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Rejection of endocrine disrupters contained in biologically treated sewage by nanofiltration

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Abstract−Two commercial nanofiltration membranes, NF-1 (low salt rejection) and NF-3 (medium salt rejection), were used for basic experiments on the rejection of endocrine disrupters of 17βestradiol, *p*-nonylphenol, bisphenol A and their mixed solution. Nanofiltration membrane experiments were carried out under low trans-membrane pressure of 0.5 MPa as the operating condition. For the two nanofiltration membranes, the rejection factor was high when the pH of each feed solution was not adjusted. Based on the results of the nanofiltration membrane experiments, four commercial nanofiltration membranes--NF-1, NF-2 (medium salt rejection), NF-3 and NF-4 (high salt rejection)--were used for the rejection of endocrine disrupters contained in biologically treated sewage. The biologically treated sewage concentration of 0.039-0.055 μg/L as 17βestradiol equivalent was reduced by each nanofiltration membrane to 0.026 μg/L (NF-1), 0.025 μg/L (NF-2), 0.003 μg/L (NF-3) and 0.009 μg/L (NF-4), as 17βestradiol equivalent, respectively. The rejection efficiency of endocrine disrupters showed the same tendency as the TOC rejection efficiency. The permeate flux of nanofiltration membranes was high in the order of NF-1, NF-3, NF-2 and NF-4.

Key words: Nanofiltration Membrane, Endocrine Disrupters, Estrogen Receptor Competition Assay, Biologically Treated Sewage

INTRODUCTION

In recent years, certain chemical substances that are artificially produced and used have caused unprecedented environmental problems, because once a chemical is absorbed in the body, it binds to an estrogen receptor and acts as if it were a female hormone [Colborn et al., 1996; Kuroda, 1998; Inoue, 1997]. It is known that substances such as 17βestradiol from human and animal excrement, and nonylphenol, which are widely used as non-ionic surfactants, have been detected with relatively high frequency in water environments. There has been concern that micro-pollutants of several chemicals in the environment are affecting human health by disrupting normal endocrine function [Tanaka, 1999; Tanghe et al., 1999; Blackburn and Waldock, 1995].

Sewage resulting from residential and commercial facilities is treated at municipal sewage treatment plants before being discharged into water environments. It is highly probable that harmful micro-pollutants that may act like estrogen are mixed with influent sewage at municipal sewage treatment plants into which various kinds of substances are flowing [Takigami et al., 1999; Fujita et al., 2000]. Therefore, the discharge of endocrine disrupters (EDs) into public waters, rivers and estuaries must be minimized. However, current sewage treatment processes cannot completely remove harmful micro-pollutants such as nonylphenol etc.

Many kinds of nanofiltration membranes, which lie between ultrafiltration membranes and reverse osmosis membranes as molecular weight cut-off, are often used in ultrapure water and water purification [Prakorn et al., 2004; James et al., 1997; Fu et al., 1994; Itoh et al., 2000; Trebouet et al., 1999]. And because their rejection efficiency varies depending not only on the operation pressure but also the pH level of the feed solution, and the properties of the solute which will attempt to reject, it is expected to reject harmful micro-pollutants which have been considered inapplicable [Kim et al., 2006; Childress and Elimelech, 2000; Braghetta et al., 1997].

The objective of this study was to investigate rejection property of the three endocrine disrupters (17βestradiol, nonylphenol and bisphenol A) with nanofiltration membranes at a low pressure operation. Based on the results, the rejection efficiency of endocrine disrupters contained in biologically treated sewage was also investigated.

MATERIALS AND METHODS

1. Nanofiltration Membrane

The experiments used a membrane treatment apparatus (RUM-10, Nitto Denko, Japan) consisting of a feed storage tank, a flat sheet type membrane test cell (C10-T, Nitto Denko, Japan), a magnetic type pressurization gear pump (Tuthill, U.S.), a flowmeter and a pressure gauge, etc. The cross flow filtration method was adopted for experimental apparatus because it is easy to perform similar to

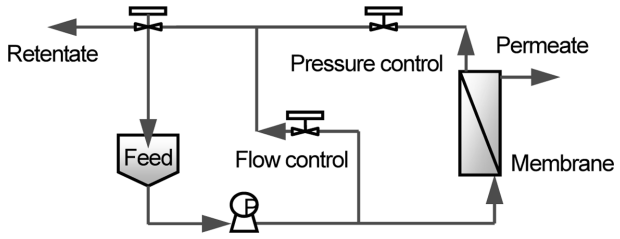

Fig. 1. A schematic diagram of nanofiltration membrane process.

