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Effect of engineered environment on microbial community structure in biofilter and biofilm on reverse osmosis membrane

Sanghyun Jeong^{a, b, c, 1}, Kyungjin Cho^{d, 1}, Dawoon Jeong^{d, e}, Seockheon Lee^d, TorOve Leiknes^b,
Saravanamuthu Vigneswaran^{c, *}, Hyokwan Bae^{f, *}

^a Graduate School of Water Resources, Sungkyunkwan University (SKKU), 2066, Seobu-ro, Jangan-gu, Suwon-si, Gyeonggi-do, 16419, Republic of Korea

^b Biological and Environmental Science & Engineering (BESE), Water Desalination and Reuse Center (WDRC), King Abdullah University of Science and Technology (KAUST), Thuwal 23955-6900, Saudi Arabia

^c Faculty of Engineering and IT, University of Technology, Sydney (UTS), PO Box 123, Broadway, NSW 2007, Australia

^d Center for Water Resource Cycle Research, Korea Institute of Science and Technology, 39-1 Hawolgok-Dong, Seongbuk-Gu, Seoul 136-791, Republic of Korea

^e Chemical and Biomolecular Engineering, Yonsei University, 50 Yonsei-ro, Seodaemun-Gu, Seoul 120-749, Republic of Korea

^f Department of Civil and Environmental Engineering, Pusan National University, 63 Busandeahak-ro, Geumjeong-Gu, Busan 46241, Republic of Korea

¹ Co-first authors (Sanghyun Jeong and Kyungjin Cho) contributed equally to this work.

^{*} Corresponding authors: Saravanamuthu Vigneswaran (Tel.: +61-2- 9514-2641; Email: Saravanamuthu.Vigneswaran@uts.edu.au), Hyokwan Bae (Tel.: +82-51-510-2392; Email: hyokwan.bae@pusan.ac.kr)

Abstract

Four dual media filters (DMFs) were operated in a biofiltration mode with different engineered environments (DMF I and II: coagulation with/without acidification and DMF III and IV: without/with chlorination). Designed biofilm enrichment reactors (BERs) containing the removable

reverse osmosis (RO) coupons, were connected at the end of the DMFs in parallel to analyze the biofilm on the RO membrane by DMF effluents. Filtration performances were evaluated in terms of dissolved organic carbon (DOC) and assimilable organic carbon (AOC). Organic foulants on the RO membrane were also quantified and fractionized. The bacterial community structures in liquid (seawater and effluent) and biofilm (DMF and RO) samples were analyzed using 454-pyrosequencing. The DMF IV fed with the chlorinated seawater demonstrated the highest reductions of DOC including LMW-N as well as AOC among the other DMFs. The DMF IV was also effective in reducing organic foulants on the RO membrane surface. The bacterial community structure was grouped according to the sample phase (i.e., liquid and biofilm samples), sampling location (i.e., DMF and RO samples), and chlorination (chlorinated and non-chlorinated samples). In particular, the biofilm community in the DMF IV differed from the other DMF treatments, suggesting that chlorination exerted as stronger selective pressure than pH adjustment or coagulation on the biofilm community. In the DMF IV, several chemoorganotrophic chlorine-resistant biofilm-forming bacteria such as *Hyphomonas*, *Erythrobacter*, and *Sphingomonas* were predominant, and they may enhance organic carbon degradation efficiency. Diverse halophilic or halotolerant organic degraders were also found in other DMFs (i.e., DMF I, II, and III). Various kinds of dominant biofilm-forming bacteria were also investigated in RO membrane samples; the results provided possible candidates that cause biofouling when DMF process is applied as the pretreatment option for the RO process.

Keywords: Biofilm; Biofiltration; Chlorination; Coagulation; Seawater reverse osmosis; Microbial community structure

Abbreviations

AOC: Assimilable organic carbon; ATP: Adenosine tri-phosphate; BB: Building blocks; BER: Biofilm Enrichment Reactor; BP: Biopolymers; DMF; Dual media filter; DOC: Dissolved organic

53 carbon; HS: Humic substances; LC-OCD: Liquid chromatography with organic carbon detection;
54 LMW-N: Low molecular weight neutrals; NGS: New generation sequencing; PA: Polyamide; PAC:
55 Powder activated carbon; qPCR: Quantitative polymerase chain reaction; RO: Reverse osmosis;
56 RSW: Raw Seawater; SWRO: Seawater reverse osmosis

1. Introduction

At present, seawater RO (SWRO) is the leading and preferred technology for desalination and is the benchmark for comparison with any new desalination technologies. SWRO desalination consisted of the intake, pretreatment, post-treatment, and brine discharge stages. Of these stages, pretreatment of raw seawater before it is fed into the RO process accounts for most total energy use in SWRO (Dreizin et al., 2008). Pretreatment systems are generally installed upstream of RO membranes to: firstly, reduce the inorganic load of colloidal and particulate matter reaching the membranes; and secondly, minimize or delay associated operational problems. An effective pretreatment strategy is a crucial requirement for reducing the fouling rate and cost-effective desalination (Ghaffour et al., 2013). However, biofouling can occur extensively on the membranes even after feed stream has been pretreated and disinfectants such as chlorine are added (Matin et al., 2011). Biofouling (or biofilm forming) potential of the feed water depends on concentration and speciation of the microorganisms, content of easily biodegradable compounds, concentration and composition of nutrients and water temperature (Xue et al., 2014).

