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Attachment of selenium to a reverse osmosis membrane to inhibit biofilm formation of *S. aureus*

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ABSTRACT

Previous experiments have shown that when selenium was attached to a surface, biofilm development on the surface was inhibited. Selenium is a catalytic producer of superoxide radicals via oxygen reduction. The superoxide radicals can cause damage to the outer membrane of bacterial cells that frequently results in cell death. Therefore, we propose selenium attachment to an RO membrane surface as a biofouling inhibition technique. Selenium was attached to the surface of RO membranes via monomer (selenocystamine) and polymer (aceto acetoxy ethyl methacrylate) attachment mechanisms. Using confocal microscopy and *Staphylococcus aureus* cell counting to evaluate *S. aureus* biofilm growth, the number of attached *S. aureus* cells was seen to be significantly reduced on RO membranes coated with selenium. While both selenium coatings had similar *S. aureus* inhibition, the selenium coatings had much different impacts on permeate flux. The selenocystamine attachment method maintained a higher permeate flux compared to the AAEMA attachment method due to AAEMA requiring a harsh attachment procedure. Ultimately, attached selenocystamine showed great potential to serve as a biofouling inhibitor by reducing attached *S. aureus* growth on RO membranes without excessive permeate flux loss.

1. Introduction

As water demands increase and potable water source availability decreases, reverse osmosis (RO) is becoming more popular for sea water desalination and treated wastewater reclamation [1]. In 2005, 50% of all desalination capacity had been operating for less than five years [2]. While the overall cost of production of water has decreased, the major operational costs of energy and RO membrane replacement remain the same [3]. Both of these costs are associated with RO membrane fouling. As foulants build up on the RO membrane surface, greater energy levels are required to maintain production rates until ultimately the RO membrane must be replaced.

While the cause of fouling is ultimately feed water dependant, the major RO membrane foulant types are inorganic salts [4,5], organic material [6,7], colloidal particles [8–11], and microbial growth. Growth of microbial species on the RO membrane surface differs from other foulants due to the complexities associated with multispecies ecosystems that are able to grow and adjust to changing environmental conditions.

Microbiological fouling, or biofouling, is a significant problem occurring at RO treatment plants. Complete biofilm coverage of an RO membrane occurred after only three days of system operation [11]. Biofilm formation on RO membranes may be divided into three steps: transport of microbial cells to the RO membrane surface, attachment of the individual cells to the RO membrane, and microbial population growth and maturation. Transport of the cells to the RO membrane surface is encouraged via convection as water passes toward, and ultimately through, the RO membrane. Once on the surface, cells initially attach via surface–surface attractions [7,12,13] which are later aided by extracellular polysaccharide (EPS) compounds excreted by the cell [1,14]. EPS also encourage attachment of other cells by providing suitable surface characteristics. A thick biofilm layer forms as the number of attached bacterial cells significantly increases both by continued planktonic cell attachment and cell growth by attached cells. The mature biofilm consists of multiple microbial species connected by complex EPS matrix that is extremely difficult to remove without significant treatment operations.

Due to the numerous operational problems associated with biofouling, a variety of biofilm control techniques have been proposed [14]. A common method for limiting RO membrane biofouling is feed water pretreatment, which typically consists of either biocide addition to kill bacterial cells or removal of bacterial growth factors to limit microbial growth [15]. However, neither biocides nor nutrient removal is able to kill bacterial cells on the RO

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membrane. Oxidative biocides (chlorine, ozone, etc.) must be removed before reaching the RO unit to prevent RO membrane damage of PA RO membranes. This results in there being no residual bacterial inhibition at the RO membrane surface [16]. Removing bacterial growth factors does not prevent biofilm growth as bacteria are able to establish biofilm even in low nutrient growth environments [14].

Modifying the surface of the RO membrane has been proposed as a passive biofilm control alternative. Most surface modifications consist of altering surface characteristics to decrease the rate of initial bacterial attachment including modifying an RO membrane's hydrophobicity, surface roughness, and surface charge [12,17–19]. While surface modifications altering the attraction between the RO membrane and bacterial cell have successfully slowed initial attachment of bacteria, the coating effects would be minimal once a biofilm was established. Therefore, any surface modification that inhibits biofilm growth on the surface would be more desirable than one that merely delays biofilm attachment. Such surface modifications damage or kill bacterial cells before they form a biofilm on the surface. Several bacterial cell killing coatings have been proposed. Attachment of polycationic chains to a microfiltration membrane inhibited *Escherichai coli* attachment by cellular membrane disruption [20]. Bacterial cell killing by the polycationic chains is consistent with other Gram negative inhibiting guanidine polymers. TiO_2 nanoparticles have also been proposed for their ability to catalyze the production of reactive oxygen species such as hydrogen peroxide and hydroxyl radicals when excited by UV light [21].

