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N. Qureshi[a]; H. P. Blaschek[a]
[a] BIOTECHNOLOGY & BIOENGINEERING GROUP, DEPARTMENT OF FOOD SCIENCE & HUMAN NUTRITION, UNIVERSITY OF ILLINOIS AT URBANA-CHAMPAIGN, URBANA, ILLINOIS, USA

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Fouling Studies of a Pervaporation Membrane with Commercial Fermentation Media and Fermentation Broth of Hyper-Butanol-Producing *Clostridium beijerinckii* BA101

N. QURESHI* and H. P. BLASCHEK
BIOTECHNOLOGY & BIOENGINEERING GROUP
DEPARTMENT OF FOOD SCIENCE & HUMAN NUTRITION
UNIVERSITY OF ILLINOIS AT URBANA-CHAMPAIGN
1207 W GREGORY DRIVE, URBANA, ILLINOIS 61801, USA

ABSTRACT

Fouling studies of a pervaporation membrane (area based on internal tube diameter 0.08 m^2) were carried out in batch mode using model butanol solution in distilled water, commercial fermentation media (P2 and corn steep liquor, CSL), and P2 and CSL fermentation broth. Butanol fermentations were conducted using semidefined P2 medium, CSL medium (a low cost commercial nutrient medium), and the *Clostridium beijerinckii* BA101 hyper-butanol-producing mutant strain. The rates of removal of butanol from the commercial fermentation media (P2 and CSL) were similar to the rates of butanol removal from butanol solution in water. The rates of removal of butanol from the CSL fermentation broth were higher than the rates of butanol removal from butanol solution in water and fermentation media. Butanol removal experiments were run from 14 to 240 hours using different media and fermentation broth, and it was concluded that the pervaporation membrane did not foul under the conditions employed. The selectivities of butanol ranged from 20 to 55 when feed butanol ranged from 33.0 to 3.33 g/L. The total flux was 25–45 $g/m^2 \cdot h$ using air as the sweep gas. In the permeate, the butanol concentration ranged from 122.9 to 458 g/L.

Key Words. Fouling; *Clostridium beijerinckii* BA101; Butanol; Fermentation media; Fermentation broth

* To whom correspondence should be addressed.

INTRODUCTION

Pervaporation is a technique which uses selective membranes to remove organic volatiles (1, 2). It has been increasingly applied to remove butanol (and acetone, ethanol; ABE) from the fermentation broth of clostridia to reduce end-product inhibition in batch (3, 4), fed batch (5), and continuous fermentors (6). As a result, higher rates of sugar utilization and hence higher rates of butanol production have been achieved in these fermentation systems due to significantly reduced end-product inhibition. Additionally, the cultures have been reported not to be affected by pervaporation. However, new strains need to be tested prior to commercial application. Pervaporation, in contrast to distillation (where water is removed as the top product and butanol is recovered as the bottom product) removes butanol selectively, leaving behind water, residual sugar, and nutrients for further recycle. Additionally, pervaporation reduces the waste disposal streams. This technology could be used commercially to recover butanol from fermentation broth if cheap, durable, and stable membranes become available. It should be noted that the final fermentation broth of our hyper-butanol-producing strain contains approximately 963 g/L water and 33 g/L total solvents.

Although improved fermentations have been reported when employing pervaporation, there is little information on fouling of the pervaporation membranes when using *Clostridium acetobutylicum* or *C. beijerinckii* and commercial fermentation media. Fermentation media are often complex and may result in membrane fouling. Fouling of ultrafiltration membranes with the fermentation medium is common and has been extensively studied (7–10). Fouling is defined as a reduction in the rate of permeation ($g/m^2 \cdot h$) with time of membrane operation. Corn steep liquor (CSL) is a cheap source of nutrients and is usually recommended as a fermentation nutrient supplement in order to reduce the cost of production of chemicals (11, 12). Since CSL is complex in nature, it is likely that it may foul the membrane.

Recently, in an attempt to economize the process of butanol production by fermentation, a hyper-butanol-producing strain of *C. beijerinckii* BA101 was developed and characterized in this laboratory (13). Under optimized environmental conditions this strain produces ca. 20 g/L butanol and over 32 g/L total solvents in batch fermentations (14). Furthermore, pilot-plant scale reactors with 200 L capacity have been successfully run using CSL as a low cost nutrient source (12). In this report, our objective was to study the fouling of pervaporation membranes when using P2 and CSL media, and spent fermentation broth containing the newly developed hyper-butanol-producing *C. beijerinckii* BA101.

