Journal of Industrial and Engineering Chemistry xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Journal of Industrial and Engineering Chemistry

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

Development of a quorum quenching-column to control biofouling in reverse osmosis water treatment processes

Seonki Lee[a], Huijuan Xu[a], Scott A. Rice[b,c], Tzyy Haur Chong[a,d,**], Hyun-Suk Oh[e,*]

[a] Singapore Membrane Technology Centre, Nanyang Environment and Water Research Institute, Nanyang Technological University, Singapore
[b] Singapore Centre for Environmental Life Sciences Engineering, Nanyang Technological University, Singapore
[c] The School of Biological Sciences, Nanyang Technological University, Singapore
[d] School of Civil and Environmental Engineering, Nanyang Technological University, Singapore
[e] Department of Environmental Engineering, Seoul National University of Science & Technology, Republic of Korea

ARTICLE INFO

Article history:
Received 8 April 2020
Received in revised form 7 October 2020
Accepted 26 October 2020
Available online xxx

Keywords:
Quorum sensing
Quorum quenching
Biofouling
Reverse osmosis
Water reclamation

ABSTRACT

Biofouling is recognized as one of the most problematic fouling types in reverse osmosis (RO) processes and lead to high energy requirements and operating costs. Over the past decade, many studies on membrane bioreactor (MBR) systems for wastewater applications demonstrated that disrupting cell-cell communications among bacteria, called quorum quenching (QQ), is a promising approach to inhibit biofouling of membranes. Here, we developed the QQ-column as a novel strategy to control biofouling in RO systems. The QQ-column was prepared by incorporating a recombinant bacterial QQ strain into hydrogel beads and embedding these beads inside a column. The QQ-column was installed upstream of the RO module to degrade *N*-acyl homoserine lactone, a quorum sensing (QS) signal, from the feed in a laboratory-scale RO system operating in total recycle mode. The QQ-column reduced the concentrations of signal molecules by ~29% in an RO system and mitigated biofilm formation (38.6% reduction of cell number) on the membrane, consequently reducing the transmembrane pressure by 50.1%. These results demonstrate that integrating QQ bacteria into columns is a practical method to control biofouling in RO systems.

Introduction

The increasing demand for high quality reclaimed water has spurred interest in applying reverse osmosis (RO) for municipal and industrial wastewater reclamation and reuse. RO process is attractive over other water treatment technologies because of its high contaminant rejection, sustainability, small footprint, and facile operation [1–3]. However, despite these many advantages, membrane fouling remains a major challenge for RO technology. Specifically, biofouling causes serious operational problems such as decreased membrane permeability, deteriorated permeate quality, and increased pressure drop across the feed channel, which necessitate frequent chemical cleaning of the membranes, shortened membrane lifespan, and increased energy consumption [4].

With respect to pretreatment, low-pressure membrane processes such as microfiltration (MF) and ultrafiltration (UF) that effectively remove microorganisms have been widely applied [5]. Alternatively, the effluent of a membrane bioreactor (MBR) has been directly used as the RO feed [3,6]. Yet even with such pretreatments, substantial biofouling is often observed on the RO membrane within a few months of operation [7]. Such biofouling arises because MF/UF pretreatment or MBR processes do not completely eliminate organic matter (small-sized organics in particular) from wastewater, which allows the microorganisms present in a non-sterile RO system to form a biofilm on the RO membranes. Membrane technologies based on high retention such as nanofiltration membrane bioreactors (NFMBRs) or osmotic membrane bioreactors (OMBRs) would be more effective in reducing biofouling in subsequent RO, but they still exhibit limitations in practical applications [8–11].

To address the challenge presented by biofouling, a variety of technologies have been studied for cleaning the biofouled RO membranes. Chemical cleaning processes have been periodically applied to restore the performance of biofouled RO membranes [12,13]. However, current chemical cleaning technologies have

* Corresponding author.
** Corresponding author.
E-mail addresses: THChong@ntu.edu.sg (T.H. Chong), hyunsukoh@seoultech.ac.kr (H.-S. Oh).

https://doi.org/10.1016/j.jiec.2020.10.037
1226-086X/

several problems, which include reduced permeate production due to system shutdown during membrane cleaning, membrane damage, high costs, and environmental pollution. Alternative approaches to chemical cleaning include degrading the biofilm extracellular polymeric substances (EPS) by using enzymes such as proteases and lipases, which are associated with low toxicities and are environmentally friendly [14,15]. However, such enzymatic technologies typically have considerable shortcomings due to insufficient enzyme activity and/or stability as well as high cost.

In 2009, Yeon et al. demonstrated a correlation between quorum sensing (QS) and the accumulation of biomass on membranes and further showed that quorum quenching (QQ), the inhibition of QS, delayed biofouling in an MBR for wastewater treatment [16]. Since then, several successful applications of QQ in MBRs for biofouling control have been reported [17–23]. In particular, the entrapment of QQ bacteria that produce QQ enzymes into carriers was considered as a practical application method for submerged MBRs. This method exploits the capability for QQ bacteria to survive and grow in bioreactors, without competition from other bacterial species, to thereby maintain the QQ effect [17,21]. QQ degrades QS signal molecules that otherwise induce the production of QS-regulated EPS, the key material for biofilm formation. Thus, QQ suppresses biofilm formation on the membrane surface.