Recently, biofiltration has been employed as a pretreatment for SWRO since biofouling potential can be reduced through adsorption of organic matter onto filter media. Biodegradation of the organic matter was enabled by microorganisms developed in the filter (Jeong et al., 2014). Dual media filtration (DMF) is installed as a primary pretreatment in most SWRO desalination plants because of its ease to operate (Voutchkov, 2010). DMF facilitated to obtain good quality feed water having very low turbidities and to achieve a feed silt density index (SDI) of less than 5, which is considered acceptable for RO system (Mitrouli et al., 2008). When high concentrations of organic matter or turbidity loads are encountered, coagulation is incorporated to retain the particulate and colloidal matter in DMF (Villacorte et al., 2015). In addition, acidification (or pH adjustment to acidic level)

has been selected to help the hydrolytic polymerization in coagulation prior to DMF (Sutzkover-Gutman and Hasson, 2010).

However, if DMF is operated in the biofiltration mode in desalination plants, it is expected that organic fouling can be further reduced, in addition to the particulate removal. A previous study demonstrated the feasibility of rapid sand filter as a biofilter, and they observed a significant reduction in the amount of organic carbon from the source water with the outgrowth of a microbial population and biofilm development on the filter bed medium (Bar-Zeev et al., 2012). The combination of deep bed filtration (packed with sand and/or anthracite) and in-line flocculation was also used to test the potential of biofilter for SWRO pretreatment (Jeong and Vigneswaran, 2013). It required a month to create a bio-stabilization stage with seawater. Hence, further promoting biological activity in DMF as a biofilter should be studied to ensure that biofiltration operates effectively.

Biofouling reduction using DMF may be achieved by choosing the appropriate operational conditions (Jeong et al., 2016b), and the advanced understanding of microbial role in biofilters. Generally the DMF process operates with coagulation technique but it is not effective in reducing the organic and biofoulant (Matin et al., 2011). In the SWRO process, pre-chlorination is normally used and it is employed to the seawater intake to prevent biological growth during the pretreatment stage in desalination systems (Nguyen et al., 2012). However, chlorination can also increase the AOC, which promotes the re-growth of biofilm-forming bacteria. It can be speculated that pre-chlorination enhances the removal of organics during the DMF process by encouraging the growth of biofilm-forming bacteria despite its biocide effect. However, not many studies have been done on pre-chlorination with DMF in the SWRO process in mind. For this reason, this study evaluated the effects of various kinds of pretreatment processes including pre-chlorine treatment on DMF performance.

Understanding the biofilm-forming microbial community in the SWRO process will help for biofilm characterization and process optimization (e.g., the modification of RO surface chemistry design or the development of cleaning method) (Cho et al., 2016, Manes et al., 2011). In the SWRO process, the biofilm-forming bacterial community is dependent on both seawater conditions (or environmental conditions) and engineering factors of the pretreatment process (Lee and Kim, 2011). However, previous studies indicated that engineering factors have more significant influences on the biofilm community structure because they can provide a strong selective pressure rather than natural conditions (Ahn et al., 2009, Xia et al., 2012). In fact, a drastic bacterial community shift has been found in the SWRO pretreatment processes. A significantly different biofilm community was formed in the membrane bioreactors used for seawater pretreatment depending on the use of powdered activated carbon (PAC); *Maricoccus* was the dominant biofilm genus in the PAC-free state, whereas *Thiothrix*-like bacteria was the most dominant biofilm bacteria in the PAC-added state (Jeong et al., 2016a). Changes in bacterial community structure were also reported in DMF process; the bacterial community structure of permeate clearly shifted along with the DMF operation, which was corresponded with the decrease of the permeability (Jeong et al., 2016b). It can therefore be suggested that the types of pretreatment determined the bacterial community structure, indicating the need to investigate the process specifically. However, our knowledge of the impact of the DMF operational conditions (or engineering factors) is still limited. In particular, to the best of our knowledge, a combined approach using chlorination (pre-chlorination-DMF) has never been studied in detail.

This study investigated the effects of different combinations of DMF processes including coagulation (with/without pH adjustment)-DMF, and with/without chlorination-DMF, in terms of the DMF performance and bacterial community structure. The evaluation of DMF performance was conducted based on the reduction of dissolved organic carbon (DOC) and assimilable organic carbon (AOC).

Laboratory-designed biofilm enrichment reactors (BER) fixing the RO coupons were connected at the end of the DMFs operated in parallel to create a biofilm on the RO membrane formed by DMF effluent. Performance of DMF was evaluated in terms of the reduction of dissolved organic carbon (DOC) and assimilable organic carbon (AOC). Organic foulants on the RO membrane were also quantified and fractionized. The bacterial community structure was analyzed using the next generation sequencing (NGS) method because it can effectively generate huge amount of sequences within a short period of time so that a deeper understanding of the microbial communities is possible.

2. Materials and methods

2.1 DMF operation

DMF operation was conducted on-site with continuous feeding of raw seawater (RSW, which was pre-filtered through 140 μ m) at the Sydney Institute of Marine Science at Chowder Bay, Sydney in Australia for 30 d. Prior to starting the main experiment, the DMF operation was stabilized for one week. During this stabilization period, RSW was fed into DMFs without any chemical dosing.

Fig. 1 presents a schematic diagram of the experimental set-up (four DMF columns). The DMF columns (diameter = 0.02 m and effective height = 0.90 m; packed with sand (0.25 m at the bottom) and anthracite (0.65 m above the sand layer)) were operated in parallel in different operational conditions. A preliminary test was conducted to optimize the filtration velocity, filtration time and backwashing frequency and intensity in order to operate the filters in the biofiltration mode (Jeong et al., 2016b). The monitoring of samples collected at various filtration times at different operating conditions revealed that DMF with 24h-backwash interval and 2min-backwashing duration (at a backwashing velocity of 20 m/h) was effective in promoting biological growth on DMF, which functioned as a biofilter.