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EXPERIMENTAL

Membrane and Membrane Operation

A thin wall silicone tubing (wall thickness 0.6 mm, inside diameter 3.4 mm) was purchased from Fisher Scientific, and a membrane module was made from it. The silicone tubing (7.62 m) was cut into five equal pieces (1.52 m each). One end of each tube was connected to a common inlet while the other end was connected to a common outlet using two small glass bulbs. These bulbs had a single inlet and five outets. The membrane was then enclosed in a circular mode (airtight) in a 2-L glass vessel. The inside volume of the membrane was 250 mL and the membrane area based on inside diameter was 0.08 m^2. Five hundred milliliters of model butanol solution, fermentation medium, or fermentation broth was circulated through the membrane at a flow rate of 300–500 mL/min using an easy load II Masterflex pump (Cole-Palmer Instrument Company, Vernon Hills, IL). The experiments were conducted at a feed temperature of 41 ± 1°C (membrane feed out temperature 40.5 ± 0.5°C). The temperature of the feed solution was controlled using a magnetic stir bar/hot plate supplied by Corning. Figure 1 shows a simple schematic diagram of the experimental setup. Chemically resistant tubing (PharMed 65) was used for the feed pump. Sweep gas (air) was

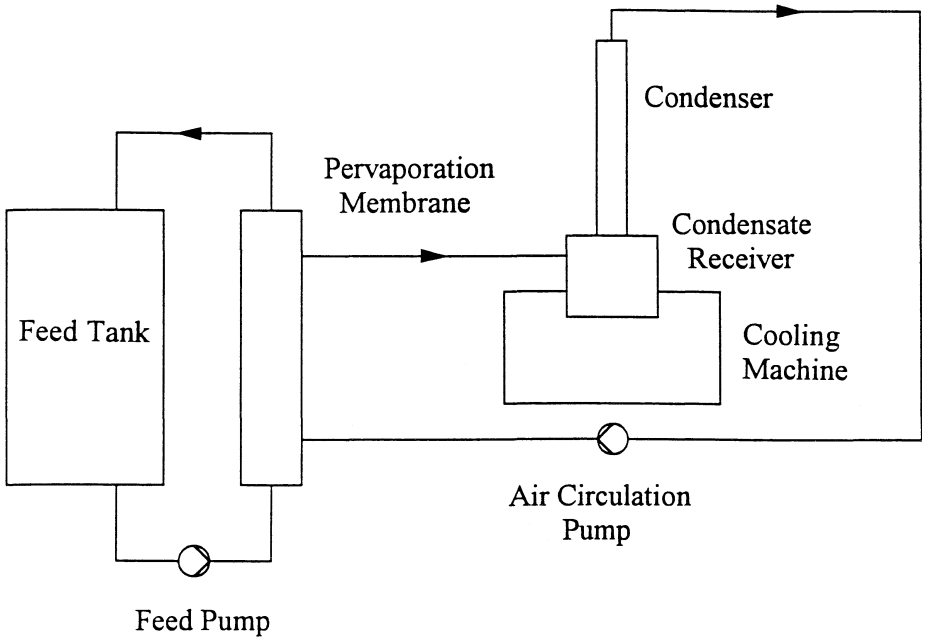

FIG. 1 A simple schematic diagram of butanol removal by pervaporation.

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circulated through the shell side of the module at a rate of 120 L/h using a blower (Gast Manufacturing Corporation; Regenair, Benton Harbor, MI) in order to remove diffused butanol vapors. The sweep gas was cooled in the condenser to condense butanol vapors, which were collected in order to measure selectivity and flux.

