Dynamics of microbial communities in an integrated ultrafiltration-reverse osmosis desalination pilot plant located at Arabian Gulf

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Abstract

This study demonstrated the use of high-throughput sequencing to assess the efficacy of an integrated Ultrafiltration (UF)-Reverse Osmosis (RO) desalination pilot plant located at the Arabian Gulf, and to identify potential microbial-associated problems that may arise in this plant. When integrated into the desalination treatment system, the UF membranes were able to serve as a good pretreatment strategy to delay RO fouling by achieving up to 1.96-log removal of cells from the seawater. Consequently, the differential pressure of the RO membrane remained around 1 bar for the entire 6-month study, suggesting no significant biofouling performance issue identified for this RO system. Examples of microbial populations effectively removed by the UF membranes from the feed waters included *Nitrosoarchaeum limnia* and phototrophic eukaryotes. Microbial-associated problems observed in this pilot plant included the presence of *Pseudomonas* spp. in coexistence with *Desulfovibrio* spp. These two bacterial populations can reduce sulfate and produce hydrogen sulfide, which would in turn cause corrosion problems or compromise membrane integrities. Chemical-enhanced backwashing (CEB) can be used as an effective strategy to minimize the associated microbial problems by removing bacterial populations including sulfate-reducers from the UF membranes.

Keywords: High-throughput sequencing; biofouling; chemical-enhanced backwashing; *Pseudomonas*; sulfate-reducers

1. Introduction

Since the first implementation of desalination technology about 20-30 years ago, the Gulf Cooperation Council (GCC) countries have become increasingly reliant on the Arabian Gulf as a source for their desalinated water. Majority of the desalination plants in the GCC countries utilize Multi Stage Flash Distillation (MSF) but it is projected that the number of desalination plants utilizing reverse osmosis (RO) membranes will increase in the future as the technology becomes more affordable [1, 2]. Compared to MSF, RO membrane-based systems are designed with a series of pretreatment steps as RO membranes can become adversely affected by rapid fouling. The problem is further compounded by the complexity of the Arabian Gulf waters, which tend to have high salinity, high organic content, high temperatures and microbiological activity due to limited exchange of seawater with the open sea. The culmination of these factors would mean that RO membranes are subjected to high fouling risks.

Given that the fouling rates of the RO membrane are strongly linked to the quality of feed water [3], an effective pretreatment method is needed to prolong the operating longevity of RO membranes and to ensure the economic sustainability of seawater reverse osmosis (SWRO) desalination plants. Conventional pretreatment systems that have been widely used include flocculation/coagulation and dual media/sand filtration [4-6] but these systems are generally not able to provide a stable silt density index (SDI) value in the RO feed water [5]. Changing sea water conditions result in high total suspended solids (TSS) which can result in breakthrough during conventional pretreatment and will adversely impact the performance of downstream RO systems. Furthermore, large amount of chemicals have to be consumed to achieve good agglomeration of particulates for effective removal by dual-media filtration [6]. Several studies have concluded that ultrafiltration (UF) membranes can produce pretreated water quality that is more superior to that obtained from conventional pretreatment systems [4, 5].

Prior assessment of UF as a pretreatment emphasizes on the ability to lower the SDI value in feed water and the removal of cell counts [5, 7]. However, there remain three main questions that need to be explored in depth. Firstly, microbial populations that are removed by the UF should be assessed as specific bacterial populations can play an important role in the biofouling of the SWRO membranes [8]. Secondly, for a UF system to be successful, the accumulated foulants on the membrane should be monitored to determine if they are effectively removed by either backwashing or chemically-enhanced backwashing (CEB) [9]. Thirdly, although UF has achieved high bacteria removal, regrowth can occur in the system downstream of the UF due to remaining nutrients in the feed water and is very relevant to the fouling of RO membranes. High-throughput sequencing technology can be used to address all three questions by providing insights on the dynamics of microbial populations on the membranes and in the water samples collected along the desalination process. However, most of these studies were performed on seawater in other geographical locations which has water quality distinct from that in the Arabian Gulf [8, 10-12] and limited studies were conducted on the Arabian Gulf waters [13].

This study aims to utilize high-throughput sequencing to assess the efficacy of UF membranes as pretreatment in a SWRO plant desalinating seawater from the Arabian Gulf. Water samples were collected throughout the desalination process to provide comparative analyses on the microbial populations that were removed by the UF membranes. This study also aims to identify potential microbial-associated problems that may arise in this integrated UF-RO desalination plant. The microbial populations attached on the UF membranes before and after CEB were identified and compared to those attached on the RO membranes.

