Does Chlorination of Seawater Reverse Osmosis Membranes Control Biofouling?

Muhammad Tariq Khan, Pei-Ying Hong, Nabil Nada, Jean Philippe Croue

PII: S0043-1354(15)00219-5
DOI: 10.1016/j.watres.2015.03.029
Reference: WR 11225

To appear in: Water Research

Received Date: 17 December 2014
Revised Date: 26 March 2015
Accepted Date: 27 March 2015

Please cite this article as: Khan, M.T., Hong, P.-Y., Nada, N., Croue, J.P., Does Chlorination of Seawater Reverse Osmosis Membranes Control Biofouling?, Water Research (2015), doi: 10.1016/j.watres.2015.03.029.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.
Does Chlorination of Seawater Reverse Osmosis Membranes Control Biofouling?

Muhammad Tariq Khan\textsuperscript{a}, Pei-Ying Hong\textsuperscript{a}, Nabil Nada\textsuperscript{b}, and Jean Philippe Croue\textsuperscript{a,c,*}.

\textsuperscript{a} Water Desalination and Reuse Research Center, King Abdullah University of Science and Technology, Thuwal 23955-6900, Kingdom of Saudi Arabia.

\textsuperscript{b} NOMAC-Saudi Arabia.

\textsuperscript{c} Curtin Water Quality Research Centre, Curtin University Perth WA Australia.

*Corresponding Author. Tel.: +61 8 9266 9793; E-mail address: jean-philippe.croue@curtin.edu.au

Abstract

Biofouling is the major problem of reverse osmosis (RO) membranes used for desalting seawater (SW). The use of chlorine is a conventional and common practice to control/prevent biofouling. Unlike polyamide RO membranes, cellulose triacetate (CTA) RO membranes display a high chlorine tolerance. Due to this characteristic, CTA membranes are used in most of the RO plants located in the Middle East region where the elevated seawater temperature and water quality promote the risk of membrane biofouling. However, there is no detailed study on the investigation/characterization of CTA-RO membrane fouling. In this investigation, the fouling profile of a full–scale SWRO desalination plant operating with not only continuous chlorination of raw seawater but also intermittent chlorination of CTA-RO membranes was studied. Detailed water quality and membrane fouling analyses were conducted. Profiles of microbiological, inorganic, and organic constituents of analysed fouling layers were extensively discussed. Our results clearly identified biofilm development on these membranes. The incapability of chlorination on preventing biofilm formation on SWRO membranes could be assigned to its failure in effectively reaching throughout the different regions of the permeators. This failure could have occurred due to three main factors: plugging of membrane fibers, chlorine consumption by organics accumulated on the front side fibers, or chlorine adaptation of certain bacterial populations.

Keywords: Biofouling; Chlorination; Cellulose Triacetate Membranes; Seawater Reverse Osmosis Desalination

1. Introduction

Reverse osmosis (RO) is currently the most widely used desalination technology. Reliance on RO is increasing worldwide due to continuous technological improvements, resulting in substantial cost reductions (Lee et al., 2011). Polymeric RO membranes have
always dominated the desalination market due to promising contaminant rejection capabilities (Lee et al., 2011). Presently, two main types of polymeric RO membranes exist in the market: CTA membranes and PA thin film composite (PA–TFC) membranes. CTA membranes are available as fine hollow fibers wound into an element of a layered and mutually crossed configuration, whilst PA–TFC membranes have a flat sheet physical structure and their elements have spiral wound configuration.

Biofouling of polymeric membranes impacts the operation and economic aspects of seawater reverse osmosis (SWRO) desalination processes (Khedr, 2002). Chlorination of feed water is a commonly used strategy to inhibit biofilm formation in RO systems (Nguyen et al., 2012). However, PA membranes, which are the most commonly used type of RO membranes, cannot withstand chlorine exposure even at low ppm concentrations. On the other hand, CTA membranes exhibit some resistance against chlorine at concentrations of up to 5 mg Cl\textsubscript{2}/l and 0.2 mg Cl\textsubscript{2}/l for short and long exposure times, respectively (Kumano, 2012; Kumano and Fujiwara, 2008). This chlorine tolerance feature of CTA membranes allows the use of chlorine inside RO membrane vessels. Previous studies have claimed that CTA–RO membrane exposure to chlorine would prevent biofouling (Johnson and Busch, 2010; Kumano and Fujiwara, 2008).

Many studies conducted on fouling analysis of PA–TFC membranes can be found in the literature. However, only few investigations (Butt et al., 1995, 1997a, 1997b, 1997c; Farooque et al., 2005; Fujiwara and Matsuyama, 2008; Saeed et al., 2000) have focused on fouling problems of CTA–RO membranes. Additionally, the membrane performance data has been used to characterize the fouling intensity (Butt et al., 1997c; Fujiwara and Matsuyama, 2008; Saeed et al., 2004) without supporting analysis of fouling layer and
also without investigating the efficacy of chlorination as anti-biofouling strategy. In some cases, if the analysis of fouling layer was performed, the approach remained limited to microscopic methods (Butt et al., 1997b; Farooque et al., 2005). Some of these studies only used simple coulometric, chromatographic, and/or spectroscopic analytical techniques (Butt et al., 1995, 1997a, 1997b). Organics, which have been reported as the major fraction of the fouling material isolated from these membranes (Butt et al., 1997b), have never been well characterized. Similarly, an analysis of microbial communities found in water samples and in the membrane fouling layer of these plants has not been conducted.

In this project, the efficacy of chlorination as anti–biofouling strategy was studied by investigating the fouling profile and scenario developed on a full–scale SWRO desalination plant operating with not only continuous chlorination of raw seawater but also intermittent chlorination of CTA-RO membranes. Detailed water quality and membrane fouling analyses were performed by using advanced analytical techniques including Fourier transform infrared (FTIR) spectrometry, pyrolysis gas chromatography coupled with mass spectrometry (Pyrolysis/GC–MS), $^{13}$C-nuclear magnetic resonance (NMR) spectrometry, inductively coupled plasma optical emission spectrometry (ICP–OES), elemental analysis, adenosine–5’–triphosphate (ATP) content analysis, and $16S$ rRNA gene-based sequencing of microbial community.

2. Materials and Methods
2.1. Plant Design and Setup

The studied SWRO desalination plant, located on the Red Sea coast in Saudi Arabia, has a capacity of 216,000 m$^3$/d water production. It comprises two stages of RO filtration. In the first stage, pressure vessels operated at feed water pressure of 65–69 bar and permeate flux of 1.16 LMH were equipped with 280 mm diameter and 2 m long elements/permeators of CTA–RO hollow fine fiber (HFF) membranes (outer diameter: 165 µm; and inner diameter: 70 µm). Permeate of the 1$^{st}$ stage enters a 2$^{nd}$ stage filtration system equipped with brackish water (BW) RO membranes (spirally wound PA–TFC membranes).

