Understanding microbial assembly on seawater reverse osmosis membranes to facilitate evaluation of seawater pretreatment options

Abdullah H.A. Dehwah[1,2], Hong Cheng[1], Thomas M. Missimer[3*], Pei-Ying Hong[1*]

1. [a] King Abdullah University of Science and Technology (KAUST), Water Desalination and Reuse Center (WDRC), Biological and Environmental Sciences & Engineering Division (BESE), Thuwal, 23955-6900, Saudi Arabia
2. Desalination Technologies Research Institute (DTRI), Saline Water Conversion Corporation (SWCC), P.O. Box 8328, Al-Jubail 31951, Saudi Arabia
3. Emergent Technologies Institute, U.A. Whitaker College of Engineering, Florida Gulf Coast University, 16301 Innovation Lane, Fort Myers, FL, 33913, United States

*Corresponding author:

Pei-Ying Hong

Email: peiying.hong@kaust.edu.sa; Phone: +966-12-8082218

Thomas Missimer

Email:tmissimer@fgcu.edu; Phone: +1-239-7454538

Abstract

Membrane biofouling is the primary cause of inefficiency in seawater reverse osmosis desalination. The identification and subsequent removal of causative microorganisms would therefore be beneficial. To achieve this aim, the assembly of microorganisms onto the reverse osmosis membranes was first modeled to reveal a niche-selective process. Specifically, bacterial genera *Hyphomonas*, *Muricauda*, *Bacillus* and *Pseudoruegaria* were detected in occurrence frequency higher than predicted, and likely play a role in biofouling due to production of exopolymers. Subsequently, four different pretreatment systems, namely ultrafiltration (UF) membranes, intake wells, dual media filtration (DMF) and cartridge filters (CF), were evaluated for their log removal efficiencies of these four genera. UF outperformed the others in removing the potential biofouling-associated genera, but intake wells achieved a higher log removal of cell densities. Microbial regrowth, as denoted by an increase in cell numbers, was consistently observed within the CF. Using well intakes provides the highest degree of pretreatment in removing total cells in a chemical-free manner, while UF is the next best process to remove bacteria and organic carbon compounds most responsible for membrane biofouling.

Keywords: assembly model; biofouling; amplicon sequencing; log removal

1. Introduction

Exacerbating rates of global water depletion have incentivized countries to explore seawater desalination as an alternative source for freshwater [1]. Seawater can be converted into freshwater by removing salt content either by means of thermal distillation or membrane-based desalination. Although only about 1% of the world's current water supply is produced through desalination, it is projected that by 2025, about 14% of global water will be provided by desalination [2]. Specifically, desalination by means of seawater reverse osmosis (SWRO) membranes accounts for about 65% of the global capacity, and will increasingly be adopted by countries that aim to produce desalinated waters for municipal use [2].

Although RO desalination produces high quality potable water, the membranes are generally very sensitive to feedwater quality and are particularly prone to biofouling [3, 4]. The accumulation of the foulant layer decreases the permeate flux, compromises the overall efficiency of the desalination plant, and hence, requires placing the system offline for membrane cleaning [5]. Even after cleaning the membrane, it was observed that membrane function never recovers to its full effectiveness and tends to further decline with subsequent cleanings [6]. Hence, biofouling has a significant impact on SWRO treatment cost [7].

To mitigate this challenge, various types of pretreatment processes are operated before seawater enters the SWRO membranes [8, 9]. Larger debris is removed by some type of traveling screen system. Conventional pretreatment systems include dual media filters (DMF) and cartridge filters (CF). Dual media filters (DMF) have differing designs with layers of anthracite, sand, pebbles and gravels to provide physical filtration of the raw seawater [9]. Water flow through can be downwards (most systems) or upwards (Tampa Bay Water SWRO Plant)

66 depending on the objectives of the pretreatment system. CF provides for removal of particle sizes
67 of 1, 2, 5, 10 and 25 µm, with the most frequently used size being 5 µm [9], and occurs directly
68 upstream of the membrane process to protect it from particulate entry. Both types of mixed
69 media filtration can be used with chemical coagulants (e.g. $FeCl_3$) to cause particle aggregation
70 to facilitate removal of smaller particles and aggregated bacteria. In some modern pretreatment
71 systems where potential algal blooms occur, a dissolved air flotation (DAF) system can occur
72 after large debris removal. Ferric chloride is commonly added to the inflowing water to cause
73 flocculation of particulate matter. The DAF system is then followed by mixed media filtration
74 and then cartridge filters.

75 Another pretreatment strategy that works based on physical separation and removal is to use
76 ultrafiltration (UF) to produce water quality that is superior to that obtained from DMF and CF.
77 However, the small pore size associated with UF membranes requires higher energy costs to
78 maintain the needed permeate flux compared to a CF or DMF system. In recent years, subsurface
79 intakes, including wells and seabed galleries, have been used as an alternative environmentally
80 friendly pretreatment system [10, 11] . Intake wells rely on indigenous geological media to
81 provide physical filtration of particulates, adsorption, and biological degradation of organics in
82 the raw seawater, much like that of DMF and CF. SWRO systems operating with well intakes
83 tend not to use any chemical coagulants [10, 12].

84 A limited number of systematic studies have been conducted to evaluate these pretreatment
85 systems for the changes in the microbial community along the treatment process [13-20]. A more
86 focused evaluation on their overall removal efficacies of bacteria should be made. Specifically,
87 these pretreatment systems should be evaluated for their removal efficiencies of microbial
88 populations that may contribute to the reverse osmosis membrane biofouling. However, it is not

known if the fouling process on SWRO membranes follow a neutral assembly model or a niche selection system. A neutral assembly system assumes a random stochastic process where any microorganisms may contribute to the attachment process, and will be replaced rapidly by another microorganism should it die off or be detached from the biofilm matrix [21, 22]. Hence, no single bacterium plays an important role in the biofouling process. In contrast, a niche selection model presumes that a particular bacterial group outcompetes the others in attaching onto the membrane, hence playing a bigger contribution on the fouling process which was found by some researchers [23, 24]. The repercussions of determining which model best describes the RO membrane fouling process is that the pretreatment systems can then be evaluated accordingly. For example, if the RO membrane fouling follows a neutral assembly model, then the pretreatment system that achieves the highest log removal for the total cell density, regardless of what type of bacterial population is removed, would be preferable. Alternatively, if the RO membrane fouling follows a niche selection model, a pretreatment system that effectively removes those causative bacterial populations would be more effective in delaying RO membrane fouling. This study therefore aims to first determine the assembly model for a fouled SWRO membrane. Second, the four pretreatment systems, namely, the subsurface seabed, DMF, CF and UF are further evaluated for their removal efficiencies of microbial communities, with emphasis made on log removal of cell counts depending on the outcome of the assembly model.

