Leda Katebian, E.I.T
Ph.D. Candidate

Telephone: 949-374-6760 Email: lkatebia@gmail.com

Education

University of California Irvine (UC Irvine)

Doctor of Philosophy	January 2016 *(Expected)*
Major:	Engineering
Concentration:	Environmental Engineering
Preliminary Exam:	May 2012
Committee Members:	Dr. Sunny Jiang, Civil & Environmental Engineering Department Dr. Diego Rosso, Civil & Environmental Engineering Department Dr. Soroosh Sorooshian, Civil & Environmental Engineering Department
Qualifying Exam:	December 2013
Qualifying Topic:	Membrane Biofouling Prevention using Quorum Sensing Inhibitors
Committee Chair	Dr. Sunny Jiang, Civil & Environmental Engineering Department
Committee Members:	Dr. William Cooper, Civil & Environmental Engineering Department Dr. Diego Rosso, Civil & Environmental Engineering Department Dr. Kristen Davis, Civil & Environmental Engineering Department Dr. Hung Nguyen, Chemical Engineering Department

University of California Irvine

Master of Science	March 2012
Major:	Engineering
Concentration:	Environmental Engineering
GPA:	3.965
Thesis Topic:	Marine Biofilm Formation and Its Responses to Periodic Hyperosmotic Shock on a Flat Sheet Membrane Surface
Committee Chair:	Dr. Sunny Jiang, Civil & Environmental Engineering Department
Committee Members:	Dr. William Cooper, Civil & Environmental Engineering Department Dr. Diego Rosso, Civil & Environmental Engineering Department

University of California Irvine

Bachelor of Science	June 2010
Major:	Chemical Engineering
Specialization:	Environmental Engineering
GPA:	3.31
Research Topic:	Marine Biofilm Inhibition using Hyperosmotic Shock
Research Advisor:	Dr. Sunny Jiang, Civil & Environmental Engineering Department

Certification

Engineering-In-Training (EIT/FE), certification number 145361, April 2012

Research Experience

Researcher Feb. 2015-Jan. 2016
Geology and Planetary Sciences, California Institute of Technology

- Incorporated quorum sensing (QS) inhibitors, vanillin and cinnamaldehyde onto the RO membrane surface using a chemical deposition method in order to improve the membrane anti-fouling potential for seawater desalination.
- This work is supported under the Dow Resnick Bridge Program.

Laboratory Safety Representative, Sept. 2014-Dec. 2015
Civil & Environmental Engineering, UC Irvine

- Worked with Environmental Health and Safety to make sure Dr. Sunny Jiang's lab was in compliance with safety regulations ranging from to hazardous chemicals and earthquake preparedness.
- Responsible for going over standard operating procedures for incoming lab members.

2012 Sustainability Science Team, Environmental Institute, UC Irvine Sept. 2012-2014

- Worked in a multidisciplinary team of five doctoral students to study the Salton Sea's water quality and hydrological cycle. Investigation included which desalination technology will be able to restore and sustain salinity levels as well as determined the sustained profitability of desalination. Lastly, the project anticipated the potential coalitions and incentives to help gain political and financial support to assist Salton Sea's stakeholders.
- *Specific Role:* Determined multi-effect distillation with thermal vapor compression is the best desalination method to restore the Salton Sea salinity level.

EPA People, Prosperity, Planet (P3) Grant Aug. 2011-May 2012
***Microbial Desalination Fuel Cell as a Sustainable Technology for Renewable Water and Power* (SU836030), Co-Investigator, UC Irvine**

- Worked in an interdisciplinary team to develop a microbial desalination fuel cell (MDFC) as a pre-treatment to the reverse osmosis system for seawater desalination.
- *Specific Role:* Supervised undergraduate students for the scalability analysis of MDFC for seawater desalination plants using MATLAB software.
- Website: http://jianglab.eng.uci.edu/epap3/

Researcher Assistant, Civil & Environmental Engineering, UC Irvine Fall 2008-Jan. 2016

- *Ph.D. Research*: Characterized the role of QS in marine biofilm and effect of QS inhibitors to reduce RO and FO membrane biofouling for seawater desalination.
  - Carried out high-pressure RO and FO membrane experimental studies in Murdoch University in Western Australia in March and August 2013.
  - Supervised a chemical engineering undergraduate student to investigate biofilm response to QS inhibitors using a crystal violet microtiter plate assay.
- *B.S./M.S. Research*: Characterized biofilm development on a flat sheet membrane by developing a bench-scale biofilm detector system operated in a dead-end filtration mode. Demonstrated periodic hyperosmotic shocks are an effective strategy to reduce biofouling.

Industry Experience

Engineering Intern, GHD, Irvine CA December 2013-May 2014

- Assisted in monitoring a pretreatment pilot operation for Carlsbad Desalination plant including a two-week algal simulation run.
- Sampled various locations of the pilot to analyze water quality of feed water, pre-treated water, and reverse osmosis permeate stream.

Engineering Assistant March 2007-Sep. 2009
TechCom International Corporation, Irvine, CA

- Provided assistance in performing Instrumentation & Controls loop uncertainty analysis for nuclear power generation industry.

Academic Experience

CEE 160 Guest Lecturer, Civil & Environmental Engineering, UC Irvine May 22, 2015

- Lectured on reverse osmosis membrane technology and membrane desalination unit process.

CEE 169 Teaching Assistant, Civil & Environmental Engineering, UC Irvine Fall 2014

- Lead laboratory sections for Environmental Microbiology for Engineers.
- Course description: Fundamental and applied principles of microbiology. Structures and functions of microorganisms, the microbiology of water, wastewater and soil used in environmental engineering, and the impact of microorganisms on human and environmental health.

CEE 160 Teaching Assistant, Civil & Environmental Engineering, UC Irvine Spring 2014

- Lead discussion sections for Introduction to Environmental Engineering.
- Lectured for one class on desalination technologies and membrane desalination unit processes.
- Course description: Introduction to environmental processes in air and water, mass balances, and transport phenomena. Fundamentals of water quality engineering including water and wastewater treatment.

CEE 160 Reader, Civil & Environmental Engineering, UC Irvine Spring 2013

- Graded exams and design project for Introduction to Environmental Engineering.
- The course description is same above for CEE 160.

CEE 167 Reader, Civil & Environmental Engineering, UC Irvine Winter 2013

- Graded exams and lab reports and assisted in applying i-clicker technology for Coastal Ecology.
- Course description: Examines the ecological processes of the coastal environment. Investigates the causes of coastal ecosystem degradation and strategies to restore the ecosystem balance or prevent further coastal ecosystem health degradation.

Graduate Student Representative Sep. 2011-March 2012
Civil and Environmental Engineering, UC Irvine

- Created and distributed market surveys at professional conferences and to engineering firms to assess the demand for an online M.S. Environmental Engineering program at UC Irvine.

CEE 160 Teaching Assistant, Civil & Environmental Engineering, UC Irvine Spring 2011

- Lead discussion sections for Introduction to Environmental Engineering.
- The course description is the same as above for CEE 160.