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Table 1. Characteristics of nanofiltration membranes (data from the manufacturer)

Items	Nanofiltration membranes			
	NF-1	NF-2	NF-3	NF-4
Rejection (%)				
NaCl	10.0	50.0	60.0	93.0
Sucrose	5.0	36.0	98.0	99.0
Test condition				
Conc. (%)	0.20	0.20	0.15	0.15
Pressure	0.5 MPa	1.0 MPa	1.5 MPa	1.5 MPa
pH range	7.0			
Configuration	Flat sheet			
Area (cm^2)	60			
Charge	Negative			
Materials	SPS	SPS	PVA	PVA

SPS; Sulfonated/Polysulfonate
PVA; Polyvinylalcohol/Polyamides

that of spiral membrane modules that are widely used in full-scale membrane treatment plants. Fig. 1 shows a schematic diagram of the nanofiltration apparatus. Nanofiltration membrane surface area was 60 cm^2 (46 mm by 180 mm). Membrane experiments used four kinds of nanofiltration membranes with different salt rejection rates. Table 1 shows the properties of the nanofiltration membranes.

2. Municipal Sewage

The biologically treated sewage was collected from an operating pilot plant with the conventional activated sludge process at municipal sewage treatment plant K in east central Japan, and stored in a sample storage room which was maintained at 10 °C. Septum cap vials cleaned with methanol (analytical grade, Wako pure chemical industries, Japan) were used to collect the sewage in order to prevent its contamination by adhering impurities. The biologically treated sewage was filtered with a 0.45 μm membrane filter to remove constituents such as organic substances and colloidal matters that would obstruct membrane operation before supplying the biologically treated sewage to the membrane process.

3. Experimental Method

The nanofiltration membrane was completely rinsed with ion exchange water and made to permeate the membrane for approximately 10 minutes before the membrane experiment was started, then water samples were filtered. All membrane experiments were carried out at the operating pressure of 0.5 MPa in a constant temperature room at 20 °C.

Before starting the membrane experiment using biologically treated sewage, the rejection efficiency of nanofiltration membranes, NF-1 and NF-3 to reject 17βestradiol (analytical grade, Wako pure chemical industries, Japan), *p*-nonylphenol (analytical grade, Kanto chemicals, Japan), bisphenol A (analytical grade, Kanto chemicals, Japan), and their mixed solution was tested by changing their pH to pH 6.5 (unadjusted), pH 9, pH 7 and pH 5. The standard solution concentrations of 17βestradiol, *p*-nonylphenol and bisphenol A were 250 μM, 500 μM and 50 mM, respectively, and their standard solutions were made with ethanol (analytical grade, Wako pure chemical industries, Japan). These standard solutions were diluted in stages; then 50% inhibition concentration (IC_{50}) was determined based on the fluorescence polarization obtained by the estrogen receptor competition assay as described below. The membrane experiment was carried out with the feed solution concentration set at close to 50% inhibition concentration.

Biologically treated sewage, which is stored in a sample storage room as described above, was provided for use in the membrane experiment after it was restored to room temperature. After 2.5 liters of the biologically treated sewage that had been filtered using a 0.45 μm membrane filter (ADVANTEC, Japan) was placed in the storage tank, a pressurization pump was performed at the constant operation pressure of 0.5 MPa and flow rate was 1.0 L/min corresponding to a cross flow velocity of approximately 1.5 cm/s until 1 liter of membrane permeate was obtained. The above experiment was considered as one cycle, and each time one cycle was completed, the membrane was rinsed with ion exchange water and compared with the ion exchange water permeate flux of a new membrane. The solute rejection efficiency was calculated by the following equation. Because water temperature rose gradually during the membrane experiment, permeate flux was obtained by the equation: (the permeate at experimental temperature)/temperature conversion coefficient ($1.03^{(T-25)}$).

$$\text{Rejection}\ (\%) = \{1 \cdot (2 \cdot C)/(C_o + C_l)\} \times 100$$

C_o : concentration of feed solution (raw water)
C_l : concentration of retentate solution
C : concentration of permeate solution

4. Estrogen Activity Measurement Method

The estrogen receptor competition assay and its assay kit (Pan Vera, U.S.) were used to measure estrogen activity to perform overall assessment of endocrine disrupters [Kondo et al., 1999]. Endocrine disrupters added to a mixed liquid of human recombinant estrogen receptor (hERα) and fluorescence estrogen (FES1) cause a competitive reaction concerning receptor combination between the fluorescence estrogen and the endocrine disrupter, which changes the degree of polarization of the fluorescence estrogen that is inhibited from binding. The estrogen receptor competition assay measures change with a fluorescence polarization analyzer (Full-Range Beacon 2000, Pan Vera, U.S.).

