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The impact of diatoms on the biofouling of seawater reverse osmosis membranes in a model cross-flow system

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HIGHLIGHTS

- Assess propensity of diatoms to foul RO membranes.
- The TEP concentration of feed water remained constant throughout the experiment.
- Microbial interactions should be considered when investigating biofilm formation in the future.

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ABSTRACT

Diatoms are well known contributors to the biofouling of seawater reverse osmosis (SWRO) membranes however there have been no studies on the isolation of specific diatoms from native sea water and their subsequent impact in a laboratory scale cross-flow situation. SWRO feed tank water was analyzed for microorganisms, nutrients and transparent exopolymer substances (TEPs). Subsequently, the diatom genus, *Pseudo-nitzschia* sp., was isolated and used in a laboratory scale cross-flow system as a model phytoplankton species for TEP production. The biofilm produced after 7 days was then characterized, as well as the nutrients and TEP. The average quantity of TEP found on the biofouled membrane was 28.09 ± 3.40 μg XG eq L^{-1}, which is lower than that of the feed water over the duration of the cross-flow experiment (3.5–5.1 × 10^3 μg XG eq L^{-1}). Proteins, lipids and polysaccharide biofilm compounds were present on the RO membranes, as well as diatoms and bacteria. The total abundance of pico-phytoplankton increased over the experimental period with proliferation of *Prochlorococcus* being the major contributor to this increase.

1. Introduction

Reverse osmosis (RO) membrane technology is used in the desalination industry to produce high-purity water that is microbiologically safe and biologically stable [1,2]. However, RO membranes are subject to biofouling which leads to (1) a decline in membrane performance [3,4], (2) a reduction in system efficiency [3,4] and (3) a reduction in the lifetime of the membranes [5]. Given the complexity of biofilm formation in marine (saline) environments, it is essential to better understand the main precursors of biofouling, particularly if prevention is to be achieved.

Transparent exopolymer particles (TEPs) have been identified as one of the main precursors of biofouling in desalination membranes [5]. TEP comprises of sticky organic microgels with varying size range from 0.4 μm to >200 μm, including exopolymeric substances (EPS), made of polysaccharides and of other constituents (i.e., proteins, uronic acids, sulfates) [6]. Various studies have shown that microalgae produce EPS [6–10] and that in the ocean these biopolymers play a crucial role in the formation of marine gels, marine snow and biofilms [11–14].

Important contributors to EPS in the ocean are diatoms, a group of microalgae [12]. Interestingly, the chemical composition and quantity of EPS produced by diatoms vary with irradiance (i.e., light/dark exposure; [15]), nutrient limitation (i.e., limitation in nitrogen or phosphorus; [16]) and cell growth stage [17]. The production and structural characteristics of EPS also vary depending on the diatom species, with a production ranging from 1 mg·L^{-1} to 27.5 mg·L^{-1} [7,13]. However, even if diatoms are known to produce EPS, and are present in most

Abbreviations: SWRO, seawater reverse osmosis; TEPs, transparent exopolymer substances; RO, reverse osmosis; EPS, exopolymeric substances; TFC, thin film composite; SEM, scanning electron microscopy; ATR-FTIR, attenuated total reflectance-Fourier transform infrared; TFF, tangential flow filtration; PES, polyethersulfone; μg XG eq L^{-1}, μg of xanthan gum equivalent per liter; HDNA, high DNA.

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aquatic environments, only one study has so far focused on the role of diatoms in the biofouling of RO membranes [18].

Recently, Balzano et al. [19] worked on the assessment of seasonal fluctuations of microbial communities in a seawater reverse osmosis (SWRO) desalination plant (Penneshaw, Kangaroo Island, South Australia). Diatoms were found to be present in the desalination system throughout the year and 16 diatom species were regularly observed in the RO feed tank, even after pre-treatment [19]. Diatoms, like those observed in the RO feed tank, can live in microbiota with bacteria and form biofilms [20,21].

Here, the diatom species *Pseudo-nitzschia* sp. was isolated from the RO feed tank of the SWRO Penneshaw Desalination Plant (Kangaroo Island, South Australia) and used as a phytoplankton model species for TEP production. Its propensity to form biofilms on commercial thin film composite (TFC) SWRO membranes was assessed using a laboratory-scale cross-flow system. Microbial communities, nutrients and TEP were analyzed using flow cytometry, light microscopy, flow cell injection and colorimetry. All biofilms formed were characterized using scanning electron microscopy (SEM), attenuated total reflectance-Fourier transform infrared ((ATR-FTIR) spectroscopy and colorimetry.

2. Materiel and methods

2.1. Culture of Pseudo-nitzschia sp.

The diatom, *Pseudo-nitzschia* sp., was isolated from the RO feed tank of the SWRO desalination plant in Penneshaw (Kangaroo Island, South Australia) through serial dilutions in 24 well plates containing f/2 medium, according to Guillard et al. [22]. Diatom isolates were subsequently inoculated in larger volumes of f/2 medium until 50 mL cultures were established. The cultures were maintained at 20 °C under a 12/12 h light/dark cycle.

