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Evaluation of biofouling potential of microorganism using flow field-flow fractionation (Fl-FFF)

Seongbeen Lim, Sangyoup Lee, Soohoon Choi, Jihee Moon, Seungkwan Hong

Abstract

The adhesion property of Pseudomonas putida on reverse osmosis (RO) membranes was systematically investigated using the asymmetrical flow field-flow fractionation (AsFl-FFF). The adhesion of P. putida on two different RO membranes was investigated by varying the salt concentration of carrier solution to evaluate the effect of ionic strengths and membrane characteristics on the biofouling potential of RO membranes. The elution peak in terms of peak retention time and area obtained from AsFl-FFF analysis was used to evaluate the adhesion tendency of P. putida under different solution ionic conditions. Results showed that P. putida was favorably attached to RO membranes at higher ionic strengths. Hydrophobic RO membrane exhibited more adhesive property to P. putida compared to the tested hydrophilic membrane under the lower ionic strength condition. The effect of solution ionic strength on the adhesion tendency was more influential than membrane characteristics. In addition, the influence of ionic strength variation on adhesion tendency was more sensitive to hydrophilic membranes than hydrophobic membranes.

1. Introduction

The deposition of undesirable materials on the membrane surface and/or pores may reduce the permeate flux and operation efficiency in reverse osmosis (RO) membrane technology, which is called fouling. Though the demand for RO technology is tremendously increasing as an alternative technology solving water problem, membrane fouling still acted as the major obstruction to limit RO membrane application in water treatment, wastewater reclamation and even in desalination. Among various foulants, microorganisms are one of the major foulants in most of the RO membrane processes, that is, biofouling [1–3]. Biofouling on the membrane surface is composed of two principle mechanisms. The primary mechanism is related to the attachment of microorganisms to the membrane surface as a single or group. After the initial attachment stage, the microorganisms proliferate and form a biofilm over the membrane surface [4,5]. Biofouling can be caused by numerous microorganisms including bacteria, algae and fungi [6], and moreover, biofouling is difficult to predict due to the numerous different fouling characteristics caused by various microorganisms. Comparing with the existing studies on other foulants such as yeast, proteins, colloids and natural organic matter (NOM), research on biofouling is still insufficient, particularly with respect to the attachment of microorganisms and its influence on the membrane fouling [7–10].

Traditional methods to investigate biofouling have been attached to the biofilms with respect to its detection and analysis. Biofilms can be detected in a direct way by membrane autopsies [11], or crossflow filtration test combining with microscopic methods to directly observe the fouling processes [12]. Indirect methods of detection include the observation of membrane performances in terms of permeate flux and pressure changes [13], the enumeration of microorganisms and other bio-foulants in the feed water [14], and the use of inline sensors [15]. The analyses of biofilms on membranes are approached from numerous points of view: the specific components such as thickness [16] or concentration [17], cellular components [18] or the estimation of the activities [19] have been approached.

Meanwhile the flow field-flow fractionation (Fl-FFF) proposed in this study for biofouling analysis has mainly been used as an analytical tool for the separation and characterization of various solutes. For example, the separation of bio-particle like Escherichia coli [20] was conducted in the early stages followed by protein analysis [18]. In appliance to viruses, their size and diffusivity were characterized based on their comparatively small size [21,22]. Additional applications have been shown in medical field with respect to immunoassays [23] and size distribution for pharmaceutical particles or virus-like nano particles [24,25]. However, studies on examination of membrane fouling using Fl-FFF have recently been put to effort, which was associated with the accumulation and/or adhesion of particles and some dissolved organic matter (DOM) onto the membrane surface. The Fl-FFF has been used in fouling experiments due to its similarity to the flow scheme of an actual crossflow membrane filtration as well as
its flexibility to test various membranes under different physico-chemical operating condition. Wright used Fl-FFF to estimate the interaction between colloids and UF membranes by investigating the effects of ionic strengths and cross flow intensity on membrane fouling [26]. Fl-FFF was also utilized to verify the effects of ionic strength and membrane charge on the fouling of DOM including NOM and wastewater effluent organic matter (EfOM) [27,28].