Pretreatment of raw seawater includes: continuous chlorination of seawater (0.7 – 1.0 mg/l) at the intake point while maintaining the residual chlorine concentration before SWRO inlet in the range of 0.25 – 0.30 mg/l, pH adjustment to 7.0 with sulphuric acid ($\text{H}_2\text{SO}_4$), addition of ferric chloride ($\text{FeCl}_3$; 6 mg/l) as coagulant and a cationic polymeric flocculant (0.1 – 0.5 mg/l), dual media filter (DMF) containing a 600 mm thick layer of 1.4 – 2.5 mm size anthracite particles and a 900 mm thick layer of 0.7 – 1.4 mm size sand particles, micro cartridge filtration (MCF, 10µm nominal pore size), and de-chlorination with sodium bisulfite (SBS, 1.5 – 2.5 mg/l) just prior to SWRO membrane filtration. SWRO membranes were also exposed to chlorine dosage (0.25 – 0.30 mg/l) by stopping SBS dosing in the feed stream for 1 hour after every seven hours of operation (Figure 1). Despite the routine disinfection of SWRO membranes with intermittent chlorination, citric acid cleaning was also implemented when a 10-15% decrease in permeate flux was observed.
2.2. Sampling plan

Two sampling campaigns were performed: $T_1$ (conducted in May 2012) and $T_2$ (conducted in October 2012). Both membranes and water samples collected by triplicates from various points in the pretreatment train were analysed. All the chlorine containing water samples were immediately de-chlorinated by adding SBS solution at a final concentration of 2–3 mg/L (stoichiometrically, 1.98 mg of SBS are required for the de-chlorination of 1 mg of free chlorine as HOCl). Water samples collected for flow cytometric (FCM) analysis of phytoplankton and bacterial cells were fixed by adding aqueous glutaraldehyde solution (Polyscience, cat# 1909–10) at a final concentration of 2% in volume.

Based on the flow path of feed water stream inside the CTA-RO module, three regions were identified: feed, middle, and brine (Figure 2). Foulant material was isolated from fouled membrane fibers by ultrasonication (48 kHz, 50W) of fibers immersed in Milli-Q water (resistivity of 18.2 MΩ.cm at 25 °C) for 30 seconds. Following this extraction procedure, foulant material was obtained as dry powder through a lyophilization process.

2.2.1. 1st Campaign, May 2012 ($T_1$)

During the 1st campaign (i.e., $T_1$) a fouled SWRO module ($T_1$–M) operated for 23 months was autopsied (Table 1). Membrane fibers were collected from feed to middle regions (Figure 2) where the intensity of fouling was visually higher. Water sampling points included intake water (raw seawater), after Cl₂, before DMF (DMF Inlet), after DMF (DMF outlet), SWRO Inlet (after MCF and SBS), SWRO permeate, and SWRO Brine.

2.2.2. 2nd Campaign, October 2012 ($T_2$)
During the 2nd campaign (i.e., T2), two SWRO modules were autopsied: one after operating for 1 month (T2–fouled membrane/module A, T2–MA), the other after operating for 27 months (T2–fouled membrane/module B, T2–MB) (Table 1).

Like the previous campaign, water samples were collected from the same locations with the addition of two more samples, i.e., raw seawater collected before chlorination and water after MCF step.

Citric acid cleaning of T2–MA was performed right before harvesting from the vessel, whereas the other two membrane elements were subjected to cleaning approximately 4 months before the autopsy was conducted.

2.3. Methods

Fouling load, ATP contents, Scanning Electron Microscopy (SEM), FT–IR, Pyro/GC–MS, solid state $^{13}$C–NMR spectroscopy, ICP–OES, CHN and phylogenetic analyses were performed to characterize the membrane fouling material. Additionally, water quality was examined by pH, conductivity, turbidity, Silt Density Index (SDI$_{15}$), Total/Dissolved Organic Carbon (TOC/DOC), Size Exclusion Liquid Chromatography coupled with an Organic Carbon Detector (LC-OCD), ATP, Heterotrophic Plate Count (HPC), and Flow Cytometry (FCM) analyses/measurements. All these analytical procedures have been discussed in Khan et al., 2013a; 2013b. Details of some analytical procedures exclusively implemented during this study (i.e., acid dialysis, FCM analysis, and pyrosequencing data processing) are described here after.
a) Cleaning of foulant material

Foulant suspensions collected in dialysis tubes (1000 Dalton MWCO; Spectra/Por 7 by Spectrum labs, USA) were dialyzed against a 1% w/v oxalic acid (OA) solution (Sigma–Aldrich, cat# 241172–50G) to remove inorganics causing analytical interferences. This dialysis practice is efficient in removing iron from foulant material. T1–M foulant sample was also subjected to dialysis with 1% w/v hydrofluoric acid (HFA) solutions (Sigma–Aldrich, cat# 339261–800ML) to remove silicates (Figure 2).

b) Flow cytometric analysis

Flow cytometric analysis was conducted to count the microbial cells in unit volume of water. Algal and bacterial communities were quantified with a FACS Verse flow cytometer (Becton Dickinson, Belgium). For the algal cell count, chlorophyll present in cells were excited with a blue laser (488 nm) while the orange (570 nm) and red (690 nm) fluorescence emission were measured to count phytoplankton (cyanobacteria from pico and nano–eukaryotes, respectively). For bacterial cell count, 1 mL of seawater was stained with 2 µl of SYBR® Green–I (SG) stock solution (Invitrogen, USA) and incubated in the dark at room temperature for 10 min. The blue laser (488 nm) was used to excite the SG while the green fluorescence (520 nm) signals were collected for quantification. Low Nucleic Acid (LNA) and High Nucleic Acid (HNA) bacterial counts were determined as previously described by Servais et al., 2003. Fluorescent beads of 1.0 µm diameter (Polysciences, Inc., Europe) were added to each sample for analysis validation purposes.
c) Pyrosequencing data analysis

Pyrosequencing of 16S rRNA genes of DNA, extracted from water and membrane samples, was performed as mentioned in Khan et al., 2013a; 2013b. Among water samples, only T1–water samples were subjected to pyrosequencing analysis. Pyrosequencing data was analysed as follows. A total of 206,210 raw sequence reads were obtained and checked for their quality based on previously described procedures (Hong et al., 2012). Ribosomal Database Project (RDP) classifier was used for taxonomical assignments of the 16S rRNA gene sequences at a 95% confidence level (Cole et al., 2009). Hierarchical assignment files were downloaded from RDP Classifier and the relative abundance of sequences assigned at the respective genus and uncategorized groups were collated for principal component analysis (PCA) and similarity analysis. Both PCA and Bray–Curtis similarity analysis with square root transformation were conducted by Primer–E v5.2.4.

3. Results and Discussion

3.1. Water Quality Analysis

3.1.1. Physicochemical characterization

TOC/DOC values of raw seawater (i.e., 1.86/1.68 mg/L) were higher than at other sites/plants studied along the Red sea coast (i.e., 1-1.2mg/L (Khan et al., 2013a; 2013b)). SDI15 (>>5) and turbidity (3.8 NTU) values also revealed that the fouling potential of this source water was higher than at other sites. Other physicochemical water quality parameters, i.e., pH and conductivity (cond.) values were similar to any other typical Red Sea water sample (Table 2).
The decrease in TOC/DOC in water sample before DMF/after coagulant addition was due to coagulation/flocculation taking place in the sampling bottle (Table 2). Some coagulant flocs we observed settled at the bottom of sampling bottles. As shown by the LC–OCD profiles (Figure 3), removed organics mainly belong to the biopolymer fraction. A part of humic–like substances was also removed after coagulant injection. Further decrease in biopolymers can be observed after DMF. Surprisingly, TOC/DOC contents increased in T₁–after SBS sample. LC–OCD chromatogram confirmed an increase of the signal in the region assigned to medium to lower molecular weight organics that might be attributed to a malfunction of MCF. To further investigate the possible reason of this TOC/DOC increase, one more sample was collected immediately after MCF (i.e., before SBS dosing point) during T₂ sampling campaign. Although T₂–after SBS sample showed little increase in LC–OCD signals (i.e., similarly to T₁–after SBS sample) (Figure 3), no evident increase in TOC/DOC was observed in the water samples either after MCF or after SBS treatment points. These observations suggest that most likely at the time of T₁ sampling, MCF was in or near the saturation state and started leaching organics (i.e., organic material trapped in or produced from biological activity growing in these filters).

