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Novel insights into the role of *Pseudomonas* quinolone signal in the control of reverse osmosis membrane biofouling

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ABSTRACT

The role of the *Pseudomonas* quinolone signal (PQS) pathway in the control of biofouling in reverse osmosis membrane filtration was examined by inoculating *Pseudomonas aeruginosa* into feed water to simulate biofouling. Various concentrations of PQS (2-heptyl-3-hydroxy-4-quinolone) and an anthranilate analog (methyl anthranilate) were used as the activator and inhibitor of the PQS pathway, respectively. Membrane performance results showed that addition of exogenous PQS caused severe decreases in permeate flux and salt rejection and increase in irreversible membrane fouling. In contrast, the presence of methyl anthranilate mitigated the declines in permeate flux and salt rejection by biofouling and significantly reduced the total fouling resistance and hence shorten the membrane cleaning periods. Biofilm characterization demonstrated that PQS aggravated membrane biofouling by promoting initial bacterial attachment, increasing levels of the extracellular polymeric substances (EPS), total organic carbon (TOC) and extracellular DNA, and changing the mature biofilm structure from a mushroom shape to a denser flat shape. In contrast, addition of methyl anthranilate increased protein adsorption during the initial biofouling stage through electrostatic interactions, but significantly inhibited biofilm growth via reducing levels of EPS and TOC, as well as total cell number in the biofilm. These results provide evidence that PQS pathway plays an important role in biofilm formation and an anthranilate analog, methyl anthranilate, could be a novel agent to control RO membrane biofouling. Therefore, PQS synthetic pathway and precursors of quorum sensing signals may provide viable targets for biofouling control in practical RO membranes applications.

1. Introduction

In recent decades, reverse osmosis (RO) membrane has been widely used in seawater desalination, wastewater reclamation, and drinking water treatment [1]. However, membrane fouling issues including inorganic fouling, organic fouling, colloidal fouling and biofouling have increased operational costs and impacted the produced water quality [2]. Of these issues, biofouling is the most difficult to control because of the excessive rate of biofilm formation on the membrane surface and consequent membrane performance deterioration [3,4]. Moreover, bacteria living in biofilms exhibit higher resistance against antibiotics than planktonic cultures. Therefore, many strategies have been developed to deal with biofilm formation on membrane surfaces, such as pretreatment, membrane surface modification, feed water chlorination and chemical cleaning [5,6]. However, all these techniques have limitations and cause membrane deterioration [7]. Alternatively, a novel biochemical method based on quorum sensing (QS), has attracted significant attention in biofouling control; the advantages of this approach are its high efficiency and that it imposes low selection pressure, thus preventing the development of bacterial resistance [8,9].

QS refers to the cell-to-cell signals used by microorganisms to sense cell density; once these signals reach a critical threshold, specific sets of genes are expressed in response [10]. QS has been shown to regulate a wide range of biological processes such as biofilm formation, bioluminescence, and virulence factor secretion [11]. Comprehensive studies have found that interfering with this cell density-dependent communication mechanism constitutes a promising strategy for controlling biofilm formation on membrane surfaces [12,13]. Biofilm formation on the membrane was reduced by acylase as it inhibits the activity of N-acylhomoserine lactone (AHL) which is a signal molecule of QS [14,15].

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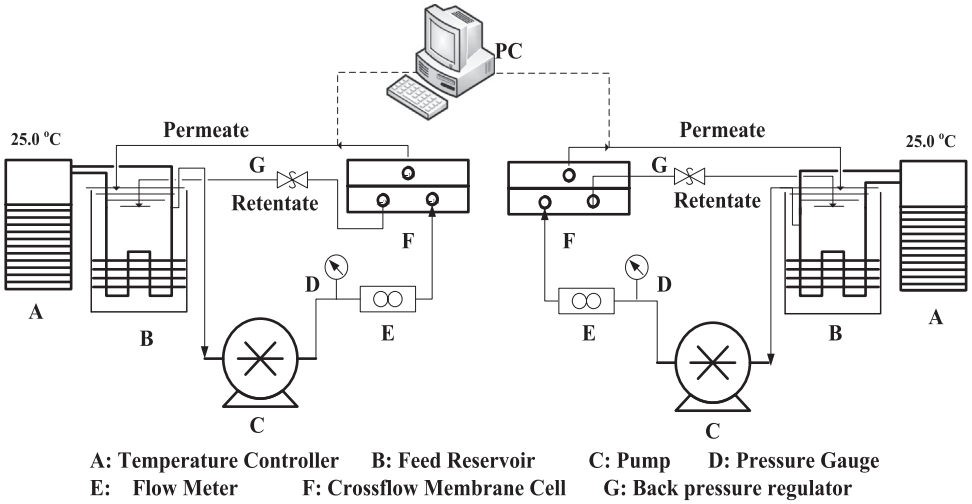

Fig. 1. Cross-flow RO membrane filtration system.

Thus, further investigations aimed at inhibitor development based on quorum sensing are of paramount importance.