Skills

- C++
- MATLAB
- Pro II
- EES
- Microsoft Office
- Scanning Electron Microscopy
- Confocal Laser Scanning Microscopy
- Liquid Chromatography-Mass Spectrometry
- Raman Microscopy
- Goniometer

Awards/Fellowships

School of Henry Samueli's Academic Year Fellowship, UC Irvine Academic Year 2014- 2015

2014 Affordable Desalination Collaboration (ADC) Fellowship, AMTA Summer 2014

2014 Thomas R. Camp Scholarship, AWWA & CDM Smith Summer 2014

Summer Graduate Research Fellowship, Civil & Environmental Engineering, UC Irvine Summer 2014; 2013; 2012; 2011

Winter Graduate Research Fellowship, Civil & Environmental Engineering, UC Irvine Winter 2011

Undergraduate Research Opportunities Program Grant/Fellowship (UROP), UC Irvine Fall 2009- Spring 2010

Summer Undergraduate Research Program Grant/Fellowship, UC Irvine Summer 2009

UROP Grant/Fellowship, UC Irvine Fall 2008- Spring 2009

Publication

L. Katebian, S.C. Jiang, Marine bacterial biofilm formation and its responses to periodic hyperosmotic stress on a flat sheet membrane for seawater desalination pretreatment, J. Mem. Sci. 425-426 (2013) 182–189.

L. Katebian, E. Gomez, L. Skillman, D. Lim G. Ho, S.C. Jiang, Inhibiting Quorum Sensing Pathways to Mitigate RO Membrane Biofouling for Seawater Desalination, Desalination. *(Submitted)*

Conference Publication

L. Katebian, E. Gomez, L. Skillman, D. Lim G. Ho, S.C. Jiang, Mitigating Seawater Desalination RO Membrane using Quorum Sensing Inhibitors, AMTA-AWWA Membrane Technology Conference, Orlando, Florida, March 2015

Presentations

Oral Presentations	L. Katebian, S.C. Jiang, Mitigating Seawater Desalination RO Membrane Biofouling using Quorum Sensing Inhibitors, AMTA-AWWA Membrane Technology Conference, Orlando, Florida, March 2015
	L. Katebian, S.C. Jiang, Quorum Sensing Inhibitors to Prevent Seawater Desalination Membrane Biofouling**,** Water Reuse and Desalination Conference, Las Vegas, Nevada, May 2014
	L. Katebian, S.C. Jiang, Seawater Desalination Membrane Biofouling Presentations Prevention using Quorum Sensing Inhibitors, Graduate Student Symposium, UC Irvine, Nov. 2013
	L. Katebian, S.C. Jiang, Inhibiting Membrane Fouling using Quorum Quenchers for Seawater Desalination, Graduate Student Symposium, UC Irvine, Dec. 2012
	L. Katebian, S.C. Jiang, Preventing Membrane Fouling, Graduate Student Symposium, UC Irvine, Dec. 2011
	L. Katebian, S.C. Jiang, Preventing Membrane Fouling by Osmosis Stress, IWA on Natural Organic Matter, Costa Mesa, CA, July 2011
Expo	L. Tseng, J.C. Gellers, L. Jiang, M. Jeung, L. Katebian, K. Lim, H, Wang, E. Glenn, S. Huang, X. Huang, A. Karman; T. Tu; Y. Wu, S.C. Jiang, Microbial Desalination Fuel Cell as a Sustainable Technology for Renewable Water and Power, EPA P3 Expo, Washington, DC, April 2012
Poster Presentations	L. Katebian, S.C. Jiang, The Effectiveness of Osmosis Stress on the Removal of Biofilm, American Society for Microbiology, San Diego, CA, May 2010
	L. Katebian, S.C. Jiang, Effectiveness of Osmotic Shock on Marine Biofilm removal, UROP Symposium, UC Irvine, May 2010
	L. Katebian, S.C. Jiang, Marine Biofilm Removal using Osmosis Stress, Symposium, UC Irvine, May 2009

Professional Societies & Organizations

American Water Works
Academy of Environmental Engineering Scientists & Professors
American Membrane Technology Association
International Desalination Association

Journal of Membrane Science 425-426 (2013) 182–189

Contents lists available at SciVerse ScienceDirect

Journal of Membrane Science

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

Marine bacterial biofilm formation and its responses to periodic hyperosmotic stress on a flat sheet membrane for seawater desalination pretreatment

Leda Katebian, Sunny C. Jiang *

Civil and Environmental Engineering, 716 E Engineering Tower, University of California, Irvine, CA 92697, USA

ARTICLE INFO

Article history:
Received 23 May 2012
Received in revised form
31 July 2012
Accepted 15 August 2012
Available online 12 September 2012

Keywords:
Marine biofilm
Biofouling
Hyperosmotic stress
Seawater desalination

ABSTRACT

Cartridge and membrane biofouling is a significant challenge for the seawater desalination industry. Current cleaning methods remain inefficient or potentially damaging to the membrane. This research characterized marine bacterial biofilm formation and further examined if periodic hyperosmotic shocks to the surface of a filter membrane would reduce bacterial biofilm and prevent membrane fouling. A lab-scale biofouling detector system was developed using an eight-channel pump to deliver simultaneous flow rates through eight 5 μm pore size, 25 mm diameter nitrocellulose membrane filters. A marine *Alteromonas* strain isolated from a desalination pilot plant was used as the model biofouling agent. The results showed the 30% NaCl shock produced a hyperosmotic stress that maintained the membrane permeability and flow rate while the control and DI H_2O treated filters did not. Confocal Laser Scanning Microscopy results illustrated that the periodic 30% NaCl shocks slowed the biofilm maturation process by inducing cell mortality and reducing the biofilm thickness. Scanning Electron Microscopy results showed the salinity shock also reduced the coverage of extracellular polysaccharides in the treated biofilm matrix.

1. Introduction

As water demands and water shortage concerns rise from increasing population growth and climate changes, seawater desalination using reverse osmosis membranes (SWRO) is emerging as an important alternative source to produce high quality potable water. In the United States, the reverse osmosis (RO) membrane accounts for 70% of the total desalination capacity whereas it accounts for 53% globally [1]. The RO membrane is a semi-permeable membrane, operated in a cross-flow mode that achieves salt rejections greater than 99%. SWRO plants consist of the following unit systems: intake, pretreatment, RO, post-treatment, and discharge processes.

The main goal of the pretreatment process is to protect the RO membranes by reducing the fouling propensity of seawater [2]. Conventional pretreatment uses cartridge filters operated in a dead-end mode to remove particulate matter that is 5–10 μm in size as the last line of defense before the RO unit. New pretreatment incorporates microfiltration [MF] or ultrafiltration [UF] as a more defined barrier to the RO unit because it reduces silt density index (SDI) to less than 2, lowers turbidity to less than 0.05 NTU, and often eliminates the need for cartridge filtration [3]. MF membranes reduce turbidity and remove suspended solids and bacteria that range in size from 0.1–10 μm using either dead-end or cross-flow modes [1,2]. UF membranes also operate in either mode to remove high molecular weight dissolved organic compounds and some viruses that range from 0.005–0.1 μm in size [1,2]. The type of membrane selected for pretreatment depends on the turbidity, biological matter, and total dissolved solids present in the seawater.

A crucial obstacle for the cartridge and membrane filtration industry is biofouling because it causes a decrease in membrane flux, an increase in operational pressure, and an increase in the frequency of membrane cleanings, which incurs a higher energy demand [4,5]. Biofouling is caused by the attachment and growth of bacteria and accumulation of the bacterial metabolic products such as extracellular polysaccharides (EPS), proteins, and lipids on the membrane surface [6–8]. After the bacterial cells deposit onto the surface, minimal amounts of nutrients are sufficient enough for cells to produce biofilm [9]. Based on the specific bacteria characteristics and stages in biofilm growth, biofilm distribution on the surface ranges from uneven, discontinuous colonies to bulky, continuous films [10].

Generally, membranes are cleaned when the permeate flow rate, applied pressure, or product water quality changes by 10–15%. MF and UF membranes operated in cross-flow mode undergo chemical cleaning consisting of aggressive acidic chemicals (pH=2.0) such as

* Corresponding author. Tel.: +1 949 824 5527; fax: +1 949 824 3672.
E-mail address: sjiang@uci.edu (S.C. Jiang).