3.1.2. Microbiological characterization

Active biomass in the water samples was determined by ATP and HPC analyses. The values of these two parameters, which are indicators of active microbial cells in raw SW were slightly higher (Table 2) than the values determined at other Red Sea sites (Khan et al., 2013a; 2013b).

Flow cytometric (FCM) analyses were performed to determine the bacterial and phytoplankton cell counts in the water samples. While taking into account analytical error
due to non–living particles and a narrow difference between background of free stain and intensity of signal of stain bound to cellular DNA, any value of detected cell count ≤200 cells/ml was considered negligible (Table 2).

Bacterial cells were counted and classified as low DNA (LNA) and high DNA (HNA) containing cells while using SYBR® Green–I as DNA stain. Most researchers have reported HNA as actively growing cells (Lebaron et al., 2001). Total bacterial cell count in the raw seawater was in the order of $10^6$ to $10^7$ cells/ml, which is comparable to previously reported bacterial cell count values of Red Sea water (Khan et al., 2013a).

Bacterial cell count was not decreased to a large extent right after chlorination in both $T_1$ and $T_2$ water samples. This observation might be due to immediate preservation/fixing of samples and short Cl$_2$ contact time to destroy the cell structures. Total number of bacterial cells was in the order of $10^5$ to $10^6$ with LNA and HNA cell count of 3.1x$10^5$ and 8.7x$10^5$ cells/mL in $T_1$–raw seawater; and 9.1x$10^4$ and 8.1x$10^5$ in $T_2$–raw seawater samples, respectively (Table 2). However, in all other samples, bacterial cell counts significantly dropped probably due to relatively longer chlorine reaction time and other downstream pretreatment steps, i.e., coagulation and filtration. It should be noted that the cell count increased in $T_1$–after MCF and SBS samples. Similar to the TOC/DOC increase, the increase in bacterial cell count might also be attributed to their detachment from MCF and/or rapid growth of bacteria following the addition of a de–chlorinating agent, i.e., SBS. Such increase in bacterial cell count was not observed in $T_2$–after MCF and $T_2$–SBS samples. This finding support the hypothesis that during $T_1$ sampling campaign, the MCF was not working properly and instead of improving the water quality it was probably serving as source of microbial and organic contamination.
Phytoplankton cell count in raw seawater was $4.4 \times 10^4$ cells/mL, a value comparable to the others previously detected in water at different sites in the Red Sea (not shown). After chlorination, all $T_1$ and $T_2$ water samples had a phytoplankton cell count <100 cells/ml. Phytoplankton cells were more severely impacted by chlorination than bacterial cells.

**Bacterial populations in water samples**

Phylum *Proteobacteria* was predominant in all water samples, accounting for 58% to 97.7% of the total microbial community (Figure 4A). In addition, phylum *Cyanobacteria* was also abundant in the seawater intake (20.8%) and in the DMF inlet (9.9%), but was gradually removed from the suspended water microbial community as the water moved through the pretreatment train and into the RO brine. The phylum *Proteobacteria* was mainly comprised of unclassified *Proteobacteria*, *Alphaproteobacteria*, and *Gammaproteobacteria* (Figure 4B). Furthermore, bacterial populations that were present in at least four of the five water samples included various unclassified groups as well as genera *Ralstonia*, *Methylophilus*, *Methylobacterium*, *Kordiimonas*, and *Streptococcus*, each at an average relative abundance of 2.6%, 0.32%, 0.41%, 0.38% and 0.15%, respectively. Among them, the relative abundance of unclassified *Hyphomonadaceae* increased along the pretreatment train from 0.75% in the seawater intake to 11.5% in the water sampled after SBS. Similarly, a high relative abundance of unclassified *Rhodobacteraceae* (20.6%) was also observed in the RO brine sample and this relative abundance was at least 5.8–fold higher than that observed in the water sampled after SBS (Figure 4B). These observations suggest that certain bacterial populations may have different removal efficiencies in the pretreatment train and by the RO membrane.
The difference between the physicochemical and microbiological traits of T₁ and T₂ water sample, especially raw seawater samples, might be attributed to seasonal effects.

3.2. Membrane Fouling Analysis

3.2.1 Analysis of fouling intensity and foulant nature

Fouling load, calculated by weighing the dried foulant material recovered from membrane fibers, was measured as 1.01 ± 0.09 g/m² for T₁–M (sampled from feed to middle region of the module). For both T₂ modules, fouling load was relatively high at feed region fibers, i.e., 0.21 ± 0.07 and 1.07 ± 0.08 g/m² for T₂–MA and T₂–MB feed region fibers, respectively, and then it was gradually decreased towards brine region fibers (Figure 5).

ATP analysis was performed to detect active biomass/biofilm present on membrane surface. ATP content values for all membranes were quite low as compared to commonly reported values for bio-fouled RO membranes (i.e., 20–45,000 pg/cm² (Vrouwenvelder and van der Kooij, 2001)), however, they provided significant information about microbial activity on these membrane fibers. ATP content in the fouling layer of T₁–M was measured as 5.44 ± 0.37 pg/cm². ATP content varied at the three filtration regions in T₂-modules. In the case of T₂–MB, ATP concentration was 3.32 ± 0.21, 8.12 ± 0.81, and 0.97 ± 0.11 pg/cm² in the feed, middle, and brine regions, respectively (Figure 5). Despite exhibiting lower mass of foulant material per unit surface area, the middle region fibers showed significantly higher active biomass (i.e., relatively more developed biofilm) than the feed region. Significant biological activity was detected in the fouling layer accumulated on brine side fibers, where the fouling load was very low. A higher
contribution of microbial activity in the fouling of these fibers was confirmed from the calculated values of their ATP contents present per unit mass of the fouling layer (Figure 5). These biofilm forming bacteria were either resilient enough to survive the chlorine stress or they might have been shielded from the chlorine disinfection. This finding proved that intermittent chlorine (0.25 – 0.30 mg/L) exposure is not capable of totally eradicating biofilm formation in RO module; a situation that has also been observed in chlorinated drinking water distribution systems (Hwang et al., 2012; Mathieu et al., 2009).

The relatively effective development of biomass in the posterior regions of T2–MB might be explained by poor or no penetration of Cl₂, which resulted in no or little impact on microbial growth. Plugging of fibers, due to limited free space between them, has been pointed out as one of the major operational problems of HFF modules (Gorenflo et al., 2005). Plugging of fibers may create dead zones where biofilm formation might have occurred without being affected by chlorine. Another reason of low penetration of chlorine might be the oxidant demand of the organics accumulated in the front side fibers.

In the case of T2–MA, both fouling load and active biomass per unit surface area uniformly decreased from its front to rear end fibers (Figure 5). In contrast to T2–MB, significantly lower ATP contents and a continuous decrease in ATP values across the feed to brine sides of this permeator/module suggest that intermittent chlorine was able to uniformly reach all the fibers. Uniform distribution of chlorine might have been favored by relatively less plugging issues in this module which was operated for only 1 month. Low plugging/fouling of this permeator was verified by low fouling load values. The
same reason (i.e., better/uniform penetration of fed chemical agents at early stages of fouling) may justify the better efficacy of the cleaning agent.

