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Improved permselectivity of forward osmosis membranes for efficient concentration of pretreated rice straw and bioethanol production

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Abstract

The hydrothermal pretreatment of rice straw generates a liquid fraction that contains low concentrations of sugars, as well as fermentation inhibitors which are not favourable for efficient bioethanol production. For the development of a forward osmosis (FO) process to concentrate sugars and remove fermentation inhibitors, we fabricated polyamide-polysulfone (PA-PSf) membranes treated with NaClO solution. The NaClO treatment improved the water permeability (*A* value) and the permselectivity of fermentation inhibitors against sugars. Maximum separation of xylose from furfural and acetic acid was achieved when the treated membranes had *A* values of 5.5–7 $\mathrm{L{\cdot}m^{-2}{\cdot}h^{-1}{\cdot}bar^{-1}}$. Using an optimized membrane with *A* value of approximately 6 $\mathrm{L{\cdot}m^{-2}{\cdot}h^{-1}{\cdot}bar^{-1}}$, a liquid fraction from hydrothermally pretreated rice straw was concentrated in a one-step FO process to increase sugar concentrations and decrease fermentation inhibitor concentrations. The ethanol concentration and yield (ethanol/total sugar) by xylose-utilizing *Saccharomyces cerevisiae* reached 18.0 g/L and 0.40, respectively, which were much higher than those obtained with commercial FO membranes. These results clearly indicate the possibility of introducing a simple and low-cost FO process into bioethanol production from lignocellulosic biomass by incorporating NaClO treatment to FO membrane.

Keywords: forward osmosis membrane; permselectivity; NaClO treatment; concentration of rice straw; bioethanol

1. Introduction

Nowadays, approximately 80% of the energy required for human life is derived from non-renewable fossil fuels [1]. To cope with resource depletion and future population increases, sustainable alternative transportation fuels are in high demand, and strong interest is now directed toward biofuels, such as bioethanol. Since bioethanol does not increase the amount of carbon dioxide in the atmosphere by its combustion, it is considered as a clean energy source. In contrast to ethanol produced from food (e.g., corn), bioethanol is produced from waste material and thereby allows extensive benefits to be drawn from agricultural products. Rice straw, a kind of agricultural waste, is an abundant lignocellulosic biomass in Japan [2,3]. The usage of rice straw in bioethanol production was investigated, although rice straw is composed of heterogeneous complex of carbohydrate polymers (predominantly containing cellulose, hemicellulose, and lignin) [2,4,5]. To obtain the final bioethanol product, several steps are necessary: pretreatment (to fractionate the structural components and increase enzymatic digestibility) [6], enzymatic glycation, fermentation of derived sugars, and recovery and purification of ethanol. After hydrothermal pretreatment with liquid hot water (160−220 °C) and pressure, which can avoid corrosion problems incurred with acid or alkaline solutions, a liquid fraction is obtained after separating the solid fraction. The liquid fraction contains not only hemicellulose-derived saccharides but various by-products, such as acetic acid, formic acid, furfural, 5-hydroxymethyl furfural (HMF), and aromatic lignin degradation compounds [3]. It has been reported that these by-products negatively affect microbial metabolism during fermentation [3]. Previously, using the liquid fraction obtained after hydrothermal pretreatment of rice straw, the polysaccharides composed of glucose, xylose, and fructose were hydrolyzed by hemicellulase and fermented by a recombinant xylose-utilizing strain of *Saccharomyces cerevisiae* (*S. cerevisiae*) [7][8]. However, because of the low saccharides concentration (1–2 wt%) in the liquid fraction, the concentration of produced ethanol was extremely low. As a result, it is then necessary to concentrate the ethanol to high concentration in the purification process for practical use. As is well known, ethanol purification is an energy-consuming process, even if energy-saving separation processes (hybrid distillation, membrane-based separation) are used [9]. To save the energy during the ethanol purification process, it is helpful to produce a more concentrated ethanol solution in the fermentation process [10].

Membrane-based separations are relatively simple processes and can be easily scaled up. They have been utilized in various processes of biorefining and bioenergy production, such as separation and purification of individual molecules, enzyme recovery from hydrolysis processes, and in membrane bioreactors for bioenergy and chemical production [11]. Nanofiltration (NF) and forward osmosis (FO) have also been proposed to concentrate the liquid fraction from lignocellulosic biomass. The NF process is driven by applied pressure (gas or hydraulic pressure) that allows for the physical separation and retention of particles. A commercial NF membrane has been utilized to remove fermentation inhibitors from the liquid fraction from rice straw and has proven effective for increasing bioethanol production [4,12]. However, the associated energy cost is not low, because a significantly high pressure (> 20 bar) is required as the driving force for concentration. In addition, it is difficult to concentrate the liquid fraction from rice straw, considering the high osmotic pressure of the liquid fraction, the limited pressure resistance of the membrane, and membrane fouling.

On the other hand, FO is a process that is driven by osmotic pressure, and has been used to treat industrial wastewaters [13], reclaim wastewater [14], and desalinate seawater [15,16]. FO has high potential as part of an energy-saving sugar concentration process, and it can achieve a higher concentration ratio than NF by choosing a powerful draw solution (DS) with high osmotic pressure. However, there are still few FO membranes that are available to use on the liquid fraction from rice straw. In our previous study, for the first time, FO was used to concentrate the liquid fraction from rice straw during bioethanol production, and achieved a high concentration ratio (8 times) with a commercial FO membrane, TFC-ES (Hydration Technology Innovation) [17]. This work indicated that FO had an advantage over NF by reaching a higher concentration without the application of high pressure. However, this system produced a liquid with very low ethanol concentration because of the residual fermentation inhibitors after the sugar concentration process. Therefore, it is highly desirable to develop an FO membrane that has high permselectivity for fermentation inhibitors against sugar to avoid the suppression of ethanol production in subsequent process.

In this study, we investigated an active layer treatment to achieve an optimal FO membrane which gives adequately concentrated liquid fraction from rice straw and has a high permselectivity of fermentation inhibitors for further fermentation and bioethanol production. Herein, an extremely dense polyamide (PA) active layer was prepared on a commercial polysulfone (PSf) support substrate and then treated by NaClO solutions of different concentrations. The permselectivity of each treated membrane was investigated and an optimum treatment condition was established. Then, the performance of each developed membrane was further investigated by ethanol fermentation using xylose-utilizing *S. cerevisiae*.

2. Experimental

2.1. Materials and chemicals

All chemicals were of analytical grade and used without further purification, and provided by Wako Pure Chemical Industries, Osaka, Japan unless otherwise stated. Ultrapure water was supplied by a Milli-Q ultrapure water system (Millipore, Bedford, MA, USA). 1,3-Phenylenediamine (MPD, >98%, from Tokyo Chemical Industry Co., Tokyo, Japan), sodium dodecyl sulfate (SDS, 95%), (±)-10-camphorsulfonic acid (CSA, >98%, from Tokyo Chemical Industry Co.), triethylamine (TEA), and 1,3,5-benzenetricarbonyl trichloride (TMC, >98%) were used for active layer preparation [18,19]. Sodium hypochlorite pentahydrate ($NaClO \cdot 5H_2O$) and NaOH were used for active layer treatment [20,21], and HCl was used for pH adjustment. For the membrane performance tests of reverse osmosis (RO) and FO, the aqueous solutions of NaCl, $MgSO_4$, xylose, furfural (from Sigma-Aldrich Co., St. Louis, MO, USA) and acetic acid were used as feed solution. For FO test, a liquid fraction obtained after hydrothermal pretreatment of rice straw (from Mitsubishi Heavy Industries, Tokyo, Japan) was used as a sugar solution. Yeast extract (from Becton Dickinson and Co., Sparks, MD, USA), yeast nitrogen base without amino acids (from Becton Dickinson and Co.), glucose (from Nacalai Tesque, Kyoto, Japan), and peptone (from Becton Dickinson and Co.) were used for cultivation of *S. cerevisiae*.