Condenser and Cooling Machine

A coolant was circulated through the condenser jacket at a flow rate of 600 mL/min. The temperature of the coolant (50% v/v, ethylene glycol) was maintained at 0 to −15°C, and it was confirmed that in this temperature range there was no effect on the amount of condensate recovered. The cooling machine (refrigerating circulator) was obtained from Beckman Instruments Inc., Palo Alto, CA (GeneLine cooler). A glass condenser (62 × 600 mm; inside cooling surface area 1292 cm^2) was used to condense the butanol vapors. A round-bottom ground socket (34/45) conical flask was used to collect the condensate from the condenser. The condensate was removed intermittently from the flask using a miniperistaltic pump (Fisher Scientific, Fair Lawn, NJ) and weighed in order to determine flux.

Fermentation

Culture and Culture Propagation

Details of the culture and media [P2 and CSL (also called CSW)] have been published elsewhere (12, 13). Spores of *C. beijerinckii* BA101 were stored at 4°C in sterile distilled water. The spores (0.1 mL) of *C. beijerinckii* BA101 were heat shocked in TGY (10 mL) medium for 10 minutes as described earlier (13), followed by incubation at 35 ± 1°C anaerobically (85% N_2, 10% CO_2, and 5% H_2) in a Coy anaerobic chamber (Coy Laboratory Products, Inc., Ann Arbor, MI). Following 16 hours, 5–8 mL of an actively growing culture was inoculated into 100 mL P2 medium containing 60–80 g/L glucose and 1 g/L yeast extract (Difco Laboratories, Detroit, MI). P2, TGY, and CSL media were kept in the anaerobic chamber for 24 hours prior to inoculation. TGY and P2 media were sterilized in an autoclave at 121°C for 15 minutes. Minerals, vitamins, and acetate buffer stock solutions of P2 medium were sterilized using a 0.2-μm micropore filter and added to the medium after cooling to room temperature. An inoculum for the CSL fermentation was prepared in P2 medium. The fermentations were conducted at 35°C for 48–72 hours in an anaerobic chamber. During this time the cells grew and produced about 23–26 g/L solvents.

Butanol Production in P2 Medium and CSL Medium

Butanol was produced in 500 mL P2 medium in 750 mL screw-capped bottles. For CSL fermentation, 500 mL glucose solution (60–80 g/L) was pre-

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pared and autoclaved at 121°C for 15 minutes followed by cooling anaerobically to room temperature. After cooling, 100 mL filter sterilized CSL was added. The pH of CSL was adjusted to 6.8 using NaOH pellets prior to sterilization. To CSL medium, 0.012 g/L hepta-hydrate ferrous sulfate filter sterile solution was added as the iron source. CSL medium was inoculated with 30–48 mL of actively growing *C. beijerinckii* cells in P2 medium. Cell growth and fermentation was carried out at 35°C for 72 hours anaerobically. All of the butanol fermentation studies reported here were conducted in an anaerobic chamber without any agitation. The pH of the culture was not controlled as the solventogenic culture regulates its own pH at 5–5.3. The final fermentation broths (P2 and CSL) were employed for the membrane fouling studies. The terms "broth" and "media" are used for post-fermentation and pre-fermentation of the fermentation media, respectively.

ANALYSES

Acetone, butanol, ethanol, acetic acid, and butyric acid were measured using a Hewlett-Packard Gas Chromatograph 6890 equipped with a flame ionization detector. A capillary column (crosslinked FFAP; dimension 30 m $\times$ 0.53 mm and film thickness 1 μm) was used for these measurements. Glucose was measured enzymatically using a Sigma Diagnostics Kit, Glucose HK (Sigma Chemicals, St. Louis, MO). For these determinations, absorbances were measured at 340 nm using a Beckman DU-40 Spectrophotometer. Cell concentration was determined by measuring the optical density at 540 nm. Selectivity was calculated as $\alpha = [y/(1-y)]/[x/(1-x)]$, where x and y are butanol weight fractions in feed and permeate, respectively. Flux is given as W/Ah, where W is weight of permeate (including solvent/s and water), A is membrane area, and h is time period during which permeate W was collected.