Giddings first suggested the theory of FFF in the 1960s [29]. The asymmetrical version of the Fl-FFF (AsFl-FFF) was first introduced in 1987 [30,31]. AsFl-FFF consists of only a single permeable wall at the bottom of the channel, which differs from the symmetrical Fl-FFF system. That is, the upper porous wall in the symmetrical Fl-FFF is replaced by a solid wall that is impermeable to the carrier liquid. A channel flow is parallel to the permeable wall while a cross flow perpendicular to the permeable wall is generated by the back pumps [32]. AsFl-FFF has the following advantages over the symmetrical Fl-FFF: a simpler installation and easier check during experiment through the visible upper wall [33]. A schematic representation is shown in Fig. 1. AsFl-FFF has the same analysis range as the symmetrical Fl-FFF. The carrier solution that acts as the cross flow must enter the channel through a slot on the tip of the channel because of the absence of a depletion wall. It is driven by the pressure differential across the membrane-frit assembly formed by an external back pump [34].

The purpose of this study was to use a novel approach to investigate the initial adhesion of Pseudomonas putida on the membrane surface by using the AsFl-FFF. P. putida was selected as the model microorganism according to the report on the frequent occurrence in the Veolia Water plant [35]. The AsFl-FFF was used to examine the adhesion property of P. putida on different membranes. Two different RO membranes were used in this study with various salt concentrations as carrier solution for AsFl-FFF analysis. Through this study, the initial attachments of bio-foulants were observed by the AsFl-FFF under similar flow schematic conditions to the crossflow filtration used in the actual membrane filtration processes. Moreover, as well as the understanding of the initial attachment characteristics of bio-foulants to the AsFl-FFF, it can provide a time-efficient analytical method to predict biofouling without any membrane filtration test using a bench- or pilot-scale experiment.

2. Materials and methods

2.1. Membrane

Two different RO membranes and one UF membrane were used for the AsFl-FFF experiment. The UF membrane was made of polyether sulfone (PES) purchased from Postnova analytics (German), with a dimension of 26 cm in length and 2 cm in width. Two RO membranes were SW-30HR and TM-820 manufactured by Dow chemical and Toray, respectively. RO membranes were cut from an 8 inch spiral RO membranes were SW-30HR and TM-820 manufactured by Dow chemical and wastewater effluent organic matter (EfOM) [27,28].

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2.2. Fouling

P. putida was chosen as a model bio-foulant regarding as the most representative foulant according to a practical desalination plant study in Daesan Korea [35] (Veolia Water Solution & Technologies Korea in Daesan, Chungnam, Korea). The representative bio-foulant was observed from the fouled RO membrane through membrane autopsy analyses, which was randomly sampled from three different locations in the membrane unit. Through the separation and analysis of the bio-foulants, the Gram negative aerobic bacteria P. putida was selected as the model bio-foulant in this study.

The strain was cultivated in growth media prepared from reagent grade chemical. For batch experiments, a fresh single colony of P. putida pre-grown on Luria-Bertani (LB) agar at 37 °C was inoculated in 10 ml LB broth and incubated for 18 h under vigorous agitation (160 rpm) at 37 °C. The overnight grown cultures were collected by centrifugation (3000 rpm, 10 min.) and rinsed three times with 20-ml phosphate buffered saline (PBS) solution to remove the nutrient. Then, P. putida was diluted to the concentration of 0.0001 (Optical Density 600 nm) approximately 1.0 by fluorescence excitation-emission matrix analysis [36]. SBR buffer was used to remove the nutrient. SBR buffer solution is composed of 2.7 M NaCl, 54 mM KCl, 86 mM Na2HPO4, and 28 mM KH2PO4 with pH 7.2.