2.2. Membrane preparation

A commercial PSf membrane (CF30K, Nitto Denko, Osaka, Japan) was used as support substrate for the thin film composite (TFC)-FO membrane. The PA active layer was formed on the top of commercial PSf membrane by interfacial polymerization [18,19]. MPD (2 wt%), SDS (0.25 wt%), CSA (4 wt%), and TEA (2 wt%) were dissolved in Milli-Q water and stirred at room temperature (25 °C) to prepare the amine aqueous solution. Acid chloride solution was prepared by dissolving TMC (0.15%) in hexane. After dropping amine aqueous solution onto the PSf substrate for 60 s, excess solution was removed completely. Then acid chloride solution was added for 30 s, and drained out for 30 s. After the interfacial polymerization, the membrane was heat-treated at 100 °C for 5 min. The membrane was rinsed with pure water and stored in pure water at 4 °C before use.

For active layer treatment, the prepared membrane was immersed in NaClO aqueous solutions (pH adjusted to 7.0) with different concentrations for 1 h, and then immersed to a 0.1 M NaOH aqueous solution for 16 h [20, 21]. After that, the membrane was washed with Milli-Q water and stored in pure water at 4 °C.

2.3. Membrane characterization

Surface morphology was observed by field emission (FE)-scanning electron microscopy (SEM) to determine the active layer structure. The membranes before and after NaClO treatment were freeze-dried (FD-1000, Eyela, Japan) and treated with OsO_4 sputtering for observation by FE-SEM (JSM-7500F, JEOL, Japan). Changes in chemical structure were analyzed by Fourier transform infrared spectroscopy (FTIR-8100A, Shimadzu, Japan). X-Ray photoelectron spectroscopy (XPS; ESCA-3400, Shimadzu, Japan) was also used to investigate the chemical composition of the treated membrane surface.

2.4. Reverse osmosis (RO) test

All experiments were done with an laboratory-scale crossflow RO system reported previously [22] to assess the performance of the treated membrane. The effective membrane area was 10.18 cm^2 and the transmembrane pressure difference was 10 bar. A variable speed pump (NPL-120, Nihon Seimitsu Kagaku, Tokyo) was used to provide the feed solution (FS) at a flow rate of 10 ml/min. Six different feed solutions were used to investigate the water permeability and the selective solute permeability: Milli-Q water, and aqueous solutions of NaCl (2 g/L), $MgSO_4$ (2 g/L), xylose (1.5, 2 g/L), furfural (1.7, 5.2, 20.8 mM), and acetic acid (30 mM, pH 4.6). Water permeability was determined by measuring the permeated water mass every minute. The water flux (J_w^{RO}, $L \cdot m^{-2} \cdot h^{-1}$) and the water permeability coefficient (A, $L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$) of the membrane were calculated with Eqs. (1) and (2), respectively.

$$J_w^{RO} = V / (A_m \cdot \Delta t) \qquad (1)$$

$$A = J_w^{RO} / \Delta P \qquad (2)$$

where V (L) is the volume of permeated water, A_m (m^2) is effective membrane area, ΔP (bar) is transmembrane pressure difference and Δt (h) is filtration time.

The solute rejection ratio ($R, \%$) was defined as:

$$R = (1 - C_{ip} / C_{if}) \times 100 \qquad (3)$$

where C_{if} and C_{ip} are the respective concentrations of the feed solution and of the permeate solution of substance i, respectively. In this study, NaCl and $MgSO_4$ were used as solutes to investigate membrane performance and these salts were selected as an appropriate DS solute. In addition, xylose was selected as a target monomeric sugar, and furfural and acetic acid were selected as target fermentation inhibitors to study the selective permeability between sugars and inhibitors. The rejection of NaCl and $MgSO_4$ was calculated by measuring the concentration using a compact conductivity meter (LAQUAtwin B-771, Horiba). Xylose rejection was calculated by measuring the concentration using high-performance liquid chromatography (HPLC, Shimadzu, Kyoto, Japan), and furfural and acetic acid rejection were quantified by measuring the concentration using a gas chromatography–mass spectrometry (GC–MS, QP2010Plus, Shimadzu, Kyoto, Japan) (see Sect. 2.4).

2.5. Forward osmosis (FO) test

A laboratory-scale crossflow FO system (Figure S1) [22] was used to concentrate two types of sugar solutions. One is a synthetic sugar solution that contained 20 g/L xylose, 0.5 mM furfural and

30 mM acetic acid. The synthetic sugar solution was used to check the selective permeability between monosaccharide and fermentation inhibitors. In this case, a saturated $MgSO_4$ (2 L) aqueous solution was employed as DS. Another one is a liquid fraction obtained after hydrothermal pretreatment of rice straw. The liquid fraction was concentrated to confirm the selectivity and used for ethanol fermentation later. For this fraction, rice straw was pretreated with hot water at a specified temperature (160–220 °C) at a pressure below 100 bar. The liquid fraction (pH 4.6) was obtained by filtering the pretreated rice straw through a mesh filter and was used as a source of hemicellulosic material [2,3]. The composition of liquid fraction is listed in Table S1. The liquid fraction from rice straw (250 g) was used as FS, and saturated $MgSO_4$ (2 L) aqueous solution was used as DS.

In both experiments, a small gear pump (GPU-3, As One) was used to circulate FS in a closed loop at a crossflow velocity of 0.5 L/min, and a low-pressure pump (Aquatec CDP-8841) was used to circulate the DS at a crossflow velocity of 0.7 L/min. FS and DS were kept uniform by magnetic stirring of the solutions. The prepared FO membrane was settled in a custom-built flat membrane test cell in active layer facing feed solution (AL-FS) mode with an effective membrane area of 43 cm^2.

The water flux was determined by monitoring the mass change in FS. The water flux (J_w^{FO}, $L \cdot m^{-2} \cdot h^{-1}$) and the reverse solute flux from DS ($MgSO_4$) (J_s, $mmol \cdot m^{-2} \cdot h^{-1}$) through the membrane were calculated with Eqs. (4) and (5), respectively.

$$J_w^{FO} = \Delta V / (A_m \cdot \Delta t) \qquad (4)$$

$$J_s = \Delta n / (A_m \cdot \Delta t) \qquad (5)$$

where $\Delta V / \Delta t$ ($L \cdot h^{-1}$) and $\Delta n / \Delta t$ ($mol \cdot h^{-1}$) were obtained from the weight change and the change in electrical conductivity in FS, respectively.