2. Methods

2.1. Sampling sites and pretreatment description

Four types of pretreatment systems utilized by five different desalination plants located in Saudi Arabia were included for analysis in this study (Fig. 1). The first examined pretreatment systems were intake wells used for seawater reverse osmosis (SWRO) plants located on the Red Sea at the North Obhor (site A), Jeddah Corniche (site B) and South Jeddah Corniche (site C) sites. The detailed description of these three studied sites were provided in an earlier study [10] . The second examined pretreatment system is dual media filter (DMF), used after the subsurface intake wells at site A [10] and in a separate SWRO plant (site D) located on the Red Sea coast in Saudi Arabia [20]. The third examined pretreatment system is micro cartridge filtration, CF, which provides a filtration size ranging from 5 µm to 25 µm. CFs were used at sites A and D after the DMF. A double CF system (the first CF has filtration size of 25 µm, and the second CF has filtration size of 5 µm) was utilized at site B after the subsurface intake wells. At site C, CF was used after UF. The fourth examined pretreatment system was ultrafiltration (UF) utilized by a pilot-scale desalination plant, site E, located in Jubail, Saudi Arabia [15]. UF system in site C was not included for sequencing analysis in this study as site C has a mesh system preceding the UF that would complicate determination of which bacterial populations were removed solely by UF. More details on the operating parameters of each pretreatment options are provided in Table 1. In addition, fouled RO membranes from 1st and 4th modules of site E were also sampled for their biomass based on procedures described earlier [15]. Access to fouled RO membranes from the remaining sites were not provided, and therefore not included in this study.

Water samples were collected before and after each type of pretreatment system, and filtered through a 0.4 µm Whatman Nucleopore^{TM} track-etched polycarbonate membrane filter (GE

Healthcare Life Sciences, Little Chalfont, Buckinghamshire, UK). Biomass retained on the polycarbonate filters was stored at -20 ^{o}C until DNA extraction and 16S rRNA gene-based amplicon sequencing.

2.2. Flow cytometry to determine total cell counts

Total cells in water samples were determined by flow cytometry either on Accuri C6 or BD FACSVerse (BD Bioscience, NJ, US) based on protocol described previously [10, 15, 20]. Briefly, samples were stained with 100X SYBR green (Thermo Fisher Scientific, MA, US) at a volumetric ratio of 100: 1 (i.e., for every 1 mL of samples, 10 uL of SYBR green was used to stain cells). The suspension was then incubated in 35 ^{o}C and in the dark for 10 min before flow cytometry. 50 µL aliquots of stained samples were injected with a 35 µL/min flow rate to enumerate the total cells. Log removal values (LRV) of total cells are calculated based on Equation 1:

$$LRV = Log_{10}(N_{inflow}/N_{outflow}) \quad \textbf{---- Equation 1}$$

2.3. DNA extraction and 16S rRNA gene-based amplicon sequencing

DNA extraction and 16S rRNA gene-based amplicon sequencing for sites A, B and C were newly performed for this study, while that for sites D and E were performed in earlier studies [15, 19, 25]. Specifically, all biomass collected from sites A, B, C and E were extracted using UltraClean® Soil DNA isolation kit (MoBio, Carlsbad, CA, US) based on a modified protocol described earlier [26]. The modified protocol combines enzymatic, chemical and physical lysis to ensure comprehensive extraction of bacteria and archaea that may have different cell wall structures. Samples collected from site D were extracted for DNA by another research group, which although used a different extraction kit, relied on similar combination of enzymatic,

chemical and physical lysis [25]. PCR amplification of the 16S rRNA genes was performed with 515F (5'- Illumina overhang- GTGYCAGCMGCCGCGGTAA- 3') and 907R (5'- Illumina overhang- CCCCGYCAATTCMTTTRAGT- 3') for sites A, B, C and E. For site D, PCR amplification of the 16S rRNA genes was performed with 27F (5′-AGAGTTTGATCMTGGCTCAG-3') and a reverse primer 519R (5′ -GTNTTACNGCGGCKGCTG – 3'). All amplicons were of the anticipated size of approximately 500 bp, and the negative control had no amplification. Samples from sites A, B and C were newly sequenced by KAUST Core lab on Illumina MiSeq for this study. Samples collected from sites D and E were amplicon sequenced as described earlier [15, 20]. All high-throughput sequencing files newly obtained for this study were deposited in the Short Read Archive (SRA) of the European Nucleotide Archive (ENA) under study accession number PRJEB32161.

2.4. High-throughput sequencing data analysis

Amplicon sequences newly obtained for this study had a Phred score >30 and sequencing length >280 nt. Primers, adaptors, and index sequences were removed. All new sequencing data (for sites A to C) and downloaded raw data (for sites D and E) were identified and removed for their chimeras by UCHIME algorithm [27]. Chimera-free sequences were then analyzed through two approaches. The first approach was to analyze for their taxonomical assignment using Ribosomal Database Project (RDP) Classifier at 95% confidence level with copy number adjustment [28]. Relative abundance at the genus level was calculated for each sample. Absolute abundance of specific genera present in a particular sample is estimated by multiplying the relative abundance of that genus against the total number of cells obtained by flow cytometry for that sample.

177 Parallel to the above analysis, chimera-free sequences within a single sample dataset were
178 also aligned for homology against each other based on the Infernal aligner prior to complete
179 linkage clustering [29]. The cluster file is then input into the RDP Pipeline to determine Shannon
180 diversity index (H) and Chao1 index.

181 In the second approach, sequence files were identified as unique operational taxonomic units
182 (OTUs). Briefly, chimera-free sequences were combined with an in-house written Perl script.
183 The combined sequence was then sorted for unique OTUs at 97% 16S rRNA gene similarity
184 using CD-Hit program to cluster and compare the nucleotide sequences [30]. Relative abundance
185 was calculated. Taxonomy classification was conducted using QIIME open-source
186 bioinformatics pipeline [31] based on RDP database.

187 **2.5. Neutral assembly modeling for seawater reverse osmosis membranes**

188 To assess the role of neutral process in the assembly of the seawater desalination reverse
189 osmosis membranes, the Sloan neutral model [21, 22] was examined to fit the relative abundance
190 of the rarefied OTUs in the untreated seawater and fouled reverse osmosis membranes sampled
191 from site E. All samples were sub-sampled at same sequence depth. After the fitting, OTUs from
192 the pool were subsequently sorted into three partitions depending on whether they occurred more
193 frequently than ('above' partition), within ('neutral' partition), or less frequently than ('below'
194 partition) the 95% confidence interval of the Sloan neutral model predictions [32]. The taxa
195 above the partition indicates they were actively being selected for, while taxa below the partition
196 indicates that they were actively being selected against. The goodness of fit for the Sloan neutral
197 community model was evaluated using the root mean square error (RMSE) and the generalized
198 R-squared (R^2 = 1-the sum of squares of residuals/the total sum of squares) [33]. A higher R^2
199 value (maximum value of 1) implies that a neutral process of dispersal and ecological drift

200 contributes more towards community assembly, whereas a low R^2 value (e.g., ca. 0.2) implies
201 poor fitting and other processes (i.e., selective growth/attachment) contributing to the community
202 assembly [34, 35].

