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Characterization of biofilm bacteria isolated from two distinct seawater reverse osmosis systems in Saudi Arabia

Mohamed O. Saeed[a,*], W.L. Teng[b], Ibrahim A. Al-Tisan[a], Mohammed A. Namazi[a]

[a]*Saline Water Desalination Research Institute (SWDRI), Saline Water Conversion Corporation (SWCC), P.O. Box 8328, Al-Jubail 31951, Kingdom of Saudi Arabia*
Email: rdc@swcc.gov.sa
[b]*Technolgy and Water Quality Office, PUB, 82 Toh Guan Road East, WaterHub 608576 Singapore, Singapore*

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ABSTRACT

A study on type and tolerance of biofilm bacteria from two seawater reverse osmosis (SWRO) plants sourced from same lagoon but with different types of membrane and pretreatment regimens was carried out. One system uses hollow fine fiber seawater RO membranes of cellulose triacetate with membranes arranged in one element per vessel and the other system uses spiral wound seawater RO membranes of polyamide (PA/thin film composite) with membranes arranged in two elements per vessel. The first system (system-1) uses chlorine with acidification, coagulation, and media filtration followed by micron cartridge filtration. The second system (system-2) uses only acidification and micron cartridge filtration without chlorination. Three different bacterial isolates were found predominantly in membranes belonging to system-1 while nine bacteria species were isolated from membranes in system-2, two species from the feed side and seven species from the brine side elements, respectively. All bacterial isolates were identified by sequencing the 16S rRNA genes. The matrix-assisted laser desorption/ionization-time of flight mass spectrometry, despite good spectra, could not provide any reliable identification, indicating the limitation of the existing database for the identification of environmental isolates. The identified species were distinct to each SWRO plant; Alphaproteobacteria was the only common class in both systems, while Bacteroidetes seemed unique to the brine side element of system-2. To devise practical control measures for biofouling, tolerance tests were carried out on the biofilm isolates. All of the isolates were sensitive to a solution of 2% citric acid at pH 4.0 and a few isolates were also susceptible to osmotic shock in distilled water for varying time ranging from 3 to $\leqslant$ 24 h. On the contrary, exposure to chlorine and high salt concentration did not seem to have adverse impact on most of the isolates, making osmotic shock in hypotonic medium, a plausible alternative to control biofilm bacteria growth on SWRO membranes.

Keywords: SWRO membranes; Pretreatment; Biofilm Bacteria; Control Measures

1. Introduction

The two major modes of desalination are thermal-based processes such as multi-stage flash (MSF) distillation and membrane-based processes using reverse osmosis (RO) [1]. In the past decade, RO desalination technology has gone through a remarkable transformation and has gained widespread acceptance which is evident from the increased share of RO desalination

*Corresponding author.

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facilities worldwide. In 2008, RO systems alone provided 61% of the world installed desalination capacity [2]. Saudi Arabia is second only to the USA in installed membrane capacity of ~4.7 millions m^3/d. This significant growth in RO desalination technology is due to advancement in membrane technologies, pretreatment and energy saving devices, favorable energy to water ratio compared to MSF, and lower water production cost due to coupling of MSF with RO such as di– and tri-hybrid coupling [3,4].

Despite the advancement in membrane technology and plant operational conditions, fouling of RO membranes remains a key challenge to the performance of seawater reverse osmosis (SWRO) plants, in particular biofouling. Biofouling itself was reported to account for more than 10% of a SWRO plant's operational cost [5]. The biofouling problem in membrane desalination plants located along the Red Sea and the Gulf coasts is further augmented by the hot climate which is conducive for microbial growth. As a result, an alternative beach well intake system had been sought for one such RO plant in order to overcome chronic membrane fouling [6]. The minimization of membrane biofouling is therefore of paramount importance to ensure sustainability of this water supply.

The source water for seawater desalination contains diverse microbial species that could be deposited onto the SWRO membrane surface since pretreatment to prepare the feed water for membranes does not remove all species. Subsequent growth of these micro-organisms results in accumulation of biofilms on the membrane surface including the plastic spacer material which are difficult to eliminate. There is therefore scientific and technical merit to determine the microbial community in these fouling causing biofilms and to explore conditions that could suppress the development of these species on the RO membrane surface.

Microbial identification using traditional methods that are based on phenotypic characteristics and biochemical properties are generally laborious, time-consuming, and require pure cultures. The latter is most challenging since a vast majority of bacteria are non-culturable and can only be characterized by molecular techniques. The development of Matrix-assisted laser desorption/ionization–time of flight mass spectrometry (MALDI-TOF) mass spectrometry methods for the characterization of large molecules leads to chemotaxonomic classification of bacteria with surprising ease. As such, MALDI-TOF mass spectrometry in addition to 16S rRNA gene sequencing was applied for the identification of biofilm bacteria isolated in this study. Besides identification of the microbial community, it is perhaps more important from the perspectives of RO operators to investigate means to control the development of these biofilm isolates in order to alleviate membrane biofouling problems in the long term. This is particularly true because biofouling is practically unavoidable. As such, the tolerance of these biofilm bacteria to varying chemicals and growth conditions was also examined in this study as stepping stones to devise more effective measures to mitigate membrane biofouling.