In addition, because the analysis of liquid fraction which contains many kinds of hemicellulose-derived sugars and other substances is extremely difficult during the membrane separation operation, the degree of sugar concentration was monitored based on membrane water flux that was calculated from water weight change. The sugar concentration C_{sugar} was calculated with Eq. (6).

$$C_{sugar} = n_0 / (V_0 - \Delta V) \qquad (6)$$

where n_0 (mol) and V_0 (L) were the initial total mole of sugar and the initial solution volume of FS, respectively.

To analyze the actual sugar content in FS after enzymatic glycation, the concentration of monosaccharides (xylose, glucose, and fructose) was measured by HPLC using a Shim-pack SPR-Pb column (Shimadzu, Kyoto, Japan) and an RID-10A refractive index detector (Shimadzu). Fermentation inhibitors were measured by GC–MS. A DB-FFAP column (Agilent, Palo Alto, CA, USA) was used for analysis of acetic acid and formic acid, while a CP-SIL 8 CB low bleed/MS column was used for analysis of furfural, HMF, vanillin, and syring aldehyde.

2.6. Ethanol fermentation

S. cerevisiae strain MN8140X/TF-TF harboring a plUX1X2XK plasmid (URA3, intracellular co-expression of xylose reductase and xylitol dehydrogenase from *Pichia stipites* and xylulokinase from

S. cerevisiae genes) was used for ethanol fermentation [2,3]. *S. cerevisiae* strains were aerobically precultivated for 24 h at 30 °C and 150 rpm in 5 mL of synthetic defined medium (10 g/L yeast extract, 6.7 g/L yeast nitrogen base without amino acids, 20 g/L glucose), and then cultivated for 72 h in 500 mL of YPX medium (10 g/L yeast extract, 20 g/L peptone, 20 g/L xylose) at 30 °C and 150 rpm [4,12]. The cells were collected by centrifugation at 3000 g_0 at 4 °C for 10 min and washed twice with Milli-Q water. The pH of the liquid fraction of the hot-water pretreated rice straw after membrane concentration was adjusted to 5.5 using 10 M NaOH. *S. cerevisiae* was inoculated into solutions containing yeast extract (final conc. 10 g/L), peptone (final conc. 20 g/L) and concentrated liquid fraction by the FO test which was hydrolyzed with 8 g/L hemicellulase (G-Amano, Amano Enzyme, Japan) in advance. The initial concentration of *S. cerevisiae* was 50 g/L of wet cells, corresponding to 10 g/L of dry cells. Ethanol fermentation was performed under oxygen-limited conditions at 35 °C, with mild agitation, in 50-mL bottles equipped with an outlet for CO_2. Sampling was conducted at 0, 3, 6, 9, 24, and 48 h [4,12].

3. Results and discussion

3.1. Characterization of membranes before and after active layer treatment

PA-PSf membranes were treated with NaClO solutions of different concentration (500 and 2500 ppm) to control the permselectivity between sugars (such as glucose, xylose, and fructose) and fermentation inhibitors (such as acetic acid, formic acid, furfural, HMF, vanillin, and syringaldehyde) in the sugar solution. Untreated PA-PSf membrane was used as control. The surface morphologies of membranes with and without NaClO treatment were observed by SEM (Figure 1). All figures showed a rough and dense polyamide layer formed by interfacial polymerization. The surface of the untreated membrane appeared to have little ridges, while the treated surfaces had more ridges and the polyamide active layer of the membrane became rougher by NaClO treatment. This observation aligns with previous research showing that exposure of a polyamide surface to chlorinated solution causes conversion of hydrogen bonding C(=O)N−H functional groups to C(=O)N−Cl groups, resulting in a loss of H-bonding and increases in rotational freedom and flexibility of the polymer chains [23]. It appears that a broad ridge was cut off by immersing into NaClO solution. This cut-off effect was accelerated after NaOH immersion considering the resolution of small polyamide pieces with low molecular weight. The destruction of a broad polyamide network increased the size of channels for permeation and resulted in higher water permeation.

To confirm the structural changes of PA layers, ATR-FTIR spectroscopy was performed on the PA-PSf membranes treated by different NaClO solutions. The peak assignments for the ATR-FTIR spectra of these PA membranes were reported in detail elsewhere [24]. As shown in Figure 2, the fully aromatic (FA) amide signature peaks at wave numbers 1541, 1609, and 1663 cm^{-1} indicated obvious changes after NaClO treatment. The amide II band (1541 cm^{-1}) for N−H in-plane bending and N−C stretching vibration and the aromatic amide peak (1609 cm^{-1}) for N−H deformation vibration and the C=C ring-stretching vibration were decreased at low NaClO concentration (500 ppm) and were drastically decreased at high NaClO concentration (2500 ppm). It is suggested that chlorine replaced the hydrogen of the amide nitrogen via electrophilic substitution in N-chlorination [18]. The amide I band (1663 cm^{-1}) showed a slight shift to higher wavenumber by NaClO treatment, which represents C=O stretching (major contributor), C−N stretching and C−C−N deformation vibration. The shift was likely caused by the breakage of hydrogen bonds between C=O and N−H groups and additional carboxyl groups by hydrolysis [18]. On the other hand, the peak at the broad wavenumber range (3700–3200 cm^{-1}) centered at 3300 cm^{-1}, which were assigned to N–H and/or O–H stretching, decreased after NaClO treatment. To confirm the reduction of the FA amide signature peaks, the ratio of amide II band, aromatic amide peak and amide I band to C-O-C stretching (1244 cm^{-1}) which was the signature peak of polysulfone substrate were compared in Table 1, because the height of C-O-C stretching was stable against NaClO treatment. All of the FA amide signature peaks decreased with NaClO concentration.

The surface chemical composition of the PA-PSf membrane before and after treatment by different NaClO solutions was monitored by XPS and the results are listed in Table 2. The appearance of Cl indicated that Cl remains after treatment and cannot be washed out easily. The Cl/N ratio increased with NaClO concentration. This result is consistent with observations made by ATR-FTIR spectroscopy. Both results imply that the replacement of the hydrogen of the amide nitrogen with Cl.

3.2. RO performance evaluation

3.2.1. Water permeability changes with NaClO concentration

To illustrate the changes in intrinsic membrane properties, water permeability coefficient (*A*, $L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$) and solute rejection ratio (*R*, %) for untreated and treated PA-PSf membranes are presented in Figure 3 (a) and (b), respectively. Figure 3 (a) shows an example of the water permeability as a function of the effective NaClO concentration in the range of 100–2500 ppm. The water permeability increased with the increase in effective NaClO concentration. In this study, the *N,N*-diethyl-*p*-phenylenediamine (DPD) method was used to measure NaClO concentration, and effective NaClO concentration was determined by color comparison [25]. With increasing effective NaClO concentration (~1000 ppm), the *A* value reached around 7 $L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$. In the membrane chlorination procedure, hydrogen bonds between C=O and N−H groups in the network of the PA active layer was curtailed [18]. The NaOH immersion procedure then caused further breakup of the polyamide network in the active layer because of dissolution of polyamide fragments with low molecular weight. As a result, the looser PA active layer allowed water molecules to permeate more easily, and larger molecules could also permeate because of the decreased size exclusion. Therefore, NaClO treatment gave the membrane higher water permeability, as reported for commercial TFC membranes [20]. In addition, when the PA active layer was treated in concentrated NaClO solution (2000 and 2500 ppm), the *A* value tended to become constant.