Estimation of organic and inorganic fractions of foulant material isolated from membrane fibers was conducted by loss on ignition (LOI) test (Figure 6). It should be noted that the LOI test was performed on dried foulant obtained after Milli–Q dialysis followed by lyophilization process. As acid dialysis might change the relative proportion of organics and inorganics through the removal of inorganic foulants, LOI test was performed on the foulants only after desalting with Milli–Q but prior to any acid dialysis. Because LOI test requires approximately 50 mg of foulant mass, it could not be performed on the foulant collected from brine region fibers of $T_2$–membranes where a small amount of material was recovered.

The organic material was predominant in $T_1$–M and $T_2$–MB foulants. The strong organic character and relatively high ATP content of foulant material deposited on these membranes were indicators of biofouling scenario. Conversely, $T_2$–MA foulant was characterized by a slightly higher proportion of inorganic constituents.

### 3.2.2. Elemental composition of foulants

Elemental composition was assessed using direct (SEM–EDX given as in Supplementary Information-1) and indirect (ICP–OES and CHN analyser) analytical techniques. ICP–OES analysis results (Figure 7) were in accordance with the findings of EDX analysis (SI). Fe was the most abundant element detected in all the foulant samples. Organic analysis of foulants, i.e., $^{13}$C–NMR and FT–IR spectroscopic analyses and CHN elemental analysis are affected by inorganics, especially metallic elements (e.g., Fe).
Hence, oxalic acid (OA) dialysis was applied as a foulant treatment procedure to remove the metallic ions prior to organic characterization. It was noticed that OA dialysis treatment removed very well Fe contents, however, Al and Mg were not removed (indicated by an increase in their abundance in the dialysed foulant material). Higher Fe concentration was also detected in T2–MB with concentrations decreasing from feed to middle and brine fibers. Fe contents were approximately three times lower in the same corresponding regions of the T2–MA (Figure 7), a rational finding as citric acid cleaning of RO vessel containing this particular module was recently performed. However, the last citric acid cleaning of other modules was conducted approximately four months before being extracted from the vessel. Nevertheless, the presence of Fe at these high concentrations in all foulant samples can be assigned to FeCl₃ dosing in the pretreatment line. In addition to negative impact on membrane performance as individual foulant, in ferric hydroxide forms (neutral or cationic form), Fe can also play a major role in the formation of organic fouling layers (i.e., adsorption and complexation phenomena).

With the exception of T2-MB-brine foulant, calcium (Ca) was observed near 10 mg/g in all foulant samples. Moreover, relative abundance of Ca was increased from the front to the rear end region samples. In case of T2-MB brine samples, this increase was relatively intense (i.e., Ca was 82 mg/g of the foulant), suggesting precipitation/scaling of Ca-containing compounds or the presence of organic material with high Ca complexing property.

Aluminium (Al), silicon (Si), and magnesium (Mg) were three major inorganic elements detected at high concentrations after Fe, especially in T2–MA foulant material (Figure 7). Relatively higher abundance of Al, Si, and Mg in relatively low fouled and citric acid
cleaned membrane (T$_2$–MA) suggested no or minor removal of these elements by acid
cleaning. A similar result has been observed when the oxalic acid dialysis was performed
with T$_1$M foulant. The higher Al and Si concentrations were attributable to the presence
of alumino–silicates which might be contributing in membrane fouling phenomenon,
especially during initial phases (i.e., plugging of fibers). Significant fouling of CTA-RO
membranes by alumino-silicates has also been reported by Butt et al., 1995, 1997a,
1997b.

In the case of T$_1$–M foulant (before and after OA and HFA dialysis), CHN analysis
showed low C/N ratios (i.e., 6.78 and 7.64, respectively) that could be attributed to a
significant incorporation of proteinaceous structures. This result showed that dialysis
procedures had minor impact on C/N ratios. An increase in the relative abundance of all
the organic elements, i.e., CHN after OA dialysis and HFA dialysis, is due to the removal
of inorganics, mainly Fe and Si species, respectively (Figure 8). All the foulant samples
of T$_2$ campaign were subjected to CHN analysis after OA dialysis. In case of both sets of
T$_2$–foulant samples, C and N contents increased along the feed water flow path, i.e., from
front/feed to brine side. A continuous decrease in C/N atomic ratio can be observed from
feed to brine side (Figure 8), result that could be interpreted as an increase in nitrogen
containing structures in the foulant of fibers located behind the feed region. Previously,
similar low C/N values (i.e., 4–8) have been reported for marine bacterial cells (Bhosle,
2004; Müller, 1977) and actively growing biofilms (Bhosle, 2004; Khan et al., 2013a,
2013b).
3.2.3. Biopolymers Characterization

The resolution of FT-IR and $^{13}$C-NMR spectra (Figure 9 and 10) was considerably improved after OA/HFA dialysis. This finding confirmed the importance of inorganic constituent removal for biopolymers characterization.

IR bands that are characteristic of biogenic material, i.e., proteinaceous structures (amide A, B, I, II and III bands), aminosugars (methyl of N-acetyl group band), sugars (O-alkyl group band) and fatty acids (carboxyl and alkyl groups bands), were recorded in all foulants. Moreover, IR band (at 1238 cm$^{-1}$) attributable to phosphodiester linkages of nucleic acids (Naumann et al., 1996) was also detected in all foulants. Significantly higher intensities of these bands of biogenic material suggested a considerable presence of microbial activity/growth, i.e., biofouling, in the treatment system. An increase in intensities of bands assigned to organic functional groups, especially of those attributed to sugars/aminosugars, were improved for both T$_2$–MA and T$_2$–MB foulants (analysed after OA dialysis) from feed to brine side foulants. In case of T$_2$–MB profiles, it can be visually assessed that ratio among area/height of polysaccharides/proteins peaks was remarkably increased in the brine region foulant. We could not see this relative increase in the intensity of polysaccharide peak due to overlapping silica/silicate peak. These results are in agreement with collective findings of ATP and LOI analyses, suggesting relatively higher occurrence of biofilm on the rear end fibers, i.e., middle and brine regions of the permeators. Bands for alumino–silicates/silica were observed in the spectra of T$_1$–M foulant material and T$_2$–MA foulant material. Also, a band at 1238 cm$^{-1}$ could be attributed to inorganic phosphates.
Solid state $^{13}$C–NMR spectroscopic analyses were conducted to further explore the nature of the organic component of foulant material. This analysis of T$_1$–M foulant was performed before and after OA dialysis. After observing a positive effect on the removal of inorganics (i.e., mainly Fe) on the spectrum of T$_1$–M foulant sample, all the T$_2$–membrane foulants were subjected to OA dialysis prior to $^{13}$C–NMR spectroscopic analysis. $^{13}$C–NMR spectra (Figure 10) are discussed in the light of data published by Leenheer, 2009, and Khan et al., 2014; Khan et al., 2013b.

NMR bands for carbon atoms present in the branched and cyclic structures, which are feature of biogenic matter, were detected (near 20 ppm, 39 ppm and 43 ppm) in all foulants. Moreover, bands at 31 ppm and 171 ppm were derived, at least partly, from alkyl chain methylene and carboxyl group carbons, respectively, of fatty acids. These two bands might also be originated from proteins containing aliphatic amino acids with long side chain structures. Proteins were indicated by N–C peak at 51 ppm. Aromatic carbon band centred at 129 ppm would be indicative of the presence of aromatic amino acids. Carbohydrates are indicated by secondary alcohol (O–Alkyl) and anomeric carbon (Di–O–Alkyl) bands at 72 ppm and 102 ppm, respectively. The band at 20 ppm was attributable to methyl–C of N–acetyl group of aminosugars.