0376-7388/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.memsci.2012.08.027

citric acid, phosphoric acid, sulfuric acid, and hydrochloric acid to dissolve inorganic precipitates and inorganic constituents in the biofilm matrix [11–13]. In addition, basic solutions (pH=12.0) such as sodium lauryl sulfate and sodium hydroxide are used to dissolve organic deposits [11–13]. MF and UF membranes operated in dead-end mode use a backwash cycle with air injection to remove the fouling layer in addition to chemical cleaning while cartridge filters do not undergo cleaning. For a typical SWRO plant's operating costs, membrane replacement accounts for 5%, maintenance and parts accounts for 7%, and chemicals (consumables) account for 3% [14]. Therefore, it is imperative to find efficient, low cost, and environmentally friendly methods to treat membrane biofouling in the pre-treatment process for SWRO plants.

Hyperosmotic stress may be used as an alternative method to control biofilm growth because it inhibits a variety of bacterial physiological processes, such as nutrient uptake or DNA replication by diminishing the cytoplasmic water activity [15,16]. Presently, hyperosmotic shock is used as an 'insider's tip' in some of the membrane operations with varying results, but little research has been conducted to understand the mechanism of membrane recovery. Lee and Elimelech [17] used alginate and natural organic matter to show that salt cleaning was effective in the recovery of EPS-fouled RO membrane by changing the structure of the EPS cross-linked gel layer and inducing the breakup of calcium-foulant bonds as well as the calcium bridging between foulant molecules. Chen and Stewart [18] demonstrated 0.3 M NaCl reduced *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* biofilm growth by 56.7% using a continuous flow annular reactor. *Brazie* et al. [16] showed 0.5 M NaCl decreased *Pseudomonas aeruginosa* biofilm thickness by 50% and biofilm formation was reduced by 66.7% by using a microtiter dish assay. These previous studies used either well-characterized lab bacterial strains that adapted to the low salinity environment or pure chemical components of EPS as the model systems, which are neither a good representation of marine biofilm nor membrane recovery upon salt cleaning. Therefore, the goal of this research was to investigate the effect of hyperosmotic stress on reducing marine biofilm formed by bacterial isolates from a Southern California desalination pilot plant and improving membrane permeability. The development of membrane biofilm, which underwent periodic hyperosmotic shocks were investigated in a well-controlled laboratory scale system operated in dead-end filtration mode.

2. Experimental

2.1. Bacterial strains and biofilm production characterization

Four biofilm producing bacteria used in this study were isolated from the SWRO membranes and cartridge filters in 2009 from the Carlsbad Desalination Pilot Plant in Carlsbad, CA. B1 and B4 were previously determined to be *Shewanella sp.* while B2 and B3 were identified as *Alteromonas sp.* based on their 16S rRNA genes [19].

An optical density and a crystal violet (CV) assay were conducted to determine the principal biofoulant based on bacterial growth and biofilm production. Briefly, each isolate was inoculated into 5 ml of an artificial seawater medium (ASWJP) with 2.5 g/L peptone, 0.5 g/L yeast (ASWJP+1/2PY) as previously reported [20] and incubated at 21 °C for 24 h on a shaker. Next, the bacteria culture was diluted 1:100 in ASWJP. The dilution (200 μl) was inoculated into each well in a 96-well microtiter plate and incubated for 24 h at 21 °C. The optical cell density was measured at 550 nm wavelength using SOFTmax PRO program (Molecular Devices). After removing the supernatant and rinsing the plate using PBS (phosphate-buffered saline; pH=8.0), 100 μl of 99% methanol was added to each well for 15 min. The cells were stained using 100 μl of 0.5% CV for 20 min, the bound CV was released using 150 μl of 33% acetic acid, and the biofilm production was measured at 590 nm as previously described [22].

2.2. Effect of hyperosmotic stress on bacterial mortality

To examine the bacterial response to hyperosmotic shock, each individual isolate was cultured in 5 ml of ASWJP+1/2PY for 24 h at 21 °C. The next day, the bacteria were collected onto a 0.45 μm polycarbonate membrane filter (MF-Millipore) on a filtration tower to remove the nutrient medium by applying a gentle vacuum. Hyperosmotic shock was applied by adding 5 ml of 30% NaCl solution to the filtration tower. Additionally, 5 ml of ASWJP was applied to the surface of a control filter. The bacteria on the filters were immersed in the solutions for 15 to 30 min prior to draining by applying a gentle vacuum. The treatment and control filters were then transferred to a culture tube with 5 ml of ASWJP and vortexed for 1–2 min to elude the bacteria from the filters. A series of 10-fold dilutions were prepared and 100 μl were plated onto ASWJP+1/2PY agar plates using a spread plate method. After 24 h incubation at 21 °C, the individual bacterial colonies on the plates were enumerated and the mean mortality rates were calculated as:

$$Mortality\% = \frac{No.\ of\ Control\ Colonies - No.\ of\ Treatment\ Colonies}{No.\ of\ Control\ Colonies} \times 100\%$$

2.3. Membrane fouling study

To investigate the bacterial biofilm fouling on a flat sheet membrane in a dead-end filtration mode, the well-controlled biofilm fouling-detector system was set up as shown in Fig. 1. The system comprised of an 8-channel pump (Masterflex) to deliver simultaneous flow rates in a re-circulation mode. Eight 25 mm diameter in-line filter holders (Fischer Scientific) that contained 5 μm pore-size nitrocellulose membrane filters (MF-Milipore) were installed in each of the four treatment and four control channels.

Seawater collected from Newport Beach Pier, CA was filtered through 0.45 μm pore-size polycarbonate membrane filters (MF-Milipore) to remove the majority of bacteria and planktons in the seawater. The filtrate (1.3L) in the feed tank was seeded with 10 ml of an overnight culture of bacterial isolate, B2 that grew in ASWJP+1/2PY at 21 °C to accelerate membrane biofilm growth. The feed solution was mixed continuously on a stir plate (Thermo Fisher Scientific) set to a gentle mixing speed and was pumped through eight biofouling monitoring filter membranes in a dead-end flow mode (Fig. 1). The pump was set to generate a permeate flow rate of 20 ml/min through each channel, and an initial pressure differential of zero between the treatments and controls. The system, covered in black plastic to prevent light exposure, was run in a batch mode and no additional feed solution was added through the duration of the experiment.

Periodic shock using 30% NaCl or deionized (DI) H_2O to the filter surface was introduced at 12 h intervals to the treatment channels while no shock was applied to the control channels. DI H_2O was used to confirm 30% NaCl was producing a hyperosmotic shock rather than perturbing the filter membrane. The shock was introduced manually by injecting 5 ml of treatment solution into each injection valves after stopping the normal pump flow (Fig. 1). After a 10 min contact time with the treatment solution, the pump was restarted to flush out the treatment solution to the waste tank and then the system was returned back to recirculation mode. The flow rate, pressure differential, and the temperature of the feed tank were taken prior to each shock.