2. Materials and methods

2.1. Desalination plant treatment process and sampling points

Sampling was performed in a pilot-scale desalination plant located in Jubail, Saudi Arabia (Figure 1). The desalination plant utilized an integrated ultrafiltration (UF)-reverse osmosis (RO) system to produce freshwater. The seawater intake line was located1.5 km from the coastline and took in seawater from the Arabian Gulf at a depth of 5 m below surface. Seawater was first dosed with an approximate concentration of 0.2 mg/L residual chlorine, and screened by three 130 µm strainers operating in parallel. The filtered seawater was then stored in a buffer UF feed tank, and pumped through two parallel modules of outside-in (O-I) hydrophilic-modified PVDF UF modules, with a nominal pore diameter of 0.03 µm. The UF modules were backwashed for a short duration of < 2 min at every hour interval, and chemically cleaned with 350 mg/L sodium hypochlorite for 10-15 min at every 24 h interval. The UF filtrate from both modules was collected in a RO feed tank where 1 mg/L industrial-grade antiscalant and sodium metabisulfite (SMBS, $Na_2S_2O_5$) were dosed. The water was then pumped through 5 µm cartridge filters towards a dual-stage RO pressure vessel that has 4 module elements in each stage. The UF membranes were operated at a flux of 70-85 L/m^2/h (LMH). The polyamide thin-film composite RO membrane was continuously operated at 15.2 LMH and at 50% recovery for almost 6 months without any noticeable increment in the differential pressure, scaling or biofouling on the RO membranes. The differential pressure for the RO was stable at approximately 1 bar.

2.2. Sample Collection and Preparation

Two sampling trips were performed on 2 and 23 December 2013. For each trip, water samples were aseptically collected in 10 L bottles at six locations along the treatment process. The samples were denoted on Figure 1 and included raw seawater, chlorine-dosed seawater prior to UF, filtrate after UF, antiscalant- and SMBS-dosed filtrate prior to RO, RO permeate after 1st

stage RO pressure vessel, and the combined RO permeate from 1st and 2nd stage RO pressure vessels. Water samples were stored at 4 °C prior to subsequent analyses. UF membranes, both fouled and chemically cleaned, as well as the 1st, 4th and 8th module element of the 1st stage RO membranes were autopsied on-site on 23 December 2013. Approximately 500 g of capillary UF fibers from the mid and bottom sections of the cartridge were individually placed into aseptic collection bag. Approximately 6 sheets, each of dimensions 40 x 40 cm of flat sheet RO membranes were collected from the feed, mid and brine section of the 1st, 4th and 8th module element. After cutting subsections of the RO membranes, the entire 1st, 4th and 8th module elements were flushed through with water, and collected for the respective foulant concentrates. All samples were immediately shipped to laboratory in KAUST, and were stored at 4 ^{o}C for 1-2 days prior to further preparation and analyses as detailed in sections 2.3 through 2.6.

2.3. Chemical water quality and membrane foulant load analysis

Non-particulate organic carbon (NPOC) was measured by the high-temperature catalytic oxidation (HTCO) method using a commercially available automatic TOC-V_{CPH} analyzer (Shimadzu, Japan). Samples were pre-filtered through 0.4 µm syringe filters and used for analysis. One blank deionized water and positive control of known NPOC concentration were analyzed along with the samples following the methodology recommended by Shimadzu. Foulant load analysis on the RO membranes was conducted as described previously [14].

2.4. Enumeration of microorganisms

Flow cytometric analysis was performed on BD Accuri™ C6 flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA). Water samples were diluted by 50-fold in 1X PBS, and stained in a final concentration of 1X Invitrogen SYBR® Green nucleic acid stain (Thermo Fisher Scientific, Carlsbad, CA, USA) based on protocols described by manufacturer. Briefly, 1000 µL of 50-fold