Pseudomonas aeruginosa (*P. aeruginosa*), ubiquitous in aquatic water, is routinely selected as a model strain in membrane biofouling studies [16]. This bacterium has two quorum-sensing systems (Las and Rhl), which utilize acyl homoserine lactones (AHLs) comprised of N-(3-oxododecanoyl)-L-homoserine lactone (3-oxo-C_{12}-HSL) and N-butyryl-L-homoserine lactone (C_4-HSL) as their intercellular signals [17]. Until now, various quorum quenching compounds and enzymes with different mechanisms have been widely used to reduce AHL-mediated quorum sensing and thus prevent bacterial biofilms [18,19]. In addition to AHLs, *P. aeruginosa* also produces a secondary metabolite, 2-heptyl-3-hydroxy-4-quinolone (referred to as the *Pseudomonas* quinolone signal [PQS]), which has also been shown to play an important role in regulating biofilm formation. Recently, Calfee et al. [20] identified the PQS biosynthetic pathway; they demonstrated that anthranilate was a precursor of PQS and that an anthranilate analog (methyl anthranilate) can inhibit PQS production. Aendekerk et al. [21] reported that the addition of exogenous PQS can overcome the growth defect of *P. aeruginosa* pump mutants. PQS has also been found to have an impact on biofilm structure. *P. aeruginosa* biofilms grown in the presence of high levels of QS inhibitors are flatter than untreated control biofilms [22]. Aleksic et al. [23] identified new quinoline derivatives with strong anti-QS activity inhibiting biofilm formation and without bactericidal effect. In summary, QS interference via PQS is considered a promising and novel approach for the development of anti-virulence and anti-biofilm compounds [24,25]. However, most studies of PQS-dependent cell-to-cell communication regulation in *P. aeruginosa* biofilms have focused on biological aspects and limited information is available regarding the impact of PQS pathway interference on membrane biofouling control in hydrodynamic membrane filtration processes.

Therefore, we aimed to elucidate the role of PQS pathway in *P. aeruginosa* biofouling control by applying an activator (PQS, 2-heptyl-3-hydroxy-4-quinolone) and an inhibitor (methyl anthranilate) of the PQS pathway in cross-flow RO membrane filtration. The influences of PQS pathway interference on permeate flux, salt rejection, membrane cleaning, and biofilm properties were examined to gain a better understanding of the underlying relationship between the PQS pathway and biofouling control. Biofilm characterization included extracellular polymeric substances (EPS) concentration, total biomass, cell viability, and total cells measurements and observation of biofilm structure. Furthermore, initial bacterial attachment and subsequent biofilm formation were both examined to further elucidate the interference process of PQS and methyl anthranilate.

2. Materials and methods

2.1. Chemicals and strains

An artificial wastewater feed solution consisting of 1.16 mM $C_6H_5Na_3O_7{\cdot}2H_2O$, 0.94 mM NH_4Cl, 0.45 mM KH_2PO_4, 0.5 mM $CaCl_2{\cdot}2H_2O$, 0.5 mM $NaHCO_3$, 2.0 mM NaCl, and 0.6 mM $MgSO_4{\cdot}7H_2O$ was freshly prepared prior to every experiment [26]; the final pH of this solution was 6.9. PQS (2-heptyl-3-hydroxy-4-quinolone) and an anthranilate analog (methyl anthranilate) were purchased from Sigma-Aldrich and were dissolved in methanol prior to the experiments. The model strain *P. aeruginosa* (ATCC 27853) was grown in 90 mL of Luria-Bertani (LB) broth overnight (optical density of 1 at 600 nm) to a final concentration of 10^9 cells/mL. Next, 10 mL of the suspension was centrifuged for 10 min at 8000 rpm and then re-suspended in the synthetic water using for three replicates. The washed *P. aeruginosa* cells were inoculated into a feed reservoir containing 1 L of synthetic wastewater to achieve an initial cell concentration of 10^7 cells/mL for the biofouling protocol.

2.2. Laboratory-scale membrane system and biofouling protocol

A cross-flow membrane system using a flat sheet membrane with an effective area of 24 cm^2 was used for the fouling experiments (Fig. 1). A flat-sheet reverse osmosis membrane (CSM, PA, RE1812-50) was utilized in this study. The constant operating conditions for all of the fouling experiments were transmembrane pressure (TMP) of 0.8 MPa, cross-flow velocity of 1.3 m/s, and temperature 25 °C in the lab-scale membrane system (Re ≈ 130). Permeate flux was calculated using a digital balance connected to a personal computer. The permeate and retentate were circulated back to the feed tank to maintain a constant feed water quality. Prior to inserting clean membranes into the membrane cell, membrane system was disinfected with recirculation of 0.5% NaClO for 2 h and then recirculation of 95% ethanol for 1 h. A new membrane was used for each experiment and was compacted with DI water for 12 h until it attained a constant permeate flux, followed by the initial baseline performance for 2 h. The initial permeate flux of the RO membrane in this study was measured as approximately 15.4 L/m^2h. Short-term accelerated biofouling tests of the RO membrane system were performed for 30 h, as described in a previous publication [26]. In order to study the impact of the PQS pathway on biofouling control, three sets of biofouling protocols were conducted in this study. The first biofouling set was carried out without any chemicals related to the PQS pathway; this was regarded as the control experiment. PQS production has been shown to be inhibited by anthranilate analogs (e.g. methyl

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