RESULTS AND DISCUSSION

Initially a 34.8 g/L butanol solution in distilled water was used to examine pervaporation and determine the rate of butanol removal through the membrane. It took 33 hours to reduce the butanol concentration to 4.9 g/L in the feed tank (Fig. 2). As the concentration of butanol in the flask decreased, so did the rate of removal. A 34.7 g/L butanol solution was also prepared in P2 medium, and the ability of the membrane to remove butanol from this solution was examined. To examine the possibility of fouling of the pervaporation membrane with CSL, a 34.8 g/L butanol solution was also prepared in CSL medium. CSL is complex in nature and would be expected to foul the pervaporation membrane. We routinely use CSL in our fermentation media to im-

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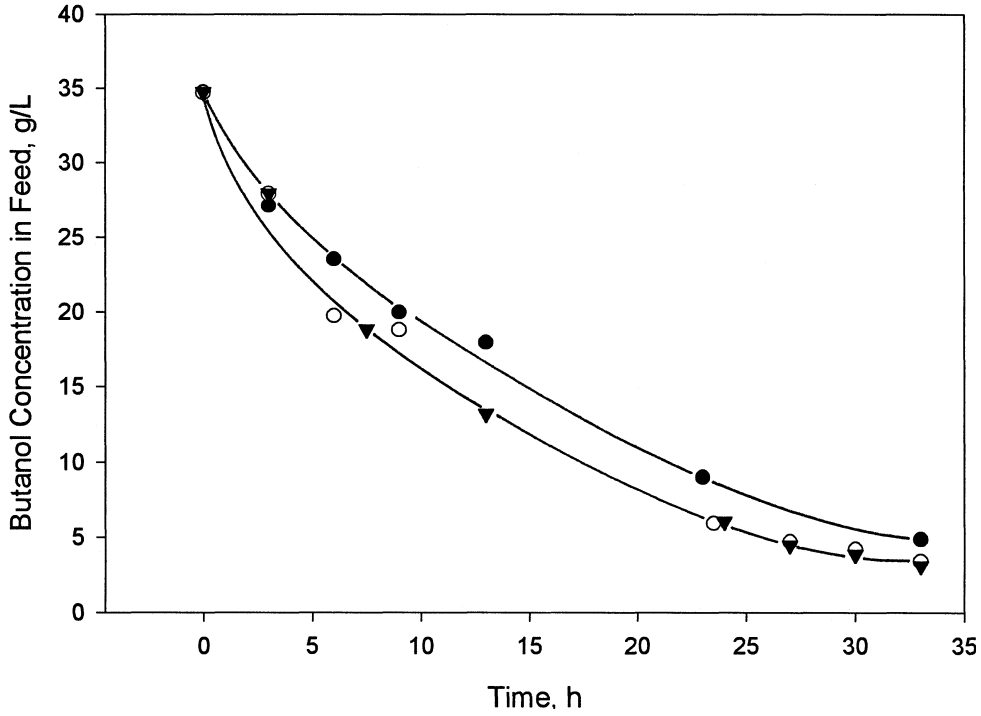

FIG. 2 Removal of butanol from butanol–water solution, and uninoculated P2 and CSL media by pervaporation: (●) butanol–water solution, (○) P2 medium, (▼) CSL medium.

prove the economics of butanol production. The removal of butanol from CSL medium was very similar to that observed for P2 medium. The rates of butanol removal from these model solutions are shown in Table 1. At low concentrations of butanol in the feed tank the rates of removal were low due to a low

TABLE 1
Rates of Removal of Butanol from Model Solutions and Fermentation Broth at Different Butanol Concentrations

Butanol concentration (g/L)	Butanol in water (g/L·h)	Butanol in P2 or CSL medium (g/L·h)	Butanol in CSL fermentation broth (g/L·h)	Butanol in P2 fermentation broth (g/L·h)
29.3	1.79	1.82		
20.0	1.23	1.24		
13.5	0.93	0.93	2.50	1.64
6.3	0.58	0.58	0.92	0.58
3.0	0.14	0.12	0.56	—[a]

[a]Not measured.

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concentration gradient across the membrane. A comparison of the data from experiments demonstrated that neither P2 nor CSL medium fouled the membrane. Since CSL was filtered through a 0.2-μm filter, the possibility of fouling the membrane by suspended solids was eliminated. Pervaporation membrane fouling has been shown to occur due to suspended solids (8, 10), chemicals, and macromolecules (15).