diluted water samples were incubated in the dark for 10 min at 35 ^{o}C, then stained with 10 µL of SYBR® Green and incubated further for 10 min at 35 ºC before measurement. Quantitative PCR (qPCR) was also performed to determine copy numbers of *Pseudomonas aeruginosa* based on primer assays Pae-F (5'- CCGACTGACGCCAACGA -3') and Pae-R (5'- CGACCCTACCTCCCACGAAT -3'). These primers target toxin A synthesis regulating regA gene of *P. aeruginosa* [15]. qPCR standards for regA gene was prepared by individually cloning the gene amplicons into Invitrogen pCR^{TM}4-TOPO® TA vector (Thermo Fisher Scientific, Carlsbad, CA, USA). Plasmid DNA was extracted using PureYield^{TM} Plasmid Miniprep System (Promega, Madison, WI, USA). The extracted plasmid was sequenced to verify the oligonucleotide sequences of gene insert, and quantified for its copy numbers per µL. A 5-points standard curve was generated using a serial dilution within the range of 10^{10} to 10^{2} copies/µL. Amplifications to obtain standard curves, test amplifications and negative blanks were run in duplicates. Each reaction volume of 20 µL contained 10 µL of Applied Biosystems® FAST SYBR Green master mix (Thermo Fisher Scientific, Carlsbad, CA, USA), 0.4 µL of each primer (10 µM), 1 µL of DNA template and 8.2 µL of molecular biology grade water. The reactions for amplification were carried out using the standard curve (AQ) assay and standard mode on Applied Biosystems® SDS version 2.3 software (Thermo Fisher Scientific, Carlsbad, CA, USA). The protocol includes 40 cycles of 1 s denaturation at 95 ^{o}C and 60 s of annealing and extension at 60 ^{o}C. Dissociation curve analysis was included to detect non-specific amplification. The C_q value was automatically defined by the software as the cycle number at which fluorescence passes the detection threshold. Standard curve for each tested gene was obtained by plotting the C_q value for each dilution point against the log-transformed concentration of each dilution. The amplification efficiency of the regA gene primer assays was 1.89, and the R-squared value was

0.98. All negative controls have C_q values > 35 and samples were deemed to have non-detectable abundance of regA genes if the C_q in the sample was > 33.

2.5. Microbial enrichment

Microbial enrichment was performed when the presence of sulfate-reducing bacteria was suspected. Approximately 1 g of the capillary UF fibers collected at the top, mid and bottom section of UF cartridge, as well as 4 x 2 cm of flat sheet RO membranes collected from feed, mid and brine sections of RO cartridge were individually placed into centrifuge tubes containing 30 mL of sterile Difco marine broth 2216 (BD Biosciences, Franklin Lakes, NJ, USA). The cultures were placed in a 37 ^{o}C static incubator for 48 h to enrich for the microbial consortium. After 48 h, the cultures were observed for their phenotypic traits and 1 mL of cell culture was centrifuged in a 1.5 mL microcentrifuge tube at 12,000 rpm for 10 min to obtain a cell pellet for DNA extraction.

2.6. Sample preparation and DNA extraction

Two liters of raw seawater and chlorinated seawater were individually filtered through 0.4 µm Whatman Nuclepore^{TM} track-etched polycarbonate membrane filters (GE Healthcare Life Sciences, Little Chalfont, Buckinghamshire, UK). All other water samples were filtered through 0.4 µm polycarbonate filter in 10 L volume. To prepare the UF membranes for DNA extraction, a total of 1 g of hollow fiber UF membranes was aseptically cut and suspended in 5 mL of 1X PBS. The suspension was then vortexed briefly to dislodge loosely bound biomass, and ultrasonicated with amplitude intensity of 25% for 5 min (Qsonica, Newtown, CT, USA). The supernatant was recovered and centrifuged at 12,000 rpm for 10 min to obtain cell pellet. The flat-sheet RO membranes were swabbed with sterile cotton tips to recover attached biomass. Approximately 12 x 22 cm and 23 x 30 cm of membrane surface areas were swabbed for the 1st

and 4th module element, respectively. The swabbed cotton tips were suspended in 5 mL of 1X PBS, vortex briefly, ultrasonicated and centrifuged in a similar manner as described earlier. No swabbing was performed for the membranes harvested from the 8th module element as biomass was not apparent on these membranes. Instead, 1 mL of biomass collected from flushing the entire 8th module element was centrifuged at 12,000 rpm for 10 min to obtain cell pellet for DNA extraction. Biomass on the polycarbonate filters as well as cell pellets from membranes and enrichment cultures were extracted for their total genomic DNA using the UltraClean® Soil DNA Isolation Kit (MoBio, Carlsbad, CA, USA) using protocol described previously [16].