We anticipate that QQ could potently mitigate biofouling in RO systems in which microbial deposition is difficult to avoid. In our previous study, two QQ strategies were tested that integrated QQ bacteria into the RO system; one was to continuously inject QQ bacteria into the RO feed stream and the other was to inject the QQ bacteria downstream of an RO loop operated in total recycle mode [24]. Both strategies delayed the increase of transmembrane pressure (TMP) and demonstrated the potential application of the QQ technique in an RO system, although these approaches had shortcomings. Continuous injection of QQ bacteria into the RO feed stream not only significantly increases operating costs but also can lead to the deposition of the QQ organisms onto the RO membrane, which can accumulate on the membrane over long term operation and become biofoulants. Injecting QQ bacteria downstream of the RO module, which avoids the deposition of QQ bacteria on the membrane, showed comparatively better performance for the RO system operating in total recycle mode. However, this method is not applicable to full-scale RO systems in a single-pass mode, because in such systems any QQ bacteria that are injected downstream of the RO module would not affect the biofouling of the RO membrane. Therefore, it is necessary to develop a practical method that integrates QQ technology into the RO process operating in single-pass mode.

In this study, we developed a promising QQ strategy in which a QQ-column is installed upstream of the RO module for biofouling control. The high-pressure tolerant QQ-column was designed to entrap QQ bacteria inside, degrading QS signal molecules from the RO feed stream and preventing QQ bacteria from entering the RO module. Immobilization of QQ bacteria onto the column proceeded in two steps: first, the QQ bacteria were entrapped in the hydrogel beads, and then the QQ-beads were embedded inside the QQ-column. The QQ-column was tested in a flow cell system to interrogate its ability to inhibit biofilm formation by a model microorganism under single-pass and non-filtration conditions. Subsequently, the QQ-column was installed upstream of a RO module in a lab-scale RO system to test its anti-biofouling effect.

Experimental

Bacterial strains and growth conditions

Pantoea stewartii R067d (NCBI accession no. KC252900) [24,25] was used as a model biofouling bacterium in this study. *E. coli* Top10-Control and *E. coli* Top10-AiiO were used as control and QQ bacteria, respectively [24]. *N*-acyl homoserine lactones (AHLs), which are QS signaling molecules of *P. stewartii* were detected and quantified by a reporter strain, *Agrobacterium tumefaciens* A136 [26]. All strains were routinely cultured in Luria-Bertani (LB) broth (Difco, Becton Dickinson, Singapore) at 30 °C. Kanamycin (50 μg/mL) and ampicillin (100 μg/mL) were supplemented into the growth medium of both control and QQ bacteria whenever necessary. Spectinomycin (50 μg/mL) and tetracycline (4.5 μg/mL) were supplemented into the growth medium of *A. tumefaciens* A136.

Preparation of QQ-beads and QQ-column

Control-beads and QQ-beads were prepared as described previously [19]. Briefly, *E. coli* TOP10-Control and TOP10-AiiO were incubated separately in nutrient broth medium (Difco, Becton Dickinson; beef extract 3.0 g/L and peptone 0.5 g/L) overnight at 30 °C and were subsequently centrifuged (7512 *g* for 5 min) and re-suspended in sterile MilliQ water. At the same time, a mixture of polyvinyl alcohol (Sigma Aldrich, USA) and sodium alginate (Thermo Fisher Scientific, Singapore) was prepared and mixed with re-suspended *E. coli* TOP10-Control or TOP10-AiiO solution. The final concentration of the mixed solution was 2.7–3.0 mg dried cells/g. The mixed solution was then transferred dropwise into a solution containing $CaCl_2$ (4 wt%)

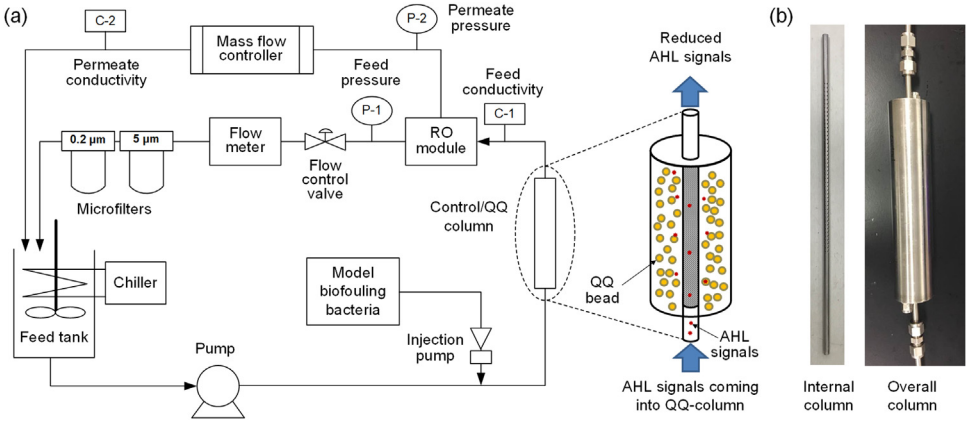

Fig. 1. Operation of laboratory-scale RO system with a QQ-column. (a) Schematic diagram of the RO system and (b) photographs of an internal column and fully assembled QQ-column.

and boric acid (7 wt%), and subsequently into a solution of 0.5 M sodium sulfate to form hydrogel beads *via* cross-linking reactions. Approximately 120 mL of control-beads and QQ-beads were loaded into each column (referred to as the control-column and QQ-column, respectively), specifically into the annular space between the internal (id, 5 mm; od, 7 mm) and external (id, 40 mm; od, 42 mm; length, 190 mm) stainless-steel columns (Fig. 1). The internal column was designed to have 180 holes (diameter: 2 mm), which allows mass transfer between the feed stream and QQ-beads for AHL degradation and prevents the flow of beads into the RO module.

