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Does Chlorination of Seawater Reverse Osmosis Membranes Control Biofouling?

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

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

^b NOMAC-Saudi Arabia.

^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

34 always dominated the desalination market due to promising contaminant rejection
35 capabilities (Lee et al., 2011). Presently, two main types of polymeric RO membranes
36 exist in the market: CTA membranes and PA thin film composite (PA-TFC) membranes.
37 CTA membranes are available as fine hollow fibers wound into an element of a layered
38 and mutually crossed configuration, whilst PA-TFC membranes have a flat sheet
39 physical structure and their elements have spiral wound configuration.

40 Biofouling of polymeric membranes impacts the operation and economic aspects of
41 seawater reverse osmosis (SWRO) desalination processes (Khedr, 2002). Chlorination of
42 feed water is a commonly used strategy to inhibit biofilm formation in RO systems
43 (Nguyen et al., 2012). However, PA membranes, which are the most commonly used type
44 of RO membranes, cannot withstand chlorine exposure even at low ppm concentrations.
45 On the other hand, CTA membranes exhibit some resistance against chlorine at
46 concentrations of up to 5 mg Cl_2/l and 0.2 mg Cl_2/l for short and long exposure times,
47 respectively (Kumano, 2012; Kumano and Fujiwara, 2008). This chlorine tolerance
48 feature of CTA membranes allows the use of chlorine inside RO membrane vessels.
49 Previous studies have claimed that CTA-RO membrane exposure to chlorine would
50 prevent biofouling (Johnson and Busch, 2010; Kumano and Fujiwara, 2008).

51 Many studies conducted on fouling analysis of PA-TFC membranes can be found in the
52 literature. However, only few investigations (Butt et al., 1995, 1997a, 1997b, 1997c;
53 Farooque et al., 2005; Fujiwara and Matsuyama, 2008; Saeed et al., 2000) have focused
54 on fouling problems of CTA-RO membranes. Additionally, the membrane performance
55 data has been used to characterize the fouling intensity (Butt et al., 1997c; Fujiwara and
56 Matsuyama, 2008; Saeed et al., 2004) without supporting analysis of fouling layer and

57 also without investigating the efficacy of chlorination as anti-biofouling strategy. In some
58 cases, if the analysis of fouling layer was performed, the approach remained limited to
59 microscopic methods (Butt et al., 1997b; Farooque et al., 2005). Some of these studies
60 only used simple coulometric, chromatographic, and/or spectroscopic analytical
61 techniques (Butt et al., 1995, 1997a, 1997b). Organics, which have been reported as the
62 major fraction of the fouling material isolated from these membranes (Butt et al., 1997b),
63 have never been well characterized. Similarly, an analysis of microbial communities
64 found in water samples and in the membrane fouling layer of these plants has not been
65 conducted.

66 In this project, the efficacy of chlorination as anti-biofouling strategy was studied by
67 investigating the fouling profile and scenario developed on a full-scale SWRO
68 desalination plant operating with not only continuous chlorination of raw seawater but
69 also intermittent chlorination of CTA-RO membranes. Detailed water quality and
70 membrane fouling analyses were performed by using advanced analytical techniques
71 including Fourier transform infrared (FTIR) spectrometry, pyrolysis gas chromatography
72 coupled with mass spectrometry (Pyrolysis/GC-MS), ^{13}C -nuclear magnetic resonance
73 (NMR) spectrometry, inductively coupled plasma optical emission spectrometry
74 (ICP-OES), elemental analysis, adenosine-5'-triphosphate (ATP) content analysis, and
75 16S rRNA gene-based sequencing of microbial community.

76 **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 m³/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 1st stage enters a 2nd 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 (H₂SO₄), addition of ferric chloride (FeCl₃; 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.

