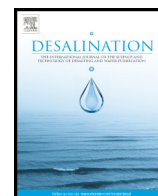




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Investigation of compounds that degrade biofilm polysaccharides on reverse osmosis membranes from a full scale desalination plant to alleviate biofouling

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HIGHLIGHTS

- SNP and xanthine oxidase performed better than DBNPA for permeability recovery.
- Xanthine oxidase was more effective than SNP in decreasing biovolume.
- DBNPA reduced the number of live cells but was ineffective in decreasing biovolume.
- Free radical generators may remove biofilms by physical breakdown of polysaccharides.

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ABSTRACT

Biofouling of seawater reverse osmosis (SWRO) membranes in desalination processes causes increased energy consumption and operating costs. In spite of pre-treatment systems, polymeric materials are deposited on the membranes along with bacteria and other particles. Bacteria grow and produce further polymers (extracellular polymeric substances; EPS) *in situ* forming a recalcitrant biofilm. Current membrane cleaning protocols aim to kill the bacteria but do not necessarily remove the associated polysaccharides which appear to reduce permeate permeability. Few studies have investigated the removal of both biofilm bacteria and associated polysaccharides. There is some evidence that compounds which produce free radicals can degrade polysaccharides; and the previous studies in our laboratory have suggested that they can reduce polysaccharide adhesion and the effects of membrane fouling. In this study, we compared the effect of two free radical producing systems with the currently accepted control agent, a biodegradable biocide, 2,2-dibromo-3-nitrilopropionamide (DBNPA). The free radical generating systems were sodium nitroprusside (SNP) that spontaneously releases nitric oxide free radical and a xanthine oxidase enzyme with a hypoxanthine substrate to release a superoxide radical. Experiments were conducted on the fouled membranes collected following membrane unit replacements at a full scale seawater desalination plant in Western Australia. Both free radical generating compounds improved permeate flow in a bench scale cross-flow RO system compared to the biocide without damaging membrane structures. The CLSM analysis suggested the biofilm was thinner but also less compact. A lectin bioassay supported the conclusion that the free radicals had a direct effect on the biofilm polysaccharides, not just the biofilm cells.

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1. Introduction

Biofouling in seawater reverse osmosis (SWRO) systems is a continuing problem. Several studies have shown that microbial fouling is a major contributor to biofouling which significantly increases energy consumption and the associated cost of water production [1–4]. Biofilms are formed by microbes which change from a free living planktonic state to a sessile stage attached to the membrane surface where

they produce an extracellular matrix. The extracellular matrix is composed of 90% water and 10% extracellular polymeric substances, or EPS [5]. Structural components of EPS matrix include proteins, lipids, humic substances and polysaccharides. Of these, polysaccharides constitute a major component of the biofilm matrix and are crucial in maintaining the physical integrity of biofilm matrix [6,7]. So far, just over 30 different biofilm matrix polysaccharides have been characterized [5]. Mutant strains of bacteria unable to synthesize polysaccharides, were less efficient in forming biofilms and more susceptible to biocides [8].

Control of biofouling has been an ongoing challenge [9], and in most trials bacterial biofilms are recalcitrant to removal either by developing resistance to biocides or by failure to dislodge from the membrane

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surface [10]. Previous researchers have used several strategies to either prevent biofilm formation on membranes or disperse and in turn remove mature biofilms. Some of the methods to alleviate biofouling on RO membranes include physical flushing or cleaning of membranes, addition of chemicals compounds targeting the bacterial cells and/or extracellular matrix, and modification of membrane surface and structure [11]. However, each has disadvantages [11]. Current clean in place (CIP) practices are often ineffective due to incorrect chemical selection; incomplete penetration of biofilm layers; poor cleaning practice with respect to pH, temperature, contact time; improper recirculation flow rates and incomplete removal of biomass [12]. The repeated use of biocides may also cause bacterial resistance by modification of the bacterial cell envelope reducing biocide permeability, production of enzymes to degrade the biocides, or acquiring other biocide resistance genes [12, 13]. Biofilm control strategies using enzymes to degrade the EPS matrix include glycosidases, proteases and deoxyribonucleases, but such enzymes target specific strains and their efficacy in complex multispecies biofilms has not been established [14]. In addition, enzymes are typically expensive and there are other practical difficulties in treatment and flushing of membranes on an industrial scale. There is therefore still an urgent need for more efficient and cost effective methods to remove biofilms and alleviate biofouling in SWRO processes.

Although polysaccharides form a large proportion of the EPS matrix, their contribution to biofilms is largely ignored and most removal strategies target the bacterial cells themselves. Methods to disrupt the physical integrity of the polysaccharides should loosen and dislodge both the EPS matrix and biofilm cells. Previous studies investigating the effect of free radicals (particularly nitric oxide donors) on single species biofilms [15–18], focused on their role in cell signaling and programmed cell death causing biofilm cells to transform into planktonic cells. In this study, we suggest free radicals have a mucolytic action and break down the biofilm polysaccharides leading to physical dislodgement of the slime layer that is necessary for improving separation performance. Our previous work has shown free radical generating compounds reduce both polysaccharide adhesion and the effects of membrane fouling, improving permeate flux [19]. Therefore, in this study, we selected two free radical producing systems: sodium nitroprusside (SNP) that spontaneously releases nitric oxide free radical and a xanthine oxidase enzyme with a hypoxanthine substrate to release a superoxide radical, for studying their impacts on the treatment of fouled SWRO membranes. They were compared with the currently accepted control agent, a biodegradable biocide, 2,2-dibromo-3-nitropropionamide (DBNPA). By comparing the three treatments using RO filtration and additional characterization techniques, we found both free radical producing systems were superior to the commonly used biocide DBNPA and reduced polysaccharide adhesion and membrane fouling without compromising membrane salt rejection.