2.7. Barcoded PCR amplification for Ion Torrent sequencing

Ion Torrent PGM™ sequencing was conducted to examine the microbial communities of all collected samples except those from RO permeate. This is because the amount of DNA extracted from 10 L of RO permeates was below detection limit of Invitrogen Qubit® 2.0 fluorometer (Thermo Fisher Scientific, Carlsbad, CA, USA) and therefore not analyzed further for the microbial communities. PCR for 16S rRNA gene-based Ion PGM™ sequencing was carried out using the barcoded 515F: (5′-Barcode-GTGYCAGCMGCCGCGGTA-3′) and reverse 909R: 5′-CCCCGYCAATTCMTTTRAGT-3′) primers. PCR reaction mixtures were prepared by pipetting 0.3 µL of 0.025 U/µL Ex Taq polymerase (Takara Bio, Dalian, China), 20 µL of 2X Epicentre Biotechnologies FailSafe™ Premix F (Illumina, Madison, WI, USA), 0.8 µL each of 10 µM each of forward and reverse primer, 17.6 µL H_2O and 16 ng of DNA template. The thermal cycling program used included an initial denaturation stage at 95 °C for 3 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 45 s and extension at 72 °C for 60 s, and then a final extension stage at 72 °C for 10 min. Amplicons were excised from gels and purified with the Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA). Purified

amplicons were submitted to KAUST Genomics Core lab for Ion Torrent PGM™ sequencing on two 314 chips.

2.8. Data analysis

All sequences with Phred score > 20 were sorted based on the barcodes to form a total of 62 sample libraries. Sequences were then trimmed off for the primers, barcodes and adaptor sequences. Trimmed sequences that were less than 350 nt in length were removed by the KAUST Bioinformatics Team. The sequences were then further checked for chimeras on UCHIME [17] by referencing to a core reference set that was downloaded from Greengenes (i.e., gold strains gg16 – aligned.fasta, last modified on 19 March 2011). A total of 3030,336 sequences were obtained for the 62 libraries. RDP Classifier was used for taxonomical assignments of the aligned 16S rRNA sequences at 95% confidence level [18]. The hierarchical text files were downloaded and filtered on Microsoft Excel to denote the number of sequences assigned to each individual group of unclassified bacteria and bacterial genus. The relative abundance was calculated by dividing the number of sequences assigned to that group with the total number of sequences obtained for that sample. The relative abundances of all bacterial groups were collated into a single Excel worksheet for all samples, and imported into Primer-E v 5.4 to calculate square-root-transformed Bray-Curtis similarity matrix. Bray-Curtis is a statistic used to quantify the extent of similarities or dissimilarities in bacterial composition and abundance among different sample sets [19, 20]. All chimera-removed fasta files were also combined together with an in-house written Perl Script, and the collated file was used to identify for unique OTUs at 97% 16S rRNA gene similarity using CD-Hit [21]. The output file denotes the relative abundance of each unique OTU in each barcoded sample, and the nucleotide sequence of each OTU. To identify the phylogenetic affiliation of the OTU, the nucleotide

sequence was BLASTN against the NCBI nucleotide (nr/nt) collection database. Chimera-free sequences were aligned using the RDP Infernal Aligner, and were submitted for clustering analysis to generate rarefaction curves [22]. Microbial richness for each membrane sample was denoted from the rarefaction curves based on a defined sequencing depth of 10460 sequences. Rarefaction curves for water and membrane samples were shown in Figure 2.

3. Results

3.1. Water quality

Non-particulate organic carbon (NPOC) of the feed water ranged from 2.0 to 4.0 mg/L. The UF filtrate was successful in reducing the NPOC content prior to feeding the RO system as the RO permeate recorded NPOC values between 0.11 to 0.15mg/L. The mean SDI value for the feed water throughout operation was 5.67 ± 0.55, and with an average TDS of $4.38 \times 10^4 \pm 1.54 \times 10^3$ mg/L. UF as a pretreatment system reduced the SDI of incoming feed water to 2.73 ± 0.52 (Figure 3A). Flow cytometry counting revealed that the feed water contained an average 3.72×10^9 cells/L, and that the cell counts in the chlorinated feed water prior to UF remained at 4.37×10^9 cells/L. The UF membrane along with the 5 µm cartridge filter was able to achieve a 1.96-log removal of the microbial cells, effectively lowering the cell count in the UF permeate to 4.87×10^7 cells/L (Figure 3B). However, because SYBR® Green which was used to stain the cells prior to flow cytometry do not differentiate between dead and live cells, the proportion of such cells remained unknown before and after the UF pretreatment process.