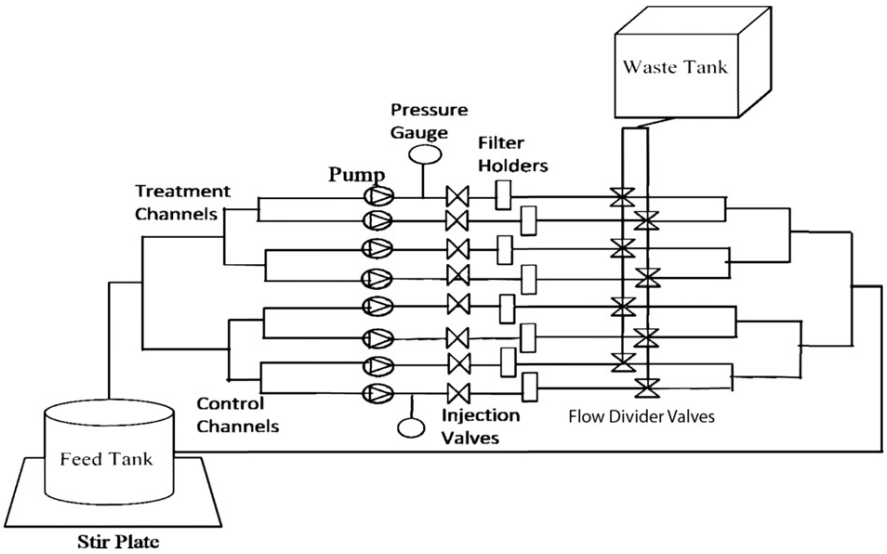

Fig. 1. Schematic of biofouling detector system.

Additionally, the normalized flow rates were calculated as the average treatment flow rate divided by the average control flow rate. A set of treatment and control filters were removed after each shock to analyze the biofilm and cell density on the filters.

2.4. Biofilm thickness and cell density analysis using confocal laser scanning microscopy (CLSM)

LSM 510 Meta Two-Photon CLSM (Zeiss) was used to analyze biofilm thickness and live and dead cells on the filter membranes. The cells were stained using SYTO 9 green fluorescence and propidium iodide following the procedures from the LIVE/DEAD Biofilm Viability Kit (FilmTracer™). The Argon laser was used to observe live cells at a wavelength of 488 nm and dead cells at a wavelength of 514 nm. The emitted light was collected through a 500–550 IR filter for live cells and 650–750 IR for dead cells. Prior to cell counts and biofilm thickness determination, the blank filters with and without staining were tested to make sure that the blank filter does not produce auto-fluorescence or take up the stain to interfere with the enumeration of stained cells. Five images were taken along the horizontal diameter of the filter membrane to avoid bias due to the uneven biofilm distribution on the filter membrane. The images were compiled and processed using Image J software (http://imagej.nih.gov/ij) to render a 3D composite image to show the spatial distribution of the live and dead cells in the biofilm along horizontal (coverage) and vertical (thickness) distribution on the filter membrane as indicated by the fluorescence intensity.

To establish if a significant difference existed between the biofilm thickness on the control and treatment filters, a paired two-sample mean T-test using a 95% confidence level was performed. The mean difference (MD) and the p value were reported for the significant differences.

2.5. Biofilm morphology analysis using scanning electron microscopy (SEM)

The biofilm morphology on the filters was analyzed using the XL-30 SEM (Philips/FEI). The filters were post-fixed using 0.5% osmium tetroxide in 0.1 M cacodylate buffer for 30 min, rinsed with distilled water, and underwent a series of dehydration steps where they were immersed for 10 min in 20% ethanol, 50% ethanol, 70% ethanol, and 100% ethanol. The filters were dried overnight in a desiccator with anhydrous $CaSO_4$ at 21 °C. Then, the biofilm on the filter membranes was coated with a mixed gold/palladium target using the 7620 sputter coater (VG/Polaron). For SEM observation, a working distance of 13 mm, a spot size of 3, and an accelerating voltage of 10.0 kV was applied.

3. Results

3.1. Effect of hyperosmotic stress on bacterial mortality

Fig. 2 shows the optical cell density and biofilm density by the CV assay for B1, B2, B3, and B4 bacterium after a 48 h incubation in ASWJP+½ PY. All four marine bacterial isolates grew well in the seawater medium, but B2 had the highest biofilm production among the isolates tested. The bacterial responses to the 30% NaCl shock are summarized in Table 1. The mean mortality rates for all bacteria increased as the exposure time to the salt solution increased from 15 to 30 min. The *Shewanella* strain, B1 was the most susceptible to the hyperosmotic shock with a greater than 99.5% mortality rate at 30 min contact time. The *Alteromonas* isolates, B2 and B3 were least susceptible to the 30% NaCl shock for both contact times. The cell mortality rate was less than 70% for B2 at 30 min contact time. Since B2 was relatively more resistant to the 30% NaCl shock and produced the most biofilm among the four bacterial isolates tested, it was chosen for the subsequent study to investigate the effectiveness of periodic hyperosmotic shocks to the recovery of membrane permeability. Additionally, the *Alteromonas* strain, B2, belongs to the class γ-Proteobacteria, which is one of the dominant types of bacteria present in the seawater intake and on RO membranes in SWRO plants [5,19,21].

3.2. Effect of hyperosmotic shocks on membrane normalized flow rate

The biofilm fouling-detector experiments showed significant changes in the normalized flow rate in membranes treated with periodic hyperosmotic shocks. The normalized flow rate in Fig. 3, provided a comparison of the trends of membrane permeability during the course of the two experimental trials using 30% NaCl

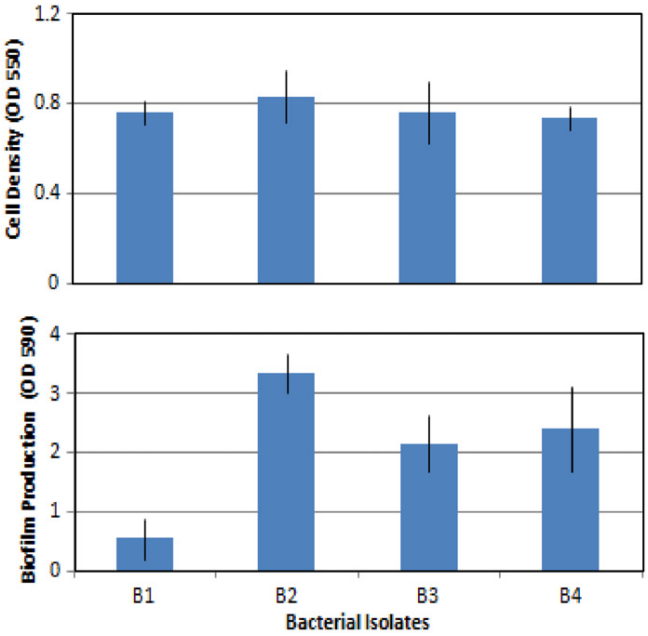

Fig. 2. Cell density and biofilm production by four different marine bacterial isolates. The cell density of the bacterial culture was measured at an optical density (OD) of 550 nm wavelength. The biofilm density was determined after staining with crystal violet (CV) at OD of 590 nm.

Table 1
Mean bacterial mortality rates expressed as a percentage of the control.

