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Ethanol production in a continuous fermentation/membrane pervaporation system

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Abstract The productivity of ethanol fermentation processes, predominantly based on batch operation in the U.S. fuel ethanol industry, could be improved by adoption of continuous processing technology. In this study, a conventional yeast fermentation was coupled to a flat-plate membrane pervaporation unit to recover continuously an enriched ethanol stream from the fermentation broth. The process employed a concentrated dextrose feed stream controlled by the flow rate of permeate from the pervaporation unit via liquid-level control in the fermentor. The pervaporation module contained 0.1 m^2 commercially available polydimethylsiloxane membrane and consistently produced a permeate of 20%–23% (w/w) ethanol while maintaining a level of 4%–6% ethanol in a stirred-tank fermentor. The system exhibited excellent operational stability. During continuous operation with cell densities of 15–23 g/l, ethanol productivities of 4.9–7.8 $g l^{-1} h^{-1}$ were achieved utilizing feed streams of 269–619 g/l glucose. Pervaporation flux and ethanol selectivities were 0.31–0.79 $l m^{-2} h^{-1}$ and 1.8–6.5 respectively.

Introduction

The current demand for fuel ethanol in the U.S. is approximately 10^9 US gallons/year (3.8×10^9 l) (Hoffman 1995) and is expected to double within 5 years, primarily because of its use as a fuel oxygenate in mandated automotive-emission-reduction programs. In the U.S., ethanol is produced predominantly from starch-based grains, mainly corn, by the traditional alcoholic fermentation employing *Saccharomyces cerevisae*. Research into improvement of current processes for ethanol production generally falls into two areas: development of continuous fermentation systems and increasing the energy efficiency of ethanol recovery and purification. Continuous fermentation systems with high cell loadings can result in greatly improved volumetric productivities and, thus, reduced capital costs for fermentor vessels. One technique employed in continuous fermentation processes is the integration of an ethanol-recovery step with fermentation to minimize product inhibition of the fermenting organism. In laboratory studies, continuous ethanol removal from fermentation broths has been accomplished by vacuum distillation (Cysewski and Wilke 1977), solvent extraction (Minier and Goma 1982; Kollerup and Daugulis 1986), and membrane pervaporation (Shabtai et al. 1991; Shabtai and Mandel 1993; Mori and Inaba 1990; Groot et al. 1992). Proposed processes are many and varied and often include cell-recycling operations or cell immobilization.

Pervaporation is a unit operation in which two components are separated through the combination of a difference in permeation rates through a non-porous, semi-permeable membrane and an evaporative phase change between the upstream and downstream sides of the membrane. The downstream or permeate side of the membrane is usually maintained under vacuum. Pervaporation is a low-temperature, low-pressure unit operation and, because of the nature of the vapor liquid equilibrium of ethanol water, has a built-in selectivity for ethanol at low concentrations. The development of pervaporation technology began in the 1950s (Binning et al. 1961). It is now used commercially for solvent dehydration operations (Fleming 1992). Excellent reviews of general pervaporation theory and applications (Fleming and Slater 1992) and selective permeation of organics, including ethanol (Beaumelle et al. 1993) are available.