The *A* value always showed a sensitive response to the treatment conditions. It has been reported that the treatment condition (treatment time, NaClO concentration and pH value) affected their membrane permeation [20, 21]. In this study, we kept the treatment time for 1 h and the value of pH at 7, and focused on the effect of NaClO concentration. The treated membrane showed similar tendency of the water permeability against effective NaClO concentration with the previous studies. For the effect of NaClO concentration, the *A* value of treated membranes linearly increased with NaClO concentration below 1000 ppm. On the other hand, the *A* value of membranes treated with high NaClO concentration (>2000 ppm) deviated from the straight line and was nearly constant (Figure 3(a)). This is probably because the effect of treatment on membrane surface was heterogeneous. It is also possible that the PA-PSf membrane became overly loose, and thus, compaction could occur in the active layer during an overnight water filtration under 11 bar prior to the experiment. Consequently, the *A* value was used as a new standard to distinguish membranes treated by different NaClO solutions. As shown in Figure 3 (b), solute rejections of NaCl and $MgSO_4$ showed similar moderate declines at low *A* (meaning low treatment degree) and showed sharply declines with *A* value over approximately 8.4 $L{\cdot}m^{-2}{\cdot}h^{-1}{\cdot}bar^{-1}$. Furthermore, $MgSO_4$ showed higher rejection than NaCl, consistent with the Donnan exclusion mechanism.

3.2.2. Rejection of monomeric sugar and fermentation inhibitors

To investigate the effect of NaClO treatment and to establish the optimum treatment condition for permselectivity between sugar and fermentation inhibitors, a synthetic feed solution containing monomeric sugar and fermentation inhibitor was prepared. Xylose was selected as the synthetic monomeric sugar because xylose was smallest among monomeric sugars in the real liquid fractions from hydrothermal pretreatment of rice straw. Furfural and acetic acid were selected as typical non-ionic and ionic fermentation inhibitors, respectively because of their high concentrations in real liquid fractions.

The rejection of various solutes was checked under an applied pressure of 10 bar by monitoring solute concentration on the permeate side, and the permselectivity for furfural and acetic acid against xylose were investigated. The same membranes were used to check the rejections between monomeric sugar (xylose) and each fermentation inhibitor (furfural or acetic acid). Figure 4 (a) shows the rejections of xylose and furfural, and Fig. 4 (b) the rejections of xylose and acetic acid. As shown in Figure 4 (a), high rejection of xylose over 95 % was retained for *A* values below 8 $L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$, and extremely high rejection was achieved for *A* values below 5.5 $L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$. On the other hand, low rejection of furfural was obtained around *A* values of 5.5 $L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$, which reflects good permeability of furfural. These results suggest that *A* values in the range of 5.5–8.5 $L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$ allows high xylose rejection and low furfural rejection. Similarly, the results shown in Figure 4 (b) show high xylose rejection for *A* values below 8 $L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$, and good selective performance between xylose and acetic acid was achieved for the *A* value range of 5–7 $L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$. The results shown in Figure 4 suggest that an *A* value range of 5.5–7 $L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$ is optimal for high permselectivity of both furfural and acetic acid. Consequently, a membrane with high permselectivity for fermentation inhibitors could be obtained by NaClO treatment with solutions of 300–550 ppm.

3.3. FO concentration process

3.3.1. Confirmation of FO performance

In the test of FO performance, Milli-Q water was used as FS, while saturated $MgSO_4$ solution or NaCl solution with the same osmotic pressure (3000 mOsmol/kg) was employed as DS in order to investigate the effects of the NaClO treatment to the membranes on their water flux and reverse salt flux. The fundamental membrane performance, water flux (J_w^{FO}) and reverse salt flux (J_s) are summarized in Table S2. The results for J_w^{FO} show that water were transported from FS to DS at a rate of at least 1.92 $L \cdot m^{-2} \cdot h^{-1}$ [(PA-PSf (Untreated) in the case of $MgSO_4$ as a DS]. Compared to untreated membranes, treated membranes always showed a higher water flux because of the loose PA layer. Water flux showed no obvious change with increased NaClO concentration when $MgSO_4$ was used as the DS. However, with NaCl solution as the DS, water flux reduced slightly when using PA-PSf (NaClO-1000) compared to PA-PSf (NaClO-500), which was attributed to DS leakage. Here, PA-PSf (NaClO-1000) and PA-PSf (NaClO-500) indicate PA-PSf treated with 1000 ppm NaClO and 500 ppm NaClO. Moreover, $MgSO_4$ solution gave a much lower J_S value than NaCl, because of its larger molecule size and divalent charge. As a result, even though the water flux in case of using $MgSO_4$ was lower than that in case of using NaCl, $MgSO_4$ was selected as DS to avoid the effect on concentrated sugar solution fermentation caused by DS leakage.

3.3.2. Concentration of synthetic sugar solution

The permselectivity of the treated membranes for furfural and acetic acid against xylose was investigated in FO mode. Synthetic sugar solution (500 mL; 20 g/L xylose, 0.5 mM furfural, 30 mM acetic acid; these concentrations are the same as actual concentrations in liquid fraction after hydrothermal pretreatment of rice straw) was concentrated in a laboratory-scale crossflow FO system using saturated $MgSO_4$ (2 L) aqueous solution as the DS. The time course of each component concentration in the FS was measured and shown in Figure 5. The membrane treated with 350 ppm NaClO solution drastically concentrated xylose from 22.9 g/L to 52.4 g/L. Most of the furfural permeated through the treated membrane and the furfural concentration remained constant even though water permeated from FS to DS, whereas the acetic acid concentration gradually increased. The increase of acetic acid concentration would be caused by the electrostatic repulsion between acetic acid and the negatively charged polyamide layer of the treated membrane [13]. Thus, it is assumed that a loosely structured active layer with less negative charge would be ideal for separation of xylose and acetic acid.

3.3.3. Concentration of liquid fraction from hydrothermal pretreatment of rice straw

Untreated PA-PSf membrane and two others that were treated with 350 and 400 ppm NaClO solution were denoted as PA-PSf (Untreated), PA-PSf (NaClO-350), and PA-PSf (NaClO-400), respectively. Liquid fraction (250 g) of hydrothermally pretreated rice straw was placed in the FS side and concentrated until its weight reduced to about 50 g. The sugar concentration of FS was calculated assuming no sugar transport to DS. For PA-PSf (NaClO-350), the time course of water flux and FS concentration ratio defined by (sugar concentration at time, t / initial sugar concentration) are shown in Figure 6.

As shown in Figure 7 and Table 3, almost all of the fermentation inhibitors concentrations remained unchanged in the case of PA-PSf (NaClO-350) and PA-PSf (NaClO-400), while all the fermentation inhibitors became concentrated with time in the case of using PA-PSf (Untreated). In addition, as for PA-PSf (NaClO-350) and PA-PSf (NaClO-400), the concentration ratio of each fermentation inhibitor was lower than that for total sugars (glucose + xylose + fructose) (Table 3). Thus, it can be considered that NaClO treatment was effective for removal of fermentation inhibitors while retaining sugar in the solution. In comparing the separation of fermentation inhibitors with previous studies, PA-PSf (NaClO-350) showed better removal of fermentation inhibitor than the commercial FO membrane (TFC-ES), and comparable separation performance with a commercial NF membrane (ESNA3) (Table 3). Ultimately, enhanced removal of fermentation inhibitors by NaClO-treated membranes will lead to higher ethanol productivity.

