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

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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.

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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 are 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

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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 crossflow 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 wound unit to fit the FFF channel. Both RO membranes were made of polyamide. In order to prepare the experiment, every membrane was soaked into distilled water for 24 h before inserting into the AsFl-FFF channel. The general properties of the membranes are listed in Table 1.

2.2. Foulants

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 OD_{600} (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 Na_2HPO_4, and 28 mM KH_2PO_4 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 (NaN_3) (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

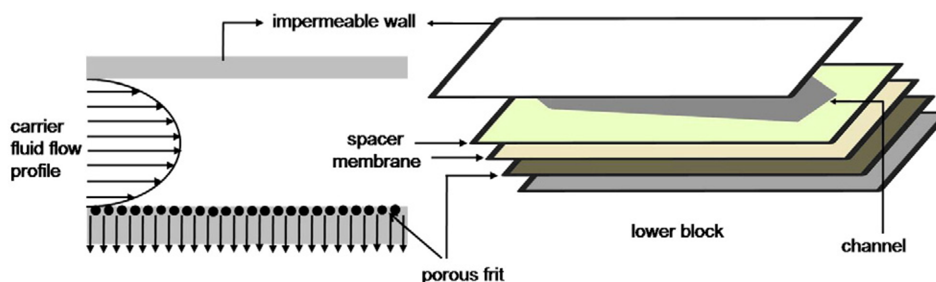

Fig. 1. A schematic of AsFl-FFF channel structure.

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