During the experimental period, temperature, pH and turbidity of RSW were 23.3 ± 0.7 °C, 7.8 ± 0.2 , and 0.15 ± 0.08 NTU, respectively. Filtration velocity in all four DMF columns was at 10 m/h (52.3 mL/min). Ferric sulfate solution (Orica Chemical, Australia) was used as a primary coagulant. Coagulation aid was Floquat FL 4526 PWG (SNF, Australia), which is Polydiallyldimethylammonium chloride (PoluDADMAC). The operations of four DMF columns are summarized in **Table 1**.

2.2 Biofilm enrichment reactor (BER)

As shown in **Fig. 1**, BERs were installed at the end of each DMF column to obtain biofilms on the RO membrane coupons. A flat sheet thin-film composite polyamide (PA) RO membrane (RE8040-SHF, Toray Chemical, Republic of Korea) was utilized. 12 RO coupons (1cm x 2cm size) were fixed in the holder of each cylindrical BER column. BER columns were placed horizontally on the table and RO membrane coupons were installed vertically in the BER columns to prevent from the effect of gravity affecting biofilm growth on the membranes. DMF effluents were passed continuously through the BER columns. The RO coupons were taken away during the DMF backwashing periods every 5 d for further analysis.

2.3 Organic analyses

2.3.1 Dissolved organic carbon (DOC)

Organic reduction by DMF and organic fouling on RO membrane coupons were measured in terms of DOC concentration. This reduction was measured using DOC-LABOR Liquid Chromatography - Organic Carbon Detector (LC-OCD). A software program (ChromCALC DOC-LABOR, Karlsruhe, Germany) identified different organic fractions from the LC-OCD chromatogram obtained. DOC in seawater mainly contains biopolymers (BP), humic substances (HS or humics), building blocks (BB)

and low molecular weight neutrals (LMW-N) and acids (LMW-A) (Huber et al., 2011). The analysis was conducted using dual column with 180 min retention time. Procedures concerning the LC-OCD have previously been detailed in previous study (Jeong et al., 2013b). The organic foulant on the RO membrane coupon was extracted using mild sonication at 10W for 30 min to avoid the denaturing of organic matter. To do this, RO membrane coupons were cut into small parts and put into a 50mL tube containing 20 mL of 1x phosphate buffered saline.

2.3.2 Assimilable organic carbon (AOC)

AOC can be used as an indicator to quantify the relative biofouling potential or biofilm formation of water samples. AOC concentration in feed water (seawater) and filtered water samples were monitored every 5 d. This study used a bioluminescence method with *Vibrio fischeri* MJ-1 strain (Jeong et al., 2013c). All the samples including the chlorinated samples taken from DMF IV were free chlorine quenched using sodium bisulfite added at 110% of the stoichiometric dosage (it was around one drop) to ensure the no detectable chlorine residual prior to AOC test. A standard curve established in a previous study (Jeong et al., 2013c) was employed. Glucose served as a model AOC compound since better cell growth can be obtained even at low concentration ($\sim 0.1 \mu\text{g-C/L}$). Thus, AOC concentration is expressed as $\mu\text{g-C glucose equivalents/L}$.

2.4 Microbial analyses

Filter medium samples on the top (0.75m from the bottom of the filter) and middle (0.45m from the bottom) layers of DMFs were taken through sampling ports every 5 d (both layers contained mostly anthracite and small portions of sand). Once the media samples were collected they were kept in a box containing ice prior to microbial analyses.

For ATP analysis, the DMF media were homogenized by vortex mixing for 1 min. The media was placed in a beaker with 1x phosphorus buffered saline (PBS). The beaker was sonicated to extract the

biofilm on the media. The mild sonication was carried out in an ultrasonic bath (Powersonic 420, Thermoline Scientific, 300 W) for a short time (10 min).

2.4.1 Adenosine tri-phosphate (ATP)

ATP was used as an indicator of biological activity in DMF effluent samples and in biofilm on media and BacTiter-Glo Microbial Assay kit was used for ATP measurement. A 96-well luminometer (Wallac 1420 VICTOR2™ multilabel, multitask plate reader (PerkinElmer Inc., US)) was utilized for the measurement of luminescence produced from ATP reaction at room temperature. The procedure was followed according to the manufacturer's guidelines.

2.4.2 DNA extraction

DNA samples on the media of DMF and fouled RO membrane were extracted by a modified CTAB-PEG protocol (Jeong et al. 2014). DNA was extracted from 1 mL (cm³) media sample at the speed of 13,000 g for 5 min. To extract the surface bound community from the fouled RO membrane taken every 5 d, a piece of membrane (1 cm x 2 cm) that was cut into small pieces was put into 2 mL tubes and glass beads were added. Similarly, 1 mL of DMF media sample was put into 2 mL tubes.

2.4.3 454 pyrosequencing

The 454 pyrosequencing analysis was conducted to reveal the bacterial community structure. Two combined universal bacterial primers conjugated with adapter, A-27F (5'- GAGTT TGATC MTGGC TCAG -3') or B-518R (5'- WTTAC CGCGG CTGCT GG -3'), were applied to amplify the bacterial 16S rRNA gene sequence (Ko et al., 2016). The PCR amplification was executed as follows: initial denaturation at 94 °C for 3 min; 35 cycles of denaturation at 94 °C for 15 s per cycle, annealing at 55 °C for 45 s and extension at 72 °C for 1 min; and a final extension at 72 °C lasting 8 min. Following PCR purification, the sequencing run was done using a Genome Sequencer FLX plus instrument (454 Life Sciences). The quality control for the obtained 16S rRNA gene sequences was conducted according to the advice of Macrogen Ltd. (Seoul, Republic of Korea) as described

previously (Shin et al., 2016). The refined sequences were clustered using MOTHUR with CD-HIT-OTU based on the 3% genetic cut-off as operational taxonomic units (OTUs). To identify the microorganisms using the SILVA 16S rRNA gene database, a taxonomic assignment was undertaken. Overall, twenty four genomic DNA samples were tested for seawater, DMF media, RO membrane, and effluent. The sample list for the 454 pyrosequencing analysis is given in **Table 2**.

