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Different bacterial species and their extracellular polymeric substances (EPSs) significantly affected reverse osmosis (RO) membrane fouling potentials in wastewater reclamation

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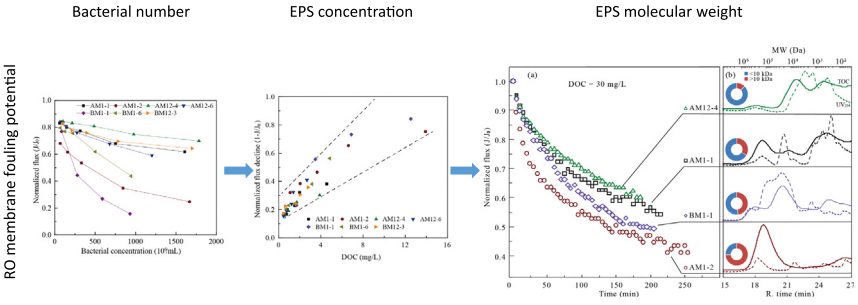
HIGHLIGHTS

- Dissolved EPS rather than bacterial number mainly contributes to membrane fouling.
- High-MW fraction in EPSs contributed dominantly to RO membrane fouling.
- The matters in the EEM region V may be related to the strong membrane fouling potential.

GRAPHICAL ABSTRACT



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ABSTRACT

Biofouling represents the "Achilles' heel" for reverse osmosis (RO) processes due to the growth of bacteria and their production of extracellular polymeric substances (EPSs). Although the microbial community structure on the RO membrane has been analysed previously, the bacterial species with a high potential of causing RO membrane fouling have not yet been identified clearly. The key components in EPSs causing RO membrane fouling have not been revealed either. In this study, seven different bacterial species were isolated from fouled RO membranes, and their EPSs were analysed in terms of the content of polysaccharides and proteins, fluorescence characteristics and molecular weight (MW) distributions. The membrane fouling potentials of these bacterial species and EPSs were evaluated based on normalized flux decline. Generally, under the same growth conditions, bacterial species with higher EPS concentrations, rather than higher cell numbers, resulted in more severe flux decline. The flux decline showed an apparent positive correlation with the EPS concentration, indicating that the concentration of EPS rather than the bacterial number mainly contributed to biofouling. Furthermore, it was found that the MW distribution was the key factor affecting the RO membrane fouling potential of EPSs from different bacterial species. With the increase in the percentage of the high-MW fraction (>10 kDa) in the EPSs from 12.6% to 74.4%, the normalized flux decline increased from 0.4 to 0.59. The components in EPSs with a MW over 10 kDa were also separated by the ultrafiltration membrane and were proven to have a higher membrane fouling potential.

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1. Introduction

With the growing demand of high-quality reclaimed water for applications with close human contact (e.g., toilet flushing, car washing and recreational uses), reverse osmosis (RO) process has been increasingly applied to wastewater reclamation because of the high quality of its permeate, stability of the system and ease of automatic operation (Bartels et al., 2005; Bellona and Drewes, 2007; del Pino and Durham, 1999; Singh, 2011). However, membrane fouling can induce multiple adverse effects on the performance of the RO system, such as the decrease in permeate production, deterioration of permeate quality and reduction in module lifespan. These problems have constrained the expansion and extensive application of the RO process (Flemming, 1997; Johir et al., 2009; Schneider et al., 2005).

RO membrane fouling can be classified into 4 kinds: colloids and particles deposition, inorganic scaling, organic fouling and biofouling, which can occur simultaneously and interact with each other (Greenlee et al., 2009; Vrouwenvelder and Van der Kooij, 2001). Compared with other kinds of fouling, biofouling, which represents the "Achilles' heel" of the RO process, is much more difficult to prevent. Although all the pollutant could be removed efficiently by pretreatment, microorganisms can regrow and recover rapidly. Even if the microorganism removal efficiency can be as high as 99.99%, there are still sufficient cells remaining that can survive and grow using biodegradable substances in water (Flemming et al., 1997). Moreover, even alkali-acid chemical cleaning could not kill all the bacteria on RO membranes (Yu et al., 2017b).

Biofouling occurs through a series of events including cell attachment, cell growth and production of extracellular polymeric substances (EPSs) (Matin et al., 2011). The initial event of cell attachment has been found to be related to physicochemical factors such as cell type, hydrophobic interactions, motility, surface charge characteristics and surface features (Camesano and Logan, 1998; Gannon et al., 1991; Matin et al., 2011; Schäfer et al., 1998; Vigeant et al., 2002; Walker et al., 2004). After the attachment, cells begin to multiply and produce EPSs using soluble and sorbed nutrients in the feed water (Matin et al., 2011). Therefore, EPSs have been regarded as the main structural component of biofilms, making up 50–80% of the organic matter of biofilms (De Beer and Stoodley, 2006). In addition, the permeate flux decline is caused by hydraulic resistance and is mainly attributed to the EPSs surrounding the bacterial cells (Herzberg and Elimelech, 2007; Herzberg et al., 2009). In terms of the composition of EPSs, polysaccharides are more likely to accumulate on the RO membrane than are proteins and DNA (Herzberg et al., 2009). Other research indicated that alginate fouled the RO membrane more severely than did bovine serum albumin (BSA) (Ang and Elimelech, 2007; Li et al., 2007).