Butanol fermentation broth is usually viscous in nature due to the fact that it contains polysaccharides and cells (16) which may foul the membrane. To examine this possibility, fermentations were carried out using P2 and CSL media. The dry weight cell concentration in the fermentation broth was 2.0–3.0 g/L (wet weight 6.7–10 g/L). Following a 72-hour fermentation, *C. beijerinckii* BA101 produced 26.4 g/L total solvents in P2 medium and 23.9 g/L total solvents in CSL medium. This is similar to the results obtained previously for this strain (12, 13). The concentration of glucose, individual solvents, and solvent yield at the end of fermentation are shown in Table 2. The removal of butanol from P2 and CSL fermentation broth over time is shown in Fig. 3. Cells were not removed from the fermentation broth prior to pervaporation. The rates of removal of butanol from the two fermentation broth are presented in Table 1. Interestingly, the rates of butanol removal from the fermentation broth are not lower than the rates of removal from either butanol–water solution or butanol–P2/CSL medium.

Other than butanol, the fermentation broth also contained acetone, ethanol, acetic, and butyric acid (Table 2). Acetone and ethanol must also be removed from the fermentation broth for economic reasons. The pervaporation membrane removed both acetone and ethanol (Table 3). Selectivities for acetone

TABLE 2
Production of Various Products Following Growth of *C. beijerinckii* BA101 in P2 and CSL Media

	P2 fermentation medium (g/L)	CSL fermentation medium (g/L)
Acetone	5.8	5.4
Butanol	20.2	17.8
Ethanol	0.3	0.6
Acetic acid	0.1	0.7
Butyric acid	0.8	0.7
Total solvents	26.4	23.9
Total acids	0.8	1.4
Initial glucose	65.9	68.1
Final glucose	1.6	5.3
Solvent yield	0.41	0.38

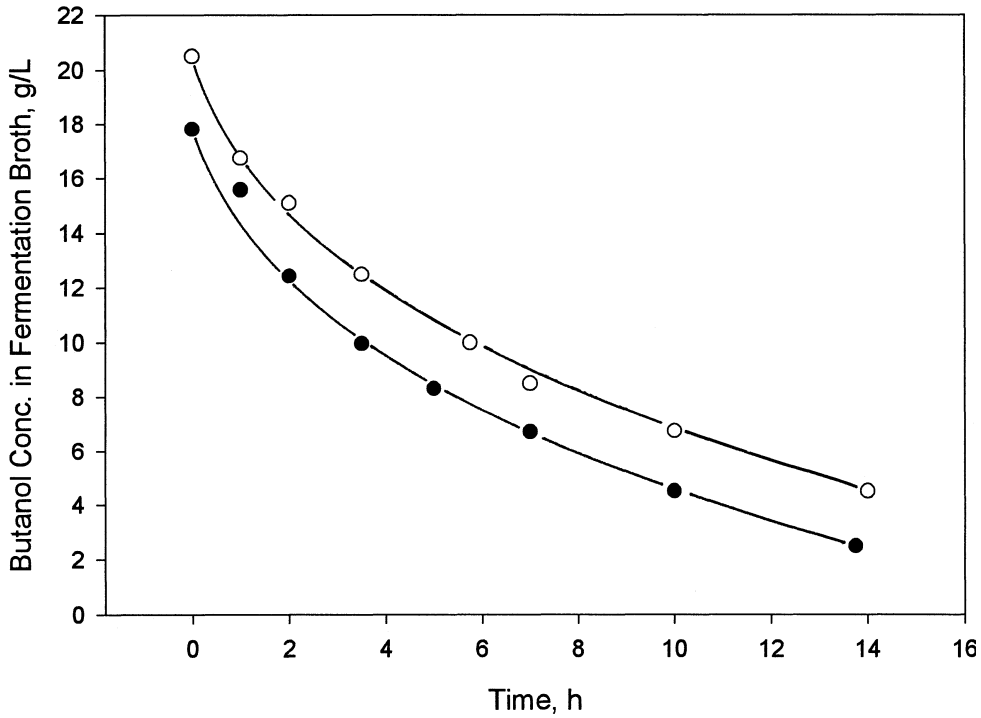

FIG. 3 Removal of butanol from P2 and CSL medium fermentation broth by pervaporation: (○) P2 medium fermentation broth, (●) CSL medium fermentation broth.

ranged from 4.54 to 7.61, while that for ethanol ranged from 3.31 to 5.85. These results suggest that the membrane is selective for both acetone and ethanol. Acetic and butyric acids were identified only in the first pervaporate sample, and their selectivities were 1.51 and 0.48, respectively.

