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Biofilm formation on RO membranes: the impact of seawater pretreatment

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ABSTRACT

Membrane biofouling is an acute problem that interferes with filtration and pressure-driven desalination processes. In this study, ultrafiltration (UF) and membrane bioreactor (MBR) system were examined for their potential use in the removal of organic matter from seawater as pretreatment for reverse osmosis (RO) desalination. The study showed that MBR treatment equipped with UF decreases total organic carbon, polysaccharides, and biofouling potential of RO membrane in comparison to feed seawater after UF treatment alone. Bacteria in the feed water and in the MBR system were characterized. The most abundant heterotrophic bacteria nourished from organic substances present in the MBR system belonging to the *Alphaproteobacteria* and *Gammaproteobacteria* classes increased from ~40% in seawater to ~60% in the MBR. These results indicate that pretreatment using a seawater MBR system can improve RO feed water quality and reduce the biofouling potential of RO membranes.

Keywords: Biodegradable organic matter; Biofouling; Membrane bioreactor; Reverse osmosis

1. Introduction

One of the most acute problems in the world in general and in arid zones in particular is the scarcity of potable water. A potential solution with great promise is seawater or brackish groundwater desalination, in a process where reverse osmosis (RO) and forward osmosis membranes are used to separate the salts from the water [1–4]. These membranes, however, are susceptible to damage, as four main types of fouling can compromise their surfaces: scaling, organic fouling, colloidal fouling, and biofouling [3–6]. While the first three types of foulants have been intensively investigated yielding relatively simple methods to remove them by chemical and physical means [7], biofouling, the most problematic type of fouling, is also

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the one with the least amount of information regarding remediation methodologies [8]. Biofouling is defined as the accumulation of biomass on a surface by microorganism growth and/or deposition to an extent that causes system operational problems [9–11]. Investigation of the fouling process on the RO membrane revealed that the microorganisms settle on a membrane during the first hours of operation, but only when a certain threshold of interference is exceeded, biofouling takes place [5].

Biofilm formation on a membrane is caused by the interaction of water-borne microorganisms with the membrane in a multi-step process. The process begins with the rapid adsorption of dissolved organic matter, transparent exopolymer particles (TEP), and extracellular polymeric substances (EPS) to the membrane surface to form a layer that enhances subsequent microbial adhesion, and thus had a high fouling potential on RO membranes [12–18]. During microbial adhesion to the conditioned surface, the adhered cells grow and produce EPS that are mainly composed of polysaccharides and proteins. The final step is maturation of microcolonies into an EPS-encased mature biofilm. At this point, the accumulated biomass is recognized as a biofilm [19], a structure that enables the individual microorganisms that produced the biofilm to passively increase their nutrient consumption from the surrounding water [20].

Feed water organic matter content, necessary to support biofilm growth, can be effectively reduced to prevent fouling by removing the organic substances before they come in contact with the RO membrane [16,21]. Thus for RO membrane systems, feed waters with high biological activity require extensive pretreatment to lower the potential of biofouling development inside the RO membrane module [11]. Microfiltration (MF) or ultrafiltration (UF) alone, commonly used as pretreatment to RO membranes [22–24], cannot eliminate supply biodegradable substances dissolved in the fed seawater and subsequent membrane biofouling. Thus, the removal of biodegradable organics as well as microbial inactivation at the same time would be effective to control the biofouling [25].

A viable pretreatment system that can minimize the fouling problem by reducing the levels of organic carbon and nutrients in the feed water is a membrane bioreactor (MBR) [16,21]. The MBR system comprises two processes, a biological process and MF or UF. In the biological process, microorganisms are nourished by the organic matter present in the seawater, thus reducing organic carbon level and levels of other nutrients such as nitrogen, phosphorous, and iron, among others. Use of both MBR and RO methods in tandem for advanced wastewater or seawater treatment is currently not common. Recently, a dual MBR–RO process has been studied in municipal wastewater reuse to augment potentially high-quality water supplies [16,21,26–29]. Previous study that tested MBR system in the pretreatment of RO feed seawater showed that in the short-term experimental runs enabled higher flow rates relative to non-treated water. Its long-term effects included the removal of biological matter from the feed water and biofouling control [30].

In this study, biofouling potential of seawater pretreated with MBR system on RO membrane was examined. In addition, the microorganisms in the feed water and in the MBR system were characterized.