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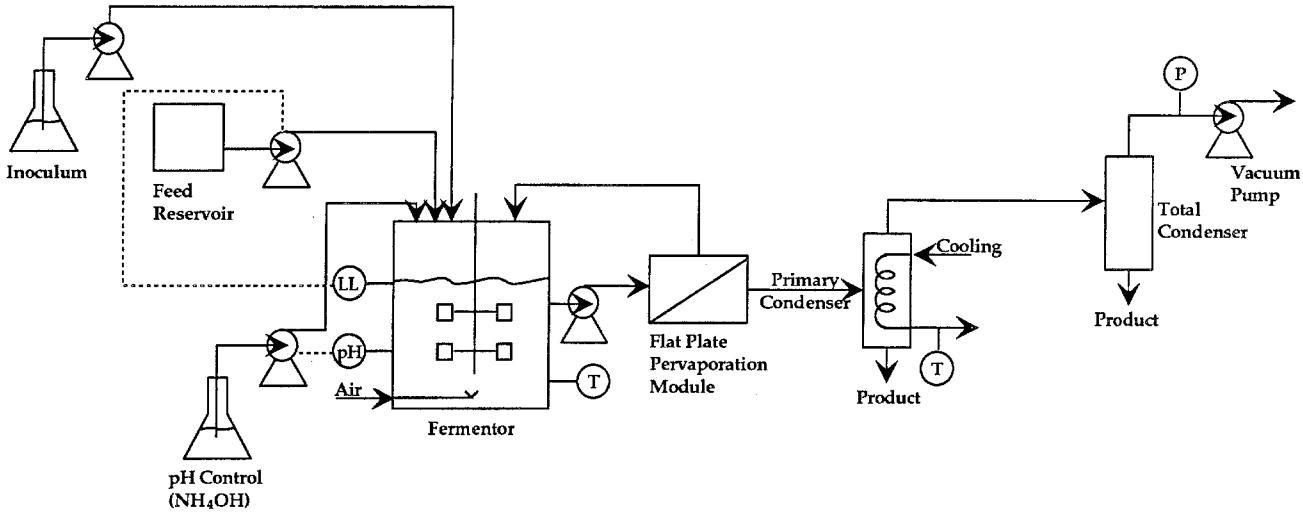

Fig. 1 Schematic of apparatus for continuous fermentation/pervaporation experiments

Most previous studies of coupled fermentation/pervaporation processes refered to earlier have utilized conditions (e.g., immobilized cell fermentors, novel fermenting organisms) often far removed from those in current industrial processes. To allow for better comparison to existing industrial technology, fermentation conditions such as reactor design and fermenting organism in this study were chosen to closely resemble current industry practice. In addition, the pervaporation operation was accomplished with a commercially available membrane. The specific objective was to achieve a stable, continuous fermentation by recovery of an enriched ethanol stream from the fermentation broth by pervaporation with minimal loss of ethanol yield or cell productivity.

Materials and methods

Organism/medium

The fermenting organism was the yeast *Saccharomyces cerevisiae*, ATCC 4126 (American Type Culture Collection, Rockville, Md.). The medium was prepared in tap water and had the following composition (g/l): yeast extract 12.75, ammonium chloride 1.98, calcium chloride 0.09, magnesium sulfate heptahydrate 0.18. Glucose was supplied as a commercial dextrose, Cerelose (Corn Products, a division of CPC Intl, Summit-Argo, Ill.) to an initial fermentor concentration of 150 g/l glucose.

Fermentor/pervaporation system

A schematic diagram of the experimental apparatus used in this study is presented in Fig. 1. Fermentations were carried out in a 3-l bench-top fermentor (Omni-Culture, Virtis Co., Gardiner, N.Y.) with a working volume of 1.5 l. Experimental conditions were an air flow rate of 0.033–0.10 vvm, agitation at 100 rpm, a temperature of $35 \pm 1°$ C and pH controlled at 5.0 ± 0.05 with 1 M ammonium hydroxide. The liquid level in the fermentor was maintained by a modified foam control probe, which controlled addition of a concentrated glucose feed via a pump during operation of the pervaporation module.

The pervaporation module was a flat-plate design with membranes housed between stainless-steel plates, $28 \times 23 \times 2.5$ cm thick. The membranes were assembled in the module with the Millipore Pellicon Cassette System (Millipore Corp., Bedford, Mass), which allowed for parallel flow across multiple membranes. Pervaporation was accomplished with MPF-50 membranes from Membrane Products, Kiryat Weizmann Ltd. (Rehovot, Israel). These are composite membranes incorporating a 2-μm polydimethylsiloxane layer on an asymmetric polysulfone support. The module was fitted with five membranes with a total membrane area of 0.1 m^2. Fermentation broth was circulated across the feed side of the membrane at a flow rate of 1.5 l/min by a peristaltic pump. A vacuum pump maintained a vacuum of 1–4 mm Hg (130–530 Pa) on the downstream (permeate) side. Primary and total condensers collected the ethanol-rich permeate. The primary condenser employed a circulating coolant at $-17°$ C. The total condenser utilized a solid CO_2/acetone mixture. Temperatures and vacuum were monitored by thermocouples and an electronic vacuum gauge (Vacuum Research Corp., Pittsburgh, Pa.) respectively, connected to a digital data-acquisition system. The unit was sterilized by circulation of a 70% ethanol solution through the feed side, followed by rinsing with sterile distilled water.