2. Materials and methods

2.1. Experimental SWRO plants

Both SWRO plants are sourced from two different intake pits out of a common Gulf coastal lagoon. The first plant (system-1) is a commercial SWRO plant with a design production capacity of 24mgd. The plant utilizes chlorine-tolerant membrane (Toyobo HJ 9155Pi) of cellulose triacetate with hollow fine fiber configuration and size $9.5 \times 65''$, with single element per vessel. Source water is chlorinated at the intake followed by acid and ferric chloride dosing before filtration through a dual media filter and a micron cartridge filter. Membranes receive intermittent chlorination once every 8 h. Membrane recovery is 30%. The second plant (system-2) is a pilot scale unit with a capacity of ~4mgd. The plant uses spiral wound (SW) thin film composite SWRO membrane (Hydranautics SWC1-4040) of polyamide and size $4'' \times 40''$, with two elements per vessel. Raw seawater only passes through cartridge filter after acid dosing without any chlorination treatment. Membrane recovery is also 30%.

2.2. Isolation of membrane biofilm bacteria

The elements were disassembled from their operation racks and membrane autopsies were carried out using sterile tools and aseptic techniques. Sample of fibers from the hollow fine fiber (HFF) membrane of system-1 was collectively retrieved as mixed fibers from the outer, middle, and innermost membrane layers. Excised pieces of the SW membranes of system-2 were obtained from both the feed and brine side membrane elements. These membrane samples were then gently rinsed to remove loosely attached bacteria before performing isolation of biofilm bacteria. The suspensions of biofilm bacteria derived from the membrane samples were then plated onto Marine agar 2216 (Biomed Diagnostics) and colonies presented with distinct morphologies were plated onto new Marine agar for isolation of pure cultures. Pure bacterial isolates were harvested in two aliquots sus-

Table 1
List of test conditions for bacterial tolerance tests

Test factors	Test media	Exposure time (h) and incubation
Osmotic pressure	5 ml volumes of 10, 15, 20, 25 and 30% (w/v) of concentrated salt solutions[a]	12, 24 and 48 h incubation at 30°C for 7 days
	5 ml volumes of distilled water	3, 5, 8 and 24-h incubation at 30°C for 7 days
Temperature	Marine agar cultures	4, 10, 12,15,17, 30, 37, 40 and 42°C for 7 days
pH	5 ml volumes of Marine broth adjusted to a series of pH ranging from 4 to 11	1, 2, 3, 4, 6, 8, 12, 24, 48, 72, and 96-h Incubation at 30°C for 7 days
Chlorine	5 ml volumes of 0.5 and 1.0 mg/L of calcium hypochlorite in artificial seawater	1, 2, 3, 5, 8, 12, 24 and 48-h incubation at 30°C for 7 days
Citric acid	5 ml volumes of 2% citric acid adjusted[b] to pH 4	1, 2 and 3-h incubation at 30°C for 7 days

[a]Concentrated salt solutions were prepared by adding specified percentages of table salts into filtered seawater.
[b]Adjusted by dropwise addition of 35% ammonium hydroxide.

pended in either phosphate buffered saline or distilled water. Ethanol was added to aliquots in distilled water to a concentration of 70% for downstream MALDI-TOF analysis. All of the pure cultures were kept frozen at −40°C until use.

2.3. Identification of biofilm bacteria

The isolated biofilm bacteria were identified by sequencing the 16S rRNA genes and analyzing the cell extract using MALDI-TOF mass spectrometry. To amplify the 16S rRNA genes, crude genomic DNA was first extracted from pure biofilm bacteria isolates by suspending the cells from an agar culture into 100 µl of DNAse-free water, boiling for 10 min, and centrifuging at 11,000 g for 10 min at room temperature. The DNA in the supernatant was then used directly as template in PCR amplification. PCR amplification was conducted using *Taq* DNA polymerase (Promega) and a 0.5 µM concentration of each primer. The almost full-length 16S rRNA genes were amplified using the universal bacterial primers E8f (5′-AGA GTT TGA TCA TGG CTC AG-3′) and U1510r (5′-CGG TTA CCT TGT TAC GAC TT-3′) [7]. DNA sequencing was performed on an ABI's 3730XL DNA Analyzers (AITbiotech) and the elucidated DNA sequences were subjected to BLAST searches in the GenBank database to identify best-matched species. In MALDI-TOF analysis, bacterial isolates suspended in 70% ethanol solutions were extracted using formic acid–ethanol extraction (Bruker Dalton Lab GmbH), and MALDI Biotyper RTC Instrument (Microflex) set at linear positive mode was used to generate the individual spectrometry spectrum. As a control, two isolates derived from a freshwater drinking well were included in the analysis to reveal any shortcoming of the MALDI database in identifying marine bacteria.

