Title

Block copolymer pervaporation membrane for in situ product removal during acetone–butanol–ethanol fermentation

Authors

Shin, Chaeyoung
Baer, Zachary C
Chen, X Chelsea
et al.

Publication Date

2015-06-01

DOI

10.1016/j.memsci.2015.03.005

Peer reviewed

Journal of Membrane Science 484 (2015) 57–63

Contents lists available at ScienceDirect

Journal of Membrane Science

journal homepage: www.elsevier.com/locate/memsci

Block copolymer pervaporation membrane for in situ product removal during acetone–butanol–ethanol fermentation

Chaeyoung Shin [a,b,1], Zachary C. Baer [a,b,1], X. Chelsea Chen [c], A. Evren Ozcam [a,b], Douglas S. Clark [a,b,*], Nitash P. Balsara [a,b,c,**]

[a] Department of Chemical and Biomolecular Engineering, University of California, Berkeley, CA 94720, USA
[b] Energy Biosciences Institute, University of California, Berkeley, CA 94704, USA
[c] Material Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA

ARTICLE INFO

Article history:
Received 1 December 2014
Received in revised form
27 February 2015
Accepted 2 March 2015
Available online 11 March 2015

Keywords:
Pervaporation
Block copolymer membrane
ABE fermentation
In situ product removal

ABSTRACT

We address two major challenges facing commercialization of acetone–butanol–ethanol (ABE) fermentation: product inhibition and low productivity. We studied a polystyrene-*b*-polydimethylsiloxane-*b*-polystyrene (SDS) triblock copolymer membrane for selective removal of butanol from aqueous solutions by pervaporation. The SDS membrane exhibited higher permeabilities than a commercially available cross-linked polydimethylsiloxane (PDMS) membrane. Both types of pervaporation membrane were also used for in situ product removal of ABE biofuels in *Clostridium acetobutylicum* fermentations operated in a semi-continuous mode. Membrane performance and its effect on the fermentation process were assessed by measuring flux, OD_{600} and concentrations of different components in the fermenter as a function of time. Volumetric ABE productivity increased from 0.45 g/(L h) in simple batch fermentation to 0.66 g/(L h) in the case of pervaporative-fermentation with the PDMS membrane. A further increase in productivity to 0.94 g/(L h) was obtained in the case of pervaporative-fermentation with the SDS membrane. Overall, total ABE production improved by a factor of three, viable fermentation time increased by a factor of two, and cell density increased by a factor of 2.5 upon applying SDS membrane pervaporation, relative to the batch process.

Published by Elsevier B.V.

1. Introduction

There is considerable effort underway to replace fossil fuels with biofuels produced from renewable resources [1]. Biobutanol is more attractive than first generation bioethanol because it has higher energy density, lower miscibility with water, and lower vapor pressure. *Clostridium acetobutylicum*, *Clostridium beijerinckii*, and *Clostridium saccharoperbutylacetonicum* are commonly used microbes for producing biobutanol. In addition to butanol, however, these microbes also produce acetone and ethanol. Acetone-butanol-ethanol fermentation (ABE fermentation) by *C. acetobutylicum* occurs in two phases: an acidogenesis phase wherein the microbes mainly produce acetic acid and butyric acid, followed by a solventogenesis phase wherein the microbes mainly produce ABE [1–3].

Two significant challenges facing commercialization of ABE fermentation are: (1) product inhibition (this means that the products of fermentation are toxic to the microorganisms), and (2) low ABE productivity. ABE fermentation normally stops when the total ABE concentration is 2 wt% [2]. Conventional production of biofuel is carried out in a batch process. In the case of ABE fermentation, biofuel is only produced during the second phase of batch fermentation. Afterwards, the fuel is typically separated from the reaction broth by distillation. The availability of in situ product removal methods will lead to better utilization of the microorganisms and higher volumetric productivities, and may ultimately enable continuous biofuel production [4,5]. Methods for in situ product removal include liquid-liquid extraction [6,7], adsorption [8], and pervaporation [9–13]. However, none of these technologies has been scaled-up for industrial use.

The purpose of this study is to compare different pervaporation membranes for continuous ABE fermentation at high cell densities. Pervaporation has advantages over other technologies in that it has better selectivity toward the ABE and is less invasive to the cells [14,15]. However, the flux of biofuels through currently available membranes is low, and this limits the efficacy of the separation process. The membrane material most widely used for

* Corresponding author at: Department of Chemical and Biomolecular Engineering, University of California, Berkeley, CA 94720, USA. Tel.: +1 510 642 2408.
** Corresponding author at: Department of Chemical and Biomolecular Engineering, University of California, Berkeley, CA 94720, USA. Tel.: +1 510 642 8973.
E-mail addresses: dsc@berkeley.edu (D.S. Clark), nbalsara@berkeley.edu (N.P. Balsara).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.memsci.2015.03.005
0376-7388/Published by Elsevier B.V.

biofuel purification is polydimethylsiloxane (PDMS) [8–13]. For example, Van Hecke et al. [12] attached a pervaporation module with a PDMS membrane to a two-stage chemostat, and increased total ABE productivity from 0.13 g/(L h) to 0.30 g/(L h). From an industrial point of view, it would be desirable to retain the cells in the reactor and remove only the fuel. Also, higher culture densities promote greater productivities [16]. The possibility of using a PDMS membrane for this mode of operation was recently explored by Li et al. [13], who concluded that fermentation productivity is improved when assisted by pervaporation.

