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Effect of microbial community structure on organic removal and biofouling in membrane adsorption bioreactor used in seawater pretreatment

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Abstract

Membrane bioreactors (MBRs) were operated on-site for 56 d with different powdered activated carbon (PAC) dosages of 0, 1.5 and 5.0 g/L to pretreat seawater for reverse osmosis desalination. It was hypothesized that PAC would stimulate adsorption and biological degradation of organic compounds. The microbial communities responsible for biofouling on microfiltration (MF) membranes and biological organic removal in MBR were assessed using terminal restriction fragment length polymorphism fingerprinting and 454-pyrosequencing. The PAC addition improved assimilable organic carbon removal (53-59%), and resulted in reduced biofouling development on MF (> 50%) with only a marginal development in trans-membrane pressure. Interestingly, the

bacterial community composition was significantly differentiated by the PAC addition. *Cyanobacterium*, *Pelagibaca* and *Maricoccus* were dominant in the PAC-free conditions, while *Thiothrix* and *Sphingomonas* were presumably responsible for the better reactor performances in PAC-added conditions. In contrast, the archaeal communities were consistent with predominance of *Candidatus Nitrosopumilus*. These data therefore show that the addition of PAC can improve MBR performance by developing different bacterial species, controlling AOC and associated biofouling on the membranes.

Keywords: Biofouling; Membrane bioreactor; Microbial community composition; Powder activated carbon; Seawater; Sequencing

Abbreviations

AOC: Assimilable organic carbon; CA: Correspondence analysis; DOC: Dissolved organic carbon; LC-OCD: Liquid chromatography with organic carbon detection; MBR: Membrane bio-reactor; MF: Microfiltration; NMDS: Non-metric multidimensional scaling (NMDS); PAC: Powder activated carbon; RO: Reverse osmosis; SMABR: Submerged membrane adsorption bio-reactor; SMBR: Submerged membrane bio-reactor; SW: Seawater; SWRO: Seawater reverse osmosis; TMP: Trans-membrane pressure; T-RFLP: Terminal restriction fragment length polymorphism

1. Introduction

Biofouling remains one of the most difficult challenges in seawater reverse osmosis (SWRO) technology. One major effect of biofouling, through the development of a biofilm on RO membranes, is an increase in membrane resistance and a reduction in performance such as water permeation and rejection of solutes. The development of biofilms in RO modules is affected by the biofouling potential in the feed water, which is influenced by the concentration of bioavailable organic

compounds and the presence of biofilm-forming bacteria [1, 2]. The integration of microfiltration (MF) with physico-chemical treatments such as adsorption and/or coagulation could significantly reduce organic matter in seawater, especially biopolymer and humic materials, which are associated with membrane fouling. This reduces the amount of organic foulants reaching the RO membrane and alleviates the problem of flux decline during RO operation. Also, bacterial cell numbers and cell viability decline significantly, indicating reduction of biofouling potential through the separation and the inactivation of microbes [2].

In this regard, biological pretreatment systems such as biofiltration [3] and a membrane bioreactor (MBR) [4], utilizing biological degradation have been tested for SWRO. However, biofouling is also a problem for MF membranes in the pre-treatment MBR [4]. For these pretreatment systems, it has been observed that biofouling causes the trans-membrane pressure (TMP) to increase when the submerged MBR was operated at a constant flux [5]. In one study, the *ex situ* observation by confocal laser scanning microscopy (CLSM) demonstrated that biofilms formed on the MF membranes, and they were correlated with reduction in system performance [6]. Thus, the control of biofouling is also required in the MBR system when used as pretreatment for SWRO.

To reduce fouling in the MBR system, powder activated carbon (PAC) in suspension has been used in previous studies. PAC is a porous adsorbent with a significant surface area to volume ratio and facilitates the adsorption of macromolecules in an amended MBR (PAC-MBR) system [7]. The PAC also becomes colonised by microorganisms that can utilise the adsorbed compounds for growth. In this way, the simultaneous adsorption and biodegradation of those macromolecules, rather than in a single biological process, reflects an advantage of the PAC-MBR system over conventional MBR [8]. Adding PAC can also improve membrane filtration by altering the floc characteristics in the bulk mixture and lowering the foulant concentrations [9].

Recently, the PAC-MBR system has been developed as a new pretreatment option for SWRO [4, 10] and is referred to here as a submerged membrane adsorption bioreactor (SMABR). Microorganisms

were found to colonise on the PAC and this was hypothesised to increase biodegradation of the organic substances previously adsorbed. PAC thus has several benefits including enhanced scouring and initial coating of the membrane surface by PAC particles and adsorption of organic foulants with subsequent biodegradation [10]. However, the key microbial community species, which mainly remove organic matter in the SWRO process, remain largely not well understood.

The growth and species composition of microbial communities on SMABR may play a crucial role in removing organics from seawater [10]. It is also likely that the efficiency of biological treatment strategies could be improved through a thorough and complete understanding of microorganisms and their metabolism when growing on a biological activated carbon (BAC) filter [11]. The study of the bacterial community composition on the biofouled SWRO membrane revealed that the predominant microorganisms were α -proteobacteria as well as Firmicutes, Actinobacter, Planctomycetes and Bacteroidetes phyla [12, 13]. Analytical methods for DNA sequencing have improved and become significantly less expensive over the last 30 years allowing for increasingly detailed analysis of microbial communities. New developments enable meta-community sequencing based approaches, such as through barcode tagged sequencing of 16S rRNA genes amplified from the environment, without having to isolate individual organisms.

Technological advances such as pyrosequencing enable rapid characterization of microbial communities at a greater sequence depth than Sanger sequencing [14]. In addition, fingerprinting techniques such as terminal restriction fragment length polymorphism (T-RFLP) that produce readily interpreted patterns of microbial community diversity serve as useful tools for investigating the relative abundance and diversity of microbial communities, and can complement or supplement meta-community sequencing based approaches [15]. T-RFLP-based community assessment was used in this study to characterize the community composition and to correlate with the composition to see how well the system performed.

In this study, MBR (or submerged MBR; SMBR) and SMABR were used as a biological pretreatment to SWRO and their performances were evaluated in terms of organic removal, especially assimilable organic carbon (AOC), and TMP increase. T-RFLP and 454-pyrosequencing technologies were then employed to elucidate the composition of microbial communities responsible for biofouling of the MF membranes in MBR and removing organic compounds in SMABR.