2.4.4 Statistical analysis of 454 pyrosequencing data

Two statistical analyses were performed to visualize how the similarity of bacterial community structures is differentiated among the samples; i) non-metric multidimensional scaling (NMDS) and ii) clustering analysis (CA). Both statistical analyses were conducted based on Bray-Curtis distance using PC-ORD v.5.0, MjM software (Gleneden Beach, OR). The relative abundance of bacterial OTUs was used for the statistical analyses. The significance of the NMDS result was assessed on the basis of the statistical values (i.e., stress value < 20 and instability value $< 10^{-4}$) (Quintana et al., 2010).

3. Results and discussion

3.1 Organic reduction by DMF biofilter

3.1.1 DOC reduction

Organic reduction by DMF biofilters was evaluated in terms of the concentration of DOC and organic fractions. DOC concentration of RSW was 1.43 ± 0.24 mg/L, including 0.07 ± 0.06 mg/L of BP, 0.58 ± 0.04 mg/L of HS, 0.04 ± 0.02 mg/L of BB and 0.80 ± 0.24 mg/L of LMW-N, during the entire operational time (it was monitored every 2-3 days). The DOC fraction in RSW consisted mainly of HS (39%) and LMW-N (55%).

The overall efficiency of DMF biofilters in the removing DOC (during an operation lasting 30 d) was in the following order (**Table 3**): DMF IV (25.14%) > DMF II (21.83%) $\approx$ DMF I (19.77%) > DMF III (10.23%). The amount of BP removed by DMF biofilters was very small, which was probably due to biological activity in the DMF biofilters (BP produced from microbes during their growth), or DMF did not effectively work as a barrier to BP. It even increased slightly for the DMF III (0.07 ± 0.06 to 0.09 ± 0.04 mg/L). The efficiency of DMF biofilters in the removing BB was also very poor. BB is broken down organic materials from HS and it is occasionally increased depending on biological activity (Jeong et al., 2013b). Here, it should be noted that these two organic compounds (BP and BB) are in relatively small amounts compared to HS and LMW-N in RSW. A small amount of HS was removed by DMFs I and II operated with coagulation. For example, DMF I reduced only around $9 \pm 8\%$ of HS from RSW. This is because the pH adjustment improved the coagulation performance in DMF I. In the case of LMW-N, all the DMF biofilters indicated significant removal efficiency. In particular, DMF IV resulted in more than 50% removal of LMW-N from RSW (from 0.80 ± 0.24 to 0.37 ± 0.11 mg/L). The reduction trend of LMW-N was very similar to that of total DOC (DMF IV: 53.36% > DMF II: 38.16% > DMF I: 35.03% > DMF III > 26.81%), since LMW-N is the highest portion (55%) of DOC in seawater. However, the removal efficiency by DMF II was slightly higher than DMF I, suggesting that DMF II provides more favourable biological growth conditions than DMF I. This indicates that LMW-N is closely related to biological activity and biological utilization of organics in DMF biofilters.

Moreover, as can be seen in **Fig. 2**, DOC removal by DMF biofilters increased with operational time. After 15 d of operation, DOC removal by DMF biofilters improved remarkably and it remained constant afterwards. In particular, DMF IV demonstrated the highest efficiency in DOC removal from the initial period of operation indicating that chlorinated seawater affects the growth of specific microbial community structures in the biofilter, which may help with organic assimilation. Further

examination of the role of microbes responsible for organic reduction must to be carried out to optimize or maximize the applicability of biofilter as a pretreatment to seawater.

3.1.2 AOC (or biofouling potential) reduction

The result of AOC reduction can support the carbon source utilization by biological activity in the biofilter and furthermore, AOC measurement can be considered as a biofouling potential. AOC concentration of RSW was 63.03 ± 6.67 $\mu\text{g-C}$ glucose equivalent/L during the entire operational period (**Fig. 3**). AOC removal increased with the operational time, especially after 15 d when DMF proved to be an effective biofilter. DMF III removed the AOC from RSW marginally (8.35%) while DMF IV's AOC removal efficiency was 45.36%. DMF IV produced the most biological safe effluent for RO. AOC concentration in DMF IV effluent after the 15th day was maintained at around 30 $\mu\text{g-C}$ glucose equivalent/L, while AOC concentration in DMF IV effluent ranged from 57.99 to 28.47 $\mu\text{g-C}$ glucose equivalent/L during the entire operational period. AOC removal by DMFs I and II was similar but in DMF I it was slightly higher than DMF II (DMF I: 25.65% > DMF II: 22.97%).