A shortcoming of PDMS is that it is a soft rubber. Increasing the rigidity of PDMS membranes is usually accomplished by increasing cross-linking density. In this paper, we use block copolymer self-assembly to improve the mechanical properties of PDMS-based membranes. Polystyrene (PS) blocks are covalently bonded at the ends of PDMS chains to produce a polystyrene-*b*-polydimethylsiloxane-*b*-polystyrene (SDS) triblock copolymer. Microphase separation results in the formation of mechanically rigid PS cylinders in a PDMS matrix. Thin films of SDS were coated onto a commercial polyethersulfone support and used in a pervaporation module that was attached to an ABE fermentation reactor. The fermentation was started in batch mode until the acidogenesis phase was completed. The reactor was then operated in a continuous mode with a feed stream comprising concentrated medium and an ABE product stream separated by pervaporation. Advantages due to the high flux of ABE through the SDS membrane are quantified by repeating the same experiment with a commercially available PDMS membrane in the pervaporation module.

2. Experimental

2.1. Membrane preparation

A SDS copolymer with PS block molecular weights of 22 kg/mol and PDMS block molecular weight of 104 kg/mol was purchased from Polymer Source. 60 wt% of the sample was the SDS triblock copolymer, 30 wt% was the polystyrene-*b*-polydimethylsiloxane diblock copolymer, and 9.3 wt% was PS homopolymer (Viscotek GPC, Malvern). The polydispersity index of the polymer was 1.3 and the volume fraction of PDMS was 72% in the triblock copolymer. The same polymer was used in reference [17] A supporting membrane (Biomax PBHK100205), purchased from Millipore, consisted of a porous polyethersulfone layer with a pore size cutoff of 100 kg/mol, and a non-woven polyester layer beneath the polyethersulfone. 1 g of SDS was dissolved in 20 mL of cyclohexane (Sigma Aldrich, used as received). The supporting membrane was cut into a 10×10 cm^2 square and attached onto a 3 in diameter silicon wafer using double-sided tape, with the polyethersulfone layer facing upward. The silicon wafer with the supporting membrane attached was placed on a spin coater, and 6 mL of the SDS/cyclohexane solution was placed on the membrane, thoroughly covering the entire area of the membrane. The polymer was spin-coated at 300 rpm for 40 s. The membrane was then dried at room temperature for a day. A commercially available supported PDMS membrane was purchased from Pervatech. Each pervaporation experiment was conducted on a different piece of circular SDS or PDMS membrane (area = 37 cm^2).

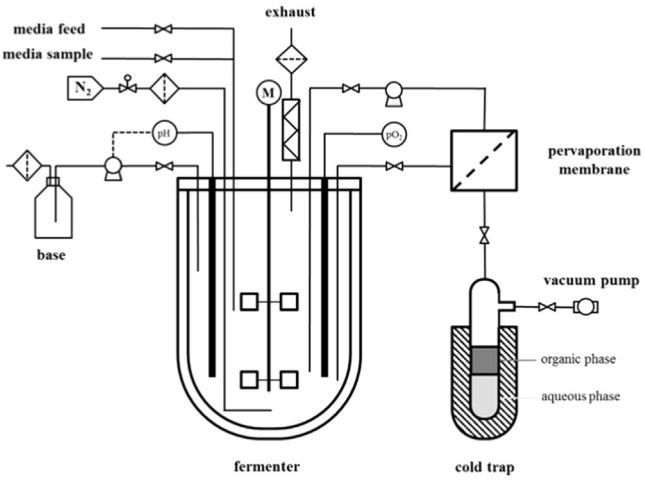

Fig. 1. Schematic diagram of the pervaporative-fermentation setup.

2.2. Scanning and transmission electron microscopy

Cross-sectional scanning electron microscopy (SEM) samples were obtained by cryo-fracturing the membranes with support in liquid nitrogen. Samples were sputter coated with 5 nm of Au before imaging. SEM images were obtained on a Zeiss ULTRA 55 analytical SEM operating at 5 kV.

Thin transmission electron microscopy (TEM) samples with thicknesses of approximately 120 nm were microtomed at −120 °C on a Leica EM FC6 and picked up on lacey carbon coated copper grids (Electron Microscopy Sciences). TEM experiments were conducted on a Philips CM 200 FEG using acceleration voltage of 200 keV. Double tilt series images were collected in the angle range −60–60° for each tilt series. Exposure time for image collection was set to 1 s. Fiducial gold with 5 nm diameters were deposited on the sample to facilitate alignment of the tilt series images. Alignment and reconstruction were done using the IMOD tomographic reconstruction software package. The reconstructed tomogram was segmented and colored using Avizo Fire.

2.3. Aqueous butanol pervaporation experiments

Pervaporation experiments with 2 wt% aqueous butanol solutions were conducted on a bench top unit manufactured by Sulzer Chemtech, as described in references [17,18]. The SDS and PDMS membranes were placed in a membrane holding module and the butanol solution feed was pumped across the membrane at a rate of 3 L/min. The membrane temperature was maintained at 37 °C. On the permeate side of the membrane, a vacuum of ~2 mbar was applied using a vacuum pump (Welch, model 2014) and the permeate stream was condensed in a cold trap using dry ice/isopropanol at −70 °C. The permeate was collected in a cold trap for 30–60 min. The permeate phase-separates into a butanol-rich phase and a water-rich phase. After measuring the mass, the permeate was diluted with water to form a single phase solution and the ABE concentrations were measured by high performance liquid chromatography (HPLC) using a Prominence UFLC instrument (Shimazu). The compositions of both the feed and permeate streams were monitored by HPLC as a function of time. Average values of four separate permeate collections are presented.