It is also noteworthy that formic acid (low molecular weight) was apparently concentrated while the concentration of acetic acid (high molecular weight) remained near constant (Figure 7 (b) and (c)). To investigate the influence of negative surface charge (Figure S2) on the permeability of formic acid and acetic acid, their rejections were measured under different pH conditions as shown in Figure S3. It was found that the rejection of these acids depended on the pH values. The rejection of acetic acid was higher than that of formic acid at pH 3.7, but became lower at pH higher than 3.7. It can be considered that rejection was dominated by molecular weight at low pH (< 3.7) and dominated by electrostatic repulsion at high pH (> 6.6). This explanation can be applied to the concentration experiment of real sugar solution. Since the pH of the sugar solution was 4.6, acetic acid showed lower rejection than formic acid, which means that acetic acid tended to permeate more easily than formic acid. Therefore, in the process of concentrating the liquid fraction of hydrothermally pretreated rice straw, formic acid was concentrated slightly because of electrostatic repulsion while acetic acid showed almost no change in concentration.

3.3.4. Ethanol fermentation

The liquid fraction of hydrothermally pretreated rice straw, which was concentrated by PA-PSf (NaClO-350) or PA-PSf (NaClO-400), was used to investigate the effect of NaClO treatment on ethanol productivity. Concentrated liquid fractions obtained by the FO test were fermented by a xylose-assimilating *S. cerevisiae* MN8140X/TF-TF after 1 day of enzymatic glycation. Figure 8 shows the concentration change of sugars and fermentation products (glycerol and xylitol) in concentrated liquid fractions with PA-PSf (Untreated) and PA-PSf (NaClO-350). Compared to the case of PA-PSf (Untreated), xylose was totally consumed when the liquid fraction from rice straw was concentrated by PA-PSf (NaClO-350). According to the previous report [3], fermentation inhibitors reduce ethanol productivity because of a decline in xylose consumption. This result suggested that the removal of fermentation inhibitors using treated membranes was effective for complete conversion of xylose to alcohol products.

The apparent ethanol yields, defined as the ratio of the amount of ethanol produced to the sum of glucose, xylose, and fructose consumed [4], are summarized in Table 4. The ideal ethanol yield is 0.51, considering the chemical reaction equation for the three monomeric sugars [12]. As part of the FO test, real sugar solutions were concentrated by PA-PSf (NaClO-350) or PA-PSf (NaClO-400) and then fermented. An ethanol yield of 18 g/L was achieved, and this was four times higher than the concentration achieved (4.83 g/L) without any concentration. Although the ethanol yield increased up to 0.39 and 0.40, respectively, which are similar to the value for the original liquid fraction (0.38), it is apparent that the usage of the treated membranes did not decrease the original ethanol productivity. In the case of commercial FO membrane (TFC-ES), even though it is successful in achieving a high sugar concentration (118 g/L), the ethanol yield was extremely low (0.01), which was attributed to a large amount of fermentation inhibitor remaining in the concentrated liquid fraction (Table 4). It is obvious that the homemade NaClO-treated PA-PSf membranes prepared in this work were much more effective in removing fermentation inhibitors than commercial TFC-ES membrane.

It is meaningful to compare the yield and ethanol concentration in the case of concentration by the treated membranes with the performance using commercial NF membrane (ESNA3) [17]. In the case of 1-cycle NF method, the original real sugar solution was concentrated in a single-step NF operation. The ethanol yield of 0.31 (Table 4) was lower than the data in this work using PA-PSf (NaClO-350) or PA-PSf (NaClO-400) because of the increased content of fermentation inhibitors. In the case of 3-cycle

NF method, dilution was used twice in up-concentration process between three NF concentrations. The ethanol yield in the case of concentration by the FO method using NaClO-treated membranes was similar to that obtained by the 3-cycle NF method. Therefore, it can be considered that it is effective to upconcentrate sugar solution with fewer steps and achieve comparable ethanol productivity when NaClO-treated membranes are applied for the FO process.

Thus, NaClO-treated PA-PSf membranes can achieve high ethanol productivity without any other additional operations, such as dilution. These results can contribute to the generation of bioethanol with lower production costs by using a simplified process.

4. Conclusions

Polyamide-polysulfone membranes were treated with NaClO solution to improve the performance of FO process to concentrate sugars and remove fermentation inhibitors. The optimum treatment condition was established to improve the water permeability (*A* value) and permselectivity of the fermentation inhibitors (furfural and acetic acid) against xylose. In the FO concentration of the real liquid fraction of the hydrothermally pretreated rice straw, sugars were selectively concentrated, while all of the fermentation inhibitors were permeated through the membrane and showed almost no change in concentration. The permselectivity of fermentation inhibitors was higher than that of commercial FO membrane (TFC-ES). Finally, xylose-assimilating *S. cerevisiae* produced 18.0 g/L of ethanol and achieved a high ethanol yield (0.40) for the sugar solution concentrated by the FO process.

Compared to other processes including multistage NF membranes reported before, adequately concentrated sugar solutions with low levels of fermentation inhibitors can be generated in a one-step concentration by a FO process using NaClO-treated PA-PSf membranes. It is apparent that PA-PSf membrane treated with NaClO can expand the possibilities for bioethanol production from lignocellulosic biomass.

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Figure captions

Figure 1 The surface of the PA-PSf membrane before and after NaClO treatment, observed by SEM. (a, b) Untreated membrane, (c, d) membrane treated with 500 ppm NaClO, (e, f) membrane treated with 2500 ppm NaClO

Figure 2 ATR−FTIR spectra of the PA-PSf membrane before and after NaClO treatment: (a) untreated and (b, c) treated with 500 ppm and 2500 ppm NaClO

Figure 3 Intrinsic membrane property changes by NaClO treatment: (a) Correlation between *A* value and effective NaClO concentration, (b) Correlation between rejections of solutes against *A* value.