Contact time [min]	*Shewanella sp.*		*Alteromonas sp.*	
	B1 (%)	**B4** (%)	**B2** (%)	**B3** (%)
15	80.0	75.0	67.5 ± 4.9	34.0 ± 22.6
30	99.5 ± 0.7	95.0 ± 7.1	69.0	72.2

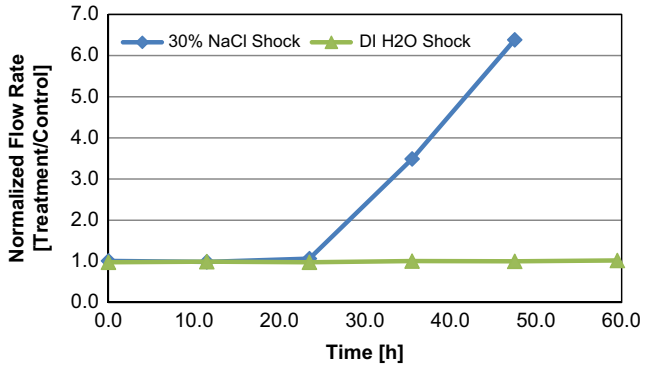

Fig. 3. The normalized flow rate through 30% NaCl and DI H_2O treated membranes, respectively. The normalized flow rate was 6.5 times higher in the membrane treated with 30% NaCl than that treated with DI H_2O.

and DI H_2O shock, respectively. There was no detectable change in membrane permeability based on the normalized flow rate between the membrane treated with the 30% NaCl solution, DI H_2O, and the control membrane within the first 24 h of the experiment. From 24 to 48 h, there was a 6.5 fold increase in the normalized flow rate from the 30% NaCl treated filter membrane, which corresponded to a 17 ml/min difference between the saline treated and control flow rates. The membrane

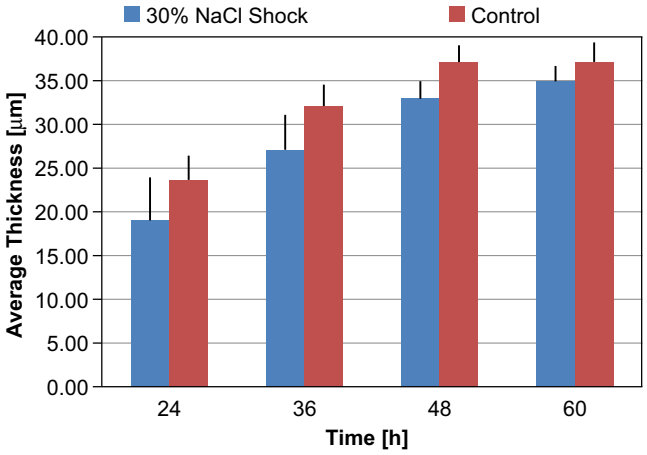

Fig. 4. The average B2 biofilm thickness (μm) on the 30% NaCl treated and control filter membranes determined using CLSM.

treated with periodic DI H_2O shock did not show an improved flow rate in comparison with the control membrane. This suggests the 30% NaCl shock produced a hyperosmotic stress that maintained the permeability and flow rate instead of perturbing the membrane surface.

3.3. Membrane biofilm thickness

Fig. 4 shows that the mean *Alteromonas* biofilm thickness measurements by CLSM increased on both the 30% NaCl treated and control filters from 24 to 48 h. However, the biofilm was significantly less on the 30% NaCl treated membrane in comparison with the control membrane at 36 h (MD=5; $p=0.03$) and 48 h (MD=4.19; $p=0.02$) based on the paired two-sample mean T-test. The biofilm thickness on the treated and control filters was not statistically significantly different at 24 and 60 h. However, thinner biofilm was still observed on the NaCl treated filter than on the control filter. The biofilm thickness on the control filter did not change between 48 and 60 h due to a leak in the in-line filter holder from the pressure build-up as a result of the increased biofilm formation.

3.4. Biofilm structure and composition

The Image J 3D composite images (Fig. 5) were analyzed to characterize the structure and spatial distribution of the live and dead cells in the biofilm matrix on the membrane surface. The 3D image is composed of multiple biofilm layers from bottom to top, artificially divided as approximately 100 μm in height shown along the *y*-axis (Fig. 5). The *x*-axis corresponded to the bottom layer of the biofilm formation along the horizontal diameter of the filter membrane. Thus, from 24 to 48 h as the biofilm coverage and thickness increased, the dimensions of the *x*- and *y*-axis increased. Additionally, the *z*-axis measured the fluorescence intensity, an indication of cell density on the membrane. The results showed the biofilm structure and composition were similar on treated and control membranes at 24 h (Fig. 5a and b). However, dead cells (red color) dominated the biofilm bottom layer on 30% NaCl treated membrane at 36 h while few dead cells were observed in the control membrane biofilm (Fig. 5c and d), implying the effectiveness of the 30% NaCl shock at bacterial mortality. Analyses of biofilm structure and composition on control membranes also revealed the stages of biofilm formation. Although a healthy layer of *Alteromonas* biofilm was established within 24 h of filtration, the biofilm coverage on the surface of the

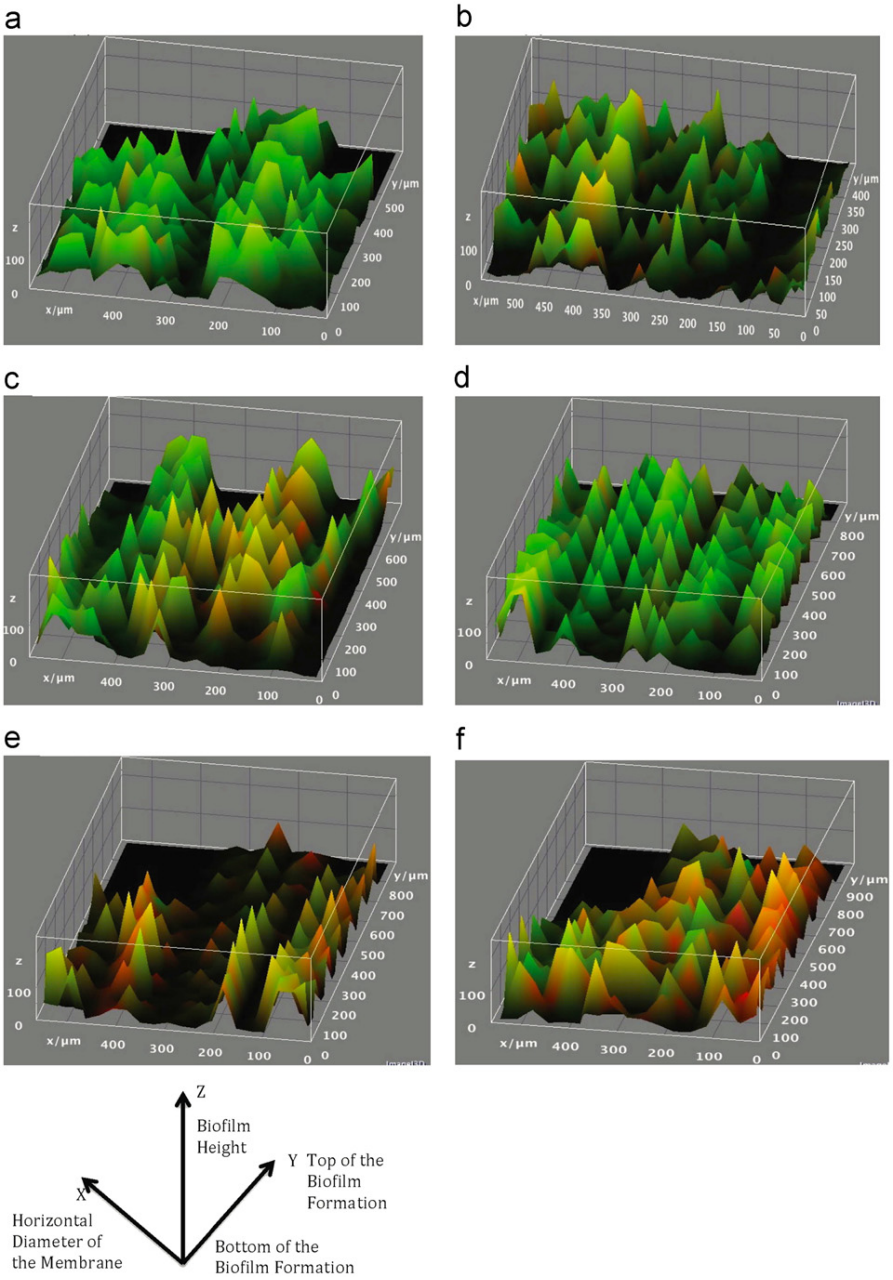

Fig. 5. The 3D composite images of the biofilm formation on (a) the 30% NaCl treated filter at 24 h, (b) the control filter at 24 h, (c) the 30% NaCl treated filter at 36 h, (d) the control filter at 36 h, (e) the 30% NaCl treated filter at 48 h, and (f) the control filter at 48 h. The *x*-axis of the image shows the biofilm along the horizontal diameter of the filter membrane. The 3D image is composed of multiple biofilm layers along the *y*-axis. The dimensions of the *x*- and *y*-axis are automatically determined by Image J based on the thickness of the biofilm. Thus, the dimensions alter as the biofilm formation changes on the filter membrane surface. The *z*-axis measures the fluorescence intensity of live and dead cells.