RO operation

Two laboratory-scale RO systems with either a control-column (Control-RO) or a QQ-column (QQ-RO) with an operating volume of 10 L were used in total recycle mode as described previously [24]. Single-pass operation for the RO system in this laboratory study was impractical because an excessively large volume of the feed solution (MilliQ water, nutrient broth, and salts) was necessary to meet the volumetric flow requirement. In each RO system, the control- or QQ-column was installed upstream of the RO module in Control-RO or QQ-RO, respectively (Fig. 1). A RO membrane with an effective area of 0.0045 m^2 (Filmtec TW-30, DOW Chemical Co., USA) was installed in each stainless-steel RO module, which had a flow channel size of $150 \times 30 \times 0.8$ mm (L × W × H). The feed solution was maintained at 25 ± 1 °C by a stirrer (IKA, Germany) and a chiller (Polyscience, USA) in each feed tank and was delivered by a high-pressure diaphragm pump (Hydra-Cell, Wanner Engineering Inc., USA) at a cross-flow velocity of 0.28 m/s (equivalent to 400 mL/min). A solution of model biofouling bacteria (OD600 of 0.1) was injected continuously into the flow line upstream of QQ-column at a dilution ratio of 1:800. The bacterial solution was prepared in one bottle and then injected into two parallel RO systems to prevent the experiment from being affected by differences in microbial activity that may occur in each cultivation. The system pressure was set to 21 bar, and the pressures of the feed and permeate streams were monitored by using digital pressure gauges (Ashcroft, USA). A mass-flow controller (Model 5882, Brooks Instrument, USA) was used to maintain the flux at 30 L/m^2/h (LMH) throughout the experiment. In addition, two microfilters in series (pore sizes of 5 and 0.2 μm, KAREI, Thailand) were installed downstream of the RO module to prevent the inflow of bacteria into the feed tank. Therefore, the concentration of bacteria in the feed stream (*i.e.*, entering the RO module) was held constant throughout the RO operation. NaCl and nutrient broth were added into the feed tank at final concentrations of 2 g/L and 24 mg/L, respectively, to simulate the salt and organic concentrations of the feed in the RO reclamation process [27]. The feed solution was replenished twice daily by replacing old feed with fresh feed. Dissolved organic carbon (DOC) was measured using a total organic carbon (TOC) analyzer (Model TOC-VWS, Shimadzu, Singapore) to ensure that the concentration of nutrients remained equal in each parallel unit.

Flow cell test

The flow cell test was designed to determine the effect of QQ-column on biofilm formation (*i.e.*, bacterial attachment) of *P. stewartii* when the system is operated in single-pass (non-recycle) mode without permeate production. An overnight culture of *P. stewartii* (OD_{600} 1.0; ~10^9 CFU/mL) was diluted 1000 fold in a 2 g/L NaCl solution containing 24 mg/L of nutrient broth. This solution was added to a batch reactor with a stirring apparatus that was connected to either the control- or QQ-column. Two flow cell modules that were connected in series were installed to the effluent side of each column (Fig. 2). Identical RO membranes with effective areas of 2.6 cm × 8 cm were placed into each flow cell module. A peristaltic pump was used to maintain a constant flow rate of 1.0 mL/min, which was 400-fold lower than in RO operation. After 18 h of operation, the RO membrane was removed from the module for autopsy. The quantities of attached cells were assessed *via* adenosine triphosphate (ATP) and cell counting assays. The QQ activity of QQ-column was evaluated at the beginning and the end of the flow cell test, as detailed in the following section.

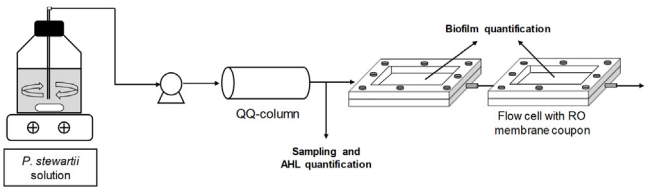

Fig. 2. Schematic diagram of the configuration of components used in a flow cell test.

AHL extraction and quantification

The RO concentrate was periodically collected to monitor the AHL levels in the RO system during operation. The collected sample was filtered through a 0.45 μm syringe membrane and the filtrate was maintained at −40 °C before extraction of the AHLs. AHLs were extracted from 30 mL of RO concentrate twice with 30 mL of ethyl acetate, which was subsequently dried using a concentrator (CentriVap Concentration Systems, Labconco, USA) and resuspended in 50 μL of methanol to achieve an overall concentration factor of 600 fold. AHLs were quantified by bioluminescence by using a reporter strain, *A. tumefaciens* A136, and Beta-Glo® Assay System (Promega, USA) [28]. Briefly, 5 μL of the sample was added to 95 μL of A136 culture solution (OD_{600} = 0.1) in a 96-well plate and incubated at 30°C for 90 min. Then, 30 μL of Beta-Glo solution was added to quantify the β-galactosidase produced by the reporter strain. After 40 min, luminescence was measured by a luminometer (Synergy 2, Biotek®, USA). The quantities of AHLs was evaluated based on the calibration curve of standard 3-oxo-hexanoyl-homoserine lactone (3OC6-HSL), which is the dominant AHL produced by *P. stewartii* [29].

QQ activity of QQ-beads and QQ-column

The stability of the QQ-beads was investigated by measuring the QQ activity of the beads before and after use in the RO system. The QQ activity of the beads was defined by the degradation rate of 3OC6-HSL (Sigma Aldrich, USA) in a 2 g/L NaCl solution. The standard AHL solution was prepared by adding 3OC6-HSL to a final concentration of 1 μM in 20 mL of 2 g/l NaCl solution, and then adding 50 QQ-beads to the resulting solution. The beads and solution were incubated with orbital shaking (50 rpm) at room temperature and the 3OC6-HSL concentration was measured by bioluminescence as described above. In addition, the viability of cells entrapped in beads was confirmed by using confocal laser scanning microscopy (CLSM). The beads were stained with *Bac*Light live/dead kit (Molecular Probes, USA) and were imaged by CLSM (LSM880, Zeiss, Germany) with a 10× objective len.