2.4. Fermentation

All fermentations were carried out with *Clostridium acetobutylicum* ATCC824 purchased from the American Type Culture Collection (Manassas, VA, USA). *C. acetobutylicum* cultures were inoculated and cultivated in clostridia growth medium (CGM, in g /L: glucose 70, yeast extract 5, ammonium acetate 2, sodium chloride 1, potassium phosphate monobasic 0.75, potassium phosphate dibasic 0.75, L-cysteine-hydrochloride monohydrate 0.5, magnesium sulfate heptahydrate 0.1, ferrous sulfate heptahydrate 0.01, manganese sulfate monohydrate 0.01). All cultures were

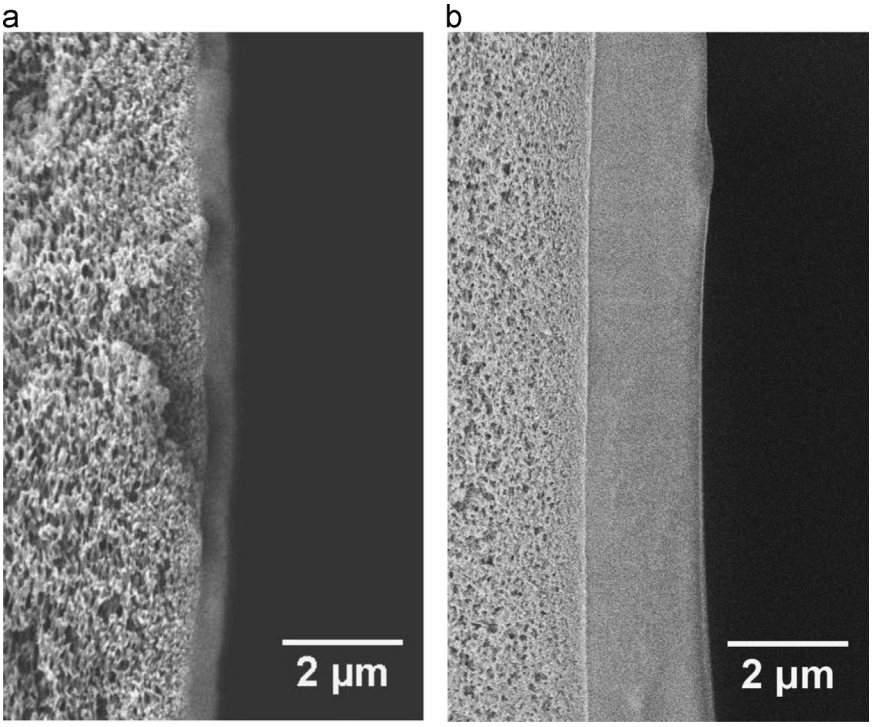

Fig. 2. Scanning electron microscopy image of the (a) PDMS and (b) SDS membrane cross-sections.

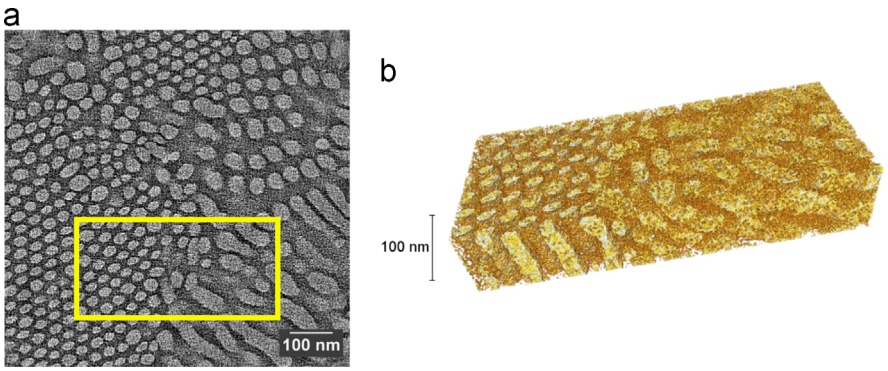

Fig. 3. (a) Transmission electron microscopy image of a free-standing SDS membrane. (b) Reconstructed 3D image of region inside the yellow box in (a).

Table 1
Data from the binary butanol/water pervaporation experiment is shown from the SDS membrane and the PDMS membrane. Flux of each component was measured and the separation factor, permeability, and selectivity were calculated.

membrane material	Thickness (μm)	Butanol flux (g/m^2 h)	Water flux (g/m^2 h)	Separation factor β	Butanol permeability $\times 10^{12}$ (mol m/ m^2 s Pa)	Water permeability $\times 10^{12}$ (mol m/ m^2 s Pa)	Selectivity α
SDS	2	420	1600	24	13	8.2	1.6
PDMS	0.5	170	1100	16	1.3	1.3	1.0

maintained in CGM with 25 wt% glycerol for long-term storage at −80 °C. Fermentation precultures were started by the addition of 0.5 mL glycerol stock in 10 mL of CGM and incubated at 37 °C overnight.

