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Ability of a perfluoropolymer membrane to tolerate by-products of ethanol fermentation broth from dilute acid-pretreated rice straw

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ABSTRACT

A perfluoropolymer (PFP) membrane has been prepared for use in vapor permeation to separate aqueous ethanol mixtures produced from rice straw with xylose-assimilating recombinant *Saccharomyces cerevisiae*. PFP membranes commonly have been used for dehydration process and possess good selectivity and high permeances. The effects of by-products during dilute acid pretreatment, addition of yeast extract, and ethanol fermentation on PFP membrane performance were investigated. While feeding mixtures of ethanol (90 wt%) in water, to which individual by-products (0.1–2 g/L) were added, the PFP membrane demonstrated no clear change in permeation rate (439–507 g m^{-2} h^{-1}) or separation factor (14.9–23.5) from 2 to 4 h of the process. The PFP membrane also showed no clear change in permeation rate (751–859 g m^{-2} h^{-1}) or separation factor (12.5–13.8) while feeding the mixture (final ethanol conc.: 61 wt%) of ethanol and distillation of the fermentation broth using a suspended fraction of dilute acid-pretreated rice straw for 20 h. These results suggest that the PFP membrane can tolerate actual distillation liquids from ethanol fermentation broth obtained from lignocellulosic biomass pretreated with dilute acid.

1. Introduction

Combustion of fossil fuel is increasing the atmospheric carbon dioxide level, raising interest in finding a more sustainable energy source [1]. Ethanol produced from biomass is one alternative to petroleum-derived transportation fuels. In Japan, approximately 9.6 megatons of rice straw are produced as agricultural residue annually [2], making efficient production and recovery of ethanol from rice straw an attractive goal.

Rice straw is mainly composed of cellulose, hemicellulose, and lignin [3]. Rice straw typically includes glucose, xylose, arabinose, mannose, and galactose [4]. Therefore, the ethanol production process from rice straw involves three steps [5]. First, polymeric substrates are degraded into monosaccharides with chemical or enzymatic treatment. Second, the sugars are converted into ethanol by bacteria or yeast (usually the strain *Saccharomyces cerevisiae*). Finally, the ethanol is purified from fermentation broth by processes such as adsorption, distillation, and liquid–liquid extraction [6,7]. Among these processes, dehydration of water–ethanol mixtures using a vapor permeation process is well-developed method [8,9]. In the vapor permeation process, the feed solution is vaporized first and then permeated through the membrane that has a hydrophilic nature and selectively transports water [7]. This process concentrates the ethanol in the feed solution.

Dilute acid hydrolysis of rice straw has been applied successfully to the pretreatment of ethanol fermentation [4,10]. However, harsh conditions in the dilute acid pretreatment cause extensive biomass decomposition that generates compounds such as acetic acid, formic acid, furfural, 5-hydroxymethyl furfural (5-HMF), vanillin, and syringaldehyde, in addition to xylose and glucose [10]. Other by-products found in the following ethanol fermentation process can affect membrane performance, even in small amounts [1,11]. Therefore, an effective membrane should be capable of tolerating these by-products for a long period of time. However, the effect of individual by-products on membrane performance during vapor permeation has not yet been clarified.

Here, by-products from ethanol fermentation after dilute acid pretreatment of rice straw were analyzed in detail. A perfluoropolymer (PFP) membrane was utilized for vapor permeation because PFP membranes are commonly used to separate water/ethanol mixtures and are reported to have good selectivities and high

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permeances [12]. The aim of this study was to investigate the effect of individual by-products from dilute acid pretreatment of rice straw and ethanol fermentation on PFP membrane performance. The PFP membrane was used as a selective membrane on actual distillation liquid of fermentation broth from rice straw dilute acid-pretreated for 20 h, to investigate the effect of by-products on permeation rate and separation factors.

2. Materials and methods

2.1. Acid pretreatment of rice straw

Rice straw (Nipponbare) was harvested in 2009 in Kasai, Hongo, Japan. Naturally dried rice straw was shredded to a length less than 2 mm. Rice straw (100 g/L) was immersed in 1% (v/v) sulfuric acid solution and incubated at 180 °C for 45 min as described previously [10]. The solution of acid-pretreated rice straw was filtered through a membrane (No. 1, Advantec, Osaka, Japan), and the residue on the filter and the filtrate were considered the solid fraction and suspended fraction, respectively.