Recent studies proposed selenium as an antimicrobial agent that inhibits the development of bacterial biofilm on a surface [22,23]. Selenium inhibits the development of bacterial biofilm by acting as a catalyst for redox reactions involving reactive oxygen species. Selenium is a micronutrient for eukaryotic cells necessary for glutathione peroxidase production [24]. Glutathione peroxidase protects cell components from hydrogen peroxide (H_2O_2) by reducing H_2O_2 to water [23]. During H_2O_2 reduction, glutathione acts as an electron donor and is oxidized. Independent of glutathione peroxidase, selenium compounds can catalytically oxidize glutathione with oxygen acting as the electron acceptor [25]. The dissolved oxygen is converted to superoxide (O_2^-), a free radical that can reduce nearby compounds or form other reactive oxygen species such as hydrogen peroxide or a hydroxyl radical (•OH) [22,24]. All of these reactive oxygen species are capable of attacking various compounds located in bacterial outer membranes. This selenium induced damage of the bacterial outer membrane commonly leads to cell lysis [23]. Therefore, continuous superoxide production by attached selenium on a surface prevents bacterial attachment and inhibits biofilm development.

The objective of this paper is to evaluate the efficacy of selenium coated RO membranes in inhibiting the development of bacterial biofilm. Two separate coating methods were utilized. Using both quantitative and qualitative assays, we examined the effectiveness of the attached selenium on RO membranes to inhibit biofilm development by the Gram positive bacteria *Staphylococcus aureus*. In addition, the secondary impacts of the selenium coating on RO membranes were examined by measuring permeate flow rate and salt rejection.

2. Materials and methods

2.1. Experiment supplies

2.1.1. Chemicals

All chemicals used during the experiment were reagent grade (Fisher Scientific).

2.1.2. RO membranes

RO membranes were purchased from GE Osmonics (Minnetonka, MN). CA-CE cellulose acetate (CA) and PA-AK polyamide (PA) RO membranes were used in all experiments for comparison purposes.

2.2. Selenium attachment

RO membrane surfaces are generally nonreactive with few reactive functional groups for convenient selenium attachment. Therefore, two different selenium attachment methods were investigated: polymer and monomer.

2.2.1. Polymer attachment (Se-AAEMA)

Selenium polymer attachment utilized aceto acetoxy ethyl methacrylate (AAEMA). The AAEMA was previously modified by covalent attachment of a selenium atom to the acetate end of the AAEMA molecule with an organic linking molecule intermediary, giving a working stock concentration of 22% Se (selenium mass/total mass). The Se-AAEMA solution was diluted from the 22% Se stock to a 1% Se-AAEMA working concentration with unmodified AAEMA. The surface of samples was immersed in the 1% Se-AAEMA solution, sprayed with 3% H_2O_2, and left in a 66 °C oven until dry. During drying, Se-AAEMA molecules formed a polymer by creating cross linkages between Se-AAEMA molecules and the RO membrane surface via Van der Waals forces and hydrogen bonding. RO membranes coated with Se-AAEMA were submerged in dimethyl sulfoxide (DMSO) and quickly rinsed with ultrapure water to remove any unattached Se-AAEMA monomers.

2.2.2. Monomer attachment (selenocystamine)

Selenium monometer attachment consisted of attaching the amine group of selenocystamine to the RO membrane surface after surface activation. Surface activation was achieved by exposing the RO membranes to ethylene oxide gas, generating free hydroxyl groups on the RO membrane surface. The hydroxyl groups were oxidized to carboxyl groups by submersing the RO membranes in 1 g mL^{-1} $KMnO_4$ for 24 h. Crosslinking agents, 3.2 g L^{-1} 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC) and 3.6 g L^{-1} N-hydroxysulfosuccinimide (sulfo-NHS), catalyzed covalent bonds between the attached carboxyl groups and 2.67 g L^{-1} selenocystamine. The RO membranes soaked in the crosslinker-selenocystamine solution for 24 h and followed by nanopure water washings to remove unattached selenocystamine. Later experiments utilized cystamine as a blocker molecule to reduce attached selenium concentrations. Both 1:1 and 1:9 [selenocystamine]:[cystamine] molar ratios were used to achieve a 50% and 90% attached selenium concentration reduction, respectively.

2.3. Biofouling experiments

2.3.1. Bacterial species

For bacterial experiments, *S. aureus* strain AH1333 was provided by Dr. Alexander Horswill of the University of Iowa. Strain AH1333 contains a pE194-based plasmid which carries the green fluorescent protein (GFP) and erythromycin resistance genes. Frozen stock of *S. aureus* AH1333 was kept at −80 °C in Luria Bertani (LB) broth with 20% glycerol. Cultures were grown from the frozen stock to LB broth supplemented with 10 μg L^{-1} erythromycin. The culture was grown overnight at 37 °C under shaking conditions (200 rpm).

