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Biofouling of reverse-osmosis membranes during tertiary wastewater desalination: Microbial community composition

*Ashraf Al Ashhab, Moshe Herzberg, Osnat Gillor**

Zuckerberg Institute for Water Research, Blaustein Institutes for Desert Research, Ben Gurion University of the Negev, Sede-Boqer Campus, 84990, Israel

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ABSTRACT

Reverse-osmosis (RO) desalination is frequently used for the production of high-quality water from tertiary treated wastewater (TTWW). However, the RO desalination process is often hampered by biofouling, including membrane conditioning, microbial adhesion, and biofilm growth. The vast majority of biofilm exploration concentrated on the role of bacteria in biofouling neglecting additional microbial contributors, i.e., fungi and archaea. To better understand the RO biofouling process, bacterial, archaeal and fungal diversity was characterized in a laboratory-scale RO desalination plant exploring the TTWW (RO feed), the RO membrane and the RO feed tube biofilms. We sequenced 77,400 fragments of the ribosome small subunit-encoding gene (16S and 18S rRNA) to identify the microbial community members in these matrices. Our results suggest that the bacterial, archaeal but not fungal community significantly differ from the RO membrane biofouling layer to the feedwater and tube biofilm ($P < 0.01$). Moreover, the RO membrane supported a more diverse community compared to the communities monitored in the feedwater and the biofilm attached to the RO feedwater tube. The tube biofilm was dominated by Actinobacteria (91.2 ± 4.6%), while the Proteobacteria phylum dominated the feedwater and RO membrane (at relative abundance of 92.3 ± 4.4% and 71.5 ± 8.3%, respectively), albeit comprising different members. The archaea communities were dominated by Crenarchaeota (53.0 ± 6.9%, 32.5 ± 7.2% and 69%, respectively) and Euryarchaeota (43.3 ± 6.3%, 23.2 ± 4.8% and 24%, respectively) in all three matrices, though the communities' composition differed. But the fungal communities composition was similar in all matrices, dominated by Ascomycota (97.6 ± 2.7%). Our results suggest that the RO membrane is a selective surface, supporting unique bacterial, and to a lesser extent archaeal communities, yet it does not select for a fungal community.

Abbreviations: RO, Reverse osmosis; TTWW, Tertiary treated wastewater; UF, Ultrafiltration; HG-MBR, Hybrid growth membrane bioreactor; SEM, Scanning electron microscope; ICP-OES, Inductively coupled plasma-optical emission spectrometry; TNA, Total nucleic acid; PCR, Polymerase chain reaction; bTEFAP, Bacteria tag-encoded FLX amplicon pyrosequencing; aTEFAP, Archaea tag-encoded FLX amplicon pyrosequencing; fTEFAP, Fungi tag-encoded FLX amplicon pyrosequencing; OTU, Operational taxonomic unit; ANOSIM, Analysis of similarity; PCA, Principal component analysis; H′, Shannon-Weaver index; S, Richness estimator, chao1 index.

* *Corresponding author*. Tel.: +972 8 6596986; fax: +972 8 6596909.
E-mail addresses: ashraf.ashhab@gmail.com (A. Al Ashhab), herzberg@bgu.ac.il (M. Herzberg), gilloro@bgu.ac.il, osnat.gillor@gmail.com (O. Gillor).

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1. Introduction

Reverse-osmosis (RO) membranes are used for reclamation of saline water (seawater or brackish water) and tertiary treated wastewater (TTWW) effluents. The main technical impasse in the application of RO technology is fouling of the membrane during operation (Baker and Dudley, 1998; Flemming, 1997). One type of fouling is biofouling, resulting from microbial biofilm growth caused by deposition, attachment and proliferation of microorganisms on the membrane surface (Pang et al., 2005). Biofouling has been observed within a first few hours of RO operation (Lee et al., 2010; Chen et al., 2004), causing an increase in hydraulic resistance, reduced permeate flux and an increase in salt passage (Herzberg and Elimelech, 2007; Hoek and Elimelech, 2003). The removal of biofouling layers has been tested using a variety of chemical cleaning agents; however, these have been found to affect only the top biofilm surface (Bereschenko et al., 2011).