2.3. Carrier solutions

Sodium chloride (NaCl) was used as the salt in order to adjust ionic strength of carrier solution for AsFl-FFF analyses. Conductivity of carrier solutions is listed in Table 2. 0.1 mM of sodium azide (NaN3) (Fisher Scientific) was added to every carrier solution to prevent bacterial growth. All other chemicals used in this study were of analytical grade without further purification. DI water was prepared by an Easy pure RO system (D-7429-33 Labscience, South Korea) with a conductivity of 18 μS. Solutions were mixed for a minimum of 2–3 h before injecting into the AsFl-FFF system.

2.4. Asymmetrical flow field-flow fractionation

AsFl-FFF uses two independent flow streams within a thin channel: one is perpendicular flow to the membrane called a crossflow, and the other is channel flow parallel to the membrane. A cross flow acts a field forcing to separate a target substance within the channel: it is a vertical stream to the membrane surface in Fl-FFF channel, which should be discriminated against the crossflow filtration in membrane process. A channel flow has a parabolic pattern with the highest velocity at the center of the channel. When the microorganism is introduced to the Fl-FFF channel, they will be positioned in an equilibrium state between the applied field force and solute diffusion characteristics during focusing step. Then, the microorganism will be transported along the channel at the corresponding velocity within the parabolic channel flow stream. Then, it

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![Fig. 1. A schematic of AsFl-FFF channel structure.](image-url)
can be separated based on its diffusion property related to the size, generally. In Fl-FFF, however, this diffusion property can be affected by the interaction between substance and membrane as well as between the substances. Thus, different in retention time distribution obtained at given operating condition in Fl-FFF analysis can reflect the membrane characteristics as well as diffusion properties of substance.

Two HPLC pumps were used to operate both the channel and cross flows of the AsFl-FFF system. Samples were injected into the channel via 25 μm injection loop. The AsFl-FFF has a channel with dimensions of 2 cm in width, 29.5 cm in length, and a thickness of 0.02 cm. Fractionated sample from AsFl-FFF channel was detected by UV spectrometer at a wavelength of 254 nm. A bubble trapping device was connected to the HPLC pumps to prevent any air bubbles from flowing into the system. Operating control, data acquisition and analysis were accomplished by a computer system with a commercialized program provided by Postnova Analytics.

### 2.5. Bench scale membrane filtration test for biofouling analysis

Biofouling experiments were performed with a laboratory-scale cross flow filtration unit. The membrane filtration test unit consists of a cross flow membrane cell, high-pressure pump (Hyundai Heavy Industries), feed tank, chiller equipped with a temperature control system (LAB House), flow meter, back pressure regulator, pressure gauge and a data acquisition system (CAS Corporation) interfaced with a computer. The membrane channel was rectangular with dimensions of 3 cm × 14 cm, and a channel thickness of 0.3 cm. Before each experiment, the RO unit was operated with DI water under low pressures to remove any trace materials in the system. And after each experiment the test unit was disinfected by circulating 95% alcohol in order to eliminate the residual microorganisms. The membrane was compacted with DI water at pressure 35–41 b, cross flow velocity of 0.3 L/min for 10–14 h until the constant permeate flux was attained (14.7 μm/s) for stabilization. After permeate, data acquisition, 1/100 diluted LB broth and 10 times diluted bacterial suspension adjusted for OD600 of about 0.1 were inoculated into the feed reservoir.