The amount and composition of EPSs produced by different bacterial species varied widely. Thus, to control biofouling of the RO membrane, it is important to identify the typical bacterial species with high capacity to produce EPSs and cause biofouling. To date, many studies have studied RO biofouling by molecular techniques to reveal bacterial communities on the fouled RO membrane (Ayache et al., 2013; Khan et al., 2015; Luo et al., 2017; Yu et al., 2017b). Although the phylum Proteobacteria is usually dominant in the diversity studies from RO membranes, variations in operating conditions and cleaning strategies lead to differences in community structure at the genus level (Sánchez, 2018). Therefore, studies using pure-cultured bacteria may be helpful for acquiring knowledge on the key bacteria responsible for biofouling.

Some researchers tried to analyse the biofilm formation potentials of different pure-cultured bacterial species in microtiter plates (Pang et al., 2005). However, the growth in plates could not reflect membrane fouling potentials faithfully; therefore, it is better to directly evaluate membrane fouling potential under defined filtration conditions (Habimana et al., 2014; Ishizaki et al., 2016). Previous studies on RO membrane fouling potentials usually focused on model bacteria (e.g., *Pseudomonas aeruginosa* and *Pseudomonas putida*) (Ferrando et al., 2017; Herzberg and Elimelech, 2007; Herzberg et al., 2009; Saeki et al., 2016). In addition, Gutman et al. (2014) clarified the positive contribution of *Sphingomonas* and its glycosphingolipids after Bereschenko et al. (2010) found the dominant role of *Sphingomonas* on biofilm formation. Therefore, to find bacterial species with a high potential of causing RO membrane fouling, more information is required by isolating bacterial species from RO membranes and extracting their EPSs.

Consequently, the objectives of this study are to compare the RO membrane fouling potentials of different fouling bacterial species and their EPSs and identify the dominating factors on flux decline. As such, the study evaluated seven bacterial species isolated from fouled RO membranes in a wastewater reclamation plant and directly evaluated their membrane fouling potential in terms of normalized flux decline using a lab-scale membrane filtration unit. Furthermore, the membrane fouling potentials of their EPSs were also evaluated in parallel. The composition of the EPSs was analysed in detail to access the correlation between EPS composition and their RO membrane fouling potentials.

2. Materials and methods

2.1. Isolation of bacterial species

Four fouled RO membranes were collected from a full-scale wastewater reclamation plant in Beijing, China, where microfiltration (MF) was used as a pretreatment prior to a two-stage RO process. The characteristics of the RO membranes are listed in Supplementary Table 1. Foulants from 4 membrane samples were suspended and serially diluted with phosphate-buffered saline (PBS), spread on R2A agar plates (Sigma-Aldrich, USA), and incubated at 25 °C. Bacterial colonies were streaked 3 times on R2A agar plates to obtain a pure colony. Cultures were maintained on R2A agar plates at 25 °C, and stocks were preserved in sterile 0.85% (w/v) saline supplemented with 15% (v/v) glycerol at −80 °C.

For 16S rRNA gene sequencing, DNA was extracted from strains and purified using standard methods (Ausubel et al., 1992). The 16S rRNA gene was amplified by PCR with two universal primers (27f, 5′-AGAG TTTGATCCTGGCTCAG-3′; 1492r, 5′-GGCTACCTTGTTACGACTT-3′). The PCR products were purified using the TIANgel Midi Purification kit and sequenced by an automated DNA sequencer (model ABI3730XL; Applied BioSystems) at Tsingke Co., Ltd., Beijing, China. All sequence similarities were analysed on the EzTaxon server (Kim et al., 2012).

The growth curve of the bacteria based on cultivation in R2A broth medium at 25 °C was measured by optical density at 600 nm (OD_{600}). The size of bacteria was estimated by scanning electron microscopy (SEM, Sirion 200, FEI). The bacterial cells were fixed, dehydrated and coated with a layer of gold approximately 10 nm thick according to the method described by Huo et al. (2016).

2.2. EPS extraction and analysis

Each strain was cultivated in R2A broth medium at 25 °C until the stationary growth phase. The cells in 1 L R2A broth were collected and rinsed by ultrapure water to remove residual medium and then were resuspended with 100 mL ultrapure water. The EPSs were extracted using the method described by Zhang et al. (2016) with modifications. Overall, the resuspended cells were subjected to heat treatment at 45 °C for 1 h. The suspension was centrifuged at 12,000*g* and 4 °C for 20 min. The supernatant filtered through a 0.2 μm membrane (Whatman, England) was regarded as EPS.

The amount of EPSs was measured using dissolved organic carbon (DOC, TOC-VCPH, Shimadzu). The carbohydrate and protein concentrations of the EPSs were measured using the anthrone method with glucose as the standard (Rondel et al., 2013) and the Lowry method with BSA as the standard (Frølund et al., 1996), respectively. A fluorescence spectrophotometer (F-7000, Hitachi) was used to measure the excitation emission matrix (EEM) spectra of EPS with the method described