2. Materials and methods

2.1 Experimental design and performance

In this study, a SMBR was operated at 10 L/m²h (LMH) without adding PAC. At the same time, two SMABR systems were run with different PAC doses (SMABR-1.5 and SMABR-5.0; 1.5g/L and 5.0g/L PAC initially added, respectively) at 30 LMH. High fouling of SMBR (without PAC addition) even at 10LMH and low fouling of SMABR (with PAC addition) at high flux of 30LMH led us to perform the subsequent SMABR at high flux of 30 LMH. The SMABR experiment at lower flux will be performed in our future study. The long-term operation of SMABR revealed the synergistic effects of PAC adsorption/biodegradation and membrane rejection in removing organic matter [10]. With PAC addition, the submerged membranes did not become clogged. This was due to fact that PAC removes the majority of organics prior to their entry to the membranes. The membrane functioned mainly to retain the PAC and other suspended solids. Hence, SMABR functions like a biofilm reactor. In both SMABR reactors, the amount of PAC replaced was 1.5% on a daily basis (based on total volume of reactor), which corresponds to a 66 d residence time (note: SMBR contained only small amount of sludge, which was not activated sludge but accumulated from suspended solids including naturally present and grown biomass). The SMBR and SMABRs (**Fig. 1**) were operated at the Sydney Institute of Marine Science, Chowder Bay in Sydney, Australia with continuous feeding of seawater for 56d. Daily backwashing was performed with MBR permeate (filtered water) withdrawn using a peristaltic pump at 30LMH (water flux) for 2 min. The

specification of MF membrane and detailed experimental design have been documented elsewhere [10].

The membrane-filtered effluent was obtained by suction using a pump connected to the membrane modules. The TMP development (in kPa) was monitored by a pressure gauge. While the SMBR was in operation, a gradual increase in TMP was observed followed by a rapid increase with fouling. In this study, a newly developed bioluminescence method was used to measure AOC concentration of seawater and pretreated seawater samples [10, 16]. This method used *Vibrio fischeri* MJ-1 and glucose was employed as an AOC standard carbon compound in this test. AOC measurement procedure has been provided in other studies [10, 16]. The AOC method is an indicative measure of the biological growth potential of water (or biofouling potential) since it represents the fraction of labile DOC that is readily assimilated and utilized by microorganisms resulting in increased biomass concentration. Subsequently, in this study, the extent of biological organic availability was monitored in terms of changes in AOC concentration. After 14 d of pre-acclimation, mixed liquor suspended solids (MLSS) in SMBR remained less than 2,500($\pm$ 1230) mg/L during the entire operation time. It should be noted that no sludge was added during the acclimation period and the MLSS became constant after 14 d of acclimation. However, the MLSS in SMABR-1.5 and SMABR-5.0 was 3350($\pm$ 1380) mg/L and 4440($\pm$ 950) mg/L, respectively. Thus, organic loading rate of SMBR and SMABR was nearly same at 0.11 $\pm$ 0.04 and 0.14 $\pm$ 0.02 g DOC/g MLSS-d, respectively (even though they were operated at different fluxes of 10 LMH and 30 LMH). Therefore, different fluxes used in SMBR and SMABR may not affect the microbial community composition related to organic utilization.

2.2 Sample preparation

Mixed liquid samples (including PAC or sludge) from reactors and hollow fiber membranes (at different locations) were collected every 8 d during the experimental period. Membrane samples

were cut from the middle (M) and both sides (B) of the hollow fiber bundle. For the SMBR, M and B samples were separated since there was severe fouling on the membrane, while M and B parts were quantified together (M) in the SMABR due to the lower amount of fouling biomass. As a result, biomass samples collected in M and B portions of membrane in SMABRs were not separated and analyzed together. DNA samples of the floccular biomass in the reactor and from fouled hollow fiber membranes were separately extracted immediately after sampling using a modified CTAB-PEG protocol. This procedure has been explained in other studies [10]. **Table 1** lists the samples used in the microbial community analyses.

2.3 Terminal restriction fragment length polymorphism (T-RFLP)

2.3.1 DNA Amplification

A ~550 base pair region of DNA encoding a section of the 16S ribosomal subunit was amplified using a 6-FAM-labelled 27F (5'-AGAGTTTGATCMTGGCTCAG-3') primer and an unlabelled 519R (5'-GWATTACCGCGGCKGCTG-3') primer (IDT DNA, Baulkham Hills, NSW, Australia). The reaction mix contained 50- μ L EconoTaq PLUS GREEN master mix (Lucigen, Wisconsin, USA), 0.3 μ M of each primer, 2 μ L of sample template DNA and 42 μ L of molecular-grade water. The cycling conditions included an initial denaturing step of 96°C for 3 min, then 30 cycles of 96°C for 60 s, 55°C for 60 s, 72°C for 1 min before a final extension at 72°C for 10 min and holding at 4°C. The samples were purified using the Genomic DNA Clean & Concentrator™ (DCC™) kit (Ref Zymo) according to the manufacturer's instructions.

2.3.2 Restriction Endonuclease Digestion

The purified amplicon was then digested for 3 h at 37°C in a reaction containing 1 μ L of Buffer 4, 100 ng of purified DNA, 10 U *Msp*I restriction enzyme and molecular-grade water in a 10 μ L total reaction volume (New England Biolabs, Massachusetts, USA).

2.3.3 T-RFLP Determination

Samples of 1-3 μ L, with a total of 5-10 ng of DNA, were analyzed at the Ramaciotti Centre for Genomics (University of New South Wales, Australia) for fragment analysis. The analysis was done on an Applied Biosystems AB3730 DNA Analyzer (Applied Biosystems, California, USA) using a GeneScan LIZ-1200 size standard (Applied Biosystems, California, USA).

2.3.4 Peak Detection

PeakScanner v1.0 (Applied Biosystems, California, USA) was used to identify fragments and their size was compared to the LIZ-1200 size standard. The baseline minimum threshold was set to 1 fluorescent unit for the blue sample channel, 500 for the orange size standard channel and 5000 for the other colour channels. This allowed all noise from the sample channel to pass through for later filtering. The labels and “raw” data were exported to the correct compatible formatting required by the T-REX T-RFLP Analysis tool [17].

2.3.5 Noise-Filtering, Terminal-Restriction Fragment Alignment

The exported raw data and label files from PeakScanner were uploaded to the T-REX T-RFLP analysis tool. T-REX systematically selects “true” peaks by repeatedly comparing the peaks in the sample to the standard deviation of all peaks multiplied by a user-selectable multiplier and removing those that do not exceed it. The multiplier selected for the blue channel data here was 3 and the other channels remained at the default of 1, as no data for these channels was uploaded.

