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

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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.

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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

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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).

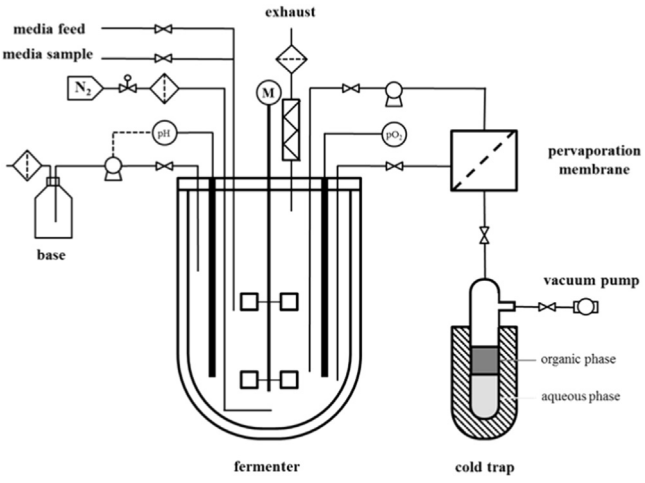

Fig. 1. Schematic diagram of the pervaporative-fermentation setup.

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