



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



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### 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.

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

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## 1. Introduction

At present, seawater reverse osmosis (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, the 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. Organic foulants on the RO membrane were 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  $\mu\text{m}$ ) 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

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