The test procedure was as follows. A test compound of 2 μl was added to 48 μl of buffer solution, then 50 μl of mixed liquid (FES1 and hERα) was added to make capacity of 100 μl. For positive control (corresponding to 0% inhibition), 50 μl of mixed liquid and 2 μl of dimethyl sulfoxide (analytical grade, Wako pure chemical Industries, Japan) were added to 48 μl of buffer solution to make capacity of 100 μl. For negative control (corresponding to 100% inhibition), 10 μl of fluorescence estrogen and 2 μl of dimethyl sulfoxide were added to 88 μl of buffer solution to make capacity of 100 μl. Then sample solutions were left to stand for reaction for 60 minutes at room temperature and their polarization was measured with the fluorescence polarization analyzer. The inhibition rate was calculated by solving the following equation for the polarization of each sample. Then, the strength of estrogen activity of the samples was converted to 17βestradiol equivalent based on a 50% inhibition concentration obtained from the competition curve. Fig. 2 shows a result. The horizontal axis is 17βestradiol standard concentration and the vertical axis is inhibition rates. 50% inhibition concentration of the

17βestradiol was approximately 6.5 nM (1.8 μg/L).

$$\text{Inhibition (\%)} = (A_o - A)/(A_o - A_{100})$$

A : Test compound polarization (mP)
A_o : Positive control polarization (mP)
A_{100}: Negative control polarization (mP)

Fluorescence substances possibly existing in samples may obstruct endocrine disrupter measurement of biologically treated sewage when with the fluorescence polarization analyzer. Therefore, the samples were adjusted by the solid phase extraction method to remove fluorescence substances during pretreatment of them. The sample was extracted by dichloromethane (analytical grade, Kanto chemicals, Japan) using the Sep-Pak C18 cartridge (Waters, U.S.); then dimethyl sulfoxide was added to it to make analysis samples. In the case of membrane experiments using biologically treated sewage, total organic carbon (TOC) was also measured with the TOC analyzer (TOC-5000, Shimazu, Japan).

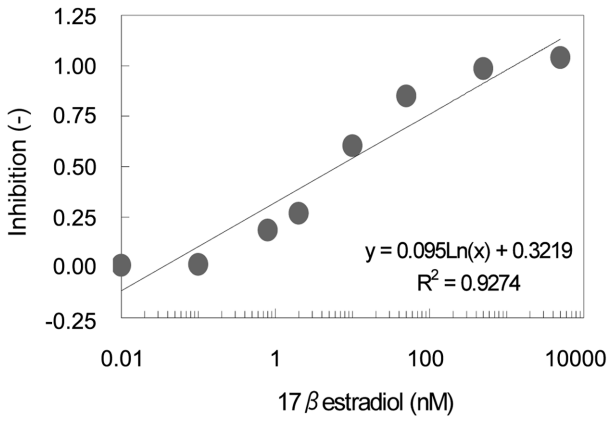

Fig. 2. Competition assay against 17βestradiol.

Table 2. Estrogenic activity of endocrine disrupters

Conc. (nM)	17βestradiol		*p*-nonylphenol		Bisphenol A	
	P†	I††	P	I	P	I
1000000					94.0	0.875
500000					101.9	0.843
200000					134.0	0.715
100000					152.0	0.643
20000					233.7	0.317
10000			129.6	0.733	264.8	0.193
5000	77.4	1.040	162.6	0.601	288.6	0.097
500	90.0	0.985	277.1	0.143	320.2	−0.029
50	121.3	0.850	330.9	−0.072		
10	178.7	0.602	328.5	−0.062		
2	256.3	0.267	331.0	−0.072		
1			351.1	−0.152		
0.8	275.5	0.184				
0.1	314.7	0.015	356.4	−0.174		
0.01	315.8	0.010	357.0	−0.176		