100 **2.2. Sampling plan**

101 Two sampling campaigns were performed: T_1 (conducted in May 2012) and T_2
 102 (conducted in October 2012). Both membranes and water samples collected by triplicates
 103 from various points in the pretreatment train were analysed. All the chlorine containing
 104 water samples were immediately de-chlorinated by adding SBS solution at a final
 105 concentration of 2–3 mg/L (stoichiometrically, 1.98 mg of SBS are required for the de-
 106 chlorination of 1 mg of free chlorine as HOCl). Water samples collected for flow
 107 cytometric (FCM) analysis of phytoplankton and bacterial cells were fixed by adding
 108 aqueous glutaraldehyde solution (Polyscience, cat# 1909–10) at a final concentration of
 109 2% in volume.

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

115 **2.2.1. *1st Campaign, May 2012 (T_1)***

116 During the 1st campaign (i.e., T_1) a fouled SWRO module (T_1 –M) operated for 23 months
 117 was autopsied (Table 1). Membrane fibers were collected from feed to middle regions
 118 (Figure 2) where the intensity of fouling was visually higher. Water sampling points
 119 included intake water (raw seawater), after Cl_2 , before DMF (DMF Inlet), after DMF
 120 (DMF outlet), SWRO Inlet (after MCF and SBS), SWRO permeate, and SWRO Brine.

121 **2.2.2. *2nd Campaign, October 2012 (T_2)***

122 During the 2nd campaign (i.e., T₂), two SWRO modules were autopsied: one after
123 operating for 1 month (T₂-fouled membrane/module A, T₂-MA), the other after
124 operating for 27 months (T₂-fouled membrane/module B, T₂-MB) (Table 1).

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

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

131 **2.3. Methods**

132 Fouling load, ATP contents, Scanning Electron Microscopy (SEM), FT-IR, Pyro/GC-
133 MS, solid state ¹³C-NMR spectroscopy, ICP-OES, CHN and phylogenetic analyses were
134 performed to characterize the membrane fouling material. Additionally, water quality was
135 examined by pH, conductivity, turbidity, Silt Density Index (SDI₁₅), Total/Dissolved
136 Organic Carbon (TOC/DOC), Size Exclusion Liquid Chromatography coupled with an
137 Organic Carbon Detector (LC-OCD), ATP, Heterotrophic Plate Count (HPC), and Flow
138 Cytometry (FCM) analyses/measurements. All these analytical procedures have been
139 discussed in Khan et al., 2013a; 2013b. Details of some analytical procedures exclusively
140 implemented during this study (i.e., acid dialysis, FCM analysis, and pyrosequencing data
141 processing) are described here after.

142 **a) *Cleaning of foulant material***

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

149 **b) *Flow cytometric analysis***

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

163 ***c) Pyrosequencing data analysis***

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

176 **3. Results and Discussion**

177 **3.1. Water Quality Analysis**

178 **3.1.1. Physicochemical characterization**

179 TOC/DOC values of raw seawater (i.e., 1.86/1.68 mg/L) were higher than at other
 180 sites/plants studied along the Red sea coast (i.e., 1-1.2mg/L (Khan et al., 2013a; 2013b)).
 181 SDI₁₅ (>>5) and turbidity (3.8 NTU) values also revealed that the fouling potential of this
 182 source water was higher than at other sites. Other physicochemical water quality
 183 parameters, i.e., pH and conductivity (cond.) values were similar to any other typical Red
 184 Sea water sample (Table 2).

185 The decrease in TOC/DOC in water sample before DMF/after coagulant addition was due
 186 to coagulation/flocculation taking place in the sampling bottle (Table 2). Some coagulant
 187 flocs we observed settled at the bottom of sampling bottles. As shown by the LC–OCD
 188 profiles (Figure 3), removed organics mainly belong to the biopolymer fraction. A part of
 189 humic–like substances was also removed after coagulant injection. Further decrease in
 190 biopolymers can be observed after DMF. Surprisingly, TOC/DOC contents increased in
 191 T_1 –after SBS sample. LC–OCD chromatogram confirmed an increase of the signal in the
 192 region assigned to medium to lower molecular weight organics that might be attributed to
 193 a malfunction of MCF. To further investigate the possible reason of this TOC/DOC
 194 increase, one more sample was collected immediately after MCF (i.e., before SBS dosing
 195 point) during T_2 sampling campaign. Although T_2 –after SBS sample showed little
 196 increase in LC–OCD signals (i.e., similarly to T_1 –after SBS sample) (Figure 3), no
 197 evident increase in TOC/DOC was observed in the water samples either after MCF or
 198 after SBS treatment points. These observations suggest that most likely at the time of T_1
 199 sampling, MCF was in or near the saturation state and started leaching organics (i.e.,
 200 organic material trapped in or produced from biological activity growing in these filters).