2. Materials and methods

2.1. Seawater source and experimental setup

Seawater samples were collected from the entrance ports of the Ashkelon desalination plant on the Israeli coast of the Mediterranean Sea and transported in water tanks to the laboratory. Experiments were carried out using a custom MBR system constructed in our laboratory (Fig. 1). The system is comprised of a 500 L feed tank connected to 30 L MBR tank supplied with a constant flow of air to optimize biomass growth. In the MBR tank, there is a PVDF hollow (outside-in-filtration) UF membrane (ZW-1, Zenon-GE, Canada) with a nominal pore size of 0.04 μm [31]. In addition to the MBR system, feed water was filtered using a UF membrane (ZW-1, Zenon-GE, Canada) with a nominal pore size of 0.04 μm.

The permeate flow rate from MBR and UF (standalone) systems was 42 mL/min, which corresponds to an average flow velocity of 21 cm/min in a polypropylene tube with a 16 mm diameter. Under this laminar flow condition, wall shear rate was ~1.75 s^{-1}. Biofilms were cultivated on RO membrane attached to the internal side of the permeate tubes walls in both systems under this conditions, promoting biofilm growth.

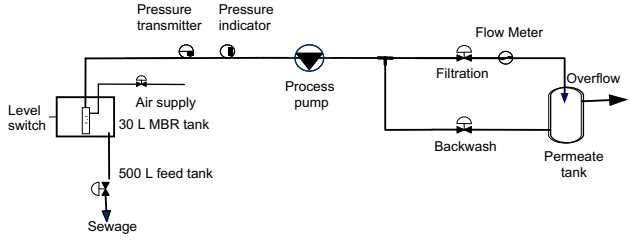

Fig. 1. Experimental setup including the MBR system.

2.2. Total organic carbon (TOC) measurement

Because seawater has high salt concentrations and low TOC, 150 mL samples were filtered through a 0.2 μm membrane using a vacuum pump and then evaporated to concentrate the volume of the samples 10-fold to 15 mL. The samples were dialyzed for 48 h against distilled water (molecular weight cut-off, 6,000–8,000) to remove excess salts. Distilled water (0.5 L) was changed at least five times at 9 h intervals. TOC was determined using the Apollo 9000 TOC combustion analyzer (Tekmar-Dohrmann, Cincinnati, USA).

2.3. Polysaccharide component analysis

The polysaccharide content in EPS of the biofilms was analyzed colorimetrically according to Dubois et al. [32] with sodium alginate as the standard (10–100 mg/L).

2.4. Nucleic acid extraction

Samples (feed water, MBR water, and permeate water) of 1.5 L were filtered through a 0.45 μm membrane. Total genomic DNA from membrane samples were extracted using a ZR Soil Microbe DNA isolation kit (Zymo Research Corp., Orange, CA), and DNA concentrations were determined by an ND-1000 UV–Vis Spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE) and stored at −20˚C.

2.5. PCR amplification of 16S rRNA gene fragments

16S rRNA gene fragments were amplified by PCR with a Biometra TGradient thermocycler (Biometra, Göttingen, Germany), using the common universal forward 8F (AGAGTTTGATYMTGGCTCAG) and reverse 907R (CCGTCAATTCMTTTGAGTTT) set of primers. Reaction mixtures included a 12.5 μL Reddy-Mix (PCR Master mix containing 1.5 mM $MgCl_2$ and 0.2 mM concentrations of each deoxynucleoside triphosphate) (ABgene, Surrey, UK), 1 pmol each of the forward and reverse primers, 1–2 μL of the sample preparation, plus water for a total volume of 25 μL. An initial denaturation-hot start of 4 min at 95˚C was followed by 30 cycles of the following incubation pattern: 94˚C for 30 s, 54˚C for 30 s, and 72˚C for 60 s. The procedure was completed with a final elongation step at 72˚C for 20 min.