†; polarization
††; inhibition

RESULTS AND DISCUSSION

1. Rejection Property of Endocrine Disrupting Chemicals

Table 2 shows the fluorescence polarization and inhibition rate for concentrations of each endocrine disrupter. Based on a 50% inhibition concentration, the concentrations of 17βestradiol, *p*-nonylphenol and bisphenol A in test solution were set at 100 nM, 5,000 nM and 10,000 nM, respectively. The pH of solutions of 17βestradiol, *p*-nonylphenol, bisphenol A and their mixed solution were varied to pH 6.5 (unadjusted), pH 9, pH 7 and pH 5 to examine the endocrine disrupters rejection efficiency of nanofiltration membranes NF-1 and NF-3 with different salt rejection rates. Fig. 3 shows the results.

The NF-1 membrane showed negative rejection efficiency against 17βestradiol regardless of the variance of pH, while the NF-3 membrane showed rejection efficiency of 70% or higher and, especially when pH was not adjusted, 87.6% of the highest rejection efficiency was shown. The NF-1 membrane showed a rejection efficiency of as low as 32.8% against *p*-nonylphenol at pH 5, and 71.8% at pH 6.5. The rejection efficiency of *p*-nonylphenol of the NF-3 membrane ranged between 49% and 59%, narrower than that of the NF-1 membrane. The NF-1 membrane revealed a narrow range of rejection efficiency against bisphenol A with different pH values, which was as low as 10% or less. The rejection efficiency of the NF-3 membrane was 50% overall and was a little higher for the solution without pH adjustment.

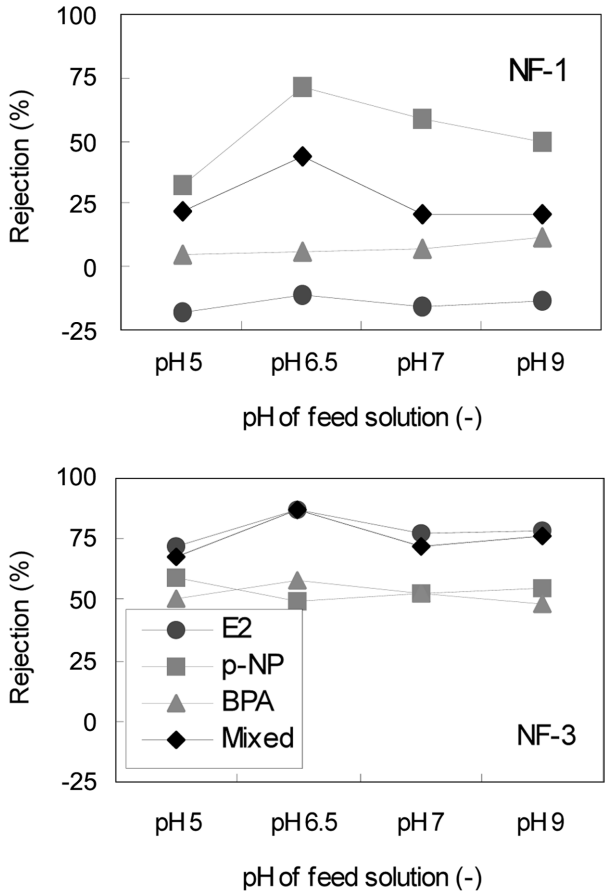

Fig. 3. Rejection of 17βestradiol, *p*-nonylphenol bisphenol A and their mixed solution.

Regarding the rejection efficiency of mixed solution of the above three substances (represented by 17βestradiol equivalent), the NF-1 membrane showed a rejection efficiency of 43.5% at pH 6.5, almost twice as high as those with pH adjustment. NF-3 also showed the highest rejection efficiency of 87.1% at pH 6.5. Both nanofiltration membranes used for the experiment showed their highest rejection efficiency against chemical substances such as 17βestradiol, *p*-nonylphenol and bisphenol A that are suspected of acting like estrogen when pH of the solution was not adjusted. For the rejection efficiency of the mixed solutions of three endocrine disrupters, both nanofiltration membranes showed little dependence on variance of pH and showed relatively high rejection efficiency at pH 6.5.

When nanofiltration membranes reject solute in a solution, the repulsion force against the membrane may reject electrically equivalent dissociated ionic substances due to Donnan exclusion near surface of the membrane in which negatively charged functional groups are fixed. It is probable that the solute rejection efficiency is low in the low pH range where no particular dissociation occurs and high in the high pH range [Hagiwara and Hashimoto, 1972]. Because the variance of pH values of solutions of 17βestradiol and *p*-nonylphenol used for the membrane experiment from pH 5 to pH 9 caused little change in their rejection efficiency, it is assumed that there is little apparent dissociation and solutes exist in the molecular state in solutions.