201 3.1.2. Microbiological characterization

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

206 Flow cytometric (FCM) analyses were performed to determine the bacterial and
 207 phytoplankton cell counts in the water samples. While taking into account analytical error

208 due to non-living particles and a narrow difference between background of free stain and
209 intensity of signal of stain bound to cellular DNA, any value of detected cell count ≤ 200
210 cells/ml was considered negligible (Table 2).

211 Bacterial cells were counted and classified as low DNA (LNA) and high DNA (HNA)
212 containing cells while using SYBR[®] Green-I as DNA stain. Most researchers have
213 reported HNA as actively growing cells (Lebaron et al., 2001). Total bacterial cell count
214 in the raw seawater was in the order of 10^6 to 10^7 cells/ml, which is comparable to
215 previously reported bacterial cell count values of Red Sea water (Khan et al., 2013a).
216 Bacterial cell count was not decreased to a large extent right after chlorination in both T_1
217 and T_2 water samples. This observation might be due to immediate preservation/fixing of
218 samples and short Cl_2 contact time to destroy the cell structures. Total number of
219 bacterial cells was in the order of 10^5 to 10^6 with LNA and HNA cell count of 3.1×10^5
220 and 8.7×10^5 cells/mL in T_1 -raw seawater; and 9.1×10^4 and 8.1×10^5 in T_2 -raw seawater
221 samples, respectively (Table 2). However, in all other samples, bacterial cell counts
222 significantly dropped probably due to relatively longer chlorine reaction time and other
223 downstream pretreatment steps, i.e., coagulation and filtration. It should be noted that the
224 cell count increased in T_1 -after MCF and SBS samples. Similar to the TOC/DOC
225 increase, the increase in bacterial cell count might also be attributed to their detachment
226 from MCF and/or rapid growth of bacteria following the addition of a de-chlorinating
227 agent, i.e., SBS. Such increase in bacterial cell count was not observed in T_2 -after MCF
228 and T_2 -SBS samples. This finding support the hypothesis that during T_1 sampling
229 campaign, the MCF was not working properly and instead of improving the water quality
230 it was probably serving as source of microbial and organic contamination.

Phytoplankton cell count in raw seawater was 4.4×10^4 cells/mL, a value comparable to the others previously detected in water at different sites in the Red Sea (not shown). After chlorination, all T₁ and T₂ 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

275 contribution of microbial activity in the fouling of these fibers was confirmed from the
276 calculated values of their ATP contents present per unit mass of the fouling layer (Figure
277 5). These biofilm forming bacteria were either resilient enough to survive the chlorine
278 stress or they might have been shielded from the chlorine disinfection. This finding
279 proved that intermittent chlorine (0.25 – 0.30 mg/L) exposure is not capable of totally
280 eradicating biofilm formation in RO module; a situation that has also been observed in
281 chlorinated drinking water distribution systems (Hwang et al., 2012; Mathieu et al.,
282 2009).

283 The relatively effective development of biomass in the posterior regions of T₂-MB might
284 be explained by poor or no penetration of Cl₂, which resulted in no or little impact on
285 microbial growth. Plugging of fibers, due to limited free space between them, has been
286 pointed out as one of the major operational problems of HFF modules (Gorenflo et al.,
287 2005). Plugging of fibers may create dead zones where biofilm formation might have
288 occurred without being affected by chlorine. Another reason of low penetration of
289 chlorine might be the oxidant demand of the organics accumulated in the front side
290 fibers.