3.2. Microbial populations removed by UF

The abundance and diversity of bacterial populations in water samples were determined by high-throughput sequencing. Dissimilarities in the microbial communities were evaluated on a multidimensional scaling plot (MDS). Microbial communities in the non-chlorinated and

chlorinated feed water samples shared 62.3% Bray-Curtis similarity and clustered together in the MDS plot as Group A waters (Figure 4). After the UF pretreatment, the average Bray-Curtis similarity between the microbial communities in the feed water (i.e., Group A waters) and those after UF (i.e., Group B waters) decreased to 39.5%, suggesting that a portion of the microbial communities in the feed water was effectively removed by the UF membranes. At a sequencing depth of 10460 reads, the average microbial richness in feed water showed a significant reduction from 1711 OTUs to 872 OTUs in the UF filtrates (One-way ANOVA, $F = 6.01$, $P = 0.04$). To further evaluate which bacterial populations were removed, all sequences were evaluated for their phylogenetic identities at the OTU level, and then compared for their relative abundances in the feed water samples and UF filtrate. Six OTUs (i.e., OTU153, OTU211, OTU397, OTU428, OTU534 and OTU984) identified to be uncultured *Nitrosoarchaeum limnia*, uncultured *Proteobacteria*, uncultured bacterium and phototrophic eukaryotes were significantly more abundant in the feed waters than the UF permeate (t-test, $P = 0.05$). Furthermore, these OTUs were present in high relative abundance on the UF membranes, ranging from 0.17 to 24.9% of the total microbial community (Table 1). In contrast, they were either absent or present in low relative abundance of up to 0.25% of the total microbial communities in the 1st RO module element. These observations indicate that certain bacterial populations, in particular the 6 above-mentioned OTUs, were effectively removed by the UF membranes.

3.3. Distinct differences in microbial community on UF and RO

The average microbial richness on UF and RO membranes was 740 and 828 OTUs, respectively, detected at a sequencing depth of 10460 reads, and were not significantly different (One-way ANOVA, $F = 0.31$, $P = 0.59$). However, microbial communities attached on the UF and RO membranes were distinctly different and shared a low average 30.9% Bray-Curtis similarity.

Compared with the RO, bacterial OTUs related to *Planococcus* spp., *Aeromonas* spp., *Pseudomonas* spp. and an uncultured bacterium likely to be *Acinetobacter* were present in up to 21-fold higher abundance on the UF membranes. Specifically, these OTUs each account for an average 3.2%, 6.9%, 7.6% and 20.9% of the total microbial community on the non-cleaned and cleaned UF membranes (Table 1). In contrast, these OTUs were only detected at average 0.004%, 0.01%, 0.02% and 0.28% of the total microbial community on the RO membranes, suggesting that majority of these OTUs were already removed by the UF membranes. Instead, OTUs related to *Ruegeria*, *Planctomycete*, *Erythrobacter* and several other uncultured bacterium associated with *Alphaproteobacteria* were more than 15-fold higher in relative abundance on the RO membranes than on the UF. Among them, the OTUs associated with *Ruegeria* sp. and *Erythrobacter* sp. each accounted for 15% of the total microbial communities on the RO membranes (Table 1). Microbial populations on the 1st and 4th RO modules shared an average 63.5% Bray-Curtis similarity but both modules were only 51.2% similar to the microbial populations on the 8th module, hence clustering apart as shown on the MDS plot (Figure 4). Bacterial OTUs identified to be *Erythrobacter* spp. decreased from 24.7% in the 1st to 9.1% in the 4th and to 0.23% in the 8th RO module. In addition, uncultured bacterial strains that belong to *Gammaproteobacteria* and *Alphaproteobacteria* collectively accounted for a relative abundance of 18% the total microbial community in the 1st RO module but this relative abundance decreased to 3.7% in the 4th and 0.14% in the 8th module. Fouling load analysis was performed, and it was observed that the 1st module element exhibited a high fouling load of 3.11 g/m^2. Fouling load decreased from 1.64 g/m^2 in the 4th module element to 0.37 g/m^2 in the 8th module. The organic percentage in the 1st, 4th and 8th module element accounted for 85.1%, 64.9% and

63.6% of the total foulant material, respectively. These analyses suggested that majority of the organic contents was already removed by the 1st and 4th module RO element.

3.4. Cleaning effect on UF membranes

Chemical-enhanced backwash (CEB) resulted in a shift in the microbial communities attached on the middle and brine section of the UF membranes (Figure 5). Specifically, the CEB-cleaned membranes shared only 55.2% Bray-Curtis similarity from the non-cleaned membranes and hence clustered apart on the MDS plot. Unclassified *Rhizobiales* decreased by 76.8-fold from an average relative abundance of 37.1% to 1.25% of the total microbial community on a cleaned UF membrane (Figure 5). Similarly, bacterial groups within unclassified *Proteobacteria*, specifically *Alphaproteobacteria*, decreased from an average relative abundance of 10.2% to 0.3% of the total microbial community after CEB was performed (Figure 5). In contrast, bacterial genera *Aeromonas*, *Pseudomonas* and *Acinetobacter* increased by more than 2.9-fold in their relative abundance after CEB (Figure 5). For example, *Aeromonas* spp. and *Pseudomonas* spp. accounted for 3.8% and 1.8% of the total microbial community on a non-cleaned UF membrane, respectively. After CEB, the respective relative abundance increased to 11.4% and 6.5% of the total microbial community on a cleaned membrane (Figure 5). This suggested a slight enrichment of certain genera relative to other microbial communities on the cleaned membranes.