Procedures

The fermentor was inoculated with an active 12-h-old yeast culture in a volume equivalent to 10% of the fermentor volume. When the ethanol level reached 5% in the broth, the pervaporation module was started along with liquid-level control. The only material that left the fermentor did so via the pervaporation module, balanced by periodic additions from the feed reservoir via a pump controlled by the level control. The total volume in the fermentor/pervaporation system was 1.5 l with a 0.15-l hold-up in the pervaporation loop.

In some experiments, the membranes were periodically washed with warm (37° C) sterile distilled water. After a 5-min rinse, the washing solution was recirculated across the feed side of the membranes for 30 min. The system was run for 10 min and the permeate

Table 1 Effect of operating parameters on pervaporation flux. The feed was 8% ethanol. Means are calculated from three replicates per treatment

Temperature (°C)	Feed flow rate (ml/min)	Flux ($l\,m^{-2}\,h^{-1}$) Vacuum			
		150 mmHg		1 mm Hg	
		Mean	SD	Mean	SD
25	65	0.88	0.26	1.12	0.06
25	312	1.16	0.12	1.71	0.25
45	65	1.63	0.07	2.51	0.09
45	312	2.34	0.15	3.23	0.21

Table 2 Effect of operating parameters on pervaporation selectivity. The feed was 8% ethanol. Means are calculated from three replicates per treatment

Temperature (°C)	Feed flow rate (ml/min)	Ethanol selectivity Vacuum			
		150 mmHg		1 mm Hg	
		Mean	SD	Mean	SD
25	65	1.70	0.40	1.31	0.23
25	312	1.39	0.39	1.81	0.98
45	65	1.82	0.47	2.07	0.19
45	312	2.11	0.22	1.81	0.03

discarded before data collection was resumed. Typically, the membranes were washed every 10–14 h of operation.

Analytical

Glucose analyses were performed with a YSI model 2000 glucose analyzer. Ethanol concentrations were determined by capillary gas chromatography on a HP 5890 gas chromatograph (Hewlett Packard, Avondale, Pa.) by direct aqueous injection onto a 30 m fused silica column (Supelco, Bellefonte, Pa.). Cell concentrations were reported as dry weights after drying at 100° C.

Results

Membrane characterization

The performance of the MPF-50 membranes over the range of operating conditions expected was initially established in experiments using model solutions of ethanol/distilled water and one membrane in the pervaporation module. A factorial series of experiments was conducted to determine the effects of the major pervaporation operating variables, temperature, vacuum level, and feed flow rate, on the membrane flux and ethanol selectivity. Selectivity is defined as the ratio of the ethanol and water concentrations (%, w/w) in the permeate divided by this same ratio calculated for the feed stream. The results are presented in Tables 1 and 2. These results showed that temperature, feed flow rate, and vacuum level significantly affect ($P < 0.05$) the pervaporation flux (Table 1) while, of these variables, only temperature has a significant impact ($P < 0.05$) on the ethanol selectivity (Table 2). Thus, conditions favoring ethanol recovery were high temperature, high feed-flow rate, and low vacuum. However, because of the coupling of the fermentation and pervaporation steps in this study, the temperature was restricted to that of the fermentation, 35° C.

Continuous fermentation/pervaporation

Experiments employing the pervaporation module for continuous removal of ethanol from the fermentation broth began as batch experiments with an initial glucose concentration of 150 g/l. Results of these experiments are presented in Table 3. The glucose consumption and ethanol production rates, r_S and r_P, respectively, were calculated from mass balances on these components in sampling periods subsequent to the beginning of pervaporation operation in which glucose was not limiting. The cell yield, $Y_{X/S}$, was calculated from data in the time interval immediately following commencement of pervaporation operation. The product yield, $Y_{P/S}$, was calculated from the equation $Y_{P/S} = r_P/r_S$. All of these kinetic parameters were calculated over discrete sampling periods, typically 2 h, and then averaged to calculate overall values for the experiment or regions within an experiment.