The QQ activity of the QQ-column was also evaluated in the flow cell system (Fig. 2), in which the concentration of AHLs was much lower (~10 nM) than in the activity test. Samples were collected from the feed and effluent of the QQ-column at the beginning (0 h) and the end (18 h) of a flow cell operation in which the flow rate was 1.0 mL/min. AHLs were extracted from the samples and

concentrated ~22 fold before being quantified (as equivalents of 3OC6-HSL) as described above. Experiments were performed using flow rates ranging from 1.0 to 5.0 mL/min to investigate the change in QQ activity with flow rate. The percentage removal of AHLs was calculated by the following formula: Percentage removal (%) = (1 - $[AHLs]_{QQ}$ / $[AHLs]_{control}$), where $[AHLs]_{control}$ and $[AHLs]_{QQ}$ denote the concentration of 3OC6-HSL equivalent in the effluent of control- and QQ-column, respectively.

Membrane autopsy

Fouled RO membranes were removed from both QQ and control RO modules after 87 h operation. The middle sections of fouled membranes (2 cm × 2 cm) were cut and stained with dyes, including *Bac*Light live/dead kit (Molecular Probes) and Calcofluor white (Sigma Aldrich, USA) to enable visualization of bacterial cells and EPS in the biofilm, respectively, by using CLSM. Z stack (3D) CLSM images were obtained from six different locations and average biovolumes were calculated using IMARIS software (Bitplane, Switzerland).

The RO biofilm was detached from the remaining part of the fouled membrane (11 cm × 3 cm) by scraping the membrane with cotton-tipped applicators and re-suspending the adhered biological debris in a 2 g/L NaCl solution. The amount of biofilm was determined by ATP and cell counting assays. ATP content was measured using a portable lumitester C-110 and Kikkoman kit (Kikkoman, Japan) while cell counts were determined by using a flow cytometer (BD, USA) with SYTO9 and PI dyes (Molecular Probes, USA). Additionally, cell viability was calculated as the percentage of live cells relative to total cells obtained from cell counting assay.

The EPS in biofilm was extracted by the formaldehyde plus NaOH method [30,31]. Briefly, 12 μL of formaldehyde (36.5%; Sigma Aldrich, USA) was added to 10 mL of the biofilm solution and the mixture was then incubated for 1 h at 4°C. Next, 0.8 mL of 1 N NaOH was added to the solution, followed by an incubation for 3 h at 4°C. Finally, the solution was centrifuged at 4696 *g* for 20 min and the supernatant was collected and stored at 4°C until analysis. The composition of extracted EPS was characterized by excitation-emission matrix (EEM) analysis. Fluorescence intensities were measured by a fluorescence spectrophotometer (Agilent, USA) at excitation and emission wavelengths ranging from 220 to 450 nm and from 280 to 550 nm, respectively. EPS was classified into five components based on fluorescence behavior: aromatic protein I (Ex < 250 nm, Em < 330 nm), aromatic protein II (Ex < 250 nm, Em < 380 nm), fulvic acid-like matters (Ex < 250 nm, Em > 380 nm), microbial byproduct-like matters (Ex = 250–280 nm, Em < 380 nm), and humic acid-like matters (Ex > 250 nm, Em > 380 nm). A fluorescence regional integration analysis [32] was performed to compare each component of EPS extracted from the control biofilm and the QQ biofilm. In addition, polysaccharides and proteins were quantified by a phenol-sulfuric acid method [33] and bicinchoninic acid (BCA) assay (Thermo Scientific Inc., USA), respectively.

Results and discussion

The effect of QQ on biofilm formation in a flow cell system

Biofilms formed during flow cell operation without permeate production were experimentally interrogated to understand the influence of QQ on biofilm formation. After operating the flow cell for 18 h, biofilm formation was quantified by measuring cell count and ATP in the biomass scraped from the surfaces of membrane coupons. Biofilms from flow system with QQ-column showed reductions in bacterial cell counts and ATP by ~21% and 34%, respectively, *versus* the control system (Figs. 3a and b). To investigate whether the anti-biofilm effect in the QQ-column system arose from the inhibition of QS, the concentrations of AHLs were measured at the beginning and after 18 h of flow cell operation. The AHL concentration in the feed, which was 8.2 nM at the beginning of operation, increased to 27.2 nM after 18 h due to the growth of *P. stewartii* in the feed bottle. Relative to this feed concentration, the QQ-column reduced the level of QS signals by 54.2% at 0 h and 51.6% at 18 h, while the control-column had little effect (17.1% reduction at 0 h and 3.7% reduction at 18 h) (Fig. 4a). Thus, the QQ-column effectively lowered the QS levels in the feed stream and mitigated biofilm formation by *P. stewartii* in the flow cell system under single-pass (non-recycle) operation. Koutsoudis et al. reported that *P. stewartii* mutants that lack AHL signal synthase (EsaI) or AHL receptor (EsaR) failed to produce biofilms while the QS wild-type strain yielded biofilms with 3D architectures within 24 h [34]. Though not explicitly tested here, these behaviors support that the reduction of QS signal molecules (presumably by degradation) in the feed stream by the QQ-column prevents the accumulation of QS molecules in the *P. stewartii* colony on the RO membrane, thereby retarding biofilm development. The effect of the flow rate on the degradation of QS signal molecules was further investigated at flow rates ranging from 1.0 to 5.0 mL/min (Fig. 4b). The results show that, in single-pass operation, increasing the flow rate decreased the degradation of signal molecules, likely due to the lower residence time of signal molecules in the QQ-column. Considering that the flow rate in RO systems is ~100 fold higher than that in our flow cell test, the QQ-column seems to require further improvement, *e.g.*, modified column design to improve internal mixing or to increase residence time to a range that does not cause a pronounced pressure drop.