2.6. Clone library construction and sequencing

PCR products were purified by electrophoresis through a 1% agarose gel (Sigma), stained with ethidium bromide and visualized on a UV transilluminator. The approximately heterologous 16S rRNA gene (0.9 kp) products were excised from the gel, and the DNA was purified from the gel slice using the Ultraclean™15 DNA Purification Kit (MO Bio Laboratories). The gel-purified PCR products were cloned into the pCRII-TOPO-TA cloning vector as specified by Invitrogen (Carlsbad, CA) and transformed into BioSuper $CaCl_2$-competent HD5α *E. coli* cells (Bio-Lab, Israel) according to the manufacturer's instructions. Clones were checked for inserts by PCR amplification using M13-F and M13-R primers (Invitrogen). Sequencing with 8F forward primer for 16S rRNA genes was performed by an ABI PRISM dye terminator cycle sequencing ready reaction kit with an AmpliTaq DNA polymerase FS and DNA sequencer ABI model 373A system (Perkin-Elmer).

2.7. Sequence analysis

All rRNA gene sequences of each group were first compared with those in the GenBank database with the basic local alignment search tool BLAST network service (http://www.ncbi.nlm.nih.gov/blast/blast.cgi). Two programs available at the Ribosomal Database Project-II Web site [33], Classifier (version 1.0; assign 16S rRNA gene sequences to a taxonomical hierarchy) and Library Compare (compares two sequence libraries using the RDP Classifier), were used to find diversity on different ranks of related sequences.

For diversity analyses, sequences were grouped into OTUs (operational taxonomic units) on the basis of rRNA gene sequence similarity. First, a distance matrix was generated using the MEGA package [34]. This matrix was then fed into the DOTUR computer program with all options set to default [35].

2.8. Laser scanning confocal microscopy (LSCM)

Microbial biofilms on the RO membrane were examined after 7 and 28 d filtration using UF and MBR as pre-treatment. The RO membrane was carefully removed and cut into pieces measuring 5 × 5 mm. Live and dead cells were then stained with Syto-9 and Propidium iodide, respectively, using the live/dead BACLight bacterial stain kit L-7012 (Molecular Probes, Inc., Eugene, OR). In addition, Concanavalin A (ConA) conjugated to Alexa fluor 633 was used to stain EPS. Microscopic observation and image acquisition were performed using an LSCM Zeiss-Meta 510, which was equipped with a Zeiss dry objective LCI Plan-NeoFluar (25× magnification and a numerical aperture of 0.8).

The LSCM was equipped with detectors and filter sets for monitoring Syto9 and propidium iodide-stained cells as well as Alexa fluor 633 dye (excitation wavelengths of 488 and 633 nm, respectively). LSCM images were acquired using Imaris software with surface rendering (Bitplane, Zürich, Switzerland). The specific biovolume values ($\mu m^3/\mu m^2$) of viable/dead cell and LPS in the biofouling layer were determined by COMSTAT image-processing software [36], written as a script in Matlab 5.1 (The MathWorks, Natick, MA, USA), and equipped with an image-processing toolbox.

3. Results and discussion

3.1. Microbial diversity

The efficient reduction of TOC and nutrient levels in the MBR system depends on the quantity and composition of the microbial community [37]. We characterized the microbial community (16S rRNA gene sequences) and examined species diversity in the feed seawater relative to those that developed in the MBR system (0.5 d retention time). Water in the MBR system was sampled after 90 d of operation. The microbial population that developed in the MBR displayed a shift in its diversity compared to that in the feed seawater (Fig. 2(A) and (B)). Sequences of 16S rRNA genes of photosynthetic cyanobacteria (17%) retrieved from the feed water (Fig. 2(B)) completely disappeared from the MBR system (Fig. 2(A)) primarily because of the dark conditions prevailing in the MBR system. This resulted in chemoorganoheterotrophic bacteria replacing the photolithoautotrophic cyanobacteria. *Proteobacteria* (composed mainly by *Alpha-*, *Beta-*, and *Gammaproteobacteria* classes) was the most abundant phylum obtained in both, seawater and in the MBR system, and increased from 50% (Fig. 2(B)) to 67% (Fig. 2(A)), respectively. The abundance of *Proteobacteria* in seawater was supported by previous studies [38]. The most abundant heterotrophic bacteria nourished from organic substances present in the MBR system were *Alphaproteobacteria* and *Gammaproteobacteria*, which originated from the feed water. Recently, it was shown that RO membrane biofilm was composed mostly of *Proteobacteria* (*Alpha-*, *Beta-*, and *Gammaproteobacteria* classes) and *Bacteroidetes* (*Sphingobacteria* class), while the relative abundance of the *Actinobacteria* and *Acidobacteria* was low [39] and was comparable with bacterial composition in our MBR system (Fig. 2(A)). The development of a unique bacterial community on the RO membrane might be attributed to membrane material properties, cell-surface interactions during bacterial adhesion and which were influenced by effects of microenvironmental conditions experienced at the membrane interface, such as feed water composition, hydrodynamics, permeate flux, and conditioning layers [40]. Previous studies demonstrate the importance of *Alpha-*, *Beta-*, and *Gammaproteobacteria* in initial colonization and biofilm development on the RO membranes [41–43].

