1. Introduction

Membrane biofouling in nanofiltration and reverse osmosis applications is a major operational problem that leads to increased water production costs and potentially lower final water quality [Herzberg and Elimelech (2007); Huertas, et.al. (2008)]. Disinfection of the feed waters is the most widely used membrane biofouling control method, but has been broadly unsuccessful in preventing significant biofilm formation on membrane surfaces [Fleming (1997)]. Even with high log removal of bacteria (e.g. 4-log), significant biofouling can happen because only a few bacteria are needed to colonize a surface and subsequently form a biofilm [Fleming (1997)].

Alternate methods for biofouling control have been studied including processes to remove nutrients or bacteria, changing the physical properties of the membrane surface, and coating the surface in biocides [Al-Jaboori and Yusaf (2012); Altman, et.al. (2009)]. These passive methods aim to decrease bacterial attachment or inactivate bacteria upon attachment but have fallen short of being widely applicable, cost-effective, and/or successful at controlling biofouling [Al-Jaboori and Yusaf (2012); Altman, et.al. (2009)].

Our approach to this problem was to incorporate 2-aminoimidazoles (2-AIs, see Figure 1) into the membrane matrix. 2-AIs are non-biocidal organic molecules that actively disrupt the ability of bacteria to detect and react to environmental stimuli, which keeps bacteria in a planktonic state and prevents their attachment to surfaces [Rogers and Melander (2008); Thompson, et.al. (2012); US Patent 7,897,631 (2011); US Patent 7,906,544 (2011); US Patent 8,278,340 (2012); US Patent 8,367,713 (2013); US Patent 8,653,124 (2014)]. These 2-AIs have inhibited biofilm formation of bacteria across different orders, classes, and phyla [Rogers and Melander (2008)].

![Figure 1. General chemical structure of 2-AIs. R represents where the moiety can vary.](image-url)
The specific objectives of this research were to: (1) determine whether a 2-AI would be amenable to incorporation into water purification membranes and maintain anti-biofilm activity once incorporated, (2) incorporate a 2-AI into the matrix of a commercial membrane, and (3) evaluate the performance of the 2-AI-membrane in comparison to a control membrane lacking 2-AI in terms of water permeability, salt rejection, and biofilm inhibition.

2. Materials and Methods

2.1 Materials
All chemicals used were of ACS reagent grade or better. The 2-AI and 2-AI conjugate were synthesized in-house, and their structures and purity were confirmed by proton nuclear magnetic resonance spectroscopy (1H NMR) and mass spectrometry (MS). The commercial RO membrane, XLE, was purchased from the manufacturer (Dow Filmtec, Minneapolis, MN).

2.2 Amenability of 2-AI to incorporation into polyamide membranes and potential to inhibit biofilm
The 2-AI (where R= p-aniline) was treated with benzoyl chloride, which is an organic compound representative of a polyamide matrix component of NF/RO membranes. The 2-AI-benzoyl conjugate produced was isolated and characterized by 1H NMR. The ability for the 2-AI-benzoyl conjugate at varying concentrations to inhibit biofilm while in solution was determined using *Pseudomonas aeruginosa* (PA14) in a crystal violet biofilm inhibition assay [Richards, et.al. (2008)].

2.3 Incorporation of 2-AI and model compound into a polyamide membrane
The 2-AI and a surrogate compound were incorporated into a commercial membrane via a substitution reaction after activating the carboxylic acid groups in the polyamide membrane matrix. Each membrane was characterized using Rutherford backscattering spectrometry (RBS) and Fourier transform infrared spectrometry (FTIR), in order to evaluate and/or quantify the incorporation of each compound in the membrane matrix. The same incorporation and analysis procedures were followed using 4-iodoaniline as a surrogate for 2-AIs. 4-iodoaniline has a similar chemical structure to 2-AIs and contains iodine, which facilitates its quantification after incorporation into the polyamide membrane matrix.

2.4 Performance evaluation of 2-AI membranes
The water permeability and salt rejection of 2-AI and control membranes were evaluated using a dead-end high pressure stirred cell (Sterlitech, Kent, WA) at 200 psi of applied pressure. A sodium chloride solution was used for performance tests. To evaluate how well the 2-AI membrane inhibited biofilm formation, the surface of the 2-AI and control membrane were exposed to an aqueous solution containing PA14 and nutrients and incubated under stagnant conditions. The biofilm formation was quantified after incubation by comparing the colony forming units on the 2-AI and control membranes using a plate count method.

3. Results and Discussion

3.1 Amenability of 2-AI to incorporation into polyamide membranes and potential to inhibit biofilm
The 2-AI reacted with the benzoyl chloride to form a 2-AI-benzoyl conjugate, confirming that the 2-AI is amenable to incorporation into membranes containing terminal carboxylic acid groups. The 2-AI benzoyl conjugate inhibited PA14 biofilms significantly. The half maximal inhibitory concentration (IC50) of the 2-AI-benzoyl conjugate is 160 μM, which is orders of magnitude less than the amount of carboxylic acid groups in water purification membranes (0.2-0.6 M [Coronell, et.al. (2010)]).

### 3.2 Incorporation of 2-AI and model compound into polyamide membranes

RBS and FTIR analyses confirmed that 2-AI and the surrogate compound 4-iodoaniline were incorporated into the membrane matrices. For example, the RBS spectrum in Figure 2 shows that the membrane into which 4-iodoaniline was incorporated contained iodine (peak at ~3300 keV), whereas the control membrane did not contain any iodine, confirming that 4-iodoaniline was in fact incorporated. The 4-iodoaniline results are indicative of 2-AI concentration via incorporation using the same membrane and incorporation procedure.

![Figure 2. RBS spectra at 2800-3500 keV of a membrane where 4-iodoaniline was incorporated and a 4-iodoaniline lacking control membrane. Peak at ~3300 keV corresponds to iodine.](image)

### 3.3 Performance evaluation of 2-AI membranes

Initial results indicate no significant difference in water permeability and salt rejection between the control membrane and the 2-AI membrane when tested in dead end configuration. The 2-AI membrane inhibited PA14 biofilm formation 70-86% under stagnant conditions. The extent of biofilm inhibition depended upon the conditions under which membranes were exposed to the bacteria (e.g. incubation time, bacterial concentration).

### 4. Conclusions

A 2-AI and the corresponding model compound were successfully incorporated into a commercial polyamide RO water purification membrane. Initial results indicate that the incorporation of 2-AI does not significantly affect the water permeability or salt rejection of the membrane tested as evaluated by dead-end performance tests. The membrane with a 2-AI incorporated substantially inhibited PA14 biofilm under static conditions. This 2-AI membrane is
the first non-biocidal membrane that has inhibited biofouling by actively interfering with the biofilm formation mechanisms of bacteria.

References