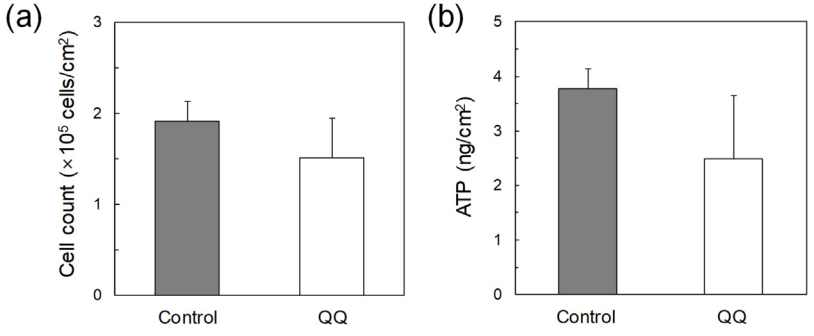

Fig. 3. Effect of the QQ-column on the biofilm formation in a flow cell test. (a) Bacterial cell count and (b) ATP concentrations were quantified from biofilms scraped from the membrane coupons after ~18 h of operation in flow cells. Error bars were defined as standard deviations (n = 2, biological replicates).

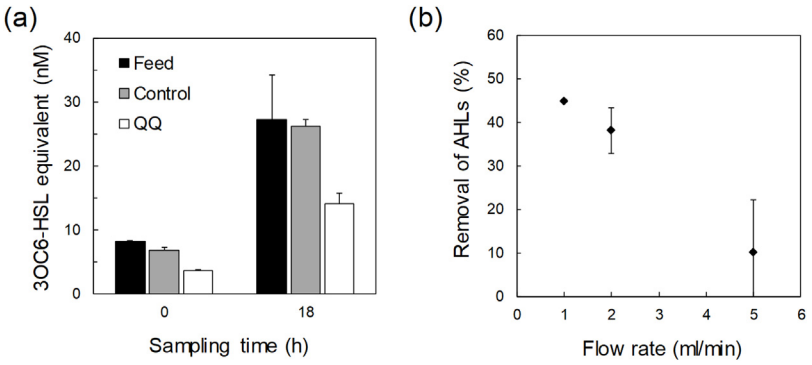

Fig. 4. Effect of the QQ-column on AHL concentrations in a flow cell test. (a) The concentrations of AHLs sampled at 0 h and after 18 h of operating the flow cell at a flow rate of 1 mL/min. (b) Removal of AHLs (%) by the QQ-column as a function of flow rate. Error bars were defined as standard deviations (n = 2, technical replicates).

Capability of QQ to control biofouling in the RO system

Control of biofouling in the RO system by the QQ-column

The capability of the QQ-column to inhibit membrane biofouling was investigated in RO systems operating in total recycle mode. The normalized TMP values (TMP/TMP_0) were monitored in both RO systems, *i.e.*, Control-RO and QQ-RO (Fig. 5). The TMP of the Control-RO increased slowly at the early stage but abruptly increased after ~74 h of operation (Fig. 5a). The TMP of the system increased by 80% with respect to the initial TMP at 115 h of operation. By contrast, the TMP of the QQ-RO increased by only 37% over the same period. In fact, the QQ-RO system operated for 143 h before reaching the same 80% increase in TMP, which represents a 24% delay compared to the Control-RO. This clearly demonstrates that the QQ-column effectively inhibited biofouling in the RO membrane during operation. The RO process was repeated with the control- and QQ-columns loaded with freshly prepared control- and QQ-beads, respectively, and the membranes were removed for autopsy after 87 h of operation, in which the TMP profiles exhibited similar trends (Fig. 5b). The fouling resistance (R_f), defined as the total resistance (R_t) during the filtration minus the resistance of the membrane (R_m), was calculated in both of these repeated RO tests. At the time when the TMP of the Control-RO increased by 80% (*i.e.*, a TMP/TMP_0 of 1.8) the fouling resistance of QQ-RO was $0.30–0.47 \times 10^{14}\ m^{-1}$, which was 56.7% lower than that of Control-RO ($0.89–0.94 \times 10^{14}\ m^{-1}$).

The concentrations of AHLs in the Control-RO and QQ-RO were measured during RO operation to establish whether the QQ-column inhibited biofilms by quenching of QS signals. The AHL levels were much lower in the QQ-RO than in the Control-RO (99 ± 27 *vs.* 70 ± 30 pM of 3OC6-HSL equivalent) (Fig. 6a). No difference was observed in the DOC of the RO concentrate in the two systems over the entire period (Fig. 6b), indicating that different fouling behaviors were not related to disparate concentrations of organic nutrients. Moreover, the salt concentrations (2800–2900 mg TDS/l) and rejections (97–98%) were similar in both systems (data not shown). The only difference between the Control-RO and QQ-RO was the presence of the AHL-lactonase (AiiO gene), which is expressed solely in the QQ population. Thus, the QQ column conclusively reduced the QS levels, which is known to reduce biofilm formation by *P. stewartii*. Yet as the flow cell results showed only marginal QQ activity at flow rates above a certain level, the design of the column needs to be improved to support higher QQ activities to be applicable in full-scale RO systems operating in single-pass mode.