In order to establish stock cultures for the experiment, *Pseudo-nitzschia* sp. was cultured in four 1 L aerated bottles containing f/2 medium, up to a final concentration of 1×10^5 cells mL^{-1}. In parallel, 36 L of seawater was filtered through a hydrosart cartridge (Sartorius Stedim, Dandenong, Australia) with a molecular weight cut-off (MWCO) of 100 kDa using Vivaflow 200 tangential flow filtration (TFF) (Sartorius, Dandenong, Australia) in combination with a Masterflex L/S peristaltic tubing pump (Cole Parmer, Chatswood, Australia) in order to remove the bacteria present in the seawater. The seawater was then filtered to remove viruses using the TFF system with a 10 kDa polyethersulfone (PES) cartridge (Sartorius, Dandenong, Australia). The four *Pseudo-nitzschia* sp. cultures were then inoculated into the 36 L of TFF filtered seawater to attain a final concentration of 1×10^4 cells mL^{-1}, as observed in situ in the RO feed tank [19].

2.2. Cross-flow experiment

TFF filtered seawater (40 L), containing *Pseudo-nitzschia* sp., was used as feed water in a custom built laboratory cross-flow filtration system (Fig. 1). The experimental system consisted of six Sterlitech CF042 membrane cross-flow filtration cells, connected as two parallel sets of three. Water from the feed tank of the cross-flow filtration system was recirculated through the cross-flow system. The pressure was set at 500 psi and the flow at 1.5 $L \cdot min^{-1}$. DOW Filmtec® SW30HR TFC RO membranes were used for the experiments, as they were the RO membranes used at Penneshaw. The cross-flow experiments were conducted for 7 days to emulate the RO desalination process in the laboratory and to assess biofilm formation, with T_0 denoting samples at the start of the experiment and T_{7d} the end of the experiment (day 7). The pH and salinity were measured using an AquaRead multi-parameter probe. Samples were analyzed to assess (1) TEP quantification, (2) abundance of microbes and (3) nutrient concentration. After T_{7d} the RO membranes were removed from the cross-flow system and analyzed using colorimetric TEP quantification, SEM and FTIR spectroscopy.

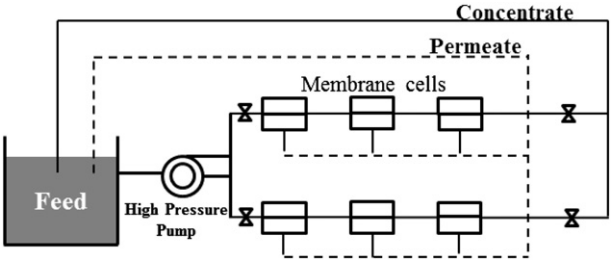

Fig. 1. Diagram of the custom built laboratory cross-flow filtration system which consisted of six Sterlitech CF042 membrane cross-flow filtration cells, connected as two parallel sets of three. Permeate and concentrate were recirculated through the cross-flow system via the feed tank. The pressure was set at 500 psi and the flow at 1.5 $L \cdot min^{-1}$.

2.3. Analysis of seawater prior to and post cross-flow experiments

2.3.1. Nutrient quantification

The concentration of dissolved silica, ammonium, orthophosphate and the combined concentrations of nitrate and nitrite (nitrate/nitrite) were measured using a Lachat Quickchem Flow Injection Analyser (FIA), following published methods [23]. 10 mL triplicates of water were sampled at T_0 and T_{7d} from the feed tank of the laboratory cross-flow system and filtered through bonnet syringe Minisart filters (0.45 μm pore size, Sartorius Stedim, Dandenong, Australia) to remove any large particles and microorganisms. Filtrates were then stored at −20 °C. Prior to analysis, the samples were thawed and then injected into the FIA (~10 mL). The method was calibrated using standard solutions prepared in 0.6 M sodium chloride, corresponding to typical seawater salinity values of 35 practical salinity units (PSU). The detection limit was 0.2 μM for SiO_2, 40 μM for NH_3, 0.02 μM for PO_4 and 0.009 μM for NO_x.

2.3.2. TEP colorimetric quantification

The determination of TEP concentration (measured in μg of xanthan gum equivalent per liter; μg XG eq L^{-1}) in the seawater samples was performed using the modified centrifugation protocol of Claquin et al. [24]. A duplicate standard curve using Xanthan gum was used to determine the concentration of TEP following Claquin et al. [24]. First, 10 mL triplicates of water were sampled at T_0 and T_{7d} from the feed tank of the laboratory cross-flow system and centrifuged at 4000 rpm for 20 min. The supernatant and pellet were separated to differentiate the TEP bound to the diatoms (pellet) and the free TEP present in solution. The supernatant was then filtered using a 0.4 μm polycarbonate filter under a gentle vacuum and then transferred into 0.2 μm filtered raw seawater (5 mL) and stored at −20 °C, as described by Klein et al. [25]. Samples were subsequently analyzed following Balzano et al. [19].

2.3.3. Abundance of bacteria and viruses

As bacteria and viruses are naturally present in phytoplankton cultures, and would also contribute to biofilm formation during the experiment, bacterial and viral counts were carried out using flow cytometry following the protocol of Schapira et al. [26] and Paterson et al. [27]. For each bacterial and viral count, 1 mL triplicates of water were sampled at T_0 and T_{7d} from the feed tank of the laboratory cross-flow system and fixed with 0.5% glutaraldehyde (Proscitech, Thuringowa, Australia), flash frozen in liquid nitrogen and stored at −80 °C until further analysis, as described in Le Lan et al. [28].

2.3.4. Abundance of pico- and nano-phytoplankton

Photosynthetic eukaryotic pico- and nano-phytoplankton (<5 μm) counts were carried out using flow cytometry following the protocol of Schapira et al. [29]. For each photosynthetic eukaryotic pico- and