2.2. GC–TOF-MS (gas chromatography–time of flight mass spectrometry) analysis

GC–TOF-MS analysis was conducted as described by Hiller et al. [13]. The fermentation liquids (10 μL) were dried under vacuum and stored at −80 °C until analysis. Dried samples were derivatized at 30 °C for 90 min with 50 μL of 20 mg/mL methoxyamine hydrochloride in pyridine and at 37 °C for 30 min after the addition of 100 μL of *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (GL Science, Tokyo, Japan). The derivatives (1 μL) were used for the GC–TOF-MS analysis (Leco Pegasus HT TOFMS, Joseph, MI, USA) conducted under the following conditions: column: CP-Sil 8 CB low bleed (30 m × 0.25 mm i.d. DF = 0.25 μm; Varian Inc., Palo Alto, CA); carrier gas: helium; flow rate: 1 mL/min; front inlet temperature: 230 °C; transfer line temperature: 250 °C; ion source temperature: 200 °C; injection volume: 1 μL; injection mode: pulsed split 1:10; pulse pressure: 20 psi; pulse duration: 1 min; oven temperature: 80 °C for 2 min, ramped to 330 °C at a rate of 15 °C/min, then 330 °C for 6 min; solvent delay: 4.0 min; electron voltage: −70 eV; and detection mode: MS detection mode, scan *m*/*z* 50–50, acquisition rate: 20 scan/s. Metabolite identification was performed by comparison of retention index and mass spectra data with those of standards using Metabolite Detector version 2.0.6.

2.3. Ethanol fermentation from the filtrate of acid pretreatment of rice straw

S. cerevisiae strain YPH499XU harboring a plUX1X2XK plasmid (URA3, intracellular co-expression of xylose reductase from *Pichia stipitis*, xylitol dehydrogenase from *P. stipitis*, and xylulokinase from *S. cerevisiae* genes) was used for ethanol fermentation with the suspended or solid fractions from dilute acid-pretreated rice straw [14]. Cultivation and collection of *S. cerevisiae* were carried out as described previously [14]. Then, *S. cerevisiae* was inoculated into solutions containing the yeast extract (final conc.: 10 g/L), peptone (final conc.: 20 g/L), and three-fifths diluted suspended fraction or solid fraction (final weight of solid rice straw: 50 g/L) followed by ethanol fermentation as described previously [10]. Additionally, 2 mL/L of cellulase SS (15 FPU/L, Nagase ChemteX, Osaka, Japan) were included in the ethanol fermentation using solid fractions. The initial pH in the mixture including the suspended fraction was adjusted to 5.0 using a $Ca(OH)_2$ solution. Ethanol fermentation using the suspended fraction or solid fraction was conducted in triplicate.

Glucose, xylose, ethanol, glycerol, and xylitol in the fermentation medium were analyzed by high-performance liquid chromatography (Shimadzu, Kyoto, Japan) using a shim-pack SPR-Pb column (Shimadzu) and a refractive index detector (model RID-10A, Shimadzu) as described previously [14]. After ethanol fermentation from suspended and solid fractions, the distillation liquid of the fermentation broth was analyzed by GC–TOF-MS as described above. The distillation liquid of the fermentation broth was also analyzed by gas chromatography–mass spectrometry (GC–MS; 2010plus, Kyoto, Japan) using the external standard method to detect acetic acid, formic acid, furfural, 5-HMF, vanillin, and syringaldehyde as described previously [10].

2.4. Vapor permeation experiment

Vapor permeation experiments were done using the apparatus described by Uragami et al. [8] (Fig. 1). The fermentation broth (500 mL) was placed in the apparatus. The parts of the feed solution and the membrane in the apparatus were heated to 105 °C, and experiments were conducted under reduced pressures. Vapor was collected as permeate in a glass trap cooled with liquid nitrogen. The ethanol composition in the feed solution and permeate was analyzed by high-performance liquid chromatography as described above. The permeation rate was determined by measuring the permeate weight at 25 °C. The separation factor was calculated from the following equation:

$$\alpha_{A/B} = \frac{Y_A/Y_B}{X_A/X_B}$$

where X_A and X_B are the weight fractions of water and ethanol, respectively, in the feed solution, and Y_A and Y_B are the weight fractions of the water and ethanol, respectively, in the permeate.

2.5. Effect of various compounds on membrane performance

Each compound identified in dilute acid pretreatment of rice straw and ethanol fermentation was added to a 95 wt% ethanol solution. This mixture was utilized as the feed solution. Compounds included were acetic acid, formic acid, furfural, 5-HMF, 4-hydroxybenzoic acid, vanillin, syringaldehyde, glycerol, xylitol, glucose, pyruvic acid, oxalic acid, isoleucine, and pyroglutamic acid. The concentration of each compound was determined by referring to the concentration in the dilute acid pretreatment and ethanol fermentation broth. Final concentration of acetic or formic acid was 2 g/L. Final concentration of glycerol or glucose was 1 g/L. Final concentration of the other compounds was 0.1 g/L. Vapor permeation experiments were conducted for 4 h. The vapor (permeate) after 2 and 4 h was sampled.

2.6. Effect of ethanol fermentation broth on the membrane

Fermentation broth for the suspended fraction from dilute acid pretreatment of rice straw was used. The fermentation broth was distilled and collected. Then, the distillation liquid of the fermentation broth was mixed with ethanol to a final ethanol concentration of 61 wt%. Vapor permeation experiments using this mixture were conducted for 20 h. Vapor (permeate) after 5, 10, 15, and 20 h was sampled.

3. Results and discussion

3.1. Ethanol fermentation from suspended and solid fractions of dilute acid-pretreated rice straw

Dilute acid-pretreated rice straw was divided into 2 fractions: a suspended and a solid fraction. The suspended and solid fractions