203

204 **3. Results**

205 **3.1. Fouling of reverse osmosis membranes in SWRO is a niche selected process**

206 A neutral assembly model was used to investigate the formation of microbial community on
207 seawater reverse osmosis membranes obtained from site E. The goodness-of-fit (R^2) value of the
208 model was 0.22 (Fig. 2), and this low R^2 value suggested that the microbial community in both
209 the feed stream and biofilm did not follow random migration, but instead was shaped by niche-
210 driven selection. Specifically, the OTUs that were located above the fit of the neutral model
211 (denoted as OTUs in the purple zone, Fig. 2) were present in a frequency higher than that
212 predicted by the model. This suggests that they were identified to occur on the fouled RO
213 membranes at a higher frequency than predicted, and likely to be preferentially selected for by
214 RO membranes to attach onto the surfaces. In contrast, the OTUs that were located below the
215 neutral model (denoted as OTUs in the green zone, Fig. 2) were present in frequency lower than
216 that predicted by the model, suggesting that these OTUs do not attach well on the RO membrane.
217 By comparing the identities of genera associated with these OTUs in both the purple and green
218 zones, and discarding those that appeared in both zones since those would signify ambiguous and
219 contradictory results, it was determined that *Hyphomonas*, *Pseudoruegeria*, *Bacillus* and
220 *Muricauda* were found consistently located above the model in the purple zone. In particular,
221 *Hyphomonas* accounted for 27.3% of the total located in the purple zone (Fig. 2). These

observations suggest that these four genera may contribute more than other genera towards seawater RO membrane fouling.

3.2. Log removal of RO-selected microbial genera varied across the pretreatment type

Given that *Hyphomonas*, *Pseudoruegeria*, *Bacillus* and *Muricauda* were selectively attached on the fouled reverse osmosis membrane and may contribute more towards RO membrane fouling, the upstream pretreatment steps (i.e., intake wells, CF, DMF, and UF) were therefore further evaluated for their log removal values of these four genera (Fig. 3). LRV were determined based on the estimated abundances of individual genera before and after pretreatment. *Pseudoruegeria* was not detected in samples collected from the pretreatment stages and no LRV could be determined for this genus. The observed removal efficiencies of the different pretreatment methods was UF > intake wells > DMF > CF. UF performed better than all other pretreatment methods, achieving 1.0, 0.2 and 1.3 log removal for *Hyphomonas*, *Bacillus* and *Muricauda*, respectively. Intake wells achieved 0.6 and 0.7-log removal for *Hyphomonas* and *Bacillus*, but supported a potential regrowth of *Muricauda*. In contrast, CF did not provide any removal, and instead resulted in a potential regrowth for all three evaluated genera.

3.3. Changes in cell abundance and top abundant genera at each sampled site

A further examination of each stage of the pretreatment system at sites A through E suggested that intake wells achieved an average 1.0 log LRV of the total cells compared to the other three pretreatment methods (Fig. 4). This reported LRV was comparable to that achieved by UF (0.8-log), and higher than that reported for DMF (0.6-log). Among the four sites that

operate CF, three experienced a positive increase in cell numbers after CF, suggesting a wide variability in CF performance from site to site (Fig. 4). Based on the removal values of the total cell numbers, the top 20 most abundant genera were further examined for their removal efficiencies by the respective pretreatment method (Fig. 3). The intake wells achieved positive log removal efficiencies of 18 of the top 20 most abundant genera, except for *Nitrosopumilus* and *Nitrososphaera*. In contrast, DMF and UF did not achieve positive log removal for 6 to 7 of the top 20 most abundant genera. CF consistently was not able to remove any bacterial genera effectively with the exception of *Pseudomonas* and *Alcanivorax*, albeit at very low log removal (< 0.4-log).

3.4. Changes in microbial diversity along the pretreatment train at each site

Despite a decrease in the total cell numbers, there was an increase in the microbial diversity, as exemplified by both Chao and H' indices, in the waters after passing through the subsurface seabed (Table 2). In contrast, microbial diversity in waters decreased after passing through DMF, CF and UF. Although microbial diversity increases after the intake wells, the total cell numbers decreased by ca. 1-log. This means that the new microbial populations added to the system by the intake wells account for a very low estimated abundance. A further examination of the top 20 most abundant genera that were not detected in the seawaters but detected after passing through subsurface seabed revealed that they are mainly indigenous populations associated with the marine environment and well water below the seabed (Table 2). Since the raw well water is held in the storage tanks for variable time periods, the microbial diversity decreased back to a level that approximates that found in the raw seawater (Table 2).

4. Discussion

Biofouling of seawater reverse osmosis is thought to be the major bottleneck in the overall sustainability of membrane-based desalination. Biofouling involves the preconditioning of the membrane and then attachment of primary microbial colonizers onto the membrane surface [36]. These microorganisms can secrete extracellular polymeric substances which further condition the membrane surface to facilitate subsequent attachment and buildup of the biofilm layer by secondary colonizers [37, 38]. Collectively, the biofilm matrix contributes to irreversible foulant layer that may be difficult to eradicate even with chemical cleaning. It is therefore inferred that by identifying the primary colonizers and devising strategies to inhibit their colonization, it would delay biofouling. However, this intervention strategy specifically targeting the primary colonizers or causative microbial agents would only be effective if the biofouling process is dominated by a niche selection process and not by the neutral assembly process.

Modeling of 16S rRNA amplicon sequencing data obtained from fouled SWRO membranes in site E suggests that biofouling indeed followed a niche selection process, and was potentially mediated by four main genera, namely *Hyphomonas*, *Muricauda*, *Bacillus* and *Pseudoruegeria*. *Hyphomonas* and *Muricauda* which belong to the class Alphaproteobacteria and Flavobacteria, respectively. Both classes of bacterial populations have been reported on fouled RO membranes in earlier studies. Khan et al. [39] examined in temporal succession the microbial communities developed on SWRO membrane, and found that a 3-week old fouled membrane was almost exclusively represented by Alphaproteobacteria. Similarly, Zhang et al. also reported 61.2% of the total microbial community on fouled SWRO membranes to be related to Alphaproteobacteria, while Flavobacteria constituted a lower percentage of the microbial

289 community compared to Alphaproteobacteria [18]. In another study, the percentage of
290 Alphaproteobacteria on fouled SWRO membranes could range from 73 to 91% of total microbial
291 community throughout the four seasons in Mediterranean Sea [16]. However, at a finer
292 taxonomical resolution, it was reported that SAR11, and not *Hyphomonas* spp., accounted for as
293 the main Alphaproteobacteria on the fouled membranes harvested from the desalination plant
294 located in Mediterranean Sea [16]. Neither was *Hyphomonas* spp. reported to be one of the
295 genera within Alphaproteobacteria recovered from the fouled SWRO membranes harvested from
296 the desalination plants located in Carlsbad, California [18]. This suggests that the four genera
297 reported in this study may not be universal fouling-causing bacterial populations. Instead, they
298 may be playing location-specific roles in fouling of SWRO membranes since all sampled sites
299 included in this study were located in Red Sea.