T-REX then defined T-RFLPs by aligning peaks with a clustering threshold of 0.5 and 1 peak per T-RF, based on a previous method [18]. T-RFLP data were exported as the area of the peaks, relativized within each sample to negate the effect of varying total DNA concentrations in the original sample. This allows the dominant T-RFLP to be determined in each sample and comparison

of this dominance between samples to be made, at the expense of determining the quantifiable concentration of the T-RF.

2.4 Pyrosequencing

The detailed microbial community structures in the SBR and SMABR-1.5 systems were identified by 454 high-throughput sequencing of the 16S rRNA gene using a Roche 454 FLX instrument (Roche, Indianapolis, IN). The pyrosequencing was performed for samples collected on the 16th day and 40th day since the composition of the microbial community, as observed from T-RFLP analysis, shifted significantly at these time points. In addition, the microbial community composition of SMABR-1.5 samples was compared with that of the SBR samples as a control trial. For this reason, the pyrosequencing was conducted for the four reactor (liquid) samples (i.e. 0-16, 0-40, 1.5-16, and 1.5-40) and two biofilm samples (i.e. 0-40-M and 1.5-40-M). One hundred ng of extracted DNA was sent to the Research and Testing Laboratory (Lubbock, TX, United States of America) for analysis of bacterial and archaeal rRNA genes using FLX amplicon pyrosequencing. For bacterial 16S rRNA gene amplification, the forward primer was constructed with the Roche A linker (5'-CCATC TCATC CCTGC GTGTC TCCGA CTCAG-3'), an 8-10 bp barcode, and the Bac28F (5'-GAGTT TGATC NTGGC TCAG-3'). The reverse primer was constructed with a biotin molecule, the Roche B linker (5'-CCTAT CCCCT GTGTG CCTTG GCAGT CTCAG-3'), and the Bac519R (5'-GTNTT ACNGC GGCKG CTG-3'). For archaeal rRNA gene amplification, YuiArchF (5'-CCCTA YGGGG YGCAS CAG-3') and Arch958R (5'-YCCGG CGTTG AMTCC AATT-3') were linked with the Roche A linker and Roche B linker, respectively. Amplification was performed in 25 µL reactions with Qiagen HotStar Taq master mix (Qiagen Inc, Valencia, California), 1 µL of 5 µM each primer, and 1 µL of template. Reactions were executed on ABI Veriti thermocyclers (Applied Biosystems, Carlsbad, California) under the following PCR profile: 95°C for 5 min, then 35 cycles of 94°C for 30

s, 54°C for 40 s, 72°C for 1 min, followed by one cycle of 72°C for 10 min and 4°C hold. Sequencing analysis followed the established manufacturer protocols (454 Life Sciences).

Any sequence that contained a low quality barcode or failed to be at least half the expected amplicon length (or 250 bp, whichever was shortest) was removed from the data pool. All sequences were then denoised using an algorithm based on USEARCH and checked for chimeras using UCHIME. The ribosomal 16S rRNA gene database in Silva was used as reference information for BLAST. Operational taxonomic unit (OTU) analysis for community richness was undertaken using the CD-HIT-OUT (<http://weizhong-lab.ucsd.edu/cd-hit-otu/>). The Shannon-Weaver diversity index and Simpson index, which represent species diversity of the samples, were evaluated by Mothur software (1.33.0) (<http://www.mothur.org/>). The OTU analysis was conducted for the bacterial and archaeal sequences, respectively.

2.5 Statistical analysis of T-RFLP and 454 pyrosequencing data

Non-metric multidimensional scaling (NMDS) and correspondence analysis (CA) were conducted to visualize the similarity of the microbial community structure among the samples in low dimensional space and to group the samples sharing the similar microbial community structure. The NMDS and CA were done based on Sorensen (Bray–Curtis) distance way using PC-ORD v.5.0, MjM software (Gleneden Beach, OR). For the statistical analysis of T-RFLP, the minor T-RFLP peaks (<5% relative abundance) were removed in order to exclude the noise peaks. The peak intensities were normalized as previously described [19]. The relative abundances of the T-RFLP peak intensities served to perform the NMDS and CA. The statistical tests concerning the pyrosequencing data were also carried out based on the relative abundance of the OTU results. Shannon index was used to determine biodiversity index in this study. It has been widely used as common ecological tool to represent microbial diversity of environmental samples; the higher value indicates higher microbial diversity [20].

2.6 Biofouling observation

The distribution of cells and polysaccharides (PS) on membranes was observed using Olympus FV-1000 confocal laser scanning microscope (CLSM) after staining with 4',6-diamidino-2-phenylindole (DAPI, Sigma–Aldrich) for nucleic acids and Concavalin A (ConA, Molecular Probes) for PS, respectively. Detailed procedures for this have been described previously [10].

3. Results and discussion

In this study, two SMABR systems were operated with two different PAC doses (1.5 g/L; *SMABR-1.5* and 5.0 g/L; *SMABR-5.0*) at a high flux of 30 LMH for a period of 56 d. The results were compared with the SBR (without any PAC being added) at a lower flux of 10 LMH. Both SMABR and SBR systems were run under critical flux condition, fouling extent will not be affected even through they were operated at two different fluxes [21]. In other words, the fouling of SMABR will be similar both at 10LMH and 30LMH as both fluxes are under critical flux of SMABR operation.

3.1 Filtration performance of SBR and SMABR

3.1.1 TMP development

Fig. 2a presents the TMP profile ($TMP_d = TMP/TMP_0$) of the SBR (operated without PAC addition at 10 LMH) and two SMABR systems (*SMABR-1.5* and *SMABR-5.0* operated at 30 LMH). In the SBR, during the initial stage of the experiment (16~24 d), the TMP_d increased sharply around 1.5times of initial TMP (TMP_0) and from 24 d onwards it remained relatively high, between 1.5 and 1.7 times of the TMP_0 . On the other hand, the TMP_d was marginal ($\approx 15\%$) in both SMABRs, even at a higher flux of 30 LMH. The addition of PAC reduced the TMP_d and the irreversible fouling

on the MF membrane. In SMABR-5.0, a higher dose (5.0 g/L) of PAC resulted in a slightly higher rate of TMP_d compared to SMABR-1.5 (SMABR-5.0=1.14 and SMABR-1.5=1.06).

3.1.2 AOC reduction

Biological organic degradation in the MBR was evaluated in terms of AOC reduction (**Fig. 2b**). During the experimental period, the AOC concentration of raw seawater (influent) was 37.3 ± 7.8 $\mu\text{g-C}$ glucose equivalents/L. In the SMBR, the AOC was not removed. In contrast, the SMABR systems showed AOC reductions of 53~59% and increased its removal efficiency over the operational time.