291 In the case of T₂-MA, both fouling load and active biomass per unit surface area
292 uniformly decreased from its front to rear end fibers (Figure 5). In contrast to T₂-MB,
293 significantly lower ATP contents and a continuous decrease in ATP values across the
294 feed to brine sides of this permeator/module suggest that intermittent chlorine was able to
295 uniformly reach all the fibers. Uniform distribution of chlorine might have been favored
296 by relatively less plugging issues in this module which was operated for only 1 month.
297 Low plugging/fouling of this permeator was verified by low fouling load values. The

298 same reason (i.e., better/uniform penetration of fed chemical agents at early stages of
299 fouling) may justify the better efficacy of the cleaning agent.

300 Estimation of organic and inorganic fractions of foulant material isolated from membrane
301 fibers was conducted by loss on ignition (LOI) test (Figure 6). It should be noted that the
302 LOI test was performed on dried foulant obtained after Milli-Q dialysis followed by
303 lyophilization process. As acid dialysis might change the relative proportion of organics
304 and inorganics through the removal of inorganic foulants, LOI test was performed on the
305 foulants only after desalting with Milli-Q but prior to any acid dialysis. Because LOI test
306 requires approximately 50 mg of foulant mass, it could not be performed on the foulant
307 collected from brine region fibers of T₂-membranes where a small amount of material
308 was recovered.

309 The organic material was predominant in T₁-M and T₂-MB foulants. The strong organic
310 character and relatively high ATP content of foulant material deposited on these
311 membranes were indicators of biofouling scenario. Conversely, T₂-MA foulant was
312 characterized by a slightly higher proportion of inorganic constituents.

313 **3.2.2. Elemental composition of foulants**

314 Elemental composition was assessed using direct (SEM-EDX given as in Supplementary
315 Information-1) and indirect (ICP-OES and CHN analyser) analytical techniques.

316 ICP-OES analysis results (Figure 7) were in accordance with the findings of EDX
317 analysis (SI). Fe was the most abundant element detected in all the foulant samples.
318 Organic analysis of foulants, i.e., ¹³C-NMR and FT-IR spectroscopic analyses and CHN
319 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

343 cleaned membrane (T₂-MA) suggested no or minor removal of these elements by acid
344 cleaning. A similar result has been observed when the oxalic acid dialysis was performed
345 with T₁M foulant. The higher Al and Si concentrations were attributable to the presence
346 of alumino-silicates which might be contributing in membrane fouling phenomenon,
347 especially during initial phases (i.e., plugging of fibers). Significant fouling of CTA-RO
348 membranes by alumino-silicates has also been reported by Butt et al., 1995, 1997a,
349 1997b.

350 In the case of T₁-M foulant (before and after OA and HFA dialysis), CHN analysis
351 showed low C/N ratios (i.e., 6.78 and 7.64, respectively) that could be attributed to a
352 significant incorporation of proteinaceous structures. This result showed that dialysis
353 procedures had minor impact on C/N ratios. An increase in the relative abundance of all
354 the organic elements, i.e., CHN after OA dialysis and HFA dialysis, is due to the removal
355 of inorganics, mainly Fe and Si species, respectively (Figure 8). All the foulant samples
356 of T₂ campaign were subjected to CHN analysis after OA dialysis. In case of both sets of
357 T₂-foulant samples, C and N contents increased along the feed water flow path, i.e., from
358 front/feed to brine side. A continuous decrease in C/N atomic ratio can be observed from
359 feed to brine side (Figure 8), result that could be interpreted as an increase in nitrogen
360 containing structures in the foulant of fibers located behind the feed region. Previously,
361 similar low C/N values (i.e., 4–8) have been reported for marine bacterial cells (Bhosle,
362 2004; Müller, 1977) and actively growing biofilms (Bhosle, 2004; Khan et al., 2013a,
363 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.

