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# Efficient butanol recovery from acetone–butanol–ethanol fermentation cultures grown on sweet sorghum juice by pervaporation using silicalite-1 membrane

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**We investigated butanol recovery by pervaporation separation, using a silicalite-1 membrane, from batch cultures of butanol-producing *Clostridium beijerinckii* SBP2 grown on sweet sorghum juice as a fermentation medium. The pervaporation system yielded 73% (w/v) butanol from intact feed cultures containing 1% (w/v) butanol, and had a butanol permeation flux of 11 g $m^{-2}$ $h^{-1}$. Upon neutralization and activated charcoal treatment of the feed cultures, butanol yield and total flux increased to 82% (w/v) and 40 g $m^{-2}$ $h^{-1}$, respectively. This system is applicable to refining processes for practical biobutanol production from a promising energy crop, sweet sorghum.**



[**Key words:** Biobutanol; Sweet sorghum; *Clostridium beijerinckii*; Separation; Membrane; Pervaporation]

Sweet sorghum (*Sorghum bicolor* (L.) Moench) is a C4 gramineous crop and is regarded as promising for biofuel production (1). It requires minimal amounts of fertilizer for its cultivation and can grow on marginal or non-arable lands for short periods (3–5 months) (2). The plants accumulate large quantities of fermentable sugars in their stems (as much as 25% of the dry weight biomass) and have a high biomass, growing to more than 3 m (2). Bioethanol production from sweet sorghum juice (SSJ) or its lignocellulose hydrolysate has therefore been studied and developed into several cost-effective production systems (3–5). Compared with ethanol, *n*-butanol (hereafter referred to as butanol unless otherwise stated) is a superior fuel candidate and has advantages such as higher caloric value, lower corrosivity, and lower volatility. Importantly, butanol can be directly used as fuel or fuel additive in modern engine systems because of its similarity to gasoline (6).

Acetone–butanol–ethanol (ABE) fermentation by clostridia has been used in a major bioproduction system for butanol (7). To compete with synthetic butanol in the chemical market, several technical and commercial challenges for the fermentative production of butanol must be surmounted, namely high feedstock cost, low butanol yield, low volumetric solvent productivities, high-energy methods involving conventional distillation for solvent recovery, and high water usage (7). As discussed above, the feedstock cost can be lowered by using sweet sorghum. However, the butanol yield of ABE fermentation is typically less than 20 g $l^{-1}$, and butanol is toxic to bacterial cells, inhibiting their growth at 12–16 g $l^{-1}$ (8). This low butanol yield is associated with high-energy costs for butanol recovery by means of conventional distillation. One approach to facilitating cost-effective recovery of butanol is the use of a pervaporation system (9). We recently reported that highly concentrated butanol solutions [>80% (w/w)] may be recovered from clostridial ABE fermentation broths cultured in laboratory growth media by pervaporation using silicalite-1 membranes and thus butanol recovery may be greatly simplified by using this membrane system (10,11). In the present study, we used a *Clostridium* isolate able to grow vigorously in SSJ and produce butanol, and investigated the potential for using a pervaporation system to facilitate efficient recovery of butanol from bacterial cultures grown on SSJ.

## MATERIALS AND METHODS

**Strain, SSJ preparation, and growth conditions** The strain used in this study was a gram-positive, endospore-forming, obligately anaerobic, rod-shaped bacterium (strain SBP2), which was isolated from an upland soil sample at the Field Science Center (Ibaraki University College of Agriculture, Ibaraki, Japan). The strain is related most closely to *Clostridium beijerinckii* JCM1390$^T$, with 99.9% similarity based on a 16S rRNA gene sequence (the DDBJ accession number for the sequence of strain SBP2 is LC005451). Strain SBP2 showed better growth in SSJ than *C. beijerinckii* JCM1390$^T$ because of its greater tolerance of phenolic compounds in SSJ (M. Kanemoto, unpublished result).

A cultivar of sweet sorghum (KCS 105) was seeded in a field at the Field Science Center in June 2009 and in June 2014 and harvested the following November. The harvested stems were immediately squeezed and the resultant juice was stored at −20 °C before use. In the fermentation experiment, SSJ was centrifuged at 10,000 × *g* for 10 min, autoclaved, and then centrifuged again to remove debris.