3.2 Biological activity in the DMF process

3.2.1 Biological activity in DMF effluents

Biological activity in DMF effluents was assessed in terms of ATP concentration at different operational times (Days 5, 10, 15, 20, 25 and 30). **Fig. 4a** shows the variation of ATP concentration in the effluents of DMF biofilters operated at different conditions as a function of time. Smaller biological activity was found in the effluents of DMFs IV and I (82.75 ± 36.00 and 69.22 ± 35.92 nM/L, respectively) compared to other two DMFs, which released more ATP to the effluents throughout the operational period. Here, it is noted that ATP concentration in a mixed culture is not an absolute value to quantify the biological activity. An average value of 173.08 ± 87.98 nM/L of ATP was

detected in DMF III effluent. On the other hand, DMF IV was operated with chlorinated seawater and it constantly produced effluent with a certain level of chlorine residual (at around 0.2 mg/L) during the entire operation. This resulted in the reduced presence of active cells in the effluent. Further it will assist in the reduction of biofilm growth in the downstream to RO unit and on the RO membrane.

3.2.2 Biological activity on DMF media

The ATP concentrations on the DMF media samples were also measured at different operational times (Days 5, 15 and 30) and locations (Top and Middle) in order to quantify the biological activity in the DMF biofilters (**Fig. 4b**). The top position of DMF biofilters possessed less active biomass compared to middle position during the entire operational period (Top: $0.62 \pm 0.06 \sim 2.26 \pm 0.23$ pg/m³ < Mid: $2.59 \pm 0.26 \sim 6.07 \pm 0.61$ pg/m³). This is probably due to backwashing, which removes blocking and clogging materials from the top layer of filter media. In this case, some biomass could be detached and removed from the media. DMF II revealed more biological activity on the media than DMF I, indicating that coagulation without acidification (in DMF II) resulted in more favorable condition to maintain a stable biological activity on the DMF biofilter. On the other hand, the acidification led to lower biological growth on the media (DMF I). In addition, biological activity on the filter media improved as an operational time proceeded, except for DMF I (15-top and 15-mid = 0.86 ± 0.19 and 3.56 ± 0.36 pg/m³, and 30-top and 30-mid = 0.69 ± 0.07 and 3.12 ± 0.31 pg/m³, respectively). A similar concentration of ATP was detected on the media of two DMFs (III and IV), showing that chlorination did not hinder the biological growth in the DMF biofilters. Nevertheless, the biological growth on the top layer of DMF IV during the initial operational period was slightly low.

3.3 Organic fouling on RO membrane

The different DMF treatment methods resulted in deposition of significantly different amounts of organic foulants on the RO membrane. **Fig. 5** shows the amount of organic foulants on the RO membrane formed by the effluents of DMF biofilters run in different operational conditions after 30 d of the operation. The total amount of organic foulants on the RO membrane with DMF III effluent was 1361.5 ± 30.4 mg-C/m². DMFs I and IV led to less organic fouling on the RO membrane (compared to other DMFs), 296.6 ± 15.4 and 266.2 ± 34.6 mg-C/m², respectively. This indicates that DMF I was formed organic fouling layer on the RO membrane more homogeneously. Interestingly, LMW-N is the highest portion (54~77%) of all the organic foulants on the RO membranes. The larger amount of organic foulants formed by DMF III (1361.5 mg-C/m²) compared to other DMFs due to mostly LMW-N. Slightly larger amounts of BP, HS and BB were found on the RO membrane fouled by DMF III effluent. In summary, the total DOC concentration on RO membrane surface was in the following order (DMF IV: 266.2 ± 34.6 mg-C/m² > DMF I: 296.6 ± 15.4 mg-C/m² > DMF II: 698.9 ± 27.7 mg-C/m² > DMF III > $1,361.5 \pm 30.4$ mg-C/m²). It can therefore be suggested that the pre-chlorination combined with DMF process (DMF IV) was the most effective in the reducing the organic foulants on the RO membranes. There was no obvious deterioration of membrane by free chlorine. Further study on the effect of initially reduced organic fouling on the biofilm formation (biofouling) in a long-term operation is required.

3.4 Bacterial community structure

3.4.1. Overall comparison

In total, 286,936 raw 16S rRNA gene amplicons were generated by the 454 pyrosequencing analysis. After the low quality sequences were screened, 96,349 of high quality 16S rRNA gene sequences

were grouped into 183 bacterial OTUs. Statistical analyses including NMDS and CA were used to evaluate the level of microbial similarity among the samples.

The 2D ordination plot and clustering dendrogram demonstrate that the microbial community structure was clearly classified into two groups; firstly, the biofilm samples (i.e., DMF media and RO membrane) and secondly, the liquid ones (i.e., seawater and DMF effluent) (**Fig. 6**). The middle sample in DMF IV (i.e., 3IVM) was positioned in a distinct location compared to the other samples and it is probably because chlorination-DMF treatment created the unique bacterial community niche (**Fig. 6a**). However, according to the dendrogram, the bacterial community structure of 3IVM was more similar to the biofilm samples; therefore, it could be classified as the group of biofilm samples (**Fig. 6b**). This clustering pattern between the biofilm and liquid samples indicates that the different biofilm communities derived from the seawater were formed on the DMF media and RO membranes. The outcomes suggest that the environmental factors resulted from the engineering process such as DMF or RO provided a strong selective pressure on the bacterial community structure rather than seawater conditions. Interestingly, the microbial similarity was more varied among the biofilm samples' group rather than the liquid samples' group (**Fig. 6b**), which indicates that the biofilm community structure was significantly affected by the types of engineered systems. For this reason, the statistical analyses on the group of the biofilm samples were done to obtain a better understanding of the microbial clustering pattern caused by the engineering factors. The middle sample of DMF IV media (i.e., 3IVM) was excluded from the additional statistical analyses because its community structure was significantly different from the other biofilm samples.