### 3. Results and discussion

#### 3.1. Standard driving conditions for AsFl-FFF analysis

AsFl-FFF was used for the initial adhesion experiment using *P. putida* as a target bio-foulant. The standard flow condition for AsFl-FFF experiment in this study was derived using the UF membrane with 1 kDa MWCO, which was commercialized PES membrane for Fl-FFF analysis by Postnova Analytics. In every analysis, the sample injection volume of *P. putida* was kept in 25 μl based on the injection loop volume. Various flow conditions were examined to get a standard operating condition for biofouling analysis which allows better separation between void and sample elution peak as well as insures reliable elution peak area for *P. putida*. The ratio of cross flow to channel flow was 0.004 in the AsFl-FFF experiment for this study, which was somewhat mild comparing with the flow ratio conducted in the RO filtration test condition described in the previous Section 2.4 (0.012). After the operating conditions were determined, every experiment was repeated more than three times for experimental reproducibility. Fig. 2 shows retention time distribution of AsFl-FFF with PES UF membrane under the standard condition obtained in this study, which was 2.5 ml/min and 0.01 ml/min for channel and cross flow, respectively (total tip flow 2.51 ml/min). The first peak called void peak in Fig. 2 showed at 5.75 min and the elution peak for *P. putida* at 10.81 min. The void peak represented samples not retaining in the elution peak during equilibration and then flowing out of the system right after focusing step. The samples fully focused and equilibrated within the parabolic velocity profile resulted in the elution peak by fractionating process containing most of the injected samples. Under the given flow conditions, the tested RO membranes (SW-30HR and TM-820) were also evaluated by the AsFl-FFF in terms of the initial attachment property of *P. putida* at the same flow conditions. The void peaks were checked for experimental accuracy in every AsFl-FFF measurement under the same flow conditions. After finishing the sample elution, the residual sample inside the channel can be rinsed off by turning off the cross flow pump, which is possible to accumulate and/or attach onto the membrane surface during fractionation.

#### 3.2. Effect of ionic strength

Ionic strength of carrier solution for the AsFl-FFF experiment was varied in order to verify of interaction between the RO membrane surface and *P. putida*. Ionic strength of carrier solution was adjusted by salt concentrations using NaCl, which were 0.1 mM, 1 mM, and 10 mM, respectively. Fig. 3 exhibits the AsFl-FFF fractograms obtained from the TM-820 RO membrane (i.e., hydrophobic RO membrane). During the AsFl-FFF fractionation, the carrier solution in the channel flows according to a parabolic velocity profile, and thus, a particle with higher diffusion coefficient also travels away from the membrane in the channel where the velocity is the faster within the velocity profile. Therefore, the longer retention time of elution peak can represent the fact that a particle has either larger size or more

### Table 1

<table>
<thead>
<tr>
<th>Membrane</th>
<th>Postnova</th>
<th>SW-30HR</th>
<th>TM-820</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contact angle (°) @T=23 ± 3 °C</td>
<td>79.73</td>
<td>24</td>
<td>79</td>
</tr>
<tr>
<td>Surface charge (mV) @pH=6.1 ± 2</td>
<td>-6.39</td>
<td>-30.11</td>
<td>-18.31</td>
</tr>
<tr>
<td>Roughness (nm)</td>
<td>8.89</td>
<td>87.31</td>
<td>77.56</td>
</tr>
<tr>
<td>Material</td>
<td>PES (polyether sulfone)</td>
<td>Polyamide</td>
<td>Polyamide</td>
</tr>
</tbody>
</table>

* Standard deviation.

### Table 2

<table>
<thead>
<tr>
<th>Conductivity (μS/cm)</th>
<th>Dow</th>
<th>Toray</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 mM NaCl+ 0.1 mM NaNO3</td>
<td>37.1</td>
<td>29.7</td>
</tr>
<tr>
<td>1 mM NaCl+ 0.1 mM NaNO3</td>
<td>133.6</td>
<td>135.3</td>
</tr>
<tr>
<td>10 mM NaCl+ 0.1 mM NaNO3</td>
<td>1060</td>
<td>1188</td>
</tr>
</tbody>
</table>

Fig. 2. Standard operating condition obtained from the Postnova membrane (PES one). (2.5 ml/min of channel flow and 0.01 ml/min of cross flow under the carrier solution containing 0.1 mM NaCl+ 0.1 mM NaNO3).
adhesive interaction with the membrane. Given the fact that the microscopic size of the \textit{P. putida} was constant in all conditions, the longer retention time of the elution peak can be resulted from the stronger adhesive interactions with the membrane. Moreover, the area of the elution peak can also represent the amount of bio-particles flowing out from the AsFI-FFF system, and thus, the smaller peak area can also stand for the more attachment of bio-particle onto the membrane surface based on the same amount of sample injection volume in terms of concentration or number of particle.