386 Solid state ^{13}C -NMR spectroscopic analyses were conducted to further explore the nature
387 of the organic component of foulant material. This analysis of T_1 -M foulant was
388 performed before and after OA dialysis. After observing a positive effect on the removal
389 of inorganics (i.e., mainly Fe) on the spectrum of T_1 -M foulant sample, all the T_2 -
390 membrane foulants were subjected to OA dialysis prior to ^{13}C -NMR spectroscopic
391 analysis. ^{13}C -NMR spectra (Figure 10) are discussed in the light of data published by
392 Leenheer, 2009, and Khan et al., 2014; Khan et al., 2013b.

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

403 Similar to IR profiles, NMR spectra also indicated that most likely the origin of organic
404 matter was microbial activity. Additionally, in the spectra of foulants collected from
405 different regions of T_2 -permeators, an evolution or increase of the intensity of some
406 specific bands was observed across the feed to brine sides. These include the bands in
407 aliphatic region and characteristic bands of aminosugars (i.e., methyl group) and
408 polysaccharides (i.e., alcohol group) originating from substances derived from microbial

409 cells and EPS of biofilm, respectively. An increase in intensities of these bands in the
410 middle and brine region foulants may correspond to more developed biofilm in these
411 regions.

412 Pyrolysis/GC–MS analyses of foulants were performed to determine the relative
413 abundance of biopolymers (Supplementary Information-2). T₁–M foulant was subjected
414 to pyrolysis/GC–MS analysis before and after OA dialysis. The profile of
415 pyrochromatograms of T₁–M foulant, before and after OA dialysis, was almost identical.
416 On the basis of this observation, T₂–foulants were pyro–analysed without performing OA
417 dialysis. Foulants from all the samples exhibited almost similar biopolymers distribution
418 with few differences.

419 In all foulant samples, the relative abundance of aromatic amino acids, i.e., tyrosine
420 (phenol and p-cresol pyrofragments) and phenylalanine (toluene and styrene
421 pyrofragments) was quite significant (Bruchet et al., 1990; Christy et al., 1998). Signals
422 of aminosugars (acetamide peak) and small chain fatty acids (C10–C18) in the
423 pyrochromatograms of foulant material were good evidence of biogenic matter (Khan et
424 al., 2013b). Significantly higher relative abundance of signal for polyhydroxybutyrates
425 (PHBs; crotonic acid peak), known as energy storage molecules of microbes released
426 under stressful condition (Dawes, 1988), detected in all samples also indicated the
427 presence of biofilm in the permeators. Relative abundance values of polysaccharide and
428 lipids/fatty acids signals increased from feed to brine region foulant samples of both T₂
429 permeators, a finding in agreement with the results of FTIR and ¹³C–NMR spectroscopic
430 analyses.

431 **3.2.4. Ultrastructural analysis**

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

439 No significant difference could be identified among the feed, middle, and brine region
440 fibers of permeators in terms of abundance of microbial structures and morphological

441 features of fouling layer. The only observed difference was the relatively more abundant
 442 inorganic salts like structures on brine side fibers.

443 **3.2.5. Microbial community analysis**

444 ***Comparison of microbial communities on T₁-M and T₁-water samples***

445 The microbial communities on T₁-M fibers shared low similarity of 18.5% with those
 446 present in the water samples and clustered apart from the suspended microbial
 447 communities (Figure 11). Within the attached microbial community, the genus
 448 *Acinetobacter* accounted for the highest relative abundance of 36.1%. The other
 449 remaining abundant bacterial populations on the T₁-M include *Ralstonia* (15.1%),
 450 *Diaphorobacter* (11.3%), unclassified Comamonadaceae (10%), *Stenotrophomonas*
 451 (8.5%), and unclassified *Enterobacteriaceae* (6.5%). These bacterial populations were
 452 usually present in lower relative abundance in the water samples, ranging from 0.3% to
 453 3.3% of total microbial community. The higher relative abundance of these bacterial
 454 populations on the membrane compared to the water samples suggests a preferential
 455 concentration and attachment of these microorganisms on the membrane surface.