Similar to IR profiles, NMR spectra also indicated that most likely the origin of organic matter was microbial activity. Additionally, in the spectra of foulants collected from different regions of T$_2$–permeators, an evolution or increase of the intensity of some specific bands was observed across the feed to brine sides. These include the bands in aliphatic region and characteristic bands of aminosugars (i.e., methyl group) and polysaccharides (i.e., alcohol group) originating from substances derived from microbial
cells and EPS of biofilm, respectively. An increase in intensities of these bands in the middle and brine region foulants may correspond to more developed biofilm in these regions.

Pyrolysis/GC–MS analyses of foulants were performed to determine the relative abundance of biopolymers (Supplementary Information-2). T$_1$–M foulant was subjected to pyrolysis/GC–MS analysis before and after OA dialysis. The profile of pyrochromatograms of T$_1$–M foulant, before and after OA dialysis, was almost identical. On the basis of this observation, T$_2$–foulants were pyro–analysed without performing OA dialysis. Foulants from all the samples exhibited almost similar biopolymers distribution with few differences.
In all foulant samples, the relative abundance of aromatic amino acids, i.e., tyrosine (phenol and p-cresol pyrofragments) and phenylalanine (toluene and styrene pyrofragments) was quite significant (Bruchet et al., 1990; Christy et al., 1998). Signals of aminosugars (acetamide peak) and small chain fatty acids (C10–C18) in the pyrochromatograms of foulant material were good evidence of biogenic matter (Khan et al., 2013b). Significantly higher relative abundance of signal for polyhydroxybutyrates (PHBs; crotonic acid peak), known as energy storage molecules of microbes released under stressful condition (Dawes, 1988), detected in all samples also indicated the presence of biofilm in the permeators. Relative abundance values of polysaccharide and lipids/fatty acids signals increased from feed to brine region foulant samples of both T2 permeators, a finding in agreement with the results of FTIR and $^{13}$C–NMR spectroscopic analyses.

3.2.4. Ultrastructural analysis

The morphology of fouling layer membrane samples was observed by freeze-drying SEM imaging technique. In the micrographs of all the membranes, microbial structures, most of them similar to bacterial cells, were observed (Supplementary Information-3). This analysis provided direct evidence of biofilm in the SWRO system of a plant where an extensive use of chlorine was conducted to prevent biofouling. Fouliants mass trapped between the walls of adjacent fibers was observed, which might have resulted in plugging of permeators.

No significant difference could be identified among the feed, middle, and brine region fibers of permeators in terms of abundance of microbial structures and morphological
features of fouling layer. The only observed difference was the relatively more abundant inorganic salts like structures on brine side fibers.

3.2.5. Microbial community analysis

Comparison of microbial communities on $T_1$–$M$ and $T_1$–water samples

The microbial communities on $T_1$–$M$ fibers shared low similarity of 18.5% with those present in the water samples and clustered apart from the suspended microbial communities (Figure 11). Within the attached microbial community, the genus Acinetobacter accounted for the highest relative abundance of 36.1%. The other remaining abundant bacterial populations on the $T_1$–$M$ include Ralstonia (15.1%), Diaphorobacter (11.3%), unclassified Comamonadaceae (10%), Stenotrophomonas (8.5%), and unclassified Enterobacteriaceae (6.5%). These bacterial populations were usually present in lower relative abundance in the water samples, ranging from 0.3% to 3.3% of total microbial community. The higher relative abundance of these bacterial populations on the membrane compared to the water samples suggests a preferential concentration and attachment of these microorganisms on the membrane surface.

Microbial community on $T_2$–Membranes

Microbial communities differed at the feed, middle, and brine ends of the $T_2$–MB (Figure 11). Specifically, the feed end shared a 65.3% similarity with the middle and brine ends, while microbial communities in the middle and brine ends shared a 79.8% similarity. The relative abundance of unclassified Alphaproteobacteria, as well as genera Janthinobacterium and Pedobacter, decreased from the feed to brine end of the membrane. In addition, genera Janthinobacterium and Pedobacter were more than 3–fold
higher in relative abundance at the feed end than the brine end, suggesting an effective removal of these bacterial populations along the membrane module (Figure 12). However, *Silicibacter, Pseudomonas* and unclassified *Rhodobacteraceae* were present at more than 1.9–fold higher relative abundances on the brine end than feed end of the module (Figure 12). Fiber plugging and/or consumption of chlorine by the organic foulants accumulated on the feed region fibers may have lowered the concentration of chlorine at the middle and brine end of the modules. An increase in the relative abundance of *Silicibacter, Pseudomonas*, and unclassified *Rhodobacteraceae* at the middle and brine ends may indicate that these bacterial populations are able to survive with lower residual chlorine concentration. ATP analyses of the membrane recorded 2.4–fold increase in the ATP concentration at the middle end relative to the feed end. This increase in ATP concentration coincides with an increase in the relative abundance of *Silicibacter* and unclassified *Rhodobacteraceae* from the feed end to the brine end of the module, and likely suggests that these two bacterial populations may be resistant to the residual chlorine and remained biologically active on the membrane. Although the high-throughput sequencing performed in this study does not allow bacterial identification at the species level due to the short read length; previous studies have shown that species within the genus *Pseudomonas* overexpresses production of polysaccharide in the extracellular polymeric matrix as a defensive mechanism against residual chlorine (Grobe et al., 2001; Xue et al., 2013). Furthermore, sugar-like trehalose can be produced by *Pseudomonas* as a compatible solute in response to increasing salinity from the feed to the brine end of the module (Diab et al., 2006). The overexpression of polysaccharide by *Pseudomonas*, as well as the increase in its relative abundance, are therefore likely to
account for the relatively higher concentration of sugars detected on the fibers located at the brine end of the module.

The microbial communities at different spatial locations of the T₂–MA fibers clustered apart from those on the T₂–MB fibers (Figure 11). Specifically, these microbial communities on the T₂–MA fibers only shared a 30.2% similarity with those on the T₂–MB fibers, suggesting that either the citric acid cleaning procedure might have disrupted the microbial community or the microbial composition might have changed with increasing age of the biofoulant layer.

_Pseudomonas_ spp. was present in high relative abundance of 24.9%, 5.8%, and 64% of the total microbial community in the feed, middle, and brine end of the T₂–MA, respectively (Table 3). Furthermore, spore–forming unclassified _Bacillales_ were detected at a high relative abundance in the feed end of T₂–MA (3.6%). Gram–positive _Streptococcus_ and _Staphylococcus_ were also detected at a relative abundance of 10.3% and 2.7% (Table 3). _Acidocella_, a genus associated with acidophiles, were present at 3.1% of total microbial community. These observations indicate that certain bacterial populations (e.g. spore–formers, gram–positive bacteria and acidophiles) were likely able to withstand the cleaning process.

### 3.2.6. Fouling dynamics and mechanism

Continuous trend of increase in the relative abundance of aminosugars and fatty acids in the foulant material collected from lead to rear end region fibers is a strong evidence of higher microbial activity occurred in the posterior region of the module. This increasing trend in the relative abundance of the mentioned biogenic material is highlighted by
consistent FTIR, $^{13}$C-NMR, and Pyro/GC-MS results. Additionally, a parallel rise in the relative abundance of sugars might be considered as an evidence of the increasing maturity level of the biofilm (i.e., biofilm with more released extracellular polymeric substances, EPS, which is most likely composed of higher sugar contents). This interpretation is in accordance with the findings of Bhaskar and Bhosle, 2005, showing polysaccharides as the most abundant component of the marine microbial EPS (Bhaskar and Bhosle, 2005). CHN elemental analysis suggested that relative abundance of nitrogen containing substances in the foulant material was increasing from feed to brine region. This trend might have been caused by nitrogen present in non proteinaceous structure (e.g., nitrates). Significantly higher relative abundance of biopolymers, especially proteins, but lower relative presence of sugars in the foulant material of feed region fibers, also highlighted the occurrence of microbial growth, but this activity could not result in the formation of a mature biofilm. Most likely the cells were able to grow when the RO vessel was not subjected to chlorination (i.e., 1 hour of chlorination after 7 hours in absence of residual chlorine), however, the formation of mature biofilm could not have been completed due to the intermittent chlorination. This cycle of formation and decay of immature biofilm is probable to recur over time with significant impact of the chemical and microbiological composition of the fouling layer.