TABLE 3
Removal of Acetone, Ethanol, Acetic Acid, and Butyric Acid from P2 Medium Fermentation Broth of *C. beijerinckii* BA101 Using Pervaporation[a]

Time, (h)	AC_2O concentration in feed (g/L)	AC_2O selectivity	EtOH concentration in feed (g/L)	EtOH selectivity	HAc concentration in feed (g/L)	HAc selectivity	HBu concentration in feed (g/L)	HBu selectivity	Total flux ($g/m^2 \cdot h$)
0.00	5.8		0.3		0.9		0.8		
3.00	4.6	4.54	0.3	3.31	0.7	1.51	0.9	0.48	45.62
6.20	4.4	5.48	0.3	3.64	0.6	0.00	0.3	0.00	36.13
22.40	2.7	7.61	0.2	5.85	0.7	0.00	0.3	0.00	32.21

[a] AC_2O, acetone; EtOH, ethanol; HAc, acetic acid; HBu, butyric acid.

The rates of butanol removal from CSL fermentation broth were higher than those for P2 fermentation broth, butanol in water, and CSL medium (Table 1). P2 medium is a rich source of nutrients compared to CSL and results in higher cell concentration and cellular by-products. The cellular by-products along with the cells may be a reason for the reduced rate of butanol removal from P2 medium fermentation broth. These experiments with P2/CSL fermentation broth suggested that the membrane was not fouled with *C. beijerinckii* BA101 fermentation broth. It may be that neither the cells polarized the membrane nor chemicals present in fermentation broth fouled it. Concentration polarization reduces the flux rate through the membrane.

In order to compare butanol selectivities, additional experiments were run at different butanol concentrations in the feed tank, and butanol selectivities and flux rates were calculated for five different experiments (butanol solution in water, butanol in P2 and CSL media, and P2 and CSL fermentation broth). The butanol selectivities are plotted in Fig. 4(a). At low butanol concentration in the feed, butanol selectivities are high, and at high butanol concentration in the feed they are low. This is because of the selectivity and feed butanol weight fraction (x) relationship. At low values of x, high values of selectivity are achieved, and at high values of x low values of selectivity are obtained. Several authors have mentioned this type of relationship (17–19). The curve represents data on butanol in water, butanol in P2 medium, and butanol in CSL medium and P2 medium fermentation broth. The data points for the CSL fermentation broth are scattered on both sides of the curve. It is not clear why there was so much variability in the butanol selectivities obtained from the CSL fermentation broth. The butanol selectivities ranged from 20 to 55 over the concentration range of 33.0 to 3.33 g/L.

Flux values over the same butanol concentration range were 45 to 25 g/m^2·h (Fig. 4b). Flux, as is the case with selectivity, is a function of butanol concentration in feed. A higher feed butanol concentration results in a higher flux, and a lower feed butanol concentration results in lower flux. The flux is high for a silicone membrane, when comparing membrane thickness and operational conditions (20). Membrane thickness, vacuum or sweep gas, butanol concentration, and temperature all affect butanol flux through the membrane (20). An examination of the flux data shows that the membrane was not fouled with P2 or CSL fermentation media or fermentation broth.

The pervaporate butanol concentrations (instant) at the different feed butanol concentrations from the above five experiments are plotted in Fig. 5. The pervaporate butanol concentration ranged from 458 to 122.9 g/L depending upon the butanol concentration in the feed. The amount of water in the permeate is calculated to be from 434.6 to 848.3 g/L, respectively, at the above permeate butanol concentrations. At less than 4 g/Lbutanol in the feed, butanol concentration in the pervaporate was over 120 g/L, and at 34 g/L bu-