Regarding the nanofiltration membranes experiment to reject endocrine disrupters, the NF-1 membrane maintained a lower salt rejection rate property than that of the NF-3 membrane. This indicates that 17βestradiol is permeated through the membrane easily and it lowered the rejection efficiency. For *p*-nonylphenol, however, the NF-1 membrane showed a higher rejection efficiency than the NF-3 membrane. Therefore, it is difficult to evaluate their rejection efficiencies based only on the molecular weight fraction of each substances, and it is presumed that there are factors other than chemical properties of substances and membrane materials that affect solute rejection efficiency [Braghetta et al., 1997; Maria et al., 1992; Mulder, 1997]. In other words, the solute separation by membranes is related to whether the solvent (water) or the solute is selectively adsorbed by the membrane materials [Hagiwara and Hashimoto, 1972].

Based on the results of the rejection experiment for 17βestradiol, *p*-nonylphenol, bisphenol A and their mixed solution revealed that test solution with unadjusted pH showed high rejection efficiency. The membrane experiment was performed using biologically treated sewage without pH adjustment and using the two types of nanofiltration membranes plus two additional types of nanofiltration membranes with different salt rejection rates, NF-2 and NF-4.

2. Rejection Property of Endocrine Disrupters Contained in Biologically Treated Sewage

17βestradiol equivalent in the biologically treated sewage used for the membrane experiment ranged between 0.039 μg/L and 0.055 μg/L. The 17βestradiol equivalent of the biologically treated sewage was filtered with a 0.45 μm membrane, which was used as test solution for the membrane experiment, ranging between 0.035 μg/L and 0.053 μg/L . Therefore, there were hardly rejected endocrine disrupters only by micro-filtration performed as pretreatment. Fig. 4 shows the rejection efficiency of the four nanofiltration membranes

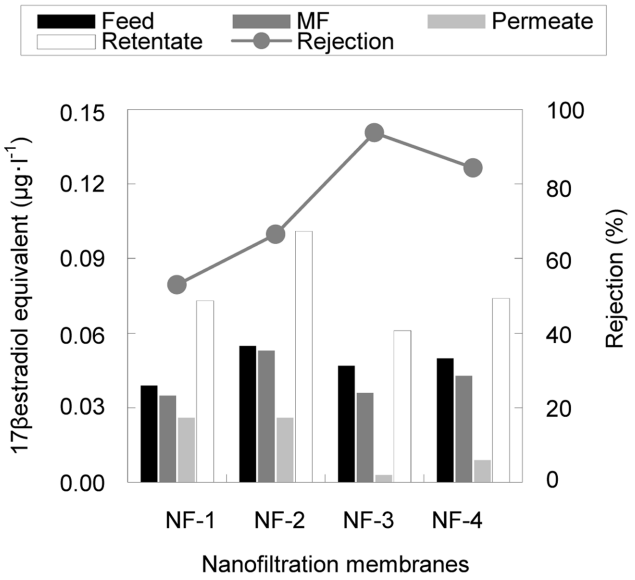

Fig. 4. Rejection of endocrine disrupters contained in biologically treated sewage by using four nanofiltration membranes.

with different salt rejection rates to reject endocrine disrupters in filtered biologically treated sewage. The NF-1 membrane lowered 17βestradiol equivalent from 0.035 μg/L in filtered biologically treated sewage to 0.026 μg/L in the solution that permeated the membrane, indicating a rejection efficiency of 53% (calculated by averaging 17βestradiol equivalent in filtered biologically treated sewage and in the retentate water). The NF-2 membrane lowered 17βestradiol equivalent from 0.053 μg/L in filtered biologically treated sewage to 0.025 μg/L in the solution that permeated the membrane, indicating a rejection efficiency of 66.5%. The rejection efficiency of the NF-1 membrane with a low salt rejection rate was about 10% higher than that when the NF-1 membrane filtered a mixed solution of endocrine disrupters, regardless of the low-pressure opera-