The DOC concentrations in both systems gradually decrease from ~9.6 to ~1.3 mg DOC/l over time, although the feed solution was replenished twice daily. This reduction in DOC arose because the *P. stewartii* bacteria, which were dosed into the feed stream, accumulated in the microfilter cartridges and increased organic consumption over time. The concentration of AHLs concomitantly decreased over time, though exhibited more variation than DOC. A similar trend was observed in our previous study, in which AHL concentrations decreased substantially with DOC over time [24]. Based on these observations, it is speculated that AHL production closely relates to the organic concentration such as assimilable organic carbon, which is a key parameter of biofouling in RO systems [35].

Bacterial cells and EPS on the fouled RO membrane

Fouled membranes obtained from both the Control-RO and QQ-RO after 87 h of operation (Fig. 5b) were visualized by confocal microscopy. CLSM observations show that the biofilm from the QQ-RO system was smaller and more porous *versus* that of the Control-

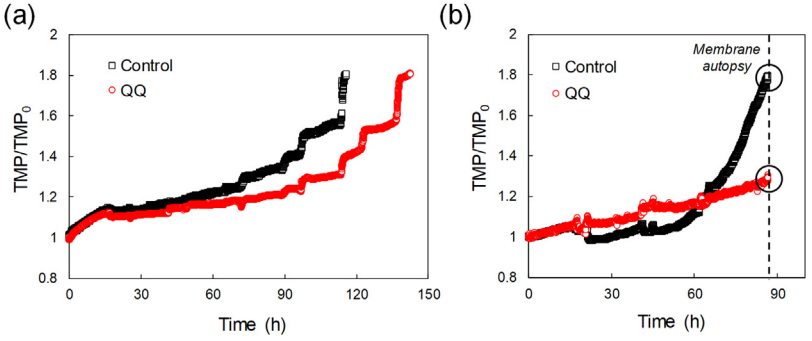

Fig. 5. TMP profiles of the Control-RO and QQ-RO. (a) A comparison of the two RO systems in which each was operated until the TMP increased by 80% from the initial value at 0 h. (b) A comparison of the two RO reactors in which each was operated for the same amount of time, as determined by the time at which the TMP of the Control-RO reached an 80% increase *versus* the initial value at 0 h.

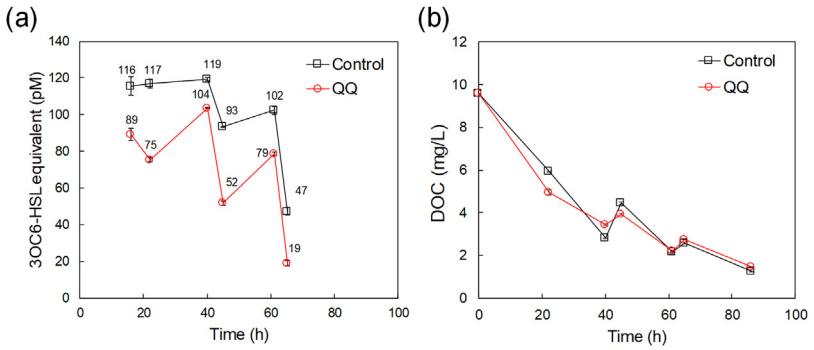

Fig. 6. AHL (a) and DOC (b) concentrations in the Control-RO and QQ-RO. Error bars were defined as standard deviations (n = 2, technical replicates).

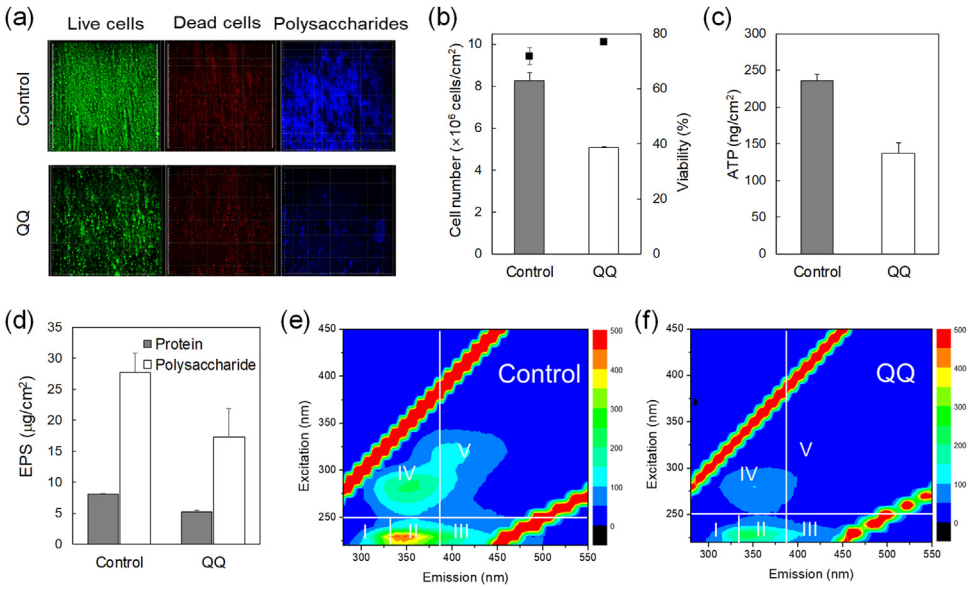

Fig. 7. Autopsy of biofouled RO membranes acquired from the Control-RO and QQ-RO after 87 h of operation: (a) CLSM images (Image size: 1415 × 1415 μm), (b) cell number (bar) and viability (dot), (c) ATP concentration, (d) protein and polysaccharide concentrations, and (e-f) EEM matrix of EPS. Error bars were defined as standard deviations (n = 2, technical replicates).

Table 1
Biovolumes of live cells, dead cells, and polysaccharides calculated from CLSM images (n = 6, technical replicates).