300 Nevertheless, this study provides a proof-of-concept of an approach to first determine the
301 assembly process of microorganisms onto SWRO membrane fouling, and then evaluate the
302 pretreatment options for the removal of those microorganisms contributing the most to fouling.
303 For example, in this study, it was elucidated SWRO membrane biofouling follows a niche
304 selective process. The foremost criteria when evaluating the pretreatment options would
305 therefore be the log removal values of *Hyphomonas*, *Muricauda*, *Bacillus* and *Pseudoruegeria* –
306 the four genera preferentially selected for by RO membranes. In particular, *Hyphomonas* spp.
307 were found to be very good biofilm formers, typically forming granular aggregates or were
308 found adhered on the walls of glass culture bottles [40]. A similar observation was made for the
309 Flavobacteria which appear to be major bacterial colonizers on transparent exopolymeric
310 particles (TEP) [41]. The concentration of TEP, which comprise extracellular polymeric
311 substances (EPS), correlate to RO membrane fouling in a pilot-scale desalination plant in Saudi

Arabia [42]. This suggests that both *Hyphomonas* and *Muricauda* play an important role in the biofouling, likely through exopolymer production that would contribute to increased attachment of other bacteria like *Bacillus*. Hence, the pretreatment options that achieve the highest log removal values of *Hyphomonas* and *Muricauda* – the fouling causative bacteria - would serve to delay RO membrane fouling more effectively than those pretreatment options that achieved only low removal values.

However, perhaps concerning is that among all evaluated pretreatment, CF results in microbial regrowth, as denoted by the increase in cell numbers, of most predominant bacterial populations. In an early study [42], the authors attribute this increase in cell numbers to the way pretreatment system was operated – possibly because sodium bisulfate (SBS) was added to quench residual chlorine prior CF and thus deactivated bacteria recover their activity. Alternatively, phosphate-based antiscalant typically used in pretreatment may have enhanced the bioactivity, since phosphate limitation is proposed as a way to control biofouling [43]. Chemical addition was found to also correlate with biofouling potential in the full scale desalination plant.

Similarly, chemical addition may also account for regrowth and increase in certain bacterial populations observed in DMF permeate streams. DMF is generally coupled with ferric-based coagulants so that suspended particulates including bacterial contaminants would aggregate and be removed effectively by DMF. It is likely that the iron-based coagulants can also contribute to the regrowth events since the typical concentration of $FeCl_3$ coagulants used are about 0.25 to 5 mg/L [44]. This is lower than the toxic concentrations of 300 uM (i.e., 180 mg/L) determined for *Pseudomonas syringae* [45]. Low concentrations of iron have also been found to lead to significant increase in *Escherichia coli* in oligotrophic environment as iron is an essential element for bacteria to sustain its metabolic pathways, amino acids and nucleic acid synthesis

[46, 47]. In contrast, despite the use of the same chemicals in the UF system, regrowth was not apparent in UF permeate likely because the small pore sizes would have rejected these microbes. Alternatively, the intake wells do not require addition of chemicals and yet were able to achieve LRV comparable to that of the UF. Specifically, it was previously demonstrated to result in significant reduction in TEP and organic constituents in the seawater [10, 12]. This minimizes the potential for chemical-induced microbial regrowth events and costs associated with chemical addition. However, it is noted that all of the pretreatment options did not manage to achieve a total removal of both the causative bacterial genera (Figure 3) and total cells (Figure 4), and hence a complete eradication of RO membrane fouling would not be possible. Instead, all the examined pretreatment options, particularly that of UF and intake wells can serve to delay biofouling by achieving high removal values of the causative bacterial genera selected for by the RO membranes.

Given the projected needs for freshwater by an increasing exponential rate of population growth, and the exacerbating water scarcity in many arid countries, turning to seawater as a source of drinking water would be increasingly adopted. The use of intake wells and/or UF as an appropriate pretreatment system prior RO can be considered in places where the local geology of the site would permit good removal as is the case observed for sites A through C in this study.

5. Conclusions

This study demonstrates that certain bacterial populations (e.g. *Hyphomonas, Muricauda, Pseudoruegeria, Bacillus*) are selectively attached on seawater reverse osmosis membranes, likely due to their ability to form an adhesive exopolymer that conditioned the membranes to

facilitate subsequent biofilm formation. Pretreatment systems that achieved high removal values of these four genera are therefore more favorable. It was observed that UF achieved the best removal values for all three bacterial genera followed by intake wells. CF, in contrast, had higher cell counts in the CF permeate, likely due to the use of Fe-based chemical coagulants that supported microbial regrowth and the > 5 um pore size that does not reject bacterial cells effectively. Despite the good performance of UF in removing the bacterial populations that would detrimentally affect RO, UF by itself is also a membrane and can be prone to fouling. Routine replacement costs for UF units may add on to the operational costs of desalination plants [48], and may not be an optimal option for cost-conscious utilities. Hence, considering the collective information obtained from this study in terms of removal efficiency, associated costs and use of chemicals, intake wells and/or UF may be more optimal options compared to DMF and CF.

Acknowledgement

This study is funded through KAUST Competitive Research Grant URF/1/2982-01-01 awarded to P.-Y.H.

373 **Table 1.** Operating details of pretreatment options at studied sites.

374

Site	Pretreatment	Chemicals	Reference
A	Well intake + dual media filter + cartridge filter (5 µm)	No chlorination, no coagulation, only antiscalants	[10]
B	Well intake + cartridge filter (25 µm) + cartridge filter (5 µm)	No chlorination, no coagulation, only antiscalants	[10]
C	Well intake + mesh filter (100 µm) + UF membrane + cartridge filter (25 µm) + cartridge filter (5 µm)	No chlorination, no coagulation, only antiscalants, CIP cleaning for UF membrane with citric acids (once per week)	[10]
D	Dual media filter + cartridge filter (10 µm)	Continuous chlorination for intake water, antiscalants, coagulant, cationic polymeric flocculant, dechlorination	[20]
E	130 µm strainers + UF membrane+ cartridge filter (5 µm)	Chlorination for intake water, antiscalant and sodium metabisulfite were added, UF membrane were back washed for 2minutes every hour and it was cleaned with sodium hypochlorite for 10–15 min. at every 24 hours	[15]

375

Table 2. Diversity indices obtained at different stages of the pretreatment train in Sites A through E.