3.1.3 Membrane biofouling

Fouled membrane samples were analyzed using CLSM to quantify the abundance of cells and polysaccharide (PS) during different operational periods (**Fig. 2c**) (CLSM images can be found elsewhere [10]). Biofilm formation was observed on the SMBR membranes, beginning on the 8th day and average biovolumes of cells and PS were 7.56 ± 0.21 $\mu\text{m}^3/\mu\text{m}^2$ and 9.40 ± 1.21 $\mu\text{m}^3/\mu\text{m}^2$, respectively, during the initial 16 d. After 16 d of operation, the number of cells as well as the PS increased significantly. This is correlated to the TMP_d trend, which revealed that TMP_d might be caused by biofouling on the membrane in SMBR. In contrast, there was less biofilm formation on membranes in the SMABRs. In particular, the amount of PS detected was considerably less in the SMABR relative to the SMBR (PS from 40 d to 56 d: SMABR-1.5 = 3.48 ± 0.54 $\mu\text{m}^3/\mu\text{m}^2$ and SMABR-5.0 = 4.20 ± 0.78 $\mu\text{m}^3/\mu\text{m}^2$). This suggests that there was not a substantial difference in the amount of biofilm that formed on the membranes in the SMABR.

3.2 Comparison of community diversity using T-RFLP

The differences in the microbial community structure obtained from the T-RFLP analysis were examined and visualized using NMDS and CA. Raw profiles of T-RFLP are given in **Fig. S1** as a

supplementary data. The 2D ordination plot of the NMDS analysis shows that the microbial communities of the SMBR were distinct from the SMABR community (**Fig. 3**). The instability and stress values of the 2D plot were evaluated to 1.0×10^{-5} and 18.3, respectively. These indicate that the NMDS results were statistically valid as described in previous studies (i.e. stress value < 20, instability value < 0.001) [19]. The NMDS and CA results show that the bacterial community structure is clearly classified into two primary clusters, SMBR and SMABR groups (**Fig. 3** and **Fig. 4**). In addition, the bacterial communities of the biofilms (on the membrane samples) were well clustered as a function of time (**Fig. 4**). The results imply that the addition of PAC in MBR (SMABR) was a significant factor that was correlated to a change in the microbial community compared to the SMBR. In a previous study on biofilters used to treat seawater, the granular activated carbon biofilter contained a distinct microbial community, consisting of diverse heterotrophs while the anthracite biofilter was mainly composed of sulfur-oxidizing and reducing bacteria as well as alkalitrophic heterotrophs [22]. Thus, the activated carbon biofilter process can be suitable for removing AOC because it provides favorable conditions to the functional bacterial community that degrades residual AOC in the engineered system. Therefore, the specific bacterial community selected for by adding PAC in MBR may contribute to its filtration performance and reduction of organic compound. In fact, SMBR removed only a small amount of DOC from seawater (less than 10%). On the other hand, significant DOC removal was achieved by SMABRs (SMABR-1.5 = $64.9 \pm 10.4\%$; and SMABR-5.0 = $67.2 \pm 8.2\%$) during the entire run. Especially, the DOC removal increased (by around 75%) as biological activity increased. Although the PAC addition in SMABR helped to achieve significant DOC removal, it varied only marginally with the increase in PAC dose (from 1.5g/L to 5.0g/L) in the long-term run. Bio-carriers or biological activated carbon (BAC) systems can mitigate the biofilm formation in the SWRO process by removing the biofoulants through adsorption and biodegradation [22]. However, some samples, such as 0-32, 0-16-M, 1.5-2, and 1.5-56, were not clustered. This may be attributed to operational issues. For example, at the 16th and 32th days, SMBR underwent high

TMP development and required more backwashing to recover the TMP, which may have had an impact on the microbial community.

3.2.1 Changes in community composition in the SMBR

The species composition and distribution of the microbial community in the SMBR and SMABR systems is more clearly identified by a dendrogram of CA (**Fig. 3**). The dendrogram provides the nearest neighbours, which makes the relationships between the samples more clear (**Fig. 4**). After 24 d, the microbial community composition differed from the initial stage of operation (**Fig. 3**) and was clustered by the operational time, where samples from the 2nd day to 24th day and from the 32nd day to 48th day were evident (**Fig. 4**). These showed that there was a change occurred in the microbial community in the SMBR before and after the 24 d of operation. It implies that this dynamic shift in the bacterial community is closely related to the rapid increase in the TMP ratio, which was observed between days 16 and 24 (**Fig. 2**). Additionally, at this time, the AOC removal was also reduced and more biofilm was observed on the membranes. The samples were also well separated based on whether they were collected from the reactor (liquid) or from the membranes (biofilms), suggesting that some selection was made for a biofilm specific community on the membranes. On the 40th day of operation, severe biofouling on the membranes was observed (**Fig. 2**). The biofilm samples from days 32 to 48 cluster together. This may also indicate that microbial community was associated with the rapid increase in TMP (**Fig. 4**).

3.2.2 Changes in community composition in the SMABR

For the SMABR, the biofilm samples were grouped together for the SMABR samples (i.e. 1.5-40-M, 1.5-20-M, 5.0-40-M, 5.0-8-M, and 5.0-56-M), except for 1.5-8-M and 5.0-24-M. These data suggest that there was not a significant change in the biofilm community during the operation of the SMABR. Similarly, the performance of the SMABR remained stable across these time points and lower

biofouling on the membrane was observed compared to the SBR. On the other hand, the increased fouling and the associated rapid TMP rise of the SBR could suggest that the microbial community blocks the membrane pores. When the microbial communities were compared between the SMABR-1.5 and SMABR-5.0 samples, there were no differences in the communities, suggesting there are no differences in selection for fouling organisms between the two treatments. It also showed that PAC dose level had no significant effect on the bacterial community structure. The restriction fragment patterns obtained from the T-RFLP analysis are limited in resolution and cannot directly identify the organisms present in the communities. Therefore, 16S based pyrosequencing of the communities was undertaken to better resolve differences between samples and to identify the organisms involved. Given the similarities for the SMABR-1.5 and SMABR-5.0 communities, only the SMABR-1.5 samples were sequenced, along with the SBR samples.