2.4. Tolerance tests

The tolerance of the isolated biofilm bacteria towards varying chemical and growth conditions was examined in this study according to the test conditions summarized in Table 1. The viability of the biofilm isolates after exposure to the stated conditions with the exception of temperature was examined by plating five replicates of 10 µl cultures onto fresh Marine agar incubated at 30°C up to 7 days.

3. Results

Membrane autopsies were performed on RO elements derived from two SWRO plants using distinct membrane types and pretreatment regimens. System-1 utilized cellulose tri-acetate (CTA) type of hollow fine fiber membranes and, in contrast, system-2 utilized spiral wound polyamide RO membranes. System-2 did not apply any chlorination neither media filtration when compared with System-1. A total of 12 bacterial species were isolated and cultured from three SWRO elements belonging to these two SWRO systems in this study (Table 2). All bacterial isolates were identified by sequencing the 16S rRNA genes, with at least 98% identity at DNA sequence level. On the contrary, MALDI-TOF mass spectrometry could not provide any reliable identification for all the biofilm isolates with the exception of two known isolates previously identified as *Pseudomonas aeruginosa* and *Pseudomonas alcaligenes*, indicating the limitation of the existing database for the identification of environmental isolates.

Three culturable bacteria species belonging to alphaproteobacteria phylum were identified in system-1. *Roseovarius* sp. was the most dominant isolate amongst the three species. The remaining nine species

Table 2
Identification and tolerance tests for bacteria associated with biofilm formation in membranes of two seawater reverse osmosis systems sourced from Gulf coast water of Saudi Arabia

Isolates	Osmotic shock survival (h)		Temp.[b] range (°C)	Lethal pH[c] value and exposure time (h)	Survival in chlorine[d] 1 mg/L (48 h)	Survival in 2% citric acid (1 h)
	Salt (%)	Distilled[a] water				
System-1[e]:						
Roseovious mucosus	24 h/30	<5	4[w]–40	4.0(1 h)/10(8 h)	– (+ at 0.5 mg/L, 1 h)	–
Ruegeria sp.	24 h/30	≥24	15[w]–40	4.0(3 h)/10(3 h)	+	–
Erythrobacter sp.	24 h/30	<3	15[w]–42	4.0(2)/10(3 h)	+	–
System-2[f]:						
A. Feed side						
Curtobacterium sp.	48 h/30	≥48	10[w]–42	11/(6), 10/(12)	+	–
Sphingomonas sp.	48 h/30	≥48	10[w]–42	4.0(3 h)/11(6 h)	+	–
B. Brine side						
Winogradskyella	48 h/30	≥48	4–42	4.5(3 h)/10(3 h)	+	–
Bacillus sp.-1[g]	48 h/20	<8	4[w]–42	4.0(6 h)/11(6 h)	+	–
Gaetbulibacter sp.[h]	24 h/30	<24	4[w]–40	4.0(6 h)/11(3 h)	+	–
Flexibacter sp.	48 h/30	≥48	17–42	4.0(3 h)/10(6 h)	–	–
Kangiella sp.[i]	24 h/30	<24	15[w]–42[w]	4.0(2 h)/11(12 h)	+ (3 h)	–
Bacillus sp.-2	48 h/30	≥48	15[w]–42[w]	4.0(12 h)/11(48 h)	+	–
Hyphomonas sp.	48 h/30	≥48	15[w]–42[w]	4.0(8 h)/10(12 h)	+	–

[a]conductivity 19 μS/cm.
[b]w = weak growth.
[c]First number is the lowest pH value followed by lethal exposure time in parenthesis, and second number after the slash is highest lethal pH value followed by lethal exposure time.
[d]All isolates except *Roseovious mucosus* survived 48-h exposure to 0.5 mg/L.
[e]System-1: HFF CTA membrane with intermittent chlorination and one membrane element per pressure vessel.
[f]System-2: SW PA Thin Film Composite membrane without chlorination and two membrane elements per pressure vessel.
[g]Did not survive 48 h—exposure to 25% salt concentration.
[h]Did not survive 48 h—exposure to 20% salt concentration.
[i]Did not survive 48 h—exposure to 20% salt concentration.

were isolated from system-2. In system-2, two SWRO elements were arranged within a single vessel. Seven culturable bacterial species were isolated from the brine side element, while only two species were identified in the feed side element. The identified species represented various phyla and were distinct to both systems as well as to both elements of system-2. Bacteroidetes and Firmicutes were the most common phyla represented amongst the bacterial species isolated from the brine side element.

