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Use of the composite membrane of poly(ether-block-amide) and carbon nanotubes (CNTs) in a pervaporation system incorporated with fermentation for butanol production by *Clostridium acetobutylicum*

Hong-Wei Yen *, Zhi-Heng Chen, I-Kuan Yang

Department of Chemical and Materials Engineering, Tunghai University, Taichung, Taiwan

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ABSTRACT

Fermentation incorporated with pervaporation was regarded as an efficient way to relieve the feedback inhibition of butanol in acetone–butanol–ethanol (ABE) fermentation. The addition of CNTs (carbon nanotubes) to PEBA (poly(ether-block-amide)) could greatly enhance the removal flux of solvents (acetone, butanol and ethanol) in a model solution test. The butanol removing rate results in a 61% increase in the batch with PEBA + CNTs (5%) membrane compared with that of the batch with PEBA alone. Besides the increase of removal flux, the addition of CNTs enforces the mechanical strength of the pervaporation membrane, which leads to more resistance for a longer operational time. The combination of a 5-L fermenter with the pervaporation membrane of PEBA + CNTs (10%) indicates a 20% increase both in productivity and yield compared to using PEBA. In conclusion, the addition of CNTs to a PEBA pervaporation membrane has great potential when applied in the ABE fermentation industry.

1. Introduction

Biobutanol production through biological routes (such as the pathway of acetone–butanol–ethanol, ABE) is regarded as a good alternate additive compared to bioethanol (Dürre, 2011; Green, 2011). However, ABE fermentation is known to be subject to a strong end-product inhibition (mainly coming from butanol). Butanol is highly toxic to microorganisms and for this reason less than 20 g/L of butanol is produced during regular batch fermentation (García et al., 2011; Ni and Sun, 2009; Zheng et al., 2009). Therefore, the application of in situ separation technologies (such as gas stripping, adsorption and pervaporation) are often adopted in the fermentation operation to overcome the toxicity problem and increase the final concentration of butanol (Nielsen and Prather, 2009; Qureshi and Blaschek, 2001; Qureshi et al., 2005).

Among all of the separation techniques, pervaporation is a rapidly developing membrane technology which acts as an energy-efficient process for separating liquid mixtures (e.g. azeotropic mixtures and mixtures with similar volatilities) that are difficult to separate by conventional methods (Jianga et al., 2009; Li et al., 2011a,b; Qureshi et al., 2001). Pervaporation is a typical membrane technique which allows selective removal of volatiles from a model solution/fermentation broth. The volatile or organic component is solubilized into the membrane, followed by diffusion through the membrane and recovery by condensation on the permeate side of the membrane (Smitha et al., 2004). In this process, a phase change from liquid to vapor occurs. The mechanism by which a volatile/organic component is removed by pervaporation is widely accepted and referred to as solution–diffusion.

A model of solution–diffusion was proposed to describe the separation mechanism of pervaporation, including three steps: sorption, diffusion and desorption (El-Zanati et al., 2006; Fouad and Feng, 2008). The effectiveness of separation in pervaporation is quantized by two parameters: selectivity, a measure of selective removal of volatiles; and flux, the rate at which the organic/volatile passes through the membrane per membrane area. Many studies have been cited in prior literature describing the ABE fermentation process being incorporated with different membrane materials used for pervaporation techniques (El-Zanati et al., 2006; Li et al., 2011a,b; Qureshi et al., 2005). Among all of the materials, PEBA (poly(ether-block-amide)) used as the pervaporation material has been examined for its performance of butanol separation in the model solution (Liu et al., 2005; Mandal and Bhattacharya, 2006).

Except for the conventional polymeric membrane, one current trend that has emerged in membrane-based separation is a move to the fabrication of mixed matrix membranes (MMM) (Ismail et al., 2009). MMM is a heterogeneous membrane consisting of an inorganic filler embedded in a polymer matrix and can be made into flat sheets and hollow-fibers. It is noted that MMMs obviously possess very promising properties that have remarkably surpassed others. An interesting alternative is MMM prepared with carbon

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* Corresponding author. Fax: +886 4 23590009.
E-mail address: hwyen@thu.edu.tw (H.-W. Yen).

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nanotubes (CNTs), which is an inorganic filler material that differs substantially from those molecular sieves as the inorganic disperse phase. Single walled CNTs (SWNTs) have outer diameters in the range of 1–3 nm, with inner diameters of 0.4–2.4 nm. Multi-walled CNTs (MWCNTs) can have outer diameters ranging from ~2 nm (double-walled nanotubes) up to ~100 nm with tens of walls (Sears et al., 2010). CNTs exhibit remarkable electrical and thermal conductivity and are one of the strongest fibers known. These properties, combined with their nanoscale dimensions, have led to their intense study for a wide range of applications.

The operation of polymeric membranes is often limited by the so-called trade-off curve between permeability and selectivity. Nevertheless, the addition of CNTs could be helpful in breaking the trade-off curve (Sae-Khow and Mitra, 2010; Sears et al., 2010). Peng and his colleagues had dispersed CNTs in poly(vinyl alcohol) by using β-cyclodextrin to form a hybrid membrane of poly(vinyl alcohol)/carbon nanotube. These membranes were applied to pervaporation separation of benzene/cyclohexane mixtures (50/50 wt.%), and showed excellent pervaporation properties (Peng et al., 2007).

This study aimed to investigate the application of the composite membrane of PEBAs and CNTs incorporated with a 5-L fermenter to increase butanol production by enhancing the butanol removal rate through pervaporation separation. The effects of PEBA composite membrane and carbon nanotubes (CNTs) at 5 and 10 wt.% were examined, respectively. As far as the authors know, this is the first report using PEBA/CNTs in a pervaporation system incorporating a 5-L fermenter for butanol production.