	Live cells ($\mu m^2/\mu m^3$)	Dead cells ($\mu m^2/\mu m^3$)	Total cells ($\mu m^2/\mu m^3$)	Polysaccharides ($\mu m^2/\mu m^3$)
Control biofilm	4.82 ± 1.14	0.90 ± 0.18	5.72 ± 1.12	3.72 ± 1.83
QQ biofilm	1.98 ± 0.50	0.73 ± 0.21	2.72 ± 0.51	0.60 ± 0.24

RO (Fig. 7a). The biovolumes of live cells, dead cells, and polysaccharides 58.9, 18.9, and 83.9% lower, respectively, in the QQ biofilm than in the control biofilm (Table 1). Furthermore, cell counts and ATP in the QQ biofilm were 38.6 and 42.1% lower, respectively, than in the control biofilm (Figs. 7b and c). These values are consistent with the biovolumes determined from the CLSM images. Moreover, lower quantities of proteins (5.3 $\mu g/cm^2$) and polysaccharides (17.3 $\mu g/cm^2$) were observed in the biofilms formed in the QQ-RO compared to those from the Control-RO (8.1 and 27.7 $\mu g/cm^2$, respectively) (Fig. 7d). This is supported by the EEM analysis, which showed lower fluorescence intensity for the EPS extracted from the QQ biofilm. In particular, the volumetric fluorescence intensity for all five regions of fluorescence excitation-emission was 47–53% of those measured from the control biofilm (Fig. 7e and f, and Table 2). These results indicated that QQ considerably reduced the quantities of bacterial cells and EPS formed on the RO membrane surface, which was associated with a delayed TMP rise. Therefore, the QQ-column appears to lower the QS levels in the RO system, which may lead to the suppressed expression of QS genes related to biofilm formation and a fouling layer with low hydraulic resistance. Cell viability in the biofilm was not considerably different between the Control-RO and QQ-RO

Table 2
Volumetric fluorescence intensities of EPSs extracted from control and QQ biofilms.

Intensity/cm^2	Control biofilm	QQ biofilm
Aromatic protein (I)	98,244	51,586
Aromatic protein (II)	251,415	128,578
Fulvic acid-like substances (III)	121,072	63,395
Microbial byproduct-like substances (IV)	88,018	41,591
Humic acid-like substances (V)	24,640	11,497

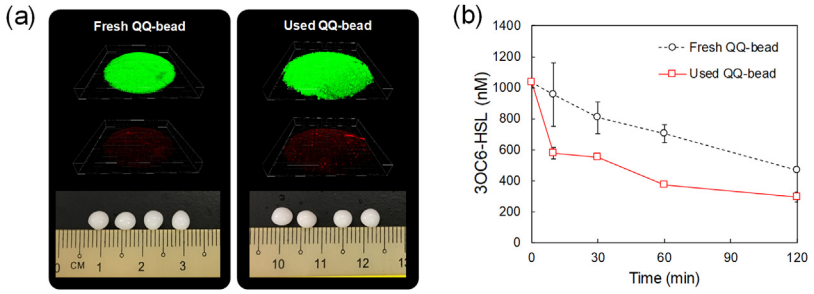

Fig. 8. Comparisons of (a) bacterial viability and (b) QQ activity in QQ-beads before operation ("fresh QQ-bead") and after 87 h of operation ("used QQ-bead") in an RO system.

(71.8% *vs.* 77.0%) (Fig. 7b), which is anticipated because QQ does not kill cells or impede cell growth [36].

Stability of QQ column

The stability of QQ-beads was determined by investigating bacterial viability and QQ activity of beads sampled from a QQ-column after 87 h of RO operation. QQ-beads sampled after RO operation showed no significant difference in size and shape as well as the viabilities of entrapped QQ bacteria (Fig. 8a) when compared to QQ-beads prior to operation. In addition, the QQ-beads showed 30% improved QQ activities after the RO operation (Fig. 8b), which may result from the growth of QQ bacteria or the accumulation of QQ enzymes inside the beads during operation. These results establish that QQ beads are mechanically and functionally stable over 87 h of RO operation, though additional tests over longer operation periods are required for future applications. But overall, the work in this study demonstrates the potential of QQ-column as a robust and potent strategy to control RO biofouling.

Conclusions

We have developed the QQ-column, which consists of hydrogel beads that incorporate QQ bacteria, as a novel strategy to control biofouling in RO processes for water reclamation. The anti-biofouling efficacy of the QQ-column was compared to that of the control-column in laboratory-scale RO systems operating in a total recycle mode. The QQ-column installed upstream of the RO module removed QS signals from the feed by ~29%, which led to a delay of TMP increase compared to the RO system with a control-column. The membrane autopsy following operation revealed that the biofilm formed in the QQ-RO system was more porous and had 38.6, 34.6, and 37.5% lower bacterial cells, proteins, and polysaccharides, respectively, than that of the Control-RO, resulting in 67.6% lower fouling resistance. Moreover, QQ did not negatively impact the salt rejection. These demonstrate the potential for the QQ-column to improve control over biofouling in RO systems, which would increase water production and reduce operating costs. Further designs of the QQ-column needs to focus on enhancing the anti-biofouling potency with the goal of translating the technology into full-scale RO systems.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This study was supported by the Research Fund for a new professor by the SeoulTech (Seoul National University of Science and Technology). We also acknowledge financial support from the Singapore Centre for Environmental Life Sciences Engineering, whose research is supported by the National Research Foundation Singapore, Ministry of Education, Nanyang Technological University and National University of Singapore, under its Research Centre of Excellence Programme. The funding support from the Economic Development Board (EDB) of Singapore to the Singapore Membrane Technology Centre (SMTC), Nanyang Environment & Water Research Institute (NEWRI), Nanyang Technological University (NTU) is also acknowledged.