As an initial study to device control measures for membrane biofouling, the degree of tolerance towards different chemical and physical conditions was examined for all the biofilm isolates (Table 2). Majority of the species grew best under mesophilic temperatures ranging between 22 and 32°C and the favorable pH range was 6–8 (data not shown). In terms of osmotic pressure, all isolates could survive at least 24 h of exposure to filtered seawater supplemented with 20% table salt. The species that could survive exposure to 30% concentrated salt solution could also tolerate the hypotonic condition in distilled water for < 24 h. The remaining species tolerated less well with the hypotonic condition of distilled water. All of the isolates with the exception of *Roseovarius* sp., *Flexibacter* sp., and *Kangiella* sp. easily tolerated 1 mg/L of chlorine for 48 h, but none of the biofilm isolates were able to survive after 1 h exposure to the 2% citric acid solution. The latter is used as the cleaning agent to remove $CaCO_3$, metal oxides, and inorganic colloids according to Toyobo's membrane cleaning procedure.

4. Discussion

In this study, we reported the isolation and cultivation of 12 bacterial species derived from three RO elements belonging to two SWRO plants that used

distinct membrane types and pretreatment regimens. Most species were isolated from the SWRO element located in the brine side of the membrane vessel in system-2. This SWRO system did not receive any conventional pretreatments such as chlorination, coagulation, and media filtration compared to system-1. The media filtration applied in system-1 served as a biofilm incubator that helped to remove nutrients from the RO feed water essentially forming an effective barrier to limit the number and growth of microorganisms on the RO membrane surface located downstream. This lack of media filtration in system-2 possibly resulted in more species cultured from its RO elements, especially for the brine side element since the feed water reaching the second element is usually more concentrated in terms of bacteria, suspended matter, or any other component.

Culturable species identified in RO elements reportedly comprised a limited number of different bacterial genera [8,9]. The few genera reported in this study belonged mostly to Alphaproteobacteria and Bacteroidetes phyla and the identified species were unique to each autopsied RO element. Majority of the isolates appeared as mucoid or slimy on solid media and isolates such as *Erythrobacter* sp., *Kangiella* sp., *Bacillus* sp., and *Hyphomonas* sp. attached very strongly to the agar surface. The latter is also known to form adherent prosthecate cell in addition to planktonic swarming cells. The extracellular polymeric substances synthesized by this group of bacteria are either only expressed as hold fast at one pole of the cell or constitutively expressed and surrounds the whole cell [10]. The propensity to produce slimes by these isolates could contribute significantly to the overall formation and accumulation of biofilms on RO membrane surface.

Besides slime production, the operating conditions of the SWRO plants would allow the identified isolates to survive and possibly grow on membrane surface. The optimal temperature (22–32˚C) and pH (6–8) for growing the isolates in the laboratory were well within the prevailing temperatures of the intake bay which ranged between 15˚C in winter (January) and 36˚C in the summer (August) and pH of both the source water (pH ~ 8.0) and feed water to membranes (pH ~ 6.0). In a recent study, the concentrated salt was explored as an environmentally friendly approach for membrane cleaning [11]. However, the results in this study showed that marine biofilm bacteria could be extremely halophytic which would render salt cleaning ineffective. Interestingly, distilled water was more effective than salt in controlling growth of some isolates and therefore, could provide a better alternative for membrane cleaning.

Lastly, bacteria from both systems tolerated residual chlorine of 1 mg/L which is the maximum limit specified for feed water in system-1. As such, chlorination in system-1 may have no added advantage compared to system-2. In reality, this maximum residual chlorine is rarely attained especially in shallow and open intake lagoons. Nevertheless, many SWRO plants still benefited from this practice of chlorination. The effect of chlorine in controlling biofouling is likely not through the killing of biofilm bacteria, but in disrupting the biofilm matrix which is the real plugging agent of the desalination membranes.

5. Conclusions

(1) The effect of different membranes and pretreatment regimens on type of biofilm-forming bacteria was investigated in two SWRO systems sourced from Gulf coastal water of Saudi Arabia.
(2) Twelve culturable biofilm bacteria were recovered, sequenced, and identified. All isolates were unique to each SWRO system and produced notable amount of slimes during growth on solid media.
(3) The pure cultures of the biofilm isolates tolerated a wide range of temperature, pH, and salt concentrations, but a few isolates did not survive exposure to distilled water for as short as 3 h. However, all isolates were killed by a 2% citric acid solution at pH 4.

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