	Chao	**H'**	**Trend compared to preceding stage**
Site A			
Seawater	17600.9	4.56	
After well	21272.3	5.31	↑
Holding tank	24555.4	6.13	↑
After DMF	20967.5	6.17	↓
After CF	20257.0	5.59	↓
Site B			
Seawater	27195.7	6.88	
After well	28616.1	7.34	↑
Holding tank	24341.3	5.97	↓
After CF	17371.4	5.17	↓
Site C			
Seawater	25048.5	6.03	
After well	27733.3	6.25	↑
Holding tank	26770.6	6.46	↓
After UF	22106.9	5.27	↓
After CF	22445.3	5.60	↓
Site D			
Chlorinated seawater	4200.1	3.98	
After DMF	721.3	3.51	↓
Site E			
Seawater	12640.92	5.99	
After UF	3036.2	4.78	↓
After CF	1959.9	4.40	↓

Table 2. Estimated abundance of bacterial genera that were detected in the water sampled after subsurface seabed but were not detected in the original untreated seawater.

Bacterial genera	**Estimated abundance (cells/mL)**
Halobaculum	1.0
Gp16	1.0
Rothia	1.1
Chelativorans	2.1
Thalassospira	2.1
Tranquillimonas	2.5
Unclassified Thaumarchaeota	2.8
Woesearchaeota Incertae Sedis AR17	3.1
Woesearchaeota Incertae Sedis AR16	3.4
Proteiniborus	3.4
Actinomyces	3.5
Nitratireductor	3.9
Halolamina	4.8
Rhodanobacter	5.2
Woesearchaeota Incertae Sedis AR18	5.4
Candidatus *Scalindua*	5.9
Halorubrum	7.2
Unclassified Candidatus Brocadiaceae	17.2
Spongiibacter	28.1
Halogeometricum	58.4

Figure legends

Figure 1. Illustration of the pretreatment at all five sites listed in this study. * denotes the point at which water samples were obtained for analysis.

Figure 2. Fit of the neutral model. The predicted occurrence frequencies for seawater influent and biofilm community. OTUs that occur more frequently than predicted by the model are shown in purple while those that occur less frequently than predicted are shown in green. The relative abundance of the OTUs in each outlier group were indicated in pie charts. Red dash lines represent 95% confidence intervals around the model prediction (blue line).

Figure 3. Heat map depicting the log removal values of different bacterial groups by the respective pretreatment system.

Figure 4. Changes in the cell abundance, denoted as grey circles, determined at different sampling points of Sites A to E. Log removal values achieved by each stage are listed in the tables. Negative LRV denotes an increase in cell density at the treatment system, with higher cell count than that measured at preceding stage.

References

[1] G. Amy, N. Ghaffour, Z.Y. Li, L. Francis, R.V. Linares, T. Missimer, S. Lattemann, Membrane-based seawater desalination: Present and future prospects, Desalination, 401 (2017) 16-21.

[2] G. Runte, Seawater and brackish water Desalination - MST052D, in, BCC Research, Wellesley, MA, USA, 2016.

[3] A. Matin, Z. Khan, S.M.J. Zaidi, M.C. Boyce, Biofouling in reverse osmosis membranes for seawater desalination: Phenomena and prevention, Desalination, 281 (2011) 1-16.

[4] T. Nguyen, F.A. Roddick, L. Fan, Biofouling of water treatment membranes: a review of the underlying causes, monitoring techniques and control measures, Membranes (Basel), 2 (2012) 804-840.

[5] N. Dhakal, Controlling biofouling in seawater reverse osmosis membrane systems, 2nd ed., CRC Press, Leiden, the Netherlands, 2017.

[6] L.H. Kim, A. Jang, H.W. Yu, S.J. Kim, I.S. Kim, Effect of chemical cleaning on membrane biofouling in seawater reverse osmosis processes, Desalin Water Treat, 33 (2011) 289-294.

[7] N. Ghaffour, T.M. Missimer, G.L. Amy, Technical review and evaluation of the economics of water desalination: Current and future challenges for better water supply sustainability, Desalination, 309 (2013) 197-207.

[8] R. Valavala, J. Sohn, J. Han, N. Her, Y. Yoon, Pretreatment in Reverse Osmosis Seawater Desalination: A Short Review, Environmental Engineering Research, 16 (2011) 205-212.

[9] N. Voutchkov, Pretreatment for reverse osmosis desalination, 1st ed., Elsevier, Amsterdam, the Netherlands, 2017.

[10] A.H.A. Dehwah, T.M. Missimer, Subsurface intake systems: Green choice for improving feed water quality at SWRO desalination plants, Jeddah, Saudi Arabia, Water Res, 88 (2016) 216-224.

[11] T.M. Missimer, N. Ghaffour, A.H.A. Dehwah, R. Rachman, R.G. Maliva, G. Amy, Subsurface intakes for seawater reverse osmosis facilities: Capacity limitation, water quality improvement, and economics, Desalination, 322 (2013) 37-51.

[12] A.H.A. Dehwah, S. Li, S. Al-Mashharawi, H. Winters, T.M. Missimer, Changes in feedwater organic matter concentrations based on intake type and pretreatment processes at SWRO facilities, Red Sea, Saudi Arabia, Desalination, 360 (2015) 19-27.

[13] A. Belila, J. El-Chakhtoura, N. Otaibi, G. Muyzer, G. Gonzalez-Gil, P.E. Saikaly, M.C.M. van Loosdrecht, J.S. Vrouwenvelder, Bacterial community structure and variation in a full-scale seawater desalination plant for drinking water production, Water Res, 94 (2016) 62-72.

[14] L.A. Bereschenko, G.H. Heilig, M.M. Nederlof, M.C. van Loosdrecht, A.J. Stams, G.J. Euverink, Molecular characterization of the bacterial communities in the different compartments of a full-scale reverse-osmosis water purification plant, Appl Environ Microbiol, 74 (2008) 5297-5304.

[15] P.Y. Hong, N. Moosa, J. Mink, Dynamics of microbial communities in an integrated ultrafiltration-reverse osmosis desalination pilot plant located at theArabian Gulf, Desalin Water Treat, 57 (2016) 16310-16323.