LB and tryptic soy broth (TSB) bacteria media were prepared as recommended by the manufacturer (Fisher Scientific). Phosphate buffer solution (PBS) was prepared by dissolving 8.0 g mL^{-1} NaCl, 0.2 g mL^{-1} KCl, 0.24 g mL^{-1} $KHPO_4$, and 1.44 g mL^{-1} Na_2HPO_4 in

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nanopure water. All media and solutions were sterilized by autoclaving before use.

2.3.2. Biofilm development on RO membranes

A 1 mL of an overnight culture of *S. aureus* strain AH1333 was pelleted, resuspended in 1 mL of sterile PBS by vortexing, and diluted with PBS to an optical density (OD) of 0.2 at 600 nm. The diluted culture further diluted by 10^{-3} concentration. The 10^{-3} dilution typically yields approximately 10^4 colony forming unit (CFU) mL^{-1}.

To sterilize the RO membrane surface prior to *S. aureus* AH1333 exposure, both sides of control and selenium coated RO membranes were exposed to ultraviolet (UV) light with a 254 nm wavelength for approximately 30 min. RO membrane disks were aseptically standardized to a 6.0 mm diameter disk using a hole punch providing a total surface area of 56.5 mm^2. Six replicates of control RO membranes and experimental selenium coated RO membranes were tested. Three of the disks were used for cell counting, two disks were used for microscopy, and the remaining disk was utilized for redundancy in case of contamination or handling issues. RO membrane disks were individually placed in separate wells of a microtiter plate. One milliliter of bacterial media consisting of 989 μL 1:25 dilution of TSB, 10 μL of the 10^{-3} *S. aureus* solution, and 1 μL of 10 g L^{-1} glutathione was added to each well. The glutathione was added to encourage superoxide production. Glutathione act as a reducing agent to initiate superoxide production. The 24-well microtiter plate was incubated at 37 °C with gentle shaking for 24 h. The disks were then removed and washed twice with sterile PBS to remove all loosely attached cells.

2.3.3. Quantitative analysis of S. aureus biofilm on RO membranes

The number of viable cells attached to the RO membrane surface were determined using the spot plating procedure. The membranes were first removed from the microtiter plates and gently rinsed with PBS to remove all loosely attached cells.

Each disk was then placed in 1.5 mL microcentrifuge tubes containing 1.0 mL sterile PBS. All tubes were sonicated for 2 min followed by 1 min of vigorous vortexing to detach attached biofilm cells. The 1.0 mL PBS solution containing the detached cells was serially diluted 1:10 in sterile PBS. A 10 μL aliquot of each dilution was spotted ($n = 3$) on LB agar plates and the plates were incubated at 37 °C for 24 h. The number of colonies were counted to determine the CFU per disk.

2.3.4. Microscopy

Two of the remaining control and selenium coated RO membrane disks from the 24-well plate exposure were separately analyzed by confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM). CLSM imaging was performed immediately after the experiments were completed to ensure maximum emission. CLSM images were obtained using an Olympus IX 71 upright microscope at the Texas Tech University Health Science Center at 30× magnification. Images were acquired from the CLSM using the Fluoview (Olympus America). Imaging analysis was conducted as previously described [26].

2.4. RO membrane performance analysis

Permeate flow rate and salt rejection were determined using a small, dead-end flow cell. The flow cell fits an RO membrane coupon 5 cm × 5 cm with an operational surface area of 5.20 cm^2. RO membranes were allowed to soak overnight in nanopure water to reach saturation before testing. Nanopure water and a salt water solution were used in flux and salt rejection experiments, respectively. Feed water tanks containing the appropriate solution were pressurized and maintained at 100 psi using a high pressure nitrogen tank.

2.4.1. RO membrane flux procedure

During nanopure flux experiments, ultrapure water that passed through the RO membrane coupon in the flow cell was collected in a jar on a laboratory balance (Omega) located below the flow cell. Mass readings from the balance were collected every 30 min and used to calculate permeate flow rate. Ultrapure water was run through the RO membrane for an initial compression period until equilibrium flow was reached. Equilibrium flow was reached when the permeate flow rate varied less than 1% for five consecutive 30-min time steps. Equilibrium flow rate was the average of the five consecutive time steps.

2.4.2. RO membrane salt rejection procedure

Salt rejection experiments were designed to provide continuity between initial small scale flow rate experiments and rejection studies rather than maximizing salt rejection. Maintaining the operational pressure at 100 psi allowed for a continuous RO membrane coupon performance analysis despite operating below optimal treatment conditions, resulting in greater salt passage.

After equilibrium flux of nanopure water had been determined, the dead-end flow cell was removed from the ultrapure water feed tank. Any remaining water in the flow cell was removed by pipetting. The flow cell was then attached to a salt water feed tank containing 400 mL of 2000 mg L^{-1} NaCl solution. A 2 cm stir bar was also inserted into the salt water feed tank and spun at 500 rpm to maintain a homogenous concentration throughout the feed tank. Permeate flow was collected on a balance and used to calculate the permeate flow rate. Permeate stream conductivity enable the calculation of salt rejection by the RO membrane. Conductivity of permeate stream was measured using a conductivity probe and meter (Hach).