The 2D plot and the dendrogram for the biofilm samples are presented in **Fig. 7**. Interestingly, the biofilm community structure was classified into three groups (i.e., Sub-group I: non-chlorinated DMF media samples; Sub-group II: chlorinated DMF media and RO membrane samples; and Sub-group III: non-chlorinated RO membrane samples). This clustering pattern provides some

meaningful insights. The biofilm community structure was differentiated according to the sampling location (Sub-group I vs. Sub-groups II and III) except DMF IV (**Fig. 7**). The results imply that the biofilm community structure depended on the sampling location (i.e., DMF media or RO membranes). It was reported that the biofilm community structure was determined by the sampling locations in the SWRO process (Levi et al., 2016). Additionally, the major biofilm community on the DMF media was completely differed from that of the cartridge filter treating the DMF effluent (Jeong et al., 2016b). Therefore, it could be speculated that the media types contributed to the formation of different biofilm communities.

Although the biofilm community structure was primarily classified by the sampling position, an exceptional clustering pattern was observed for the chlorinated biofilm samples; they were in fact clustered regardless of sampling location (i.e., DMF media: 3IVT and RO membranes: IV5, IV15, and IV30) (**Fig. 7**). In the chlorinated condition, the specific bacterial group having chlorine resistance could survive in the biofilter media because most bacteria are susceptible to free residual chlorine higher than 0.2 mg/L (Farenhorst et al., 2017); for this reason, chlorination has been regarded as the strong engineering factor exerting the selective pressure on the microbial community. According to a previous study (Shi et al., 2013), chlorination reduced the relative abundance of Bacteroidetes or Actinobacteria in drinking water treatment plant, whereas it increased the relative abundance of Proteobacteria. In this study, the biofilm community structure was also clearly classified by the presence (Sub-group II) or absence (Sub-groups I and III) of the chlorination. The results indicate that chlorination contributed to the distinct biofilm community structure dominating both DMF media and RO membrane samples (**Fig. 7**). Therefore, pre-chlorination with DMF (DMF IV) might employ strong selective pressure on the biofilm community structure rather than the sampling location or other DMF treatment methods such as coagulation with/without pH adjustment.

3.4.2. In-depth information of bacterial community structures

Analysis of bacterial community structures in biological or membrane processes is necessary to understand their roles in utilizing nutrients (or organic carbon) and fouling. In particular, the dominant biofilm communities in DMF media and RO membranes could be regarded as the functional organic carbon degrader and possible biofouling-causing bacteria in SWRO processes, respectively. For this reason, an in-depth discussion was initiated based on the dominant bacteria. The dominant bacterial OTUs were identified at the genus level and correlated with certain physiological characteristics, for example, salt tolerance, substrate type, and biofilm-forming potential. The bacterial information of the dominant bacterial OTU is listed in **Table S1-S4**. As described in section 3.4.1, the bacterial community structure was grouped with the sample phase, sampling location, and chlorination. Therefore, the bacterial community structure was discussed in detail in terms of: i) liquid samples, ii) DMF IV biofilm samples (which was operated under chlorination), iii) other DMF biofilm samples (i.e., DMF I, II, and III), and iv) RO membrane biofilm samples.

For the liquid sample, genus *Dehalococcoides* completely dominated all liquid samples (both seawater and effluent) with high relative abundance (>75.9%) (**Table S1, S4**). *Dehalococcoides*, strict anaerobic chemotrophic bacteria, has been mainly discovered in strict anoxic conditions such as sediment or aquifers (Löffler et al., 2013). Some strains belonging to *Dehalococcoides* have been isolated from marine sediment or can grow under saline conditions (Empadinhas et al., 2004); therefore, this bacterium may originate from intake seawater. The dominance of *Dehalococcoides* in all liquid samples implies that the bacterial composition of effluent was not affected by the types of DMF treatment methods, not the seawater (or feed water).

For the DMF samples, the biofilm community structure of DMF IV was different from the other DMF biofilm samples (**Fig. 6, 7**). The dominant biofilm bacteria in DMF IV may have biofilm-forming potential and chlorine resistance because the residual chlorine concentration of DMF IV was

maintained at > 0.2 mg/L. In fact, *Hyphomonas* (23.8%; relative abundance in each sample), which was the most dominant in DMF 3IVT, is known as marine biofilm-forming and chlorine resistant bacteria (Choi et al., 2010) (**Table S2**). Genus *Erythrobacter* (12.8%) has been also discovered in marine biofilm (Cho et al., 2016) or chlorine stress condition (Soto-Giron et al., 2016). Because these bacteria have chemoorganotrophic metabolism (Brenner and Krieg, 2005), they may contribute to the reduction of organic compounds in DMF IV. However, *Trichodesmium* (61.7%) and *Nitrospira* (19.5%) which were the dominant species in DMF 3IVM have photosynthetic (Capone et al., 1997) and chemolithotrophic (Brenner and Krieg, 2005) growth characteristics. Both bacteria therefore did not function as an organic carbon degrader. In DMF 3IVT, *Sphingomonas* (5.8%) was probably the major organic carbon degrader because this genus has been found to have: firstly, biofilm-forming and AOC removal potential (Jeong et al., 2016a); and secondly, chlorine resistance (Sun et al., 2013). In this study, the organic removal efficiency in DMF effluent and organic fouling reduction on RO membrane were the most efficient in DMF IV (**Figs. 3 and 5**). Therefore, it could be suggested that the chemoorganotrophic biofilm communities having chlorine resistance such as *Hyphomonas*, *Erythrobacter*, and *Sphingomonas* were strong organic degrader under chlorinated seawater conditions.