3.5. Potential microbial problems

Because genus *Pseudomonas* increased in its relative abundance after the CEB cleaning, qPCR was further conducted to determine if *P. aeruginosa,* an opportunistic pathogenic species within the genus *Pseudomonas*, may be present in the UF permeate and RO feed waters. The average copy numbers of *P. aeruginosa* detected was at a low concentration of 1.27×10^2 copies/L of UF permeate and RO feed (Figure 6), and unlikely to impose any potential health concerns. However,

P. aeruginosa had been known to reduce sodium thiosulfate to form hydrogen sulfide [23], a corrosive gas that may affect the integrity of the membranes. As such, a further enrichment for the microbial consortium on both UF and RO membranes via cultivation in synthetic marine broth was conducted to test for the presence of *P. aeruginosa* and other sulfate-reducing bacteria (i.e., bacterial populations with the ability to produce hydrogen sulfide). Black coloration of the bacterial cultures was observed after incubation, along with the presence of pungent-smelling hydrogen sulfide gas in the head space. The presence of hydrogen sulfide gas was further verified by reaction with filter paper soaked in 1% w/v lead acetate (Figure 7A). High-throughput sequencing was performed on all enrichment cultures to determine if sulfate-reducing bacteria were present. Known sulfate-reducers like *Pseudomonas* and *Desulfovibrio* spp. were detected in the black enrichment cultures. In contrast, only one of the enrichment cultures that did not turn black was detected positive for *Pseudomonas* spp. (Figure 7B). All other enrichment cultures that did not turn black were absent of both *Pseudomonas* spp. and *Desulfovibrio* spp.

4. Discussion

Given the complexity of the Arabian Gulf waters, an effective pretreatment method is needed to prolong the operating longevity of RO membranes and to ensure the economic sustainability of SWRO desalination plants in this region. Monitoring the efficacy of SWRO pretreatment technologies is traditionally based on conventional water quality parameters including turbidity, SDI, conductivity, suspended solids and TOC, as well as enumeration of microorganisms. When evaluated based on these parameters, the UF examined in this study, which had a pore size of 0.03 µm, was able to reduce the SDI of the feed water and achieve up to 1.96-log removal of bacterial cells. This in turn accounted for the RO membrane to be continuously operated for almost 6 months at a differential pressure of 1 bar and without any noticeable fouling issues.

Examples of microorganisms that were preferentially removed by the UF in this study included phototrophic eukaryotes which were of bigger size than bacterial cells (i.e., typically ranging from 1 to 2 µm in length and < 1 µm in diameter) and that of the UF pores (Figure 8). These cells were hence likely to be rejected by the UF membranes by size exclusion. Alternatively, prior study had shown that bacterial cells which possessed both twitching and swarming motilities could preferentially attach onto membrane surfaces [24]. Bacterial populations such as uncultivated *Nitrosoarchaeaum limnia* are motile with archaellum that is functionally similar to that of a bacterial flagellum [25]. Such cells including *Nitrosoarchaeum limnia* were likely to be rejected by the UF because their motilities aided in migration and subsequent attachment onto the UF membranes. This in turn accounted for their high relative abundance on the UF membranes and a corresponding lower abundance in the UF permeate (Table 1).

However, none of the microfiltration or ultrafiltration pretreatments studied thus far was able to achieve a 100% removal of bacterial cells regardless of the water matrix [7, 26, 27]. It is possible that the high flux and the corresponding transmembrane pressure sustained during the operation forced the bacterial cells through the UF membrane pores. Furthermore, marine microbial community also consists of small bacterioplanktons termed as ultramicrobials (i.e., < 0.1 μm^3 in cell volume) which may be able to pass through the UF membranes to reach the RO stages [28-30]. While there was microbial removal by the UF membrane, regrowth should also be expected. Nutrients are still available in the water, especially after chlorination where lower molecular weight organic carbon is increased and become relatively easier for the bacterial cells to assimilate [31, 32]. Continual bacterial growth is therefore expected on equipment, pipe and tank surfaces that were not easily sanitized throughout the pilot plant operations. A portion of such regrowth may have dislodged into the UF permeate, contributing to the cell counts that

reached the RO stages. While removal by the UF pretreatment was far from complete, it was sufficient to minimize any significant biofouling issues in the RO throughout the entire 6 months study period.