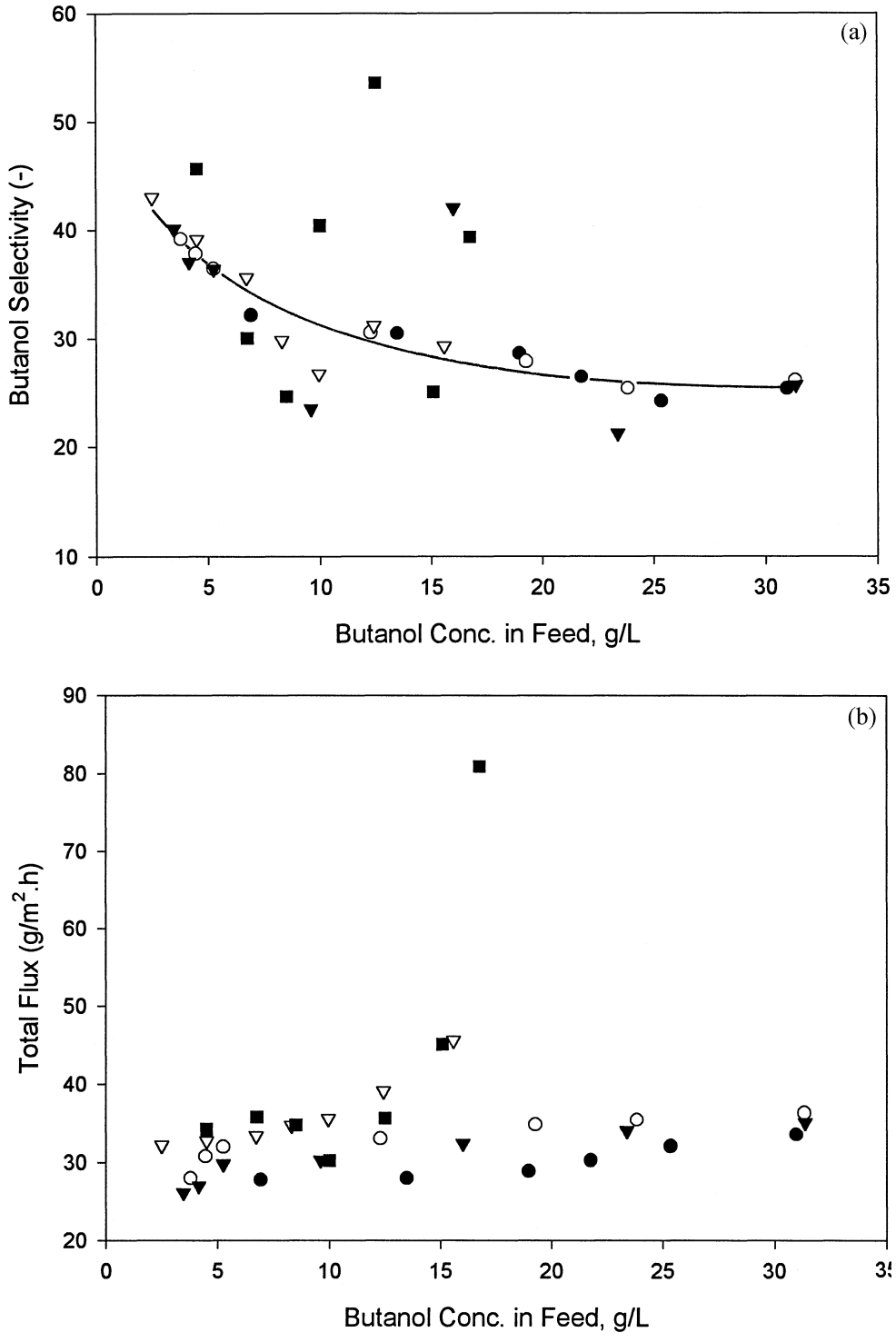

FIG. 4 Butanol selectivities (a) and total flux (b) at different butanol concentrations: (●) butanol–water solution, (○) P2 medium–butanol, (▼) CSL medium–butanol, (▽) P2 medium fermentation broth, (■) CSL medium fermentation broth.