Fig. 3 shows two main changes in the elution peaks as the ionic strength of carrier solution increased. First, the retention time of the peak was delayed with the increase of ionic strength in carrier solution. The later elution peak with the ionic strength increase can be resulted from the increase of the attractive interaction between the particle and the membrane. It can be supposed that the electrostatic double layer repulsion between microorganisms and the tested membrane is reduced as the solution ionic strength increases. Higher ionic strength of the carrier solution compresses the electric double layer of the membrane, and it results of the \textit{P. putida} particles to get closer to the membrane surface where the hydraulic velocity of flow stream is slower within the Fl-FFF channel. The second was the peak height and area of elution peak which decreased significantly as ionic strength increased. Likewise the peak retention time delay as previously mentioned, the reduced electrical repulsive force between the bio-particle and the membrane surface can give the more opportunity to interact bio-particle with membrane with solution ionic strength increase. This can result in the membrane fouling which is getting severe with increasing electrolyte (NaCl) concentration according to the previous study by Hong and Elimelech [36]. Consequently, both the area and retention time of elution peak show that \textit{P. putida} can be easily attached to the RO membrane under the higher ionic strength condition, which causes biofouling in RO membrane process.

3.3. Effect of membrane characteristics

In order to evaluate the effect of the membrane characteristics on adhesion tendency of \textit{P. putida}, both the hydrophilic (SW-30HR) and hydrophobic membranes (TM-820) were tested through AsFI-FFF experiments by varying the ionic strength of carrier solutions (see Fig. 4). When the carrier solution contains 0.1 mM in ionic strength, retention time at the peak maximum \textit{P. putida} with the hydrophilic membrane, SW-30HR was 12.52 min while the hydrophobic one TM-820 resulted in the peak maximum at 12.90 min showing longer elution time at the same flow condition in Fig. 4(a). Under the same operating condition with the diluted carrier solution, the difference in retention time of \textit{P. putida} can be originated from the hydrophobicity of the tested membrane. The tested RO membranes in this study were almost similar but only hydrophobicity was different. It can be inferred from the figure that the longer elution time is resulted from the higher interaction of the \textit{pseudomonas putida} and the membranes because the \textit{pseudomonas putida} is located closer to the membrane walls in the Fl-FFF channel as already discussed in Sect 3.2.

Acknowledging the fact that both peak was obtained under the same chemical and flow conditions for Fig. 4(a), the \textit{P. putida} revealed the stronger attractive interaction with TM-820, that is, hydrophobic membrane, under the solution condition containing relatively diluted salt concentration. It can be supposed that the hydrophobic \textit{P. putida} can be located much closer to the hydrophobic membrane than to the...
hydrophilic one, resulting in more opportunities of causing mem-
brane fouling compared to the hydrophilic membrane case under the
given condition used in this study.

However, the different characteristics of the tested membranes
resulted in very distinctive aspect in the elution peak from the AsFi-
FFF experiment as the ionic strength of carrier solution increased. In a
carrier solution containing 1 mM NaCl, SW-30HR showed an elution
peak at 9.31–12.93 min, but TM-820 showed an elution peak at
14.46 min (see Fig. 4(b)). The hydrophobic membrane exhibited the
retention time delay with small decrease of the peak area as already
discussed in Section 3.2. However, the hydrophilic one, SW-30HR
showed the elution peak which was too broad to decide the retention
time peak maximum with significant peak area reduction. Moreover,
unlike the hydrophobic membrane, SW-30HR didn’t reveal the remark-
able peak time delay as the solution ionic strength increased.

Finally, there was no elution peak under the highest ionic strength
condition of 10 mM NaCl from SW-30HR membrane (see Fig. 4(c)).