Figure 4 Rejections of (a) xylose and furfural and (b) xylose and acetic acid as a function of A value. Open symbols represent single-component feed solution of each xylose and two inhibitors and closed symbols represent mixed feed solution consists of xylose and one kind of inhibitor. ○●: xylose (2 g/L), furfural (20.8 mM), acetic acid (30 mM), △▲: xylose (1.5 g/L), furfural (5.2 mM), □■: xylose (2 g/L), furfural (1.7 mM), acetic acid (30 mM)

Figure 5 Time course of each component concentration in FS side via PA-PSf membrane treated by 350 ppm NaClO during synthetic sugar solution concentration experiment

Figure 6 Water flux and FS concentration ratio as a function of concentration time [PA-PSf (NaClO-350)]

Figure 7 Time course of six fermentation inhibitors concentrations in FS side by using homemade FO membrane: (a) PA-PSf (Untreated), (b) PA-PSf (NaClO-350), and (c) PA-PSf (NaClO-400)

Figure 8 Concentration changes of sugars and fermentation products in liquid fractions of hydrothermally pretreated rice straw concentrated by the membrane during fermentation by *S. cerevisiae* MN8140X/TF-TF. Membrane: (a) PA-PSf (Untreated), (b) PA-PSf (NaClO-350)

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Table 1 The ratio of amide II band, aromatic amide peak and amide I band to C-O-C stretching of the PA-PSf membranes before and after NaClO treatment

Membrane	Amide II	Aromatic amide	Amide I
PA-PSf (Untreated)	0.31	0.33	0.31
PA-PSf (NaClO-500)	0.20	0.21	0.25
PA-PSf (NaClO-2500)	0.15	0.16	0.20

Table 2 Surface chemical composition of the PA-PSf membranes before and after NaClO treatment

Membrane	C [%]	O [%]	N [%]	Cl [%]	Cl/N
PA-PSf (Untreated)	73.7	15.1	11.2	-	-
PA-PSf (NaClO-500)	70.4	14.0	10.5	5.13	0.49
PA-PSf (NaClO-2500)	69.5	13.3	10.2	7.05	0.69

Table 3 Concentration ratios of total sugar and six fermentation inhibitors after FO concentration against concentrations in original liquid fraction from hydrothermal pretreatment of rice straw

Membrane	Process	Concentration ratio (divided by the initial concentration)							[Ref.]
		Acetic acid	Formic acid	Furfural	5-HMF	Vanillin	Syring aldehyde	Total sugar	
PA-PSf (Untreated)	FO	2.3	1.4	1.8	2.7	3.3	1.8	3.1	This study
PA-PSf (NaClO-350)	FO	1.0	2.1	0.7	1.3	2.5	2.7	3.6	This study
PA-PSf (NaClO-400)	FO	1.2	2.3	1.0	1.8	2.9	3.5	3.6	This study
TFC-ES	FO	5.8	2.1	4.7	2.8	-	-	7.5	[17]
ESNA3	NF	1.2	1.4	1.5	1.8	2.5	1.2	3.6	[17]

Liquid fraction from hydrothermal pretreatment of rice straw was concentrated 5 times in this study.

Table 4 Summary of ethanol concentration and yield for liquid fraction from hydrothermal pretreatment of rice straw after concentration by different membrane processes

Membrane	Process	Concentration [g/L]		Yield	[Ref.]
		Total sugar	Ethanol		
PA-PSf (NaClO-350) a)	FO	56.4	17.6	0.39	This study
PA-PSf (NaClO-400) a)	FO	56.2	18.0	0.40	This study
TFC-ES a)	FO	118	1.08	0.01	[17]
ESNA3 b)	NF×1	67.6	18.5	0.31	[17]
ESNA3 c)	NF×3	65.7	22.0	0.42	[17]

a) FO method: Original⇒FO⇒Enzymatic glycation⇒Ethanol fermentation

b) NF method (1 cycle): Original⇒NF⇒Enzymatic glycation⇒Ethanol fermentation

c) NF method (3 cycle): Original⇒NF⇒dilution⇒NF⇒dilution⇒NF⇒Enzymatic glycation⇒Ethanol fermentation

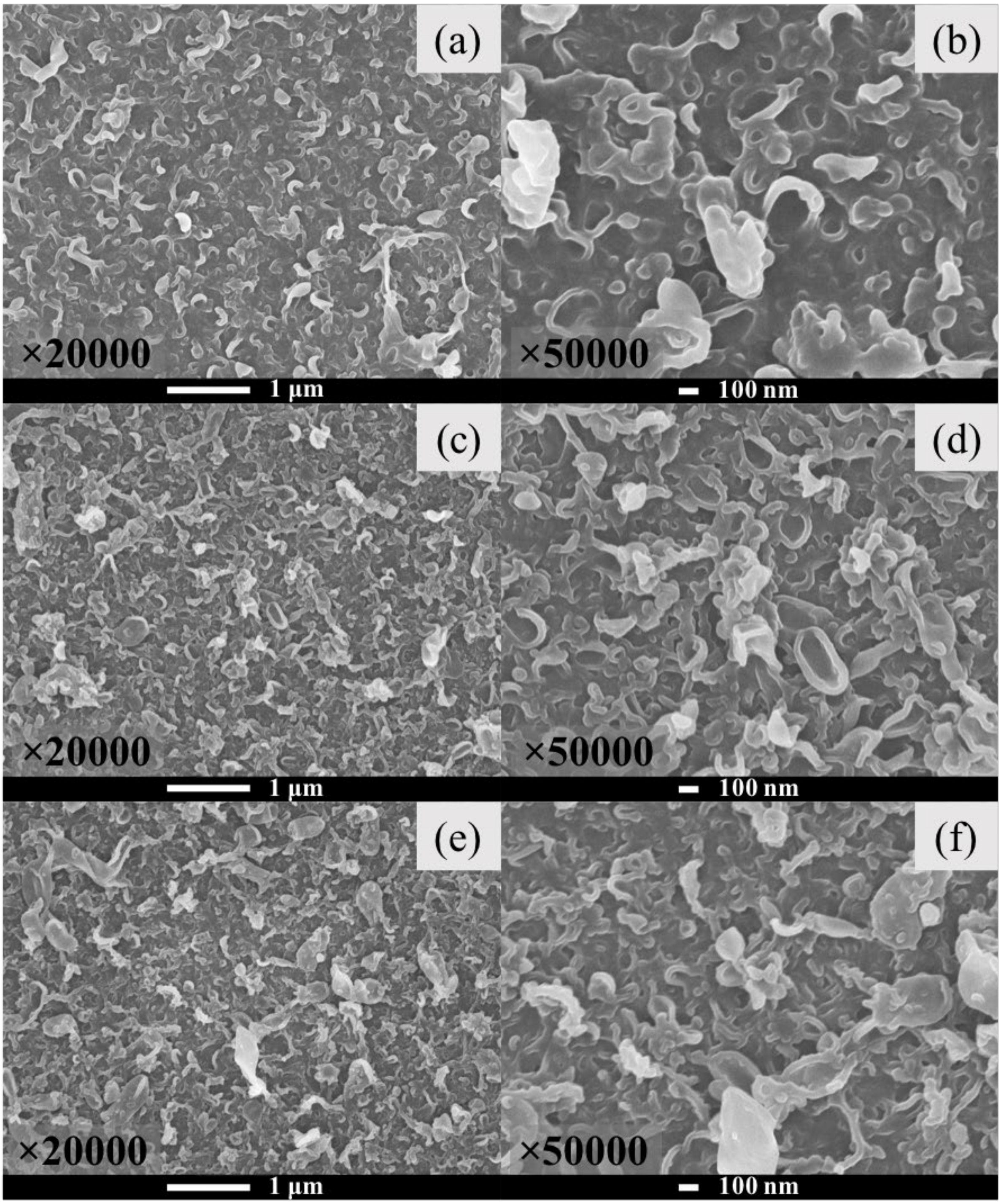
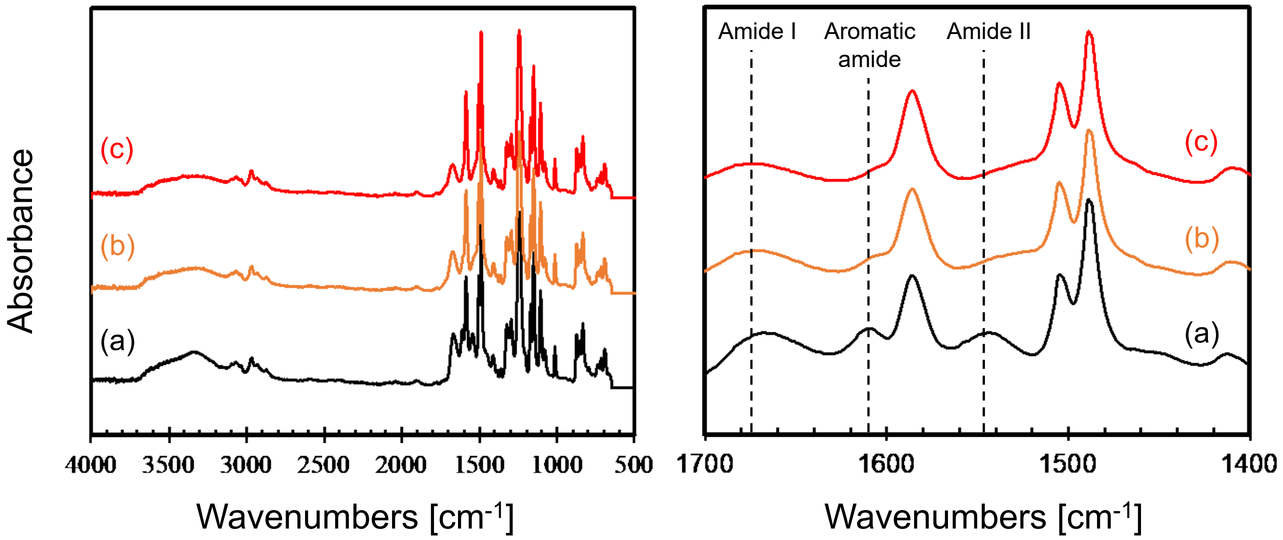