3.3 Bacterial community structure from 454-pyrosequencing analysis

3.3.1 Overall comparison of the bacterial community structure between the SBR and SMABR

For the SBR and SMABR-1.5 systems, 454-pyrosequencing generated 17,646 bacterial sequences, which were assigned to 159 OTUs based on the 3% genetic distance cutoff. The results were evaluated and visualized by CA analysis (**Fig. 5**). The results demonstrated that the bacterial community was clearly different between the SBR and SMABR, which were also observed based on the T-RFLP analysis. The bacterial communities present in the liquid phase (in the reactor) of the SBR were substantially different for days 16 and 40 (**Fig. 5**). In contrast, the microbial communities in the liquid samples for the SMABR were almost 100% similar between days 16 and 40 and there was only a slight difference in the biofouling community on day 40 relative to the organisms found in the liquid phase. Further work needs to be undertaken to fully characterize the changes in microbial community composition under these conditions and to explain the similarities

and differences observed. Interestingly, statistical analysis demonstrated that the clustering patterns of the bacterial community (i.e. SBR versus SMABR) were similar for the T-RFLP and pyrosequencing results (**Fig. 4** and **Fig. 5**). Thus, T-RFLP, together with NMDS/CA based analysis, could be employed as an effective screening method when the community structures of a large number of samples need to be investigated. It would therefore be expected that this approach could reduce the analytical cost of pyrosequencing analysis.

3.3.2 Dominant bacteria in the liquid phase of the reactors

The dominant bacterial groups were identified and correlated to the major functions for growth such as carbon source, salt tolerance, biofilm-forming potential, and other unique characteristics (i.e. substrate affinity and sulfur utilization), respectively. **Table 2** shows the dominant bacterial OTUs (B-OTU) with the closest match at the Genus level for each sample. Based on the results, the dominant B-OTUs were significantly different between the SBR and SMABR-1.5 in the liquid phase. However, the dominant B-OTUs in SMABR-1.5 were relatively low and different with the time elapsed rather than those of the SBR.

For the SBR, the dominant bacterial OTU in the reactor (liquid phase) changed from *Cyanobacterium* (42.5%) at the 16th day to *Pelagibaca* (18.0%) at the 40th day. Cyanobacteria are photoautotrophs and some can be salt tolerant. The genus *Pelagibaca*, which is chemoheterotrophic and slightly halophilic bacteria, reportedly utilizes a range of carbonous materials as a sole carbon source [23]. In addition, *Pelagibaca bermudensis*, a type strain of *Pelagibaca*, can grow under a wide range of conditions including temperatures between 10-40°C, pH 5.0-10.5, and 0.25-15 % NaCl, which can cover the reactor operating conditions. Similarly, *Phycisphaera mikurensis*, a type strain of *Phycisphaera*, which was present in the SBR at approximately 12% of the community, can also utilize a variety of substrates for its growth [24]. While these organisms are reported to have the ability to utilize a range of carbon sources, the AOC in the SBR system did not decrease. This

is probably because the absolute number of biomass in SMBR was not enough to reduce the AOC. Further work would be required to understand if those organisms are capable of utilizing the carbon sources present in the seawater, or whether the *Cyanobacterium* produces and secretes significant amounts of AOC as part of its autotrophic metabolism.

For the SMABR-1.5, the dominant community members were *Thiothrix* (28-60.4%) and *Sphingomonas* (5-48%) as given in **Table 2**. The genus *Thiothrix*, filamentous sulfur-oxidizing bacteria (SOB), has been discovered in diverse habitats such as marine, sulfide-containing waters, and activated-sludge wastewater treatment plants [25]. Members of this genus can grow at a wide variety of temperatures (i.e. cold to hot spring) and have diverse metabolic pathways (i.e. facultative autotrophs, chemoorganotrophs, and mixotrophs). The PAC had a sulfur content of ~1.0 % wt, which could promote the dominant growth of *Thiothrix*. Additionally, the high biofilm-forming potential of *Thiothrix* would also encourage significant growth on the surface of PAC [26].

Interestingly, the relative abundance of *Sphingomonas* dramatically increased from 4.9% at the 16th day to 47.6% at the 40th day in the liquid phase and this correlates with the reduction AOC. *Sphingomonas* species can assimilate a wide variety of sugars and glucose for growth and many thrive in low-nutrient conditions [27]. For example, *Sphingopyxis* (formerly *Sphingomonas*) *alaskensis* represented the dominant biomass and nutrient cycling contributor in low nutrient marine environments (< 1 mg/L of DOC) [28]. Therefore, it is possible that *Sphingomonas* spp. could utilize the residual AOC contained with an extremely low concentration in the SMABR system. In addition, *Sphingomonas* spp. secrete the gellan exopolysaccharides that promote the initial adhesion of bacteria for biofilm formation [29]. The results suggest that *Sphingomonas* might grow well on the surface of PAC where nutrients are concentrated and this could be followed up by subsequent studies using Fluorescent in situ Hybridization (FISH) probes based on these observations.

3.3.3 Dominant bacterial fouling on the membranes

The dominant bacteria on the SMBR membrane, which were distinct from the liquid phase samples were *Maricoccus* spp. (54%) compared to *Thiothrix* (28%) and *Alcanivorax* (14%) for biofilms formed on the membranes of the SMABR although the latter was found at 4% in the SMBR samples as well. *Maricoccus* was only recently isolated and hence, there is limited information on this organism [30] and therefore, it is unclear why it might dominate in the samples collected from the SMBR. *Alcanivorax*, which is a halophilic oil degrading bacteria, has been detected in upper layers of the sea, oil-contaminated conditions and coastal waters. The *Alcanivorax* spp. was also found in biofilms formed on SWRO membranes [31] and hence may be a common biofouling organism for seawater systems.

3.4 Archaeal community composition

A total of 23,223 archaeal sequences were generated from the SMBR and SMABR-1.5 samples. The archaeal sequences were assigned to 14 OTUs based on the 3% genetic distance cutoff and the community diversity was visualized using NMDS and CA plots (**Fig. 6**). In contrast to the bacterial communities, the archaeal communities showed no clustering based on bioreactor type (**Fig. 5** and **Fig. 6**). The majority of OTUs were ascribed to members of the candidate genus *Nitrosopumilus* (**Table 3**) and hence may explain why there is no clear difference in the reactor communities. *Nitrosopumilus* are ammonia-oxidizing archaea (AOA) that convert ammonia into nitrite [32]. The dominance of AOA has been frequently reported in seawater because oceanic conditions, such as low levels of ammonia and oxygen, are ideal for the growth of this archaeal group [33]. In particular, some microbial species belonging to *Nitrosopumilus* can grow even at an extremely low level of ammonia (<10 nM) that is near the limit to maintain its survival. This group of organisms may be relevant for seawater bioreactors as it was previously reported that AOA dominated an aerobic reactor fed with seawater [34]. In addition, *Nitrosopumilus* has been frequently detected in the attached growth systems such as membrane biofilm reactor or biofilter process, which supports the

dominant role of *Nitrosopumilus* in the biofilm samples [35]. Therefore, *Nitrosopumilus* may be grown in the SMBR and SMABR systems by utilizing the residual ammonia under raw seawater. It will be important to further investigate this dominance of AOA, particularly with respect to the issue of nitrification.