To prepare the feed solutions for the pervaporation experiments, bacterial cultures were grown at 35 °C in an anaerobic chamber (ANX-6, Hirasawa Works, Tokyo, Japan) maintained with a gas mixture of 80% $N_2$, 10% $H_2$, and 10% $CO_2$. Precultures were grown for 24 h in 100 ml of PYG medium (12) [grams per liter: Bacto tryptone

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(Becton, Dickinson and Company [BD], Franklin Lakes, NJ, USA), 1.0; Bacto yeast extract (BD), 5.0; glucose, 20.0; $Na_2SO_4$, 0.18; $K_2HPO_4$, 3.48; biotin, 0.01; *p*-aminobenzoic acid, 0.01; L-cysteine hydrochloride, 0.5]. The medium was supplemented with 1 ml of a mineral stock solution described by George et al. (12). The medium was adjusted to pH 6.8 with HCl. The culture inoculated with one-third strength SSJ (SSJ/3; 1.8 l) containing 0.03% (w/v) L-cysteine hydrochloride. The stationary-phase cultures were centrifuged at 3800 × *g* for 10 min and the supernatants passed through nitrocellulose membrane filters (pore size: 0.8 μm; Advantec, Tokyo, Japan). The resulting solutions were used in the pervaporation experiments.

**Preparation of silicalite-1 membrane** Tubular silicalite-1 membranes were hydrothermally synthesized on a tubular, porous stainless-steel support, as described previously (13). There were two process; seeding silicalite crystals and hydrothermal synthesis. In brief, silicalite-1 seed crystals were suspended at 1 g $l^{-1}$ in acetone, and a tubular porous stainless-steel support was coated with silicalite-1 seed crystals in an electrophoretic deposition bath at 100 V for 5 min. The hydrothermal synthesis was carried out at 170 °C for 48 h in autoclave entered the hydrothermal synthesis gel ($SiO_2$: tetra-*n*-propyl ammonium bromide: NaOH: $H_2O$ = 1: 0.005: 0.05: 100; aging at 27 °C for 3 h 40 min) and the seeded tubular support. The obtained membrane was calcined at 375 °C for 60 h for eliminating the $TPA^+$.

**Pervaporation performance and adsorption on silicalite powder** Batch pervaporation experiments using the silicalite-1 membranes were carried out at 60 °C with homogeneous agitation at 500 rpm. All experimental data were obtained at steady state. The total permeation flux and selectivity ($\alpha$) for each compound was calculated using the following equations:

$$\text{Total permeation flux} = \text{permeate } (g)/[\text{membrane area } (m^2) \times \text{permeation time } (h)] \quad (1)$$

$$\alpha = [y/(1-y)]/[x/(1-x)] \quad (2)$$

where $x$ and $y$ are the weight fractions of the compound in the feed and permeate samples, respectively.

To examine the effect of neutralization and charcoal treatment of the feed solution on pervaporation performance, the feed solutions were neutralized with 1 M NaOH and then treated with activated charcoal (0–200 g $l^{-1}$; Sigma–Aldrich, St. Louis, MO, USA; C-4386) for 10 min at room temperature. Feed butanol concentrations were adjusted to and maintained at an arbitrary concentration by adding pure commercial butanol during the pervaporation experiments.

Adsorption of phenolic compounds in the SSJ to the silicalite-1 powder was monitored by suspending 0.2 g of silicalite powder in 2 ml of SSJ fermentation culture at 60 °C for 24 h, with stirring at 120 rpm. The amount of adsorbed phenolic compound was calculated from the difference between the concentrations before and after contact with the silicalite powder.

**Analytical methods** Sugars, alcohols, and organic acids in the SSJ and bacterial cultures were determined by high-performance liquid chromatography using a Shodex Super Sugar SH1011 column, as described previously (5). Butanol in the feed and permeate solutions was quantified by gas chromatography (7820A, Agilent Technologies, Palo Alto, CA, USA) equipped with a thermal conductivity detector. Quantification was done by direct aqueous injection at 220 °C into a 2 m Gaskuropack 56 packed column (GL Sciences, Tokyo, Japan) at 200 °C. Total phenolic compounds in the SSJ were estimated calorimetrically using Folin–Ciocalteu reagent and gallic acid as the standard (14). Protein was determined with a Bio-Rad protein assay kit (Bio-Rad Laboratories, Hercules, CA, USA).

## RESULTS AND DISCUSSION

**Pervaporation performance with silicalite-1 membrane** Eleven silicalite-1 membrane samples were prepared and tested for pervaporation performance, with binary butanol/water mixtures (1.0 ± 0.1% [w/v]) as the feed. Highly concentrated butanol solutions (80.0 ± 4.5% [w/v]) were recovered from the feed. The butanol selectivity ($\alpha$) and total permeation flux were 440 ± 146 and 67.3 ± 23.7 g $m^{-2}$ $h^{-1}$, respectively. To examine the system's performance with lower butanol concentrations in the feed, eight randomly selected membrane samples were used for the butanol/water mixtures, containing 0.1–1.1% (w/v) butanol. The permeate butanol concentration remained at about 80% (w/v) when the feed butanol concentration was >0.5% (w/v), but sharply declined for feed concentrations of <0.2% (w/v) (Fig. 1). This decline was also the case for the total flux: the relative total flux (normalized by the total flux at 1% [w/v] feed butanol) dropped down to 0.3 (Fig. 1).

