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Mitigation of biofouling in forward osmosis process by bacteria-oriented quorum quenching molecules

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ABSTRACT

In this study, the intervention of bacterial communication or quorum quenching (QQ) technique has been investigated to mitigate biofouling in forward osmosis (FO) membrane processes. The 10 mg-C/L lysate of QQ enzyme-producing *Rhodococcus* sp. BH4 successfully degraded 79% of the bacterial signal molecule (*N*-acyl homoserine lactone, AHL). In a continuous lab-scale FO experiment using *Pseudomonas aeruginosa* PAO1 as a model bio-foulant, flux recovery after physical cleaning was higher in the presence of 10 mg/L of *Rhodococcus* lysate. The retardation of biofouling in the presence of *Rhodococcus* lysate was largely due to the reduced amount of bio-volume and extracellular polymeric substances (EPS), which were reduced by 68% and 75% compared with the control. In conclusion, the application of bacteria-oriented QQ molecules could be the potential solution not only to mitigate biofouling but also to meet the economic demands.

Keywords: Biofouling; Quorum sensing; Quorum quenching; *Rhodococcus* sp.; Extracellular polymeric substances

1. Introduction

Availability of water resources is one of the most serious challenges facing humanities with increasing world population, climate change, and human activities, and water reuse and desalination are regarded as core technologies to realize diversification of water resources [1]. Membrane-based desalination processes including reverse osmosis (RO), nanofiltration (NF), and forward osmosis (FO) have been widely applied for the purpose of water reuse and desalination [2], and fouling has long been regarded as the main disadvantage in pressure-driven membrane processes such as RO and NF [3,4]. Among various fouling phenomena, biofouling is responsible for almost 40% and is difficult to be removed [5]. Extracellular polymeric substances (EPS), bacterial secretions to form a sticky biofilm matrix [6], lead to irreversible fouling [7,8]. Despite the chemical cleaning was shown to be effective in treating biofouling in FO, the usage of cleaning agents is known to damage the polyamide polymer structure of the membrane [7,9].

Recently, quorum sensing (QS), a bacterial cell-to-cell communication by signal molecules, has been acknowledged to control the formation of biofilm [10]. Signal molecules differ by bacterial species, and many bacteria utilize acyl homoserine lactone (AHL) and peptide signals as QS molecules [11]. The intervention and inhibition of QS system, or quorum quenching (QQ), has been extensively studied in membrane-based water or wastewater treatment systems. In a membrane bioreactor (MBR), the porcine kidney acylase was applied and found to be effective in reducing biofouling [12]. In addition, acylase was immobilized on NF membranes or nanofibers and showed to be highly effective in anti-biofouling [13,14]. However, the applicability of QQ enzyme in the commercial scale is limited due to their operational difficulties and high cost of enzyme. On the other hand, *Rhodococcus* sp. BH4, which produce the intracellular

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QQ enzymes such as lactonase, was fixed in a bead or vessel and effectively mitigated biofouling through relatively cheaper approaches [15,16]. However, the application of QQ bacteria through carriers in MBR system showed a low efficiency in transporting the QQ enzyme, and fixing the bacteria into the carrier itself was economically infeasible for large scale MBR application [17]. Recently, the QQ technique has been applied to RO membrane process which has lower bacterial density compared with MBR, and showed retarded increase in trans-membrane pressure [18]. It was noted that the application of QQ technique in field scale RO would be limited due to the fixation of bacteria as well as high hydraulic pressure applied in RO.

On the other hand, in FO process, naturally occurring osmotic pressure difference between concentrated draw solution and low salinity feed solution is the main driving force transporting water through the semi-permeable membrane [19]. Because of the difference in the driving force, fouling layer in FO is loose and easy to be cleaned, and thus, energy consumption for cleaning is much lower compared with that of pressure-driven RO [20]. However, fouling in FO is still problematic because of the cake-enhanced osmotic pressure (CEOP), which results in decrease in the permeate flux, requires frequent membrane cleanings and further increases the operational costs [21].

Therefore, the objective of this study is to explore the applicability of QQ technique in FO membrane process for controlling biofouling. In the batch test using *chromobacterium violaceum* CV026, we examined the lysate of *Rhodococcus* sp. BH4 as QQ molecules whether it could degrade bacterial signal molecules. Moreover, in a continuous FO biofouling test, the permeate flux was monitored whether the addition of *Rhodococcus* lysate could mitigate biofilm formation as well as weaken the biofilm structure.