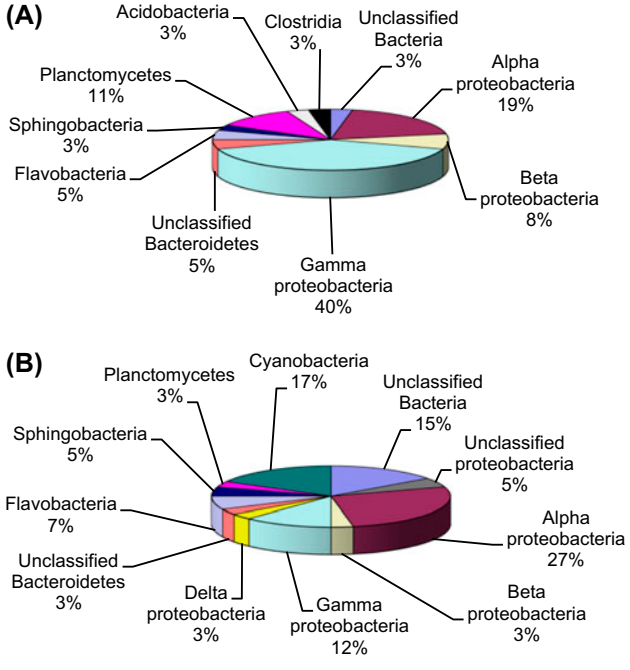

Fig. 2. Bacterial diversity in the MBR system (A) and in the feed water (B).

The overall diversity of cloned sequences was analyzed at two levels (90 and 97% similarity) using cluster analysis with DOTUR software [35]. The results are summarized in Table 1. From the feed water samples, a total of 27 OTUs were observed at the 97% similarity level (roughly representing species level) compared to about 22 OTUs obtained in the MBR system. This result was repeated for the Chao1 richness estimator and Shannon diversity index (Table 1). Similar results were also obtained for the 90% similarity level (roughly representing phylum-level diversity). A total of 20 OTUs were observed in feed water compared to an average of 16 OTUs in MBR samples. A similar trend was obtained for the Caho1 and Shannon indices (Table 1), indicating a decrease in diversity at the phylum-order level. Species diversity depends on the magnitude of the changes of the environmental gradients along which the community parameters are measured. In microhabitats with a modest supply of resources, groups of species can coexist, and each group will be at a relatively low dominance level, but species richness will be high. After biomass production reaches a

Table 1
Number of OTUs and richness estimation of 16S rRNA gene libraries from MBR and seawater. Shannon–Weaver diversity index and Chao1 richness estimator were computed using DOTUR[a]

		Richness estimators					
		No. of OTUs		Chao1 value		Shannon–Weaver index	
16S rRNA clone library	No. of clones sequenced	Cut-off 97%	Cut-off 90%	Cut-off 97%	Cut-off 90%	Cut-off 97%	Cut-off 90%
MBR-17.01	46	20	9	27.5	10.5	2.69	1.77
MBR-12.05	40	24	16	58	25	2.95	2.38
MBR-25.08	43	19	17	49.3	39	2.28	2.17
MBR-26.10	39	26	22	89.3	40.2	3.06	2.88
Feed-26.10	40	27	20	74.5	38.3	3.14	2.78

[a]Number of OTUs, Chao1 estimated richness and Shannon–Weaver diversity index are shown for both 3 and 10% differences in nucleic acid sequence alignments.