The target range within which it was desired to maintain the ethanol concentration in the fermentor was 45–65 g/l. Continuous operation in this range would maximize the ethanol concentration in the permeate, c_{PP}, while minimizing inhibition of the yeast. For Table 3, overall pervaporation fluxes and selectivities were calculated in a manner similar to that for the biokinetic parameters.

Results of a typical experiment (experiment 4 in Table 3) are displayed in Fig. 2. Stable, continuous fermentation with ethanol removal from the broth for over 50 h was achieved. Pervaporation produced a permeate with an ethanol concentration of approximately 15%. The ethanol concentration in the broth was maintained between the target range of 45–65 g/l, as it was for most experiments.

Beginning with experiment 5 of Table 3, new membranes were installed in the flat-plate membrane holder and were utilized for all subsequent experiments. In experiments 6–9, the membranes were washed every 10–14 h to determine if their performance could be maintained and their useful life extended by this procedure. Prior to these experiments, the membranes were only washed at the conclusion of the run. The major effect of the membrane washing was to improve the selectivity from a range of 1.8–2.6 (experiments 1–4) to a general range of 3.2–4.1 (experiments 6–9).

The product yields obtained in the continuous fermentation/pervaporation experiments ranged from

Table 3 Continuous fermentation/pervaporation (*PV*) experiments. c_{SF} substrate (glucose) concentration in the fermentor feed, c_{PP} ethanol concentration in the permeate of the pervaporation unit, r_S rate of substrate (glucose) utilization, r_P rate of product (ethanol) formation, $Y_{P/S}$ yield of product (ethanol) on substrate, $Y_{X/S}$ yield of biomass on substrate

			Fermentation Kinetics					Pervaporation performance		
Expt.	PV duration (h)	Aeration rate ($l l^{-1} min^{-1}$)	c_{SF} (g/l)	r_S ($g l^{-1} h^{-1}$)	r_P ($g l^{-1} h^{-1}$)	$Y_{X/S}$ (g/g)	$Y_{P/S}$ (g/g)	c_{PP} (g/l)	Flux ($l m^{-2} h^{-1}$)	Selectivity
1	26.1	0.1	259	17.3	6.7	0.043	0.39	123	0.74	2.6
2	33.5	0.1	269	18.4	6.4	0.046	0.34	111	0.73	2.2
3	46.5	0.1	269	19.4	7.8	0.049	0.40	101	0.79	1.8
			400	14.4	6.5		0.45	158	0.56	2.3
			600	19.4	6.9		0.36	162	0.65	2.4
4	44.0	0.033	269	17.0	7.7	0.047	0.45	104	0.72	2.0
			400	15.5	6.5		0.42	148	0.65	2.4
5[a]	26.0	0.033	380	15.8	6.54	0.027	0.41	171	0.51	3.7
6[b]	52.5	0.033	404	14.7	6.63	0.033	0.45	192	0.42	3.3
			608	13.4	5.41		0.40	335	0.31	6.5
7[b]	59.0	0.033	385	11.5	5.80	0.042	0.50	223	0.37	4.1
			550	15.0	4.89		0.33	216	0.37	3.7
8[b]	55.5	0.033	385	14.3	5.52	0.042	0.39	196	0.40	3.7
			584	14.1	5.24		0.37	209	0.40	3.7
9[b]	53.5	0.033	386	14.7	5.44	0.032	0.37	200	0.37	3.6
			619	12.6	6.43		0.51	201	0.38	3.2

[a] New membranes installed and utilized in all subsequent experiments
[b] Membranes washed during experiments