2.5. Selenium attachment analysis

Selenium concentration on the RO membrane surface and in solution was determined by third party sample analysis (Trace Analysis Inc.) using inductively coupled plasma. These were done in accordance with established analytical methods [27,28].

2.6. Statistical analysis

Quantitative colony counts are reported as mean ± standard deviation. Significance was determined by statistical analysis (Excel, Microsoft) with values of $P \leq 0.05$ considered significant.

3. Results and conclusions

3.1. Inhibition of S. aureus biofilm by selenium coating

The ability of attached selenium to inhibit biofilm formation on RO membranes was investigated by encouraging *S. aureus* biofilm formation on CA and PA RO membranes and evaluating selenium's biofilm inhibition efficacy. The efficacy of selenium was evaluated qualitatively by CLSM images and quantitatively by biofilm CFUs on RO membrane disks. Selenium attachment was achieved using two methods: polymer attachment of Se-AAEMA and monomer attachment of selenocystamine.

3.1.1. Quantitative analysis of selenium inhibition

Colony counting of *S. aureus* cells attached to the RO membranes showed the biofilm inhibition characteristics of the selenium coatings. Coating the RO membranes with selenium resulted

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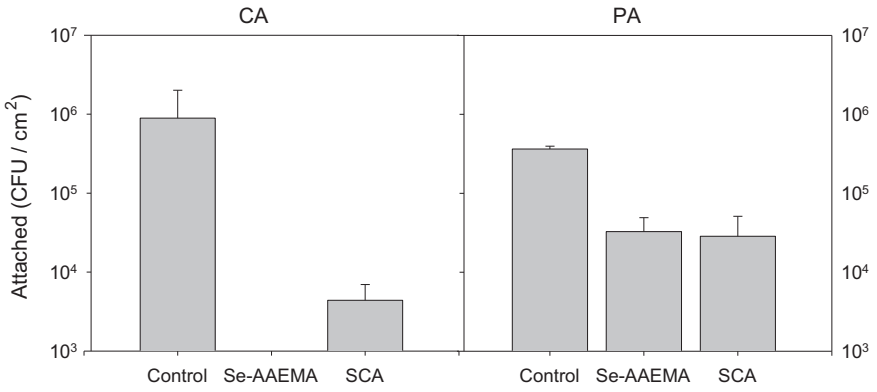

Fig. 1. *S. aureus* colony counts of attached cells on CA and PA RO membranes after 24-h exposure in dilute TSB media ($n = 9$). RO membranes were coated with selenium using either Se-AAEMA or selenocystamine (SCA).

in a significant reduction in the number of *S. aureus* CFU. As shown in Fig. 1, Se-AAEMA coated CA RO membranes completely inhibited *S. aureus* attachment and biofilm development. Selenocystamine coated CA RO membranes displayed a significant reduction ($P < 0.03$) in *S. aureus* attachment and biofilm development. CA RO membranes coated with selenocystamine showed a 2-log reduction in attached *S. aureus* CFU concentration compared to *S. aureus* biofilm on uncoated CA RO membranes.

While Se-AAEMA and selenocystamine coatings displayed different levels of inhibition on CA RO membranes, the inhibition levels by the two selenium coatings on PA RO membranes were comparable. Both selenium coatings on PA RO membranes significantly inhibited *S. aureus* attachment (Fig. 1). Se-AAEMA ($P < 0.001$) and selenocystamine ($P < 0.03$) both showed a 1-log decrease of attached *S. aureus* cells. While the Se-AAEMA coating showed a greater *S. aureus* inhibition on CA RO membranes than on PA RO membranes, the selenocystamine coating inhibition was similar for both CA and PA membranes. This is likely due to differences in surface characteristics between the CA and PA RO membranes affecting the selenium coating performance. Differences in RO membrane surface characteristics of CA and PA RO membranes did not significantly affect *S. aureus* attachment (Fig. 1) as there was no significant difference between *S. aureus* CFU concentration to uncoated PA and CA RO membranes ($P > 0.098$).

3.1.2. Qualitative analysis of selenium inhibition

CLSM images of the CA and PA RO membranes support the quantitative analysis of attachment of *S. aureus* to RO membrane surfaces. As seen in Fig. 2, all selenium coated RO membranes showed a reduced attachment of *S. aureus* (reduced GFP signal) compared with uncoated RO membranes. CA RO membranes coated with Se-AAEMA showed no GFP expressing cells consistent with no attached CFU while CA RO membranes coated with selenocystamine only showed sporadic *S. aureus* cells. PA RO membranes coated with both selenium coatings also showed only intermittent *S. aureus* cell distributions.