Nevertheless, our study provided insights to other potential problems that can arise in a SWRO plant. The addition of sodium metabisulfite is a common practice in most desalination plants as chlorination or other oxidizing biocides are utilized to suppress microbial regrowth in the RO feed, and subsequent neutralization with sodium metabisulfite is therefore required to protect the integrities of the RO membranes. Coupled with the high sulfate concentration in seawater (ca. 4700 mg/L), there is abundant sulfate in the feed water that would favor the formation of *Desulfovibrio* spp. and *Pseudomonas* spp. as constituents of biofilm matrix on membranes as observed in this study (Figure 7B). *Desulfovibrio* spp. are sulfate-reducers that can utilize sulfate or other oxidized forms of sulfur (e.g. sulfite or thiosulfate) as electron acceptors to perform anaerobic oxidative phosphorylation of organic acids or hydrogen [33, 34]. It is hypothesized that *Pseudomonas* spp. can first deplete the localized oxygen content to provide favorable conditions for *Desulfovibrio* spp. Alternatively, the *Pseudomonas* spp. can directly reduce thiosulfate to form hydrogen sulfide since they possess functional genes that allow for assimilatory and dissimilatory sulfate reduction [35]. In addition, it is likely that the extracellular matrix surrounding bacterial cells impede oxygen transfer from the bulk liquid fraction into the biofilm, hence resulting in an oxygen concentration that decreases along the depth of the biofilm matrix [36, 37]. This facilitates the establishment of anoxic and anaerobic zones within the biofilm matrix which further allows *Desulfovibrio* spp. to reduce oxidized forms of sulfate despite most desalination plants maintaining high dissolved oxygen content in the bulk liquid.

By emitting corrosive and toxic hydrogen sulfide, the sulfate-reducers have been demonstrated to play a direct role on localized pipe corrosion [38, 39]. This study further illustrated that *Pseudomonas* spp. and sulfate-reducers may be present as attached bacteria on UF and RO membranes. Upon favorable conditions, for example during intermittent operation or in stagnant waters, hydrogen sulfide may be produced that can potentially compromise the membrane integrity. A combination of potential issues related to pipe corrosion and compromised membrane integrities would be detrimental to the operation of a SWRO plant.

CEB treatment can be used as a strategy to remove foulants attached on membrane surfaces. In this study, the CEB treatment resulted in a shift in the microbial community distinct from the non-cleaned UF membranes. The shift in the microbial community included the elimination of sulfate-reducing *Desulfovibrio* on the cleaned UF membranes (Figure 7B). Microbial populations like unclassified *Rhizobiales* which were originally abundant on the non-cleaned UF membranes were also effectively removed by CEB treatment. Although there was an increase in the relative abundance of certain bacterial genera including *Pseudomonas*, *Aeromonas* and *Acinetobacter* after CEB, the short sequencing read length (~ 400 bp) did not allow for an accurate identification of bacterial OTUs at the species level. As such, qPCR approach was further conducted to identify if *Pseudomonas aeruginosa*, an opportunistic pathogenic species within the genus *Pseudomonas* would be present. qPCR revealed a low abundance of the regA genes associated with *Pseudomonas aeruginosa* in the UF permeate (Figure 6). Different *P. aeruginosa* strains contain varying multiple copies of regA gene (i.e., > 1 copy of regA gene per *P. aeruginosa* cell) [40], and this would equate to less than 3.03×10^2 cells/L of *P. aeruginosa* in the UF permeate. Given that the dosage of *P. aeruginosa* required to infect 50% of the tested mice hosts ranged from 5×10^3 till 2×10^4 cells [41], the amount of *P.*

aeruginosa in the UF permeate was lower than the required infectious dosage and should not impose any significant risk to the public health. Coincidentally, the regA gene of *P. aeruginosa* was also occasionally detected in drinking water in Netherlands at up to 4.4 x 10^3 copies/L [42], suggesting that *P. aeruginosa* may be ubiquitous in oligotrophic drinking water supplies including those potable waters generated from a desalination plant. Finally, despite an increased abundance of genus *Pseudomonas* after CEB cleaning, the black coloration of bacterial cultures was not observed for these chemically cleaned UF membranes (Figure 7B). Neither was there any detectable regA gene associated with *P. aeruginosa* on the UF membranes. This suggested that the increase in the relative abundance of genus *Pseudomonas* after CEB cleaning was due to other *Pseudomonas* species besides *P. aeruginosa*, and that CEB remained effective in removing the foulants on the UF membrane.