As described in section 3.2.1, the biofilm community structure in the other DMF treatments (i.e., DMF I, II and III) was not significantly differentiated (**Fig. 7**). In the three DMF treatment processes, four genera, namely *Sneathiella* (i.e., 3IT, 3IIT, and 3IM), *Erythrobacter* (i.e., 3IT and 3IIT), *Maricaulis* (i.e., 3IIT, 3IIIT, and 3IIM), and *Roseobacter* (i.e., 3IT, 3IIIT, and 3IIM) were commonly detected in at least two or more biofilm samples as the dominant bacteria (**Table S2**). These genera are halophilic or halotolerant bacteria and have chemoorganotrophic growth metabolisms (Brenner and Krieg, 2005, Jordan et al., 2007). Three genera - *Erythrobacter* (Cho et al., 2016), *Maricaulis* (Jeong et al., 2013a) and *Roseobacter* (Buchan et al., 2005) - have been identified in the various

marine biofilms. *Sneathiella* was discovered in filtrate derived from seawater treated by the microfiltration process (Bae et al., 2011), indicating that it would be the putative biofilm community in the SWRO process. *Phaeobacter* and *Oceanicola* were also dominant in DMF II (i.e., 3IIT) and DMF III (i.e., 3IIIT), respectively. Both genera are chemoheterotrophic marine bacteria and have been discovered in biofilm formed under high saline conditions (Ahn et al., 2009, Cho and Giovannoni, 2004, Jeong et al., 2016b) (**Table S2**). The genus *Thiohalobacter* was also dominant in the three DMF processes (i.e., 3IM, 3IIM, 3IIIM); however, this did not enhance organic carbon degradation because *Thiohalobacter* is chemolithoautotrophic sulfur-oxidizing bacteria (Sorokin et al., 2010). The DMF media (anthracite) used in this study contained ~2 wt.% of sulfur, which could facilitate its growth. Various sulfur-related bacteria have been discovered in biofilter systems packed with anthracite (Jeong et al., 2013a, Jeong et al., 2016b). Therefore, organotrophic marine biofilm communities such as *Sneathiella*, *Erythrobacter*, *Maricaulis*, *Roseobacter*, *Phaeobacter* and *Oceanicola* could be responsible for organic degradation.

For RO membrane samples, some biofilm bacteria were commonly observed as the dominant bacteria similarly to DMF samples. Interestingly, genus *Thalassospira*, which is chemoheterotrophic and halophilic biofilm-forming bacteria (López-López et al., 2002, López et al., 2006), was dominant in all RO membrane samples regardless of DMF treatment methods and operation time (**Table S3**). These results imply that genus *Thalassospira* could contribute to the initial biofilm formation as well as long-term biofilm retention. Genus *Alteromonas*, which has chemoorganotrophic metabolism, was dominant especially on RO membranes during the initial stages (i.e., I5, II5, IV5, and I15). It indicates that this biofilm-forming bacterium played an important role in initial biofilm formation. In fact, *Alteromonas* sp. predominated in the early stage of marine biofilm formation (Lee et al., 2008). In addition, some bacterial genera such as *Marinobacter* (i.e., I15, IV15, I30, III30, and IV30) and *Algisphaera* (i.e., I5, I15, IV15, and II30) were also commonly dominated RO membrane samples

(Table S3). *Marinobacter* has been discovered in the marine biofilm (López et al., 2006) but our knowledge of the biofilm forming potential of *Algisphaera* is limited because this genus was only recently isolated (Yoon et al., 2014). As illustrated in Fig. 7, the biofilm community on RO membranes was also differed according to the level of chlorination. In terms of the dominant bacterial genus, *Oceanicola* (i.e., IV5 and IV30) and *Micavibrio*-like bacteria (i.e., IV15 and IV30) were only revealed to be the dominant biofilm community in the BER combined with DMF IV. Genus *Micavibrio* is known as an obligate bacterial predator (Kadouri et al., 2007); therefore, this bacterium may contribute to the reduction of biofilm. Genus *Cyanobacterium*, which has photoautotrophic metabolism, was also dominant in DMF II (i.e., II30) and DMF III (i.e., III30). The growth of algae or *Cyanobacterium* leads to the production of easily biodegradable compounds in and seawater; therefore, this bacterium may promote the biofouling problem on the RO membrane. Taken together, several biofilm-forming bacteria such as *Thalassospira*, *Alteromonas*, *Marinobacter*, *Algisphaera*, *Oceanicola*, and *Cyanobacterium* were identified as the major biofilm-forming bacteria on the surface of RO membrane. They are most likely candidates causing the problem of biofouling in the SWRO processes when DMF processes operate in tandem.

4. Conclusions and remarks

This paper showed that engineering factors wielded much more significant influence on the bacterial community structure in DMFs operated in the biofiltration mode. The results were summarized as follow.

a) Organic reduction: DMF IV revealed the best DOC removal efficiency from the initial period of operation. AOC removal increased when operational time also increased, especially when DMF

became an effective biofilter after 10 d. AOC removal efficiency by DMF IV was 45.36% suggesting that DMF IV produced the most biologically safe effluent for RO.

b) Biological activity: It was found that DMF II provided more favorable biological growth condition rather than the other DMFs. Lower numbers of active biomass (or microorganisms) presented in the effluents of DMFs IV and I (82.75 ± 36.00 and 69.22 ± 35.92 nM/L, respectively) helped to reduce biofilm growth in the downstream to the RO unit as well as on the RO membrane, compared to DMFs II and III.

c) Fouling on RO membrane: The organic fouling of DMF IV was the lowest rather than other DMFs (i.e., 266.2 ± 34.6 mg-C/m²). Therefore, the pre-chlorination could be used as the effective and alternative treatment with DMF to reduce RO membrane organic fouling.

d) Bacterial community structure: The bacterial community structure was markedly different between the liquid samples and the biofilm samples. With reference to biofilm samples, the bacterial community structure was clustered along with sampling locations and depended on the presence or absence of pre-chlorination. Specifically, the pre-chlorination with DMF (DMF IV) served as a strong selective engineering factor on the biofilm community structure. Several biofilm-forming marine bacteria were detected on RO membrane surface; they may be strongly considered to be biofoulants in the SWRO process. The knowledge obtained in this study on the biofilm community has highlighted the role of key organic scavengers in the DMF process as well as major biofilm forming candidates on the RO membrane surface.