Fermentations at the 1.25 L and 1.0 L scale were carried out in 3-L bioreactors (Bioengineering AG, Switzerland) with a 2-L working volume. Seed cultures of CGM (100 mL) were inoculated with the preculture described above (4 mL), and grown in 150 mL anaerobic serum bottles at 37 °C until the OD_{600} (optical density at a wavelength of 600 nm) reached 2.0. 60 mL of the seed culture was used to inoculate the bioreactor. The bioreactor was equipped with an automatic controller that maintained pH ≥ 5.0 during the fermentation, using a 5 M KOH solution. Nitrogen gas was sparged into the bioreactor at a rate of 200 mL/min to maintain an anaerobic environment. To minimize losses of volatiles, specifically the loss of acetone through the gas exhaust port, a cooling condenser attached to a RTE7 water bath (4 °C) was used. Agitation in the bioreactor was set to 200 rpm and the fermentation temperature was held at 37 °C.

A Shimadzu Prominence HPLC system equipped with both an RID and DAD detector was used to analyze metabolite concentrations for glucose, acetate, butyrate, acetone, butanol, and ethanol. Samples

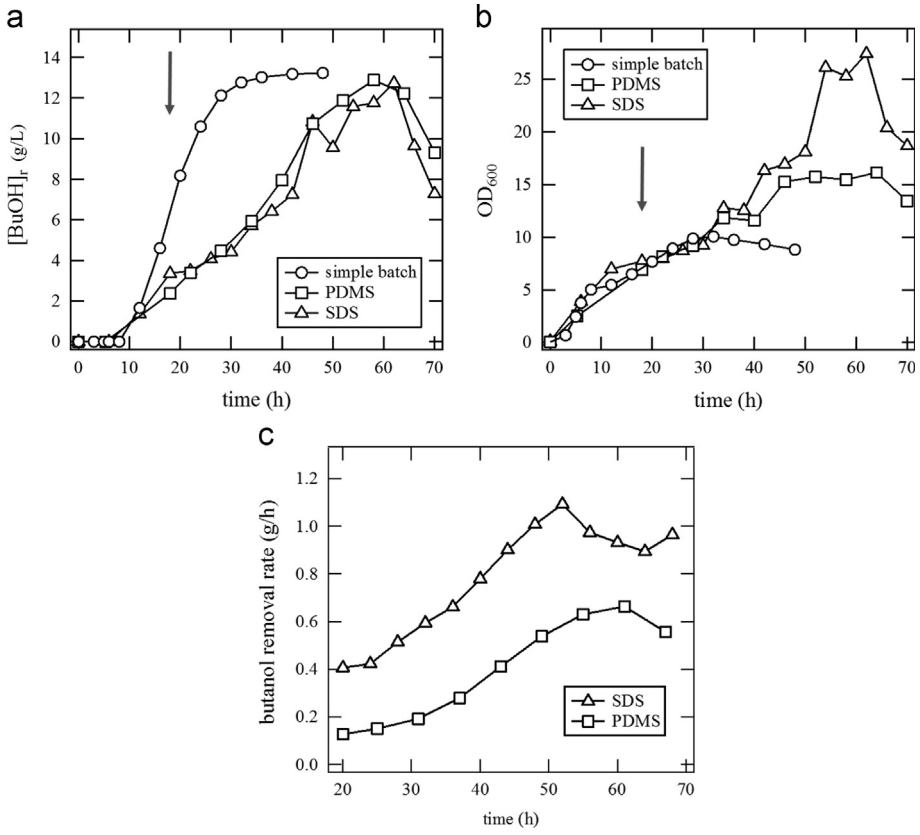

Fig. 4. (a) Butanol concentrations, (b) cell optical density, and (c) butanol removal rate through the membranes versus fermentation time for the simple batch fermentation (○), pervaporative-fermentation with PDMS membrane (□), and pervaporative-fermentation with SDS membrane (Δ). Lines in plots were drawn to guide the eye and arrows depict when the pervaporation module was initiated.

were injected onto a Bio-Rad Aminex HPX-87H column with a Cation H guard column equilibrated to 35 °C. A mobile phase of 5 mM H_2SO_4 was pumped through the column at 0.7 mL/min. Concentrations were determined based on a six-point calibration. Glucose consumption rates were determined from real-time glucose concentration measurements on a YSI Biochemistry Analyzer equipped with a glucose membrane.

The glucose concentration in the reactor was measured in real time every 4–6 h and used to determine the microbe's glucose consumption rate. Based on the calculated glucose consumption rates from the previous time points a concentrated feed (500 g/L glucose, 50 g/L yeast extract) was pulsed into the fermenter in order to maintain a glucose concentration between 5–40 g/L between time points.

2.5. Pervaporative-fermentation

The bioreactor described above was also used in the pervaporation experiments. Fig. 1 shows a schematic of the pervaporative-fermentation setup. Part of the fermentation broth was circulated into a pervaporation module that was identical to that used in the aqueous butanol pervaporation experiments described above. The main difference was that liquid nitrogen was used in the cold trap due to the volatility of acetone and ethanol.

The pervaporation module was connected after 18 h of simple batch fermentation to ensure commencement of the solventogenesis phase. This marked the start of the continuous pervaporative-fermentation experiment wherein glucose in the reactor was monitored and concentrated media was manually fed into the bioreactor. The glucose concentration in the reactor was measured every 4–6 h and the amount of glucose consumed by the microbes during that time was added in the reactor via a concentrated feed (500 g/L glucose, 50 g/L yeast extract).