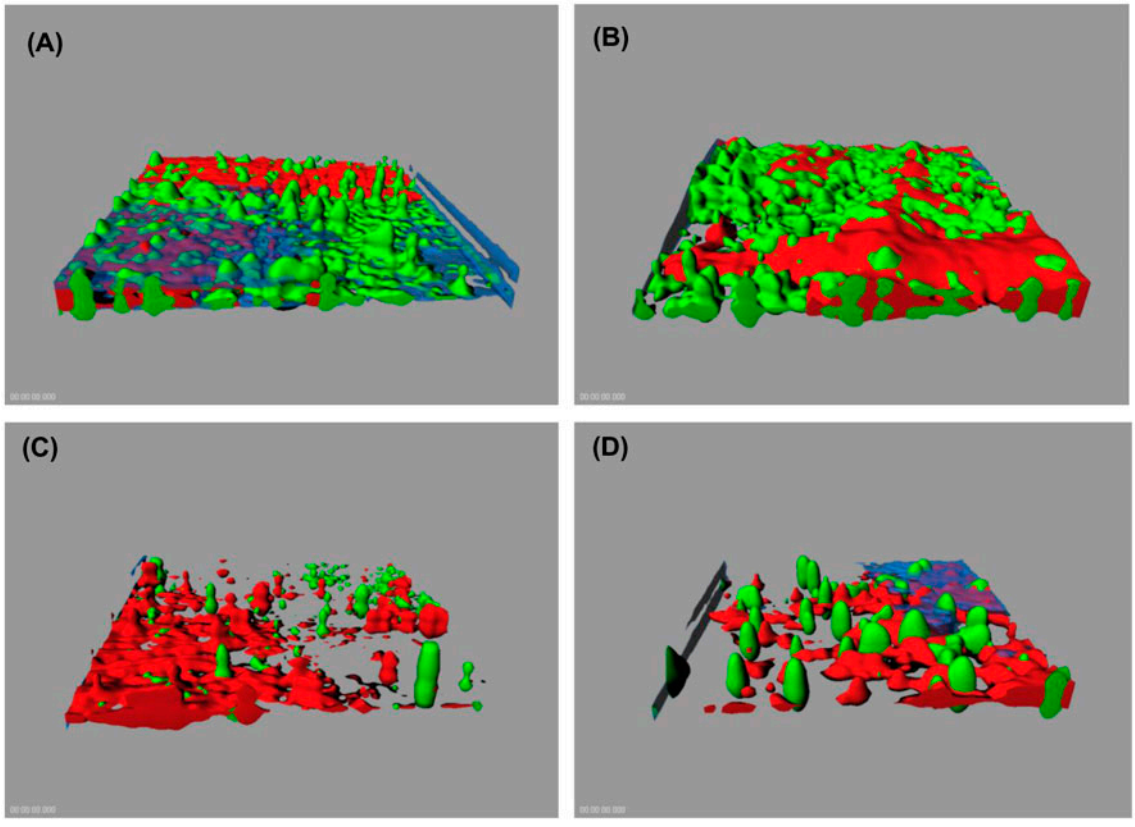

Fig. 3. Biofilm formation including live (green) and dead (red) cells and EPS (blue) on the RO membrane after two types of pretreatment: MBR and UF system, after 7 and 28 d. The images were analyzed with Imaris-Bitplane software (each image is a perspective of 1,272 × 1,272 μm). Images from UF system permeate after 7 d (A) and after 28 d (B), and from MBR system permeate after 7 d (C) and after 28 d (D).

critical range, competition becomes sufficient to eliminate less competitive species from the community. Our results displayed a decrease in bacterial diversity (at both 90 and 97% sequence similarity) in the MBR system in which the TOC concentration was lower than in feed water.

3.2. Biofouling potential

Seawater with relatively poor organic matter, microorganism contents and TOC concentrations in the range of 1.5–19 mg/L, still supports organic fouling and subsequent biofouling of RO membranes. Together with scale formation, this membrane fouling causes significant operational problems [44]. Therefore, the development of an effective pretreatment method to reduce feed seawater TOC and nutrient levels to prevent fouling is imperative. In the present study, 0.5 d retention time in the MBR system (Fig. 1) was examined in comparison to UF treatment alone. Results showed that the TOC level in the MBR permeate was decreased by approximately 43% compared to that in feed seawater after UF treatment (from 0.79 ± 0.19 to 0.34 ± 0.05 ppm, respectively). UF treatment alone did not decrease TOC concentrations in feed seawater. The 0.5 d retention time apparently enabled sufficiently long contact times between microorganisms and the organic matter in the feed water and thus the nutrients in the MBR system. Continuous use of the MBR pretreatment system was accompanied by greater DOC removal efficiency than the control membrane reactor, a finding that can be attributed to the higher chances of organic carbon coming into contact with the bacterial cells [30].