2. Materials and methods

2.1. Quantification of quorum quenching activity

2.1.1. Preparation of QQ molecules

To take QQ molecules from *Rhodococcus* sp. BH4, a high concentration (OD ≈ 3) of solution containing cells was lysed by tip sonication. The solution with cells was then sonicated using a tip sonicator (Sonics & Materials Vibracell, USA) for 1 h with an on:off cycle of 1:2 and an amplitude of 20%. Subsequently, the solution was centrifuged at 12,000 rpm for 30 min and only the supernatant was taken after the filtration through a 0.2 μm polyethersulfone filter (Corning, USA).

2.1.2. Quantification of QQ activity

N-Hexanoyl-DL-homoserine lactone (HHL, Sigma-Aldrich, USA) was used as a QS marker in this experiment. A HHL stock solution was solubilized in solution with 0.1% acetic acid and 100 mM ethyl acetate. HHL was then diluted with 10 mM potassium phosphate buffer to a final concentration of 1 μM. The AHL indicator bacteria, *Chromobacterium violaceum* CV026, was cultivated in Luria-Bertani medium for 24 h, then centrifuged and rinsed three times with 0.9% NaCl. For the evaluation of QQ activity, 0–20 mg-C/L of *Rhodococcus* lysate was supplemented into the *C. violaceum* CV026 culture, and violacein was extracted by a protocol described elsewhere [22]. Finally, the supernatant which contains violacien was extracted and the absorbance was measured using a UV spectrophotometer (Hach, USA) at 585 nm.

2.2. Lab-scale forward osmosis experiment

2.2.1. Membrane and cross-flow unit information

A commercial thin film composite FO membrane (CSM, South Korea) was used as a model membrane for continuous FO experiments. The membrane was cut into a flat sheet and stored at 4°C in DI water. The membrane was cut to a size of 9.5 cm × 4.5 cm and placed in a custom-made laboratory-scale FO test unit with inner membrane cell dimensions of 2.60 cm × 7.75 cm × 0.30 cm (W × L × H). The detailed setup of the system can be found in our previous report [23]. All FO biofouling experiments were carried out at the fixed temperature of 30°C and hydrodynamic operating conditions, where the cross flow velocities of the feed and draw solution were 12 and 7 cm/s, respectively, using two gear pumps (Longer Pump WT3000-1FA). The permeate water flux was automatically transmitted to the computer every 5 min through the digital scale (GF-4000, AND Co., Japan). Conductivity on the feed side was monitored by using a conductivity meter (Vernier LabPro, USA) to calculate the reverse salt flux.

2.2.2. FO system configuration and operation

The total volume of the feed solution was 2 L containing 0.2 mM sodium citrate (DOC = 15 mg/L) as a carbon source. For bacterial growth, the trace metal solution was added as given elsewhere [24]. To maintain the pH of the feed solution, 10 mM potassium phosphate buffer was supplemented at the initial concentration of inoculated bacteria solution of 10^7/mL. The draw solution was prepared to 0.5 M sodium chloride. Every 6 h, *Rhodococcus* lysate and sodium citrate with equal concentrations as 10 mg-C/L were added with and without the QQ system, respectively. In every 12 h, the draw solution was replaced with a fresh solution to maintain the initial osmotic pressure difference between the feed and draw solutions. When the permeate flux reached 40% of initial flux, physical cleaning was conducted with the cross flow velocity of the feed side to be 25 cm/s.

2.3. Analysis of biofilm

After staining of biofouled membrane coupons (1 cm × 1 cm) with 3.34 mM SYTO9 (Invitrogen, USA), three spots were observed through the confocal laser-scanning microscope (Zeiss LSM 5 LIVE, Germany) and the bio-volume of live cells was anlayzed using IMARIS software. To measure the mass of biofilm, biofilm was removed using silicon knife, and resuspended using 0.9% NaCl solution. Then, solution was filthered through 0.2 μm paper filter (Whatman, USA) and the difference in weight before and after the filtration of the bacteria suspended solution was defined as the biomass of the bacteria. EPS was extracted from the biofilm through a modified heat extraction method [25] and the extracted EPS

was subsequently quantified by the phenol–sulfuric acid method with glucose as the standard for carbohydrates and Bradford assay with bovine serum albumin as the standard for protein [26].