References

[1] C.R. Bartels, M. Wilf, K. Andes, J. Iong, Water Sci. Technol. 51 (2005) 473.
[2] J. Choi, J. Chung, Ind. Eng. Chem. Res. 53 (2014) 11167.
[3] J.-J. Qin, K.A. Kekre, G. Tao, M.H. Oo, M.N. Wai, T.C. Lee, B. Viswanath, H. Seah, J. Membr. Sci. 272 (2006) 70.
[4] R.A. Al-Juboori, T. Yusaf, Desalination 302 (2012) 1.
[5] R. Ordóñez, D. Hermosilla, I.S. Pío, Á. Blanco, Chem. Eng. J. 166 (2011) 88.
[6] E. Sahar, I. David, Y. Gelman, H. Chikurel, A. Aharoni, R. Messalem, A. Brenner, Desalination 273 (2011) 142.
[7] M.T. Khan, M. Busch, V.G. Molina, A.-H. Emwas, C. Aubry, J.-P. Croue, Water Res. 59 (2014) 271.
[8] W.C.L. Lay, Y. Liu, A.G. Fane, Water Res. 44 (2010) 21.
[9] G. Qiu, Y.-P. Ting, Bioresour. Technol. 150 (2013) 287.
[10] W. Luo, H.V. Phan, M. Xie, F.I. Hai, W.E. Price, M. Elimelech, L.D. Nghiem, Water Res. 109 (2017) 122.
[11] M.F. Tay, C. Liu, E.R. Cornelissen, B. Wu, T.H. Chong, Water Res. 129 (2018) 180.
[12] L.A. Bereschenko, H. Prummel, G.J.W. Euverink, A.J.M. Stams, M.C.M. van Loosdrecht, Water Res. 45 (2011) 405.
[13] S. Madaeni, S. Samieirad, Desalination 257 (2010) 80.
[14] Y.-N. Wang, C.Y. Tang, Environ. Sci. Technol. 45 (2011) 6373.
[15] M. Khan, S. Danielsen, K. Johansen, L. Lorenz, S. Nelson, A. Camper, Biofouling 30 (2014) 153.
[16] K.M. Yeon, W.S. Cheong, H.S. Oh, W.N. Lee, B.K. Hwang, C.H. Lee, H. Beyenal, Z. Lewandowski, Environ. Sci. Technol. 43 (2009) 380.
[17] H.S. Oh, K.M. Yeon, C.S. Yang, S.R. Kim, C.H. Lee, S.Y. Park, J.Y. Han, J.K. Lee, Environ. Sci. Technol. 46 (2012) 4877.
[18] W. Jiang, S. Xia, J. Liang, Z. Zhang, S.W. Hermanowicz, Water Res. 47 (2013) 187.
[19] S. Lee, S.K. Park, H. Kwon, S.H. Lee, K. Lee, C.H. Nahm, S.J. Jo, H.S. Oh, P.K. Park, K. H. Choo, C.H. Lee, T. Yi, Environ. Sci. Technol. 50 (2016) 1788.
[20] T. Ergön-Can, B. Köse-Mutlu, İ. Koyuncu, C.H. Lee, J. Membr. Sci. 525 (2017) 116.
[21] S.-R. Kim, H.-S. Oh, S.-J. Jo, K.-M. Yeon, C.-H. Lee, D.-J. Lim, C.-H. Lee, J.-K. Lee, Environ. Sci. Technol. 47 (2013) 836.
[22] K.-M. Yeon, C.-H. Lee, J. Kim, Environ. Sci. Technol. 43 (2009) 7403.
[23] S.H. Lee, S. Lee, K. Lee, C.H. Nahm, H. Kwon, H.S. Oh, Y.J. Won, K.H. Choo, C.H. Lee, P.K. Park, Environ. Sci. Technol. 50 (2016) 8596.
[24] H.S. Oh, C.H. Tan, J.H. Low, M. Rzechowicz, M.F. Siddiqui, H. Winters, S. Kjelleberg, A.G. Fane, S.A. Rice, Water Res. 112 (2017) 29.
[25] C.H. Tan, K.S. Koh, C. Xie, J. Zhang, X.H. Tan, G.P. Lee, Y. Zhou, W.J. Ng, S.A. Rice, S. Kjelleberg, Npj Biofilms Micro. 1 (2015) 15006.
[26] C. Fuqua, S.C. Winans, J. Bacteriol. 178 (1996) 435.
[27] T.H. Chong, F.S. Wong, A.G. Fane, J. Membr. Sci. 325 (2008) 840.
[28] H.S. Oh, S.R. Kim, W.S. Cheong, C.H. Lee, J.K. Lee, Appl. Microbiol. Biotechnol. 97 (2013) 10223.
[29] A. Mohanty, C.H. Tan, B. Cao, Environmental Science: Nano 3 (2016) 351.
[30] H. Liu, H.H. Fang, J. Biotechnol. 95 (2002) 249.
[31] J. Zhang, H.C. Chua, J. Zhou, A.G. Fane, J. Membr. Sci. 284 (2006) 54.
[32] W. Chen, P. Westerhoff, J.A. Leenheer, K. Booksh, Environ. Sci. Technol. 37 (2003) 5701.
[33] M. Dubois, K.A. Gilles, J.K. Hamilton, P.T. Rebers, F. Smith, Anal. Chem. 28 (1956) 350.
[34] M.D. Koutsoudis, D. Tsaltas, T.D. Minogue, S.B. von Bodman, Proc. Natl. Acad. Sci. USA 103 (2006) 5983.
[35] L. Weinrich, M. LeChevallier, C.N. Haas, Water Res. 101 (2016) 203.
[36] S. Uroz, Y. Dessaux, P. Oger, ChemBioChem 10 (2009) 205.