The fouling of RO membranes after pretreatment with the MBR system in comparison to UF treatment alone was evaluated at different time scales (7 and 28 d). Total amounts of live and dead microorganisms (Fig. 3(C) and (D)) and of polysaccharide formation (Fig. 4) on the RO membrane after pretreatment of the feed water by the MBR system equipped with UF were much lower than those from the UF system permeate alone (Fig. 3(A) and (B)). The fouling rate of RO membranes depends on amounts of organic substances in MBR pretreatment system. This rate quadrupled when fed with the high food to microorganism ratio (0.50 g/g d^{-1}) permeate when compared to those in the low ratio (0.17 g/g d^{-1}) permeate [16]. Apart from the MBR system, coagulation coupled with MF and UF membranes can be used as pretreatment for seawater RO desalination [22]. This can significantly reduce fouling of the RO membranes where soluble polysaccharides and TEP are major factors of the RO fouling.

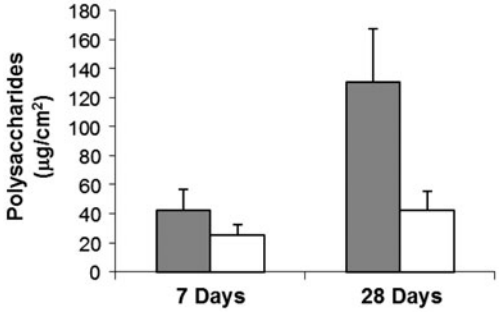

Fig. 4. Changes in quantities of polysaccharides on the RO membrane surface after pretreatment with either MBR system permeate (white bars) or UF system permeates only (gray bars).

4. Conclusions

Seawater typically contains enough organic matter and microorganisms to promote biofouling on RO membranes. Reduction of the fouling potential of seawater in RO desalination systems is critical to the viability of these systems in the extraction of fresh water. Pretreatment with the seawater MBR system for RO plant may improve effluent quality by removing organic compounds, micro-pollutants, and microorganisms in permeate water before it comes into contact with the RO membrane. The results indicate that pretreatment using a seawater MBR system improves water quality (e.g. removal of TOC and EPS) and subsequently reduces microbial colonizing and biofilm formation on the RO membrane, but before this system can be adopted as a pretreatment in seawater desalination, additional experimental, economic assessments, and further testing are needed.

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References

[1] E. Drioli, A. Criscuoli, E. Curcio, Integrated membrane operations for seawater desalination, Desalination 147 (2002) 77–81.
[2] A. Matin, Z. Khan, S.M.J. Zaidi, M.C. Boyce, Biofouling in reverse osmosis membranes for seawater desalination: Phenomena and prevention, Desalination 281 (2011) 1–16.
[3] C. Boo, M. Elimelech, S. Hong, Fouling control in a forward osmosis process integrating seawater desalination and wastewater reclamation, J. Membr. Sci. 444 (2013) 148–156.
[4] S. Phuntsho, F. Lotfi, S. Hong, D.L. Shaffer, M. Elimelech, H.K. Shon, Membrane scaling and flux decline during fertiliser-drawn forward osmosis desalination of brackish groundwater, Water Res. 57 (2014) 172–182.