2.6. Permeability calculations

The relation between molar flux of permeated species i, J_i, and permeability of species i, P_i, is given by Wijmans and Baker [19]

$$J_i = \frac{P_i}{t}\left(x_i\gamma_i p_i^{sat} - y_i p_{permeate}\right) \quad (1)$$

here t is the thickness of the membrane, x_i is the feed mole fraction, γ_i is the activity coefficient, p_i^{sat} is the saturated vapor pressure at feed conditions, y_i is the permeate mole fraction, and $p_{permeate}$ is total permeate pressure. Activity coefficients were calculated by the van Laar equation, and saturation vapor pressures of the pure components were calculated from the Antoine equation [20].

Selectivity of species i (α_i) is a measure of the enrichment of species i in comparison to water by permeation through the membrane. Subscripts B and W denote butanol and water, respectively

$$\alpha_B = P_B/P_W \quad (2)$$

Separation factor (β_i) of species i, which is used commonly to determine membrane separation performance, was calculated with the following equation:

$$\beta_B = \frac{X_B}{X_w}/\frac{Y_B}{Y_w} \quad (3)$$

here X_i and Y_i are weight concentrations of i in the feed and permeate, respectively.

3. Results and discussion

3.1. Membrane characterization

The cross-sectional structure of the supported SDS and PDMS membranes used in pervaporative-fermentation was studied by SEM and the results are shown in Fig. 2. The thickness of the commercial PDMS membrane is about 0.5 μm while that of our SDS membrane is 2 μm. The nanoscale morphology of the SDS copolymer was studied by transmission electron microtomography experiments conducted on thin sections of a free-standing membrane made by solvent casting with the same solution that was used to make the supported SDS membranes, and the results are shown in Fig. 3. The cross-sectional view in Fig. 3a shows hexagonally packed bright PS cylinders in a dark PDMS matrix. The average length of the PS cylinders is relatively short, and this is more clearly seen in the three-dimensional tomogram shown in Fig. 3b. It is likely that this short cylinder length is due to the finite polydispersity index of our copolymer. The ends of the cylinders can be regarded as topological defects, and the high concentration of topological defects may be related to the presence of uncoupled diblock copolymer and PS homopolymer in the sample and the relatively high polydispersity index of the block copolymer. Selective transport of organics through the membrane occurs primarily through the dark PDMS matrix phase in Fig. 3 [17].

Pervaporation experiments with a 2 wt% aqueous butanol solution were performed using both SDS and PDMS membranes. Butanol was chosen as the organic constituent because it is the major product and the most toxic biofuel produced in *C. acetobutylicum* fermentations [2]. Table 1 shows the results of these experiments where the flux and permeability of butanol and water through the two membranes are listed. Despite the fact that the commercial PDMS membrane is significantly thinner than the SDS membrane, the butanol flux through the SDS membrane is 2.5-fold greater than that through the PDMS membrane. The water flux through the SDS membrane is only 1.5-fold larger than that through the PDMS membrane. It is likely that the lower flux through the PDMS membrane is due to the cross-linked nature of the membrane; the PDMS chains in the SDS membrane are not cross-linked. One might expect lower flux through the SDS membranes due to the presence of rigid PS domains that do not transport butanol and water effectively. However, rapid butanol and water transport, due to the non-cross-linked nature of the PDMS chains in SDS more than compensates for this effect. Stretching of the PDMS blocks due to microphase separation may also affect transport properties of the SDS membranes [17,21]. The differences in fluxes between the membranes are manifested in the butanol and water permeability reported in Table 1. Butanol selectivity, α, is significantly higher for the SDS membrane (Table 1).

In a previous publication, we described pervaporation of aqueous ethanol mixtures through SDS membranes [17]. The thicknesses of the membranes used in that study were 100–150 μm. In contrast, the membranes used in this study were 2-μm thick. The butanol permeability through a 150-μm thick SDS membrane is 25×10^{-12} mol m/m^2 s Pa. The butanol permeability reported in Table 1 is much lower than this value. Further work is needed to identify the reasons for the observed thickness dependence of permeabilities through block copolymer membranes. Similarly, the permeabilities of 100-μm thick PDMS membranes reported in reference [22] is significantly higher than that given in Table 1 for a 0.5-μm thick membrane. Offeman and Ludvik have shown that the permeability of ethanol through PDMS membranes decreases with decreasing thickness [23].

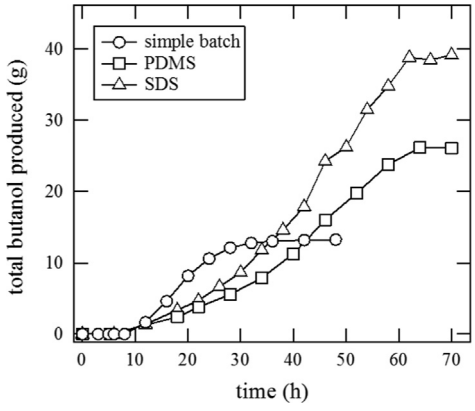

Fig. 5. Total amount of butanol produced versus fermentation time for the simple batch fermentation, pervaporative-fermentation with SDS membrane, and pervaporative-fermentation with PDMS membrane. Lines in the plot were drawn to guide the eye.