3. Results and discussion

3.1. Quantification of QQ materials

BH4 lysate showed excellent ability to degrade the bacterial signal molecules. As shown in Fig. 1, The concentration of signal molecule (AHL) was linearly hydrolyzed by *Rhodococcus* lysate tendency until 10 mg/L, then plateued at 20 mg/L. Therefore, 10 mg/L was thought to be the optimal concentration of *Rhodococcus* lysate regarding the efficiency. From the batch test, it was clearly shown that significant amount of QQ enzyme were in the lysate of *Rhodococcus* sp. BH4, thus *Rhodococcus* lysate could be used as simple and economic QQ molecules compared with purified QQ enzyme such as acylase and lactonase.

3.2. Normalized flux

To investigate the propensity of biofouling in the presence and absence of 10 mg/L of *Rhodococcus* lysate, permeate flux of FO membrane was monitored with series of physical cleaning. As shown in Fig. 2, there was no significant difference in the propensity of biofouling in the presence and absence of 10 mg/L of *Rhodococcus* lysate for the first 2 d. After the normalized flux of the whole system was reduced to 0.4, physical cleaning was conducted and second phase of operation was initiated. At the second phase, permeate flux in the absence of *Rhodococcus* lysate decreased more rapidly, and it became more severe as the increase of phase. Finally, after the third physical cleaning, flux was only recovered by 3.3%. On the other hand, the physical cleaning was more effective for the recovery of flux in the presence of *Rhodococcus* lysate (Fig. 3), and the period of physical cleaning was prolonged compared with that without the lysate (Fig. 2). These results denoted the weaker structure of biofilm formed on FO membrane surfaces due to the addition of *Rhodococcus* lysate. In addition, the similar trend in flux decline in the presence and absence of *Rhodococcus* lysate can be explained by CEOP. As particles such as microorganisms are deposited on the active layer of the FO membrane, reverse salt flux which is the salt pathway from the draw to feed side because of the difference in salt concentration, is trapped in the cake layer [23]. The osmotic pressure difference was decreased near the membrane surface and this further resulted in a decrease in the driving force of water flux [27].

3.3. Analysis of biofilm

To elucidate why there was difference in biofouling propensity and flux recovery after physical cleaning in the presence and absence of *Rhodococcus* lysate, volume of biofilm on unit area of FO membrane surface, and their EPS content were investigated. The bio-volume per unit membrane area was reduced to 68% in the presence of *Rhodococcus* lysate compared with that without *Rhodococcus* lysate as shown in Fig. 4. Thus we can conclude that the addition of QQ molecules in *Rhodococcus* lysate prevented bacteria developing dense and thick biofilm layer, and led to high cleaning efficiency.

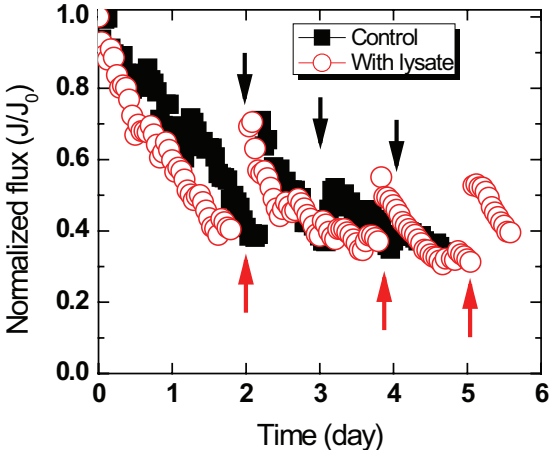

Fig. 2. Normalized flux in the presence and absence of *Rhodococcus* lysate. Note that the arrow denotes the event of physical cleaning.

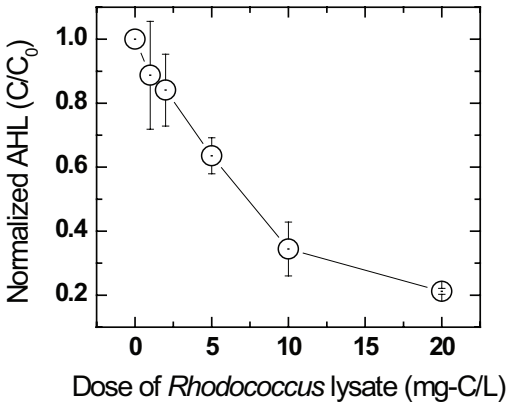

Fig. 1. Degradation of *N*-acyl homoserine lactone by *Rhodococcus* lysate.