Both the CA and PA control RO membranes showed a uniform distribution of cells across the RO membrane surface. While there appears to be a greater density of GFP expression by *S. aureus* cells on the PA RO membrane than on the CA RO membranes (Fig. 2), the similarity of the colony counts suggests this is not an indication of greater population density. The greater expression of GFP in the CLSM images may be due to spatial differences in cell density on the RO membrane disk or differences in GFP expression. Since the genes coding for the GFP are located on a plasmid, it is possible that *S. aureus* cells may have lost the pE194 plasmid during replication as there was no selective antibiotic pressure applied during the experiment. Loss of the pE194 plasmid would cease GFP expression causing CLSM images to underestimate attached growth.

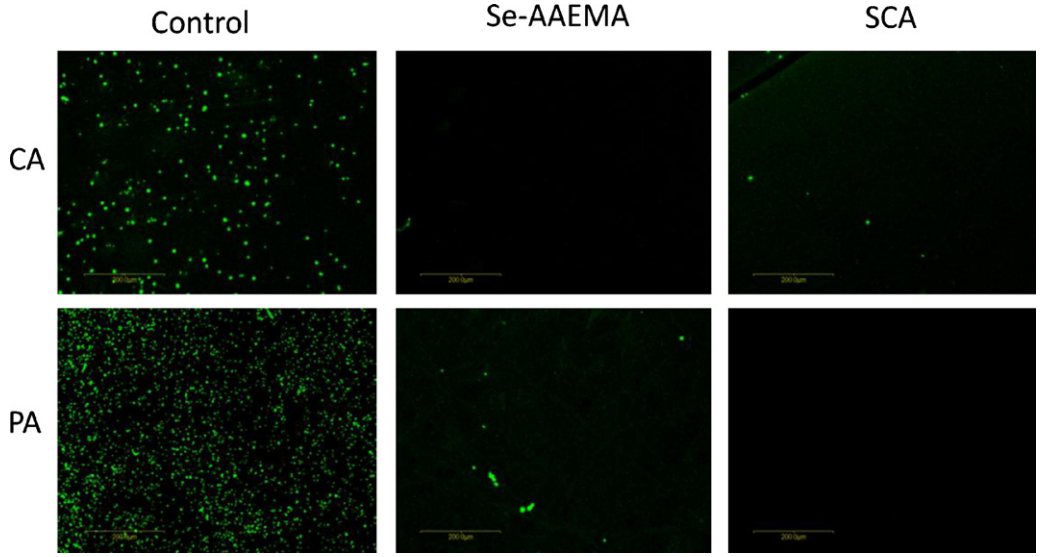

Fig. 2. Confocal laser scanning microscope (30× magnification) of RO membrane surface after 24 h exposure to *S. aureus*. RO membranes were coated with selenium using either Se-AAEMA or selenocystamine (SCA).

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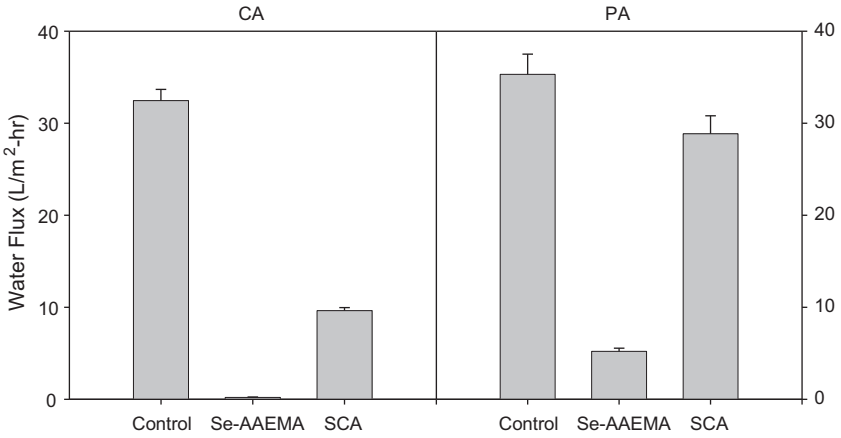

Fig. 3. Permeate flux of ultrapure water for control and selenium coated, Se-AAEMA and selenocystamine (SCA), CA and PA RO membranes ($n = 10$).

3.2. Effects of selenium coatings on RO membrane permeate flux

While both selenium coatings displayed *S. aureus* inhibition, RO membrane flux calculations showed permeate flux loss with both selenium coatings as well. Ultimately, the selenocystamine coating showed substantially better RO membrane permeate fluxes compared to the Se-AAEMA coating.

Se-AAEMA coating of CA RO membrane, which inhibited *S. aureus* attachment completely, decreased the permeate flux by 99% in comparison with uncoated CA RO membranes (Fig. 3). The Se-AAEMA coated PA RO membrane, which did not completely inhibit bacterial attachment, showed an 85% decrease in the permeate flux (Fig. 3). One possible cause for the loss of permeate flux is the Se-AAMEA polymer itself. However, further experiments revealed that the loss of permeate flux is due to the polymer attachment process. Subsequent experiments (data not shown) showed the polymerization step in which the RO membranes were left at 66 °C caused permeate flux loss as uncoated RO membranes also showed a large permeate flux loss. While decreases in the temperature during the polymerization step reduced the flux loss, the Se-AAEMA monomers were not able to polymerize and attach to the RO membrane surface at the reduced temperatures.