3.2. Pervaporative-fermentation

Results were obtained for three fermentation experiments: (1) A simple batch fermentation in which pervaporation was not used to remove the products from the bioreactor. (2) Pervaporative-fermentation with the SDS membrane. (3) Pervaporative-fermentation using the commercial PDMS membrane. The fermentation volume was 1.25 L for the three experiments.

Fig. 4a shows the butanol concentration in the bioreactor ($[BuOH]_r$) at different time points during the three fermentations. Butanol, the major product in *C. acetobutylicum* fermentations, is

Table 3
Flux of each component was measured for both SDS and PDMS membranes. $\beta_{butanol}$, $\beta_{acetone}$, and $\beta_{ethanol}$ are separation factors for butanol, acetone, and ethanol, respectively.

Membrane material	Water flux (g/m^2 h)	Butanol flux (g/m^2 h)	Acetone flux (g/m^2 h)	Ethanol flux (g/m^2 h)	$\beta_{butanol}$	$\beta_{acetone}$	$\beta_{ethanol}$
SDS	1300	220	91	23	21	22	5.8
PDMS	770	110	55	6	14	19	6.4

Table 2
Glucose consumption, ABE produced, volumetric productivity, specific productivity, and yield are measured and calculated for the simple batch fermentation, pervaporative-fermentation with the PDMS membrane, and pervaporative-fermentation with the SDS membrane. Yield is defined as (moles of glucose equivalent converted to ABE/moles of glucose consumed). The glucose equivalences were calculated assuming 1:1, 1:1, and 1:2 stoichiometric ratios for glucose to acetone, butanol, and ethanol.

Fermentation type	Glucose consumed (g)	ABE produced (g)			Volumetric productivity (g of ABE/L h)	Specific productivity (g of ABE/OD_{600} L h)	Yield (wt%)
		Acetone	Butanol	Ethanol			
Simple batch	83.45	5.83	16.56	4.80	0.45	0.091	81
PDMS	198.57	17.18	32.65	3.71	0.66	0.065	72
SDS	275.67	20.13	48.98	8.63	0.94	0.071	77

Table 4
Permeabilities of butanol and water calculated from the pervaporative-fermentation data and compared with permeability values from the binary butanol/water pervaporation experiments.

Membrane material	Model pervaporation			Fermentation pervaporation		
	Butanol permeability $\times 10^{12}$ (mol m/m^2 s Pa)	Water permeability $\times 10^{12}$ (mol m/m^2 s Pa)	Selectivity α	Butanol permeability $\times 10^{12}$ (mol m/m^2 s Pa)	Water permeability $\times 10^{12}$ (mol m/m^2 s Pa)	Selectivity α
SDS	13	8.2	1.6	8.4	6.2	1.4
PDMS	1.3	1.3	1.0	0.91	0.94	0.96

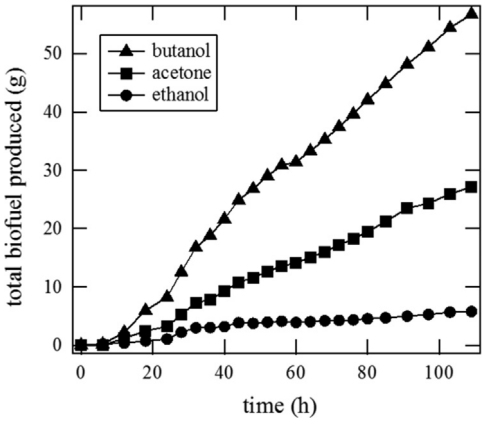

Fig. 6. Total amount of butanol (▲), acetone (■), and ethanol (●) produced versus fermentation time for a 1 L pervaporative-fermentation with the SDS membrane. Lines in the plot were drawn to guide the eye.

produced after the acidogenesis phase of cell metabolism ends, which is around 10 h into the fermentation. The arrow in Fig. 4a represents the time at which circulation to the pervaporation module was initiated in the pervaporative-fermentation experiments ($t=18$ h). The concentration of butanol at $t=18$ h ranges between 2 and 4 g/L in all experiments. In the simple batch process, $[BuOH]_r$ rises rapidly and saturates at about 13 g/L and butanol production ceases at $t=28$ h. The increase in $[BuOH]_r$ obtained during pervaporative-fermentation using both SDS and PDMS membranes are similar. Butanol concentrations in both cases increase more slowly, reaching a maximum of about 13 g/L at $t=62$ h.

Fig. 4b shows the optical density of cells (OD_{600}) at different timepoints during the three fermentations. OD_{600} values lie between 6 and 8 at $t=18$ h when circulation to the pervaporation module was initiated. In the simple batch process, OD_{600} reaches a maximum of 10 at $t=32$ h and decreases slightly at longer times due to cell death. In pervaporative-fermentation with the PDMS membrane, OD_{600} increases monotonically until $t=45$ h and saturates at a value of 15 for 46 h $\leq t \leq$ 64 h. A decrease in OD_{600} after 64 h signifies cell death and sporulation. In pervaporative-fermentation with the SDS membrane, OD_{600} increases monotonically until $t=54$ h, and saturates at a value of about 27 for 54 h $\leq t \leq$ 62 h before decreasing due to cell death. The data in Fig. 4b show that the differences in permeabilities of the SDS and PDMS membranes (Table 1) have a qualitative effect on pervaporative-fermentation. It is interesting to note that these differences occur even though the time dependence of $[BuOH]_r$ in both cases is similar (Fig. 4a).