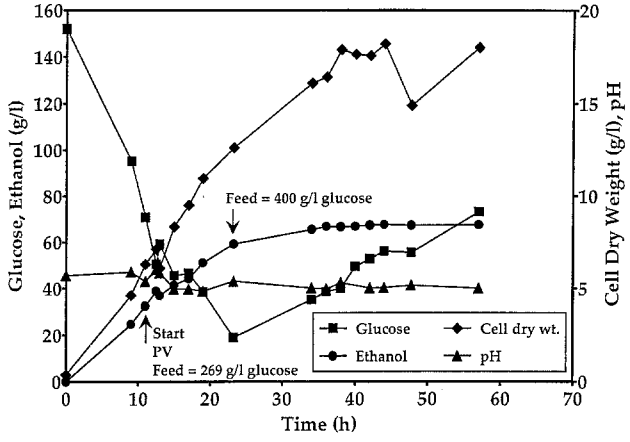

Fig. 2 Ethanol production in a continuous fermentation/membrane pervaporation system

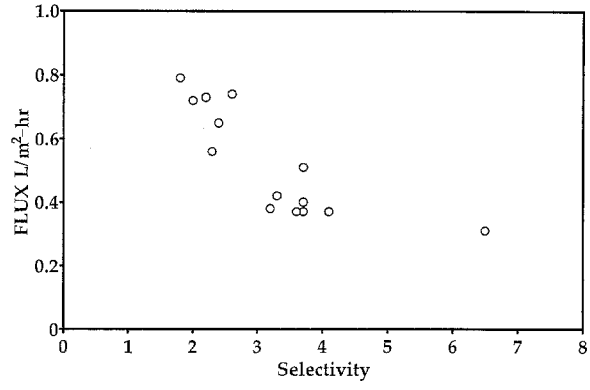

Fig. 3 Pervaporation flux versus selectivity in continuous fermentation/pervaporation experiments

0.33 to 0.51. Corresponding ethanol volumetric productivities were 4.9–7.8 $g l^{-1} h^{-1}$ in continuous fermentations lasting up to 59 h and utilizing feed solutions of 269–619 g/l glucose. The effect of reducing the aeration rate from 0.1 vvm to 0.033 vvm (experiments 1–3 compared to 4–9) did not appear to affect fermentation kinetics or yields significantly. In continuous fermentations, the aeration rate should be kept as low as possible to maximize ethanol productivity while maintaining an acceptable cell growth rate.

Fluxes through the pervaporation module during the continuous fermentation/pervaporation experiments varied from 0.31 $l m^{-2} h^{-1}$ to 0.79 $l m^{-2} h^{-1}$. As can be seen in Fig. 3, the flux is, in general, inversely proportional to the selectivity. The highest permeate concentration observed was 335 g/l (35.5 %, w/w) but with new membranes and periodic washing, the permeate concentration was consistently in the range 200–220 g/l.

Discussion

Operation of the continuous fermentation/pervaporation system was accomplished with very few problems. The additional agitation caused by the recirculation of

broth through the pervaporation module produced only minimal foaming. During experiments lasting up to 59 h, the system was able to maintain the ethanol concentration in the fermentor at a reasonably constant value in the range 40–75 g/l, usually 45–55 g/l. The system could be run in a stable and unattended manner for long periods of time. However, the long-term operation of an industrial continuous fermentation/pervaporation system would require incorporation of a bleed stream to counteract the inhibitory effects of a build-up of dissolved solids and minor fermentation products in the fermentation broth.

Owing to the evaporative cooling that occurred on the permeate side of the membrane, the temperature drop across the membrane was 11° C. Since the actual temperature at the membrane surface could not be measured, pervaporation could have occurred at a temperature lower than the reported 35° C of the bulk medium and thus affect the interpretation of the results. Furthermore, as reduced temperatures would lower the permeate flux, every attempt to mitigate the temperature drop through jacketing or module design should be undertaken.

The maximum cell concentrations achieved in the experiments were 18–23 g/l (dry weight). Because the biomass concentration was increasing gradually in these runs, it was necessary to change the glucose concentration of the feed periodically to maintain a low glucose level in the fermentor. The glucose concentration in the fermentor should be kept low to minimize its deleterious effect on the pervaporation flux. The glucose contribution to the reduction of membrane flux under actual fermentor operating conditions is the greatest of all the medium components (data not shown). Overall, the membrane flux under fermentation conditions is approximately 30%–50% of that for an ethanol/water solution. Nevertheless, stable operation of the system was accomplished at these biomass levels with glucose concentrations in the feed of up to 619 g/l.