Higher microbial activity in the rear end regions is protected against disinfection due to the inaccessibility of chlorine to these areas. This hindrance in the disinfection could be due to the phenomenon of fiber plugging and/or due to consumption of chlorine by the inorganic/organic foulants accumulated on the feed region fibers. This hypothesis is supplemented by the observation of a uniform decrease in the ATP contents, and on
contrary, a non-uniform trend of ATP contents from feed to brine side fibers in the less-fouled module (i.e., T2–MA) and more-fouled (i.e., T2–MB) module, respectively. In case of T2–MB, a lower intensity of microbial activity (lower ATP contents per unit surface area and low fouling load) in the brine side fibers than in middle side fibers might be due to the decrease in the nutrients reaching this region. The accumulation of organic and inorganic material in the front side fibers seems to play a major role in the proliferation of sustainable microbial activity/biofouling in the rear end fibers.

Some researchers have reported that under hyper-saline conditions, bacterial cell start synthesizing more sugars in order to adapt to the osmotic pressure (Roeßler and Müller, 2001). Higher sugars in the rear end fibers might also be assigned to their increased biosynthesis in response to increased salt concentration in the feed stream.

Besides its failure in controlling the biofouling, chlorination of CTA-RO membranes is known to result in some disadvantages. These include the possibility of negative impact of chlorination on the integrity of CTA-RO membrane structure. This adverse impact of chlorine is claimed to be negligible at low chlorine concentrations (approximately 0.2-0.3 mg/l) under normal operating conditions of SWRO plants. However, under certain conditions (e.g., with chlorine concentration above the mentioned limit or due to catalytic effect of metals present in the fouling layer) it can be significant. Moreover, chlorine can also react with the organic matter accumulated in the fouling layer of the membrane fibers to result in the production of toxic halogenated organic compounds. These compounds can permeate through the CTA-RO membrane or being discharged as brine into the environment, and consequently impact human health and/or environment.
4. Conclusion

Biofouling remains a substantial problem for SWRO units using CTA-RO membranes and practicing intermittent chlorine disinfection of the membrane fibers. Biofilm forming bacteria can sustain chlorine disinfection and acidic cleaning either because of their developed resistance against acidic solution or because of being protected/shielded by the fouling matrix accumulated around them. Considering the ineffectiveness of chlorine in deterring RO membrane biofouling and the high risk of generation of toxic disinfectant by products, the use of chlorination in the pretreatment stage of an SWRO is not recommended.

Acknowledgements

We thank Abdul-Hamid Emwas, Tony L Merle, Cyril Aubry and Leonardo Gutierrez for helping in the implementation of the NMR, FCM, SEM/EDX analyses and technical discussion, respectively. Additionally, authors thankfully acknowledge the support received from KAUST WDRC lab staff. All the funds for this work were provided by King Abdullah University of Science and Technology (KAUST).

References


Figure Captions

Figure 1: Schematic description of SWRO plant design. (Cl\textsubscript{2}: Chlorination, H\textsubscript{2}SO\textsubscript{4}: Sulfuric acid, FeCl\textsubscript{3}: Ferric chloride, DMF: Dual media filter, MCF: Micro cartridge filter, SWRO: seawater reverse osmosis, BWRO: Brackish water reverse osmosis, ERD: Energy recovery device).

Figure 2: Sampling of membrane fibers and extraction of foulants. a) Cross sectional view of CTA–RO element. Fibers were sampled from various regions (indicated by red rings). Green dotted lines with arrowheads show the direction of feed water flow. b) Fouled membrane fibers extracted from the module. c) Membrane fibers cleaned after extraction of foulant with ultrapure water. d) Brownish–colored powdered foulant (due to inorganics) material recovered through lyophilisation process. d) Foulant material after removal of inorganics by acid dialysis.
Figure 3: LC–OCD analysis of water samples (Mol.: molecular; MW: molecular weight; min: minutes; NOM: natural organic matter; BDOC: biodegradable dissolved organic carbon).

Figure 4: Microbial community in the water samples. (A) Relative abundance of the predominant phyla, (B) Relative abundance of the bacterial populations that were present in at least 4 of the 5 water samples (i.e., core bacterial groups).

Figure 5: Analysis of fouling intensity and assessment of active biomass. Fouling load represents the amount of foulants accumulated on unit surface area of the membrane fibers. Abundance of active biomass is presented as weight (pg) of ATP per unit surface area (cm²) of the membrane and as weight (ng) of ATP per unit weight (g) of foulant material.

Figure 6: Estimation of organic and inorganic fractions of the membrane foulant samples with loss on ignition (LOI) test.

Figure 7: Elemental analysis of foulant material. a) ICP-OES analysis of major elements present in T₁ foulant material before and after oxalic acid (OA) dialysis. b) ICP-OES analysis of major elements present in T₂ foulant material before OA dialysis.

Figure 8: Elemental analysis of foulant material. T₁ campaign foulant sample was subjected to CHN elemental analysis before and after oxalic acid (OA) and hydrofluoric acid (HFA) dialysis, whereas all T₂ campaign foulant samples were subjected to CHN elemental analysis only after OA dialysis.

Figure 9: FTIR spectroscopic analysis of foulant samples. a) Spectra of T₁ foulant samples before and after oxalic acid (OA) and hydrofluoric acid (HFA) dialysis. b) Spectra of T₂ foulant samples after OA dialysis. Y-axis (not shown) is absorbance with arbitrary units.

Figure 10: Solid state $^{13}$C–NMR spectroscopic analysis of foulant samples. a) Spectra of T₁ foulant samples before and after oxalic acid (OA) dialysis. b) Spectra of T₂ foulant samples after OA dialysis. Y-axis (not shown) is signal intensity with arbitrary units.

Figure 11: Principal component analysis (PCA) of the microbial communities in bulk water samples and on membranes.

Figure 12: Cumulative abundance of bacterial genera and unclassified groups detected on the feed, middle, and brine end of the T₂–MB fibers.

Table Captions

Table 1: Membrane sampling: Sampling region, operation time, and performance data.
Table 2: Physicochemical analysis of water samples (SW: seawater, cond.: conductivity, SDI\(_{15}\): silt density index measured after 15 minutes of filtration, TOC/DOC: total/dissolved organic carbon, ATP: Adenosine triphosphate, HPC: Heterotrophic plate count, CFU: colony forming units, LNA: low nucleic acid cells, HNA: high nucleic acid cells, SD: standard deviation. *: within the range of analytical error).