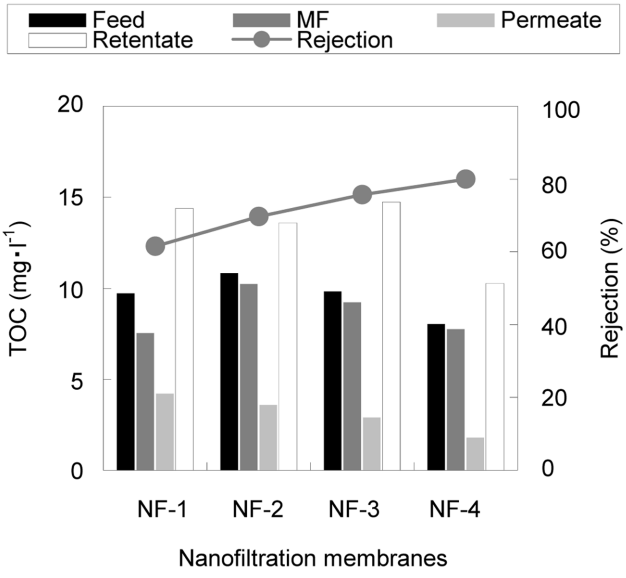

Fig. 5. TOC rejection of biologically treated sewage by using four nanofiltration membranes.

tion. Regarding rejection efficiency of the NF-2 membrane which was made of the same membrane material but had a different salt rejection rate, the rejection efficiency differed because of the high concentrations of both filtered biologically treated sewage and the retentate, although the solution permeating membrane had the same 17βestradiol equivalent as that of the NF-1 membrane.

Fig. 5, which shows TOC rejection efficiency, reveals that the NF-1 membrane lowered concentration from 7.5 mg/L in filtered biologically treated sewage to 4.2 mg/L in solution permeating the membrane and the NF-2 lowered it from 10.3 mg/L in filtered biologically treated sewage to 3.6 mg/L in solution permeating the membrane, indicating that its TOC rejection efficiency was almost equal to that of endocrine disrupters. The NF-3 membrane, which lowered 17βestradiol equivalent from 0.036 μg/L in filtered biologically treated sewage to 0.003 μg/L in solution permeating the membrane, showed the highest rejection efficiency of 94%. The NF-4 membrane, which lowered 17βestradiol equivalent from 0.043 μg/L in filtered biologically treated sewage to 0.009 μg/L in solution permeating the membrane, showed a rejection efficiency of 84%. Regarding TOC rejection efficiency, the NF-3 membrane, which lowered the concentration from 9.2 mg/L in filtered biologically treated sewage to 2.9 mg/L in solution permeating the membrane, showed a TOC rejection efficiency of 76%, while the NF-4 membrane, which lowered the concentration from 7.8 mg/L in filtered biologically treated sewage to 1.8 mg/L in solution permeating the membrane, showed a rejection rate of 80%.

Both of these nanofiltration membranes showed higher rejection efficiency than the NF-1 and NF-2 membranes. It is assumed that the difference of about 30% in endocrine disrupter rejection efficiency between the NF-3 membrane and NF-2 membrane was caused by their differing membrane materials regardless of their almost equal to nominal salt rejection rates. This assumption is also supported by the fact that differences in concentrations in solution permeating membranes were small between the NF-1 and NF-2 membranes, and between the NF-3 and NF-4 membranes, which are made of similar materials.

Fig. 6 shows the permeate flux of nanofiltration membranes in filtered biologically treated sewage. Because of its low salt rejection rate, the permeate flux of the NF-1 membrane was 2,245 L/m^2/day, 3 to 10 times larger than that of the other nanofiltration membranes. It is assumed that the NF-2 and NF-3 membranes showed a similar permeate flux of 703 L/m^2/day and 761 L/m^2/day, respectively, because of their similar salt rejection rates. Because the NF-4 membrane had the highest nominal salt rejection rate of all the nanofiltration membranes used for experiment, it adsorbed many substances that appeared to be clogging the membrane surface as shown in Fig. 7, and its permeate flux was as low as 191 L/m^2/day. Although the NF-2 and NF-3 membranes have similar salt rejection rates, the surface of the NF-3 membrane adsorbed many substances that appeared to be clogging it than that of the NF-2 membrane. However, there was little difference in the permeate flux of two nanofiltration membranes, and the NF-3 membrane showed higher efficiency to reject endocrine disrupters and TOC contained in filtered biologically treated sewage. This is assumed to be a result of the fact the NF-3 membrane is resistant to clogging even when its surface has adsorbed contaminants.

