Desalination 393 (2016) 135–143

Contents lists available at ScienceDirect

Desalination

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

Inhibiting quorum sensing pathways to mitigate seawater desalination RO membrane biofouling

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HIGHLIGHTS

- Quorum sensing inhibitors for membrane biofouling prevention were investigated.
- All biofouling bacteria examined secreted autoinducer I quorum sensing molecule.
- The quorum sensing inhibitor, vanillin reduced EPS on membrane surface by >40%.

ARTICLE INFO

Article history:
Received 2 September 2015
Received in revised form 8 January 2016
Accepted 11 January 2016
Available online 29 January 2016

Keywords:
Reverse osmosis membrane
Biofouling
Seawater desalination
Quorum sensing
Quorum sensing inhibitors

ABSTRACT

Bacterial biofilm formation, the main cause of membrane biofouling, is a crucial issue for membrane separation. Biofilm production is regulated by quorum sensing (QS) systems where bacteria secrete auto-inducers to communicate with neighboring bacteria. This research identified that several marine bacteria isolated from a desalination plant produced a low molecular weight auto-inducer 1 (AI-1) signaling molecule. AI-1 production in the mixed culture of the four different biofilm-forming marine bacteria was greater than in individual bacterial cultures. The QS inhibiting compounds, vanillin and cinnamaldehyde at 1200 mg/L significantly reduced biofilm formed by these marine bacteria by more than 79% and 70%, respectively in a microtiter plate assay. Anti-biofilm capabilities of vanillin and cinnamaldehyde were further assessed in a reverse osmosis membrane biomonitoring system using mixed bacterial cultures and native uncultured bacterial communities in natural seawater. Confocal microscopy showed vanillin (1200 mg/L) significantly reduced biofilm extracellular polysaccharides and dead cells on the membrane surface (>40%, >20%). These results indicate that QS inhibitors have the potential to remediate membrane biofouling.

1. Introduction

The amount of people residing in regions with insufficient water resources is estimated to rise from one fifth of the global population to two thirds by 2025 due to population growth and climate change [1]. Alternative water treatment processes such as coastal seawater desalination and water reuse have the potential to meet this demand by delivering high quality purified water using reverse osmosis (RO) membranes.

However, biofouling remains a significant challenge in membrane separation because it causes a reduction in membrane flux and an increase in operational pressure [2–4]. Energy loss due to membrane biofouling has been estimated to account for up to 50% of the total energy required for seawater desalination plants [5]. Biofouling consists of the attachment and subsequent growth of bacteria on the membrane surface, which secretes bacterial metabolic products including extracellular polysaccharides (EPS) [6–8]. Only less than 0.01% of the 10^6/mL of natural marine bacteria that escape the pretreatment process are sufficient enough to cause RO membrane biofouling.

Typical cleaning processes for membrane biofouling use a proprietary assortment of chemical cleaning agents to break down the biofouling layer in order to regenerate membrane permeability [9–11]. However, cleaning only partially restores the RO membrane performance for a limited time because degraded organic matter in the surrounding environment further stimulates the growth of surviving bacteria. Moreover, the remaining bacteria may develop resistance to chemical cleaning agents [12]. As a result, the increase in the frequency of membrane cleanings creates an increase in operational difficulty, a decrease in membrane life span, and raises plant operational and maintenance costs [13].

A potential method to control biofouling is to prevent biofilm formation by blocking bacterial communication in quorum sensing (QS)

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http://dx.doi.org/10.1016/j.desal.2016.01.013
0011-9164/

pathways. Bacteria utilize these pathways to regulate biofilm development and maturation. During QS, bacteria synthesize and secrete auto-inducers or signaling molecules to "communicate" with each other [14]. These auto-inducers accumulate to a threshold concentration based on the local bacterial population density [14]. After the threshold is reached, signaling molecules diffuse through cell membranes to bind to the appropriate transcription regulator in neighboring bacteria to trigger biofilm development [14]. One or more of these QS pathways may be present in bacterial species to regulate a number of specific genes [15–18].

The auto-inducer 1 (AI-1) QS system produces acyl-homoserine lactone (AHL) to regulate gram-negative (G^-) bacterial communication whereas the auto-inducer 2 (AI-2) yields a variety of signaling molecules to govern multi-species communication. In the well-studied AI-1 system, the LuxI protein initiates AHL synthesis with the LuxR protein acting as a receptor [19]. The type of AHL molecule produced is dependent on the specific bacterial species of interest. AHLs differ in degree of saturation and number of oxygen substitutions [19]. Additionally, the N-acyl chain length varies from 4 to 18 carbons bonded to a lactone ring through an amide bond [19]. In the AI-2 system, the LuxS protein synthesizes 4,5-dihydroxy-2,3-pentanedione (DPD), which spontaneously rearranges to produce the family of AI-2 QS molecules, including 4-hydroxy-5-methyl-3(2H) furanone (MHF), (2R,4S)-2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran (R-THMF), and S-THMF-borate, a furanosyl borate diester [20,21]. The detection of this auto-inducer is based on the specific AI-2 derived molecule responsible for QS, which varies in different bacterial species.