Figure 1 The surface of the PA-PSf membrane before and after NaClO treatment, observed by SEM. (a, b) Untreated membrane, (c, d) membrane treated with 500 ppm NaClO, (e, f) membrane treated with 2500 ppm NaClO



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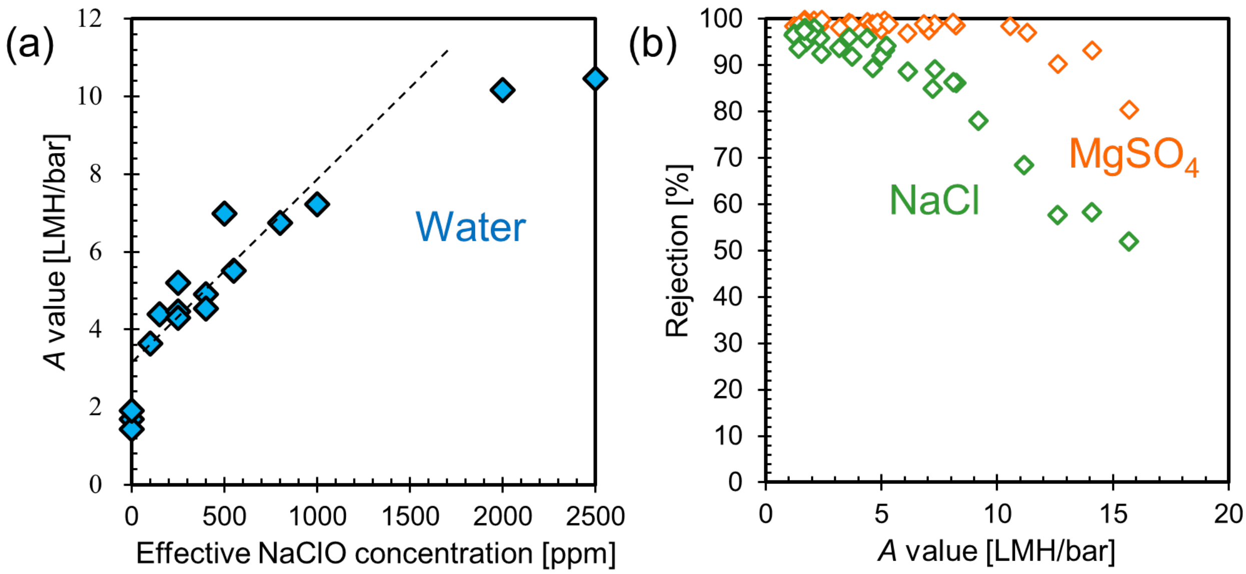

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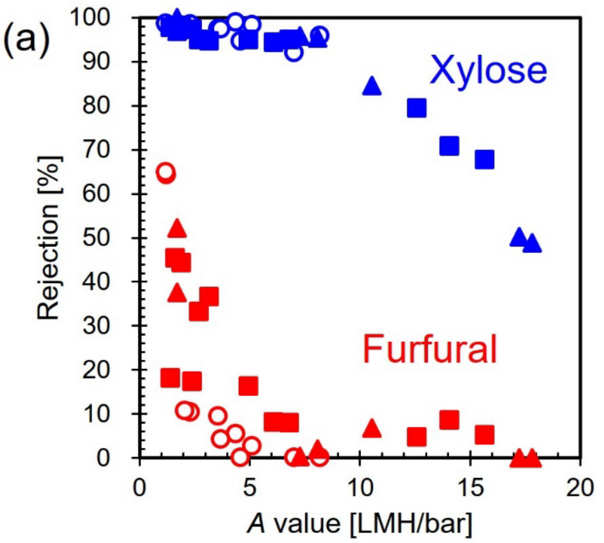

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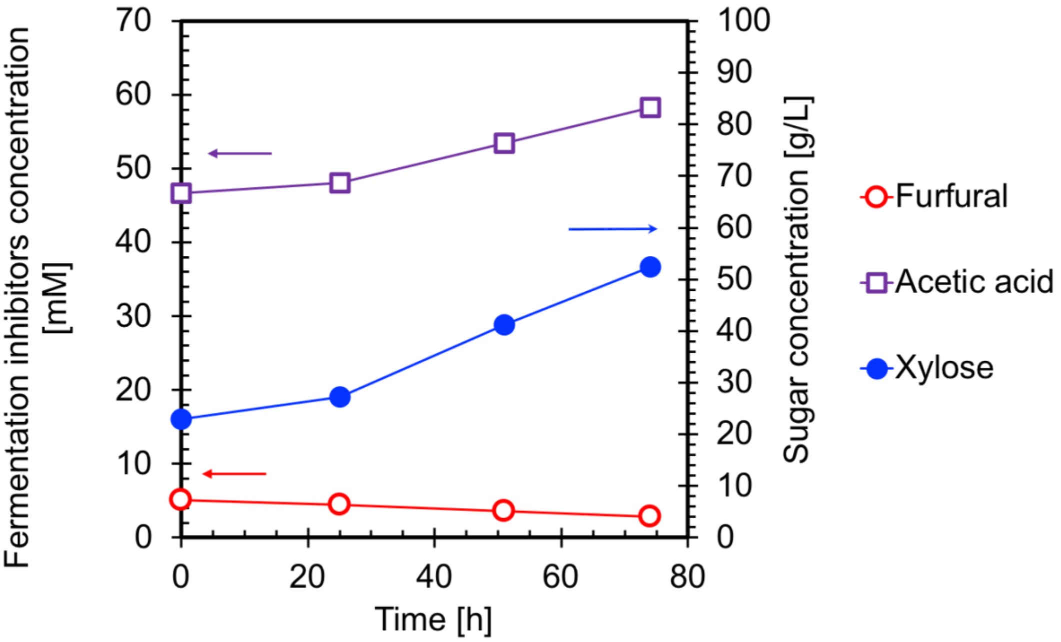