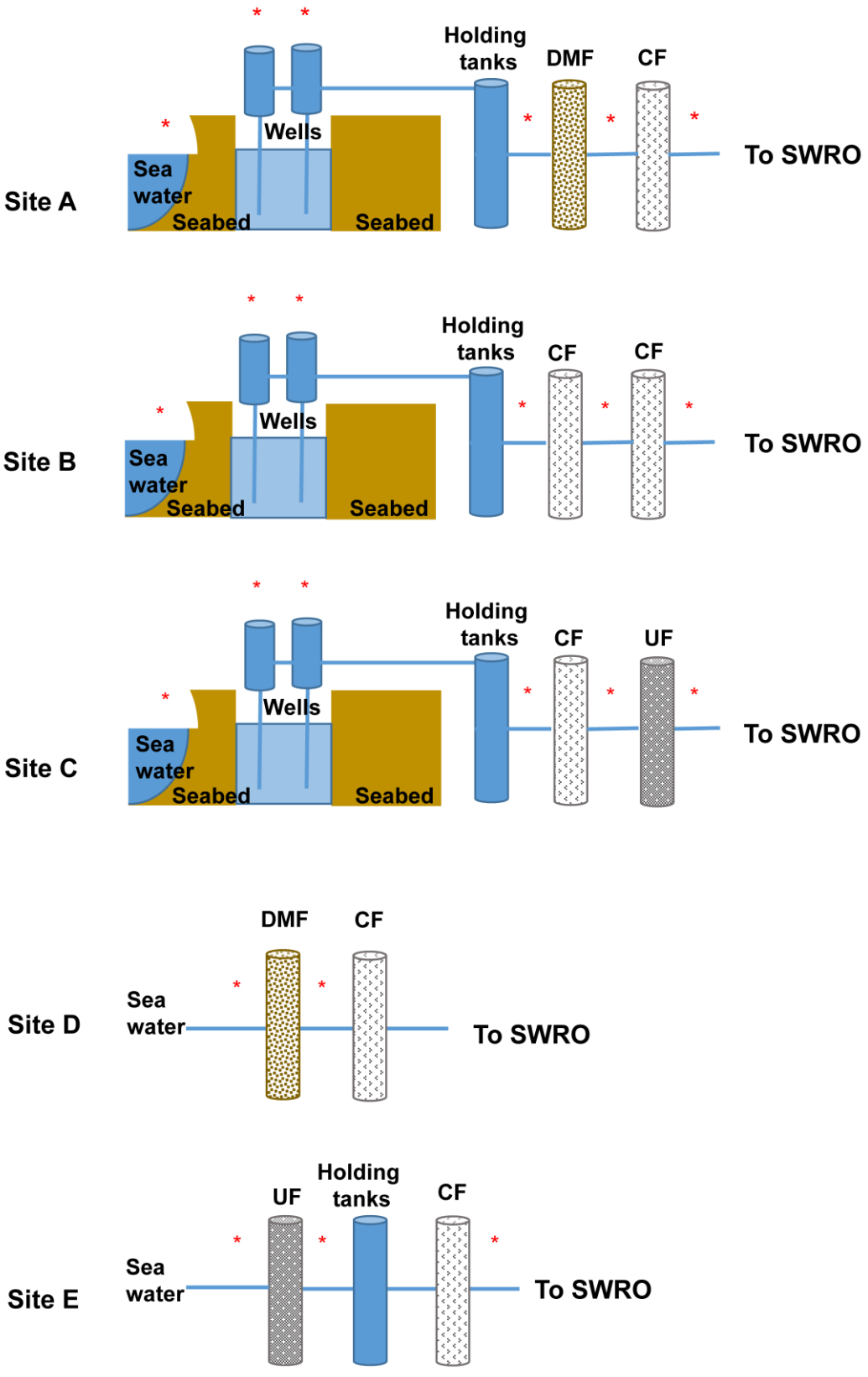
[16] A. Levi, E. Bar-Zeev, H. Elifantz, T. Berman, I. Berman-Frank, Characterization of microbial communities in water and biofilms along a large scale SWRO desalination facility: Site-specific prerequisite for biofouling treatments, Desalination, 378 (2016) 44-52.

[17] Z.U. Rehman, M. Ali, H. Iftikhar, T. Leiknes, Genome-resolved metagenomic analysis reveals roles of microbial community members in full-scale seawater reverse osmosis plant, Water Res, 149 (2019) 263-271.

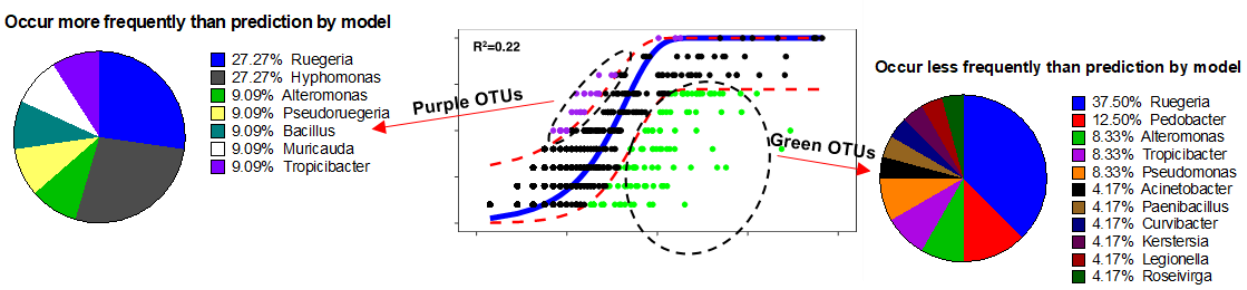
[18] M. Zhang, S. Jiang, D. Tanuwidjaja, N. Voutchkov, E.M. Hoek, B. Cai, Composition and variability of biofouling organisms in seawater reverse osmosis desalination plants, Appl Environ Microbiol, 77 (2011) 4390-4398.