4. Conclusions

In this study, the microbial community structure was investigated with reference to SMBR and SMABR systems. T-RFLP analysis was used to understand the changes in microbial community in relation to the operation of the seawater membrane bioreactors and this approach was shown to be a useful tool for this purpose. The bacterial community structure was clearly differentiated without and with PAC addition (i.e. SMBR and SMABR). However, difference in concentration of PAC used (i.e. SMABR-1.5 and SMABR-5.0) did not significantly affect the community composition. The results obtained from this study suggest that the addition of PAC selected for *Thiothrix* and *Sphingomonas* in the liquid phase and for *Maricoccus* in biofilms in the SMABR system. These differences in community composition were correlated with the reduction of AOC and maintenance of a low TMP in the SMABR. While it remains to be determined if the bacterial species observed to dominate the SMABR were responsible for AOC removal and reducing biological fouling, it was clear that the addition of PAC improved reactor performance, even when operated at a high flux.

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References

- [1] 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.
- [2] S. Jeong, L.H. Kim, S.J. Kim, T.V. Nguyen, S. Vigneswaran, I.S. Kim, Biofouling potential reductions using a membrane hybrid system as a pre-treatment to seawater reverse osmosis, *Appl. Biochem. Biotechnol.* 167(6) (2012) 1716-1727.
- [3] S. Jeong, S. Vigneswaran, Assessment of biological activity in contact flocculation filtration used as a pretreatment in seawater desalination, *Chem. Eng. J.* 228 (2013) 976-983.
- [4] S. Jeong, G. Naidu, S. Vigneswaran, Submerged membrane adsorption bioreactor as a pretreatment in seawater desalination for biofouling control, *Bioresour. Technol.* 141 (2013) 57-64.
- [5] Z. Wang, Z. Wu, X. Yin, L. Tian, Membrane fouling in a submerged membrane bioreactor (MBR) under sub-critical flux operation: membrane foulant and gel layer characterization, *J. Membr. Sci.* 325(1) (2008) 238-244.
- [6] J. Luo, J. Zhang, X. Tan, D. McDougald, G. Zhuang, A.G. Fane, S. Kjelleberg, Y. Cohen, S.A. Rice, The correlation between biofilm biopolymer composition and membrane fouling in submerged membrane bioreactors, *Biofouling* 30(9) (2014) 1093-1110.
- [7] Y. Liu, L. Wang, B. Wang, H. Cui, J. Zhang, Performance improvement of hybrid membrane bioreactor with PAC addition for water reuse, *Water Sci. Technol.* 52(10–11) (2005) 383–391.
- [8] C.A. Ng, D. Sun, M.J.K. Bashir, S.H. Wai, L.Y. Wong, H. Nisar, B. Wu, A.G. Fane, Optimization of membrane bioreactors by the addition of powdered activated carbon, *Bioresour. Technol.* 138 (2013) 38–47.
- [9] M. Remy, V. Potier, H. Temmink, W. Rulkens, Why low powdered activated carbon addition reduces membrane fouling in MBRs, *Water Res.* 44(3) (2010) 861-867.

- [10] S. Jeong, S.A. Rice, S. Vigneswaran, Long-term effect on membrane fouling in a new membrane bioreactor as a pretreatment to seawater desalination, *Bioresour. Technol.* 165 (2014) 60-68.
- [11] H. Xiang, X. Lu, L. Yin, F. Yang, G. Zhu, W. Liu, Microbial community characterization, activity analysis and purifying efficiency in a biofilter process, *J. Environ. Sci.* 25(4) (2013) 677-687.
- [12] H. Bae, H. Kim, S. Jeong, S. Lee, Changes in the relative abundance of biofilm-forming bacteria by conventional sand-filtration and microfiltration as pretreatments for seawater reverse osmosis desalination, *Desalination* 273 (2011) 258-266.
- [13] J. Lee, I.S. Kim, Microbial community in seawater reverse osmosis and rapid diagnosis of membrane biofouling, *Desalination* 273 (2011) 118-126.
- [14] M. Margulies et al., Genome sequencing in microfabricated high-density picolitre reactors. *Nature* 437(7057) (2005) 376-380.
- [15] A. Nocker, M. Burr, A. Camper, Genotypic microbial community profiling: A critical technical review, *Microb. Ecol.* 54 (2007) 276.
- [16] S. Jeong, G. Naidu, S. Vigneswaran, C.H. Ma, S.A. Rice, A rapid bioluminescence-based test of assimilable organic carbon for seawater, *Desalination* 317 (2013) 160-165.
- [17] S.W. Culman, R. Bukowski, H.G. Gauch, H. Cadillo-Quiroz, D.H. Buckley, T-REX: Software for the Processing and Analysis of T-RFLP data, *BMC Bioinformatics* 10 (2009) 171.
- [18] C.J. Smith, B.S. Danilowicz, A.K. Clear, F.J. Costello, B. Wilson, W.G. Meijer, T-Align, a web-based tool for comparison of multiple terminal restriction fragment length polymorphism profiles, *FEMS Microbiol. Ecol.* 54 (2005) 375-380.
- [19] S. Jeong, H. Bae, G. Naidu, D. Jeong, S. Lee, S. Vigneswaran, Bacterial community structure in a biofilter used as a pretreatment for seawater desalination, *Ecol. Eng.* 60 (2013) 370-381.