Table 3: Bacterial populations that were detected at relative abundance >2% of total microbial community at the feed end of the T\(_2\)-MA. N.D. denotes not detected.
Table 1: Membrane sampling: Sampling region, operation time, and performance data.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Position in the Vessel</th>
<th>Fiber Sampling Region</th>
<th>Operating Time (Months)</th>
<th>No. of Cleaning Cycles*</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 Fouled Membrane (T1-M)</td>
<td>1st</td>
<td>Feed to Middle</td>
<td>23</td>
<td>3</td>
</tr>
<tr>
<td>T2 Fouled Membrane A (T2-MA)</td>
<td>1st</td>
<td>Feed, Middle and Brine</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>T2 Fouled Membrane B (T2-MB)</td>
<td>1st</td>
<td>Feed, Middle and Brine</td>
<td>27</td>
<td>4</td>
</tr>
</tbody>
</table>

* Cleaning was conducted when the permeability was decreased by 10–15%.
Table 2: Physicochemical analysis of water samples (SW: seawater, cond.: conductivity, SDI<sub>15</sub>: silt density index measured after 15 minutes of filtration, TOC/DOC: total/dissolved organic carbon, ATP: Adenosine triphosphate, HPC: Heterotrophic plate count, CFU: colony forming units, LNA: low nucleic acid cells, HNA: high nucleic acid cells, SD: standard deviation. *: within the range of analytical error).

<table>
<thead>
<tr>
<th>Water Sample</th>
<th>pH</th>
<th>Cond. (ms/cm) (SD)</th>
<th>Turbidity (NTU) (SD)</th>
<th>SDI&lt;sub&gt;15&lt;/sub&gt; (SD)</th>
<th>TOC/DOC (mg/L) (SD)</th>
<th>ATP (ng/L) (SD)</th>
<th>HPC (CFU/mL) (SD)</th>
<th>LNA (Cells/mL) (SD)</th>
<th>HNA (Cells/mL) (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SW after Cl&lt;sub&gt;2&lt;/sub&gt;</td>
<td>8.18</td>
<td>58.9 (SD)</td>
<td>3.1 (SD)</td>
<td>&gt;&gt;5</td>
<td>1.63/1.22 (0.09/0.08)</td>
<td>31.5 (5.2)</td>
<td>1.2x10&lt;sup&gt;7&lt;/sup&gt; (5x10&lt;sup&gt;6&lt;/sup&gt;)</td>
<td>3.1x10&lt;sup&gt;3&lt;/sup&gt; (1.3x10&lt;sup&gt;3&lt;/sup&gt;)</td>
<td>8.7x10&lt;sup&gt;3&lt;/sup&gt; (2.2x10&lt;sup&gt;3&lt;/sup&gt;)</td>
</tr>
<tr>
<td>DMF inlet</td>
<td>6.53</td>
<td>59.2 (SD)</td>
<td>3.3 (SD)</td>
<td>-</td>
<td>1.12/0.87 (0.06/0.05)</td>
<td>&lt;1</td>
<td>0</td>
<td>9.5x10&lt;sup&gt;3&lt;/sup&gt; (8.1x10&lt;sup&gt;3&lt;/sup&gt;)</td>
<td>4.0x10&lt;sup&gt;4&lt;/sup&gt; (1.1x10&lt;sup&gt;5&lt;/sup&gt;)</td>
</tr>
<tr>
<td>DMF outlet</td>
<td>6.91</td>
<td>59 (SD)</td>
<td>0.15 (SD)</td>
<td>-</td>
<td>1.10/0.95 (0.06/0.05)</td>
<td>4.3 (2)</td>
<td>1.1x10&lt;sup&gt;2&lt;/sup&gt; (7x10&lt;sup&gt;1&lt;/sup&gt;)</td>
<td>6.0x10&lt;sup&gt;3&lt;/sup&gt; (9.5x10&lt;sup&gt;3&lt;/sup&gt;)</td>
<td>3.8x10&lt;sup&gt;3&lt;/sup&gt; (8.6x10&lt;sup&gt;3&lt;/sup&gt;)</td>
</tr>
<tr>
<td>After SBS</td>
<td>6.58</td>
<td>59.3 (SD)</td>
<td>0.1 (SD)</td>
<td>1.2 (SD)</td>
<td>1.27/1.13 (0.07/0.06)</td>
<td>12.6 (4)</td>
<td>1.3x10&lt;sup&gt;2&lt;/sup&gt; (6x10&lt;sup&gt;1&lt;/sup&gt;)</td>
<td>1.0x10&lt;sup&gt;4&lt;/sup&gt; (1.0x10&lt;sup&gt;4&lt;/sup&gt;)</td>
<td>6.5x10&lt;sup&gt;3&lt;/sup&gt; (97x10&lt;sup&gt;3&lt;/sup&gt;)</td>
</tr>
<tr>
<td>SWRO permeate</td>
<td>5.45</td>
<td>0.32 (SD)</td>
<td>0.16 (SD)</td>
<td>-</td>
<td>0.15/0.15 (0.02/0.02)</td>
<td>&lt;1</td>
<td>0</td>
<td>&lt;2.0x10&lt;sup&gt;7&lt;/sup&gt; (&lt;2.0x10&lt;sup&gt;7&lt;/sup&gt;)</td>
<td>&lt;2.0x10&lt;sup&gt;7&lt;/sup&gt; (&lt;2.0x10&lt;sup&gt;7&lt;/sup&gt;)</td>
</tr>
<tr>
<td>SWRO brine</td>
<td>6.53</td>
<td>89.0 (SD)</td>
<td>3.3 (SD)</td>
<td>-</td>
<td>2.08/1.71 (0.09/0.09)</td>
<td>22.53 (6)</td>
<td>1.6x10&lt;sup&gt;3&lt;/sup&gt; (1.5x10&lt;sup&gt;3&lt;/sup&gt;)</td>
<td>3.1x10&lt;sup&gt;4&lt;/sup&gt; (1.3x10&lt;sup&gt;4&lt;/sup&gt;)</td>
<td>1.9x10&lt;sup&gt;4&lt;/sup&gt; (1.1x10&lt;sup&gt;5&lt;/sup&gt;)</td>
</tr>
</tbody>
</table>

| Raw SW             | 8.22 | 54.1 (SD)         | 3.8 (SD)            | >>5                  | 1.86/1.68 (0.09/0.08) | 72.6 (5.6)     | 6.7x10<sup>3</sup> (6.0x10<sup>3</sup>) | 7.8x10<sup>3</sup> (1.9x10<sup>3</sup>) | 5.6x10<sup>3</sup> (7.5x10<sup>3</sup>) |
| SW after Cl<sub>2</sub> | 8.22 | 54.2 (SD)         | 3.3 (SD)            | -                    | 1.59/1.36 (0.08/0.07) | 7.6 (2)       | 1.5x10<sup>2</sup> (2x10<sup>1</sup>) | 9.1x10<sup>4</sup> (6.5x10<sup>4</sup>) | 8.1x10<sup>3</sup> (4.2x10<sup>3</sup>) |
| DMF inlet          | 6.55 | 54.2 (SD)         | 3.1 (SD)            | -                    | 1.26/1.05 (0.07/0.05) | <1             | 0                | 2.5x10<sup>3</sup> (7x10<sup>2</sup>) | 3.2x10<sup>4</sup> (4x10<sup>3</sup>) |
| DMF outlet         | 6.55 | 54.3 (SD)         | 0.17 (SD)           | -                    | 1.23/1.14 (0.07/0.07) | 4.3 (2)       | 1.2x10<sup>2</sup> (2x10<sup>1</sup>) | <2.0x10<sup>3</sup> (<2.0x10<sup>3</sup>) | <2.0x10<sup>3</sup> (<2.0x10<sup>3</sup>) |
| MCF outlet         | 6.51 | 54.3 (SD)         | 0.11 (SD)           | -                    | 1.22/1.14 (0.07/0.06) | <1             | 1.3x10<sup>2</sup> (6.0x10<sup>1</sup>) | <2.0x10<sup>3</sup> (<2.0x10<sup>3</sup>) | <2.0x10<sup>3</sup> (<2.0x10<sup>3</sup>) |
| After SBS          | 6.49 | 54.3 (SD)         | 0.11 (SD)           | 1.2 (SD)             | 1.23/1.15 (0.08/0.04) | 5.51 (3)      | 1.5x10<sup>2</sup> (3.5x10<sup>1</sup>) | <2.0x10<sup>2</sup> (<2.0x10<sup>2</sup>) | <2.0x10<sup>2</sup> (<2.0x10<sup>2</sup>) |
| RO permeate        | 4.94 | 0.38 (SD)         | 0.08 (SD)           | -                    | 0.21/0.21 (0.01/0.01) | <1             | 0                | <2.0x10<sup>2</sup> (<2.0x10<sup>2</sup>) | <2.0x10<sup>2</sup> (<2.0x10<sup>2</sup>) |
| RO brine           | 6.74 | 83.7 (SD)         | 0.17 (SD)           | -                    | 1.86/1.64 (0.09/0.09) | 32.53 (5)     | 1.7x10<sup>3</sup> (5.5x10<sup>2</sup>) | 1.3x10<sup>4</sup> (2x10<sup>2</sup>) | 8.0x10<sup>3</sup> (1.1x10<sup>3</sup>) |
Table 3: Bacterial populations that were detected at relative abundance >2% of total microbial community at the feed end of the T₂–MA. N.D. denotes not detected.