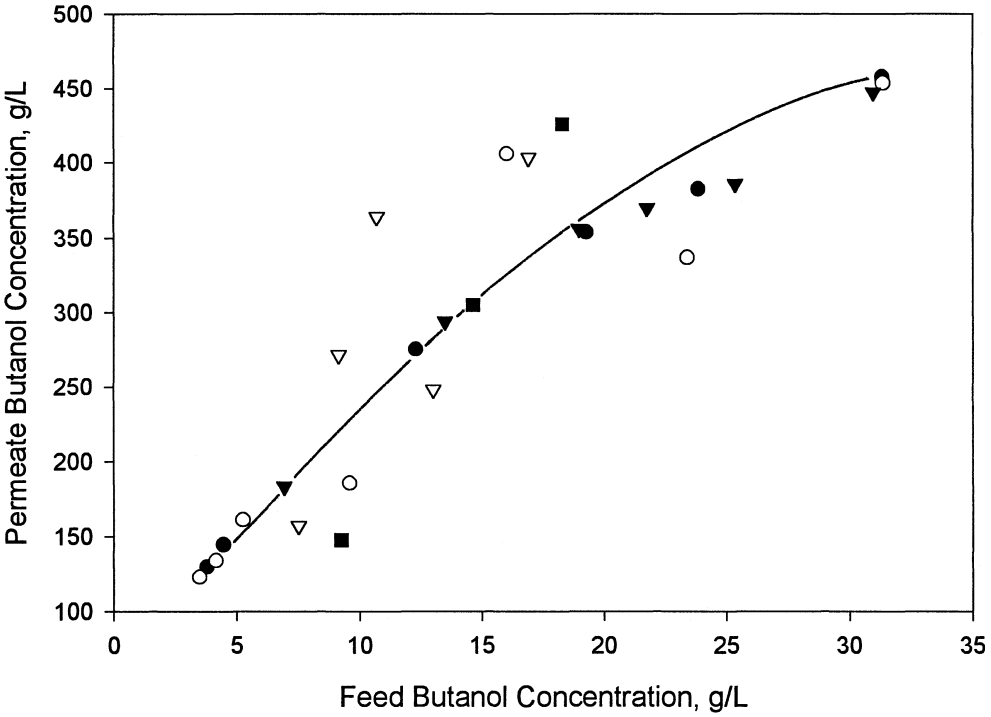

FIG. 5 Pervaporate butanol concentrations at different butanol feed concentrations: (▼) butanol–water solution, (●) P2 medium–butanol, (○) CSL medium–butanol, (■) P2 medium fermentation broth, (▽) CSL medium fermentation broth.

tanol in the feed, the pervaporate butanol concentration was 458 g/L. Above 78 g/L butanol, the permeate separated into two phases: a butanol-rich organic phase containing over 600 g/L butanol and an aqueous phase containing butanol at 78 g/L (19). To further purify butanol either from the 78 or 600 g/L phase, distillation may be the most appropriate option. This is because the membrane removes acetone and ethanol in addition to butanol.

Pervaporation membrane fouling during ethanol fermentation using *Kluyveromyces fragilis* (21) and *Clostridium thermohydrosulfuricum* (15) has been reported. Mori and Inaba (15) reported a reduction in flux by 50% using fermentation broth. Udriot et al. (21) mentioned that flux decreased by 20% in their 18-hour experiment. In contrast to Mori and Inaba (15) and Udriot et al. (21), Gundernatsch et al. (22) reported that there was no flux decrease over a period of 28 days when ethanol was produced using *Saccharomyces cerevisiae*. From these studies it appears that fouling is membrane, media, and culture specific. Our studies can not be compared with these studies because of differences in the culture and commercial media employed. We studied fouling in the batch mode using commercial fermentation media and spent fermentation broth of newly developed *C. beijerinckii* BA101. In an experiment

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the membrane was exposed to spent CSL fermentation broth for 10 days and the flux was found to be unaffected (flux range 37.5–31.4 g/m^2·h). Further pervaporation results on butanol recovery during ABE fermentation will be reported later.

CONCLUSIONS

A comparison of the butanol–water solution data when using either P2 or CSL fermentation media and P2 or CSL fermentation broth indicates that the pervaporation membrane was not fouled by the fermentation media or spent fermentation broth. *C. beijerinckii* BA101 batch fermentations using P2 and CSL media resulted in 26.4 and 23.9 g/L total solvents, respectively. Butanol selectivities ranged from 20 to 55 depending upon the butanol concentration in the feed. Total flux for the model solutions and fermentation broth ranged from 25 to 45 g/m^2·h. Butanol concentration in the pervaporate was 122.9 to 458 g/L, depending upon the concentration of butanol in the feed.

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