The selenocystamine coating showed greater permeate fluxes than the Se-AAEMA coating. Monomer attachment formed a selenium coating on the RO membrane surface without excessive damage to RO membrane performance. Selenocystamine coated CA RO membranes retained approximately 50% of the uncoated permeate flux while the PA membrane retained greater than 80% of the uncoated permeate flux. Differences in permeate flux retention between CA and PA RO membranes is likely the result of the differences in the surface characteristics of the membrane affecting the coating and permeate flux. As with the Se-AAEMA coating, the CA RO membrane coated with selenocystamine achieved greater bacterial inhibition but also displayed a greater permeate flux loss. Due to the ability of selenocystamine coated RO membranes to retain a greater permeate flux compared to Se-AAEMA coated RO membranes, further experiments were conducted to evaluate selenocystamine coating characteristics.

3.3. Attached selenium concentration of the selenocystamine coating

The amount of selenium attached to the RO membrane surface by the selenocystamine attachment method was determined using inductively coupled plasma (Trace Analysis, Lubbock, TX). Greater concentrations of attached selenium were seen on PA RO membranes compared to CA RO membranes. CA and PA RO membranes

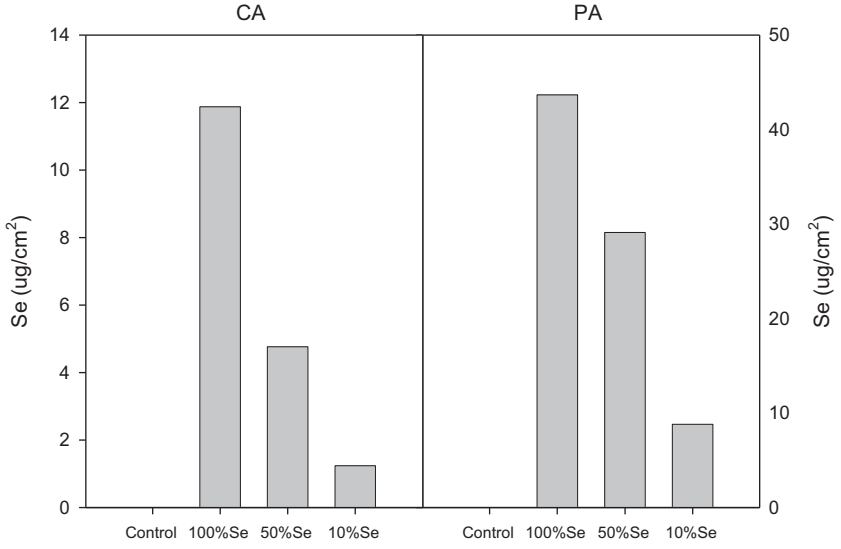

Fig. 4. Concentration of attached selenium on CA and PA RO membranes coated with selenocystamine using unmodified, full strength attachment (100% Se) or a modified attachment utilizing cystamine as a blocking compound at 50% or 10% attached Se concentration.

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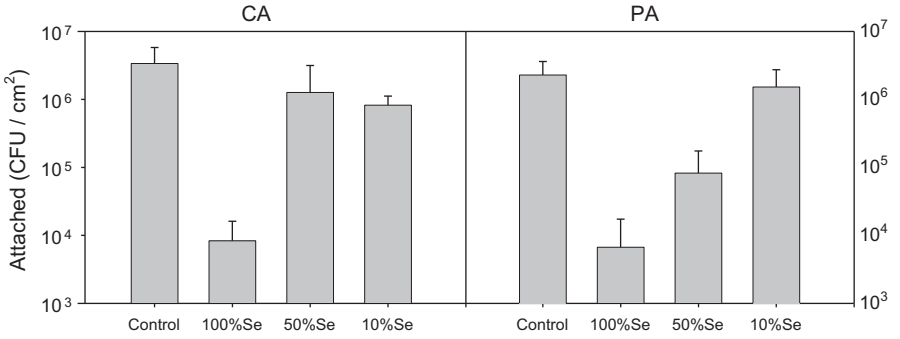

Fig. 5. *S. aureus* CFU counts of attached cells on CA and PA uncoated and selenium coated RO membranes after 24-h exposure in dilute TSB media ($n = 9$). RO membranes were coated with selenocystamine using unmodified, full strength attachment (100% Se) or a modified attachment utilizing cystamine as a blocking compound at 50% or 10% attached Se concentration.