<table>
<thead>
<tr>
<th>Bacterial populations</th>
<th>T₂-MA</th>
<th>Feed</th>
<th>Middle</th>
<th>Brine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corynebacterium</td>
<td>2.3</td>
<td>0.1</td>
<td>N.D.</td>
<td></td>
</tr>
<tr>
<td>Prevotella</td>
<td>2.6</td>
<td>0.1</td>
<td>N.D.</td>
<td></td>
</tr>
<tr>
<td>Flavobacterium</td>
<td>2.1</td>
<td>1.4</td>
<td>19.6</td>
<td></td>
</tr>
<tr>
<td>Burkholderia</td>
<td>3.8</td>
<td>0.1</td>
<td>N.D.</td>
<td></td>
</tr>
<tr>
<td>Acidocella</td>
<td>3.1</td>
<td>0.1</td>
<td>N.D.</td>
<td></td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>24.9</td>
<td>5.8</td>
<td>64.0</td>
<td></td>
</tr>
<tr>
<td>Streptococcus</td>
<td>10.3</td>
<td>0.4</td>
<td>N.D.</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus</td>
<td>2.7</td>
<td>0.1</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>Unclassified Bacillales</td>
<td>3.6</td>
<td>0.2</td>
<td>N.D.</td>
<td></td>
</tr>
<tr>
<td>Unclassified Bacteria</td>
<td>2.9</td>
<td>11.9</td>
<td>0.4</td>
<td></td>
</tr>
</tbody>
</table>
Continuous

Stopped for 1 hour after every 7 hours of operation

Figure 1: Schematic description of SWRO plant design. (Cl$_2$: Chlorination, H$_2$SO$_4$: Sulfuric acid, FeCl$_3$: Ferric chloride, DMF: Dual media filter, MCF: Micro cartridge filter, SWRO: seawater reverse osmosis, BWRO: Brackish water reverse osmosis, ERD: Energy recovery device).
Figure 2: Sampling of membrane fibers and extraction of foulants. a) Cross sectional view of CTA–RO element. Fibers were sampled from various regions (indicated by red rings). Green dotted lines with arrowheads show the direction of feed water flow. b) Fouled membrane fibers extracted from the module. c) Membrane fibers cleaned after extraction of foulant with ultrapure water. d) Brownish–colored powdered foulant (due to inorganics) material recovered through lyophilisation process. d) Foulant material after removal of inorganics by acid dialysis.
Figure 3: LC–OCD analysis of water samples (Mol.: molecular; MW: molecular weight; min: minutes; NOM: natural organic matter; BDOC: biodegradable dissolved organic carbon).
Figure 4: Microbial community in the water samples. (A) Relative abundance of the predominant phyla, (B) Relative abundance of the bacterial populations that were present in at least 4 of the 5 water samples (i.e., core bacterial groups).
Figure 5: Analysis of fouling intensity and assessment of active biomass. Fouling load represents the amount of foulants accumulated on unit surface area of the membrane fibers. Abundance of active biomass is presented as weight (pg) of ATP per unit surface area (cm$^2$) of the membrane and as weight (ng) of ATP per unit weight (g) of foulant material.
Figure 6: Estimation of organic and inorganic fractions of the membrane foulants samples with loss on ignition (LOI) test.
Figure 7: Elemental analysis of foulant material. a) ICP-OES analysis of major elements present in $T_1$ foulant material before and after oxalic acid (OA) dialysis. b) ICP-OES analysis of major elements present in $T_2$ foulant material before OA dialysis.
Figure 8: Elemental analysis of foulant material. $T_1$ campaign foulant sample was subjected to CHN elemental analysis before and after oxalic acid (OA) and hydrofluoric acid (HFA) dialysis, whereas all $T_2$ campaign foulant samples were subjected to CHN elemental analysis only after OA dialysis.
Figure 9: FTIR spectroscopic analysis of foulant samples. a) Spectra of T₁ foulant samples before and after oxalic acid (OA) and hydrofluoric acid (HFA) dialysis. b) Spectra of T₂ foulant samples after OA dialysis. Y-axis (not shown) is absorbance with arbitrary units.
Figure 10: Solid state $^{13}$C–NMR spectroscopic analysis of foulant samples. a) Spectra of $T_1$ foulant samples before and after oxalic acid (OA) dialysis. b) Spectra of $T_2$ foulant samples after OA dialysis. Y-axis (not shown) is signal intensity with arbitrary units.
Figure 11: Principal component analysis (PCA) of the microbial communities in bulk water samples and on membranes.
Figure 12: Cumulative abundance of bacterial genera and unclassified groups detected on the feed, middle, and brine end of the T₂–MB fibers.
Highlights

• Fouling mechanism of chlorine resistant seawater RO membranes is discussed.
• Significant biofouling can occur despite the chlorination of membranes.
• Initial biofilm formed by bacteria resistant to chemical stress.
• Relatively mature biofilm is developed in the posterior region membrane fibers.
SI-1

SEM–EDX elemental analysis showed Fe as the major element in the fouling material of T1–M and T2–MB. Al and Si represented the second most abundant elements in these specimens. In the case of T2–MA, the intensity of the Fe signal was significantly reduced which was attributable to recently carried citric acid cleaning. For this membrane, Al and Si were the most abundant elements (Supplementary Figure 1).
Supplementary Figure 1: SEM-EDX elemental analysis. Top) T₁–M (feed/middle side) fibers. Middle) T₂–MB (feed side) fibers. Bottom) T₂–MA (feed side) fibers.
Supplementary Figure 1: Pyro/GC–MS analysis of membrane foulants: Left) pyrochromatograms of foulant material (isolated from membranes), showing different peaks of pyrofragments of organic substances. Based on different origin, pyrofragment peaks are marked with labels of different colors. Right) pie charts showing distribution of biopolymers/organics, calculated by adding up the percent area of characteristic pyrofragment peaks of a biopolymer class.
Supplementary Figure 1: Ultrastructural analysis of membrane specimens.