[19] M.T. Khan, C.L. Manes, C. Aubry, J.P. Croue, Source water quality shaping different fouling scenarios in a full-scale desalination plant at the Red Sea, Water Res, 47 (2013) 558-568.
[20] M.T. Khan, P.Y. Hong, N. Nada, J.P. Croue, Does chlorination of seawater reverse osmosis membranes control biofouling?, Water Res, 78 (2015) 84-97.
[21] W.T. Sloan, M. Lunn, S. Woodcock, I.M. Head, S. Nee, T.P. Curtis, Quantifying the roles of immigration and chance in shaping prokaryote community structure, Environ Microbiol, 8 (2006) 732-740.
[22] W.T. Sloan, S. Woodcock, M. Lunn, I.M. Head, T.P. Curtis, Modeling taxa-abundance distributions in microbial communities using environmental sequence data, Microb Ecol, 53 (2007) 443-455.
[23] I.S. Kim, J. Lee, S.J. Kim, H.W. Yu, A. Jang, Comparative pyrosequencing analysis of bacterial community change in biofilm formed on seawater reverse osmosis membrane, Environ Technol, 35 (2014) 125-136.
[24] J. Lee, X. Ren, H.-W. Yu, S.-J. Kim, I.S. Kim, Membrane Biofouling of Seawater Reverse Osmosis Initiated by Sporogenic Bacillus Strain, Environmental Engineering Research, 15 (2010) 141-147.
[25] C.L.d.O. Manes, N. West, S. Rapenne, P. Lebaron, Dynamic bacterial communities on reverse-osmosis membranes in a full-scale desalination plant, Biofouling, 27 (2011) 47-58.
[26] P.Y. Hong, E. Wheeler, I.K. Cann, R.I. Mackie, Phylogenetic analysis of the fecal microbial community in herbivorous land and marine iguanas of the Galapagos Islands using 16S rRNA-based pyrosequencing, ISME J, 5 (2011) 1461-1470.
[27] R.C. Edgar, B.J. Haas, J.C. Clemente, C. Quince, R. Knight, UCHIME improves sensitivity and speed of chimera detection, Bioinformatics, 27 (2011) 2194-2200.
[28] Q. Wang, G.M. Garrity, J.M. Tiedje, J.R. Cole, Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy, Appl Environ Microb, 73 (2007) 5261-5267.
[29] J.R. Cole, Q. Wang, E. Cardenas, J. Fish, B. Chai, R.J. Farris, A.S. Kulam-Syed-Mohideen, D.M. McGarrell, T. Marsh, G.M. Garrity, J.M. Tiedje, The Ribosomal Database Project: improved alignments and new tools for rRNA analysis, Nucleic Acids Res, 37 (2009) D141-D145.
[30] W. Li, A. Godzik, Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences, Bioinformatics, 22 (2006) 1658-1659.
[31] J.G. Caporaso, J. Kuczynski, J. Stombaugh, K. Bittinger, F.D. Bushman, E.K. Costello, N. Fierer, A.G. Pena, J.K. Goodrich, J.I. Gordon, G.A. Huttley, S.T. Kelley, D. Knights, J.E. Koenig, R.E. Ley, C.A. Lozupone, D. McDonald, B.D. Muegge, M. Pirrung, J. Reeder, J.R. Sevinsky, P.J. Turnbaugh, W.A. Walters, J. Widmann, T. Yatsunenko, J. Zaneveld, R. Knight, QIIME allows analysis of high-throughput community sequencing data, Nat Methods, 7 (2010) 335-336.
[32] A.R. Burns, W.Z. Stephens, K. Stagaman, S. Wong, J.F. Rawls, K. Guillemin, B.J. Bohannan, Contribution of neutral processes to the assembly of gut microbial communities in the zebrafish over host development, ISME J, 10 (2016) 655-664.
[33] O. Ostman, S. Drakare, E.S. Kritzberg, S. Langenheder, J.B. Logue, E.S. Lindstrom, Regional invariance among microbial communities, Ecol Lett, 13 (2010) 118-127.
[34] A. Venkataraman, C.M. Bassis, J.M. Beck, V.B. Young, J.L. Curtis, G.B. Huffnagle, T.M. Schmidt, Application of a neutral community model to assess structuring of the human lung microbiome, MBio, 6 (2015) e02284-02214.
[35] F. Ling, R. Whitaker, M.W. LeChevallier, W.-T. Liu, Drinking water microbiome assembly induced by water stagnation, The ISME journal, 12 (2018) 1520.
[36] H.C. Flemming, Reverse osmosis membrane biofouling, Exp Therm Fluid Sci, 14 (1997) 382-391.
[37] A. Jain, N. Bhosle, Biochemical composition of the marine conditioning film: implications for bacterial adhesion, Biofouling, 25 (2009) 13-19.

[38] H. Winters, T.H. Chong, A.G. Fane, W. Krantz, M. Rzechowicz, N. Saeidi, The involvement of lectins and lectin-like humic substances in biofilm formation on RO membranes - is TEP important?, Desalination, 399 (2016) 61-68.
[39] M.T. Khan, C.L.D. Manes, C. Aubry, L. Gutierrez, J.P. Croue, Kinetic Study of Seawater Reverse Osmosis Membrane Fouling, Environ Sci Technol, 47 (2013) 10884-10894.
[40] R.M. Weiner, M. Melick, K. O'Neill, E. Quintero, Hyphomonas adhaerens sp nov.. Hyphomonas johnsonii sp nov and Hyphomonas rosenbergii sp nov., marine budding and prosthecate bacteria, Int J Syst Evol Micr, 50 (2000) 459-469.
[41] M. Simon, H.P. Grossart, B. Schweitzer, H. Ploug, Microbial ecology of organic aggregates in aquatic ecosystems, Aquat Microb Ecol, 28 (2002) 175-211.
[42] S. Li, S. Sinha, T. Leiknes, G.L. Amy, N. Ghaffour, Evaluation of potential particulate/colloidal TEP foulants on a pilot scale SWRO desalination study, Desalination, 393 (2016) 127-134.
[43] J.S. Vrouwenvelder, F. Beyer, K. Dahmani, N. Hasan, G. Galjaard, J.C. Kruithof, M.C.M. van Loosdrecht, Phosphate limitation to control biofouling, Water Res, 44 (2010) 3454-3466.
[44] K. Gaid, A large review of the pretreatment, in: R. Ying (Ed.) Expanding issues in desalination, IntechOpen 2011.
[45] B.J. Kim, J.H. Park, T.H. Park, P.A. Bronstein, D.J. Schneider, S.W. Cartinhour, M.L. Shuler, Effect of Iron Concentration on the Growth Rate of Pseudomonas syringae and the Expression of Virulence Factors in hrp-Inducing Minimal Medium, Appl Environ Microb, 75 (2009) 2720-2726.
[46] B.M.R. Appenzeller, C. Yanez, F. Jorand, J.C. Block, Advantage provided by iron for Escherichia coli growth and cultivability in drinking water, Appl Environ Microb, 71 (2005) 5621-5623.
[47] D. Grandjean, F. Jorand, H. Guilloteau, J.C. Block, Iron uptake is essential for Escherichia coli survival in drinking water, Lett Appl Microbiol, 43 (2006) 111-117.
[48] P.H. Wolf, S. Siverns, S. Monti, UF membranes for RO desalination pretreatment, Desalination, 182 (2005) 293-300.

