Desalination 429 (2018) 12–19

Contents lists available at ScienceDirect

Desalination

journal homepage: www.elsevier.com/locate/desal

Ambivalent role of calcium in the viscoelastic properties of extracellular polymeric substances and the consequent fouling of reverse osmosis membranes

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ARTICLE INFO

Keywords:
Reverse osmosis
Extracellular polymeric substances
QCM-D
Biofouling

ABSTRACT

The effects of polysaccharides composition on the interactions between EPS and RO membrane, including fouling, were studied in the presence of Ca^{2+}. EPS originated from *Pseudomonas aeruginosa* PAO1 biofilms of the wild type and its Δ*psl* isogenic mutant was used for RO membranes fouling and QCM-D adsorption experiments. For the wild type strain EPS, bridging of alginates by Ca^{2+} led to an increase in both adsorption and rigidity of the adsorbed layer. However, no change was detected for the Δ*psl* EPS adsorption and the obtained layer showed reduced rigidity, likely due to the interference of Ca^{2+} with interactions between the polysaccharides composing the EPS. In agreement with the QCM-D results, once Ca^{2+} was added, an increased in RO fouling was observed with the wild type EPS, while reduced fouling was found for the Δ*psl* mutant EPS. The presence of an ATR-FTIR peak characterized glycosidic linkage only for the fouling layer formed by the EPS of the Δ*psl* mutant in the presence of Ca^{2+}, suggested that Ca^{2+} alters the scaffold matrix of this EPS. The results of this study showed that polysaccharide composition in the EPS matrix significantly influences the interaction with Ca^{2+} as well as membrane fouling.

1. Introduction

The two main factors affecting the water production costs in reverse osmosis (RO) plants are energy and membrane replacements, comprising approximately 50% of the total water production costs [1,2]. One of the main constrains of the RO process is membrane fouling, occurring even when the feed solution carries only small amounts of salts, colloidal or dissolved matter, and microorganisms. Biofilm formation on the membrane leads to a rapid increase in hydraulic resistance through the membrane [3], enhanced concentration polarization (CP) and consequent elevation of osmotic pressure [4–6]. These phenomena in turn demands frequent membrane cleaning or replacement that leads to a sharp increase of operating costs. Thus, a thorough study of the RO systems for the improvement of plant efficiency and reduction of operation and maintenance costs is crucial.

Microbial biofilms on RO membranes are embedded in a slime matrix, through which the cells adhere to the surface [7,8]. This matrix is composed of self-produced extra-cellular polymeric substances (EPS) excreted by the sessile microorganisms [9]. In RO membrane biofouling, EPS major constituents include polysaccharides, proteins and other components, such as humic acids, nucleic acids, lipids and uronic acid in small fractions [10,11]. One important role of the EPS is to provide mechanical stability to the microbial biofilm through physicochemical interactions [3,12]. EPS composition contributes majorly to biofilm cohesion, attributed to the matrix viscoelastic properties [13–15]. Regarding membrane biofouling and the viscoelastic properties of EPS, results from previous studies showed a strong correlation between the changes in EPS adherence and viscoelastic properties and the membrane-fouling rate and EPS composition [16–18]. A different study suggested that changes in the composition of specific polysaccharides composing the matrix of a growing biofilm would provide the biofilm with distinct mechanical properties [19]. In addition to the nature of extracellular polysaccharides, a different study has shown that the presence of extracellular proteins, in particular amyloids, has a major impact onto EPS viscoelasticity. For instance, amyloid peptides influenced the deformation of EPS matrices under shear stress and

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https://doi.org/10.1016/j.desal.2017.12.006
Received 14 February 2017; Received in revised form 4 November 2017; Accepted 3 December 2017

modified its elastic response [20]. Furthermore, biofilm as well as EPS fouling layers adversely impacts RO and NF membranes performance including elevation of the longitudinal pressure drop and hydraulic resistance to permeate flux [3,21]. However, the mechanisms of biofouling and the contribution of specific EPS components, to the deterioration of RO membrane performance are not well understood.

The presence of divalent cations (e.g. Ca^{2+}, Mg^{2+}) differentially contributes to the adsorption of EPS to surfaces influencing biofilm formation and structure [10,22,23]. This contribution is associated to the amount of diverse components produced by the bacterial cells, composing the EPS, which react with divalent cations, a phenomenon known as ion bridging. For instance, the naturally charged alginate molecule makes its conformation and adherence capabilities dependent on the surrounding aquatic solution chemistry. A previous study demonstrated that increasing Ca^{2+} concentrations in a solution, containing dissolved alginate, significantly increased intermolecular adhesion forces, suggesting a specific complexation mechanism between the Ca^{2+} and alginate carboxylic groups [24].

Well-characterized bacterial strains can be a powerful tool in determining the role of EPS components on biofouling of RO membranes. Hence, even though *P. aeruginosa* PAO1 is normally found in medical systems rather than in wastewater [25], this well-characterized bacterial strain is commonly used for biofilms research [26]. PAO1 primarily produces Psl polysaccharide during biofilm formation, although is also possesses the ability to produce Alginate and Pel polysaccharides [23,27,28]. Psl polysaccharide plays an important role in *P. aeruginosa* adhesion by promoting cell-surface and intercellular interactions to both abiotic and biotic surfaces [29,30]. Likewise, Pel polysaccharide is required for both, solid surface-associated biofilm formation and pellicle formation that assembles at the air-liquid interface of a standing liquid culture [31].