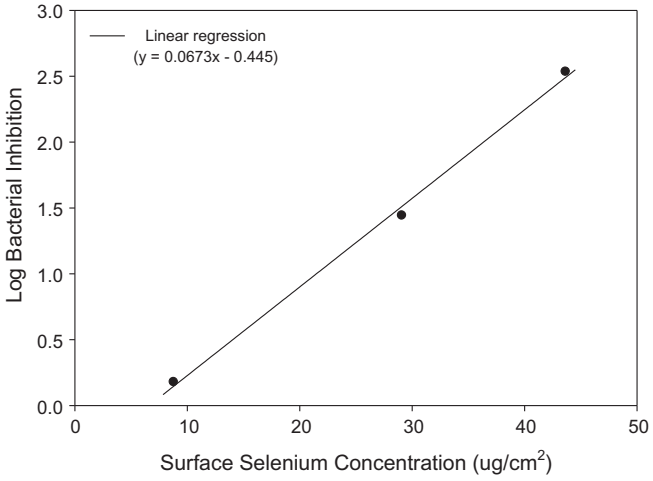

Fig. 6. *S. aureus* inhibition by selenocystamine on PA RO membranes at various attached selenium concentrations.

had an attached selenium concentration of 11.8 and 43.6 $\mu g\,cm^{-2}$, respectively (Fig. 4). As the attachment procedure was the same for both CA and PA RO membranes, differences in attached selenium concentration are likely due to differences in the number of attachment sites on the RO membrane surface. Since the selenocystamine coating process is initiated by ethylene oxide reacting with nucleophiles already present on the RO membrane surface, a greater nucleophile attachment site concentration on the PA RO

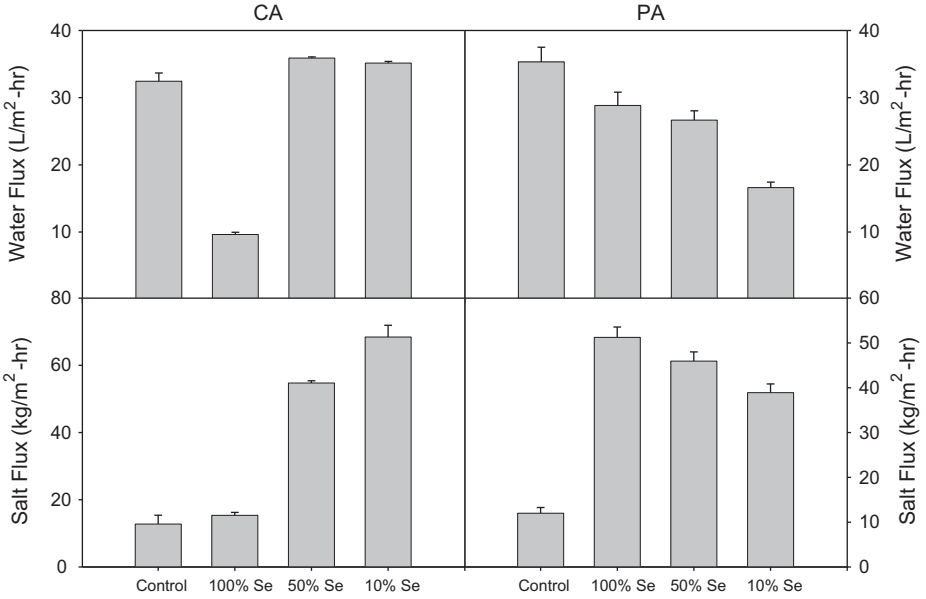

Fig. 7. Permeate water and salt flux for CA and PA uncoated and selenium coated RO membranes coated with selenocystamine using unmodified, full strength attachment (100% Se) or a modified attachment utilizing cystamine as a blocking compound at 1:1 and 1:9 [Se]:[S] molar ratios ($n = 10$).

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membrane surface would result in greater quantities of selenocystamine attached.

3.4. Blocker compound introduction successfully reduced attached selenium concentration

Since the selenocystamine attachment method resulted in high levels of attached selenium that successfully inhibited *S. aureus* biofilm formation with marginal permeate flux loss, further experiments were conducted to determine if decreasing the attached selenium concentration would increase the permeate flux.

Attached selenium concentration was decreased by introducing a blocking compound in place of selenocystamine during the selenocystamine attachment procedure. Cystamine has a similar structure to selenocystamine with an identical amine group for attachment but a noncatalytic sulfur in place of selenium. During the attachment procedure, a portion of the selenocystamine was replaced by cystamine molecules. Cystamine replacement during attachment results in selenocystamine, which contains the catalytic selenium atom, being replaced by cystamine which contains the smaller, noncatalytic sulfur atom. As described in Section 2.2.2, the attached selenium concentration expected to be reduced by 50% and 90% by altering the molar ratio of [selenocystmaine]:[cystamine] to 1:1 and 1:9, respectively. As seen in Fig. 4, attached selenium concentration on the CA and PA RO membranes were successfully decreased by 47% using the 1:1 [selenocystmaine]:[cystamine] replacement technique. Similarly, the 1:9 [selenocystmaine]:[cystamine] replacement ratio produced an 85% decrease in attached selenium concentration. Cystamine is an effective blocking compound capable of directly controling the attached selenium concentration.