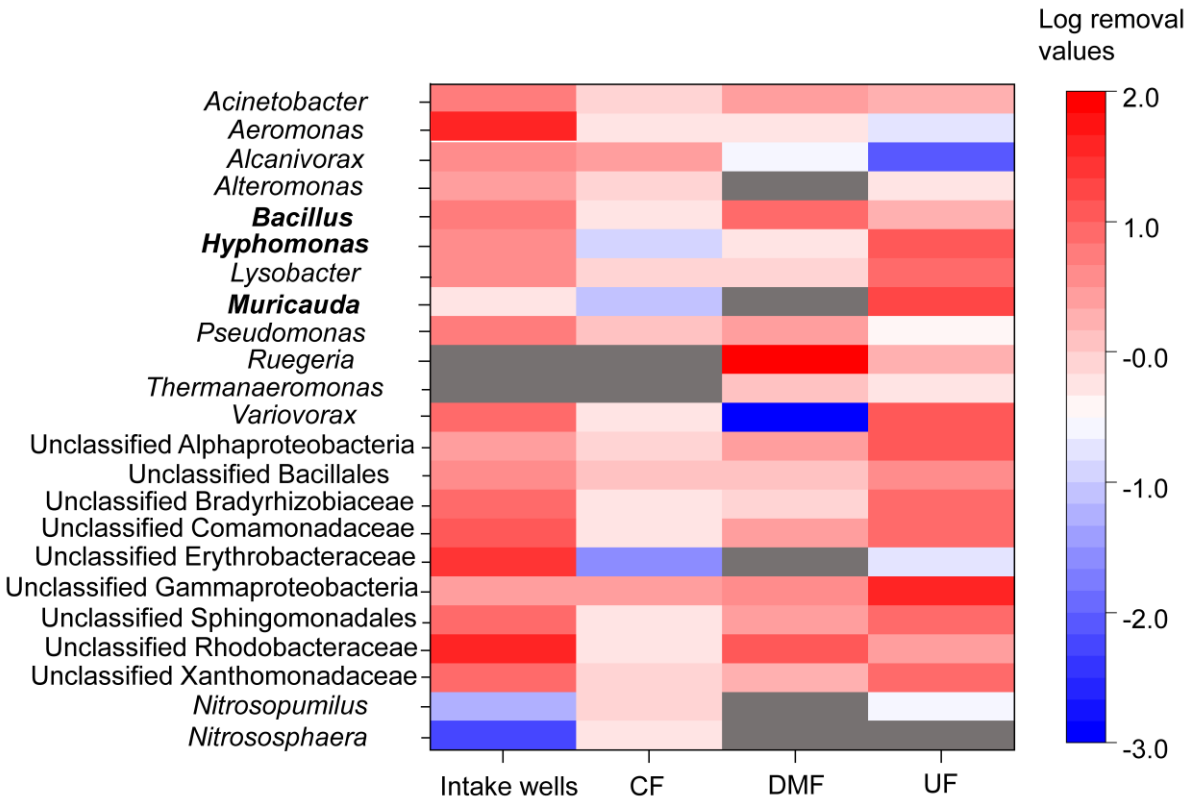

Site A
Holding tanks
DMF
CF
Wells
Sea water
Seabed
Seabed
To SWRO
Site B
Holding tanks
CF
CF
Wells
Sea water
Seabed
Seabed
To SWRO
Site C
Holding tanks
CF
UF
Wells
Sea water
Seabed
Seabed
To SWRO
Site D
DMF
CF
Sea water
To SWRO
Site E
UF
Holding tanks
CF
Sea water
To SWRO

543


Occur more frequently than prediction by model
27.27% Ruegeria
27.27% Hyphomonas
9.09% Alteromonas
9.09% Pseudoruegeria
9.09% Bacillus
9.09% Muricauda
9.09% Tropicibacter
R²=0.22
Purple OTUs
Green OTUs
Occur less frequently than prediction by model
37.50% Ruegeria
12.50% Pedobacter
8.33% Alteromonas
8.33% Tropicibacter
8.33% Pseudomonas
4.17% Acinetobacter
4.17% Paenibacillus
4.17% Curvibacter
4.17% Kerstersia
4.17% Legionella
4.17% Roseivirga

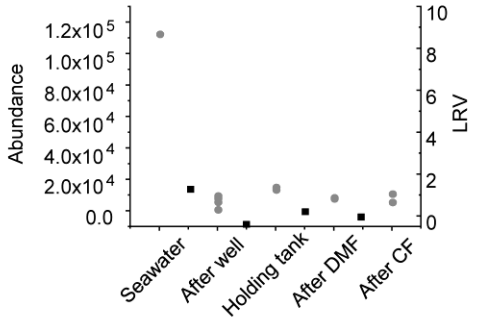
544

545

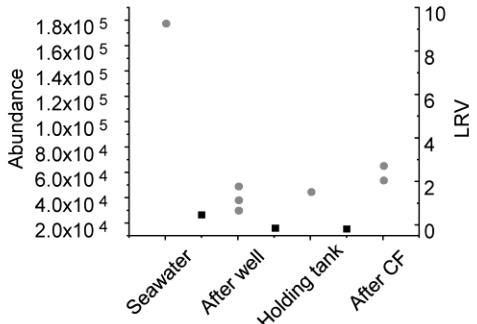

Log removal values
2.0
1.0
-0.0
-1.0
-2.0
-3.0
Acinetobacter
Aeromonas
Alcanivorax
Alteromonas
Bacillus
Hyphomonas
Lysobacter
Muricauda
Pseudomonas
Ruegeria
Thermanaeromonas
Variovorax
Unclassified Alphaproteobacteria
Unclassified Bacillales
Unclassified Bradyrhizobiaceae
Unclassified Comamonadaceae
Unclassified Erythrobacteraceae
Unclassified Gammaproteobacteria
Unclassified Sphingomonadales
Unclassified Rhodobacteraceae
Unclassified Xanthomonadaceae
Nitrosopumilus
Nitrososphaera
Intake wells
CF
DMF
UF

546

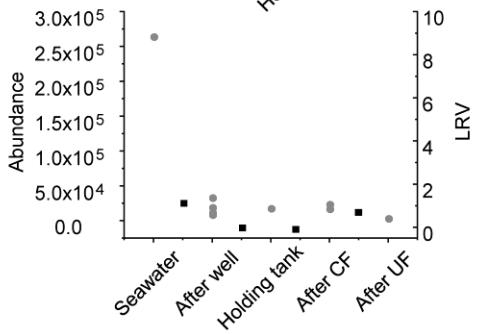
Site A



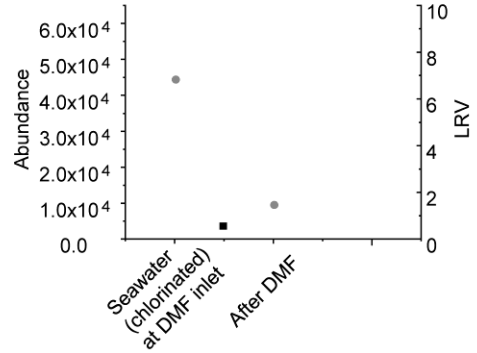
Site B



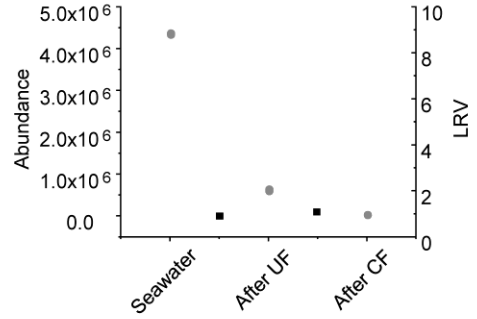
Site C



Site D



Site E



547