456 ***Microbial community on T₂-Membranes***

457 Microbial communities differed at the feed, middle, and brine ends of the T₂-MB (Figure
 458 11). Specifically, the feed end shared a 65.3% similarity with the middle and brine ends,
 459 while microbial communities in the middle and brine ends shared a 79.8% similarity. The
 460 relative abundance of unclassified *Alphaproteobacteria*, as well as genera
 461 *Janthinobacterium* and *Pedobacter*, decreased from the feed to brine end of the
 462 membrane. In addition, genera *Janthinobacterium* and *Pedobacter* were more than 3-fold

463 higher in relative abundance at the feed end than the brine end, suggesting an effective
464 removal of these bacterial populations along the membrane module (Figure 12).
465 However, *Silicibacter*, *Pseudomonas* and unclassified *Rhodobacteraceae* were present at
466 more than 1.9-fold higher relative abundances on the brine end than feed end of the
467 module (Figure 12). Fiber plugging and/or consumption of chlorine by the organic
468 foulants accumulated on the feed region fibers may have lowered the concentration of
469 chlorine at the middle and brine end of the modules. An increase in the relative
470 abundance of *Silicibacter*, *Pseudomonas*, and unclassified *Rhodobacteraceae* at the
471 middle and brine ends may indicate that these bacterial populations are able to survive
472 with lower residual chlorine concentration. ATP analyses of the membrane recorded 2.4–
473 fold increase in the ATP concentration at the middle end relative to the feed end. This
474 increase in ATP concentration coincides with an increase in the relative abundance of
475 *Silicibacter* and unclassified *Rhodobacteraceae* from the feed end to the brine end of the
476 module, and likely suggests that these two bacterial populations may be resistant to the
477 residual chlorine and remained biologically active on the membrane. Although the high-
478 throughput sequencing performed in this study does not allow bacterial identification at
479 the species level due to the short read length; previous studies have shown that species
480 within the genus *Pseudomonas* overexpresses production of polysaccharide in the
481 extracellular polymeric matrix as a defensive mechanism against residual chlorine (Grobe
482 et al., 2001; Xue et al., 2013). Furthermore, sugar-like trehalose can be produced by
483 *Pseudomonas* as a compatible solute in response to increasing salinity from the feed to
484 the brine end of the module (Diab et al., 2006). The overexpression of polysaccharide by
485 *Pseudomonas*, as well as the increase in its relative abundance, are therefore likely to

486 account for the relatively higher concentration of sugars detected on the fibers located at
487 the brine end of the module.

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

494 *Pseudomonas* spp. was present in high relative abundance of 24.9%, 5.8%, and 64% of
495 the total microbial community in the feed, middle, and brine end of the T₂-MA,
496 respectively (Table 3). Furthermore, spore-forming unclassified *Bacillales* were detected
497 at a high relative abundance in the feed end of T₂-MA (3.6%). Gram-positive
498 *Streptococcus* and *Staphylococcus* were also detected at a relative abundance of 10.3%
499 and 2.7% (Table 3). *Acidocella*, a genus associated with acidophiles, were present at
500 3.1% of total microbial community. These observations indicate that certain bacterial
501 populations (e.g. spore-formers, gram-positive bacteria and acidophiles) were likely able
502 to withstand the cleaning process.

503 3.2.6. Fouling dynamics and mechanism

504 Continuous trend of increase in the relative abundance of aminosugars and fatty acids in
505 the foulant material collected from lead to rear end region fibers is a strong evidence of
506 higher microbial activity occurred in the posterior region of the module. This increasing
507 trend in the relative abundance of the mentioned biogenic material is highlighted by

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

526 Higher microbial activity in the rear end regions is protected against disinfection due to
527 the inaccessibility of chlorine to these areas. This hindrance in the disinfection could be
528 due to the phenomenon of fiber plugging and/or due to consumption of chlorine by the
529 inorganic/organic foulants accumulated on the feed region fibers. This hypothesis is
530 supplemented by the observation of a uniform decrease in the ATP contents, and on