[5] H.C. Flemming, Biofouling in water systems—Cases, causes and countermeasures, Appl. Microbiol. Biotechnol. 59 (2002) 629–640.
[6] H. Ivnitsky, I. Katz, D. Minz, G. Volvovic, E. Shimoni, E. Kesselman, R. Semiat, C.G. Dosoretz, Bacterial community composition and structure of biofilms developing on nanofiltration membranes applied to wastewater treatment, Water Res. 41 (2007) 3924–3935.
[7] W.S. Ang, S. Lee, M. Elimelech, Chemical and physical aspects of cleaning of organic-fouled reverse osmosis membranes, J. Membr. Sci. 272 (2006) 198–210.
[8] M. Khan, S. Danielsen, K. Johansen, L. Lorenz, S. Nelson, A. Camper, Enzymatic cleaning of biofouled thin-film composite reverse osmosis (RO) membrane operated in a biofilm membrane reactor, Biofouling 30 (2014) 153–167.
[9] H.F. Ridgway, H.C. Flemming, Membrane biofouling in water treatment membrane processes, in: J. Mallevialle, P.E. Odendaal, M.R. Wiesner (Eds.), Water treatment membrane processes, Chapter 6, McGraw-Hill, New York, NY, 1996, pp. 6.1–6.62.
[10] J.S. Vrouwenvelder, D. van der Kooij, Diagnosis, prediction and prevention of biofouling of NF and RO membranes, Desalination 139 (2001) 65–71.
[11] M. Ben-Sasson, X. Lu, E. Bar-Zeev, K.R. Zodrow, S. Nejati, G. Qi, E.P. Giannelis, M. Elimelech, *In situ* formation of silver nanoparticles on thin-film composite reverse osmosis membranes for biofouling mitigation, Water Res. 62 (2014) 260–270.
[12] T. Berman, Don't fall foul of biofilm through high TEP levels, Filtr. Sep. 42(4) (2005) 30–32.
[13] E. Bar-Zeev, I. Berman-Frank, B. Liberman, E. Rahav, U. Passow, T. Berman, Transparent exopolymer particles: Potential agents for organic fouling and biofilm formation in desalination and water treatment plants, Desalin. Water Treat. 3 (2009) 136–142.
[14] 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.
[15] L.O. Villacorte, M.D. Kennedy, G.L. Amy, J.C. Schippers, The fate of transparent exopolymer particles (TEP) in integrated membrane systems: Removal through pre-treatment processes and deposition on reverse osmosis membranes, Water Res. 43 (2009) 5039–5052.
[16] B. Wu, T. Kitade, T.H. Chong, T. Uemura, A.G. Fane, Impact of membrane bioreactor operating conditions on fouling behavior of reverse osmosis membranes in MBR–RO processes, Desalination 311 (2013) 37–45.
[17] R.J. Barnes, R.R. Bandi, F. Chua, J.H. Low, T. Aung, N. Barraud, A.G. Fane, S. Kjelleberg, S.A. Rice, The roles of *Pseudomonas aeruginosa* extracellular polysaccharides in biofouling of reverse osmosis membranes and nitric oxide induced dispersal, J. Membr. Sci. 466 (2014) 161–172.
[18] S.A.A. Tabatabai, J.C. Schippers, M.D. Kennedy, Effect of coagulation on fouling potential and removal of algal organic matter in ultrafiltration pretreatment to seawater reverse osmosis, Water Res. 59 (2014) 283–294.
[19] M. Al-Ahmad, F.A. Abdul Aleem, A. Mutiri, A. Ubaisy, Biofuoling in RO membrane systems Part 1: Fundamentals and control, Desalination 132 (2000) 173–179.
[20] D. Rittschof, Fouling and natural products as antifouling. in: M. Fingerman, R. Nagabhushanam, M.-F. Thompson (Eds.), Recent Advances in Marine Biotechnology. vol. 3, Science Publishers, Inc., USA, 1999, pp. 244–249.
[21] E.L. Farias, K.J. Howe, B.M. Thomson, Effect of membrane bioreactor solids retention time on reverse osmosis membrane fouling for wastewater reuse, Water Res. 49 (2014) 53–61.
[22] S.K. Al-Mashharawi, N. Ghaffour, M. Al-Ghamdi, G.L. Amy, Evaluating the efficiency of different microfiltration and ultrafiltration membranes used as pretreatment for Red Sea water reverse osmosis desalination, Desalin. Water Treat. 51 (2013) 617–626.
[23] A. Resosudarmo, Y. Ye, P. Le-Clech, V. Chen, Analysis of UF membrane fouling mechanisms caused by organic interactions in seawater, Water Res. 47 (2013) 911–921.
[24] A.M. Bernardes, General aspects of membrane separation processes. in: A.M. Bernardes, M.A.S. Rodrigues, J.Z. Ferreira (Eds.), Electrodialysis and Water Reuse, Springer, Berlin Heidelberg, 2014, pp. 3–9.
[25] S. Jeong, G. Naidu, S. Vigneswaran, Submerged membrane adsorption bioreactor as a pretreatment in seawater desalination for biofouling control, Bioresour. Technol. 141 (2013) 57–64.
[26] A.M. Comerton, R.C. Andrews, D.M. Bagley, Evaluation of an MBR–RO system to produce high quality reuse water: Microbial control, DBP formation and nitrate, Water Res. 39 (2005) 3982–3990.
[27] J.-J. Qin, K.A. Kekre, G. Tao, M.H. Oo, M.N. Wai, T.C. Lee, B. Viswanath, H. Seah, New option of MBR–RO process for production of NEWater from domestic sewage, J. Membr. Sci. 272 (2006) 70–77.
[28] E. Dialynas, E. Diamadopoulos, Integration of a membrane bioreactor coupled with reverse osmosis for advanced treatment of municipal wastewater, Desalination 238 (2009) 302–311.
[29] D. Dolar, M. Gros, S. Rodriguez-Mozaz, J. Moreno, J. Comas, I. Rodriguez-Roda, D. Barceló, Removal of emerging contaminants from municipal wastewater with an integrated membrane system, MBR–RO, J. Hazard. Mater. 239–240 (2012) 64–69.
[30] C. Visvanathan, N. Boonthanon, A. Sathasivan, V. Jegatheesan, Pretreatment of seawater for biodegradable organic content removal using membrane bioreactor, Desalination 153 (2002) 133–140.
[31] J. Tsilogeorgis, A. Zouboulis, P. Samaras, D. Zamboulis, Application of a membrane sequencing batch reactor for landfill leachate treatment, Desalination 221 (2008) 483–493.
[32] M. DuBois, K.A. Gilles, J.K. Hamilton, P.A. Rebers, F. Smith, Colorimetric method for determination of sugars and related substances, Anal. Chem. 28 (1956) 350–356.
[33] J.R. Cole, Q. Wang, E. Cardenas, J. Fish, B. Chai, R.J. Farris, A.S. Kulam-Syed-Mohideen, D.M. McGarrell, T. Marsh, G.M. Garrity, J.M. Tiedje, The Ribosomal Database Project: Improved alignments and new tools for rRNA analysis, Nucleic Acids Res. 37 (2009) D141–D145.
[34] S. Kumar, K. Tomura, M. Nei, MEGA3: Integrated software for molecular evolutionary genetics analysis

