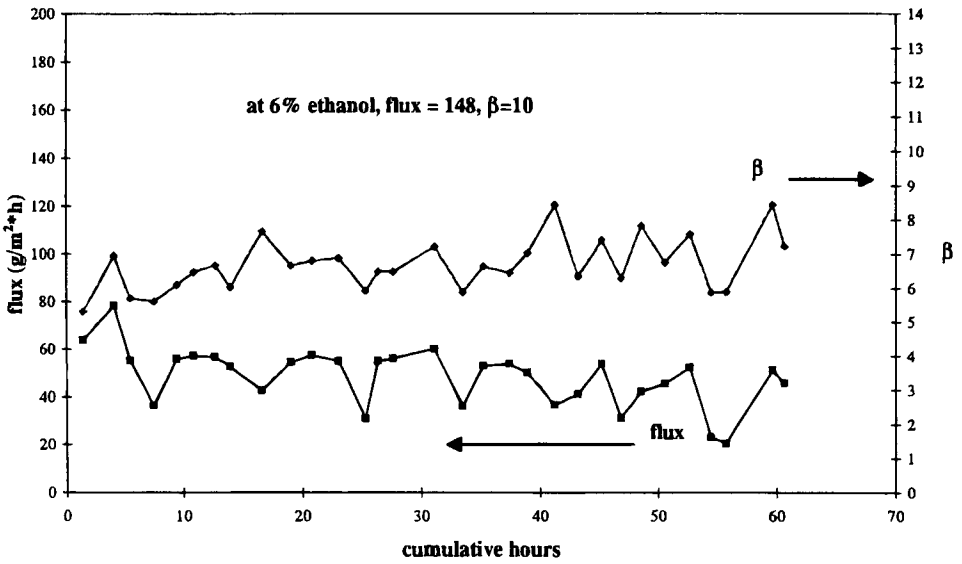


Fig. 9. PTMSP performance in extended operation with cell-free fermentation broth.

about 25% for both fermentation broths relative to the ethanol mixtures. A decline in performance was also observed with PDMS membranes; however, the relative decrease in flux and concentration factors was not as high as that observed with the PTMSP membranes. This may be attributed to the fact that the PDMS membranes were obtained from a commercial source, whereas PTMSP were developed in-house and were used unsupported in the experiments. The spent fermentation broth contains organic acids, proteins, ions, and unused nutrients in addition to product ethanol. The decrease in performance of both membranes with the fermentation broths is likely because of the mixture of components present with ethanol.

An experiment was conducted with fermentation media supplemented with 6% w/v ethanol to study the possible impact of media components on membrane performance. Results with PTMSP were inconclusive (not shown); the flux was comparable to the ethanol–water mixture, but the concentration factor was closer to that described in the previous section with fermentation broth. Our experiments so far appear to indicate a distinct loss in PTMSP performance with fermentation broths relative to ethanol–water mixtures of comparable concentrations. However, comparison of the two membranes indicates that both the flux and β with PTMSP are about 1.5-fold higher than those for similar runs with PDMS.

Analysis of prolonged operation of PTMSP with cell-free fermentation broths is shown in Fig. 9. An 8-h run was conducted each day and the system was shut off at the end of the day. The flux and concentration factor were steady overall at average values of 55 g/m^2-h and 7.0, respectively over a period of 11 d (8-h runs each day). The variations seen in the performance may be attributed to changes in ambient temperature that varied

Table 1
Summary of Pervaporation Performance

No.	Feed	Membrane	Flux, g/sq. m-hour	Average β
1	3% pure ethanol	PDMS	18	5.7
2	3% pure ethanol	PTMSP	79	13.2
3	cell-free bacterial fermentation broth (3% ethanol)	PTMSP	25	9.1
4	6% pure ethanol	PDMS	39	5.5
5	cell-free yeast fermentation broth (6% ethanol)	PDMS	33	4.5
6	6% pure ethanol	PTMSP	149	9.9
7	6% pure ethanol with 1.5% acetic acid	PTMSP	96	8.4
8	cell-free yeast fermentation broth (6% ethanol)	PTMSP	52	7.3
9	whole yeast fermentation broth (6% ethanol)	PTMSP	75	7.6

between 22 and 27°C. The temperature of operation can affect membrane performance significantly *(32,33)*. Membrane performance was observed to increase gradually to its steady level over the first hour of operation as the system reached equilibrium (not shown).

Pervaporation with Cell-Containing Broths

A single 8-h run was conducted on PTMSP with spent fermentation broth containing yeast at about 4 g/L dry cell weight. Results obtained were comparable with those of the cell-free broth. Although some cell accumulation was observed visually on the membrane at the end of the run, this did not affect the pervaporation performance. Fouling resistance of membranes is a crucial factor in the sustained operation of such units. Our results with PTMSP indicate an early promise in providing a fouling-resistant operation of pervaporation although detailed studies of fouling in sustained operation will need to be done in future.

CONCLUSION

Table 1 summarizes the results of experiments conducted with PDMS and PTMSP membranes. Pervaporation using PTMSP membranes shows a distinct advantage over conventional PDMS membranes in ethanol removal. The flux with PTMSP is about three-fold higher and the concentration factor is about twofold higher than the corresponding performance achieved with PDMS under similar conditions. The performance of PTMSP with fermentation broths shows a reduction in both flux and concentration factor relative to ethanol-water mixtures. However, the PTMSP membranes promise increased resistance to fouling in operation with cell-containing fermentation broths.

Future work will be conducted on a 4-inch pervaporation cell connected to a continuous fermentor. Modified PTMSP membranes will be studied for improved performance in pervaporation. Performance of PTMSP membranes will be studied with cell-containing streams from bioreactors for continuous removal of ethanol. The effect of operating conditions such as temperature and vacuum level will be characterized to find optimal operation. Fouling resistance of PTMSP will be investigated in more detail by conducting prolonged runs with continuous fermentation. The benefits of pervaporation in maintaining fermentor concentrations below inhibitory levels will be illustrated. The economic impacts of using pervaporation to increase fermentation productivity and to reduce energy consumption in product purifications will also be assessed.

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