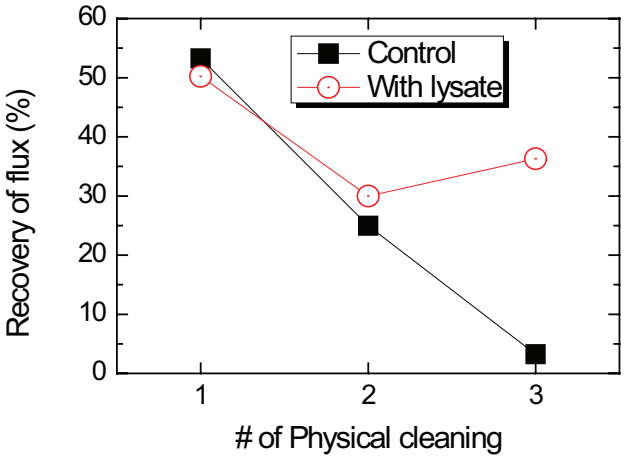

Fig. 3. Flux recovery after each trial of physical cleaning in the presence and absence of *Rhodococcus* lysate.

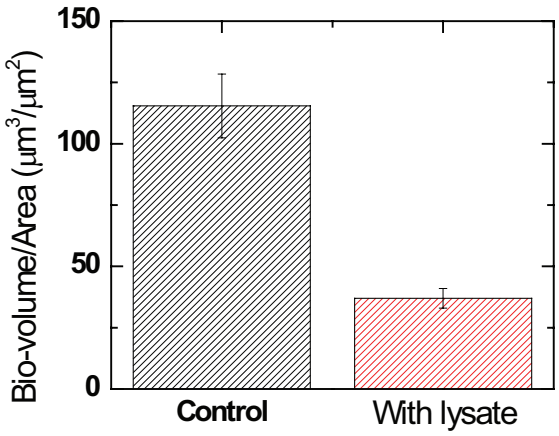

Fig. 4. Bio-volume per unit area in the presence and absence of *Rhodococcus* lysate.

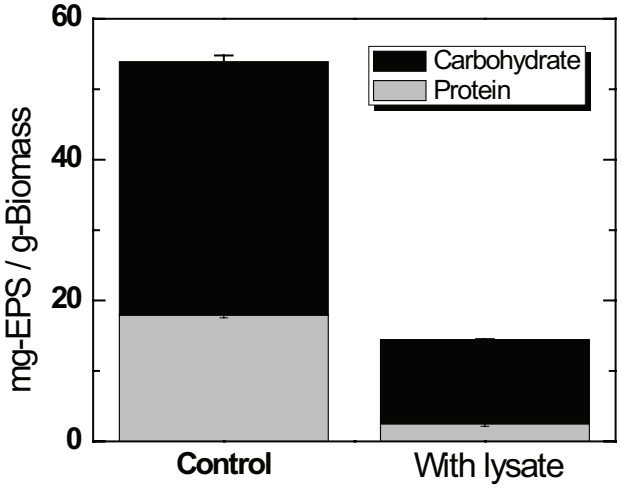

Fig. 5. The amount and composition of EPS per unit biomass in the presence and absence of *Rhodococcus* lysate.

The content and composition of bio-polymers such as EPS were evaluated in Figs. 4 and 5 because these materials had been acknowledged as main residues which induced irreversible fouling in FO membrane process [8]. In the presence of *Rhodococcus* lysate, carbohydrate and protein components of EPS were reduced to 86% and 67%, respectively, compared with the absence of *Rhodococcus* lysate. EPS are sticky materials that act as a matrix for microorganisms and makes it difficult to remove the biofilm by physical cleaning [6]. The reduction of EPS content in the presence of *Rhodococcus* lysate contributed to the formation of a looser biofilm, thus could explain higher flux recovery by physical cleaning in Fig. 3. Thus, QQ molecules effectively hindered the development of biofilm on FO membrane surface not only by reducing the bio-volume but also by providing less irreversible foulants such as EPS.

4. Conclusions

We proposed and tested the lysate of *Rhodococcus* sp. BH4 as QQ molecules to mitigate biofouling in FO processes. *Rhodococcus* lysate contained significant amount of QQ molecules which could hydrolyze signal molecules such as AHL. The addition of QQ molecules in the FO feed stream resulted in 68% less bio-volume per unit membrane area compared with the case without *Rhodococcus* lysate. Moreover, the addition of *Rhodococcus* lysate led to the reduction of carbohydrate and protein components in the EPS. The reduction of the EPS content in the presence of *Rhodococcus* lysate contributed to the formation of the weaker and thinner biofilm, which could be more easily removed by simple physical cleaning. Our findings suggested that the application of bacterial-oriented QQ molecules would be an economical alternative to promote FO systems to be operated longer with relatively high flux.

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