3.5. Decreased attached selenium concentration decreases S. aureus inhibition

By changing the attached selenium concentration on the RO membrane, varying levels of inhibition were observed. As the amount of attached selenium decreased, the CFUs of attached *S. aureus* cells increased.

Of the CA RO membranes coated with selenium, the unmodified attachment procedure showed the highest bacteria inhibition (Fig. 5). The unmodified selenocystamine attachment method reduced 91.7% of *S. aureus* CFU concentration compared to uncoated CA RO membranes ($P < 0.002$). The CA RO membranes coated with the 1:1 and 1:9 [selenocystmaine]:[cystamine] replacement procedures resulted in 62.6% and 75% *S. aureus* reduction, respectively ($P < 0.03$ and $P < 0.01$).

Similarly, the attached selenium concentration on the PA RO membranes was directly related to the inhibition of *S. aureus* cell attachment (Fig. 5). The unmodified selenocystamine coating on PA showed a 99.7% inhibition to *S. aureus* attached cells compared to uncoated PA RO membranes ($P < 0.002$). Reductions in the selenocystamine concentration resulted in a 79.5% inhibition for the 1:1 [selenocystmaine]:[cystamine] replacement procedure ($P < 0.004$) and a 33.4% reduction with the 1:9 replacement procedure ($P < 0.04$). As seen in Fig. 6, there is a strong linear relationship ($R^2 = 0.996$) between the concentration of attached selenium on PA RO membranes and the *S. aureus* inhibition.

3.6. Decreased attached selenium concentration showed no permeate flux benefits

Similarly, the amount of attached selenium impacted the permeate flux and the salt flux across the RO membrane. As with the bacterial inhibition on CA RO membranes, the water flux dropped significantly for the full unmodified selenocystamine procedure but were unaffected by the 1:1 and 1:9 procedures (Fig. 7). The PA RO membrane showed different effects of increasing selenium concentration compared to the CA RO membrane. As attached selenium concentration on the PA RO membranes increased, permeate flux decreased. Since higher attached selenium concentrations decreased PA flux but had no effect upon CA RO membranes compared to uncoated CA RO membranes, differences in flux effects of impacts on CA and PA RO membranes may be due to selenocystamine and sulfur interacting differently with the two types of RO membranes.

Ultimately, there was no benefit in decreasing the attached selenium concentration using cystamine as a blocking compound. CA RO membranes lose the majority of *S. aureus* inhibition at decreased selenium concentrations while PA RO membranes lose both *S. aureus* inhibition as well as permeate flux when attached selenium concentration decreases. The unmodified selenocystamine procedure was able to successfully inhibit *S. aureus* biofilm development on both CA and PA RO membranes without detrimentally affecting RO membrane performance.

3.7. Impact of glutathione addition

To assess the necessity of glutathione addition to jumpstart superoxide production, a side experiment was conducted [29]. *E. coli* was grown overnight at room temperature. The experimental design included *E. coli* grown with the selenium treatment with and without glutathione and *E. coli* grown without the selenium treatment with and without glutathione. Only the selenium treatment, with or without the glutathione, effectively inhibited bacterial growth. After 120 min, the survival percentage was less than 5% whereas the survival percentage for the samples without the selenium was approximately 85%. No statistically significant difference ($P < 0.05$) was observed for the selenium treatments with and without glutathione, indicating glutathione is necessary for effective kill.

4. Concluding remarks

As RO treatment becomes more widely used in wastewater and seawater recovery systems, biofouling of the RO membrane surface becomes increasingly problematic. Selenium was investigated for its ability to inhibit *S. aureus* biofilm formation and potential to decrease operational problems associated with biofouling of RO membranes. Of the two attachment mechanisms, selenocystamine showed greater potential for large scale application. While selenium attachment utilizing the polymer Se-AAEMA showed high *S. aureus* inhibition, Se-AAEMA attachment procedures detrimentally affected RO membrane flux due to high heat needed for polymerization. However, by attaching the monomer selenocystamine to the RO membrane surface, bacterial inhibition was successfully achieved while maintaining a large portion of the RO membrane permeate flux. Glutathione was added to contribute to superoxide production but subsequent experiments have shown this is not necessary to maintain inhibition. The ability of selenium to be easily attached to an RO membrane surface and inhibit attached growth on the RO membrane surface gives selenium great potential as a biofouling control agent. Selenium has numerous advantageous characteristics that make it appealing as a passive biofouling control technology, particularly the catalytic mechanism that requires no additional energy or chemical requirements to inhibit biofilm formation. By passively inhibiting bacterial growth, selenium may be utilized to decrease the destructive effects of biofouling that frequently plague RO treatment systems. The ability to significantly decrease biofouling effects could decrease energy costs, increase performance and lengthen RO membrane useful life. Future exper-

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iments will investigate the efficacy of selenium to prevent biofilm formation on RO membranes by a variety of bacterial species and growth conditions as well as the impact on RO system operations.

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