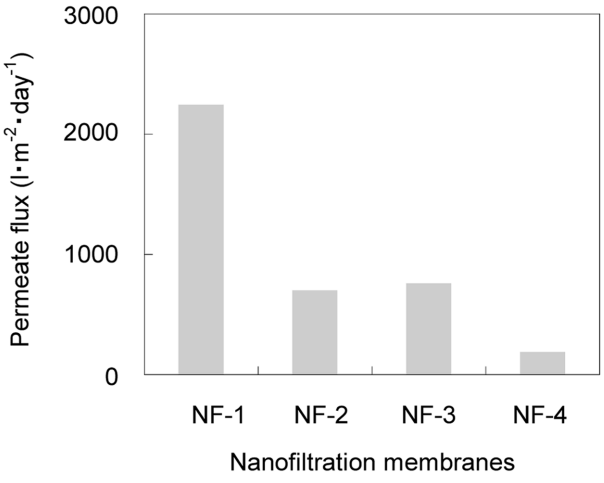

Fig. 6. Permeate flux of biologically treated sewage by using four nanofiltration membranes.

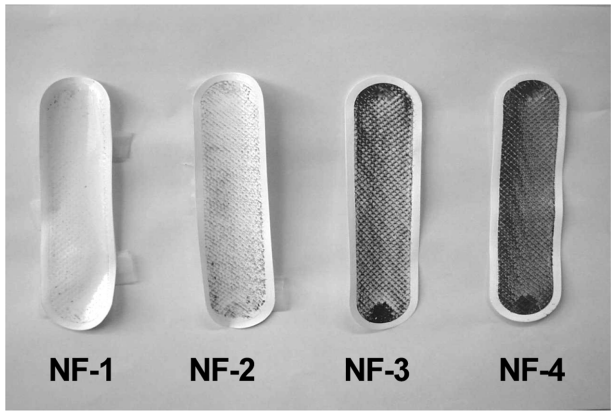

Fig. 7. Four nanofiltration membrane surface after the rejection experiments.

The criteria for selecting the membrane materials in membrane treatment operations vary according to the substance to be rejected. In the case of porous membranes such as membrane filters (microfiltration membrane) used for pretreatment, selection of the membrane material is not very important. In the case of a non-porous membrane such as a nanofiltration membrane, however, selection of the membrane material is important because of its significant effect on the membrane surface, including chemical effect, adsorption, and leakage [Hagiwara and Hashimoto, 1972; Mulder, 1997].

CONCLUSIONS

Membrane experiments were conducted to assess the efficiency of nanofiltration membranes having different salt rejection rates (NF-1, NF-2, NF-3, and NF-4) to reject 17βestradiol, *p*-nonylphenol and bisphenol A as well as endocrine disrupters contained in biologically treated sewage. The following results were obtained:

1. The NF-1 membrane with a low salt rejection rate of 10% showed a low efficiency to reject 17βestradiol and bisphenol A but a high efficiency to reject *p*-nonylphenol.
2. The NF-3 membrane with a salt rejection rate of 50% showed

higher rejection efficiency than the NF-1 membrane, especially against 17βestradiol. Neither nanofiltration membrane the NF-1 nor NF-3 showed any dependence on pH for endocrine disrupters.

3. The estrogen receptor competition assay revealed that the NF-1 and NF-2 membranes (salt rejection rate: 50%) lowered the concentration of endocrine disrupters from the range between 0.039 μg/L and 0.055 μg/L (corresponding to 17βestradiol equivalent) in biologically treated sewage to 0.026 μg/L and 0.025 μg/L, respectively, in solutions permeating the membranes, showing rejection efficiencies of 53% and 67%, respectively. The NF-3 and NF-4 membranes (salt rejection rate: 90%) made the concentration of solutions permeating the membranes 0.003 μg/L and 0.009 μg/L, respectively, showing rejection efficiencies of 94% and 84%, respectively. The endocrine disrupter rejection efficiencies of these nanofiltration membranes were similar to their TOC rejection efficiencies. The fact that the rejection efficiency varied regardless of the similar salt rejection rates and that there was little difference in concentration of solutions permeating the membranes regardless of the different salt rejection rates, clearly shows that the membrane material affects the rejection efficiency.

4. The permeate flux of the biologically treated sewage was high in the order of NF-1 membrane, NF-3 membrane, NF-2 membrane, and NF-4 membrane.

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