- [20] E.R. Reichenberger, G.M. Alexander, M.J. Perreault, J.A. Russell, R.J. Schwartzman, U. Hershberg, G. Rosen, Establishing a relationship between bacteria in the human gut and complex regional pain syndrome, *Brain, behavior, and immunity*. 29 (2013) 62-69.
- [21] S. Jeong, Y.J. Choi, T.V. Nguyen, S. Vigneswaran, T.M. Hwang, Submerged membrane hybrid systems as pretreatment in seawater reverse osmosis (SWRO): Optimisation and fouling mechanism determination, *J. Membr. Sci.* 411 (2012) 173-181.
- [22] B. McCune, J.B. Grace, D.L. Urban, *Analysis of ecological communities* (2002).
- [23] J.-C. Cho, S.J. Giovannoni, *Pelagibaca bermudensis* gen. nov., sp. nov., a novel marine bacterium within the Roseobacter clade in the order Rhodobacterales. *Int. J. Syst. Evol. Microbiol.* 56 (2006) 855-859.
- [24] Y. Fukunaga, M. Kurahashi, Y. Sakiyama, M. Ohuchi, A. Yokota, S. Harayama, *Phycisphaera mikurensis* gen. nov., sp. nov., isolated from a marine alga, and proposal of *Phycisphaeraceae* fam. nov., *Phycisphaerales* ord. nov and *Phycisphaerae* classis nov in the phylum Planctomycetes, *J. Gen. Appl. Microbiol.* 55 (2009) 267-275.
- [25] D.C. Gillan, N. Dubilier, Novel Epibiotic Thiothrix Bacterium on a Marine Amphipod, *Appl. Environ. Microbiol.* 70 (2004) 3772-3775.
- [26] M. Wietz, M.R. Hall, L. Høj, Effects of seawater ozonation on biofilm development in aquaculture tanks. *Syst. Appl. Microbiol.* 32 (2009) 266-277.
- [27] D.L. Balkwill, J.K. Fredrickson, M.F. Romine, *Sphingomonas* and Related Genera. in: M. Dworkin, S. Falkow, E. Rosenberg, K.-H. Schleifer, E. Stackebrandt (Eds.) *The Prokaryotes*, Springer, New York, (2006) pp. 605-629.
- [28] M. Eguchi, M. Ostrowski, F. Fegatella, J. Bowman, D. Nichols, T. Nishino, R. Cavicchioli, *Sphingomonas alaskensis* Strain AFO1, an Abundant Oligotrophic Ultramicrobacterium from the North Pacific, *Appl. Environ. Microbiol.* 67 (2011) 4945-4954.

- [29] J. Azeredo, R. Oliveira, The Role of Exopolymers Produced by *Sphingomonas paucimobilis* in Biofilm Formation and Composition, *Biofouling* 16 (2000) 17-27.
- [30] G. Li, Q. Lai, X. Liu, F. Sun, Y. Du, G. Li, Z. Shao, *Maricoccus atlantica* gen. nov. sp. nov., isolated from deep sea sediment of the Atlantic Ocean, *Antonie van Leeuwenhoek* 104 (2013) 1073-1081.
- [31] 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 (2013) 125-136.
- [32] M. Konneke, A.E. Bernhard, J.R. de la Torre, C.B. Walker, J.B. Waterbury, D.A. Stahl, Isolation of an autotrophic ammonia-oxidizing marine archaeon, *Nature* 437 (2005) 543-546.
- [33] R. Hatzenpichler, Diversity, Physiology, and Niche Differentiation of Ammonia-Oxidizing Archaea, *Appl. Environ. Microbiol.* 78 (2012) 7501-7510.
- [34] O. Sánchez, I. Ferrera, J.M. González, J. Mas, Assessing bacterial diversity in a seawater-processing wastewater treatment plant by 454-pyrosequencing of the 16S rRNA and amoA genes, *Microb. Biotechnol.* 6(4) (2013) 435-442.
- [35] T. Sakami, T. Andoh, T. Morita, Y. Yamamoto, Phylogenetic diversity of ammonia-oxidizing archaea and bacteria in biofilters of recirculating aquaculture systems, *Mar. Genomics* 7 (2012) 27-31.

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Table 1 List of samples used in the microbial community analyses.

Table 2 The dominant bacterial OTUs with closest genus and biodiversity index (Shannon index) in SBR and SMABR-1.5.

Table 3 The dominant archaeal OTUs with closest genus and biodiversity index (Shannon index) in SBR and SMABR-1.5.

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Table 1 List of samples used in the microbial community analyses.

T-RFLP samples				
Reactors	Operational days	In reactor	From membrane	
			Middle	Both sides
SMBR (0)	2	0-2	-	-
	8	0-8	0-8-M	0-8-B
	16	0-16*	0-16-M	0-16-B
	24	0-24	0-24-M	0-24-B
	32	0-32	0-32-M	0-32-B
	40	0-40*	0-40-M*	0-40-B
	48	0-48	0-48-M	0-48-B
	56	0-56	-	-
SMABR-1.5	2	1.5-2	-	
	8	1.5-8	1.5-8-M	
	16	1.5-16*	-	
	24	1.5-24	1.5-24M	
	32	1.5-32	-	
	40	1.5-40*	1.5-40M*	
	48	1.5-48	-	
	56	1.5-56	1.5-56-M	
SMABR-5.0	2	5.0-2	-	
	8	5.0-8	5.0-8-M	
	16	5.0-16*	-	
	24	5.0-24	5.0-24-M	
	32	5.0-32	-	
	40	5.0-40*	5.0-40-M*	
	48	5.0-48	-	
	56	5.0-56	5.0-56-M	

* Samples used in 454-pyrosequencing analysis.

Table 2 The dominant bacterial OTUs with closest genus and biodiversity index (Shannon index) in SMBR and SMABR-1.5.

Sample	Rank	OTU	Relative abundance (%)	Shannon index	Closest genus (Identity %)
0-16	1	B-OTU 3	42.5	2.34	<i>Cyanobacterium</i> (98.3)
	2	B-OTU 4	10.4		<i>Marinomonas</i> (87.3)
	3	B-OTU 5	9.9		<i>Streptococcus</i> (99.8)
	4	B-OTU 29	5.5		<i>Pseudonitzschia</i> (85.5)
	5	B-OTU 51	5.5		<i>Flavobacterium</i> (90.6)
0-40	1	B-OTU 13	18.0	3.14	<i>Pelagibaca</i> (94.5)
	2	B-OTU 7	11.8		<i>Phycisphaera</i> (86.5)
	3	B-OTU 14	8.5		<i>Phycisphaera</i> (89.2)
	4	B-OTU 57	5.9		<i>Pleurocapsa</i> (99.6)
	5	B-OTU 3	4.9		<i>Cyanobacterium</i> (98.3)
0-40-M	1	B-OTU 1	53.7	2.36	<i>Maricoccus</i> (99.8)
	2	B-OTU 8	5.0		<i>Kangiella</i> (96.3)
	3	B-OTU 15	4.1		<i>Alcanivorax</i> (99.8)
	4	B-OTU 13	3.0		<i>Pelagibaca</i> (94.5)
	5	B-OTU 12	2.5		<i>Marinobacterium</i> (89.7)
1.5-16	1	B-OTU 2	60.4	1.92	<i>Thiothrix</i> (89.5)
	2	B-OTU 19	5.7		<i>Oceanospirillum</i> (98.2)
	3	B-OTU 6	4.9		<i>Sphingomonas</i> (99.8)
	4	B-OTU 11	3.9		<i>Methylophilus</i> (95.4)
	5	B-OTU 27	2.8		<i>Methylophaga</i> (99.0)
1.5-40	1	B-OTU 6	47.6	1.48	<i>Sphingomonas</i> (99.8)
	2	B-OTU 2	32.5		<i>Thiothrix</i> (89.5)
	3	B-OTU 60	3.0		<i>Staphylococcus</i> (99.2)
	4	B-OTU 69	3.0		<i>Bradyrhizobium</i> (99.6)
	5	B-OTU 11	2.4		<i>Methylophilus</i> (95.4)
1.5-40-M	1	B-OTU 2	28.2	2.96	<i>Thiothrix</i> (89.5)
	2	B-OTU 9	14.4		<i>Alcanivorax</i> (99.8)
	3	B-OTU 3	8.2		<i>Cyanobacterium</i> (98.3)
	4	B-OTU 31	6.0		<i>Sphingomonas</i> (86.1)
	5	B-OTU 10	5.3		<i>Labrenzia</i> (99.5)