More quantitative comparisons of AsFi-FFF analysis between the
two RO membranes were conducted in Tables 3 and 4, respectively.
Table 3 shows the retention time at the peak maximum for both RO
membranes. As already discussed in Section 3.2, the more delayed
retention time was resulted from the solution ionic strength increase
in TM-820, the hydrophobic membrane in Table 3, which can be
explained by the reduction of electrostatic double layer repulsion
between the membrane surface and microorganisms. As shown in
Fig. 4, it was observed that the retention time of elution peak in the
hydrophilic SW-30HR membrane was 12.52 min under the 0.1 mM
NaCl solution, but the elution peak from SW-30HR membrane was
difficult to figure out the exact peak retention time because of broader
and smaller elution peak as the ionic strength increased. This can be
supposed to the result from the reduction of electrostatic repulsive
interaction between membrane and P. putida with the increase of
ionic strength. This can also be proven by the trend of peak area with
solution ionic strength change, listed in Table 4. Table 4 shows the
area of each elution peak which can represent the amount of P. putida
eluted from the AsFi-FFF system. In other words, if the smaller the
elution peak was under the same amount of the injected volume, the
less of the P. putida would have come from the channel. And there
have been still some residuals inside the channel, which can be
possible to attach onto the membrane surface. The peak area listed in
Table 4 exhibited that all the areas of elution peak decreased with the
increasing ionic strength both for the hydrophilic and hydrophobic
membranes. Again, the smaller peak area for the same amount of
particle in the AsFi-FFF can represent the more attachment of particle
onto the membrane surface. Thus, the broader and smaller retention
peak with the increase of ionic strength in the SW-30HR membrane
can also be explained by the severe attachment of the injected particle
to the membrane during the Fl-FFF fractionation. Of course, this
attachment is resulted from the reduction of electrostatic repulsive
interaction between the membrane and P. putida.

When comparing the tested two RO membranes, the recovery of P.
putida in the AsFi-FFF analysis was significantly reduced by increasing
the solution ionic strength rather than by changing membrane
properties. This can point out that the solution ionic strength has
more influence on the membrane fouling than the membrane charac-
teristics do (see Table 4 and Fig. 5). Under ionic strengths of 10 mM,
the peaks are less than 30% of the peak area measured under the
0.1 mM in the carrier solution. In addition, it can be seen that the
tested hydrophilic membrane, SW-30HR was more sensitive to ionic
strength changes than the hydrophobic membrane was in this study.
Consequently, it can be supposed that the membrane characteristics
have less influence with the membrane fouling in this study, especi-
ally when the membranes processes are operated under high
salt concentrations. In the current experiment, it is shown that the
effect of charge screening due to the higher salt concentrations can be
dominating membrane fouling related to the attachment in P. putida
on membrane surfaces.

Table 4

<table>
<thead>
<tr>
<th>Membrane</th>
<th>Elution peak</th>
<th>Rinse peak</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>SW-30HR</td>
<td>0.1 mM NaCl+</td>
<td>1 mM NaCl+</td>
<td>10 mM NaCl+</td>
</tr>
<tr>
<td>(SD)</td>
<td>(0.027)</td>
<td>(0.005)</td>
<td>(0.11)</td>
</tr>
<tr>
<td>Rinse peak</td>
<td>0.15</td>
<td>0.16</td>
<td>0.11</td>
</tr>
<tr>
<td>(SD)</td>
<td>(0.060)</td>
<td>(0.004)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>0.44</td>
<td>0.27</td>
<td>0.11</td>
</tr>
<tr>
<td>TM-820</td>
<td>0.30</td>
<td>0.22</td>
<td>0.09</td>
</tr>
<tr>
<td>(SD)</td>
<td>(0.031)</td>
<td>(0.010)</td>
<td></td>
</tr>
<tr>
<td>Rinse peak</td>
<td>0.06</td>
<td>0.01</td>
<td>0.05</td>
</tr>
<tr>
<td>(SD)</td>
<td>(0.002)</td>
<td>(0.008)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>0.34</td>
<td>0.23</td>
<td>0.14</td>
</tr>
</tbody>
</table>

*Standard deviation.*

3.4. Bench-scale cross flow filtration experiments

Bench-scale filtration tests were conducted to investigate the actual
fouling tendencies of the P. putida depending on hydrophobic
or hydrophilic property of the RO membranes. Both hydrophilic SW-
30HR and hydrophobic TM-820 membranes were operated in a
bench-scale test unit using feed waters containing P. putida with LB
broth. The feed waters were continuously circulated by keeping the
temperature at 25 °C and each test was operated under the constant
pressure of 41 b. Fig. 6 shows the flux decline of two tested RO
membrane, respectively. There might be three different stages where
the rate of the flux decline is different: at the first stage, initial
attachment of the bio-particles can cause pore blocking of the
membrane surfaces resulting in a steep decline in the flux. After the
initial attachment of the bio-particles, biofilms will start to develop
which decreases the flux. Finally, the biofilm reaches its full
development forming a stabilized layer on the membrane surface.