membrane was patchy as shown in Fig. 5(b). From 24 to 48 h, the biofilm coverage on the surface of membrane appeared more even and the biofilm matrix was composed of mainly live cells (green color) with clear channel systems through the surface of biofilm (Fig. 5d). The dead cells dominated the biofilm at 48 h indicating the completion of biofilm maturation and transition to detachment stage (Fig. 5f). The fluorescence profile analysis (Fig. 6) confirmed the pronounced 'peaks and valleys' area in the biofilm supporting the observation of 'channels' from the 3D composite image. The fluorescence profile also gave a quantitative result on the relative abundance of live and dead cells and revealed that dead cells were greater than live cells on treated membrane at 36 h.

3.5. SEM biofilm morphology

To further exam the biofilm morphology at the cell and matrix level, SEM images of surface biofilm were captured at each sampling time. The extensive EPS that connects the bacteria as well as the individual bacterial cells were observed on both of the treatment and control filters (Fig. 7). The cells embedded within the matrix of EPS were short rod and near spherical in

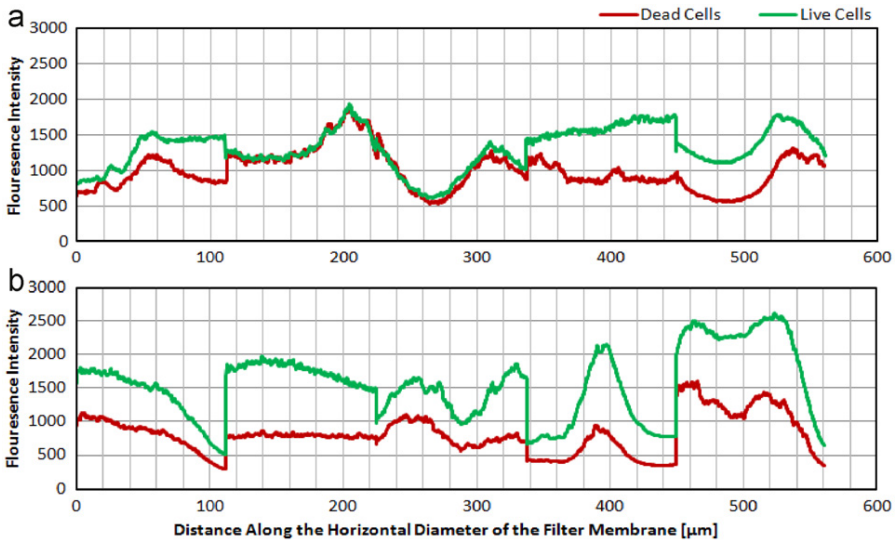

Fig. 6. The fluorescence profiles of the live and dead cells for the (a) 30% NaCl treated and (b) control filter membranes at 36 h using Image J. The profile was calculated by taking the average fluorescence intensity of the live and dead cells along the horizontal diameter of the filter membrane.

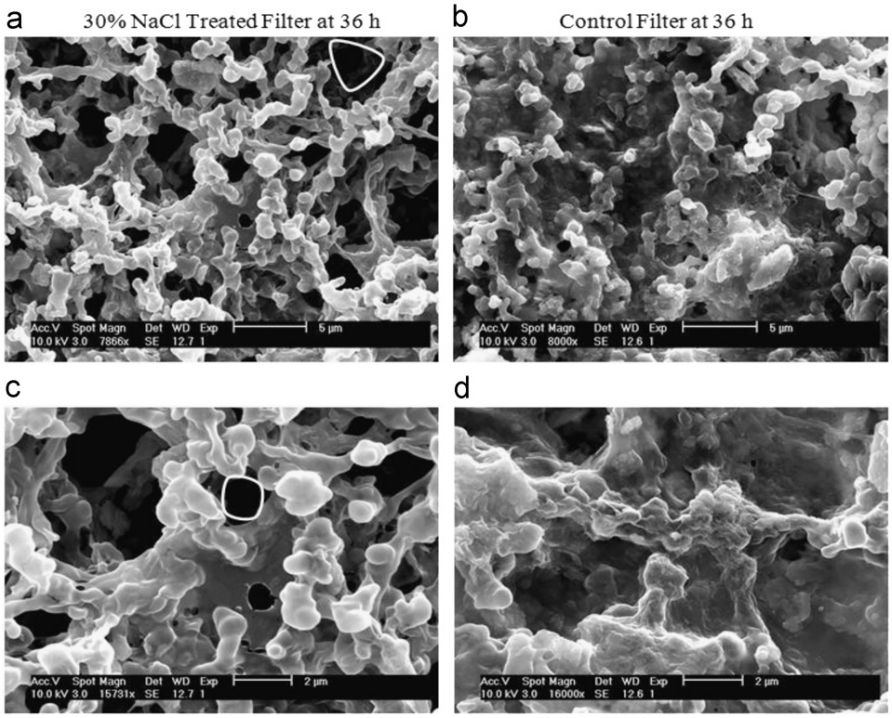

Fig. 7. SEM images show the biofilm morphology at 36 h during the experimental study for the 30% treated filter (a) (c), and the control filter (b) (d). The scale bars indicate 5 μm in image (a) and (b); 2 μm in image (c) and (d). The 'holes' between the EPS and the bacteria on the 30% NaCl treated filter membranes are depicted in (a) and (c) by the open white circles.

morphology, which is typical of *Alteromonas sp.* culture. The uniformed cell morphology suggested that *Alteromonas* seeded in the tank water was the main cause of membrane biofilm. No other type of marine bacterial morphology was obvious on the membrane surface although 0.45 μm filtered Newport Beach seawater are not completely free of native bacterial cells.

Fig. 7 shows biofilm images captured at 36 h from the treated and control membrane. The biofilm matrix on the control membrane appeared denser with fewer 'holes' between the EPS and bacterial cells (Fig. 7b and d). The biofilm surface on the control membrane was uneven as observed on the treated membrane. The 'holes' on the 30% NaCl treated membrane were more obvious, which may be responsible for the increased flow rate observed for the filtration study (Fig. 7a and c). There was no significant difference between the individual cell morphology on the treated and control filters whereas the EPS coverage seemed more extensive on the control membrane (Fig. 7c and d). The cell size remained unaltered and there was no deformation (i.e. shrinkage of cytoplasm) observed in the cell structure by SEM on the 30% NaCl treated membrane. This suggests the cell mortality was not due to cell structural damage in the high salt solution.