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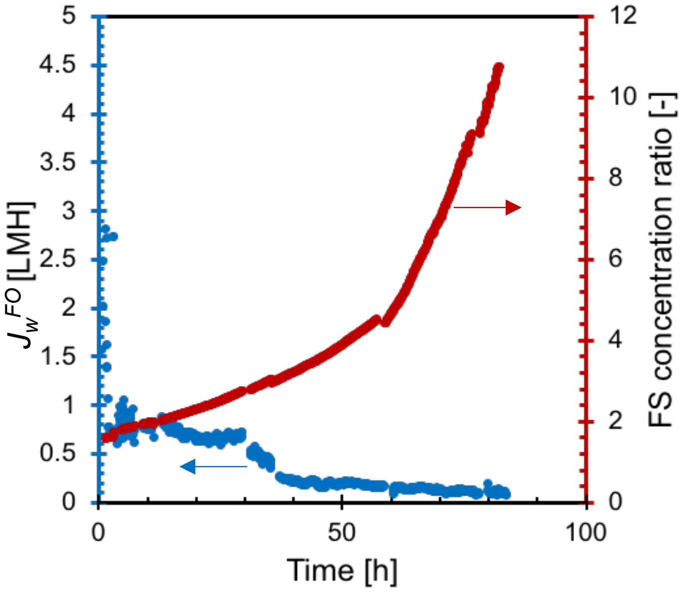

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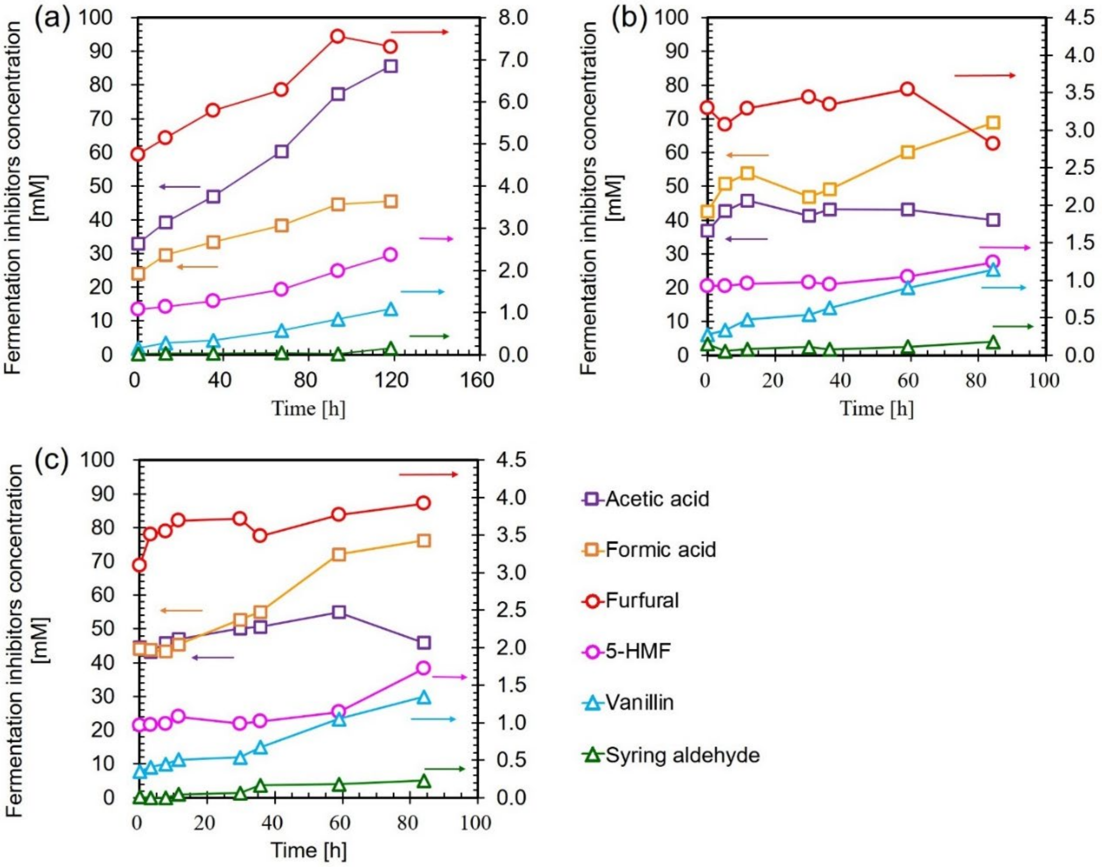

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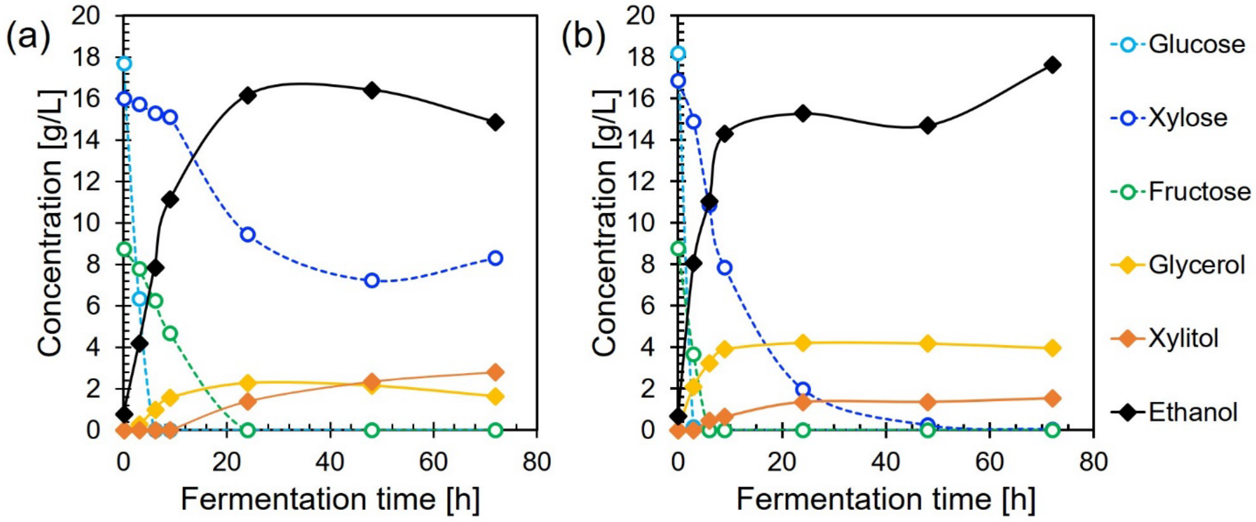

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