531 contrary, a non-uniform trend of ATP contents from feed to brine side fibers in the less-
532 fouled module (i.e., T₂–MA) and more-fouled (i.e., T₂–MB) module, respectively. In case
533 of T₂–MB, a lower intensity of microbial activity (lower ATP contents per unit surface
534 area and low fouling load) in the brine side fibers than in middle side fibers might be due
535 to the decrease in the nutrients reaching this region. The accumulation of organic and
536 inorganic material in the front side fibers seems to play a major role in the proliferation of
537 sustainable microbial activity/biofouling in the rear end fibers.

538 Some researchers have reported that under hyper-saline conditions, bacterial cell start
539 synthesizing more sugars in order to adapt to the osmotic pressure (Roeßler and Müller,
540 2001). Higher sugars in the rear end fibers might also be assigned to their increased
541 biosynthesis in response to increased salt concentration in the feed stream.

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

553 **4. Conclusion**

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

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Figure Captions

Figure 1: Schematic description of SWRO plant design. (Cl₂: Chlorination, H₂SO₄: Sulfuric acid, FeCl₃: 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. e) Foulant material after removal of inorganics by acid dialysis.

684 **Figure 3:** LC–OCD analysis of water samples (Mol.: molecular; MW: molecular weight;
 685 min: minutes; NOM: natural organic matter; BDOC: biodegradable dissolved organic
 686 carbon).

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

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

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

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

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

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

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

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

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

715

716 Table Captions

717 **Table 1:** Membrane sampling: Sampling region, operation time, and performance data.

718 **Table 2:** Physicochemical analysis of water samples (SW: seawater, cond.: conductivity,
719 SDI₁₅: silt density index measured after 15 minutes of filtration, TOC/DOC:
720 total/dissolved organic carbon, ATP: Adenosine triphosphate, HPC: Heterotrophic plate
721 count, CFU: colony forming units, LNA: low nucleic acid cells, HNA: high nucleic acid
722 cells, SD: standard deviation. *: within the range of analytical error).

723 **Table 3:** Bacterial populations that were detected at relative abundance >2% of total
724 microbial community at the feed end of the T₂-MA. N.D. denotes not detected.

Table 1: Membrane sampling: Sampling region, operation time, and performance data.

	<i>Sample</i>	<i>Position in the Vessel</i>	<i>Fiber Sampling Region</i>	<i>Operating Time (Months)</i>	<i>No. of Cleaning Cycles*</i>
T_1	<i>Fouled Membrane (T_1-M)</i>	1st	Feed to Middle	23	3
T_2	<i>Fouled Membrane A (T_2-MA)</i>	1st	Feed, Middle and Brine	1	1
	<i>Fouled Membrane B (T_2-MB)</i>	1st	Feed, Middle and Brine	27	4

* Cleaning was conducted when the permeability was decreased by 10–15%.

Table 2: Physicochemical analysis of water samples (SW: seawater, cond.: conductivity, SDI₁₅: 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).