Fig. 4c presents the butanol removal rate during pervaporative-fermentation using SDS and PDMS membranes. The advantage of higher butanol permeability of the SDS membrane is clearly seen in these data. The butanol removal rate obtained with the SDS membrane is significantly higher at all times. It appears that the higher butanol removal rate in the SDS membrane enables the cells to reach a higher density during pervaporative-fermentation.

Fig. 5 shows the total mass of butanol produced (butanol in the bioreactor and butanol removed by pervaporation) as a function of time during the three fermentations. In the simple batch fermentation, production ceased after $t=32$ h, and 13 g of butanol was produced in total. In pervaporative-fermentation with the PDMS membrane, butanol was produced steadily until $t=64$ h, and a total of 33 g of butanol was produced. In pervaporative-fermentation with the SDS membrane, production of butanol ended around $t=62$ h, and 49 g of butanol was produced in total.

Table 2 summarizes biofuel production characteristics obtained during the three fermentations. All three fermentations began with the same number of cells. Nonetheless, significant differences in the amount of glucose consumed and ABE produced are evident in the two pervaporative-fermentations. The volumetric productivity in the simple batch fermentation was 0.45 g/(L h). In the pervaporative-fermentation with the PDMS membrane, the volumetric productivity was 0.66 g/L h, and in the pervaporative-fermentation with the SDS membrane, the volumetric productivity was 0.94 g/L h. The specific productivity (g/L h OD_{600}) is similar in all cases, suggesting the increase in volumetric productivity for pervaportive-fermentations was not due to improvement of cell performance, but because of the large increase in cell population. The efficacy of glucose to ABE conversion, defined by the yield (moles of glucose equivalent converted to ABE/moles of glucose consumed), is also similar in all cases. This suggests that the underlying metabolic processes for ABE production are not affected by pervaporation. Losses of biofuels due to nitrogen sparging have not been accounted for. Acetone is the most volatile fermentation product. That the ABE compositions reported here are similar to those reported in the literature suggest that losses due to sparging are not significant.

The efficacy of the SDS and PDMS membranes during pervaporative-fermentation are quantified in Table 3 where average permeate fluxes of water and ABE are given. The fluxes are consistently higher for the SDS membrane than for the PDMS membrane. It is interesting to note that the separation factors (β) of acetone and butanol are also higher for the SDS membrane, although the ethanol separation factor was higher for the PDMS membrane.

Table 4 compares the permeabilities obtained from aqueous butanol pervaporation experiments and the pervaporative-fermentation experiments with the SDS and PDMS membranes. The fermentation broth was modeled as a binary butanol/water mixture, i.e., binary activity coefficients were used to compute the effective driving force. The measured permeabilities of both butanol and water are slightly lower in the pervaporative-fermentation experiment. It is likely that this is due to our use of binary activity coefficients in our analysis of pervaporation of the complex fermentation broth. Membrane fouling in the fermentation experiments may also be responsible for some of the deviations seen in Table 4. We observed relatively small decreases in fluxes through the membranes as fermentation proceeded (e.g., the water flux decreased by 11% and 3% for SDS and PDMS

Table 5
Data compiled from different pervaporative-fermentation experiments at 37 °C.

Reference	Liu et al. [10]	Chen et al. [11]	Van Hecke et al. [12]	Li et al. [13]	This work	
					SDS	PDMS
Microorganism	*C. acetobutylicum* XY16	*C. acetobutylicum* CICC 8012	*C. acetobutylicum* ATCC 824	*C. acetobutylicum* DP 217	*C. acetobutylicum* ATCC 824	*C. acetobutylicum* ATCC 824
Active membrane material	PDMS	PDMS	PDMS	PDMS/silicate-1	SDS	PDMS
Membrane thickness (μm)	5	8, 16	1	7	2	0.5
total Flux (g/m^2 h)	~700	784, 556	621	486	1634	941
Butanol separation factor ($\beta_{butanol}$)	9–22	10.3, 7.03	16.8–19.8	31.6	21	14
Volumetric productivity (g of ABE/L h)	–	0.314	0.37–1.13	0.97	0.94	0.66

membranes, respectively). Given the complexity of the pervaporative-fermentation experiment, the general agreement seen in Table 4 is noteworthy.

In addition to the three fermentation experiments discussed above, we conducted another pervaporative-fermentation experiment with smaller fermentation volume. Decreasing the fermentation volume to 1 L while using the same SDS membrane allowed the butanol removal rate to match the butanol production rate. Therefore, butanol concentration was maintained below the toxic level at all times during fermentation, and continuous production was observed. ABE production increased monotonically to 90 g (27 g acetone, 57 g butanol, 6 g ethanol) for 109 h until it was manually stopped (Fig. 6).

Table 5 compares the results of the present study with published results on ABE pervaporative-fermentations [10–13]. The total flux (water and ABE biofuel) obtained in our PDMS based pervaporative-fermentation is higher than that of prior studies. This may be attributed to our use of a thinner PDMS membrane (Table 5). The total flux obtained with the SDS membrane is significantly higher than in all other ABE pervaporative-fermentations. The butanol separation factor is highest when PDMS/silicalite-1 membranes are used but the addition of silicalite-1 appears to lead to a significant decrease in flux. The volumetric productivity of SDS pervaporative-fermentation is comparable to results reported by Van Hecke et al. and Li et al. We conclude, based on both our results and previously published work, that SDS membranes are more effective for in situ product removal for pervaporative ABE fermentation than cross-linked PDMS membranes.