There are various naturally occurring and synthetic QS inhibiting compounds (QSIs) that disrupt QS pathways by either degrading the signaling molecule or blocking signal production or outcompeting the signaling molecule from binding to the receptor protein [22–24]. Kojic acid, used in the cosmetic industry, and vanillin, a commonly added food-flavoring agent were identified as AI-1 QS inhibitors because both suppress or modify the AHL molecule in a LuxR based bioreporter strain [25–29]. Vanillin has further been shown to interfere or modify the AHL's structure to hinder the AHL's ability to bind to the receptor protein [25,30]. Cinnamaldehyde, another food flavoring agent and synthetic brominated furanone compounds were revealed to disrupt both the AI-1 and AI-2 QS systems by reducing the DNA binding ability of the receptor protein, LuxR [31–33]. Additionally, brominated furanone compounds have been shown to suppress the AI-2 LuxS protein [34]. Although the QSIs' ability to inhibit QS pathways has been researched extensively using bio-reporter strains or model bacterial systems for clinical and other environmental applications [27,35–41], the QSIs' potential to mitigate marine biofilm formation and seawater desalination RO membrane biofouling needs to be evaluated. This research investigated the role of QS system in desalination biofouling by examining QS production in natural bacterial isolates from biofouled seawater RO membranes. Based on the type of QS produced, several commercially available and inexpensive QSIs were selected to determine the QSI's effect on single and mixed species marine biofilm formation in a static environment. The anti-biofilm capabilities of the most effective QSIs were further evaluated in a membrane bio-monitoring system operated in cross-flow mode using native marine bacterial communities. The biofouled membranes were then examined under fluorescent microscopy to understand the biofilm structure with and without treatment by QSI.

2. Materials & methods

2.1. Bacterial strains and seawater bacterial community

A diverse group of bacteria, isolated from fouled cartridge filters and RO membranes at Carlsbad Desalination pilot plant in California (CA) and Perth Desalination plant in Western Australia were selected to characterize QS molecule production and to study biofilm formation in the presence of QSI. The four Carlsbad bacterial strains, B1 and B3 were determined to be *Shewanella* sp. while B2 and B4 were determined to be *Alteromonas* sp. in a previous study [42]. The four Australian isolates were identified as *Paracoccus* sp. (RO28), *Mycrobacterium* sp. (RO16), *Burkholderia ambifaria* (RO32), and *Bizionia* sp. (C10) by 16S rDNA sequencing [Dr. Lucy Skillman, unpublished results]. In addition, bacterial communities in natural seawater collected from Southern CA coastal sites at Dana Point, Long Beach, and Newport Beach were used without cultivation and isolation. The fresh seawater was unaltered and used directly in biofouling experiments to simulate the desalination fouling condition.

2.2. Quorum sensing inhibitors

The four QSIs selected to investigate biofilm inhibition in this study were cinnamaldehyde (CNMA), kojic acid (KJ), vanillin (VA), and a brominated furanone compound (F-30). The selection was based on: 1) the target QS molecule for biofilm inhibition (AI-1 or AI-2); 2) prior demonstration of biofilm reduction in model bacterial system; 3) commercial availability; 4) nontoxic to humans and 5) inexpensive to manufacture. CNMA (Sigma-Aldrich) was diluted in methanol and used in testing concentrations ranging from 10 to 2400 mg/L. The brominated furanone compound, F-30 also known as (5Z)-4-bromo-5-(bromomethylene)-2(5H)-furanone (Sigma-Aldrich) was dissolved in dimethyl sulfoxide (DMSO) and added to bacterial cultures or natural seawater to experimental concentrations between 10 and 30 mg/L. KJ (Sigma-Aldrich) and VA (Sigma-Aldrich) were both dissolved in deionized (DI) water and used in testing concentrations ranging from 10 to 2400 mg/L. The solvents used to dissolve each QSI were used as the control for all experiments.

2.3. Identification of bacterial AI-1 QS molecule production

AI-1 production among marine bacteria isolated from biofouled membranes was investigated because AI-1 is commonly reported among G^- bacteria and all QSIs tested in this study were able to interfere with AI-1 QS pathway. AI-1 AHL extraction was modified based on a previous study [43]. Briefly, four bacterial isolates (B1, B2, B3 and B4) from the Carlsbad plant were inoculated into an artificial seawater medium (ASWJP) with 2.5 g/L peptone, 0.5 g/L yeast (ASWJP + 1/2PY) and incubated for 24 h on a shaker at 21 °C [4,44]. A 1:100 dilution was made from the bacterial cultures into ASWJP with 1.25 g/L peptone and 0.25 g/L yeast (ASWJP + 1/4PY). The sub-cultures were incubated for 6 h on a shaker at 21 °C until bacteria reached exponential growth phase. The optical cell density was recorded at a wavelength of 600 nm (BioPhotometer, Eppendorf). The supernatants from cell cultures were collected after pelleting cells by centrifugation at 4 °C at 5500 *g* (Centrifuge, Eppendorf) for 20 min. Bacterial pellets were re-suspended in ASWJP and cell pelleted by centrifugation again to collect the supernatant. The combined supernatant from both centrifugations were extracted three times by liquid–liquid extraction with half volume HPLC-grade dichloromethane [43]. Excess water was removed by adding anhydrous magnesium sulfate to dichloromethane extracts. After removal of anhydrous magnesium sulfate by filtration, dichloromethane was evaporated using a speed vacuum concentrator at 30 °C (Refrigerated CentriVap Concentrator, LABCONCO). The extracts were dissolved in 1 ml of 1:1 of DI water: acetonitrile (HPLC-grade) and stored at −20 °C until analysis. N-butyryl-L-homoserine lactone (C4-HSL, Cayman Chemical) and N-hexanoyl-homoserine lactone (C6-HSL, Cayman Chemical) were used as the extraction control standards and were taken through the same extraction steps as described above.

The AHL concentration of each extract was analyzed using Acquity UPLC system (Waters) coupled to a Quattro Premier XE triple quadrupole mass spectrometer (MS/MS) (Waters) run in positive-ion mode. For the UPLC system, 10 μl of each sample was injected into the 2.1 × 50 mm C_{18} reversed phase column (Acquity BEH) at a flow rate