2. Methods

2.1. Strain and medium

The bacterial strain used in this study was *Clostridium acetobutylicum* BCRC 10639 (the same as ATCC 824), which was purchased from the Bioresource, Collection and Research Center (BCRC) in Taiwan. The cells were cultured in glass tubes (diameter, 3 cm; length, 15 cm) containing 25 mL of fermentation medium and purged with 50% CO_2 and 50% N_2 to ensure 100% anaerobic conditions. After being removed from the freezer, the strain was heat-shocked at 70 °C for 2 min, and then inoculated in a glass tube (3 cm OD × 15 cm high) containing 25 mL of reinforced clostridial medium (RCM), used as the seed medium, followed by an incubation period of 30 h at 37 °C.

The fermentation medium was then inoculated with seed culture at a ratio of 10% (v/v). The fermentation medium contained the following components (per liter): 80 g glucose; 0.18 g Na_2SO_4; 0.18 g K_2HPO_4; 0.01 g biotin; 0.01 g p-aminobenzoic acid; 1.0 g tryptone (Difco); 5.0 g yeast extract (Difco); and 1 mL mineral salt solution. The mineral salt solution contained the following components in 1 L of distilled water: 0.24 g of $NaMoO_4 \cdot 2H_2O$, 0.24 g of $CoCl_2 \cdot 6H_2O$, 1.5 g of $CaCl_2 \cdot 2H_2O$, 16.20 g of $FeCl_3$, 0.16 g of $CuSO_4$, 0.52 g of $ZnSO_4 \cdot 7H_2O$, 1.7 g of $MnSO_4 \cdot H_2O$, 24.58 g of $MgSO_4 \cdot 7H_2O$, and 28 mL of H_2SO_4 (6 M). The pH was adjusted to 4.8 before sterilization; the pH was not controlled during the whole fermentation process in this study.

2.2. Membrane preparation for PEBA

PEBA is a hydrophobic polyether amide polymer which slightly swells in water, absorbing preferentially aromatic compounds. PEBA 2533 (Pebax® 2533 SN01 from Atofina) was dissolved in *n*-butanol at 80 °C under vigorous stirring to form a homogenous polymer solution containing 5 wt.% of the polymer. After the polymer solution was kept at room temperature without disturbance for 1 day to remove entrapped air bubbles, it was cast onto a glass plate using a casting knife to form a film of the polymer solution with a uniform thickness. The film, together with the glass plate, was placed in an oven at 70 °C for 24 h to evaporate the solvent. Next, the dry membrane was peeled from the glass plate, followed by further drying in a vacuum oven at 50 °C for 2 days to remove any residue solvent. The thickness of the resulting dry membrane was measured by a Mitutoyo Digital Micrometer (GOTECH, GT-313-A).

2.3. The composite membrane preparation for PEBA and CNTs

Multiwalled carbon nanotubes (MWCNTs), with a commercial name of Flotube 9000, were provided by Cnano, China. The CNTs had an average diameter of 10 nm and an average length of 10 mm. The purity of Flotube 9000 was claimed as greater than 95% and the accompanied impurities were mostly metal oxides derived from the catalysts in the production process. A mixture of PEBA and CNTs at 5 or 10 wt.% was prepared directly by feeding into a ceramic drum for a 15-day ball-milling. The nanotubes were expected to be shortened and maybe broken after prolonged milling. After 15 days of ball-milling, the membranes with CNTs were prepared by pouring appropriate amounts of the solution into a smooth glass container and left in the hood overnight for evaporation of the solvent. The cast film was then placed into a vacuum oven and stored at 80 °C for 3 days in order to drive out the solvent residue and to heal the tiny cavities created by solvent evaporation. The thickness of the membranes was determined by a micrometer (Mitutoyo).

2.4. Pervaporation performance with a model solution

Pervaporation membranes (PEBA, PEBA + CNTs (5%) and PEBA + CNTs (10%)) were mounted in a pervaporation filtration frame with a total active area of 0.08 m^2 and an average thickness of 50 μm. The pervaporation membrane and the separation frame were sterilized at 121 °C for 15 min before the experiment was performed. A model solution, consisting of 4 g/L of acetone, 8 g/L of butanol, 1.33 g/L of ethanol, 3.5 g/L of acetic acid and 3.5 g/L of butyric acid, was prepared to simulate the supernatant of a fermentation broth for the investigation of pervaporation performance by different membranes. The membrane material, with a higher butanol removal flux, was to be adopted for application in subsequent fermenter experiments.

2.5. Pervaporation of fermentation broth in a 5-L fermenter

The working volume of a 5-L fermenter is 2.5 L. The initial batch fermentation process and the medium components are described as above. After 24 h batch cultivation, the operation mode was shifted to the fed-batch stage lasting for 24 h (from the 25th to the 48th hour after the inoculation) with the operation of pervaporation and glucose feeding simultaneously. The glucose feeding rate was determined by the broth removing rate through the pervaporation. After 24 h of fed-batch operation, the pervaporation and the glucose feeding both would stop till the end of fermentation process. The glucose concentration in the fermenter was maintained in the range of 10–20 g/L during the fed-batch stage. Pervaporation was performed at 37 °C, the same temperature as in the fermenter. The pervaporation module used here was the same as for the model solution test, which had the membrane area of 0.08 m^2. The broth was withdrawn from the 5-L fermenter by a peristaltic pump at a rate of 700 mL/min. The pressure on the permeate side ranged from 40 to 50 mm Hg, and the permeate gas after pervaporation was condensed using two liquid nitrogen sealed cooling traps. Samples were taken from the retentate and permeate for further product analysis.