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639 **List of Tables**640 **Table 1** Operational conditions of DMF columns used in this study.641 **Table 2** The sample list for the 454 pyrosequencing analysis in this study.642 **Table 3** Concentration of DOC and organic fractions in RSW and effluents of DMF operated
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Fig. 6 Overall visualization of the microbial similarity based on the relative abundance of bacterial OTUs. (a) 2D ordination plot. (b) clustering dendrogram.

Fig. 7 Overall visualization of microbial similarity for the biofilm samples based on relative abundance of bacterial OTUs. (a) 2D ordination plot. (b) clustering dendrogram.

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Table 1 Operational conditions of DMF columns used in this study.

DMF operations	Feed water	Coagulant	Polymer coagulant aid	pH	Chlorination
DMF I	Raw seawater (RSW) – pre- filtered 140 µm	0.68 mg of Fe ³⁺ /L of Ferric sulfate	0.28 mg/L	6.5±0.3	-
DMF II		0.68 mg of Fe ³⁺ /L of Ferric sulfate	0.28 mg/L	7.5±0.1	-
DMF III		-	-	7.8±0.1	-
DMF IV		-	-	7.5±0.3	NaOCl was added to maintain 0.2-0.4 ppm residual at DMF effluent

Table 2 The sample list for the 454 pyrosequencing analysis in this study.

DMF operations	Operation period (d)	Sample sources			
		Seawater	DMF media	RO membrane	Effluent
DMF I	5	-	-	I5	-
	15	SW15*	-	I15	-
	30	SW30*	3IT**, 3IM***	I30	EI30
DMF II	5	-	-	II5	-
	15	SW15*	-	II15	-
	30	SW30*	3IIT**, 3IIM***	II30	EII30
DMF III	5	-	-	-	-
	15	SW15*	-	-	-
	30	SW30*	3IIIT**, 3IIIM***	III30	EIII30
DMF IV	5	-	-	IV5	-
	15	SW15*	-	IV15	-
	30	SW30*	3IVT**, 3IVM***	IV30	EIV30

* Seawater samples were tested at 15 d and 30 d of operational period. The same seawater was fed into each DMF system.

** Top layer in each DMF.

*** Middle layer in each DMF.

Table 3 Concentration of DOC and organic fractions in RSW and effluents of DMF operated at different operational conditions, and the removal efficiencies. (Unit: mg/L)

		DOC	BP	HS	BB	LMW-N
RSW	(AVE.)	1.46	0.07 (4.51%)*	0.58 (39.43%)*	0.04 (2.40%)*	0.80 (54.89%)*
	(STDEV.)	0.16	0.06	0.04	0.02	0.24
DMF I	(AVE.)	1.14	0.07	0.52	0.03	0.52
	(STDEV.)	0.18	0.05	0.08	0.01	0.21
	**R %	21.83	1.27	9.28	4.76	35.03
DMF II	(AVE.)	1.17	0.07	0.56	0.03	0.50
	(STDEV.)	0.21	0.05	0.06	0.01	0.19
	**R %	19.77	-11.39	2.90	4.76	38.16
DMF III	(AVE.)	1.31	0.09	0.60	0.03	0.59
	(STDEV.)	0.15	0.04	0.07	0.01	0.20
	**R %	10.23	-40.51	-4.49	16.67	26.81
DMF IV	(AVE.)	1.09	0.07	0.61	0.03	0.37
	(STDEV.)	0.10	0.04	0.04	0.01	0.11
	**R %	25.14	-6.33	-6.67	9.52	53.36

* The percentage of individual organic fraction in DOC.

** The removal efficiencies from RSW.

Fig. 1 Schematic diagram of DMF columns operated at different conditions.

Fig. 2 Variation of DOC concentration in the effluents of DMF for different operational conditions.

Fig. 3 AOC concentration in RSW and effluents of DMF for different operational conditions.

(a)

(b)

Fig. 4 Variation of ATP concentration (a) in the effluents and (b) on the media of DMF for different operational conditions.

Fig. 5 Organic foulant and its fractions (mg of DOC/m² of RO membrane) on the RO membrane fouled by different DMF effluent samples (The concentrations of DOC were measured by 296.6±15.4 mg-C/m² for DMF I, 698.9±27.7 mg-C/m² for DMF II, 1,361.5±30.4 mg-C/m² for DMF III, and 266.2±34.6 mg-C/m² for DMFIV.)

Fig. 6 Overall visualization of the microbial similarity based on the relative abundance of bacterial OTUs. (a) 2D ordination plot. (b) clustering dendrogram.

Fig. 7 Overall visualization of microbial similarity for the biofilm samples based on relative abundance of bacterial OTUs. (a) 2D ordination plot. (b) clustering dendrogram.

Highlights

- Four dual media filters (DMFs) were operated under different engineered conditions.
- DMF with pre-chlorination treatment was effective to reduce organic matter.
- Functional biofilm bacterial structure in DMF and RO membranes were investigated.
- Pre-chlorination acted stronger selective pressure on biofilm community.