For ther long-term use of MPF-50 membranes, periodic washing would be required. Without washing, fouling of the membranes, as evidenced by decreased performance over time and loss of capability to reject other medium components, was detected. The washing procedure eliminated these problems but would increase the operational burden and decrease system productivity in an industrial process.

Several other integrated fermentation/pervaporation processes for ethanol production by yeast have recently been investigated on a small scale. In one system evaluated by Groot et al. (1992) that is similar to the present study except that a hollow-fiber pervaporation module (with an undefined commercial silicone-based membrane) was used, ethanol productivities of 9–14 $g l^{-1} h^{-1}$ were achieved at constant biomass levels of 15–27 g/l. Pervaporation fluxes of, 0.47–0.81 $l m^{-2} h^{-1}$, and selectivities of 2.5–5.7 were comparable to the results of this study. Utilizing the same membranes as in this study but in a tubular configuration, Shabtai et al. (1991) reported a pervaporation flux of approximately 0.2 $l m^{-2} h^{-1}$ and selectivity of 5 at 45° C in a coupled immobilized-yeast reactor/pervaporation operation. They also reported a decline in membrane performance over time and, in a subsequent report (Shabtai and Mandel 1993), demonstrated the importance of membrane cleaning and replacement to prevent fouling. Considering the differences in process flowsheets, bioreactors employed, configuration of the pervaporation module (hollow-fibre/tubular versus flat-plate), and operating conditions (e.g., pervaporation temperature), there is generally close agreement in the biokinetic data and pervaporation results between these investigations and the present study.

Industrial application of pervaporation for ethanol recovery from fermentation broths is dependent on several factors. Membranes with acceptable performance characteristics and low susceptibility to fouling must be developed. Condensation of the permeate (under vacuum), while it may be relatively efficient on a heat-transfer basis, would require a refrigeration system for condenser cooling water, creating additional costs over conventional cooling water. However, the economics of such a system should be judged by comparing complete processes; i.e., because of the higher ethanol productivities, a continuous fermentation/pervaporation system would require much less fermentor volume (compared to a conventional batch process), a significant savings in capital costs. In addition, distillation capital and energy costs would be lower because of a more highly concentrated feed. The balance of these savings against the membrane system costs and refrigeration system costs would largely determine the economics of pervaporation for ethanol recovery from fermentation broths.

While polydimethylsiloxane membranes possess the best ethanol permselectivity of any commercially available membranes, they are not intrinsically selective for ethanol rather than water (Blume et al. 1990); i.e., the permeability of ethanol through the membrane is less than that of water. This has several implications. The overall degree of separation will be less than that due to single-stage phase equilibrium (evaporation) thereby limiting the membrane selectivity to 5-10. Thus, to compete with distillation for ethanol recovery, membrane pervaporation under these constraints must possess advantages in capital costs and energy consumption. A recent study of the economics of integrated fermentation/membrane processes (Groot et al. 1993) concluded that, with current membrane costs and lifetimes, even pervaporation with a selectivity of 20 would not be economical. New membrane polymers or designs such as the zeolite/polydimethylsiloxane membrane reported by Hennepe et al. (1987) will most likely be required before commercial adoption of pervaporation for ethanol recovery will occur.

In summary, a conventional stirred-tank fermentor was coupled to a flat-plate pervaporation module that allowed for continuous ethanol production with recovery of an enriched ethanol stream of about 22%. At cell concentrations of 15–23 g/l during continuous operation, the ethanol productivity was 4.9–7.8 $gl^{-1}h^{-1}$ with a yield on substrate of 0.33–0.51. Periodic cleaning of the membranes improved the consistency of performance of the pervaporation operation and minimized problems due to fouling. Operation of the system at higher cell loadings through cell concentration and recycling, and use of membranes with improved operational behavior and performance characteristics, are areas of future study.

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