4. Discussion

Since the 1970s, biofilm and biofouling have been investigated on a variety of surfaces ranging from dental plaque to industrial water systems. The field of microbiology has come to accept the universality of the biofilm phenotype [23]. The key to successfully remediating the biofouling problem may hinge upon a more comprehensive understanding of the biofilm phenotype. This research worked towards this objective by characterizing the marine biofilm formation on flat sheet membrane surfaces using a marine *Alteromonas* dominated biofilm that is of significance in SWRO membrane fouling. The marine bacterial responses to the periodic hyperosmotic shock and the subsequent fouling reduction on a filter membrane surface have not been previously studied.

The model organism B2 used in the fouling study was isolated from a local desalination pilot plant and was identified as *Alteromonas,* which is one of the prevalent types of bacteria in the seawater intake and on the biofouled membrane [5,19,21]. The early characterization study indicated that this strain, B2, was a high biofilm producer and was relatively more resistant to hyperosmotic shocks. These characteristics make B2 a better representation of SWRO biofouling organisms than any other previous investigated model systems [16–18]. The bench-scale fouling study was also designed to use natural seawater that contains natural organic matter, trace nutrients and low-level of marine bacteria, which bypassed the 0.45 μm filtration to reflect the environmental condition. The enrichment of B2 in the prefiltered seawater sped up the fouling investigation. Thus, offering a well-controlled, short-term accelerated system for investigation of biofouling mechanisms and remediation strategies at the bench-scale level.

The results of this investigation showed the periodic 30% NaCl shock improved membrane permeability and reduced biofilm thickness on the membrane surface. Both the plate counts and staining assays demonstrated the 30% NaCl induced cell mortality. Cell morphological observations by SEM suggested the cell physiological responses rather than shrinkage or deformation of the cell structure was the cause of 30% NaCl induced cell death. The parallel experiment using DI water also verified that B2 cells tolerated changes in osmosis pressure. Chen and Stewart [18] suggest the biofilm removal by NaCl is not due to the osmotic effect because an isosmotic dose of sucrose does not have the same effect on biofilm removal. High concentrations of NaCl are known to cause inhibition of nutrient uptakes and DNA synthesis in bacteria [15]. Furthermore, NaCl can weaken the biofilm matrix by screening out crosslinking electrostatic interactions and break-up the bounds between foulant molecules [17]. The reduced biofilm thickness and improved permeability on the 30% NaCl treated filter is likely the result from both cell death and breakage of EPS in the biofilm matrix.

Previous investigations of cross-flow RO membrane biofouling have indicated that live and dead bacterial cells cause membrane performance deterioration by two different mechanisms [24]. The dead cells form porous cake layers that deteriorate membrane performance by hindering the back diffusion of salt, which results in an elevated osmotic pressure on the membrane surface. The live cells together with their EPS, on the other hand, contribute to the decline in membrane water flux by increasing the hydraulic resistance to permeate flow [24]. In the dead-end filtration mode using large pore-size membranes as tested in this study, diffusion of salt and osmotic pressure are not a consideration. Thus, the main cause of membrane permeability loss is due to the live cells embedded within their EPS, which causes an increase in the hydraulic resistance to the flow. We should also mention that the establishment of the marine biofilm in the dead-end filtration is initiated by the sessile bacterial growth on the membrane surface as demonstrated by the examination of the membrane surface at 24 and 36 h of the experiment. However, the formation of mature biofilm and reduction in filtration rate due to the pore clogging may cause accumulation of planktonic cells directly onto the membrane surface to form a cake layer. During the 48-h experimental period, significant bacterial growth in the feed tank was not observed (data not shown). As a result, we don't expect the bacterial cake layer formation to play an important role during the course of this investigation. Therefore, the effectiveness of the saline shocks for membrane permeability recovery is due to the destruction of EPS network and the reduction in EPS production by cell mortality. These results are an extension and a confirmation of the early study conducted by Lee and Elimelech [17] using a pure EPS component.

Biofilm formation on a membrane surface is a dynamic process that involves: cell attachment, microcolony formation and biofilm maturation [25]. Previous works have revealed striking commonalities in the structure and function of biofilms of different species [26]. The model bacterium used in this study initiated the cell attachment process within 12 h from the onset of filtration experiment. Application of the hyperosmotic shock at 12 h could not prevent biofilm formation. This was evident by the observation of similar biofilm density at 24 h on both the control and treated filter membranes.

The periodic hyperosmotic shocks were able to slow the biofilm maturation process as shown by the improved membrane permeability, the thinner biofilm thickness, and the higher number of dead cells on the treatment membrane than those on the control membrane at 36 and 48 h. This result was similar to the report for *Pseudomonas aeruginosa* biofilm responses to high NaCl osmotic shock [16], but was different from other non-halophilic cells such as *E. coli* [27]. Delaying the first hyperosmotic shock from 12 to 24 h after the onset of the filtration experiment was ineffective at slowing biofilm maturation or at improving the flow rate (data not shown). This could be due to the complete formation of microcolonies that act as a diffusion barrier to the hyperosmotic shock [28–30]. This observation may explain the variation in the effectiveness of the hyperosmotic shock for membrane recovery. Thus the application of the shock as an 'insider's tip' in membrane operation, can benefit from the development of 'a shocking regime' with an understanding of the phases of biofilm formation.

In addition to the biofilm thickness and the distribution of live and dead cells, the microscopy results illustrated the clear channels in the mature B2 biofilm on both the treatment and control filters. This observation is consistent with previous studies of the biofilm structure of non-marine bacterial origin [31] and implied the importance of channels and peaks in the biofilm architecture. These channels are postulated as 'arteries' for the transport of nutrients and oxygen within the biofilm matrix [10]. Additionally, these channels are potentially important at delivering hyperosmotic shock to cells. However, treatment at this stage was likely inefficient because biofilm maturation is associated with the reduced susceptibility of bacteria to chemical cleaning methods [32] due to the slowing down of the convective flow [33].

The investigation of periodic hyperosmotic shocks for biofilm reduction reported in this study is different from the osmotic backwash technology patented by IDE [34]. The osmotic backwash technology uses the osmotic pressure differential between feed and permeate solutions to create a flow from the permeate side to the feed side due to the forward osmosis across the RO membrane [35]. However, the mechanistic function of biofilm removal by the technology has not been investigated. Numerical simulations of the two-dimensional, transient concentration field during an osmotic backwash event indicate that a shorter pulse is significantly diluted, particularly on the membrane surface, to the point where its concentration may drop below that required for inducing osmotic flow [36]. Thus, it is critical to understand the

mechanistic function of the proposed technology for effective practical applications. The contribution of our study is at providing a theoretical explanation of the effect of hyperosmotic shock on bacterial biofilm removal, which has the potential to be applied in the desalination industry for SWRO pretreatment.

To adapt the results of this research to practical applications, the brine solution from the RO reject may be used for shock cleaning of MF or UF membrane using both forward and backwash operation to reduce the chemical consumption. The cleaning solution will have to be discharged to the waste before starting the regular filtration cycle to prevent contamination of the filtrate. Several important factors would need to be considered before this technology can be transferred to the practical application. The saline concentrate tested in this study is much higher than the SWRO reject. The brine either has to be further concentrated or amended with additional salt. Alternatively, a hyperosmotic shock regime using lower salt concentration can be investigated. Another area of further development is to understand the response of mix-culture bacteria to hyperosmotic shock since diverse bacteria cause membrane fouling and their response to hyperosmotic shock may vary greatly. Furthermore, the bacterial cell surface hydrophobicity, presence of fimbriae and flagella, and production of EPS all influence the rate and extent of attachment of microbial cells to membrane surface [23] and their removal efficiency. It is also important to note that cells are capable of adapting to the high osmotic conditions that render the inefficiency for continuous treatment [15]. Nevertheless, the research reported in this study is a first step at the development of an environmentally friendly membrane cleaning technology for improving SWRO operation.