The SSJ solution contained 8.6% (w/v) sucrose, 2.3% (w/v) glucose, 2.1% (w/v) fructose, 0.03% (w/v) phenolic compounds (gallic acid equivalent), and a trace quantity of protein (32 mg $l^{-1}$). Three runs of batch cultures of strain SBP2 on SSJ/3 (pH 5.4) yielded 0.3–0.8% (w/v) butanol, ≤0.1% (w/v) isopropanol and butyrate, and <0.1% (w/v) acetate, ethanol, and acetone. Although the acid production was very low, the pH of the cultures shifted down to 4.8 ± 0.2, probably due to the weak buffering action of SSJ. Actually, the external addition of 0.06% (w/v) commercial acetic acid to the fresh SSJ resulted in a pH decrease from 5.4 to 4.6 and further to 4.5 by the subsequent addition of 0.06% (w/v) commercial butyric acid. The residual sucrose, glucose, and fructose content of the cultures were 2.1 ± 0.5%, 0.3 ± 0.03%, and 0.3 ± 0.01% (w/v), respectively. Strain SBP2 did not produce detectable amounts of acetone from the SSJ/3 feed, and there was no detectable acetone in the permeate following pervaporation, suggesting that this strain can reduce acetone to isopropanol, probably by using secondary alcohol dehydrogenase (15). Since acetone cannot be used as a biofuel because it corrodes engine parts (16) and decreases total permeation flux during pervaporation with silicalite-1 membranes (17), the fact that strain SBP2 does not produce acetone appears to be an advantage of this method of biobutanol production.

Actual SSJ culture feed solutions containing 0.3–0.8% (w/v) butanol and those supplemented with pure commercial butanol (final concentration, 1.1% [w/v]) were examined for the pervaporation performance of the four silicalite-1 membrane samples. At > 0.8% (w/v) butanol, the permeate butanol concentration was comparable to that of the butanol/water mixtures, but was slightly lower when the feed concentration was <0.7% (w/v) (Fig. 1). This lower permeate butanol concentration seems to be explained partly by the unsaturated state of butanol adsorption onto the membrane surface due to the presence of the other fermentation products. This can expected from our previous results which showed that the amount of adsorbed butanol on the silicalite-1 membrane was lower in the feed mixture of butanol/acetone/ethanol/water than the butanol/water binary mixture (17). The data of pervaporation performance at 0.7–1.1% (w/v) feed butanol are

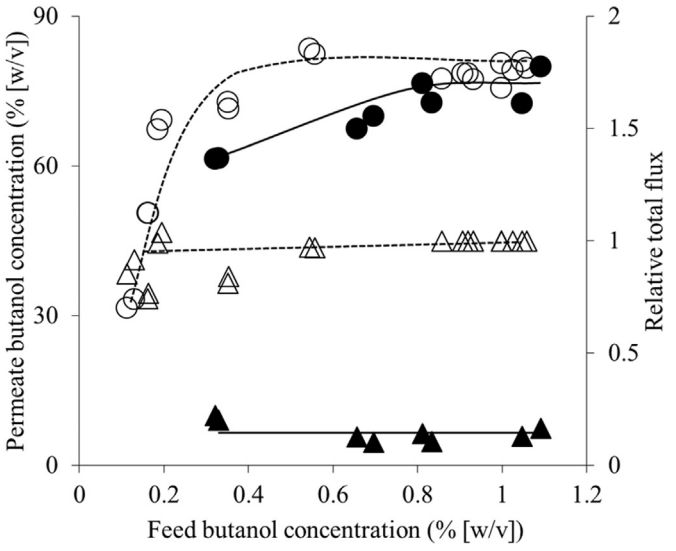


FIG. 1. Pervaporative performance of silicalite-1 membranes used for feed solutions containing various amounts of butanol. Permeate butanol concentration (circles, left axis) and relative total flux: normalized by the total flux for 1% [w/v] butanol in water (triangles, right axis). Eight membrane samples were used in 19 pervaporation experiments with binary butanol/water mixtures (open circles and triangles; two to four feed solutions per membrane) and eight experiments with the SSJ culture (closed circles and triangles; two experiments per membrane).