4. Conclusions

We demonstrate the efficacy of polystyrene-*b*-polydimethylsiloxane-*b*-polystyrene membranes for in situ product removal of biofuels by pervaporation during *C. acetobutylicum* ABE fermentations. Using pervaporative-fermentation, we were able to alleviate two major problems hindering commercialization of ABE fermentation: product inhibition and low productivity. Pervaporative-fermentation with the SDS membrane resulted in higher volumetric ABE productivity, ABE production, and cell density, when compared with the batch process and the pervaporative-fermentation using a state-of-the-art commercial PDMS membrane. Aligning the fermentation volume with the pervaporation module's capacity to remove biofuel enabled continuous ABE production at high cell density. The effective permeabilities of butanol and water obtained during pervaporative-fermentation were consistent with results from pervaporation experiments on binary butanol/water mixtures. Further work on optimizing factors such as membrane composition and thickness for pervaporative-fermentation is warranted and ongoing.

Acknowledgments

This work was supported by the Energy Biosciences Institute, University of California at Berkeley (Grant number OO3J04).

References

[1] P. Durre, Biobutanol: an attractive biofuel, Biotechnol. J. 2 (2007) 1525–1534.
[2] D.T. Jones, D.R. Woods, Acetone–butanol fermentation revisited, Microbiol. Rev. 50 (1986) 484–524.
[3] E.M. Green, et al., Genetic manipulation of acid formation pathways by gene inactivation in *Clostridium acetobutylicum* ATCC 824, Microbiology 142 (1996) 2079–2086.
[4] E.M. Green, Fermentative production of butanol – the industrial perspective, Curr. Opin. Biotechnol. 22 (2011) 337–343.
[5] D. Stark, U. Stockar, In situ product removal (ISPR) in whole cell biotechnology during the last twenty years, Adv. Biochem. Eng./Biotechnol. 80 (2003) 149–175.
[6] N. Qureshi, I.S. Maddox, Continuous production of acetone–butanol–ethanol using immobilized cells of *Clostridium acetobutylicum* and integration with product removal by liquid-liquid extraction, J. Ferment. Bioeng. 80 (1995) 185–189.
[7] S.B. Bankar, et al., Continuous two stage acetone–butanol–ethanol fermentation with integrated solvent removal using *Clostridium acetobutylicum* B5313, Bioresour. Technol. 106 (2012) 110–116.
[8] N. Qureshi, et al., Energy-efficient recovery of butanol from model solutions and fermentation broth by adsorption, Bioprocess Biosyst. Eng. 27 (2005) 215–222.
[9] L.M. Vane, A review of pervaporation for product recovery from biomass fermentation processes, J. Chem. Technol. Biotechnol. 80 (2005) 603–629.
[10] G. Liu, et al., Pervaporation performance of PDMS/ceramic composite membrane in acetone butanol ethanol (ABE) fermentation-PV coupled process, J. Memb. Sci. 373 (2011) 121–129.
[11] C. Chen, et al., Acetone–butanol–ethanol fermentation in a continuous and closed-circulating fermentation system with PDMS membrane bioreactor, Bioresour. Technol. 128 (2013) 246–251.
[12] W. Van Hecke, et al., Pervaporative recovery of ABE during continuous cultivation: enhancement of performance, Bioresour. Technol. 129 (2013) 421–429.
[13] J. Li, et al., Continuous acetone–butanol–ethanol (ABE) fermentation with in situ solvent recovery by silicalite-1 filled PDMS/PAN composite, Energy Fuels 28 (2014) 555–562.
[14] S. Li, et al., Preparation of PDMS membrane using water as solvent for pervaporation separation of butanol–water mixture, Green Chem. 15 (2013) 2180–2190.
[15] V. Garcia, et al., Challenges in biobutanol production: how to improve the efficiency? Renew. Sustain. Energy Rev. 15 (2011) 964–980.
[16] Y.S. Jang, et al., Acetone–butanol–ethanol production with high productivity using *Clostridium acetobutylicum* BKM19, Biotechnol. Bioeng. 110 (2013) 1646–1653.
[17] A.E. Ozcam, et al., Relationship between segregation strength and permeability of ethanol/water mixtures through block copolymer membranes, Macromolecules 46 (2013) 9652–9658.
[18] A.K. Jha, et al., Master curve captures the effect of domain morphology on ethanol pervaporation through block copolymer membranes, J. Memb. Sci. 401 (2012) 125–131.
[19] J.G. Wijmans, R.W. Baker, The solution-diffusion model: a review, J. Memb. Sci. 107 (1995) 1–21.
[20] J. Gmehling, et al., Vapor–Liquid Equilibrium Data Collection, Dechema, Flushing, NY, 1977.
[21] E.D. Gomez, et al., Effect of ion distribution on conductivity of block copolymer electrolytes, Nano Lett. 9 (2009) 1212–1216.
[22] L.M. Vane, et al., Factors affecting alcohol–water pervaporation performance of hydrophobic zeolite-silicone rubber mixed matrix membranes, J. Memb. Sci. 364 (2010) 102–110.
[23] R.D. Offeman, C.N. Ludvik, A novel method to fabricate high permeance, high selectivity thin-film composite membranes, J. Memb. Sci. 380 (2011) 163–170.