5. Conclusions

The periodic 30% NaCl hyperosmotic shocks reduced model bacterial biofilm on the filter membrane. These shocks maintained the initial flow rate while the control and DI H_2O treated membranes fouled during the duration of the experiment. The shocks have the potential to decrease SWRO operating costs associated with the traditional membrane cleaning methods and membrane replacement for the pre-treatment process.

Acknowledgment

This research was partially supported by the WateReuse Research Foundation (WRF08-19). We would like to thank Matt Linder for providing the cell density and biofilm production measurements. The Materials Characterization Center at UC Irvine made it possible to obtain the SEM images. The Optical Biology Core Facility at UC Irvine provided assistance for the CLSM. Dr. Adam Martiny's lab at UC Irvine provided the seawater from Newport Beach, CA used in this study.

References

[1] T. Mezher, H. Fath, Z. Abbas, A. Khaled, Techno-economic assessment and environmental impacts of desalination technologies, Desalination 266 (2011) 263–273.
[2] L.F. Greenlee, F.L. Desmond, B.D. Freeman, B. Marrot, P. Moulin, Reverse osmosis desalination: water sources, technology, and today's challenges, Wat. Res. 43 (2009) 2317–2348.
[3] J. Xu, G. Ruan, X. Chu, Y. Yao, B. Su, C. Gao, A pilot study of UF pretreatment without any chemicals for SWRO desalination in China, Desalination 207 (2007) 216–226.
[4] P.K. Abdual Azis, I. Al-Tisan, N. Saikumar, Biofouling potential and environmental factors of seawater at a desalination plant intake, Desalination 135 (2001) 69–82.
[5] J. Lee, I. Kim, Microbial community in seawater reverse osmosis and rapid diagnosis of membrane biofouling, Desalination 273 (2011) 118–126.
[6] J.W. Costerton, Overview of microbial biofilms, J. Ind. Microbiol. 15 (1995) 137–140.
[7] H.C. Flemming, G. Schaule, Biofouling on membranes–a microbiological approach, Desalination 70 (1988) 95–119.
[8] H.C. Flemming, G. Schaule, T. Griebe, J. Schmitt, A. Tamachkiarowa, Biofouling–the Achilles heel of membrane processes, Desalination 11 (1997) 215–225.
[9] M. Herzberg, S. Kang, M. Elimelech, Role of extracellular polymeric substances (EPS) in biofouling of reverse osmosis membranes, Environ. Sci. Technol. 43 (2009) 4393–4398.
[10] Z. Lendowski, H. Beyenal, Biofilms: their structure, activity, and effect on membrane filtration, in: Proceedings of IWA specialty conference 2, 2004, pp. 417–431.
[11] P.A.C. Bonné, J.A.M.H. Hofman, J.P. v.d. Hoek, Scaling control of RO membranes and direct treatment of surface water, Desalination 132 (2000) 109–119.
[12] C. Fritzmann, J. Löwenberg, T. Wintgens, T. Melin, State of-the-art of reverse osmosis desalination, Desalination 216 (2007) 1–76.
[13] F. Reverberi, A. Gorenflo, Three year operational experience of a spiral-wound SWRO system with a high fouling potential feed water, Desalination 203 (2007) 100–106.
[14] R. Semiat, Desalination: present and future, Wat. In. 25 (1) (2000) 54–65.
[15] L. Csonka, Physiological and genetic responses of bacteria to osmotic stress, Microbiol. Rev. 53 (1989) 121–147.
[16] A. Brazire, F. Diab, M. Jebbar, D. Haras, Influence of high salinity on biofilm formation and benzoate assimilation by *Pseudomonas aeruginosa*, J. Ind. Micro. Biotechnol. 34 (2007) 5–8.
[17] S. Lee, M. Elimelech, Salt cleaning of organic-fouled reverse osmosis membranes, Wat. Res. 41 (2007) 1134–1142.
[18] X. Chen, P. Stewart, Biofilm removal caused by chemical treatments, Wat. Res. 34 (2000) 4229–4233.
[19] M. Zhang, S. Jiang, D. Tanuwidjaija., N. Voutchkov, E. Hoek, B. Cai, Composition and variability of biofouling organisms in seawater reverse osmosis Desalination plants, Appl. Environ. Microbiol. 77 (2011) 4390–4398.
[20] J.H. Paul, Use of hoechst dyes 33258 and 3342 for enumeration of attached and planktonic bacteria, Appl. Environ. Microbiol. 43 (1982) 939–944.
[21] H. Bae, H. Kim, S. Jeong, S. Lee, Changes in the relative abundance of biofilm-forming bacteria by conventional sand-filtration and microfiltration as pretreatments for seawater reverse osmosis desalination, Desalination 273 (2011) 258–266.
[22] G.A. O'Toole, R. Kolter, The initiation of biofilm formation in *Pseudomonas fluorescence* WCS365 proceeds via multiple, convergent signaling pathways: a genetic analysis, Mol. Microbiol. 28 (1998) 449–461.
[23] R.M. Donlan, Biofilms: microbial life on surfaces, Emerging Infect. Dis. 8 (2002) 881–890.
[24] M. Herzberg, M. Elimelech, Biofouling of reverse osmosis membranes: role of biofilm-enhanced osmotic pressure, J. Mem. Sci. 295 (2007) 11–20.
[25] P. Watnick, R. Kolter, Biofilm, city of microbes, J. Bacteriol. 182 (2000) 2675–2679.
[26] S. Kjelleberg, M. Givskov, The Biofilm Mode of Life: Mechanisms and Adaptations, first ed., UK, 2007.
[27] N.R. Stanely, B.A. Lazazzera, Environmental signals and regulatory pathways influence biofilm formation, Mol. Microbiol. 52 (2004) 917–924.
[28] Y. Xiong, Y. Lui, Biological control of microbial attachment: a promising alterative for mitigating membrane biofouling, Appl. Microbiol. Biotechnol. 86 (2010) 825–837.
[29] A.L. Lim, R. Bai, Membrane fouling and cleaning in microfiltration of activated sludge wastewater, J. Membrane Sci. 216 (2003) 279–290.
[30] G.L. Leslie, R.P. Schneider, A.G. Fane, K.C. Marshall, C.J.D. Fell, Fouling of a microfiltration membrane by two gram-negative bacteria, Colloids Surf. A 73 (1993) 165.
[31] P. Stoodley, J.D. Boyle, I. Dodds, H.M. Lappin-Scott, Consensus model of biofilm structure, in: J.W.T. Wimpenny, P.S. Gilbert, H.M. Lappin-Scott, M. Jones (Eds.), Biofilms: community interactions and control, 1997, pp. 1–9.
[32] E. Drenkard, Antimicrobial resistance of *Pseudomonas aeruginosa* biofilms, Microbes Infec. 5 (2003) 1213–1219.
[33] G. Geesay, M.W. Stupy, P.J. Bremer, The dynamics of biofilms, Int. Biodeterior. Biodegrad. 30 (1992) 135.
[34] J.M. Arnal, B. García-Fayos, M. Sancho, Membrane Cleaning, in: R. Ning (Ed.), Expanding issues in desalination, 2010, pp. 63–75.
[35] J. Qin, M. Htun, Oo, K.A. Kekre, B. Liberman, Development of novel backwash cleaning technique for reverse osmosis in reclamation of secondary effluent, J. Mem. Sci. (2010) 8–14.
[36] G. Ramon, Y. Agnon, C. Dosoretz, Dynamics of an osmotic backwash cycle, J. Mem. Sci. 364 (2010) 157–166.