and sequence alignment, Brief Bioinf. 5 (2004) 150–163.
[35] P.D. Schloss, J. Handelsman, Introducing DOTUR, a computer program for defining operational taxonomic units and estimating species richness, Appl. Environ. Microbiol. 71 (2005) 1501–1506.
[36] A. Heydorn, A.T. Nielsen, M. Hentzer, C. Sternberg, M. Givskov, S. Molin, Quantification of biofilm structures by the novel computer program COMSTAT, Microbiol. 146 (2000) 2395–2407.
[37] M.O. Saeed, A.T. Jamaluddin, I.A. Tisan, D.A. Lawrence, M.M. Al-Amri, K. Chida, Biofouling in a seawater reverse osmosis plant on the Red Sea coast, Saudi Arabia, Desalination 128 (2000) 177–190.
[38] S.G. Acinas, J. Antón, F. Rodríguez-Valera, Diversity of free-living and attached bacteria in off-shore Western Mediterranean waters as depicted by 16S rDNA analysis, Appl. Environ. Microbiol. 65 (1999) 514–522.
[39] A. Al Ashhab, M. Herzberg, O. Gillor, Biofouling of reverse-osmosis membranes during tertiary wastewater desalination: Microbial community composition, Water Res. 50 (2014) 341–349.
[40] O. Habimana, A.J.C. Semião, E. Casey, The role of cell-surface interactions in bacterial initial adhesion and consequent biofilm formation on nanofiltration/reverse osmosis membranes, J. Membr. Sci. 454 (2014) 82–96.
[41] P. Hörsch, A. Gorenflo, C. Fuder, A. Deleage, F. Frimmel, Biofouling of ultra- and nanofiltration membranes fordrinking water treatment characterized by fluorescence *in situ* hybridization (FISH), Desalination 172 (2005) 41–52.
[42] C.M. Pang, W.-T. Liu, Community structure analysis of reverse osmosis membrane biofilms and the significance of rhizobiales bacteria in biofouling, Environ. Sci. Technol. 41 (2007) 4728–4734.
[43] L.A. Bereschenko, A.J.M. Stams, G.J.W. Euverink, M.C.M. van Loosdrecht, Biofilm formation on reverse osmosis membranes is initiated and dominated by *Sphingomonas* spp., Appl. Environ. Microbiol. 76 (2010) 2623–2632.
[44] L.I. Frebrero, A study of seawater quality in laem chabang and the evaluation of impacts of deep sea port on marine environment, AIT thesis No. EV-85-6, Thailand (1984).