In the present study, we analyzed the effects of EPS composition and solution chemistry (the presence of Ca^{2+}) on RO membrane fouling. Adsorption experiments of EPS extracted from different PAO1 biofilms differentially expressing Psl were conducted on sensors of quartz crystal microbalance with dissipation monitoring (QCM-D). These sensors were coated with aromatic polyamide, on which EPS layers were formed. The effects of different EPS compositions and the presence of Ca^{2+} on the EPS adherence and the formed EPS layer viscoelastic properties (shear modulus and shear viscosity) were analyzed. Finally, the viscoelastic properties of the EPS layers were related to their fouling extent on RO membranes and the associated permeate flux decline. We believe that under such controlled conditions—in which critical elements of both the biofilm matrix and the chemical and physical properties of the membrane surface are well defined—the mechanisms affecting membrane performance can be revealed.

2. Materials and methods

2.1. Bacterial strains and growth media

Pseudomonas aeruginosa PAO1, a well-characterized bacterial strain commonly used for biofilm research - was used in this study. In addition, a PAO1 mutant strain, which has a full deletion of the *psl* gene cluster (PAO1 Δ*psl*), was obtained from Dr. Daniel J. Wozniak of Ohio State University Medical College [30]; later this bacterial strain was characterized by Tian et al. [32]. A fresh single colony of either of the strains, pre-grown on Luria Bertani (LB) agar, was used as an inoculum for an overnight culture grown in LB broth at 30 °C with shaking (150 rpm). This overnight culture was re-diluted in 100 mL LB broth (1:100 ratio) and allowed to grow to stationary phase for 16 h for use as inoculum for biofilm growth in the flow-through columns (for later EPS extraction).

2.2. EPS extraction and preparation

For EPS extraction, biofilms of each of the variants of PAO1 were grown in a flow-through column. The 100 mL packed bed column (~2.5 cm in diameter) contained acid washed glass beads (425–600 μm in diameter, cat. #G8772 Sigma-Aldrich, St. Louis, MO). The column was wet-packed, sterilized with 70% ethanol and washed with sterilized deionized water, prior to inoculation with 100 mL of the stationary phase culture for 50 min (2 mL min^{-1}). At the end of the inoculation stage, pure LB was injected to the column for 48 h to allow biofilm growth on the beads. EPS was extracted from the biofilms of each of the PAO1 variants grown on glass beads in the columns, using a modified version of the Liu and Fang method [22] and our previous publications [33–35]. Protein concentration in the extracted EPS was determined using the Bio-Rad[a] Protein Assay (Bio-Rad, Hercules, CA) according to Bradford [36]. Polysaccharide content was determined according to Dubois et al. [37], using glucose and alginic acid as standards. EPS extracted was expressed as DOC concentration measured by using an Apollo 9000 TOC Analyzer (Teledyne Tekmar, Mason, OH).

2.3. Electrophoretic mobility

The effect of ionic strength (IS) on the electrophoretic mobility of *P. aeruginosa* EPS solutions was measured in a zeta potential analyzer (ZetaPlus 1994, Brookhaven Instruments Co., Holtsville, NY). EPS solutions were adjusted to 35 mg L^{-1} as DOC and suspended in either of the two different background solutions composed either of 10 mM NaCl or 8.5 mM NaCl and 0.5 mM $CaCl_2{\cdot}2H_2O$. The electrophoretic mobility for each sample was analyzed for 2 cycles and 5 repetitions.

2.4. RO membrane and crossflow test unit

A commercial thin film composite RO membrane ESPA-1 (Hydranautics, Oceanside, CA) was used as a model membrane for the biofouling experiments. The hydraulic resistance of the clean un-fouled membrane was determined to be 1.53 (± 0.012) × 10^{14} m^{-1} at 25 °C. The membrane was received as a flat sheet and stored in DDW at 4C. A flat-sheet, plate and frame, RO laboratory system, similar to that described in previous publications [34,38,39], was used to study the extent of RO membrane fouling by different types of extracted EPS. A detailed description of the RO unit is given in Fig. S1 (Supplementary information) and in our previous studies [33,39,40].

2.5. EPS fouling experiments of membranes

The fouling experiments were carried out with the EPS extracted from biofilms of the two *P. aeruginosa* variants, grown in a flow through column. A thorough cleaning of the unit at the beginning and end of each experiment was conducted as described previously [6]. Briefly, different cleaning solutions were recirculated in the unit as follow: (1) 0.5% sodium hypochlorite for 2 h, (2) tap water for 10 min × 2, (3) 5 mM EDTA at pH 11 for 30 min, (4) step 2, (5) 2 mM SDS at pH 11 for 30 min, (6) step 2, (7) 95% ethanol for 1 h, (8) deionized water for 10 min × 3. Following the cleaning protocol, the fouling experiments were carried out in a three-stage procedure: Firstly, the membrane was compacted with distilled water. Once the compaction of the membrane was achieved, an “osmotic” operational period of five hours with either of the background solutions, was conducted at a constant retentate flow velocity of 75 L h^{-1}, initial permeate flux 42 L m^{-2} h^{-1} and transmembrane pressure of 10 (± 1) bars. Later, 3 mg DOC L^{-1} of dissolved EPS was added to the background solution for a 16 h of fouling followed by an additional 16 h desalination of the electrolyte background solution in the absence of EPS. A temperature of 25 °C was kept constant during all the experiments. The effect of solution chemistry on the interference of RO membranes fouling, specifically the addition of Ca^{2+}, was examined by using two different background solutions: