Safer Alternatives to Prevent Biofouling in Reverse Osmosis Polyamide Membrane Systems

By

Luiz Henrique Da Silva Correa
BSc, Federal Fluminense University & University of California, Davis, 2017
MSc, University of Chemistry and Technology, Prague; IHE Delft Institute for Water Education; & Ghent University, 2020

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We acknowledge and respect the Lək̓ʷəŋən (Songhees and Esquimalt) Peoples on whose territory the university stands, and the Lək̓ʷəŋən and W̱SÁNEĆ Peoples whose historical relationships with the land continue to this day.

Supervisory Committee

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Supervisory Committee

Dr. Heather L. Buckley, Supervisor
Department of Civil Engineering and Department of Chemistry

Dr. Caetano Chang Dorea, Departmental Member
Department of Civil Engineering

Dr. Caren Helbing, Outside Member
Department of Biochemistry and Microbiology

Abstract

Biofouling is the main technical barrier to the widespread application of reverse osmosis (RO) technology in addressing worldwide water scarcity. Biofouling reduces permeate production, escalates energy demands, and exacerbates the environmental impacts associated with RO technology. To overcome these challenges, this PhD project proposed a platform to select and test safe and green anti-biofouling agents to prevent biofouling in drinking water RO system applications. The platform consisted of a screening protocol followed by a validation protocol. The proposed platform was applied to assess the applicability of nine chemicals (MIT: 2-methyl-4-isothiazolin-3-one; DBNPA: 2,2-dibromo-3-nitrilopropionamide; SBS: sodium bisulfite; SB: sodium benzoate, PE: phenoxyethanol; LAE: ethyl lauroyl arginate, PHMGH: Polyhexamethylene guanidine hydrochloride; BDMDAC: Benzyldimethyldodecyl ammonium chloride; SNP: Sodium nitroprusside) for preventing membrane biofouling in RO potable water applications. The screening protocol involved three phases: a comprehensive review, antibiofouling testing, and polyamide membrane compatibility testing. The comprehensive review investigated the applicability of the selected biocides in preventing and controlling biofouling in RO systems. It evaluated their antimicrobial efficiency, hazard levels, membrane compatibility, and suitability for drinking water treatment. Antibiofouling testing involved biofouling experiments on a Center for Disease Control (CDC; the United States) biofilm reactor with the minimum concentrations determined in microtiter plates. Confocal Scanning Laser Microscopy (CLSM) and Scanning Electron Microscopy (SEM) were used to analyze the biocides' anti-biofilm efficacies under dynamic conditions relative to the minimum biofilm inhibitory and eradication concentrations. Polyamide membrane compatibility testing assessed membrane compatibility via rapid membrane degradation tests. Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR), Atomic Force Microscopy (AFM), and SEM were used to assess the polyamide membrane damage due to exposure to biocides.

The screening protocol revealed that DBNPA and MIT pose hazards to both human health and the environment, making them unsuitable as a full-scale solution to prevent biofouling in RO potable water applications. SBS was also considered to be unsuitable due to its low antibiofilm efficacy and pH dependency. PE and SB were deemed unsuitable due to their moderate antibiofilm efficacy and incompatibility with polyamide membranes. BDMDAC was considered unsuitable for biofouling control in RO potable water applications due to its inefficacy against biofilms and common biofilm-forming microorganisms in RO systems. Further research beyond this PhD is required to assess the suitability of PHMGH and SNP for use in RO potable water applications, given their early stage of development and limited available information in the literature. Among the examined chemicals, LAE was the only biocide to successfully pass all phases of the proposed screening protocol, emerging as a promising safe chemical alternative to prevent biofouling in RO systems. LAE showed the highest efficacy against *P. aeruginosa* biofilms among the tested biocides, successfully preventing

biofilm formation by over 98% and removing existing biofilms by more than 99% from RO membranes. Additionally, rapid membrane degradation tests indicated that LAE did not cause morphological or chemical damage to the membranes. Consequently, LAE was the only biocide recommended to advance to further experiments in a RO benchtop system outlined in the validation protocol.

Therefore, considering the current need for greener alternatives to prevent biofouling in RO polyamide membrane systems in potable water applications, this PhD project has the potential to contribute to the use of RO technology for the provision of reliable, secure, and safe water supply to municipalities, industries, marginalized groups, remote work sites, and Indigenous communities. This was accomplished by establishing a platform to screen anti-biofouling agents for preventing biofouling in RO drinking water applications, alongside the identification of a promising biocide (LAE) to address membrane biofouling in RO drinking water applications.

Breaking down the PhD dissertation barrier: Making research accessible for all

This part of my PhD dissertation is dedicated to my family, friends, and fellow desalination enthusiasts. In the following pages, I will break down my entire PhD research into simpler terms, so you do not have to read through a whole PhD dissertation in civil engineering. And if you are more of a visual learner, there is a handy figure at the end of this section that sums up my PhD project and next phases.

Currently, many communities around the world are dealing with several challenges due to the lack of access to clean potable water. The application of desalination membrane technologies offers a solution to address the insufficiency in potable water supply. This is because this filtration technology has capability to produce freshwater from diverse water sources including wastewater, brackish water, and seawater. Now, you might think, with all this water covering 70% of our planet, why can't we just use desalination technologies to solve the clean water shortage worldwide? Well, desalination technology is very expensive, mainly because of this thing called biofouling. Biofouling happens when microorganisms embedded in a jelly-like material (biofilm) clog up the membranes used in the filtering process. It reduces water production, damages the membranes, and increases the energy needed to produce freshwater. To control biofouling, desalination plant operators usually use a ton of harsh chemicals, which only adds to the cost of the application of the technology. Plus, dealing with biofouling makes waste disposal trickier, further increasing the overall operating costs of desalination plants. Finding safer alternatives to prevent biofouling in desalination membrane systems is not an easy task. It is not just a complex issue; it is something that was never done effectively before. So, in my PhD research, I have come up with a platform to find greener and safer chemical solutions to prevent biofouling in desalination membrane systems.

The proposed protocol was divided into two smaller protocols: a screening protocol followed by a validation protocol. I used the proposed protocol to analyze nine different chemicals to see if they could be used to prevent biofouling in desalination membrane systems. The screening protocol had three steps: a literature review study, antibiofouling testing, and membrane compatibility testing. In our literature review, we examined various research papers, books, reports, and other sources to determine whether the selected chemicals are feasible to be used in desalination membrane technology for producing drinking water. In the antibiofouling testing, we tested if the chemicals prevent the growth of microorganisms on the surface of the membrane. In the membrane compatibility test, we tested if the chemicals damaged the membranes. Out of all the chemicals we studied, LAE (LAE stands for ethyl lauroyl arginate and it is a food preservative used in baking powders!) was the only biocide to successfully pass all phases of the proposed screening protocol,

making it a promising option to prevent biofouling in desalination membrane systems. This is because LAE has shown excellent antimicrobial properties, does not cause damage to membranes, and is considered low in hazard. But hold your horses, we're not done yet. Before we start using LAE on a big scale, we've got more tests to run (validation protocol). That's going to be the focus of my postdoctoral research. In conclusion, my PhD project marks the initial step towards an innovative green solution for water scarcity by ultimately optimizing desalination membrane technologies for widespread adoption in freshwater production.

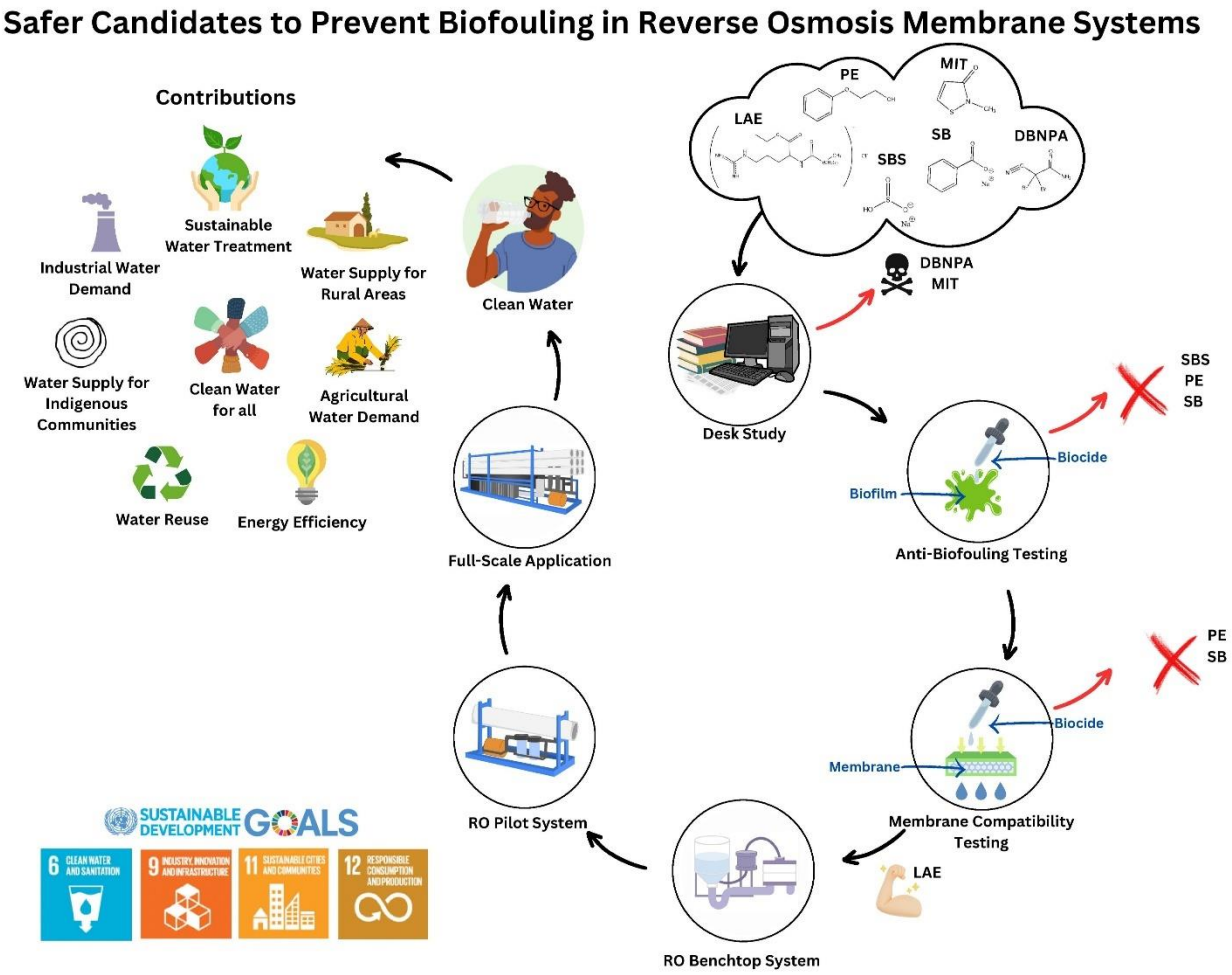

Figure – PhD research project overview

Quebrando a barreira da tese de doutorado: Tornando a pesquisa acadêmica acessível para todos (Portuguese Version)

Esta parte da minha tese de doutorado é dedicada à minha família, amigos e pessoas interessadas em tecnologias de dessalinização. Nas proxima páginas, vou explicar toda a minha pesquisa de doutorado em termos mais simples, para que você não precise ler toda uma tese de doutorado em engenharia civil. E, se você é uma pessoa que prefere figuras a textos longos, há uma figura útil no final desta seção que resume meu projeto de pesquisa e suas próximas fases.

Atualmente, ao redor do mundo, muitas comunidades estão lidando com desafios devido à falta de acesso à água potável, de modo que, a aplicação de tecnologias de membrana de dessalinização oferece uma solução para mitigar a insuficiência no fornecimento de água potável. Isso ocorre porque essa tecnologia de filtragem tem a capacidade de produzir água potável a partir de diversas fontes, incluindo águas residuais, água salobra e água do mar. Agora você provalmente deve estar pensando: com toda essa água que cobre 70% do nosso planeta, por que não podemos simplesmente usar tecnologias de dessalinização para resolver a escassez de água limpa em todo o mundo? Bem, a tecnologia de dessalinização é muito cara, principalmente por causa da bioincrustação, que é um processo que ocorre quando microorganismos envolvidos em um material gelatinoso (biofilme) entope as membranas usadas no processo de filtragem. Isso reduz a produção de água, danifica as membranas e aumenta a energia necessária para produzir água doce. Para controlar a bioincrustação, os operadores dos sistemas de dessalinização geralmente usam uma grande quantidade de produtos químicos agressivos, que aumenta ainda mais o custo da aplicação da tecnologia. Além disso, lidar com a bioincrustação torna o descarte de resíduos mais complicado, aumentando ainda mais os custos operacionais gerais das unidades de tratamento de água que usam dessalinização. Encontrar alternativas mais seguras para prevenir este processo em sistemas de dessalinização não é apenas uma questão complexa, é algo que nunca foi feito efetivamente antes. Então, na minha pesquisa de doutorado, eu desenvolvi um protocolo para encontrar soluções químicas mais verdes e seguras para prevenir a bioincrustação em sistemas de tratamento de água que utilizam membranas de dessalinização.

O protocolo geral foi dividido em dois protocolos menores: um de triagem seguido por um de validação. Eu usei o protocolo proposto para analisar nove produtos químicos diferentes para ver se eles poderiam ser usados para prevenir a bioincrustação em sistemas de membranas de dessalinização. O protocolo de triagem teve três etapas: um estudo de revisão da literatura, testes antibioincrustantes e testes de compatibilidade de membrana. Na revisão da literatura, eu e outros pesquisadores examinamos vários artigos de pesquisa, livros, relatórios e outras fontes para determinar se os produtos químicos selecionados são viáveis para serem usados na tecnologia de membranas de dessalinização para produção de água potável. Nos testes antibioincrustantes, testamos se os produtos químicos impedem o crescimento de microorganismos na superfície da membrana. No teste de

compatibilidade de membrana, testamos se os produtos químicos danificavam as membranas. De todos os produtos químicos que estudamos, o LAE (LAE significa arginato de lauroil etilo e é um conservante de alimentos usado em fermentos em pó!) foi o único biocida a passar com sucesso por todas as fases do protocolo de triagem proposto, tornando-o uma opção promissora para prevenir a bioincrustação em sistemas de membranas de dessalinização. Isso ocorre porque além de o LAE ter mostrado excelentes propriedades antimicrobianas, ele não causa danos às membranas e é considerado de baixo risco. Mas espere um pouco, ainda não terminamos. Antes de começarmos a usar o LAE em grande escala, temos mais testes para fazer (protocolo de validação). Esse será o foco da minha pesquisa de pós-doutorado. Em conclusão, meu projeto de doutorado marca o primeiro passo em direção a uma solução verde e inovadora para a escassez de água, otimizando as tecnologias de membranas de dessalinização para proporcionar a sua adoção generalizada na produção de água doce.

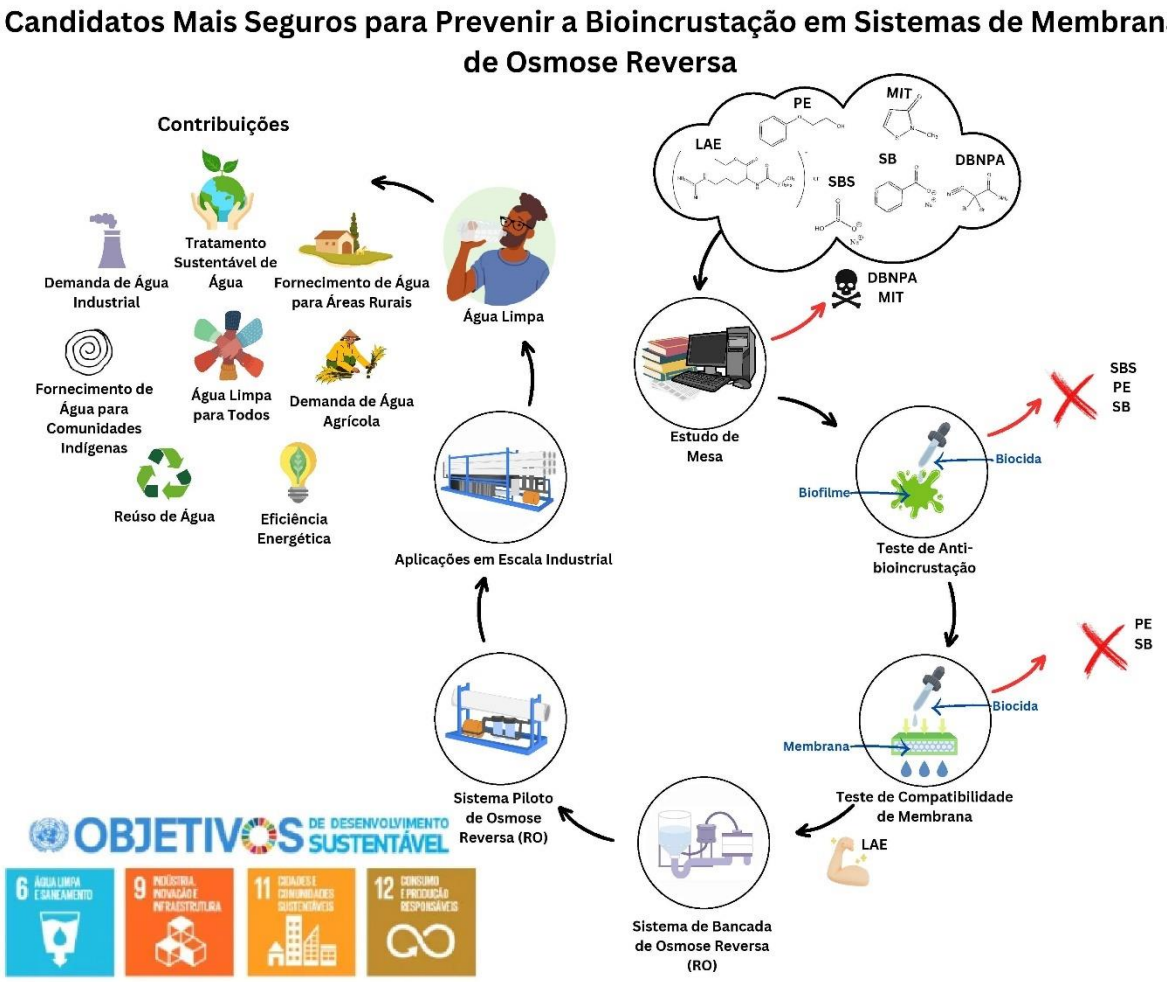

Figura – Visão geral do meu projeto de pesquisa de doutorado

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Acronyms and abbreviations

AFM	Atomic force microscopy
ANOVA	Analysis of variance
ASTM	American society for testing and materials
ATP	Adenosine triphosphate
ATR-FTIR	Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy
BDMDAC	Benzyldimethyldodecyl ammonium chloride
CDC	Center for Disease Control
CFU	Colony forming unit
CLSI	Clinical and laboratory standards institute
CLSM	Confocal scanning laser microscopy
CMIT	5-Chloro-2-methyl-4-isothiazolin-3-one
DBAM	Dibromoacetamide
DBAN	Dibromoacetonitrile
DBNPA	2,2-Dibromo-3-nitrilopropionamide
DI	Deionized
ECHA	European chemicals agency
EPS	Extracellular polymeric substance
GRAS	Generally recognized as safe
LAE	Ethyl lauroyl arginate
MBC	Minimum bactericidal concentration
MBEC	Minimum biofilm eradication concentration
MBIC	Minimum biofilm inhibitory concentration
MBNPA	Monobromonitrilopropionamide
MIC	Minimum inhibitory concentration
MIT	2-Methyl-4-isothiazolin-3-one
MOA	Mechanism of Action
NF	Nanofiltration
NO	Nitric oxide
OD	Optical Density

PE	Phenoxyethanol
PHMGH	Polyhexamethylene guanidine hydrochloride
PI	Propidium iodide
QAC	Quaternary ammonium compound
RO	Reverse osmosis
ROM	Reverse osmosis membrane
SB	Sodium benzoate
SBS	Sodium bisulfite
SDG	Sustainable development goal
SEM	Scanning electron microscopy
SMBS	Sodium metabisulfite
SNP	Sodium nitroprusside
TMP	Trans-membrane pressure
TOC	Total organic carbon
TSA	Tryptic soy agar
TSB	Tryptic soy broth
UN	United Nations
USEPA	United States environmental protection agency
USFDA	United States food and drug administration
UV	Ultraviolet radiation
WHO	World health organization

Symbols

Sa	Surface roughness
RMS	Root mean square roughness

Acknowledgements

First and foremost, I express my gratitude to God, to whom I attribute my very existence, for His unwavering faithfulness and love from the moment of my birth until now. I extend my gratitude to my mother, Elizabete, and father, Alexandre, as well as all my family members and friends, for their unconditional love and support throughout my PhD journey. A special mention to my soul twin sister Mayara for her insights, revisions, suggestions, and support throughout my PhD journey.

I would like to express my sincere admiration and gratitude to Dr. Heather Buckley. After completing my master's degree, I embarked on the search for PhD positions in the desalination field. My criteria were clear – the project had to be related to desalination, and the supervisor had to be someone I felt would be an excellent mentor. Following my interview with Dr. Heather Buckley for the Green Safe Water Lab, I knew she would be an outstanding supervisor and I was right. Her insights, mentorship, guidance, revisions, discussions, suggestions, teachings, and counseling played a crucial role in the success of my PhD project. Heather was not just a supervisor; she was an excellent mentor who provided me with the tools that were necessary to champion my PhD project. Also, Heather's assistance in finding a balance between life and work greatly benefited my mental health. I am confident that I could write a PhD dissertation to demonstrate that Heather is, without a doubt, the best PhD supervisor ever!

I extend my gratitude to my committee members, Dr. Caetano Dorea and Dr. Caren Helbing, for their guidance and insights that were crucial to elevating my PhD research. Dr. Caetano Dorea's input in the engineering aspects of my project optimized experimental procedures, and our discussions helped me to be more intentional and strategic with my designs, a skill I will carry with me forever. Caetano, você é demais! Thank you, Dr. Caren Helbing, for your insights, discussions, and recommendations. Your guidance in the microbiology aspects of my research undoubtedly elevated my PhD project. Thank you for helping an engineer to properly grow and analyze biofilms! Also, I would like to thank Dr. Rachel Scholes for contributing her expertise as the external committee member of my PhD project with valuable insights into concentrate treatment, adding another dimension to my project.

I would like to thank my friends at the Green Safe Water Lab – Christopher, Georgia, Grace, Hayley, Kirsten, Nathan, Negar, and Spencer. It was a pleasure to share my PhD journey with all of you. I appreciate your continuous support, peer reviews, insights, laughter, and coffee breaks. To my co-op students – Bethany Welsh, Danyka Thorburn, Matthew Thibodeau, Nicole Gamm, Orielle Henriquez, and Rafaela Godoy – thank you for your hard work and contributions to the project. It was an absolute pleasure being part of your journey as young researchers and serving as your mentor. This experience has enriched my PhD journey beyond the technical aspects. I also would like to extend my gratitude to Dr. Jolie Lam and all the alumni from the Green Safe Water Lab who contributed to setting up the group and co-creating the culture we have today. A special mention to Dr. Fatima Shatila, an Alumni

postdoctoral fellow in the Green Safe Water Lab, for her insights, guidance, and support throughout my PhD journey, including preparing for my candidacy exam and providing valuable input into the microbiology aspects of my research.

Furthermore, my gratitude extends to the project's industrial partners at BI Pure Water (Canada) Inc, including Mr. Jonathan Boughen and Mr. George Thorpe, for their support and insights, making our research more applicable in an industrial context. I also thank my mentors at MSR Solutions, Mr. Mike Seymour and Mr. Saman Khoddam, for strategically assigning me several desalination projects during my co-op, providing me invaluable experience for my PhD project. Special thanks to Dr. Hamed Beheshti and Mr. Ali Al-Hakim for allowing me to contribute to concentrate disposal and solar desalination projects, expanding the understanding of my PhD work.

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Dedication

I dedicate my doctoral dissertation to my beautiful mother, Elizabete Da Silva Correa.

Te amo, infinito

Chapter 1: Introduction

1.1 Background

The current global water scarcity crisis results from the inability to meet standard water demand due to an insufficiency of available freshwater[1–4]. This on-going crisis is further exacerbated by several factors such as water contamination from human activities, industrialization, climate-change-induced droughts, and a persistent rise in the global population[1,4]. According to the United Nations, 2023, insufficiency of available freshwater emerges as a global concern, with approximately one-third of the global population residing in water-stressed countries, and one-seventh lacking access to clean water[5]. Furthermore, a prediction model developed by Kuzma et al. (2023) anticipates that nearly two-thirds of the global population will experience water stress by 2050[6]. The application of desalination technologies offers a solution to address the insufficiency in potable water supply[2,4,7]. Desalination systems such as reverse osmosis (RO) membrane systems have the capability to produce freshwater from diverse sources including wastewater, brackish water, and seawater[2,7–9]. Among various desalination systems, RO polyamide membrane systems stand out as the most common technology utilized in RO water treatment applications[7,9,10]. This is because polyamide membrane systems are effective for broader pH ranges, operate at lower pressures, and exhibit higher rejection rates when compared to other desalination systems, such as cellulose acetate membrane systems[9,10]. RO polyamide membrane systems, developed in the early 1980s, play a significant role in freshwater production worldwide[2,4,7,9]. As of 2020, RO plants, predominantly using polyamide membrane systems, have achieved a daily freshwater production capacity of approximately 67 million m^3, capable of meeting the water needs of 700 million to 1.3 billion people, based on the World Health Organization's recommended standard of 50-100 liters per capita per day for the full right to water[11]. This evidence displays the great potential of polyamide membrane systems as a possible solution to address water scarcity globally.

The versatility of RO polyamide membrane technology allows its application to meet the potable water demands not only at larger scales like cities and industries but also at smaller scales such as buildings and villages[4,8,9,12–16]. For instance, previous studies showed that decentralized RO polyamide membrane systems are a robust choice for supplying clean affordable water to remote and Indigenous communities located far from water treatment plants[8]. According to the Canadian Safe Drinking Water Foundation, 2007, RO polyamide membrane systems have been successfully applied in several water-stressed areas in Canada to address local long-term potable water demands[16]. Further, decentralized RO polyamide membrane treatment units can be installed close to water users, which is critical when extreme weather events damage centralized infrastructure or pipelines[8,9,16]. RO polyamide membrane technology proves valuable not only for drinking water supply, but also for

industrial applications since it can be applied to treat wastewater, reduce pollutant fluxes to water bodies, minimize the demand for potable water through water reuse, and recover nutrients from wastewater[2,4,7–9,16–19].

1.2 The "Achilles' heel" of RO membrane technology: Biofouling

Although RO polyamide membrane technology has great potential to mitigate the challenges faced in the ongoing worldwide water crisis, biofouling poses a major technical obstacle to the application of RO technologies to provide safe, clean water[4,7,10,20–23]. This is because biofouling not only increases operational and maintenance costs of RO membrane systems but also contributes to adverse environmental impacts associated with the technology, as well as increase its energy demand[4,7,9,17–20]. Furthermore, biofouling becomes more challenging depending on the treatment context. For example, biofouling in wastewater, brackish water, and seawater RO applications is more challenging than in surface water and well water RO applications due to the high quantity of nutrients and microorganisms present in the feedwater of these applications. In a study on the main contributing factors leading to RO membrane system failure via the analysis of 150 worldwide membrane autopsies conducted by Fazel & Darton in 2001, it was concluded that biofouling is the primary cause of RO system failure, accounting for 66% of failures in RO membrane systems either as a primary or contributing factor[24]. Additionally, the study by Fazel and Darton, 2001, indicated that inorganic fouling (scaling) accounts for approximately 30% of failures in RO systems, presenting a significant limiting factor to RO technologies in providing clean water[24]. Its effects on RO systems include lower salt rejection, increased operational pressure, and reduced permeate production[9]. Scaling is a concentration phenomenon typically found in the final stages of an RO system, unlike biofouling, which occurs throughout the entire system[7,9,10]. Research and strategies to address inorganic fouling are more advanced than those for biofouling, and several effective methods are currently applied in the industry, such as the use of antiscalants, feedwater treatment, and membrane flushing[4,9,25]. However, biofouling still remains the primary technical barrier for RO technologies in providing drinking water, not only due to its detrimental effects on RO performance but also because of the lack of safe and efficient methods to address it and the limited research in this area[7,9]. Therefore, safe and green alternatives to address biofouling are needed to promote sustainable water supply via RO polyamide membrane technologies.

Before discussing the current strategies employed in the RO industry to address biofouling and investigating different means to improve them, it is crucial to understand the challenging and significant nature of membrane biofouling. Membrane biofouling consists of the accumulation of microorganisms onto a membrane surface in the form of biofilms[9,10,20]. Biofilms are microbial communities that are embedded in an extracellular polymeric substance (EPS) matrix configured in a 3D structure that is not easily removed by gentle rinsing[9,26–30]. Biofilms are primarily composed of

EPS which contributes to 90% of the biofilm matrix and microorganisms as the remaining 10%[10,28,29,31,32]. EPS is mainly composed of polysaccharides along with other components such as extracellular DNA, RNA, proteins, and lipids[9,10,28,29].

Biofilm formation is a complex process that is genetically regulated and controlled by multifactorial interactions between microorganisms with the attachment surface and environmental conditions[28–30]. Biofilm formation occurs in four distinct stages. First, pioneer microorganisms attach to a conditioning layer. Second, with continuous growth along with the production of EPS, microorganisms form microcolonies that are irreversibly attached (not removed by gentle rinsing) to a surface[27–30]. Third, further growth and diversification of microcolonies lead to macrocolonies that will result in the establishment of a mature biofilm[26–30]. The dispersion or detachment of biofilms may occur due to the disruption of the optimum environmental conditions for biofilms[26,28–30]. Biofilm composition and formation are dependent on several factors such as environmental conditions (pH, temperature, nutrient availability, osmotic pressure), surface properties (roughness, hydrophobicity, surface charge), and characteristics attributed to microbial communities (quorum sensing, genetic factors, cell membrane composition, EPS production)[4,9,10,22,28,29].

Once a biofilm has matured on the membrane surface, shear force is no longer able to remove it and the fouling is deemed irreversible, resulting in a substantial increase in the operational and maintenance costs of RO membrane systems[9,10,20,23]. Studying the impact of biofilms on RO polyamide membranes, Bereschenko et al. 2010, observed that RO polyamide membranes with a one-month old biofilm presented the same performance as 5-year-old polyamide membranes under the same operating conditions but in the absence of biofouling[33]. This observation reveals that biofouling significantly reduces the lifespan of RO membranes, consequently increasing maintenance costs due to the necessity of more frequent membrane replacements[7,9,10,20,34]. Further, mature biofilms also impact operational costs of RO systems, as a higher quantity of chemicals is required to remove biofilms from RO membranes[9,20,28,29]. The high chemical demand required to remove biofilms is attributed to the increased biocidal resistance exhibited by biofilm cells, coupled with the presence of EPS as it enhances the mechanical stability of biofilms, acts as a barrier protecting microorganisms from biocides, and creates an environment favorable for the increase of “persister” cells within the biofilm microbial communities[9,10,20,28,29].

The rise in chemical usage due to biofouling management not only escalates the costs associated with RO technology but also exacerbates one of its principal adverse environmental impacts: concentrate disposal[4,7,9,17–20,22]. The disposal of RO concentrate poses a significant environmental challenge for RO technologies, as it may result in detrimental effects on aquatic life if released into surface waters or coastal ecosystems[17–19]. The extensive use of chemicals to address biofouling directly impacts the

quality of the brine since it leads to higher concentrations of contaminants, disinfection by-products, and the presence of bacteria in the concentrate[10,17–19,28,29]. The increased concentration of hazardous chemicals in the RO concentrate makes its disposal options and treatment processes more challenging, further increasing the environmental concerns associated with RO technology[7,9,17–20].

Despite numerous efforts to reduce the energy demand of RO polyamide membrane systems, such as incorporating energy recovery devices and utilizing renewable energy sources for desalination, biofouling continues to pose challenges in achieving energy efficiency in RO polyamide membrane systems[2,7,9,20,21,23]. This is because the presence of biofilm reduces the diffusion of salt ions through the membrane, which consequently leads to an increase in the operational pressure during the membrane separation process[4,9,10,20,22,28,29]. Considering that membrane separation typically accounts for approximately 70% of the total energy demand of RO polyamide membrane systems, the increase in osmotic pressure due to biofouling is of great concern[7,9,20,35]. Biofouling not only increases energy consumption in the membrane separation process but also contributes to an overall increase in the total energy consumption of an RO plant[7,9,20,36]. When biofouling occurs, plant operators often raise the operational pressure of the RO system to maintain a constant water production rate, resulting in higher energy consumption[4,7,9,20,22]. Further, as previously mentioned, biofouling management contributes to an increase in the volume of chemicals in the concentrate. This results in an increase in the energy requirements for concentrate treatment and further increases the energy demand of RO plants[4,9,10,20,22,36]. Therefore, considering the detrimental effects of biofouling on RO systems, the development and implementation of safe and green strategies that address biofouling becomes crucial in order to promote use of RO technology for drinking water supply.

1.3 Safer alternatives to prevent biofouling in RO polyamide membrane systems

Currently, biofouling is addressed imperfectly in RO polyamide membrane systems via biofouling control programs[4,9,10,22,27]. Biofouling control programs in potable water applications mainly consist of feed water pre-chlorination followed by membrane cleaning. The disadvantages of this approach are numerous. Incompatibility of chlorine with polyamide membranes due to oxidative membrane degradation necessitates chlorine removal from the RO system with sodium bisulfite (dechlorination) after feed water pre-treatment[9,10]. Chlorine reacts with organics in the water producing carcinogenic disinfection by-products such as trihalomethanes and haloacetic acids[9,10], and also break down organic matter in a manner that can increase bioavailability and amplify biofouling[9,22]. Additionally, the chlorination of seawater, which often contains high concentrations of bromide, yields hypobromous acid which can also cause damage to RO polyamide membranes[9,10]. Although chlorine can efficiently kill a wide range of microorganisms in the RO feed water (99.9%) with low inactivation times (20-30 minutes), the small concentration of microorganisms remaining in the RO membrane vessel after dechlorination (~1000 CFU/mL) can still cause severe biofouling[9,9].

Combined chlorine (chloramines) was previously considered an alternative to chlorine for feed water pretreatment in RO systems. However, due to its membrane incompatibility, reduction of RO system performance, and slower disinfection kinetics compared to chlorine (requiring up to 100 times the contact time or 25 times the concentration of free chlorine for effective feed water pretreatment), its use is not recommended for potable water or seawater RO applications[9,10]. Instead, it is only applied in wastewater reuse applications to reduce the formation of disinfection by-products, which is necessary due to the high concentration of organics typically found in these settings[9,10]. In addition, to feed water pre-treatment by chlorine dosing, the control and removal of biofilms from RO membrane potable water applications are partially achieved via off-line shock treatments with non-oxidizing biocides such as 2,2-dibromo-3-nitrilopropionamide (DBNPA) and isothiazolones[9,10,22,27,34,37]. Due to hazard concerns to both humans and the environment, all current non-oxidizing biocides used in the RO industry for membrane cleaning cannot be applied in inline potable water applications[9,10,27,34,37].

From a sustainability lens, the current method of handling biofouling in industries and water treatment facilities is not eco-friendly or efficient since it utilizes hazardous chemicals to control biofouling instead of preventing it[9,10,27]. Further, the current techniques applied to address biofouling of RO membrane systems only offer a temporary solution and do not address the origin of the problem[9,27]. This is because biofilm removal does not reverse any damage caused by biofouling, nor inhibit biofouling from recurring in the RO system[4,9,10,22,27]. Thus, the implementation of biofouling prevention strategies using safe, effective, and green anti-biofouling chemicals should be applied to address the present challenges in membrane systems. This approach can effectively alleviate the existing challenges in membrane systems, offering a safer and greener alternative for water treatment via RO polyamide membrane systems[27]. The application of preventative techniques can decrease strain on RO systems caused by biofouling and prevent biofilms from maturing and becoming irreversibly attached to RO membranes[9,27,38]. Additionally, biofouling prevention can reduce the energy demand associated with RO technologies while minimizing its environmental impacts[7,17,36]. In terms of operational costs, preventing biofouling is less expensive than removing existing biofilms from RO membranes[9,10,27]. Therefore, safe, eco-friendly, and economic chemical alternatives to prevent biofouling are needed to promote a sustainable water supply via RO technologies[9,10,27].

Finding safer alternative chemicals to prevent biofouling in RO polyamide membrane systems involves the search for an ideal biocide. Bates (1998) defined the characteristics of an ideal biocide specifically for RO polyamide membrane applications (Figure 1)[39]. However, the currently applied biocides for polyamide membrane systems in potable water applications lack many, if not all, of these essential attributes[10]. Hence, it is essential to select a safe, green, and effective chemical candidate for preventing biofouling in RO membrane systems, ensuring that it possesses, if not all, at least most

of these attributes. The literature on biofouling prevention is limited, and tools for designing safe, green, and effective antifoulants are largely absent in an industrial context[27]. While there is no established standard protocol for testing the efficacy of new anti-biofouling agents in RO systems, methodologies for characterizing biofouling and its effects such as a) membrane autopsies, b) assessing RO performance through operational indicators (such as normalized pressure, permeate, and salt rejection measurements), and c) risk assessment are well-known[4,9,10,15,22,27,32,40]. Therefore, in an effort to advance sustainable water supply through RO technologies, the proposed PhD project aims to develop a standardized protocol to select and test green chemical alternatives for preventing biofouling in drinking water RO applications, as well as addressing durability issues related to RO polyamide membranes.

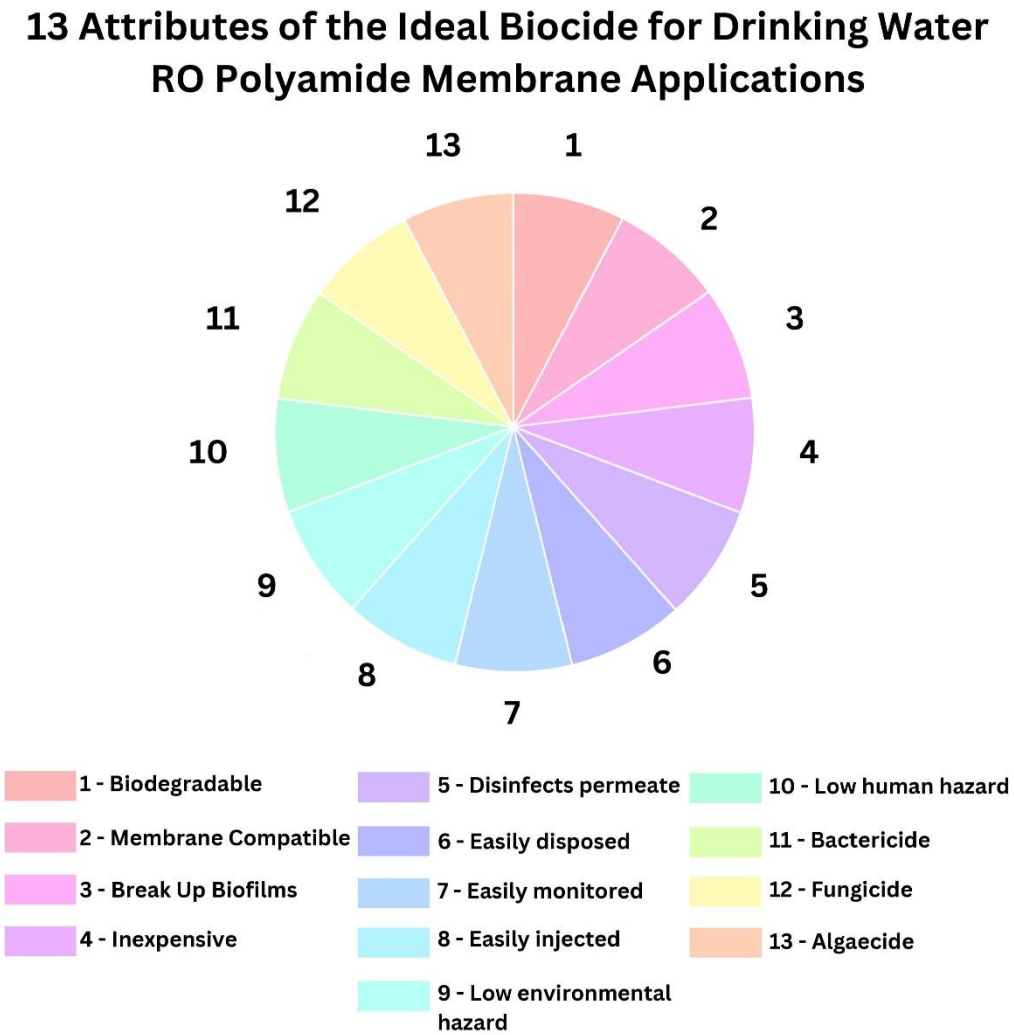

Figure 1 - 13 Attributes of an ideal biocide applicable to RO membrane applications[39].

1.4 Objectives and scope

The overall goal of this project is to develop and apply a platform on which to select and test safe and green anti-biofouling agents to prevent biofouling in drinking water reverse osmosis systems applications. The resulting new chemistries will prevent the detrimental effects of biofouling in RO systems, allowing the production of affordable and sustainable clean water for industrial and domestic use. Further, this project could be used as a guideline or standard protocol by industries and municipalities to find new anti-biofouling agents promoting sustainable water use via RO technologies. This project is the first step towards an innovative green solution for water scarcity, the dissemination of RO technology to produce fresh water, and the promotion of sustainable water use via RO technologies. The overall goal of this PhD work was addressed via the specific objectives described below.

Objective 1 – Select and evaluate the potential of a set of green chemicals to prevent biofouling in RO polyamide membrane potable water applications while considering their feasibility for practical RO application via a comprehensive literature review study, based on the attributes identified by Bates (1998)[39].

Objective 2 – Test and investigate the anti-biofouling efficacy of the biocides selected from the comprehensive review study to prevent and remove *P. aeruginosa* biofilms (a known biofilm pioneer in RO systems) from RO polyamide membranes for potential use in RO polyamide membrane potable water applications.

Objective 3 – Test and investigate the compatibility of the biocides selected from the comprehensive review and anti-biofouling efficacy study with RO polyamide membranes for potential use in RO polyamide membrane potable water applications.

Objective 4 – Propose a methodology to test and analyze the anti-biofouling efficacy and membrane compatibility of the most effective antifoulant candidates identified in the screening protocol (achieved via **Objectives 1-3**). This validation protocol (**Objective 4**) aims to bridge the transition between the screening protocol and the upscaling/optimization of the most effective identified antifoulant candidates for large-scale tests.

1.5 Dissertation outline

This PhD dissertation was prepared in a manuscript-based format, where each chapter is a manuscript either published, submitted or prepared for submission to a peer-reviewed journal. These manuscripts were prepared to specifically address the objectives outlined in Section 1.4 (Table 1). The publication status and authorship details for each manuscript are provided at the beginning of each chapter, while the author's contributions are summarized at the end of each chapter. Subsequent to the manuscript-based chapters (Chapters 2 – 5), Chapter 6 offers an overall discussion of my PhD work, exploring its broader implications, limitations, and proposing recommendations for future research.

Table 1 – Outline of the manuscripts comprising this PhD dissertation.

Chapter	Manuscript title	Objective	Protocol
Chapter 2	The application of non-oxidizing biocides to prevent biofouling in reverse osmosis polyamide membrane systems - A review	Objective 1	Screening Protocol
Chapter 3	Efficacy testing of non-oxidizing biocides for polyamide membrane biofouling prevention using a modified CDC biofilm reactor	Objective 2	Screening Protocol
Chapter 4	Rapid polyamide membrane compatibility testing of potential anti-biofouling agents for reverse osmosis membrane systems	Objective 3	Screening Protocol
Chapter 5	The application of ethyl lauryl alginate to prevent biofouling in reverse osmosis polyamide membrane systems: A benchtop study	Objective 4	Validation Protocol

Chapter 2: The application of non-oxidizing biocides to prevent biofouling in reverse osmosis polyamide membrane systems - A review

Luiz H. Da-Silva-Correa*, Hayley Smith*, Matthew C. Thibodeau*, Bethany Welsh*, and Heather L. Buckley*

*Department of Civil Engineering, Centre for Advanced Materials and Related Technologies (CAMTEC) and Institute for Integrated Energy System (IESVic), University of Victoria, Victoria, BC, Canada, V8P 5C2

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2.1 Abstract

Biofouling of polyamide membranes is one of the main barriers faced by reverse osmosis (RO) technologies to supply fresh water. Currently, biofouling is addressed by feed water pretreatment using chlorine, followed by membrane cleaning. Chlorine damages polyamide membranes and also generates harmful disinfection byproducts. Thus, safer strategies are needed to prevent biofouling in polyamide membrane systems. This review investigates the applicability of the following non-oxidizing biocides in preventing and controlling biofouling in RO systems, including their antimicrobial efficiency, hazard levels, membrane compatibility, and applicability to drinking water treatment: (1) 2,2-dibromo-3-nitrilopropionamide (DBNPA); (2) 2-methyl-4-isothiazolin-3-one (MIT); (3) sodium bisulfite (SBS), (4) phenoxyethanol (PE), (5) sodium benzoate (SB). According to this review, MIT and DBNPA present most of the features attributed to an ideal anti-biofouling chemical but also are the most hazardous biocides. Due to safety and efficacy, none of the five chemicals were determined to be the final solution to address membrane biofouling. However, alternative RO biocide research is in early development and requires further investigation via biofouling prevention studies. Therefore, future research efforts on the investigation of economic, eco-friendly, and safe antifouling agents to prevent and treat biofouling in RO systems are paramount to promote sustainable water supply in water-stressed countries.

Keywords: biocides, biofouling prevention, efficacy, polyamide membranes, reverse osmosis, safety, water treatment

2.2 Introduction

2.2.1 Reverse osmosis technology background

Water scarcity can be defined as the lack of available freshwater resources to meet standard water demand and is one of the greatest challenges of this century[1,4]. According to WHO (2017), 50–100 L of water per capita per day is needed to ensure the full realization of the right to water[11]. Eke et al. (2020) reported that water scarcity is exacerbated by many factors, such as water pollution, climate change, population growth, and industrialization [41]. According to UN-Water (2021), 2.3 billion people live in water-stressed countries, of which 733 million live in critically water-stressed countries [42]. Clean water technologies such as membrane and thermal desalination may be applied to address several long-term potable water demands[2,3,43–46]. Reverse osmosis (RO) polyamide membrane technology plays an essential role in addressing water scarcity due to its capability of producing freshwater from seawater, brackish water, and different types of wastewater[4,9]. Eke et al. (2020) found that in 2020, 16,880 desalination plants were supplying freshwater with a total worldwide capacity of 97.2 million m^3/day[41]. Also, analyzing recent growth rates of the RO desalination market, Zhao et al. (2021) predicted that the total worldwide capacity of RO plants will double between 2015 and 2025[4].

Although membrane-based techniques are important to address water scarcity, membrane systems face many challenges, such as fouling, scaling, and membrane degradation[4,10]. One of the main difficulties in RO technologies is the biofouling of polyamide membranes[4,10,22]. Fazel & Darton (2001) investigated the membrane autopsies of 150 membranes used in RO applications from all over the world[24]. The results showed that all membranes presented biofouling, of which 33% had biofouling as the main cause of systems failure. In a similar study, Pena et al. (2013) reported that 31.3% of 500 membranes presented severe biofouling causing the collapse of the membrane systems[47]. The prevalence of this challenge makes the search for novel strategies to address biofouling in polyamide membrane systems necessary to promote sustainable water supply in water-stressed countries.

2.2.2 Biofilm

A biofilm is a complex structure of microorganisms that can include bacteria, fungi, algae, and extracellular polymeric substances (EPS). EPS is primarily composed of polysaccharides and proteins[10,48]. Although the composition of a biofilm is dependent on temperature, pH, flow, osmotic pressure, and nutrient availability, approximately 90% of its mass is EPS and 10% is microorganisms [10,32]. Biofilms typically develop at solid and liquid interfaces and their formation is well characterized. The general steps of biofilm formation (Figure 2) are attachment, microcolony formation, maturation, and dispersion[48,49]. Bacteria secrete EPS, which helps additional bacteria to physically attach to the

colony, protects them from antimicrobials, and enables the bacteria in the colony to communicate via molecular signals known as quorum sensing[50]. Because biofilms form readily, become resistant to antimicrobials, and are physically difficult to remove, they pose a challenge to membrane technologies.

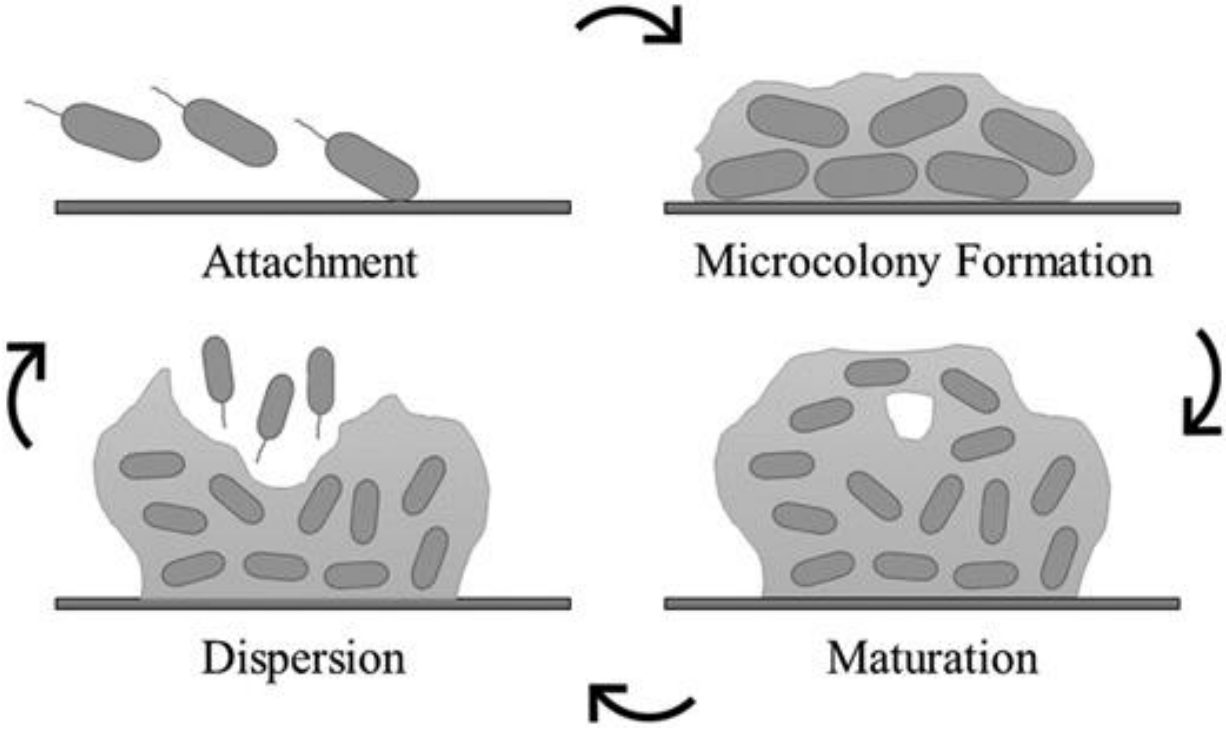


Figure 2 - Typical bacterial biofilm life cycle.

2.2.3 Reverse osmosis biofouling

RO biofouling generally occurs when bacteria in the feed water enter the system and migrate to the membrane surface, where dissolved nutrients are concentrated due to concentration polarization[51]. The design of the spiral wound elements and surface texture of the polyamide membrane create the ideal biofilm growth environment, perfect for attachment and accumulation of organisms[52]. Microorganism concentrations as low as 1,000 CFU/mL can cause severe biofouling in membrane systems[9]. Understanding the feed water composition is essential for preventing microbial growth. The source of feed water (brackish water, seawater, or potable water) impacts the growth conditions, influencing the types of microorganisms encountered including pioneer bacteria such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*[53], which are known to initiate biofilms on membrane surfaces[26]. Further, the concentration of contaminants in the water, such as total organic carbon (TOC) greater than 3 mg/L, can provide sufficient nutrients to promote bacterial build-up[9,54]. Ultimately, feed water dictates the biofouling potential of a membrane system, therefore pretreatment processes and bacteria mitigation through chemical or mechanical means are essential[9,55].

2.2.4 Effects of biofouling on RO performance

Membrane biofilm formation significantly impacts the performance of RO systems and increases operational and treatment costs[9,51]. Biofouling of RO membranes always results in an elevated pressure drop and a decrease in permeate flux[9,13]. Pressure drop is the increase in pressure differential between the feed and concentrate lines and is a direct result of the pressure loss across the membrane due to friction by foulants[9,55]. Permeate flux is the volumetric flow rate of permeate produced over the membrane area[9]. Permeate flux reduction and increased pressure differential are attributed to the increased resistance due to the low permeability of the membrane surface caused by the biofilm[56,57]. Other adverse biofouling effects include the decline in permeate quality from membrane degradation, elevated feed pressure resulting in an increase in energy requirements, and shortened membrane lifespan due to additional cleaning treatments leading to degradation and biodegradation as a result of acidic byproducts from bacteria[56,57]. Biofouling impacts on RO systems are summarized in Figure 3.

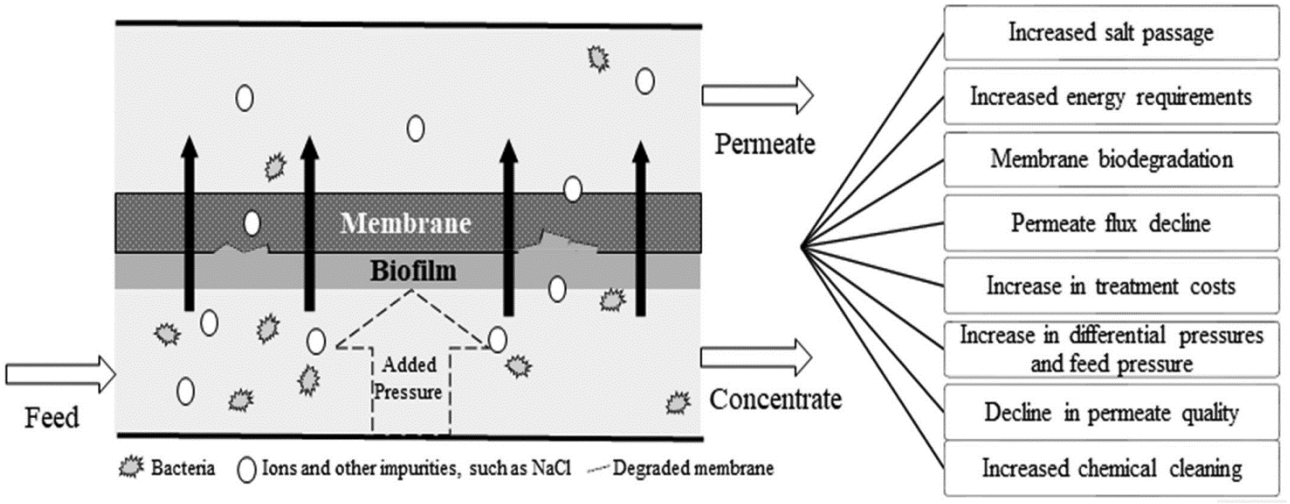


Figure 3 - Consequences of biofouling on RO systems

Techniques for monitoring and detection of biofouling in RO systems are implemented in water treatment operations to prevent biofouling impacts[54]. Monitoring practices include, but are not limited to, system performance analysis, frequent water sampling of feed water, permeate and concentrate quality, and physical inspection of the systems[56]. The biofouling indicators include a decrease in the permeate flow, stable salt rejection (percentage of influent compound retained by the RO membrane), and a large increase in differential pressure between the feed and concentrate across all stages of the RO elements [9].

2.2.5 Addressing biofouling in polyamide membrane systems: Oxidizing biocide issues

There are several strategies to address biofouling in RO systems[34]. Kucera (2019) stated that these strategies may be based on membrane modification, membrane disinfection, membrane cleaning, and bacteria or nutrient modification[10]. Currently, the effects of biofouling on the performance of RO

systems are mainly handled by disinfection and cleaning techniques. Disinfection of polyamide membrane systems is performed via chemical (e.g. biocide treatment) or physical (e.g. ultraviolet radiation) methods with the objective of killing or disabling microorganisms in the system. Then, membrane cleaning is done to remove existing biofilms[10]. RO systems are typically chemically disinfected by either shock dosing or continuously dosing biocides into the system. Continuous dosing is defined as maintaining a fixed concentration of the biocide in the feed stream of the water treatment process, whereas shock dosing is the intermittent addition of a biocide into the feed stream at higher concentrations and for a limited time[58,59]. Shock dosing is typically performed daily or weekly and can be applied on its own or in addition to continuous dosing. The aim of shock dosing is to remove any biofilm that has formed since the previous shock dose, while continuous dosing typically uses the minimum concentration of biocide necessary to maintain control of biofilm formation[59,60]. Chlorine is the most common oxidizing biocide used to address biofouling of polyamide membrane systems and is applied during feed water pretreatment (chlorination)[10,22]. This is because chemical feed water pretreatment with chlorine is effective against a wide range of microorganisms and promotes rapid cell death even at low concentrations [9]. However, the application of chlorine in RO systems is limited by many factors since it damages polyamide membranes by oxidative degradation[9,10,40], it may form harmful and carcinogenic disinfection byproducts, such as halogenated contaminants, trihalomethanes, and haloacetic acids[9,10,40], and it is not safe to handle[37]. Thus, due to the non-compatibility with polyamide membranes and corresponding hazards, chlorine has to be removed from the RO system after its application in the feed water pretreatment (dechlorination). Since free chlorine cannot be in direct contact with membranes, chlorine disinfection is only able to reduce or delay the biofouling of polyamide membranes[10,34,40]. Alternatively, non-oxidizing biocides can be applied in contact with polyamide membranes, thus the use of non-oxidizing anti-biofouling agents has been increasing in RO installations as an alternative to chlorine in many water treatment applications.

2.2.6 Biofilm prevention versus disinfection

It is important to note that disinfection and membrane cleaning techniques are only able to reduce or delay the effect of biofouling in membrane systems[34]. In order to find long-term sustainable solutions for biofouling, preventative treatments should be taken into consideration. Munla et al. (2012) showed that once formed, mature biofilms can no longer be removed by shear force and the fouling is considered to be irreversible [61]. This is because when forming biofilms, microorganisms release EPS, which holds the planktonic cells together and anchors them to the membrane surface. The EPS then becomes very difficult to remove without some sort of mechanical force, as the gel-like substance cross-links with the membrane surface and increases the mechanical stability of the biofilm[22]. In addition, once microorganisms are established into a biofilm matrix, they display different properties and express different phenotypes than those in a planktonic state, leading to increased resistance to

biocides[62]. According to Barraud et al. (2006), bacteria in biofilms can be 1,000-fold more resistant to antimicrobials than those in a planktonic state[63]. On the grounds that the biofilm matrix consists of a structure that biocides generally cannot fully penetrate, bacteria in deeper layers of the biofilm are exposed to sub-lethal levels of the biocide, resulting in optimal conditions for biocidal tolerance development[50,62]. Ultimately, biofouling prevention (1) reduces the amount of irreversible fouling, (2) reduces the formation of more resistant variants of microorganisms, and (3) ultimately requires lower biocide dosing as compared to biofouling treatment. As a result, biofilm prevention techniques are preferred to promote sustainable water treatment by RO technologies.

2.2.7 Ideal biocide to address biofouling

Bates (1998) described the characteristics of an ideal biocide to prevent and reduce biofouling in polyamide membranes[39]. The researcher identified that an ideal biocide should have the following characteristics: (1) the biocide should be compatible with polyamide membranes as well as all system components; (2) it should not pose hazards to human health and the environment; (3) the biocide should be effective against all types of microorganisms, be able to break up existing biofilms and disinfect the permeate side; (4) it needs to be biodegradable and inexpensive; and, (5) easy to handle, monitor, and inject into the membrane systems. Realistically, there is no current biocide available with all of these attributes[10]. The fact that non-oxidizing biocides may potentially be applied in contact with polyamide membranes makes them promising candidates for long-term biofouling prevention studies. However, few studies were found in the literature that assesses the application of non-oxidizing biocides to prevent and reduce biofouling in RO polyamide membrane systems and consider all of these attributes[26,34].

2.2.8 Aim and novelty of this paper

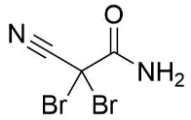
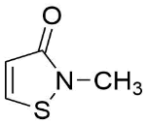
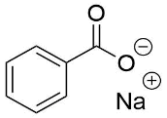
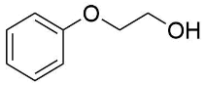
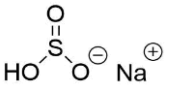
This paper intends to fill this research gap by reviewing the ability of five non-oxidizing biocides to prevent biofouling in polyamide membrane systems, considering all the attributes identified by Bates (1998), and promoting new, sustainable solutions to supply water via RO technologies[39]. Specifically, this study presents a review on (a) the applicability of five non-oxidizing biocides in preventing and controlling biofouling in RO systems, (b) information on new non-oxidizing anti-biofouling agents under development stages, (c) a comprehensive discussion and analysis on the ability of these biocides to prevent and control biofouling in regards to the efficiency and feasibility for practical RO application, as well as (d) future research recommendations.

2.2.9 Biocides

Table 2 details the five non-oxidizing biocides that are discussed in this review. Three biocides are already applied in potable water applications: (1) 2,2-dibromo-3-nitrilopropionamide – DBNPA (off-

line shock feed treatment), (2) 2-methyl-4-isothiazolin-3-one – MIT (off-line shock feed treatment and membrane cleaning), and (3) Sodium bisulfite – SBS (continuous on-line dechlorination-treatment and membrane storage)[9,10,34,64,65]. Since the biocides are currently applied in polyamide membrane systems, they are recognized as reasonable candidates for biofouling prevention in RO systems. The two remaining biocides are commonly applied as preservatives in home and personal care products and were selected due to their low associated hazards: Phenoxyethanol (PE) and Sodium benzoate (SB)[26,66–68], making them interesting candidates for biofilm prevention and control.

Table 2 - Structural and chemical formulas of non-oxidizing biocides

Biocide	Name	CAS-Number	Chemical Formula	Structural Formula
DBNPA	2,2-dibromo-3-nitropropionamide	10222-01-2	$C_3H_2Br_2N_2O$	
MIT	2-methyl-4-isothiazolin-3-one	2682-20-4	C_4H_5NOS	
SB	Sodium benzoate	532-32-1	$C_7H_5NaO_2$	
PE	Phenoxyethanol	122-99-6	$C_8H_{10}O_2$	
SBS	Sodium bisulfite	7631-90-5	$NaHSO_3$	

Each biocide is evaluated on how well it meets the criteria described by Bates (1998), including efficacy in limiting biofilm formation on RO membranes, membrane compatibility, and relative hazards[39] (Appendix A). The efficacy of each biocide is largely determined by its mechanism of action. According to Kucera (2019), biocides are characterized by their mechanism of action to kill microbiological cells or prevent growth[10]. These groups include electrophiles, with sub-categories moderate and extreme (oxidants), or membrane-active, with sub-categories lytic and protonophores (Figure 4) [69]. Electrophiles rapidly kill by entering the cell wall or by generating free radicals, which damage intracellular proteins, carbohydrates, lipids, and nucleic acids[70]. The mechanism of action of membrane-active biocides is the disruption of the microorganism's cell membrane function and structure [71]. The selection of the correct biocide depends on the type and number of bacteria [72]. Biofilms can be composed of various species, such as fungus, algae, Gram-positive bacteria, Gram-negative bacteria, and yeast, so knowledge of organisms encountered is important[72]. Selecting incorrect biocides or dosages will result in an ineffective disinfection and is expensive[72]. The hazards posed by each biocide are determined from GreenScreen assessments of the chemicals, and other data collected from the literature[73]. All biocides are evaluated on their relative risk to the environment and to human health, including hazards to natural environments, human health after consumption (for cases of potable water treatment), and human health after handling the chemicals from an operational

standpoint. With all of these attributes considered, the following sections discuss each of the five candidate non-oxidizing biocides and review their potential to prevent and remove biofouling in RO systems.

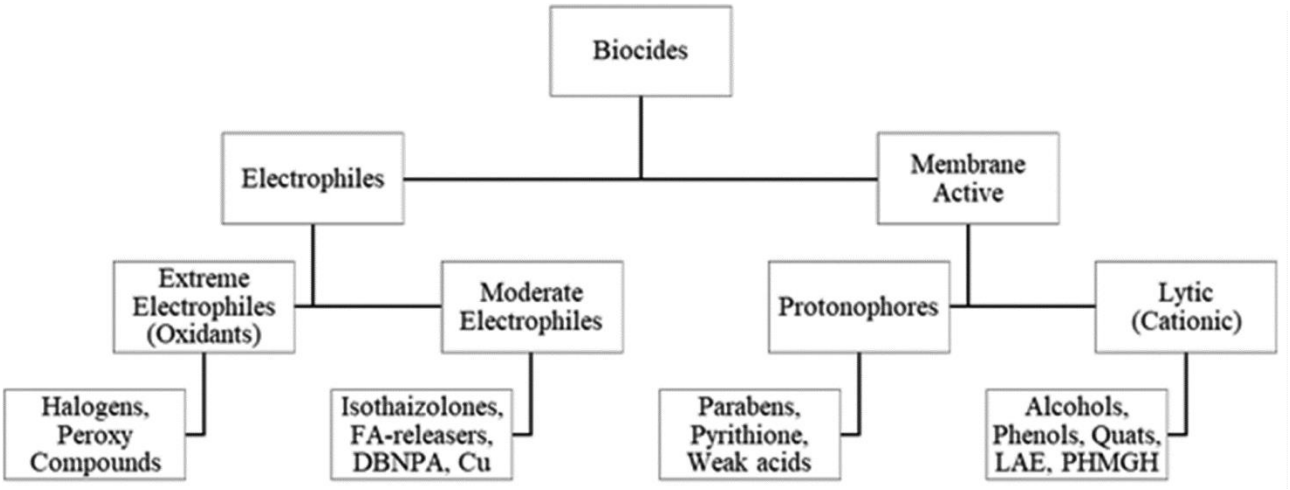


Figure 4 - Mechanism of inactivation by biocides

2.3 2,2-Dibromo-3-nitrilopropionamide (DBNPA)

2,2-dibromo-3-nitrilopropionamide (DBNPA) is a halogenated amide and has been documented as an effective non-oxidizing biocide[10,74]. In industry, DBNPA is widely used in water treatment, pulp, and paper but primarily in the oil industry to treat the water used to prepare fracturing fluids to control microbially induced corrosion of pipes[74,75]. The application of DBNPA in RO systems is common due to its compatibility with polyamide membranes and fast biocidal response[10,76]. The use of DBNPA is prohibited from inline application for potable water production, due to the harmful toxicity of the hydrolysis byproducts[58,77,78]. The typical membrane application is either by shock (intermittent) or continuous dosage and is not used for membrane storage because of the short half-life[10,54]. For instance, the hydrolysis half-life values at 25°C at pH 6.7, 7.7, and 8.0 are 37, 5.8, and 2.0 hours, respectively[79]. DBNPA acts rapidly on microorganisms, with a contact time of less than 1 h, and therefore the short half-life is generally not a concern for water treatment applications[71,80].

2.3.1 Generation

Generally, DBNPA is synthesized with the starting material cyanoacetamide (CAM) and prepared by acid-catalyzed bromination[81]. Polyethylene glycol is the preferred solvent for DBNPA, providing good product stability and solubility[82]. DBNPA is soluble in water; however, the solution is only stable in the acidic pH range[82]. Therefore, DBNPA is generally available commercially as a 20% active solution in a water/polyethylene glycol blend[10,83]. DBNPA must be stored in non-metal containers, due to incompatibility with metals[10,71]. Moreover, exposure to sunlight (UV) can degrade DBNPA[10,79], significantly reducing the efficacy of the biocide.

2.3.2 Mechanisms of action

DBNPA is an example of a moderate electrophile. Collier et al. (1990) and Slawson et al. (1990) described electrophilic agents as biocides that react covalently with cellular nucleophiles to inactivate enzymes[84,85]. Further, studies indicate that electrophiles initiate the formation of intracellular free radicals which contribute to their antimicrobial effects[86,87]. DBNPA acts by reacting with the sulfur-containing organic molecules in a bacterium cell such as glutathione or cysteine[88]. The reaction is irreversible, resulting in interruption of the transport of compounds, inhibiting the biological processes of the bacterium[74]. In summary, DBNPA prevents biofouling rapidly, by permanently attacking the microbiological cell walls.

DBNPA has a broad spectrum of inactivation, effective at treating Gram-positive and Gram-negative bacteria, yeast, and fungi[74]. However, DBNPA is not particularly effective against algae[80]. Studies found in the literature indicated that DBNPA does not penetrate EPS[34,89], and therefore DBNPA is not suitable for removing an established biofilm. For instance, Siddiqui et al. (2017) conducted a study investigating the prevention of biofilm growth and removal of established biofilms on RO membranes in a bench-top cross-flow system with high biofouling potential feed water[34]. Results indicated the continuous DBNPA dosage achieved prevention of biofilm accumulation, displaying a reduction in pressure drop. In contrast, continuous dosage to an existing biofilm was not effective at removing EPS and inactive cells or restoring the pressure drop, showing DBNPA is not suitable for curative treatment. However, the DBNPA dosage was able to prevent further biofilm formation and a further increase in pressure drop[34]. The results of the study further confirm that the application of DBNPA is effective for preventing biofouling on RO and not suitable for removing existing biofilm.

2.3.3 Anti-biofouling effect: dosage and efficacy

In RO applications, DBNPA is typically applied directly to the feed water by continuous injection or shock treatment[10,54]. The dosage concentration depends on the feed water quality, including the biofouling potential and required permeate quality[90]. A wide range of dosage intervals for continuous and shock injection are recommended, such as 2.5–10 mg/L for continuous treatment, or shock treatment at 10–30 mg/L for 1–3 h[89], every 2–7 days depending on microbial growth [90]. Moreover, DBNPA should be applied to a clean membrane for the most effective application for biofouling prevention [10]. The biocide is deactivated by reducing agents, so higher dosages are required in the presence of reducing agents, such as SBS [71]. The application of DBNPA to control biofouling is more efficient in oxidative conditions compared to reducing environments [74], and at pH lower than 8 [89]. Degradation of DBNPA by hydrolysis increases significantly with increasing temperatures and pH [79].

Microbial efficacy and dosages of biocides are generally determined by microdilution antimicrobial susceptibility tests[26,91,92]. The tests include the minimum inhibitory concentration (MIC), minimum biofilm eradication concentration (MBEC), minimum biofilm inhibitory concentration (MBIC), and minimum bactericidal concentration (MBC) [91]. According to Andrews (2001), MIC is acknowledged as the 'gold standard' for judging the antimicrobial susceptibility of organisms in the planktonic phase[93]. The author defines MIC as the lowest concentration of a biocide to inhibit the visible growth of a microorganism after incubation overnight. Further, MIC values are used by universal diagnostic laboratories to confirm antimicrobial resistance and determine the in vitro activity of new biocides. The inhibitory values of the non-oxidizing biocides against *P. aeruginosa* and *S. aureus*, common pioneer organisms in membrane systems, for DBNPA and the other selected biocides are displayed in Table 3.

Table 3 - Inhibitory concentrations of non-oxidizing biocides for *P. aeruginosa* and *S. aureus* (Biofilm pioneer microorganisms in RO systems)

Biocide	***P. aeruginosa***				***S. aureus***			
	MIC	**MBEC**	**MBC**	**MBIC**	**MIC**	**MBEC**	**MBC**	**MBIC**
	mg/L				**mg/L**			
DBNPA	80[a]	-	80[a]	-	160[a]	-	160[a]	-
MIT	30[b]	78[b]	-	22.5[b]	45[c]	-	-	-
SB	5000[b]	32,200[b]	-	25,000[b]	16,000[d]	16384[e]	32,000[d]	25,000[b]
PE	48,000[f]	120,000[b]	-	36,000[b]	1,590[b]	-	3,180[g]	-
SBS	780[h]	-	-	-	512[i]	-	512[i]	-

Note: MIC, minimum inhibitory concentration; MBEC, minimum biofilm eradication concentration; MBC, minimum bactericidal concentration; MBIC, minimum biofilm inhibitory concentration. '–': inhibitory concentration was not found in the literature. MIC: The lowest concentration of an antimicrobial that inhibits visible growth of a planktonic culture after overnight incubation. MBEC: The lowest concentration at which biofilm density is reduced by >90% when compared to control OD values. MBC: The lowest concentration of an antimicrobial that achieves a 99.9% CFU reduction of the initial inoculum of planktonic cells. MBIC: Same as MBEC but the bacterial inoculation occurs with antibiotic exposure. (Macià et al. 2014[94]; Wu et al. 2015[95]; Curtin et al. 2021[91]). Source: a - Kim & Park (2015)[40], b - Curtin (2020)[26], c - Lundov (2010)[96], d - Wang et al. (2018)[97], e - Güven & Onurdağ (2014)[98], f - Gillings (2010)[99], g - Grecka & Szweda (2021)[100], h - Penna et al. (2002)[64] and i - Frank & Patel (2007)[101].

2.3.4 Membrane compatibility

Non-oxidizing biocides, including DBNPA, are commonly used for RO biofouling control since the chemicals can be applied directly to the RO elements without the concern of damaging the membranes[71,102]. Kim & Park (2016) studied the membrane compatibility of DBNPA by completing a series of polyamide membrane destruction tests including morphological damage and oxidative damage assessments when contacted with the biocide for 24 h at high concentrations (1,000–100,000 mg/L)[103]. The study compared DBNPA to chlorine, an oxidizing biocide known to damage polyamide membranes. The researchers concluded that the polyamide surface layer was not damaged when treated with high concentrations of DBNPA, which confirms the industry practice of direct application of DBNPA on polyamide membranes.

2.3.5 Human and environmental health hazards

A summary of the human and environmental hazards levels is detailed in Tables 4 and 5. The hazard information for the biocides was collected from literature, including GreenScreen assessments and other data collected from Pharos[73,104]. The hazard levels are sourced from the Pharos database, which is based on the thresholds formed by the Globally Harmonized System of Classification and Labelling of Chemicals and additional benchmarks for endpoints by the U.S. EPA's Design for the Environment[104]. The biocides with no hazard level data do not signify the absence of hazard, but an absence of studies in literature or low-confidence results.

Table 4 - Human hazard levels of non-oxidizing biocides

Biocides	Carcinogenicity	Neurotoxicity Single Exposure	Neurotoxicity Repeated Exposure	Skin Sensitization	Respiratory Sensitization	Eye Irritation	Systemic Toxicity Single Exposure	Systemic Toxicity Repeated Exposure	Endocrine Activity
DBNPA	Low[a]	Low[b]	Low[c]	High[d]	-	High[d]	Moderate[a]	-	High[d]
MIT	Low[e]	Moderate[f]	Low[f]	Very high[e]	Low[f]	Very high[e]	Moderate[g]	Moderate[g]	Low[e]
SB	Low[e]	Low[h]	Low[h]	Low[e]	Moderate[i]	Moderate[e]	Low[h]	Low[h]	Low[e]
PE	Low[j]	Moderate[k]	Low[k]	Low[k]	-	High[k]	-	Low[k]	-
SBS	Low[m]	-	-	High[n]	High[n]	High[o]	-	-	-

Hazard levels: very low, low, moderate, high, very high. "-": data gap. Source (a - Zheng *et al*. 2021[105]; b - Min *et al*. 2019[106]; c - Chen 2012[107]; d - ECHA 2019[108]; e - Canavez *et al*. 2021[109]; f - Burnett *et al*. 2010[110]; g - Kim & Choi 2019[111]; h - CIR 2001[67]; i - SCCP 2005[112]; j - JBRC 2007[113]; k - ECHA 2015[114]; m - EFSA ANS Panel 2016[115]; n - Vally et al. 2009[116]; o - Walls *et al*. 2018[117]).

Table 5 - Environmental health hazard levels of non-oxidizing biocides

Biocides	Acute Mammalian Toxicity	Acute Aquatic Toxicity	Terrestrial Ecotoxicity	Bioaccumulation	Chronic Aquatic Toxicity	Persistence
DBNPA	Very high[a]	Very high[b]	-	-	-	-
MIT	Very high[c]	Very high[d]	Moderate[c]	Very low[e]	Very high[d]	Low[e]
SB	Low[g]	Low[g]	Moderate[g]	Low[g]	Low[g]	Low[g]
PE	Moderate[f,h]	Low[h]	-	Very low[h]	Low[h]	Very low[h]
SBS	Low[i]	Moderate[j]	-	Very low[i]	-	Low[k]

Hazard levels: very low, low, moderate, high, very high. "-": data gap. Source (a - Alexander *et al*. 2001[118]; b - Chen 2012[107]; c - Burnett *et al.* 2010[110]; d - Van Huizen *et al.* 2017[119]; e - Silva *et al.* 2020[37]; f - UNEP 2004[120]; g - WHO 2000[121]; h - ECHA 2015[114]; i - EFSA ANS Panel 2016[115]; j - Ryon *et al*. 2002[122]; k - Baker & Dudley 1998[77]).

DBNPA is a common treatment to prevent biofouling in RO for non-potable water applications[54,76]. However, allowable applications are limited due to the chemical toxicity; DBNPA has been classed as an endocrine disruptor by the European Chemicals Agency[108]. The application of DBNPA for potable water is prohibited, due to the passage (0.02%) of DBNPA into the permeate[58,77,89]. In addition, appropriate measures and risk management are required for handling DBNPA because the biocide is harmful by acute exposure by oral or inhalation pathways and is a skin irritant, skin sensitizer, and causes eye damage[108].

When DBNPA is applied to aqueous solutions, such as RO systems, hydrolysis occurs easily due to the short half-life[54]. There are two known hydrolysis pathways of DBNPA. The primary degradation products by hydrolysis are dibromoacetamide (DBAM), dibromoacetic acid, and dibromoacetonitrile (DBAN)[78,79]. Hydrolysis ends with oxalic acid which oxidizes slowly to CO_2 [74]. Dibromoacetic acid is more persistent in the environment with a half-life of 300 days [79], and DBAN is three times more toxic than DBNPA, with LC_{50} values for DBAN of 0.55 mg/L and DBNPA of 1.8 mg/L [78,123]. Due to the harmful degradation products of DBNPA, there is a concern for human and environmental impacts when DBNPA is applied to water systems. A secondary degradation pathway of DBNPA occurs when there is a high ratio of TOC to dose of DBNPA. DBNPA degrades to monobromonitrilopropionamide (MBNPA) and CAM, where MBNPA is two times less toxic compared to DBNPA, with the LC_{50} value of MBNPA as 3.4 mg/L [78,123].

To further understand the aquatic toxicology of DBNPA, Chen (2012) evaluated the chronic toxicity of the chemical in Daphnia magna and rainbow trout[107]. The researcher evaluated long-term exposure at relatively low concentrations of DBNPA, ranging from 0.005 to 0.3 ppm and 14–28 days, depending on species. The author concluded that DBNPA affected the reproduction and survival of Daphnia magna at concentrations starting at 0.053 ppm, and DBNPA affected the growth of juvenile rainbow trout at a concentration starting at 0.018 ppm after 28 days of exposure. This study showed preliminary evidence that DBNPA has negative impacts on aquatic life[107]. Overall, proper risk assessment and chemical management are essential when applying DBNPA to water systems.

2.3.6 Advantages and limitations

One of the major limitations for DBNPA as a biocide is that it is not effective at removing biofilms from polyamide membranes[34]. This is because DBNPA is only capable of preventing biofouling as it does not penetrate EPS and is most effective when applied on clean membranes[10,89]. Also, DBNPA must be used off-line for potable water or food and beverage applications[10], due to health hazard concerns of DBNPA and the harmful hydrolysis degradation products, DBAN and DBAM (Appendix A)[78,79]. DBNPA is very soluble in water, with a water solubility of 15 g/L at 20°C [82] and degrades easily due to a short half-life. DBNPA half-life decreases with increased pH, which impacts product stability and the ease of storage and transportation[76]. However, the half-life is generally not an issue

for preventing biofouling since the biocidal action occurs rapidly before hydrolysis making DBNPA an advantageous biocide for shock or continuous dosage for RO, but not effective for membrane storage[54,71,80]. Continuous injection can be more expensive in terms of treatment costs, so shock dosages are typically applied[10,54,71]. However, the relatively low concentration required for shock dosages provides a more economic biofouling prevention treatment when compared to other biocides. Overall, DBNPA is beneficial for RO membrane application due to the fast-acting and effective prevention treatment at low concentrations and compatibility with polyamide membranes[10,54,71].

2.4 Methylisothiazolinone (MIT)

2-methyl-4-isothiazolin-3-one (MIT) is a heterocyclic organosulfur compound commonly used in cooling system applications, industrial water treatment, cosmetics, and daily life products (such as detergents and paints) due to its wide-spectrum biocidal efficacy against bacteria, fungi, and algae[37,80,102,124]. MIT is applied in RO applications as a non-oxidizing biocide in cleaning events and membrane storage either alone or in combination with 5-chloro-2-methyl-4-isothiazolin-3-one (CMIT) in a CMIT/MIT ratio of 3:1 [9,84,102,125]. According to Kucera (2015), isothiazolinones are a viable choice to control biofouling in polyamide membrane systems[9]. However, although MIT applications as a non-oxidizing biocide are known to be effective in various industrial settings, most of the reports found in the literature were focused on biofouling mitigation by MIT treatment and few studies were found on its capability in preventing biofouling in RO membrane-based water treatment systems. In other words, although MIT is widely applied in RO installations, its application for biofouling prevention is not sufficiently studied.

2.4.1 Generation

The first synthesis of MIT was reported in the 1760s [126]. In this process, MIT was produced through the cyclization of cis-N-methyl-3-thiocyanatoacrylamide with an overall yield of 80% [37]. Currently, MIT is synthesized via the cyclization of amides that are produced from carboxylic acids[37]. The popularization of the mixture of MIT and CMIT as biocides in the RO industry occurred in the late 1980s [84]. One of the most commonly used CMIT/MIT biocides available today is marketed under the Kathon™ brand[80,102,103,127]. The mixture of isothiazolinones is usually marketed at an active concentration of 1.5% (1.15% of CMIT and 0.35% of MIT)[80,127,128]. Although the CMIT/MIT mixture is frequently used in RO applications, the most recent isothiazolinone biocide applications in water treatment have been based on products containing MIT as the only active ingredient[124,129,130]. This is because treatment and disposal of the RO concentrate resulting from a CMIT/MIT biocide treatment are very expensive, probably due to the high toxicity of CMIT, which is approximately one hundred times more toxic than MIT[37,124].

2.4.2 Mechanisms of action

MIT is a moderate electrophile that can enter the cell membrane by diffusion or active cell transport [10,37]. The mechanisms of action of MIT rapidly inhibit bacteria growth by impairing microbial respiration and energy production (ATP synthesis) followed by a slow cell death due to the inactivation of enzymes and irreversible cell damage by free radicals[10,37,80,131]. Within the cell, the electron-deficient N–S bond of isothiazolinone reacts with nucleophilic groups, such as thiols present in the active sites of many enzymes and in proteins, inhibiting enzymatic activity. The nucleophilic reactions between MIT and thiol-containing compounds impair microbial respiration, energy production, and other microbial functions causing growth inhibition and cellular death[10,37,84,131]. Microbial growth inhibition usually takes a few minutes and the cell death might take several hours[10,37,132]. The slow kinetics of inactivation can be overcome by increasing the biocide concentration or adding surfactants[10,133].

Notably, MIT presents high antimicrobial efficacy against a wide range of microorganisms, such as bacteria, algae, and fungi[9,102,131]. According to Frayne (2001), this antifoulant has optimal biocidal efficacy against aerobic and spore-forming bacteria at pH 6.5–9 [80]. Furthermore, Frayne (2001), indicates that MIT performs best as a fungicide and an algicide under acidic-to-slightly-alkaline pH levels[80]. These pH ranges match the operational conditions of polyamide membrane systems, making the application of MIT as an anti-biofouling agent suitable for RO systems. Williams (2006), in a study on the mechanism of action of industrial isothiazolinone biocides in water treatment applications, concluded that it is very difficult for microorganisms to build up resistance to MIT because the mechanism of action results in a wide spectrum of inhibitory pathways[131]. In the RO industry, MIT is frequently applied for both membrane storage and cleaning. This indicates that this chemical cannot only inhibit microbial growth but also it can remove mature biofilms by disrupting the EPS matrix of the biofilm[9,10,133]. Therefore, MIT is capable of preventing and controlling the biofouling [10].

2.4.3 Anti-biofouling effect: dosage and efficacy

Isothiazolones are applied in RO off-line operations for drinking water treatments at dosage rates ranging from 50 to 120 ppm (1.5% active isothiazoline) with an exposure time of 5–6 h [10,65,80]. According to Kucera (2019), the high dosages and long contact period recommended for biofouling control by MIT treatment make its application less attractive for on-line or shock treatments[10]. Williams (2007) performed various MIC studies on the antimicrobial efficacy of MIT against different microbial groups using standard MIC protocols[65]. The experiments also covered a broad range of conditions, such as pH 5–8, temperatures of 24–35°C, media (complex and defined), and incubation periods (1–7 days). The authors concluded that MIT is a highly effective biocide. The researchers also reported that MIT maintains an excellent antimicrobial efficiency against different

types of microorganisms and physical stability in wide pH (2–10) and temperature (5–60°C) ranges. This conclusion is supported by subsequent literature[37,102,134].

Although MIT is already widely applied to biofouling control in RO systems, there are no laboratory-scale or pilot-scale studies on RO membrane-based water treatment system units in the literature regarding the application of MIT to prevent biofouling of polyamide membranes. However, Curtin et al. (2021) tested the efficacy of MIT, SB, and PE in preventing and removing single-species static biofilm formation in a 96-well plates experiment using *Pseudomonas aeruginosa* (a common biofilm former in RO systems) with a final cell concentration of 1×10^6 CFU/mL [91]. To assess the antimicrobial efficacy of these biocides, minimum biofilm eradication concentrations (MBEC) and MBIC were determined based on a relative fluorescence analysis (LIVE/DEAD BacLight staining method). The experiments were performed at 37°C for 24 h. In the biofilm removal experiments, the plates contained the highest concentrations of MIT, PE, SB of 600 mg/L, 960,000 mg/L, and 250,000 mg/L, respectively, while in the biofilm prevention tests, the highest concentrations were three-quarters of these concentrations. The results indicated that MIT presented lower inhibitory concentrations as compared to SB and PE (Table 3). The researchers concluded that MIT was the most effective of the three biocides in removing and preventing biofilm, showing it to be a good model compound as a reference for biocide efficacy in RO studies.

2.4.4 Membrane compatibility

Isothiazolinone biocides such as MIT are inherently biodegradable into less hazardous compounds, inexpensive, and compatible with polyamide membranes as well as all RO system compartments[9,10,102]. Majamaa et al. (2011) performed long-term membrane compatibility tests with both new and used brackish water polyamide membranes at different temperatures (25 and 40°C)[102]. In this study, Majamaa et al. (2011) compared the membrane compatibility of a CMIT/MIT mixture with DBNPA and sodium metabisulphite (the current standard chemical used for polyamide membrane preservation and storage) at active dosage concentrations of 10 mg/L, 30 mg/L, and 3,900 mg/L, respectively[102]. The performance of the membranes was assessed on salt rejection, transmembrane pressure drop, and permeate flow after membrane storage periods of 1, 3, 6, and 12 months. The researchers concluded that both CMIT/MIT and DBNPA were compatible with polyamide membranes and their application to replace sodium metabisulphite is economically feasible with CMIT/MIT being the most economical option. Furthermore, the membrane performance results showed that CMIT/MIT and DBNPA could control microbial growth during all membrane storage periods[10,102]. Hence, MIT is compatible with polyamide membranes[9,10,65,102].

2.4.5 Human and environmental health hazards

Despite presenting remarkable biocidal efficacy in controlling biofouling in RO applications, isothiazolinones are skin sensitizers, eye irritants, ecotoxic, and allergy triggers[10,26,37,80,91,135]; thus, careful handling is required[10,37,135]. In a literature review on the toxicity profiles of isothiazolinone biocides, Silva et al. (2020) pointed out that isothiazolinone biocides present potential health hazards for industrial workers and final product users[37]. Maximum permitted concentrations of CMIT/MIT biocides and MIT biocides in cosmetics (direct human exposure) are 15 and 100 mg/L, respectively[37,136]. Workers exposed to higher CMIT/MIT concentrations (>100 mg/L) may experience harmful effects. For instance, Willi et al. (2011), in a case study on health effects of handling isothiazolinone biocides in industrial settings, reported severe skin irritation followed by allergic dermatitis of an industrial worker after handling a 3:1 ratio CMIT/MIT biocide (product concentration of 100 mg/L) applied to control biofouling in a cooling tower[135]. Besides skin irritation and allergies, human exposure to isothiazolinone biocides might cause respiratory problems[37,137,138]. Regarding the carcinogenic risk of MIT, recent studies show that MIT is not carcinogenic[109,110,128]. Kim et al. (2019) assessed the potential risks to human health of the CMIT/MIT biocide (Kathon™ brand)[128]. The authors reported that the CMIT/MIT biocide (30–300 mg/L) presented no evidence of carcinogenic effects in a 24-month drinking water study in rats. The researchers also reported that the Kathon biocide (400 mg/L) did not present dermal carcinogenic effects in mice in a 30 months experiment. Similar results were reported by Burnett et al. (2010) and Canavez et al. (2021)[109,110].

Along with human hazards, the application of isothiazolinone biocides may pose environmental health hazards[37,139]. In an extensive literature review on the safety assessment of MIT, Burnett et al. (2010) reported many acute mammalian studies which indicated that MIT may be lethal if inhaled or ingested[110]. For instance, an acute oral study showed that the median lethal doses (LD_{50}) for male and female rats were 274.6 mg and 105.7 mg/kg of body weight, respectively[110]. Aquatic toxicity studies showed that MIT is very toxic to aquatic life[10,119,140]. Van Huizen et al. (2017) investigated the effects of MIT exposure on five different planarian species, which are common flatworms found in freshwater streams, lakes, ponds, and rivers[119]. The researchers found that MIT concentrations higher than 4.5 mg/L are lethal to planarians. It also was noted that planarians exposed to MIT concentrations of approximately 1.7 mg/L presented neuromuscular and epithelial-integrity defects. Furthermore, Santos et al. (2016) assessed the effect of MIT exposure on Xenopus laevis embryos and tadpoles (the South African clawed frog) wound healing and tail regeneration to investigate the adverse effects of MIT on aquatic animals[140]. The authors concluded that exposure to MIT impairs tissue regeneration and wound repair in Xenopus laevis which may lead to lethality. In contrast to these studies, a review by Silva et al. (2020) indicated that MIT is not likely to cause long-term ecological disturbances in aquatic and terrestrial environments because MIT will not persist in the environment due to its rapid biological and chemical degradation, as well as its diversified modes and rates of dissipation and

degradation[37]. The authors also reported that in several environmental systems, the degradation of MIT will lead to malonamic, malonic, acetic, and formic acids which will be ultimately converted to CO_2 (Appendix A).

2.4.6 Advantages and limitations

The main limitations of MIT biocide treatment are its high toxicity and the long exposure time and high doses needed for controlling biofouling[10,37,125]. Silva et al. (2020) reported many hazards associated with the industrial use of MIT as a biocide such as high aquatic toxicity and adverse health effects (e.g. eye and skin irritation)[37]. Li et al. (2016) reported that the concentrate containing MIT is not easily and safely disposable, because MIT needs to be removed from the concentrate as a result of its high toxicity[125]. The researchers also reported that UV radiation and ozonation can both degrade MIT, providing a possible post-treatment approach for both the concentrate and permeate. However, this would increase the already high operational cost associated with RO systems. Furthermore, from a green chemistry perspective, toxic degradable biocides like MIT are not recommended as a safer alternative to address membrane biofouling, even when further treatment is employed. This is due to the potential human and environmental hazards these chemicals may pose throughout their entire life cycle. Safety for humans and the environment should be prioritized over all other attributes of an ideal biocide[39,141,142]. Due to these limitations, Kucera (2019) indicated that MIT might not be ideal for on-line potable water applications[10].

The main advantages of MIT as an industrial biocide rely on its excellent antimicrobial efficacy, compatibility with polyamide membranes, and distinguished biocidal performance in a good range of conditions[10,65,102]. Further, MIT is cost-competitive and very difficult to present microbial resistance[10,102]. Rapid degradation means that it is unlikely to pose a long-term threat to environmental health[65]. According to Kucera (2019), MIT treatment presents an excellent performance in preventing microbial growth in long-term membrane storage[10]. In addition, MIT is completely soluble in water which facilitates its injection in water treatment applications[110]. However, more studies need to be done to investigate long-term continuous MIT application for biofouling prevention in RO systems.

2.5 Sodium bisulfite

Sodium bisulfite is an inorganic salt, commercially used as a reducing agent in cosmetics, and as a preservative in a variety of food products and pharmaceuticals[143–145]. In industrial water treatment applications, SBS is regularly dosed as the reducing agent after chlorine disinfection to remove free chlorine from the system and is widely used as a preservative during long-term membrane storage[10,64]. The biocide has also been dosed to protect pipelines from microbially influenced corrosion and as a quenching compound to remove ozone from drinking water treatment trains[10,146]. Other applications include the preservation of water softeners and dechlorinating carbon bed filters[64]. Most SBS

solutions are about 33–37% active[10]. Due to its varied applications in water treatment systems, antimicrobial properties, and its comparatively low risk to the environment, SBS may be suitable to use as a preventative biocide in RO systems.

2.5.1 Generation

As per the following chemical reaction, SBS is formed when sodium metabisulfite (SMBS), a decomposable, white powder, is dissolved in water[147].

$$Na_2S_2O_5 + H_2O \rightarrow 2\ NaHSO_3$$

Since SMBS is readily soluble in water (3,000 g/L in water at 20°C, according to Ough & Were (2005), the compounds may be considered functionally equivalent in water treatment applications[148]. Both compounds are regularly used as preservatives and gradually oxidize and turn into sulphate due to the loss of sulfur dioxide when exposed to air[115]. In solution, SBS speciates into sufilte di-anions , bisulfite anions (HSO_3^-), sulfurous acid (H_2SO_3), and sulfur dioxide (SO_2), in proportions dependent on pH[149]. In a 10% aqueous solution of SBS, the pH range of 2.5–5.5 favors the production of sulfurous acid[115]. Solid SMBS has a typical shelf life of 4–6 months under cool, dry storage conditions[150]. A typical solution life of SBS varies with concentration. At 100,000, 200,000, and 300,000 ppm, the solution life of SBS is 1 week, 1 month, and 6 months, respectively[150].

2.5.2 Mechanisms of action

At higher concentrations, SBS can be used as a non-oxidizing antimicrobial with a relatively simple mechanism of action. SBS scavenges oxygen, effectively removing it from the surrounding environment and killing aerobic bacteria[71,151]. SBS is a cell membrane-active biocide; its strong bactericidal effect comes from inhibiting key enzymes in microorganisms, such as those involved in energy production (ATP synthesis) and bacterial metabolism[145,152]. Due to its oxygen scavenging potential, the addition of SBS effectively controls aerobic bacterial growth but simultaneously creates an environment where anaerobic bacteria can proliferate[10]. Additionally, Baker & Dudley (1998) reported that SBS would be ineffective against bacteria that can adapt to anaerobic conditions, such as sulphate-reducing bacteria[77]. SBS is described as having a bacteriostatic effect, since it prevents biological growth but does not remove existing bacteria[10]. As a result, SBS will not be effective on mature biofilms or EPS and is only effective in feed water that has a low potential for fouling. Ohara et al. (2020) reported that SBS had a strong bactericidal effect at pH 4.6 [145]. This is supported by other studies which concluded the efficacy of SBS increased in acidic conditions[64,122,153]. The effect of temperature on the efficacy of SBS was not found in the literature.

2.5.3 Anti-biofouling effect: dosage and efficacy

Sodium bisulfite is commonly used to limit microbial growth in RO systems through shock dosing and is used as the chemical additive for inhibiting biological growth during membrane storage[10,147]. For shocking dosing, 500–1,000 mg/L, or 0.05–0.1 wt% of SBS is dosed in the feed line for 30 min[10,150,154]. Shock treatment is carried out every 24 h, or when microbial growth is suspected, in order to control aerobic bacteria[150]. An SBS solution of 0.1–1% concentration is used to inhibit biofouling during membrane storage[10,64,154]. Redondo & Lomax (2001) reported that continuous dosing of up to 50 ppm SBS in the feed stream of RO plants was effective in controlling biological fouling, although this was in low-to medium-fouling potential seawater[147].

Baker & Dudley (1998) reported on the efficacy of shock dosing SBS as a biocide, stating that after 4 h of exposure at 500 ppm of SBS, there was a 75% kill noted for aerobic marine bacteria[77]. Alternatively, other data indicated kill rates of up to 99% for seawater microflora at a concentration of 500 ppm SBS with a contact time of 30 min[77]. A study by Murano et al. (2005), on the bactericidal effects of SBS at varying acidic conditions, reported that SBS did not affect the viability of the target organism (*H. pylori*) at neutral pH (7.0) and was more effective at a lower pH [153]. While studying the effects of SBS on pathogenic microorganisms in catheter lumens, Ohara et al. (2020) confirmed that, at a low pH, SBS has an evident bactericidal effect against both *P. aeruginosa* and *S. aureus*[145]. For SBS in particular, the type of microorganism present in the feed water will have a significant effect on its relative efficacy. Although SBS is effective at controlling aerobic bacteria, it creates an ideal environment for anaerobic bacteria and can be ineffective against sulphate-reducing bacteria which are able to adapt to anaerobic conditions[77]. Penna et al. (2002) listed the lowest concentration of SBS which prevents visible growth of *P. aeruginosa*, or the MIC, as 780 mg/L [64]. The MIC and MBC of SBS found for *S. aureus* was 512 mg/L. The MBEC and MBIC for either target microorganism were not found in the literature (Table 3).

Concentrations of SBS used for shock dosing and long-term storage of membranes have been largely accepted as industry standards; however, the results presented by Baker & Dudley (1998) indicate the questionable efficacy of shock dosing SBS[77]. SBS has been shown to have a strong bactericidal effect on planktonic cells and is a widely used preservative for relatively clean membranes during long-term storage, although it is not effective against existing biofilms or anaerobic bacteria.

2.5.4 Membrane compatibility

The compatibility of SBS with RO membranes and overall system components is confirmed by the regular use of the biocide as a preservative during long-term RO membrane storage, application as an agent in membrane feed water pretreatment, and common use in a variety of other water treatment processes. Wei et al. (2012) studied the effects of polysulfone ultrafiltration membrane surface

modification by the pre-adsorption of SBS and found that it improved membrane antifouling properties while increasing flux and salt rejection[154]. No reports were found in the literature as to whether the same effects would be seen with the adsorption of SBS on polyamide membranes.

Feed water quality should be carefully monitored to ensure compatibility with the proposed biocide. In cases where the feed water results in the RO membrane being fouled with heavy metals, SBS will partially convert to oxidants, resulting in membrane degradation[150]. If chlorine and SBS are being dosed consecutively in the treatment process, the SBS injection point may lead to an increase in anaerobic biological growth. In combination with chlorination, Hoeck (2017) noted a decrease in RO membrane biofouling potential when the SBS injection point was placed between dual media filters and cartridge filters, and biofouling potential increased as the injection point moved closer to the RO membranes[155]. During membrane storage, SBS scavenges oxygen from the air, producing sodium sulphate and causing a decrease in pH [10]. It is recommended to periodically change the bisulfite solution during membrane storage in order to control changes in pH so as to not lead to membrane degradation. During RO applications, the process pH after the addition of SBS should be carefully monitored in order to avoid membrane degradation. Additionally, feed water should be analyzed for heavy metals, which can react with dissolved SBS and foul the membrane. Overall, as evidenced by its wide use as a preservative during membrane storage and common application in membrane feed water pretreatment, SBS is very compatible with polyamide membranes in most cases.

2.5.5 Human and environmental health hazards

It is widely accepted that SBS in small doses is a relatively low-hazard antimicrobial, evident from its varied use in food, cosmetics, and pharmaceutical products. However, concentrated solutions are irritating to the skin, eyes, and mucous membranes, and even low doses may cause adverse reactions to individuals with sulfite allergies[156,157]. Many studies have recognized sensitivity to sulfites in some individuals and reported adverse effects after topical and oral exposure, ranging from asthma, dermatitis, and rhinoconjunctivitis to life-threatening anaphylactic and asthmatic reactions[116,158,159]. At a neutral pH, a large portion of aqueous SBS will be composed of the sulfite dianion, which is considered to be responsible for the skin sensitization potency of SBS[149]. As such, SBS requires careful handling since it can pose a high human hazard to individuals who are sensitive to the compound.

In drinking water applications, overdosing SBS may lead to higher levels of the biocide in distributed water, potentially causing human health concerns. The bactericidal effect of SBS on beneficial bacteria in the human digestive system was studied by Irwin et al. (2017), concluding that 2 h of exposure to sulfite concentrations between 250 and 500 ppm could substantially alter the gut and/or mouth microbiome[152]. In RO applications, overdosing SBS may pose an environmental hazard as excess levels of SBS in either the concentrate or permeate will scavenge oxygen when distributed to

receiving waters. The environmental hazard of SBS was reported by Ryon et al. (2002), who studied the impact on streams from the use of SBS as a dechlorinating agent[122]. The study concluded that inaccurate dosing or overfeed of SBS can create an excess of sulfite in receiving waters, potentially leading to decreases in pH and dissolved oxygen, and causing mortality in fish. As a result, according to Redondo & Lomax (2001), during shock treatments when the permeate contains 1–4% of the concentration of SBS in the feed water, an assessment of the permeate quality should be completed prior to discharge to evaluate if the permeate requires further treatment[147].

Sulfite allergies are relatively common; 1.1–4.5% of the general population are sensitive to sulfites and about 39.9% of positive test results are considered clinically relevant[158,160,161]. The risk of severe reactions in certain individuals necessitates careful handling of SBS, especially since occupational exposure is one of the most common sources responsible for the presentation of sulfite sensitivities[161,162]. If there are significant levels of SBS in distributed waters, its oxygen scavenging potential has been shown to threaten certain aquatic environments; however, SBS does not pose a significant risk to the overall environment as it quickly biodegrades[115].

2.5.6 Advantages and limitations

By reducing oxygen concentrations, SBS controls aerobic bacterial growth and effectively reduces the potential for membrane fouling. However, the efficacy of shock dosing SBS has been debated for marine bacteria and microflora, and shock dosing has been proven ineffective against microorganisms that can adapt to anaerobic conditions[77]. Additionally, the application of SBS is limited to relatively clean water, as it is ineffective against existing biofilms and high-fouling potential water[147]. SBS dosing is also not recommended in cases where the feed water contains heavy metals, as they react with SBS residual, forming oxidants and fouling the membrane[150]. The optimum dosing concentration of SBS is dependent on the type of microorganism present and varies as the composition of the feed water changes, making the optimal dosing concentration of the biocide difficult to maintain. Further, SBS is more effective at a lower pH, which is not compatible with RO system components. Methods for monitoring SBS dosage, other than monitoring the pH of the feed water and biocide solution, were not found in the literature. Overdosing SBS will potentially cause a higher residual concentration in the permeate stream, decreasing pH and dissolved oxygen content thus posing a threat to aquatic life, or killing gastrointestinal bacteria after human consumption[122,152]. As a result, treatment with SBS requires off-line dosing in potable water applications and careful monitoring of permeate and concentrate quality when distributing water to sensitive aquatic environments. A study by Majamaa et al. (2011), on the comparison of non-oxidizing biocides with SMBS, concluded that SBS is a relatively costly method for limiting biofouling in storage, due to the high concentration of chemical required and short preservation time in comparison to other non-oxidizing biocides[102].

Although comparatively costly, SBS in solution is easy to use as it can be directly injected into the feed stream during normal plant operation and can be easily stored as SMBS[150]. Additionally, SBS is formed by dissolving SMBS in water, indicating very easy generation[150]. Other advantages include that, along with limiting microbial fouling in RO systems, SBS dosing can be used to control colloidal fouling[147,163] and the acidic reaction of SBS allows for calcium carbonate control[154]. Overall, the compatibility of SBS with polyamide membranes, its widespread use as a preservative during membrane storage, and its easy application make it a worthy prospect to investigate further as a preventative biocide in RO systems.

2.6 Phenoxyethanol (PE)

2-phenoxyethanol (PE), an aromatic glycol ether, is widely used as a personal care product preservative due to its broad antimicrobial activity[68,74,164]. PE is commonly added to cosmetic and pharmaceutical products, such as hand disinfecting biocidal products and vaccines[68,165]. PE is a biocide of special interest, due to its chemical and physical properties, including effectiveness as a solvent and antimicrobial[74,166]. The preservative, naturally found in green tea, has a weaker inhibitory effect on desirable skin-resident bacteria than other cosmetic chemicals preservatives[166]; therefore, PE is used in a large range of skincare and cosmetic products, such as mascara and sunscreen creams[167–169]. Although there is no evidence of PE application to industrial water systems in literature studies, PE exhibits beneficial characteristics as a biocide and may have the potential for use on RO systems[26,91].

2.6.1 Generation

2-phenoxyethanol is manufactured by processing phenol with ethylene oxide in the presence of a catalyst under pressure and high temperatures[169]. The preservative is commonly produced in laboratories due to the importance of purity in the cosmetic and pharmaceutical industry[164]. Stability of the biocide has been demonstrated and preservative activity does not decrease with time; PE can withstand prolonged storage, transportation, or use[74].

2.6.2 Mechanisms of action

2-phenoxyethanol has a large spectrum of antimicrobial activity and is effective against Gram-negative, Gram-positive, molds, and yeast[109,170]. It is noted that PE is more effective against Gram-positive than Gram-negative bacteria[74]. The mechanism of action of PE is as a lytic membrane-active biocide[69]. It acts by disrupting the bacterial cell membrane, leading to rapid cell lysis and leakage of the cellular protein[171,172]. PE destabilizes the cell membrane by uncoupling oxidative phosphorylation, preventing respiration, and obstructing malate dehydrogenase[170], an enzyme responsible for a metabolic reaction pathway[173]. PE also acts as a biocide by using a direct inhibitory

effect on microbial DNA and RNA synthesis and increases the permeability of the cell membrane to potassium ions[174], resulting in cell death.

2.6.3 Anti-biofouling effect: dosage and efficacy

In the health and personal care industries, PE is used as a preservative or antimicrobial agent with the typical concentration range from 5,000 to 10,000 mg/L[74]. The biocidal efficacy of PE is most effective in the pH range of 4–5 [74]. According to Paulus (2005), the use of PE alone in formulations is rarely sufficient in terms of efficacy[74]. To increase the antimicrobial efficacy, PE is generally blended with other preservatives such as dibromodicyanobutane, PHB ester, iodopropinylbutylcarbamate, and formaldehyde.

The efficacy of PE, including the MIC, MBIC, and MBEC are displayed in Table 3. The inhibitory concentrations presented in Table 3 indicate that PE will be more effective at lower concentrations to prevent biofouling compared to removing the existing biofilm as indicated by the MBIC and MBEC, respectively. The information also confirms that PE is a weaker antimicrobial when compared to DBNPA or MIT and will need to be applied to RO at higher concentrations[26,40,91].

2.6.4 Membrane compatibility

There were no studies in the literature of PE being used as a biocide to prevent biofouling in water treatment applications, including RO, or for membrane storage, therefore, no studies verifying PE compatibility with RO membranes. However, due to its non-oxidizing nature, we hypothesize that PE is compatible with polyamide membranes.

2.6.5 Human and environmental health hazards

The use of PE is considered safe for consumers of all ages when applied as a preservative in cosmetic products at a maximum concentration of 10,000 mg/L[166,175]. PE is a rare sensitizer and is considered one of the most well-tolerated preservatives used in personal care products[176]. PE can cause toxic effects if inhaled, ingested, or skin contact, including skin, lung and liver irritation, kidney, and liver damage[164,170]. Moreover, the U.S. Food and Drug Administration reported that high doses of PE may cause dehydration, vomiting, nervous system problems, and diarrhea in infants and nursing mothers[177,178]. Therefore, safety precautions and appropriate personal protective equipment must be considered when handling PE concentrations over 10,000 mg/L.

In several studies, PE is confirmed as a very low hazard for environmental fate, including persistence and bioaccumulation[114,179]. Researchers Lyman et al. (1990) estimate that PE will not hydrolyze in the environment because of the absence of functional groups that hydrolyze under environmental conditions (Appendix A)[180]. In addition, acute aquatic toxicity and chronic aquatic toxicity scored

low hazard based on the LC/EC_{50} and NOEC values[114]. However, the chronic aquatic NOEC values are assigned low confidence and therefore additional testing is required. In summary, PE generally has minimal impact on the environment and aquatic life and is therefore inherently safer for water treatment applications than many other choices.

2.6.6 Advantages and limitations

A major limitation of PE involves the low biocidal efficacy, indicated by the high MIC and MBEC values which would result in higher concentrations needed when applied to RO systems[26,91]. The larger dosage will result in the application of PE to be more expensive, even though the preservative is known to be low cost in the cosmetic industry[181]. As a result of the high concentration required for application, the presence of total dissolved solids will increase, potentially exceeding water quality guidelines. Another drawback of PE is there is no evidence that PE is compatible with polyamide membranes, disinfects the permeate, or whether it is more effective at preventing or removing biofouling when dosed continuously or intermittently. Nevertheless, since PE is a non-oxidizing biocide, it can be hypothesized that PE will be compatible with polyamide membranes. The advantages of 2-phenoxyethanol include the preservative stability and solubility[74,182]. PE is soluble in water at 26,700 mg/L water at 20°C[183]. The preservative stability benefits the application for water treatment, including ease of chemical injection, storage, and versatile use including membrane storage. Other advantages of PE include low environmental and human impact, so handling and disposing of biocide waste will likely be safe when concentrations are less than 10,000 mg/L[114,166,175].

2.7 Sodium benzoate

Sodium benzoate is the sodium salt of benzoic acid. Benzoic acid is the simplest aromatic carboxylic acid and it is commonly found in nature[184]. Chipley et al. (2020) reported that SB is inexpensive and easy to obtain[185]. It is used as a preservative in cosmetic and personal care products, such as foundation, cologne, shampoo, and moisturizer[67]. It is used as an antimicrobial in food and is Generally Recognized as Safe (GRAS) by the United States Food and Drug Administration up to a concentration of 1,000 mg/L[186,187]. Many bacterial and fungal species are effectively inhibited and killed by SB[188]. SB is used in pharmacology to treat urea cycle disorders[189], acute hepatic encephalopathy[190], as an add-on drug to treat schizophrenia[191], and in combination with other compounds to treat various types of headaches[192]. SB is not used in polyamide membrane systems, but it is a promising antimicrobial because it is inexpensive, very soluble in water, and kills a variety of microorganisms.

2.7.1 Generation

The synthesis of SB consists of the neutralization of benzoic acid using sodium bicarbonate, sodium carbonate, or sodium hydroxide[121,193] as shown in Figure 5. Benzoic acid is produced by the decarboxylation of phthalic anhydride, alternatively, the chlorination of toluene to benzotrichloride, which is hydrolyzed to benzoic acid[193]. Benzoic acid can also be produced via the liquid phase oxidation of toluene[121].

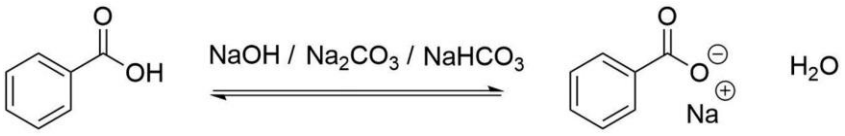


Figure 5 - Neutralization of benzoic acid

2.7.2 Mechanisms of action

Sodium benzoate is a weak acid with a pK_a of 4.2 [194]. Weak acid preservatives are more generally effective at lower pH values because a larger portion of the acid molecules is neutrally charged. If a molecule is neutrally charged, there is a higher chance of it diffusing across a cell membrane. Krebs et al. (1983) showed that benzoate acts as an antifungal against S. cerevisiae by greatly decreasing the intracellular pH[195]. At low extracellular pH, neutral benzoic acid diffuses into the intracellular space at which point the benzoic acid is subjected to physiological pH 7.4. Since the intracellular pH is higher than the pK_a of benzoic acid, it dissociates from its acidic proton. The yeast was able to maintain a physiological pH in media conditions that ranged from pH 2.5 to 7.4 in the absence of SB. The researchers noticed there was a great decrease in intracellular adenosine triphosphate (ATP) upon treatment with benzoate. Krebs et al. (1983) hypothesized that benzoate inhibited glycolytic enzymes such as phosphofructokinase[195] but this hypothesis was rejected when there was no specific blockage of glycolysis[196]. Between 60 and 120 mg/L of benzoic acid, Warth (1991) found the fermentation rate, ATP concentration, and intracellular pH of *S. cerevisiae* declined[196]. At relatively high benzoic acid concentrations, 0.12–300 mg/L, growth was fully inhibited but the cells did not rapidly die. A similar mechanism is believed to be the reason for the antibacterial effects of SB. When the intracellular pH decreases, the bacterium must pump protons out of the cell to maintain physiological pH [197]. This pumping consumes ATP which inhibits bacterial growth and can result in cell death. Based on this mechanism, it is classified as a protonophore (Figure 4). The antimicrobial activity of benzoate is effective against fungi, Gram-positive bacteria, and Gram-negative bacteria.

2.7.3 Anti-biofouling effect: dosage and efficacy

Table 3 shows the inhibitory concentrations of SB against relevant biofilm pioneer microorganisms. Karabay & Sahin (2005) reported that SB was effective against methicillin-resistant *S. aureus* (MRSA), and methicillin-sensitive *S. aureus* (MSSA) isolates in vitro. MSSA and MRSA isolates were obtained from hospitalized patients[198]. Both isolates were sensitive at SB concentrations of 32 mg/L and up. Karabay et al. (2006) also studied the activity of SB against *Enterococcus* species[188]. The authors found that the minimum concentration of SB used to inhibit 90% of microorganism growth (MIC90) against *E. faecalis* was 64 and 32 mg/L against *E. faecium*. *Salmonella* species were isolated and cultured from chicken samples by Er et al. (2014), and the antibacterial properties of preservatives were tested in microtiter plates[199]. The MIC of SB was found to range from 25 to 250 mg/L at pH 7, whereas the MBEC was 1,600–7,600 mg/L. Bacteria were consistently inhibited more effectively at lower pH. There have been no experiments using SB to prevent or treat biofouling in RO systems.

Haque et al. (2005) assessed preventing bacterial attachment and biofilm formation in fresh water using SB[200]. The authors used SB dispersed in solution and entrapped in silicone coatings over a sampling period of 28 days. The optimal concentration of dispersed SB was 700 mg/L. This concentration led to 3.3% biofilm surface coverage (79% reduction relative to control) of silicone slides after 28 days. The bacteria were isolated from fresh lake water and enriched during the challenge period of SB incubation.

2.7.4 Membrane compatibility

The direct application of SB in RO systems has not been demonstrated yet. Mohammad & Ali (2002) used SB along with other chemicals of interest to study rejection rates of polyamide nanofiltration (NF) membranes[201]. The researchers concluded that SB was consistently rejected by NF membranes and it did not reduce the salt rejection of the membranes over time for different permeate fluxes signifying no inherent membrane degradation. The results support the potential polyamide NF membrane compatibility with SB; however, no direct analysis of degradation on the polyamide surface was completed. The results reported by Mohammad & Ali (2002) indicate that SB might also be directly compatible with polyamide RO membranes since NF membranes and RO membranes are made of the same polymeric materials[201]. This is supported by the mechanism of antimicrobial action of SB, which is non-oxidizing.

2.7.5 Human and environmental health hazards

The biodegradation of SB is rapid in aerobic, aqueous conditions such as seawater[121]. No experimental data is available detailing the accumulation of SB in the environment, but it acts

similarly to benzoic acid, which poses a low environmental hazard (Appendix A). In the mitochondria, SB and glycine are combined to form hippurate, which is then cleared by the kidneys[202]. SB is metabolized into hippurate to increase hydrophilicity, which decreases the diffusion of benzoate back over the inner mitochondrial matrix. Lennerz et al. (2015) performed oral administration of the GRAS dose of SB in combination with glucose to study its effect on glucose homeostasis in humans[203]. They found no statistically significant difference in serum insulin or plasma glucose between consumption of glucose beverages with and without SB. Ingestion of SB was found to significantly influence circulating hippurate, acetyl lysine, and anthranilic acid. Benzoate caused a significant decrease in glycine. Lennerz et al. (2015) provided good insight into the acute effects of GRAS benzoate consumption, but the study does not allow us to draw conclusions about long-term exposures or doses higher than the GRAS dose[203]. Considering this evidence, SB has low human and environmental risk generally, and especially when compared to other biocides used in water treatment.

2.7.6 Advantages and limitations

Sodium benzoate is very soluble in water, up to 550,000 mg/L [200], which enables practical use in RO systems. SB is relatively inexpensive compared to other non-oxidizing biocides such as MIT and DBNPA. It is similarly priced to PE and SBS from common chemical suppliers. Depending on the microorganism PE may be more or less effective per dollar spent than SB (Table 3). SBS is less expensive than SB to achieve similar outcomes. The human and environmental hazards are low and SB is biodegradable. SB has relatively high MIC, MBIC, and MBEC values, which would require larger doses when compared to other antimicrobials to achieve similar outcomes (Table 3). This drawback can be offset by how inexpensive it is. According to Mohammad & Ali (2002), SB does not pass into the permeate, and therefore, does not disinfect it[201]. To date, SB has not been used in RO application but it is a promising biocide that poses a little relative hazard to humans and the environment (Tables 4 and 5).

2.8 Other non-oxidizing biocides

We have selected an additional four non-oxidizing biocides that are promising for use in RO applications. These biocides have not necessarily been studied in membrane applications, but based on their antimicrobial properties and compatibility, we believe they should be considered for future research and use. Limited data have been reported on these chemicals and whether they can effectively prevent biofouling. Human and environmental toxicity studies should be done on these additional biocides to determine if they are suitable for potable water uses.

2.8.1 Lauroyl arginate ethyl

Lauroyl arginate ethyl (LAE) is a cationic biocide that acts by interacting with the negatively charged membrane of bacteria, which ruins its integrity[103]. LAE was approved as a food additive by the FDA in 2005[187]. It is currently used as a preservative for food and beverages[204] and is found in some mouthwashes[205]. Kim & Park (2016) evaluated LAE for use in RO systems and found that it does not damage polyamide membranes[103]. Four bacterial species of interest (*P. aeruginosa*, *E. coli*, *S. aureus*, and *E. faecalis*) were used to test LAE and DBNPA to compare antimicrobial activities. LAE had a higher killing effect than DBNPA against every bacteria tested in suspension. LAE was also superior to DBNPA at inhibiting biofilm formation and destroying biofilms. The water flux of a polyamide membrane was not influenced by increasing concentrations of LAE except for at 100,000 mg/L which the authors proposed was caused by residual LAE on the membrane surface. Hawkins et al. (2009) studied the in vitro and in vivo metabolism of LAE in humans[206]. The authors found LAE was rapidly metabolized into naturally occurring dietary molecules and concluded it poses little to no safety risk as a food preservative. One drawback of LAE is how expensive it is[103]. Overall, because of its low toxicity, antimicrobial properties, and membrane compatibility, LAE is a very promising chemical for polyamide filtration use.

2.8.2 Polyhexamethylene guanidine hydrochloride

Polyhexamethylene guanidine hydrochloride (PHMGH) is a cationic polymer that has shown to be strongly biocidal yet has low toxicity to humans[207]. PHMG is written as an ambiguous salt form of PHMG, whereas PHMGH specifies the hydrochloride salt. Oulé et al. (2008) showed that PHMGH acts as an antimicrobial by causing tears in the cell membrane, which spills the cytoplasmic contents into the media and kills the cell[208]. The positive charge of PHMGH interacts strongly with bacterial membranes and displaces stabilizing Mg^{2+} and Ca^{2+}. Suspensions of *S. aureus* and *P. aeruginosa* were treated with various molecular structures of PHMG by Wei et al. (2009), and it was found that aqueous PHMGH had excellent antimicrobial activity when the molecular weight was above 640 Da[209]. Kim et al. (2016) subjected rats to aerosolized 100 nm PHMG-phosphate particles to study pulmonary inflammatory and fibrotic responses[210]. The researchers found inflammatory cytokines, ROS generation, fibrosis, and airway barrier injuries in the rats. Mathurin et al. (2012) tested PHMGH against several reference bacteria and fungi[211]. The researchers proposed that PHMGH was less effective against fungi compared to bacteria because of their rigid cell wall. PHMG was reacted with ethylene glycol diglycidyl ether and subsequently chemically grafted onto polyamide (PA6) membranes to study its antimicrobial and chemical properties[212]. The PHMG bonded to the polyamide membranes inhibited the growth of *E. coli* and *S. aureus* by more than 99.99%. Additionally, the study showed that PHMG did become unbonded from the membrane and leach into

the water, and the modified membranes kept their mechanical properties. It has been used in cooling water systems but not in RO systems[213].

2.8.3 Benzyldimethyldodecyl ammonium chloride

Benzyldimethyldodecyl ammonium chloride (BDMDAC) is a quaternary ammonium salt agent and cationic biocide, routinely used as a disinfectant in swimming pools and industrial cooling water systems[214,215]. BDMDAC forms an electrostatic bond with the negatively charged sites on microbial cell walls, causing cell lysis, disruption of cell-wall permeability, and reduced intake of nutrients to the cell[215]. BDMDAC has very good inactivation potential, but Gomes et al. (2014) concluded that it was unsuccessful at removing biofilms[216]. Further, BDMDAC has been found to be notoriously ineffective for Gram-negative bacteria, specifically, *P. aeruginosa*[217]. Alternatively, Gomes et al. (2021) found that BDMDAC in combination with mechanical stress was effective in removing mature biofilms, although not completely[218]. A study by Hegstad et al. (2010) reported that quaternary ammonium compounds (QACs) generally have poor degradability and that the concentration of QACs in sewage depends on the alkyl chain length, of which C12 (the alkyl chain length of BDMDAC) was the most prevalent[217]. Additionally, QACs are generally low cost but can be deactivated by hardness and have poor compatibility with polyanionic polymers[80]. The ineffectiveness of BDMDAC on the primary microbiological problem area, Gram-negative bacteria, along with the observed retention in sewers and receiving waters, limits the application of BDMDAC and other QACs as biocides in RO systems. As a result, BDMDAC is not considered a promising candidate for application as a preventative biocide in RO systems.

2.8.4 Sodium nitroprusside

Sodium nitroprusside (SNP), a nitric oxide (NO) donor compound, has a direct effect on biofilm reduction through the release of the NO free radical. SNP releases NO at picomolar and nanomolar levels, which have been shown to interact with sessile *P. aeruginosa* cells and function as a signal for the cells to transition back into a planktonic-like state, resulting in the reduction of total biofilm surface and the restoration of cell vulnerability to antimicrobials[22,219]. Alternatively, Nagaraja et al. (2017) concluded that SNP acts by degrading polysaccharides in the biofilm layer[32]. SNP is highly soluble in water (400 g/L, 25°C) and has the greatest efficacy at pH 5.0, with decreasing amounts of NO released up to pH 7.2 [32]. Multiple studies have found NO donor compounds to be effective against both Gram-positive and Gram-negative bacteria, and overall broadly effective on single-and multi-species biofilms[219,220]. This is supported by Nagaraja et al. (2017), who reported that SNP effectively dispersed a multi-species biofilm from an industrially fouled RO membrane[32]. Further, SNP was found to be more effective in recovering membrane permeability as compared to DBNPA[32]. The concentration of released NO that is effective against biofilms is several orders of magnitude below toxic levels and so should not pose a threat to humans or the environment[32,219]. Although results from

previous studies look promising, a recent paper reports that some bacterial pathogens have evolved mechanisms for NO resistance[155]. Still, NO donor compounds such as SNP, which are still in the pilot-scale of testing, can be further investigated for compatibility with RO membranes and efficacy in RO systems.

2.9 Discussion

The biofouling of polyamide membranes is one of the most challenging barriers faced by RO technologies to achieve a sustainable water supply. Membrane biofouling results in (1) an increase in the energy demand and capital cost of RO systems, (2) reduction of membrane lifespan due to frequent cleanings, and (3) loss of productivity. According to Kucera (2019), the traditional methods applied to address biofouling, feed pretreatment by chlorine disinfection, and membrane cleaning by non-oxidizing biocides, are not efficient to solve the problem of biofouling because they are not preventive techniques[10]. To overcome these issues, the introduction of new sustainable anti-biofouling agents and practices to the RO market is necessary. Based on the work presented herein, there are many potential candidates to prevent biofouling in RO systems. These biocides may come either from the non-oxidizing biocides already applied in RO installations, such as MIT, DBNPA, and SBS or low-hazard chemicals like SB and PE. However, the main limitation to finding an ideal biocide to address polyamide membrane biofouling relies on the difficulty to find antifoulants that are (a) efficient in preventing and treating biofilms, (b) compatible with membranes, (c) non-hazardous, and (d) inexpensive.

Figure 6 provides a comparison between an ideal biocide applicable to biofouling prevention and control in polyamide membrane systems and the five selected biocides discussed herein. Table 6 presents a summary of the main advantages and limitations of all anti-biofouling agents reviewed in this study in addressing biofouling prevention in RO systems. Among all five biocides discussed in detail, in terms of inhibitory concentrations (Table 3) and effectiveness against a wide spectrum of microorganisms (Figure 6), MIT presented the best antimicrobial efficiencies to prevent and disinfect membrane biofouling. On the other hand, due to the high doses needed for biofouling control and toxicity, MIT also presented the highest hazards to humans and aquatic life if compared to the other biocides, limiting its use in continuous inline water applications. DBNPA showed to be effective against biofouling prevention in clean membranes, showing a fast kill response and good compatibility with polyamide membranes. The studies discussed in this paper indicated that DBNPA is not effective against existing biofilms. It also has a short half-life of around 24 h in pH levels typical of polyamide membrane applications, and it may present adverse effects to humans and the environment (Figure 6 and Table 6). These constraints limit the long-term application of DBNPA in biofouling prevention in drinking water applications.

Table 6 - Summary of main advantages and limitations of non-oxidizing biocides for biofouling prevention in polyamide membrane systems

Biocide	Advantages	Limitations	Development Stage
DBNPA	1 - Fast biocidal activity[a] 2 - Prevents biofilms well[c]	1 - Not effective against existing biofilms[b] 2 - Short half-life at pH higher than 7[d]	Full-scale applications
MIT	1 - Effective against a wide range of microorganisms[e] 2 - Inhibits growth and kills biofilm pioneers[c]	1 - Hazardous chemical[c] 2 - RO concentrate needs to be treated[f]	Full-scale applications
SBS	1 - Good for long-term RO applications[c] 2 - effective against biofilm pioneers[c]	1 - Not effective against anaerobic bacteria[c] 2 - Expensive compared to other biocides[g]	Full-scale applications
PE	1 - Effective against biofilm pioneers[h] 2 - Low hazards[h]	1 - High doses needed for biofouling prevention[h]	Under development stage
SB	1 - Effective against biofilm pioneers[h] 2 - Inexpensive[i]	1 - Moderate doses needed for biofouling prevention[h]	Under development stage
LAE	1 - Compatible with polyamide membranes[j] 2 - Treats and prevents biofilms[j]	1 - More effective against bacteria in suspension[j]	Under development stage
PHMGH	1 - Effective against biofilm pioneers[k] 2 - Low hazards[l]	1 - Less effective against fungi[m]	Under development stage
BDMDAC	1 - Low hazards[n]	1 - Not effective in removing existing biofilms[0] 2 - Not effective against biofilm pioneers[p]	Under development stage
SNP	1 - Effective against biofilm pioneers[q] 2 - Removes existing biofilm[q]	1 - Hazardous at high concentrations[r] 2 - May lead to microbial resistance[s]	Under development stage

Source (a - Schook *et al.* 2012[89]; b - Siddiqui *et al.* 2017[34]; c - Kucera 2019[10]; d - Exner *et al.* 1973[79]; e - Silva *et al.* 2020[37]; f - Li *et al.* 2016[125]; g - Majamaa *et al.* 2011[102]; h - Curtin 2020[26]; i - Chipley *et al.* 2020[185]; j - Kim & Park 2016[103]; k - Wei *et al.* 2009[209]; l - Park *et al.* 2020[207]; m - Mathurin *et al.* 2012[211]; n - Tan *et al.* 2017[221]; o - Gomes *et al.* 2014[216], p - Hegstad *et al.* 2010[217]; q - Barraud *et al.* 2009[219]; r - Nguyen *et al.* 2012[22]; s - Hoeck 2017[155]).

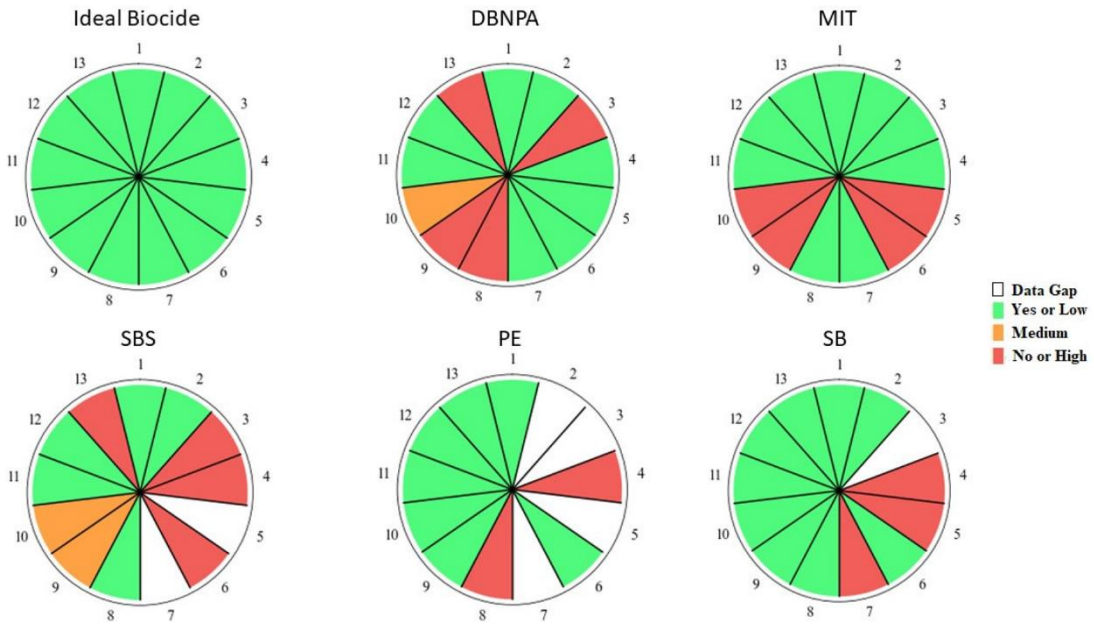


Figure 6 - Ideal biocide for RO polyamide membrane applications. White color refers to data gaps. Green refers to 'yes' or low-hazard level, orange refers to medium hazard level, and Red refers to 'no' or high hazard level.

Different from the other two commercial non-oxidizing biocides applied in the RO market (DBNPA and MIT), SBS can be applied in continuous inline drinking water applications. Nevertheless, high concentrations of SBS in the water distribution system due to overfeeding may pose adverse effects to humans if ingested, and to aquatic life. In polyamide membrane applications, SBS is the standard chemical for dechlorination and membrane storage[10]. According to the literature, SBS presented moderate antimicrobial effects against aerobic bacteria, as compared to other chemicals; however, it was not effective against anaerobic bacteria or existing biofilms (Figure 6, Tables 3 and 6). SBS is also more effective at low pH, which is a concern for RO applications because low pH levels damage polyamide membranes. The high doses and associated costs required to prevent microbial growth, along with the efficacy of the biocide restricted to low-fouling potential water, limit the application of SBS for biofouling prevention in RO systems. Further research needs to be done to determine minimum inhibitory concentrations of biofilm pioneers in RO systems for SBS as well as DBNPA (Table 3). For these reasons, the application of DBNPA, MIT, or SBS is not recommended as a permanent solution to prevent biofouling in potable water membrane system applications.

Despite the factors limiting the application of these three commercial biocides, they should still be considered in RO biofouling control and prevention studies. This is because each of these three imperfect biocides is a model of several of the features found in the ideal biocide described by Bates (1998)[39]. MIT is a good model of a biocide that presents high antimicrobial efficiency as it is concurrently effective in preventing and removing existing biofilms. DBNPA is a good reference for a fast response biocide which is effective in biofilm prevention. SBS is a model for a low-hazard chemical that is compatible with polyamide membranes and effective against biofilm pioneers. It is

important to notice that the discussion of biofouling prevention should also include biofouling treatment. As such, the search for low-hazard alternatives, simultaneously effective in biofouling prevention and biofilm removal, should be continued.

According to the results summarized in Figure 6, PE and SB presented lower human and environmental hazards as compared to MIT and DBNPA. PE was the safest chemical among all five biocides and both PE and SB are able to prevent biofilm pioneers (Figure 6 and Table 6). However, although PE and SB are not expensive products, the relatively high doses required to achieve biofouling prevention would make their application quite expensive and allowable concentrations a significant concern, limiting the use of these biocides in potable water RO applications (Figure 6 and Table 6). Furthermore, similar to SBS, PE and SB biocides are most effective at low pH, which makes their use for continuous treatment in polyamide membranes less attractive. Therefore, further studies need to be conducted to evaluate the feasibility of applying SB and PE in biofouling prevention in polyamide membrane systems.

Several promising non-oxidizing biocides in the very early stages of development were identified in this paper (Table 6). Among the promising non-oxidizing biocides discussed herein, LAE and PHMGH showed to be the most attractive candidates to prevent biofouling in RO systems due to their high effectiveness against biofilm pioneers and low associated hazards. However, comprehensive studies on membrane compatibility, human and environmental hazards, biocidal effectiveness against dynamic biofilms, and RO market readiness are necessary to determine the feasibility and applicability of those chemicals in potable water RO system applications.

In this review, many research gaps were identified in the process of selecting an effective, economic, and eco-friendly biocide applicable to polyamide membrane systems. Biofouling prevention studies are almost non-existent in the literature and unlike for the prevention of planktonic microbial growth (CLSI-M02 2018; CLSI-M07 2018; CLSI-M11 2018; CLSI-M100 2021), there are no standard protocols available to test new biocides for biofouling prevention in RO systems[222–225]. Although there is still a need for standardized protocols to test the efficacy of biocides in preventing biofouling, the metrics and figures of merit for comprehensive monitoring and diagnosis of membrane biofouling are well known[9]. A sizable body of literature exists already on different methodologies to perform (a) membrane autopsies, (b) biofilm detection, (c) impacts of biofouling on RO systems' performance, (d) risk assessment, and (e) antimicrobial efficacy analysis[9,22,26,91]. The translation of research study standard protocols to the industry will depend on the metrics selected to measure biofouling. There are various techniques available to investigate the effects of biofouling on membrane systems in an academic laboratory with spectroscopic facilities, while the majority of available research techniques may be limited in industry. Therefore, the industry applicability of standard protocols and techniques is necessary for testing the effectiveness of biocides in RO systems. Furthermore, more studies need to be done to investigate the long-term application of the selected biocides as well as new alternatives

for preventing biofouling in RO systems. With the exception of DBNPA, no studies from laboratory-scale to full-scale in RO treatment units were found in the literature investigating the use of MIT, PE, SB, and SBS to prevent biofouling in RO systems. Also, further research needs to be performed to evaluate the polyamide membrane compatibility of PE and SB. Moreover, more studies need to be conducted to determine minimum inhibitory concentrations against known biofilm pioneers (including *P. aeruginosa* and *S. aureus*) for all biocides, specifically for DBNPA and SBS.

2.10 Conclusions and prospects

The biofouling of polyamide membranes results in detrimental effects on the performance of RO systems such as an increase in the required operational pressure, a decrease of freshwater production, shortening of membrane lifetime, and increase of operational cost and energy consumption. Overcoming the existing challenges of biofouling in membrane systems is essential to promote sustainable water supply in water-stressed countries. To overcome these challenges, the introduction of new sustainable anti-biofouling agents into the RO market is necessary. This review provided a comprehensive analysis of the applicability of five non-oxidizing biocides (MIT, DBNPA, SBS, PE, and SB) to prevent biofouling in polyamide membranes to optimize RO system performance in drinking water applications. To summarize the major results for each biocide in addressing biofouling in RO systems: (1) MIT presented better antimicrobial efficiencies to prevent and disinfect biofilms in polyamide membranes when compared to other biocides; however, its use in potable water applications may pose threat to humans and the environment; (2) DBNPA showed to be very efficient in preventing biofouling, but its short half-life around neutral pH, low efficiencies against mature biofilm, and hazards limit its application in drinking water treatment by RO technologies; (3) SBS may be applicable inline RO application for potable water treatment. However, its low antimicrobial efficiency and pH effectiveness dependency bring concerns for RO applications; and (4) PE and SB were the safest biocides; however, the high doses required to prevent biofouling may limit their application in polyamide membrane separation technologies. Overall, MIT and DBNPA are excellent models for biofouling prevention studies, between them possessing most of the key features of an ideal biocide when compared to the other biocides discussed in this study. SBS provides a great reference for antimicrobial efficiency against biofilm pioneers. PE and SB provide a good model for low-hazard biocides.

Based on this review, further research efforts should focus on (a) identifying inhibitory concentrations of these biocides against biofilm pioneers such as *Pseudomonas aeruginosa* and *Staphylococcus aureus*; (b) testing the applicability of these chemistries in preventing and controlling biofouling of polyamide membranes through long-term biofouling prevention studies on laboratory-scale, pilot, and full-scale RO treatment systems; (c) verifying the compatibility with polyamide membranes through short and long-term exposure studies, and (d) developing protocols to test the efficacy of

green biocides preventing biofouling in RO systems; (e) finding better candidates for biofouling prevention. Ultimately, these studies will result in the optimization of RO system performance, minimization of operational costs and energy consumption, increase in reliability and environmental performance, and ensure the sustainable application of RO technologies in mitigating water scarcity.

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2.12 Author contributions

Luiz H. Da-Silva-Correa, Hayley Smith, Matthew C. Thibodeau, and Bethany Welsh performed this critical review with the guidance of H.L. Buckley. Luiz H. Da-Silva-Correa wrote the discussion of the information derived from the analyzed literature with assistance from Hayley Smith, Matthew C. Thibodeau, and Bethany Welsh. Luiz H. Da-Silva-Correa led manuscript preparation. H. L. Buckley provided feedback and revised the manuscript. H. L. Buckley secured funding for this project.

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Chapter 3: Efficacy testing of non-oxidizing biocides for polyamide membrane biofouling prevention using a modified CDC biofilm reactor

Luiz H. Da-Silva-Correa[a,c], Kirsten Aasen[b,c], Nicole E. Gamm[a,c], Rafaela Godoy[d], Negar Rahmati[b,c], and Heather L. Buckley[a,b,c]*

[a] Department of Civil Engineering, University of Victoria, Victoria, BC, Canada, V8P 5C2

[b] Department of Chemistry, University of Victoria, Victoria, BC, Canada, V8P 5C2

[c] Center for Advanced Materials and Related Technologies (CAMTEC), and Institute for Integrated Energy System (IESVic), University of Victoria, Victoria, BC, Canada, V8P 5C2

[d] Department of Chemistry, State University of Londrina, Londrina, PR, Brazil, 86057-970

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3.1 Preamble

In this chapter, experiments were conducted in a CDC biofilm reactor, with MBIC and MBEC determined in microtiter plates, to assess the anti-biofouling performance of biocides that passed the first stage of the screening protocol. The initial screening phase concluded that DBNPA and MIT were considered unsuitable for biofouling prevention due to their hazards to humans and the environment. However, they serve as good models for antibiofilm efficacy in biofilm prevention studies (controls). SBS, PE, and SB were considered for further testing despite flagged concerns. BDMDAC was found ineffective against biofilms and pioneer microorganisms in RO systems. Further research is needed to assess PHMGH and SNP for RO potable water systems due to limited available data. LAE was identified as a promising candidate due to its safety and excellent antimicrobial properties. Consequently, only six biocides moved to the antibiofouling testing phase: DBNPA, MIT, SBS, PE, SB, and LAE. It is important to note that PE was initially included in the CDC biofilm reactor experiments. However, membrane autopsies could not be performed with this biocide. This is because PE caused the active membrane layer to detach from the microporous support layer, suggesting membrane damage.

3.2 Abstract

Biofouling is one of the most challenging obstacles faced by reverse osmosis (RO) membrane systems to supply potable water. Currently, biofouling is imperfectly handled by RO feed water pre-chlorination, which is associated with the production of carcinogenic disinfection by-products. To propose a safer alternative to control biofouling in RO drinking water applications, this study investigates the efficacy of five biocides to prevent and remove *Pseudomonas aeruginosa* biofilms from RO membranes: 2-methyl-4-isothiazolin-3-one (MIT); 2,2-dibromo-3-nitrilopropionamide (DBNPA); sodium bisulfite (SBS); sodium benzoate (SB); and ethyl lauroyl arginate (LAE). Experiments were conducted on the United States Center for Disease Control (CDC) Biofilm Reactor with biocidal dosing estimated on 96-well microtiter plates. Confocal Scanning Laser Microscopy (CLSM) and Scanning Electron Microscopy (SEM) were used to analyze the biocides' anti-biofilm efficacies under dynamic conditions relative to minimum biofilm inhibitory and eradication concentrations. The results in this study indicated that LAE presented the best anti-biofilm efficacies in treating *P. aeruginosa* biofilms when compared to all studied biocides; it not only prevented biofilm formation (>98%) but also it effectively removed pre-established biofilms (>99%) from RO membrane coupons. Therefore, due to safety and efficacy, LAE is an excellent candidate for controlling biofouling in drinking water RO membrane systems.

Keywords: biocides, biofilm prevention, biofouling, drinking water, polyamide membranes, reverse osmosis

3.3 Introduction

Reliance on unconventional water resources is becoming increasingly necessary to bridge the water demand–supply gap. Of these sources, desalinated water is critical in relieving the global impacts of water stress, with the potential of providing a climate-independent and virtually unlimited supply of high-quality potable water[226]. Currently, reverse osmosis (RO) leads the desalination industry, boasting the highest energy efficiency of both thermal and membrane-based technologies and accounting for 69% of the installed desalination capacity[227,228]. Despite the growing amount of research and resources expended on RO technology, optimization remains elusive due to fouling – the build-up of colloidal and particulate inorganic, organic, and biological matter on the membrane's surface[41]. Biofouling, in particular, has been identified as the most difficult to control, as living cells are proliferative, and many of the stressors applied as treatment exacerbate natural defense mechanisms, such as planktonic cell attachment and secretion of extracellular polymeric substances (EPS), that support and strengthen biofilm formation[10,22]. Almost any degree of surface colonization can lead to significant reductions in permeate flux and salt rejection, which can result in substantial

economic losses as a result of reduced efficiency, a shorter membrane lifetime, and the need for physical and chemical mitigation strategies[9,10].

Presently, controlling the impacts of biofouling on RO performance is achieved through two primary means: (1) disinfection and (2) cleaning techniques[9,10,22]. Chlorine is the most commonly applied disinfectant, killing a wide range of microorganisms via oxidation at low concentrations[9,22,40]. However, its use in RO systems is limited by the polyamide membranes' susceptibility to oxidative degradation, the formation of harmful disinfection by-products, and its corrosivity and toxicity[9,10,37,40]. Membrane incompatibility, in particular, is detrimental to biocidal efficacy, as oxidative biocides must be removed prior to membrane contact. This leaves the system downstream and most critically the membrane itself, vulnerable to bacterial regrowth. Since no pretreatment method is 100% efficacious, physical cleaning techniques that dislodge foulants using mechanical force are required as secondary treatment options[9,10,22]. These techniques, such as membrane flushing, are efficacious at removing loosely bound layers but cannot remove mature biofilms and residual foulants[10,229]. Furthermore, they are only applied in response to biofilm formation – the maturity of which is marked by a significant increase in mechanical and chemical resistance[20]. This is largely due to the secretion of the EPS, which serves to protect biofilm cells and anchor them to the RO membrane, resulting in enhanced biocidal tolerance[62]. Most notably, biocides have difficulty penetrating the 3D matrix of mature biofilms and those that do are delivered to cells in sub-lethal concentrations, creating the ideal environment for the propagation of antimicrobial resistance[9,28,29,62,91]. Current methods are only able to reduce and delay biofilm formation; therefore, it is necessary to use preventative and membrane-compatibility treatments to promote sustainable RO desalination.

One approach that has demonstrated capacity for long-term biofouling mitigation in potable water treatment systems is the application of non-oxidizing biocides[9,26,103]. This class of antifouling compounds has the potential to meet the majority of the requirements for an ideal biocide listed by Bates (1998), including being low risk to human health and the environment, showing high efficacy in the prevention and removal of all types of microorganisms and providing increased compatibility with the polyamide membrane and all other system components[39]. In this study, based on the above-mentioned characteristics, five non-oxidizing biocides – 2,2 dibromo-3-nitrilopropionamide (DBNPA), 2-methyl-4-isothiazolin-3-one (MIT), sodium bisulfite (SBS), sodium benzoate (SB), and ethyl lauroyl arginate (LAE) – were selected and investigated for their ability to prevent and control biofouling in RO systems. Our previous review provided an in-depth analysis of the selected biocides and summarized important research gaps such as the identification of inhibitory concentrations and laboratory-scale biofouling prevention studies that are explored in our current work[27].

A critical characteristic of an ideal biocide is high efficacy (the ability to produce an intended result) in the prevention and removal of microorganisms[39]. A biocide's efficacy is largely subject to its

Mechanism of Action (MoA)[9,10]. Biocides in this study can be divided into two categories accordingly: electrophiles and membrane-active biocides. MIT and DBNPA are moderate electrophiles that disrupt biofilm growth by entering the cell wall of the microorganism, by diffusion or active cell transport, resulting in permanent damage to intracellular proteins, carbohydrates, lipids, and nucleic acids[9,10,34,65]. SBS, SB, and LAE are membrane-active biocides that disrupt the cell membrane function and structure[9,64,91,103]. Besides their primary MoAs, the biocides present multiple pathways that lead to cell inactivation or death[9,10,64,71,91,103]. The diverse nature of these MoAs makes it difficult for microorganisms to build biocidal resistance[9,65,71,103], increasing the longevity of biofouling mitigation.

Of the biocides in this study, three are already applied in potable water applications: MIT, DBNPA, and SBS[9,10,64,230]. MIT has been successfully implemented in both membrane cleaning and membrane storage, but the ability of MIT to prevent biofouling has not been studied[9]. DBNPA, an effective non-oxidizing biocide has been applied in RO systems; however, DBNPA may not be used directly to treat the water due to its toxicity[9,58]. SBS has been dosed as the reducing agent to remove free chlorine after chlorine disinfection and has strong antimicrobial properties. It also poses a low hazard to the environment, supporting its potential use as a preventative biocide[9,10,64]. The remaining biocides in this study, SB and LAE, are of very low risk to human and environmental health[27,66,103]. SB has been used as a preservative in cosmetic, personal care products and as an antimicrobial in food such as meat and dairy products[67,186], while LAE is a common food additive[103]. SB is inexpensive and highly soluble in water and both have demonstrated strong antimicrobial properties, making them highly suitable for potable water RO systems[103]. Despite these favorable qualities, further studies are required to prove their efficacy in inhibiting and eradicating biofilms.

In RO applications, it is essential to determine the optimal concentration of biocides. These concentrations may be referred to as the Minimum Biofilm Inhibiting Concentration (MBIC), the Minimum Biofilm Eradication Concentration (MBEC) and are typically determined under static conditions. The MBIC is the minimum concentration at which the biocide may prevent biofilm growth, while the MBEC is the minimum concentration at which the biocide may remove existing biofilms. Previous studies have reported the MBIC and MBEC values for MIT as 22.5 and 78 mg/L, respectively, and for SB as 25,000 and 32,000 mg/L, respectively[91]; however, there are no data reporting the MBIC and MBEC values for DBNPA, SBS, and LAE. Therefore, these values must be derived experimentally. The MBIC and MBEC of each biocide are critical to achieving biofilm prevention, removal, and attaining the lowest, most effective biocide dosage in the RO system. Once MBIC and MBEC values have been determined, they can be used to investigate biocidal efficacy under dynamic conditions.

In a dynamic system hydrodynamics at a surface greatly impact biofilm stability and development[231]. To this end, several standardized laboratory systems, aptly named biofilm reactors (bioreactors) are

available – each one defined by the unique range of hydrodynamic conditions they produce[232,233]. In this work, we use the Center for Disease Control (CDC) Biofilm Reactor. Originally developed by the CDC for the study of *Legionella pneumophila* disinfection in potable water systems[234], the CDC biofilm reactor is a continuously stirred tank reactor that has since demonstrated versatile and broad application in the study of biofilm growth and resistance[15,235–239]. It is well reported that high shear stress promotes adherence to surfaces and the development of a stronger EPS due to a diminishing hydrodynamic boundary layer; thereby creating biofilms that are more resistant to mechanical and chemical antifoulant strategies[231,234]. The ASTM International has approved two methods for the growth of reproducible *Pseudomonas aeruginosa* biofilms under high shear within the CDC biofilm reactor: Standard Test Method E2562 (ASTM International 2017) and Standard Practice E3161 (ASTM International 2018)[240,241]. These methods have been widely applied in the investigation of biofilm prevention and removal in RO systems through various strategies, including the addition of quorum quenching compounds[237,242], mucolytic agents[243], copper-based disinfectants[15,244,245] (Lee et al. 2017a, 2017b, 2020), and chlorinated biocides[246].

Kappachery et al. (2010), examined the application of vanillin (a natural quorum quenching compound) as a continuous feed or shock dosing treatment by exposing polyamide membranes to 1 × 10^6 CFU/mL of *Aeromonas hydrophila* for incubation periods of 1–7 days under standard CDC biofilm reactor biofilm growth conditions[237,241]. Biofilm growth in the presence of vanillin was suppressed by over 93% as measured by surface coverage, average thickness, total biomass, and total protein, that demonstrates high viability as a continuous feed treatment. However, it had no effect once the biofilm was already formed, excluding it as a feasible shock treatment. Similar results were found in their 2012 study using N-acetylcysteine, where a preliminary determination of the Minimum Inhibitory Concentration (MIC) was performed in polystyrene microtiter plates prior to efficacy testing against a multi-species culture (*A. hydrophila*, *Pseudomonas putida*, *Stenotrophomonas sp*., and *Serratia marcescens*) in the CDC biofilm reactor [243]. These investigations highlight the practicality of the CDC biofilm reactor in temporally varied experiments and its replicability in both biofilm prevention and removal studies. To study the impacts of biofouling on RO performance metrics, permeate flux, and salt rejection, Lee et al. (2020) used a laboratory-scale cross-flow RO system in conjunction with a CDC biofilm reactor [244]. The researchers noticed that the efficacies of various biocide combinations were lower in the pressurized unit due to heightened compression and densification of the EPS; however, results regarding relative biofilm inactivation efficacy were consistent across the two methods[244,245]. These works provide evidence for the relevancy and scalability of the CDC biofilm reactor as a primary testing method within this context and set a precedent for the validity of future work, such as our own.

3.3.1 Aim and novelty of the study

This study yields a preliminary assessment of green chemistries as alternative approaches for the prevention and removal of biofilms in drinking water RO membrane applications and provides a stronger understanding of potential biocidal performance in RO modules using a smaller, safer, and simpler dynamic system. Specifically, this work (a) estimates the MBEC and MBIC of non-oxidizing biocides not currently reported in the literature, including DBNPA, LAE, and SBS, using microtiter plates; and (b) tests the relative efficacy of the selected biocides, including MIT, PE, SB, DBNPA, LAE, and SBS, in removing or preventing biofilms on RO membrane coupons in a CDC biofilm reactor (CDC biofilm reactor 90, BioSurface Technologies Corporation, Bozeman, MT, USA). It is important to note that in this study each biocide is dosed, compared at their optimal concentrations (as determined by MBIC and MBEC static testing) and not at the same concentration, i.e. the relative efficacy under dynamic conditions as compared to static tests is determined. The results of this article are paramount to solving the issues of biofouling in potable water applications, such as the production of carcinogenic by-products due to chlorine-feed water pretreatment. The work presents a feasibility analysis of the application of greener strategies for biofouling control in RO systems.

3.4 Materials and methods

3.4.1 Bacterial strain and growth conditions

P. aeruginosa strain ATCC 10145™ was used in the MBIC/MBEC experiments as well as in the CDC biofilm reactor experiments because it is a well-known model biofilm organism commonly applied to study the anti-biofouling efficacy of antimicrobials applicable to RO systems[10,26,91]. This is because, in addition to *P. aeruginosa* being a known biofilm-forming pioneer organism commonly found in RO systems, *P. aeruginosa* forms a single-species biofilm that is extremely hard to treat due to its resilient nature and multi-drug resistance; making anti-biofilm efficacy studies with this microorganism relatively translatable to more complex biofilms[9,10,26,32,91,103,247–249]. The inoculum was prepared by streaking out one isolated colony of *P. aeruginosa* from a Tryptic Soy Agar (TSA) (Thermo Fisher Scientific, MA, USA) plate, which was stored at 4 °C. *P. aeruginosa* TSA plates were made from *P. aeruginosa* stocks stored at −80 °C in glycerol[91,250]. A single colony of *P. aeruginosa* was transferred to a falcon tube with 5 mL of full-strength Tryptic Soy Broth (TSB) (Thermo Fisher Scientific, MA, USA) and was incubated in an incubator shaker (VWR 1575 Incubator Shaker, orbital diameter of 1.9 cm) for 18 h at 37 °C and 200 rpm[91,250]. The bacterial cells were harvested at the exponential growth phase (OD = 1.0 [600; WPA CO 8,000 cell density meter]). In the MBIC and MBEC protocol, the overnight culture was centrifuged (3,000 rpm, 22 °C, 10 min, centrifuge model: Allegra X-12R), resuspended in 5 mL of full-strength TSB (MDL Number: MFCD00132536, Sigma-Aldrich Canada) and diluted to a concentration of approximately 10^6

CFU/mL. In the experiments performed in the CDC biofilm reactor, the overnight culture was resuspended in 5 mL of deionized (DI) water (Milli-Q IQ 700, Millipore Sigma, Darmstadt, Germany) and diluted to approximately 10^5 CFU/mL.

3.4.2 Determination of MBICs and MBECs

The two protocols applied to determine MBIC and MBEC values followed the same experimental procedure described in Curtin et al. (2021)[91]. In short, the total mean green fluorescence, after staining, associated with the total live biofilm biomass after 24 h was investigated for 12 different concentrations of each biocide. The experiments presented four experimental wells per biocide concentration, 12 wells for the positive control (MIT [CAS-Number: 2682-20-4, Sigma-Aldrich, Canada], bacteria and nutrients), 12 wells for the negative control (bacteria and nutrients), and 24 wells for the references (12 wells with nutrients only; 12 wells with nutrients and biocide)[91,250]. A second 96-well plate was used to determine the background fluorescence of the positive control (96-wells with MIT and nutrients). The references were used to remove background fluorescence[91]. The mean fluorescence values were calculated by subtracting corresponding references from the experimental wells and controls' fluorescence values[91,250]. Appendix B, Figure 19 illustrates the 96-well plate arrangement for the determination of MBICs and MBECs. Appendix B, Figure 20 illustrates the 96-well plate arrangement for the determination of the background fluorescence of MIT (nutrients and biocide). The experiments were performed in triplicate on independent weeks.

In the MBEC protocol, 100-µL aliquots of the bacterial cells, resuspended in TSB, were added to the experimental wells, controls, and references in a black-sided, clear-bottom polystyrene 96-well plate (Thermo Fisher Scientific, MA, USA) according to Appendix B, Figure 19. The plate was incubated in the incubator shaker for 24 h at 200 rpm and 37°C to allow biofilm growth[91]. After incubation, the planktonic bacterial suspension was gently removed by rinsing each well three times with a multichannel pipette (Biorad Multichannel Micropipette, eight-channel, 20–200 µL; 1660495) fitted with 200 µL of sterile DI water[91,250,251]. The rinsing step was performed by inserting the 200 µL tip slowly at 45° avoiding touching the sides and bottom of the wells[250]. After planktonic cell removal, biocides were added to the plate and incubated in the incubator shaker for 24 h at 200 rpm and 37°C (biocide treatment)[91,250]. The biocides were prepared in a separate 96-well plate (Costar 96-well Cell Culture Plate, flat bottom with low evaporation lid, polystyrene, Corning Incorporated, NY, USA) and added to the experimental, reference, and control wells to yield the following final concentrations: SB: 40,000–20 mg/L [CAS-Number: 532-32-1; Botanic Planet, ON, Canada], LAE: 20,000–10 mg/L [CAS-Number: 60372-77-2; The US Agricultural Research Service, CA, USA]; DBNPA: 3,000–1 mg/L [CAS-Number: 10222-01-2; Sigma-Aldrich, Canada]; and MIT: 2,250–1 mg/L, according to the plate arrangement presented in Appendix B, Figure 19. The plate inoculation, incubation, and biocide treatment in the MBIC protocol followed the same procedure described in the MBEC

protocol; however, biocides were co-incubated with the bacterial cells, meaning that plate inoculation and biocide treatment occurred simultaneously[91,94].

After plate incubation and biocide treatment, biofilms were stained with the LIVE stain (SYTO9; 5 μM) and DEAD stain (propidium iodide (PI), 30 μM) from the LIVE/DEAD Baclight viability kit (L 7012; Invitrogen, MA, USA). A 150-μL mixture of the stain solution diluted in DI water was added to each well slowly at 45° (avoiding the sides and bottom of the wells) using a multichannel pipette[91,250]. The plate was then incubated at room temperature in the dark for 15 min. After staining, plates were rinsed one time with DI water to remove stain residues as prescribed by the manufacturer. Finally, green (excitation wavelength: 485 nm; emission wavelength: 528 nm) and red (excitation wavelength: 485 nm; emission wavelength: 645 nm) fluorescence were measured in a plate reader (Cytation 5, Gen5 3.08 software, Biotek, VT, USA)[91]. The MBIC was determined as the minimum concentration of a biocide that can reduce more than 90% of the mean green fluorescence values of experimental wells when compared to the negative control[91,94]. In the MBIC protocol, the efficacy of the biocides in preventing biofilm formation is assessed. Similarly, the MBEC was determined as the minimum concentration of a biocide that can reduce more than 90% of the mean green fluorescence values of experimental wells when compared to the negative control[91,94]. In the MBEC protocol, the efficacy of biocide in removing a pre-established biofilm is determined.

3.4.3 Synthetic feed water and RO polyamide membranes

The synthetic feed water for biofouling experiments in the biofilm reactor was designed based on standard pH and temperature for RO studies (pH 7 and 25°C) and typical water quality of RO system influent after pretreatment, exacerbating parameters known to cause biofouling in RO systems: nutrient and microorganism concentration (*P. aeruginosa* at 10^5 CFU/mL)[9,10]. Nutrient concentration (TSB) in the feed water was set to approximately 30 mg/L of equivalent Total Organic Carbon (TOC) in the CDC biofilm reactor[249,252]. Background salinity was set to 500 mg/L (NaCl; 7647-14-5; Thermo Fisher Scientific, MA, USA)[9]. To ensure anti-biofilm efficacies, the selected biocides (LAE, MIT, DBNPA, PE, SB, and SBS [CAS-Number: 7631-90-5; Sigma-Aldrich, Canada]) were dosed at 2× MBIC values for biofilm prevention tests and 2 × MBEC values for biofilm removal studies[9,253,254]. Commercial RO polyamide membranes (TriSep, YMACM34205) were used for biofouling experiments. The RO membranes were cut into 12 mm diameter discs, sterilized with 70% ethanol, and affixed onto the coupons of the biofilm reactor with the feed side facing the center of the reactor[237,239,252,255].

3.4.4 Anti-biofouling efficacy testing in the CDC biofilm reactor

The two protocols applied for biofilm prevention and biofilm removal studies were adapted from the ASTM standard test method for quantification of 48 h *P. aeruginosa* biofilms using CDC biofilm

reactors (CDC biofilm reactor 90, BioSurface Technologies Corporation, Bozeman, MT, USA): ASTM E2562[256]. The CDC biofilm reactor contains eight rods with three coupons in each rod. Prior to the biofouling experiments, CDC biofilm reactor, tubing, and carboys were sterilized as described in previous studies[15,257]. Furthermore, RO membranes and the high-pressure pump (Optos Series 2HM, Eldex, Napa, CA, USA) were sterilized as described in Suwarno et al. (2014)[252]. The CDC biofilm reactor system was set up as depicted in Rautiola (2013)[258]. To evaluate the efficacy of the selected biocides in removing biofilms from RO membranes, 12 mm polyamide membrane coupons were affixed onto the coupons of the biofilm reactor with the feed side facing the center of the reactor using 3M double-sided tape[15]. The bioreactor was inoculated with approximately 10^5 CFU/mL of *P. aeruginosa* to grow a 24 h *P. aeruginosa* biofilm. For the first 24 h, the CDC reactor was operated in batch mode at 100 rpm, 25°C, pH 7 and an operation volume of 350 mL (TOC of 30 mg/L and NaCl of 500 mg/L). For the last 24 h, synthetic feed water solution was continuously fed to the CDC reactor at 11.7 mL/min under the same conditions to allow further biofilm development. After 48 h of growth, biofilms were treated for 24 h with a biocide concentration at 2× MBEC to test biofilm removal efficacy. Biofilm prevention experiments followed the same procedure as the biofilm removal studies with the 48 h of *P. aeruginosa* biofilm growth period done in the presence of biocides at 2× MBICs[9,26,32,237]. In other words, to test biofilm prevention efficacy, *P. aeruginosa* biofilms were grown over 48 h in the presence of biocide (prevention protocol). To test biofilm removal efficacy, the bioreactor was inoculated to grow a biofilm unencumbered over 48 h (removal protocol). After treatment, Confocal Laser Scanning Microscopy (CLSM) and Scanning Electron Microscopy (SEM) were used to visualize and quantify biofilms and bacterial cells[28,32,247]. The biofouling experiments were performed in triplicate on independent weeks.

3.4.5 Confocal scanning laser microscopy

For a quantitative analysis of biofilm prevention and removal efficacies, the LIVE/DEAD Baclight viability kit (LN 7007) was used to stain nucleic acids, differentiating intact bacterial cells (LIVE) bound to the SYTO 9 stain from ruptured bacterial cells (DEAD) bound to PI[32,91]. Furthermore, the Concanavalin A stain (C11252 Concanavalin A Alexa Fluor 488 conjugate kit) was used to quantify EPS (biofilm matrix), as it binds to polysaccharides, which are the main component of the biofilm matrix[32,247]. The membrane coupons containing biofilms were stained following the specifications of the kits discussed herein. In summary, after biocide treatment in the bioreactor, the RO membranes with biofilms were rinsed three times with DI water to remove planktonic bacteria[28,32,255,259] and then stained for 15 min at room temperature using the following dye concentrations: (i) SYTO 9 at 5 μM, (ii) PI at 30 μM, and (iii) Concavalian A at 50 μM. After staining, the RO membranes were rinsed with DI water to remove residual dyes and wet mounted on clean glass slides[32,230]. Furthermore, the biofilm matrix, as well as live and dead bacterial cells were visualized in a Zeiss LSM 880 CLSM at 20× magnification with representative areas of 531.4 μm × 531.4 μm and a resolution of 1,784 ×

1,784 pixels (16 bit)[32,238,247]. Biofilm analysis was performed in the IMARIS software from CLSM Z-stacks images collected from at least three randomly selected areas on the RO membrane (version9.8.0, Bitplane)[32,238]. In this software, the CLSM 3D images were processed and biofilm thickness, biofilm volume, biofilm appearance, and live-to-dead bacterial ratio (anti-biofilm efficacy) were quantified and analyzed as described in Heydorn et al. (2000), Donlan (2002), Lade & Paul (2015) and Nagaraja et al. (2017)[28,32,238,247].

3.4.6 Scanning electron microscopy

For a qualitative analysis and further assessment of anti-biofilm efficacy, biofilms developed on RO membrane coupons were visualized in SEM (SEM Hitachi S-4800)[251,260]. For imaging preparation, after biocide treatment, the biofilm membrane coupons were rinsed three times with DI water to remove planktonic cells, then immersed in 2.5% glutaraldehyde (CAS-Number: 111-30-8, Thermo Fisher Scientific, MA, USA) and diluted in a phosphate-buffered saline (PBS; Cytiva, Marlborough, MA, USA) solution at 4°C for 4 h for bacteria fixation. The membrane coupons were then rinsed two times with DI water. After rinsing, the biofilms were dehydrated in each of the following ascending ethanol concentrations for 5 min at room temperature: 30, 50, 70, 80, 96, and 100%[28,251,259,260]. After dehydration, the biofilms were dried with the following concentrations of hexamethyldisilazane (HMDS; CAS-number: 999-97-3, Thermo Fisher Scientific, MA, USA) for 30 min at room temperature: 50 and 100%[260]. Finally, the biofilm membrane coupons were coated with gold by sputter deposition for 120 s (Anatech Hummer VI Sputter Coater). The SEM images were taken with magnifications of 1,000 × ; 5,000 × ; and 10,000× at a voltage of 1 kV[251,260–262].

3.4.7 Statistical analysis

Statistically significant differences were analyzed using a one-way ANOVA and Student's t-test using Microsoft Excel software. Values of $p < 0.05$ were considered to be statistically significant.

3.5 Results and discussion

3.5.1 MBICs and MBECs

To find the operational biocide dosage for biofouling experiments in the CDC biofilm reactor, the MBIC and MBEC values of DBNPA, SBS, and LAE for *P. aeruginosa* were estimated. It is important to note that the MBICs and MBECs for *P. aeruginosa* for the two remaining biocides in this study were already found in the literature (MIT – MBIC: 22.5 mg/L and MBEC: 78 mg/L; SB – MBIC: 25,000 mg/L and MBEC: 32,200 mg/L)[91]. Therefore, Table 7 only presents the MBICs and MBECs of DBNPA, SBS, and LAE.