Table 3 The dominant archaeal OTUs with closest genus and biodiversity index (Shannon index) in SMBR and SMABR-1.5.

Sample	Rank	OTU	Relative abundance (%)	Shannon index	Closest genus (Similarity %)
0-16	1	A-OTU 1	87.3	0.54	<i>Candidatus Nitrosopumilus</i> (99.4)
	2	A-OTU 4	7.3		Unknown
	3	A-OTU 6	2.4		<i>Candidatus Nitrosopumilus</i> (99.7)
	4	A-OTU 3	1.1		<i>Candidatus Nitrosopumilus</i> (97.6)
	5	A-OTU 5	1.0		<i>Candidatus Nitrosopumilus</i> (98.3)
0-40	1	A-OTU 1	97.3	0.16	<i>Candidatus Nitrosopumilus</i> (99.4)
	2	A-OTU 2	1.4		<i>Candidatus Nitrosopumilus</i> (96.9)
	3	A-OTU 3	0.4		<i>Candidatus Nitrosopumilus</i> (97.6)
	4	A-OTU 12	0.4		Unknown
	5	A-OTU 13	0.4		Unknown
0-40-M	1	A-OTU 1	94.2	0.29	<i>Candidatus Nitrosopumilus</i> (99.4)
	2	A-OTU 7	3.1		Unknown
	3	A-OTU 5	1.3		<i>Candidatus Nitrosopumilus</i> (98.3)
	4	A-OTU 2	0.8		<i>Candidatus Nitrosopumilus</i> (96.9)
	5	A-OTU 3	0.6		<i>Candidatus Nitrosopumilus</i> (97.6)
1.5-16	1	A-OTU 1	95.7	0.23	<i>Candidatus Nitrosopumilus</i> (99.4)
	2	A-OTU 5	2.4		<i>Candidatus Nitrosopumilus</i> (98.3)
	3	A-OTU 3	0.8		<i>Candidatus Nitrosopumilus</i> (97.6)
	4	A-OTU 2	0.7		<i>Candidatus Nitrosopumilus</i> (96.9)
	5	A-OTU 9	0.4		<i>Candidatus Nitrosopumilus</i> (99.3)
1.5-40	1	A-OTU 1	97.1	0.17	<i>Candidatus Nitrosopumilus</i> (99.4)
	2	A-OTU 2	1.1		<i>Candidatus Nitrosopumilus</i> (96.9)
	3	A-OTU 8	0.8		<i>Candidatus Nitrosopumilus</i> (97.2)
	4	A-OTU 3	0.6		<i>Candidatus Nitrosopumilus</i> (97.6)
	5	A-OTU 14	0.4		<i>Halorubrum</i> (98.2)
1.5-40-M	1	A-OTU 1	91.7	0.41	<i>Candidatus Nitrosopumilus</i> (99.4)
	2	A-OTU 5	3.9		<i>Candidatus Nitrosopumilus</i> (98.3)
	3	A-OTU 6	1.5		<i>Candidatus Nitrosopumilus</i> (99.7)
	4	A-OTU 10	1.1		Unknown
	5	A-OTU 3	0.7		<i>Candidatus Nitrosopumilus</i> (97.6)

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Fig. 1 Conceptual diagram of SBR and SMABR.

Fig. 2 Filtration performance of SBR, SMABR-1.5 and SMABR-5.0 during 56 d (SBR operated without PAC addition at 10 LMH and SMABR-1.5 and SMABR-5.0 operated at 30 LMH); (a) Transmembrane pressure (TMP) development (TMP_d) (The first arrow indicates the point of TMP increased significantly and the second one shows the stable point after TMP increased); (b) Assimilable organic carbon (AOC) reduction; and (c) biofouling on the MF membranes in terms of the abundance of cell and polysaccharide (PS).

Fig. 3 The 2D ordination plot for the SBR and SMABR samples based on the T-RFLP results. The red and blue lines indicate the SMABR and SBR clustering groups, respectively.

Fig. 4 Dendrogram of the CA for the SBR and SMABR samples based on the T-RFLP results (For the sample description in more detail, please see **Table 1**).

Fig. 5 Dendrogram of the CA for the SBR and SMABR samples based on the bacterial sequences.

Fig. 6 Dendrogram of the CA for the SBR and SMABR samples based on the archaeal OTU results.

Fig. 1 Conceptual diagram of SMBR and SMABR.

(a)

(b)

(c)

Fig. 2 Filtration performance of SMBR, SMABR-1.5 and SMABR-5.0 during 56 d (SMBR operated without PAC addition at 10 LMH and SMABR-1.5 and SMABR-5.0 operated at 30 LMH): (a) Transmembrane pressure (TMP) development (TMP_d) (The first arrow indicates the point of TMP increased significantly and the second one shows the stable point after TMP increased); (b) Assimilable organic carbon (AOC) reduction; and (c) biofouling on the MF membranes in terms of the abundance of cell and polysaccharide (PS).

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Fig. 5 Dendrogram of the CA for the SBR and SAMBR samples based on the bacterial sequences.

○ Liquid sample in SMBR ● Membrane sample in SMBR ○ Liquid sample in SMABR ● Membrane sample in SMABR

Fig. 6 Dendrogram of the CA for the SMBR and SAMBR samples based on the archaeal OTU results.

Research Highlights

- Microbial community structure in a MBR as a pretreatment for SWRO was studied.
- Adding PAC differentiated a bacterial community compared to the conventional MBR.
- Addition of PAC was also associated with significantly reduced AOC and biofouling.