This study demonstrated the use of molecular-based approaches to track the microbial dynamics along the desalination treatment process, and provided insights to potential microbial-associated problems that may arise in a desalination plant receiving water from Arabian Gulf. Future studies should look into samples collected throughout the year so as to ensure a consistent removal of microbial populations by the UF membranes despite seasonal variations.

5. Conclusion

In summary, this study demonstrates that the UF membrane can be used as an effective pretreatment to achieve 1.96-log removal of bacterial cells and a delayed RO fouling. Microbial populations were removed by the UF membranes to result in a distinct change in the microbial community profiles between the feed water and the UF permeate. Predominant bacterial populations that were attached on the UF membranes were removed effectively by the CEB

treatment. Another advantage of CEB was the removal of sulfate-reducing bacteria from the UF membranes. *Pseudomonas* in coexistence with *Desulfovibrio* can reduce sulfate and produce hydrogen sulfide, which would in turn cause corrosion problems or compromise the membrane integrities. Despite an increase in the relative abundance of genera *Pseudomonas*, *Aeromonas* and *Acinetobacter* on the UF membranes after CEB treatment, enrichment cultures of CEB-cleaned UF membranes were negative for sulfate-reducing bacteria and hydrogen sulfide production. Furthermore, the average copy numbers of *P. aeruginosa* was only present at a low concentration in UF permeate and RO feed. This suggested that the increase in the relative abundance of genus *Pseudomonas* after CEB cleaning was due to other *Pseudomonas* species besides *P. aeruginosa*, and that CEB remained effective in removing the foulants on the UF membrane.

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Table 1. Relative abundance of selected operational taxonomic units (OTUs) identified at 97% 16S rRNA gene similarity **(A)** in the Group A and B water samples and on UF and RO membranes, **(B)** on the UF membranes in significantly higher abundance than on RO membranes, and **(C)** on the RO membranes in significantly higher abundance than on UF membranes.

OTU name	Potential matched identity	Average relative abundance (%)			
		Group A water	UF membranes	Group B water	RO membranes
(A) OTUs effectively removed by UF membranes					
OTU153	Uncultured bacterium (HM596422)	14.4	24.9	1.11	0.25
OTU211	Uncultured *Proteobacteria* (HM596417)	5.5	13.1	0.52	0.13
OTU397	Candidatus *Nitrosoarchaeum limnia* (KC357794)	1.3	0.56	0.09	0.004
OTU428	Uncultured phototrophic eukaryote (FJ649249)	2.0	0.17	0.00	0.00
OTU534	Uncultured *Proteobacteria* (GQ274230)	1.4	0.37	0.08	0.00
OTU984	Candidatus *Nitrosoarchaeum limnia* (KC357794)	9.9	5.08	0.78	0.013
(B) OTUs that are present on UF membranes at relative abundance > 1% and significantly more abundant than on RO membranes					
OTU31	*Planococcus* sp. (NR113814)	0.003	3.24	0.66	0.004
OTU74	Uncultured *Acinetobacter* sp. (LC002937)	0.00	20.9	0.08	0.28
OTU246	Uncultured *Aeromonas*	0.02	6.91	1.37	0.01

	(KJ870940)				
OTU475	*Pseudomonas* sp. (AF500620)	0.03	7.59	1.49	0.02
(C) OTUs that are present on RO membranes at relative abundance > 1% and significantly more abundant than on UF membranes					
OTU24	*Ruegeria* sp. (KJ732938)	0.45	0.29	1.56	15.4
OTU98	Uncultured *Alphaproteobacteria* (JN594697)	0.04	0.09	0.13	2.41
OTU114	*Planctomycete* sp. (JF443758)	0.00	0.02	0.01	2.63
OTU115	Uncultured bacterium (JQ178843)	0.020	0.03	0.05	8.11
OTU192	*Erythrobacter* sp. (KJ732879)	0.35	0.24	0.19	15.0
OTU232	*Planctomycete* sp. (JF443758)	0.002	0.01	0.02	1.66
OTU235	Uncultured bacterium (AB424921)	0.002	0.02	0.02	1.84
OTU286	Uncultured bacterium (JX022678)	0.16	0.04	5.67	2.68
OTU304	Uncultured bacterium (JQ178843)	0.05	0.03	0.04	4.22
OTU319	Uncultured *Alphaproteobacteria* (JQ516349)	0.58	0.64	3.55	5.09