Hydrophobic TM-820 membrane showed a greater flux decline in the
first step of the experiment which can prove more adhesive
characteristics against the bio-foulants tested in this study, than the
hydrophilic SW-30HR membrane did. This can agree with the results
in Table 3 and Fig. 4 where the P. putida had a longer retention time
with the hydrophobic TM-820 membranes. Under the lower ionic
strength condition, P. putida was more affected by attractive
interaction with lower diffusivity away from the tested hydrophobic
membrane surface from the AsFi-FFF experiment. In the second stage,
the flux in the two membranes showed a similar decline rate where
the developments of biofilms were constant regardless to the surface
characteristics. And at the final stage, the maximum flux decline was
approximately equal in the two membranes. This may contribute to
the fact that the impact and amount of the full development
of biofilms are irrelevant to the hydrophilic or hydrophobic properties.

However, the time of the maximum flux decline occurred was shorter
for the hydrophobic TM-820 membrane, which was related to the
tendency of the initial fouling. Through this filtration test results, it

Table 3

<table>
<thead>
<tr>
<th>Retention time of Pseudomonas putida at the peak maximum obtained by AsFi-FFF with different membranes and under various carrier solution ionic strengths.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retention time (min) (standard deviation)</td>
</tr>
<tr>
<td>SW-30HR</td>
</tr>
<tr>
<td>0.1 mM NaCl+ + 0.1 mM NaN3</td>
</tr>
<tr>
<td>1 mM NaCl+ + 0.1 mM NaN3</td>
</tr>
<tr>
<td>10 mM NaCl+ + 0.1 mM NaN3</td>
</tr>
</tbody>
</table>
Factors affecting the mechanisms can be successfully determined by AsFl-FFF analysis. The results showed in this study suggest that the tendency with respect to the solution ionic strength was more carrier solution. The results also showed that the variation in adhesion and hydrophobicity were somewhat masked by high salinity of the strength where the membrane characteristics such as surface charge adhesion tendency was much less pronounced at the higher ionic influence of membrane characteristics on the adhesion tendency was much less pronounced at the higher ionic strength where the membrane characteristics such as surface charge and hydrophobicity were somewhat masked by high salinity of the carrier solution. The results also showed that the variation in adhesion tendency with respect to the solution ionic strength was more susceptible for the hydrophilic membranes than the hydrophobic membranes. The results showed in this study suggest that the mechanisms of bio-foulant adhesion on RO membranes and key factors affecting the mechanisms can be successfully determined by AsFl-FFF analysis.

4. Conclusions

In this study, it was demonstrated that the adhesion tendency of bio-foulants could be systematically investigated by AsFl-FFF analysis. It was found that the adhesion of \textit{P. putida} on the RO membranes was getting stronger with increasing the ionic strength of carrier solution due to the enhanced hydrophobic interaction as well as reduced electrostatic repulsion. It was also found that the adhesion of \textit{P. putida} on the hydrophobic membrane was more favorable than the hydrophilic membrane due to the stronger hydrophobic interaction. Furthermore, the influence of membrane characteristics on the adhesion tendency was much less pronounced at the higher ionic strength where the membrane characteristics such as surface charge and hydrophobicity were somewhat masked by high salinity of the carrier solution. The results also showed that the variation in adhesion tendency with respect to the solution ionic strength was more susceptible for the hydrophilic membranes than the hydrophobic membranes. The results showed in this study suggest that the mechanisms of bio-foulant adhesion on RO membranes and key factors affecting the mechanisms can be successfully determined by AsFl-FFF analysis.

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