	Water Sample	pH	Cond. (ms/cm) (SD)	Turbidity (NTU) (SD)	SDI ₁₅ (SD)	TOC/DOC (mg/L) (SD)	ATP (ng/L) (SD)	HPC (CFU/mL) (SD)	LNA (Cells/mL) (SD)	HNA (Cells/mL) (SD)
T ₁	SW after Cl ₂	8.18	58.9	3.1	>>5	1.63/1.22 (0.09/0.08)	31.5 (5.2)	1.2x10 ² (5.0 x10)	3.1x10 ⁵ (1.3x10 ³)	8.7x10 ⁵ (2.2x10 ³)
	DMF inlet	6.53	59.2	3.3	-	1.12/0.87 (0.06/0.05)	<1	0	9.5x10 ³ (8.1x10)	4.0x10 ⁴ (1.1x10 ²)
	DMF outlet	6.91	59	0.15	-	1.10/0.95 (0.06/0.05)	4.3 (2)	1.1x10 ² (7.0 x10)	6.0x10 ³ (9.5x10)	3.8x10 ³ (8.6x10)
	After SBS	6.58	59.3	0.1	1.2	1.27/1.13 (0.07/0.06)	12.6 (4)	1.3x10 ² (6.0 x10)	1.0x10 ⁴ (1.0x10 ²)	6.5x10 ³ (97x10)
	SWRO permeate	5.45	0.32	0.16	-	0.15/0.15 (0.02/0.02)	<1	0	< 2.0x10 ² *	< 2.0x10 ² *
	SWRO brine	6.53	89.0	3.3	-	2.08/1.71 (0.09/0.09)	22.53 (6)	1.6x10 ³ (1.5x10)	3.1x10 ⁴ (1.3x10 ²)	1.9x10 ⁴ (1.1x10 ²)
T ₂	Raw SW	8.22	54.1	3.8	>>5	1.86/1.68 (0.09/0.08)	72.6 (5.6)	6.7 x10 ³ (6.0 x10 ²)	7.8 x10 ⁵ (1.9x10 ⁴)	5.6 x10 ⁵ (7.5 x10 ⁴)
	SW after Cl ₂	8.22	54.2	3.3		1.59/1.36 (0.08/0.07)	7.6 (2)	1.5 x10 ² (2. x10)	9.1 x10 ⁴ (6.5 x10 ³)	8.1 x10 ⁵ (4.2 x10 ⁴)
	DMF inlet	6.55	54.2	3.1		1.26/1.05 (0.07/0.05)	<1	0	2.5 x10 ³ (7.0 x10 ²)	3.2x10 ⁴ (4.0 x10 ³)
	DMF outlet	6.55	54.3	0.17		1.23/1.14 (0.07/0.07)	4.3 (2)	1.2 x10 ² (2.1 x10)	< 2.0x10 ² *	< 2.0x10 ² *
	MCF outlet	6.51	54.3	0.11	-	1.22/1.14 (0.07/0.06)	<1	1.3 x10 ² (6.0 x10)	< 2.0x10 ² *	< 2.0x10 ² *
	After SBS	6.49	54.3	0.11	1.2	1.23/1.15 (0.08/0.04)	5.51 (3)	1.5 x10 ² (3.5 x10 ²)	< 2.0x10 ² *	< 2.0x10 ² *
	RO permeate	4.94	0.38	0.08	-	0.21/0.21 (0.01/0.01)	<1	0	< 2.0x10 ² *	< 2.0x10 ² *
	RO brine	6.74	83.7	0.17	-	1.86/1.64 (0.09/0.09)	32.53 (5)	1.7 x10 ³ (5.5 x10)	1.3x10 ⁴ (2.0 x10 ³)	8.0x10 ³ (1.1 x10 ³)

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.

Bacterial populations	T ₂ -MA		
	Feed	Middle	Brine
	Relative abundance (%)		
<i>Corynebacterium</i>	2.3	0.1	N.D.
<i>Prevotella</i>	2.6	0.1	N.D.
<i>Flavobacterium</i>	2.1	1.4	19.6
<i>Burkholderia</i>	3.8	0.1	N.D.
<i>Acidocella</i>	3.1	0.1	N.D.
<i>Pseudomonas</i>	24.9	5.8	64.0
<i>Streptococcus</i>	10.3	0.4	N.D.
<i>Staphylococcus</i>	2.7	0.1	0.1
Unclassified <i>Bacillales</i>	3.6	0.2	N.D.
Unclassified Bacteria	2.9	11.9	0.4

Figure 1: Schematic description of SWRO plant design. (Cl_2 : Chlorination, H_2SO_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 (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 foulants 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 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) T_1 -M (feed/middle side) fibers. Middle) T_2 -MB (feed side) fibers. Bottom) T_2 -MA (feed side) fibers.

SI-2

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.