Industrially fouled membranes were chosen as the model membranes in this study. The biofilms on these membranes comprise of a diverse multispecies bacterial community and are formed as a result of years of exposure to varying environmental factors operational in the plant. Under laboratory conditions, it is near impossible to artificially foul a membrane to this effect owing to the time constraints. Therefore the effect of chemical treatments on these biofilms in bench scale experiments would be a good representative of their effect if used in large scale. A recent study suggests that under high pressure SWRO membrane productivity does not vary significantly with change in width or number of sheets [20]. The reuse of autopsied spirally wound membranes in the form of flat sheet membranes in the laboratory may therefore be considered as a good model.

2. Materials and methods

2.1. Bench scale reverse osmosis separation

In the bench scale reverse osmosis experiments, membranes were placed with their active layer facing the feed solution in a Sterilittech

CF042 cross-flow RO cell of dimensions 2.28 mm slot depth, 39 mm slot width and 42 cm² membrane surface area; in which the solution was pumped by a hydra-cell pump (B Line Pumps Pty. Ltd. AU). Typically, 6 L of feed solution, artificial sea water (ASW) made up with sea salts (Sigma-Aldrich) was used in all runs. The concentration of sea salts used was 40 g/L of DI water as per the manufacturer's instructions. The ionic composition of reconstituted seawater is as follows: chloride – 19,290 mg/L, sodium – 10,780 mg/L, sulfate – 2660 mg/L, potassium – 420 mg/L, calcium – 400 mg/L, carbonate (bicarbonate) – 200 mg/L, strontium – 8.8 mg/L, boron – 5.6 mg/L, bromide – 56 mg/L, iodide – 0.24 mg/L, lithium – 0.3 mg/L, fluoride – 1.0 mg/L, magnesium – 1320 mg/L, and trace elements – <0.5 mg/L. During the experiments, a cross-flow velocity of 8.5 cm/s, selected according to literature [21,22]; and pressure of 600 psi were maintained constantly (22 °C–23 °C). After a given period of time, the permeate produced during the RO process was collected in a container on a digital balance (A & D Australasia Pty. Ltd, AU); weight change was monitored by a laboratory computer for permeability calculation (Eq. (1)). Experiments were carried out in triplicate for each of the treatments and control run; the average values of permeability were calculated, all of which exhibited <25% of variation. Moreover, to examine the membrane integrity especially after the exposure to free radical generating compounds, the conductivity of feed water and permeate was measured during the treatment for calculating salt rejection. We found the treatment by using free radical generating compound did not vary salt rejection throughout the whole process.

Polyamide thin film composite RO membranes (Dow Filmtac SW30) were collected following autopsy of spiral-wound membrane units at the end of their useful life (5–7 years use), provided by a full scale RO plant in Western Australia. The pattern and severity of membrane fouling varied across units, according to their location within the plant. Membranes were selected with approximately average fouling from those autopsied. Of the 14 membrane sheets autopsied, samples that exhibited homogenous uniform surface fouling were used in order to minimize variations arising due to heterogeneity of biofilms. Membranes were stored at 4 °C in sealed bags under moist conditions. Before test, sections of the membrane were cut and soaked for 2 h in deionized (DI) water; this was proven to not affect the fouling layer itself but removing residual salts from the membrane, which otherwise affected salt levels and conductivity in the permeate. Afterwards, the pre-soaked fouled membrane was placed into the Sterilittech CF042 cross-flow RO cell under a constant pressure of 600 psi and a flow rate of 0.54 L/min to stabilize the water fluxes in the filtration process. Following that, the membrane was taken out of the cell, placed in a clean Petri dish, and its surface was treated with 1 mL of different compounds, namely, 200 ppm DBNPA (Dow), 0.1 mM SNP (Sigma-Aldrich) and 1 unit xanthine oxidase (Sigma) plus 100 µM hypoxanthine (Sigma). All the three compounds were constituted in sterile artificial seawater. The pH of ASW and treatment solutions were measured with a pH meter (Hanna 8521) and recorded as follows: ASW – 8.10, DBNPA – 7.92, SNP – 7.35, xanthine oxidase plus hypoxanthine – 7.01. For comparison, the control sample was treated by ASW in a similar manner to the aforementioned samples. All membrane treatments were carried out at room temperature with the exception of the use of xanthine oxidase which was carried out at 37 °C. After 1 h treatment, the membranes were gently rinsed with ASW, taking care not to disturb the biofilm, placed back to the RO system and the run resumed for a further RO experiment. After separation, the membrane was stored moist at 4 °C in a sealed container for further analyses, including CLSM, TGA, and FTIR. The membranes, treated by ASW, 200 ppm DBNPA, 0.1 mM SNP and 1 unit xanthine oxidase plus 100 µM hypoxanthine, were denoted as RO-F, RO-D, RO-S, and RO-X, respectively. For comparison, a clean membrane without prior use at the SWRO plant was taken and referred to RO-C.

The water permeability of membrane sample (P_i) was calculated as:

$$P_i = \frac{V}{A \times t \times \Delta P} \quad (1)$$

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