Two approaches are typically employed to tackle the biofouling problem; one by improving operational conditions and feedwater pretreatment, and the other by membrane surface modifications. However, previous studies investigated the bacterial composition of RO membranes biofilms have suggested that membrane surface properties (Baek et al., 2011) and feedwater pretreatment (Herzberg et al., 2010) play a minor role in RO biofilm development and composition. Two major bacteria phyla were reported to dominate the RO membrane biofilms regardless of feedwater characteristics: Proteobacteria and Bacteroidetes (Ayache et al., 2013; Herzberg et al., 2010; Ivnitsky et al., 2007; Pang and Liu, 2007; Bereschenko et al., 2008, 2010; Baba et al., 2009; Hörsch et al., 2005). Such bacterial biofilm composition was found to be unique to the RO membrane, different from other compartments of the RO unit, such as the UF feedwater tank or feedwater. The unique bacterial biofilm is initially colonized by *Sphingomonas* (a genus of the α-Proteobacteria) and later by other members of the Proteobacteria and Bacteroidetes phyla (Ayache et al., 2013; Bereschenko et al., 2008, 2010; Baba et al., 2009). Yet, only two studies investigated the presence of other microorganisms in addition to bacteria, pointing out that fungi and other eukaryotic microorganisms might instigate RO membrane biofilm formation (Flemming, 1997). The fungi genera *Fusarium* and *Phialophora* were found to colonize RO cellulose acetate membranes (Ho et al., 1983). However, archaea presence was never investigated in the biofouling layer of the RO membrane.

Microbial communities in RO systems have traditionally been investigated using molecular techniques such as fluorescence *in situ* hybridization (Bereschenko et al., 2010), terminal restriction fragment length polymorphism (Chiellini et al., 2012), and PCR based denaturing gradient gel electrophoresis (Baba et al., 2009). The recently emerging deep-sequencing techniques provide a powerful tool for characterizing microbial diversity in detail (Liu et al., 2007; Siqueira Jr. et al., 2012). This novel tool has been used once to investigate the bacterial community in an RO system (Ayache et al., 2013), yet the contribution of other microorganisms to the biofouling is not yet known. Here, we aim to take advantage of deep sequencing to gain a better insight into the origins, diversity and composition of biofilms formed in TTWW RO systems by investigating its microbial community including the bacteria, archaea and fungi. We predict that the RO membrane provide a unique surface for biofilm development, and support a unique microbial composition, distinct from the communities found in the RO feedwater or on the biofilm attached to the RO feed tube.

2. Materials and methods

2.1. RO membrane unit operation

A laboratory-scale RO unit (Fig. 1) was fed with ultrafiltration (UF) permeate of a hybrid growth membrane bioreactor (HG-MBR) that treats municipal effluents, as reported previously (Ying et al., 2012). A commercial thin-film composite RO membrane, ESPA-1 (Hydranautics, Oceanside, CA, USA), was used as a model membrane for the biofouling experiments. The RO unit was operated continuously till the permeate flux decreased to 60% of its initial flux value, under a constant pressure of 10 bar and cross-flow velocity of 15.58 cm/s corresponding to a shear rate of 370.4 s^{-1}. Biofouling experiments were performed in two separate replicates.

2.2. Sampling

2.2.1. TTWW (RO feed-water)

RO feedwater (100 l) was collected in four sterile 25-l plastic containers at the beginning, first and second weeks and at the end of each experiment. The effluent samples were composited and concentrated to approximately 100 ml using a disposable UF F200NR filter (Fresenius Medical, Bad Homburg, Germany) based on protocols described by Leskinen and Lim (2008) and Rajal et al. (2007), and held at 4 °C until DNA extraction, within 24 h.

2.2.2. RO feed tube

Biofilm growing on the RO feed tube (polypropylene, L/S 25, MasterFlex, Vermon Hills, Illinois, USA) was chosen to represent biofilm formed under feed-solution conditions without the unique environment closer to the RO membrane (higher pressure, perpendicular flow, polarization of salts and nutrients, and different physical surface properties). The feed-tube biofilm was collected at the end of each experiment and stored at −80 °C for DNA extraction.

2.2.3. RO membrane

At the end of each experiment, 0.5 cm^2 of the fouled RO membrane was collected, fixed, dehydrated and coated with an approx. 10–15 nm layer of gold for analysis with scanning electron microscope (SEM) (Philips XL30, Andover, MA, USA) following previously reported protocol (Herzberg et al., 2010; Fox and Demaree Jr., 1999) and the rest was stored at −80 °C for DNA extraction.

2.3. Chemical analyses

Samples from both RO permeate and feedwater were collected at all stages of each experiment. Each experiment was