Table 7 - MBICs and MBECs of DBNPA, SBS, and LAE for *Pseudomonas aeruginosa*

	DBNPA	SBS	LAE
MBIC (mg/L)	125	1,667	63
MBEC (mg/L)	375	2,500	5,000

Note: MBIC, minimum biofilm inhibitory concentration; and MBEC, minimum biofilm eradication concentration. The MBIC and MBEC values were determined to be significantly different from the growth control (Reference, Appendix B, Figures 19-20) via Student's t-test ($p < 0.05$). Each MBEC and MBIC value in the triplicate experiments showed not to be significantly different from each other via ANOVA ($p > 0.05$) in the respective biocide-associated experiments. MBIC (Appendix B, Figures 21–24) and MBEC (Appendix B, Figures 25–28) graphs can be found in Appendix B.

LAE and DBNPA presented the lowest MBICs among the studied biocides, with values of 63 mg/L (0.15 mM) and 125 mg/L (0.50 mM), respectively. Compared with SBS, which presented an MBIC value of 1,667 mg/L (16.05 mM), both chemicals demonstrate high efficacy in preventing *P. aeruginosa* biofilms under static conditions. This result is unsurprising, as DBNPA and LAE function under similar MoAs, mainly killing microorganisms by damaging their cell membrane[9,103,263]. Although SBS is also a cell membrane-active biocide, its main mechanism of action is through oxygen scavenging, which often requires higher concentrations to be effective. For instance, the MIC of SBS for *P. aeruginosa* is 780 mg/L – more than 10 times the MICs of DBNPA and LAE[10,64,103]. It should also be noted that the MBIC of LAE was approximately two times lower than that of DBNPA. This relationship is best supported by Kim & Park (2016), which found the same correlation between the MIC of LAE (31.3 mg/L) and the MIC of DBNPA (62.5 mg/L) for *P. aeruginosa*[103]. These results suggest that LAE is more effective at inhibiting biofilm formation than DBNPA (Kim & Park 2016), a performance difference that can be primarily attributed to the cationic nature of LAE, which strengthens interactions with negatively charged surfaces of microorganisms and facilitates disturbance of cell membrane structure and potential[103].

In general, all the MBIC values were lower than the MBEC values for their respective biocides. DBNPA had the lowest MBEC value (375 mg/L or 1.55 mM) out of the tested biocides followed by SBS with MBEC of 2,500 mg/L (24.05 mM) and LAE with a MBEC of 5,000 mg/L (11.90 mM) (Table 7). The same trend was observed by Curtin et al. (2021) and Güven & Kaynak Onurdağ (2014)[91,98]. These results indicate that preventing biofilm formation requires less biocide than removing an established biofilm. A possible explanation for this trend is that in the MBIC protocol, the biofilms were exposed to the biocides in an earlier stage of development, while in the MBEC protocol, the biocides were applied to mature biofilms (strong biofilms). This is because mature biofilms present a higher resistance to antimicrobials for several reasons, such as the lower diffusion rate of antimicrobials into the biofilm due to a more developed biofilm matrix and the increased presence of persisters in the biofilm microbial community[28,29,91].

3.6 Anti-biofouling efficacy testing in the CDC biofilm reactor

3.6.1 Confocal laser scanning microscopy

CLSM analyses were carried out in order to investigate the anti-biofilm efficacy of several non-oxidizing biocides in preventing and removing *P. aeruginosa* biofilms on commercial RO membranes. *P. aeruginosa* is a common biofilm pioneer in RO systems. For this experiment, 48 h *P. aeruginosa* biofilms were grown on polyamide RO membranes in a CDC biofilm reactor under conditions designed to mimic conditions in RO systems for potable water applications. For the prevention protocol, the biofilms were developed from the beginning of the experiment in the presence of biocides at two times their corresponding MBIC values for 24 h. For the biofilm removal protocol, the biofilms were treated with biocides at two times their corresponding MBEC values after the 48-h *P. aeruginosa* biofilm was established. The CLSM analysis compared biofilm quantification parameters between untreated biofilms and treated biofilms. The biocides' anti-biofilm efficacy was quantified and analyzed in terms of biofilm appearance, biovolume, biofilm thickness, and live-to-dead bacterial ratio as described in Heydorn et al. (2000), Lee et al. (2017a), Nagaraja et al. (2017) and Werner et al. (2019)[15,32,239,247]. Figure 7 shows the reconstructed CLSM images of 48 h of *P. aeruginosa* biofilms grown in the CDC biofilm reactor (biofilm appearance) as a result of the treatment with each biocide. Table 8 summarizes the biocides' anti-biofilm efficacy metrics for all biocides.

Table 8 - Anti-biofilm efficacy metrics

	Metrics	Untreated	MIT	DBNPA	SBS	SB	LAE
P	Biocide concentration (mg/L)	-	45	250	3,334	50,000	126
	Biocide concentration (mM)	-	0.4	1.0	32.1	347.2	0.3
	Biovolume ($\mu m^3/\mu m^2$)	6.88 ± 0.78	0.05 ± 0.01	0.70 ± 0.28	0.14 ± 0.03	0.60 ± 0.22	0.08 ± 0.02
	Biofilm thickness (μm)	13.4 ± 1.9	8.6 ± 2.3	7.4 ± 2.4	7.7 ± 1.5	7.8 ± 0.9	8.4 ± 2.7
	Live to dead bacterial ratio	54.18 ± 8.80	0.43 ± 0.29	0.13 ± 0.04	0.14 ± 0.03	0.22 ± 0.05	0.21 ± 0.09
	Biofilm prevention efficacy (%)	-	99.3 ± 0.1	89.9 ± 4.0	98.0 ± 0.4	91.2 ± 3.2	98.9 ± 0.3
R	Biocide concentration (mg/L)	-	156	750	5,000	64,400	10,000
	Biocide concentration (mM)	-	1.4	3.1	48.1	447.2	23.8
	Biovolume ($\mu m^3/\mu m^2$)	6.88 ± 0.78	0.16 ± 0.06	0.31 ± 0.03	1.26 ± 0.27	0.25 ± 0.10	0.03 ± 0.01
	Biofilm thickness (μm)	13.4 ± 1.9	7.7 ± 1.0	10.6 ± 0.4	13.7 ± 0.3	7.2 ± 0.4	9.1 ± 2.1
	Live to dead bacterial ratio	54.18 ± 8.80	0.26 ± 0.01	0.20 ± 0.02	0.51 ± 0.13	0.39 ± 0.22	0.45 ± 0.08
	Biofilm removal efficacy (%)	-	95.7 ± 0.9	95.5 ± 0.4	81.7 ± 4.0	96.4 ± 1.5	99.6 ± 0.2

Note: P, Prevention; and R, Removal. Calculated by IMARIS 9.8.0 from CLSM images. Values are shown as mean ± standard deviation, obtained from independent triplicate experiments. In the biofilm prevention protocol, the biocides were dosed at 2× MBIC values. In the biofilm removal protocol, the biocides were dosed at 2× MBEC values.

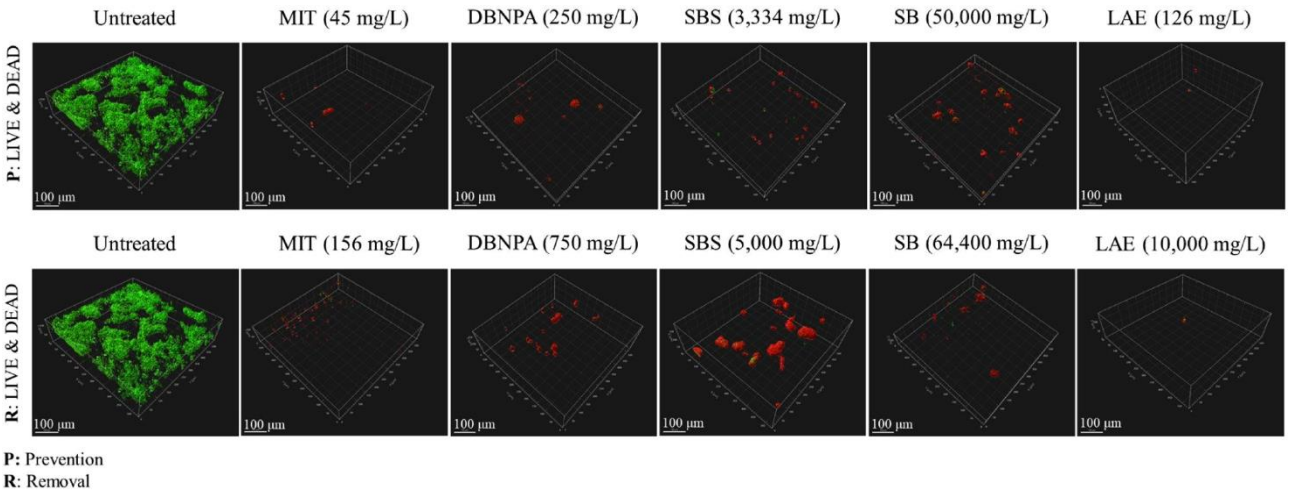

Figure 7 - Reconstructed CLSM images of 48-h *Pseudomonas aeruginosa* biofilms. P: Biofilm Prevention (co-incubation of 48-h *P. aeruginosa* biofilms with biocides at 2× MBIC values). R: Biofilm Removal (treatment of 48-h pre-established *P. aeruginosa* biofilms with biocides at 2× MBEC values). In live and dead, green refers to live biofilm biomass and red refers to-dead biofilm biomass. Images were captured at magnification 20×.

From the results shown in Figure 7 and Table 8, it is clear that the 48 h untreated *P. aeruginosa* biofilms were dominated by green fluorescence, indicating the viability of microorganisms. This observation is confirmed by the high live-to-dead bacterial ratio of 54.18 ± 8.80 observed for the untreated biofilms, indicating a higher proportion of live biofilm cells in the sample. Furthermore, the untreated biofilms were characterized by very dense biofilms with biovolume and biofilm thickness of 6.88 ± 0.78 $\mu m^3/\mu m^2$ and 13.4 ± 1.9 µm, respectively, confirming that mature biofilms were successfully established in the CDC reactor.

Overall, the tested biocides presented high efficacy in preventing *P. aeruginosa* biofilms. Values of biovolume, biofilm thickness, and live-to-dead bacterial ratio for all biocides were significantly decreased when compared to the untreated biofilms ($p < 0.05$; Student's t-test) (Table 8). MIT and LAE had the highest biofilm prevention efficacies of 99.3 ± 0.1% and 98.9 ± 0.3%, respectively, followed by SBS (98.0 ± 0.4%), SB (91.2 ± 3.2%), and DBNPA (89.9 ± 4.0%) (Table 8). Ideal anti-biofouling candidates applicable to RO systems should be able to prevent biofilms on RO membranes as well as remove them. According to the results displayed in Figure 7 and Table 8, LAE was the most effective biocide in removing established *P. aeruginosa* biofilms from RO membranes with an efficacy of 99.6 ± 0.2%, followed by SB (96.4 ± 1.5%), MIT (95.7 ± 0.9%), DBNPA (95.5 ± 0.4%), and SBS (81.7 ± 4.0%). All the anti-biofilm efficacy metrics were significantly reduced when compared to the control samples ($p < 0.05$; Student's t-test) in the biofilm removal experiments in the bioreactor, except for biofilm thickness in the SBS treatment (Table 8). This indicates that SBS could not significantly reduce the thickness of biofilms when compared to untreated biofilms in the removal experiments. In general, all the treated biofilms were dominated by red fluorescence with very low live-to-dead bacterial ratio values (<1), indicating a low proportion of living biofilm cells on the

membrane samples (Figure 7 and Table 8). Furthermore, all the treated biofilms were less dense than the untreated biofilms, with biovolume values varying from 1.26 ± 0.27 to 0.03 ± 0.01 $\mu m^3/\mu m^2$ in both protocols (prevention and removal) (Table 8).

MIT and DBNPA are biocides commonly used to control biofouling in offline RO water treatment applications. The MIT treatment in the prevention protocol was able to reduce the biofilm volume and biofilm thickness to 0.05 ± 0.01 $\mu m^3/\mu m^2$ and 8.6 ± 2.3 μm, respectively. In the removal protocol, MIT was able to reduce the biofilm volume and biofilm thickness to 0.16 ± 0.06 $\mu m^3/\mu m^2$ and 7.7 ± 1.0 μm, respectively. It is no surprise that MIT showed remarkable efficacy in preventing (99.3 ± 0.1%, Table 8) and removing (95.7 ± 0.9%, Table 8) *P. aeruginosa* biofilms from RO membranes with relatively low biocide concentrations (Figure 7). In part, this can be attributed to the ability of MIT to not only disrupt the biofilm matrix but also present a wide spectrum of inhibitory pathways that may lead to the inhibition of enzyme activity, cell membrane damage, and impairment of microbial respiration[9,37,65,91]. Based on the CLSM images and the anti-biofilm efficacy metrics displayed in Table 8, DBNPA is less effective than MIT in treating *P. aeruginosa* biofilms on RO membranes. DBNPA-treated biofilms had the highest average biovolume value in the prevention experiments (0.70 ± 0.28 $\mu m^3/\mu m^2$) and the second-highest average biovolume value in the removal experiments (0.31 ± 0.03 $\mu m^3/\mu m^2$). Although MIT and DBNPA presented biofilm prevention and removal efficacies up to approximately 99%, human and environmental health hazards attributed to the use of these biocides limit their applications in inline potable water applications[9,10,27]. These characteristics suggest that MIT and DBNPA should only be used as models for anti-biofilm efficacy in biofouling studies.

SBS is also a commercial biocide commonly used in RO applications. Due to its low risk to humans and the environment, SBS is usually applied to limit microbial growth in long-term RO membrane storage and to remove chlorine after feed water pretreatment in RO membrane modules for drinking water applications[9,10]. According to Figure 7 and Table 8, the SBS treatment resulted in a strong inhibition of biofilm formation with a biofilm prevention efficacy of 98.0 ± 0.4%. However, SBS was the least efficacious (81.7 ± 4.0%) of all the studied biocides in removing existing *P. aeruginosa* biofilms, only reducing biofilm volume to 1.26 ± 0.27 $\mu m^3/\mu m^2$. This result suggests that SBS was not able to disrupt the biofilm matrix, as supported by previous studies[9,10,64]. Therefore, SBS is unlikely to be the most optimal biocide for RO drinking water applications.

LAE and SB are biocides that present low risk to humans and the environment but have yet to be investigated for their application in RO systems[27,66,91,103]. Both biocides are applied as food preservatives and have several features ideal for RO potable water applications, such as excellent antimicrobial properties, high solubility in water, low hazard, and high biodegradability[27,91,103]. According to Table 8, SB presented moderate efficacies in inhibiting (91.2 ± 3.2%) and removing (96.4 ± 1.5%) *P. aeruginosa* biofilms compared to the other studied biocides. The moderate anti-

biofilm efficacies observed for SB are likely correlated to pH, which was kept at 7 – a typical pH in RO water treatment applications but much higher than the effective pH for SB (pH 4)[9,66,91]. Consequently, high doses were required to achieve even moderate anti-biofilm efficacies. This suggests that SB may not be suitable for addressing biofouling in RO systems, as its application could be quite expensive and its allowable concentration is a cause for concern.

On the other hand, LAE presented excellent anti-biofilm properties while requiring relatively low biocide doses. In general, it was noted that LAE was more effective than all commercial biocides (MIT, DBNPA, and SBS) in treating *P. aeruginosa* biofilms on RO membranes (Table 8 and Figure 7). This observation is best supported by Kim & Park (2016), who compared the anti-biofilm effect of LAE with two commercial biocides (DBNPA and chlorine) at concentrations up to 62.5 mg/L against four different types of single-species biofilms (*Escherichia coli*, *P. aeruginosa*, *Staphylococcus aureus*, and *Enterococcus faecalis*) in a complex growth medium through a static biofilm assay[103]. The researchers concluded that LAE showed superior biofilm prevention and removal efficacies against all tested single biofilms when compared to DBNPA and chlorine[103]. Furthermore, LAE was the most efficacious biocide in removing existing biofilms from RO membranes among all studied biocides (99.6 ± 0.2%) and the second-best biocide in inhibiting biofilm formation (98.9 ± 0.3%). Sun et al. (2022), studied the effects of LAE on the detachment of 24 h *P. aeruginosa* biofilms grown in an in vitro flow cell system. The researchers observed that exposure of biofilms to LAE at concentrations up to 42 mg/L for 1 h was responsible for a biofilm removal of approximately 68%. The results reported by Sun et al. (2022), support the high anti-biofilm efficacies observed for LAE in our study and highlight the importance of appropriate biocide concentrations in the treatment of biofilms[264]. The superior anti-biofilm properties of LAE are possibly attributed to the fact that LAE can not only disrupt biofilm matrix, but also it inhibits biofilm formation by several mechanisms such as quorum sensing inhibition and cell membrane damage[103,264,265]. Therefore, considering its high efficacy, not only for biofilm removal but also for biofilm inhibition and safety, LAE is an excellent candidate to address biofouling in RO drinking water applications.

Interestingly, biofilms grown in the CDC biofilm reactor exhibited higher tolerance to biocide treatments when compared to the treated biofilms in the 96-well plate experiments. The biocides were dosed in the bioreactor at concentrations two times higher than their respective MBIC and MBEC values with the goal of guaranteeing treatment efficacies greater than 90%. However, not all biocides achieve this efficacy. For instance, SBS presented a biofilm removal efficacy of 81.7 ± 4.0% and DBNPA presented a biofilm prevention efficacy of 89.9 ± 4.0% (Table 8). This is most likely a consequence of harsher hydrodynamic conditions within the CDC biofilm reactor that directly impact biofilm growth[28,29,235]. Biofilms grown in the CDC biofilm reactor develop under a heightened cross-flow velocity – a condition that increases cell interactions at the surface but limits nutrient transfer

throughout the matrix – resulting in enhanced secretion of EPS and, therefore, microcolonies of greater thickness, density, and complexity[216,235]. These architectural characteristics influence microbial metabolism through the imposition of diffusion limits, controlling a biofilm's response to biocidal treatment[62]. Another possible explanation for the discrepancy in anti-biofilm efficacies between static and dynamic techniques is the difference in attachment layers. Donlan (2002) reported that rough surfaces increase microbial colonization and usually result in stronger biofilms[28]. Since RO membranes present surfaces with higher roughness than the microtiter plates[28,29,235], it is unsurprising that biocidal resistance was elevated within the CDC biofilm reactor. The results from the present study emphasize the importance of testing biocides in conditions akin to those found in RO system applications, as they have a major effect on the treatment of biofilms.

3.6.2 Scanning electron microscopy

SEM was used to analyze morphological changes in biofilm structures upon exposure to biocides in the CDC biofilm reactor experiments, as well as to validate CLSM results. Figure 8 shows the SEM micrographs of 48 h *P. aeruginosa* biofilms developed in the biofilm prevention protocol. Figure 9 displays the SEM images of 48 h *P. aeruginosa* biofilms developed in the biofilm removal protocol. As shown in Figures 8 and 9, untreated biofilms presented healthy, smooth, rod-shaped bacterial cells with uniform size, and distribution[133,239]. Unsurprisingly, biofilms treated with biocides had a high number of damaged bacterial cells. In Figure 8, the results of the prevention protocol revealed a thin monolayer of EPS remaining on the RO membranes and no visible biofilm cells. This result was consistent across all biocides, with the exception of SB, which presented a thick layer of EPS. In line with CLSM results, the removal protocol was less effective at treating biofilms, exhibiting a significant number of damaged biofilm cells and a thicker biofilm matrix. Damage, characterized by an unstructured cell shape, appeared as elongated or shrunken cells with visible holes in the cell membranes – similar observations to those found by Gomes & Mergulhão (2017), which studied the effect of biocides on cell morphology[261].

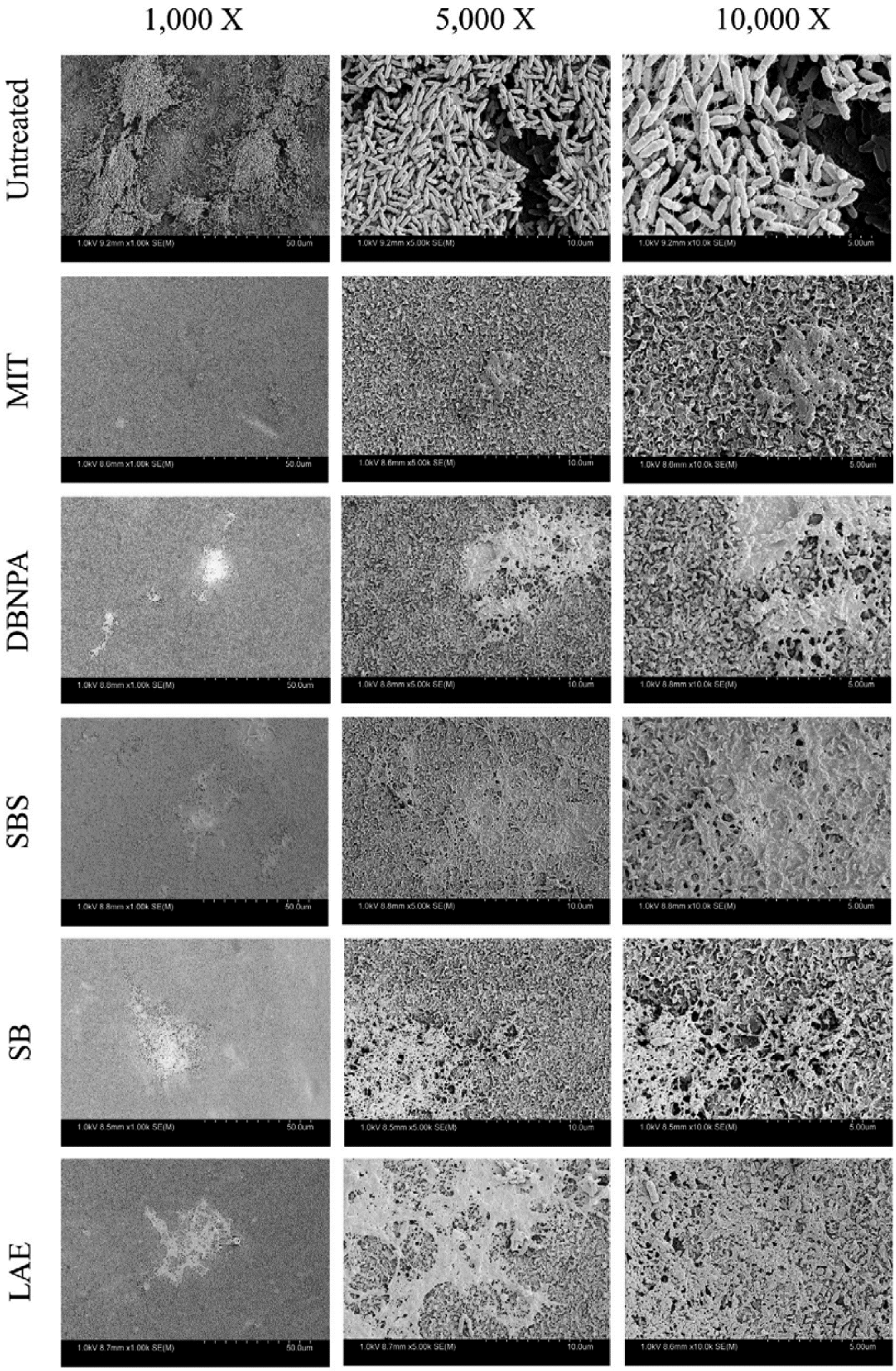

Figure 8 - Representative SEM images of 48-h *Pseudomonas aeruginosa* biofilms, co-incubated with biocides at 2× MBIC values, on polyamide RO membranes (biofilm prevention). Scales bars represent 50.0 μm (at 1,000×), 10.0 μm (at 5,000×), and 5.00 μm (at 10,000×).

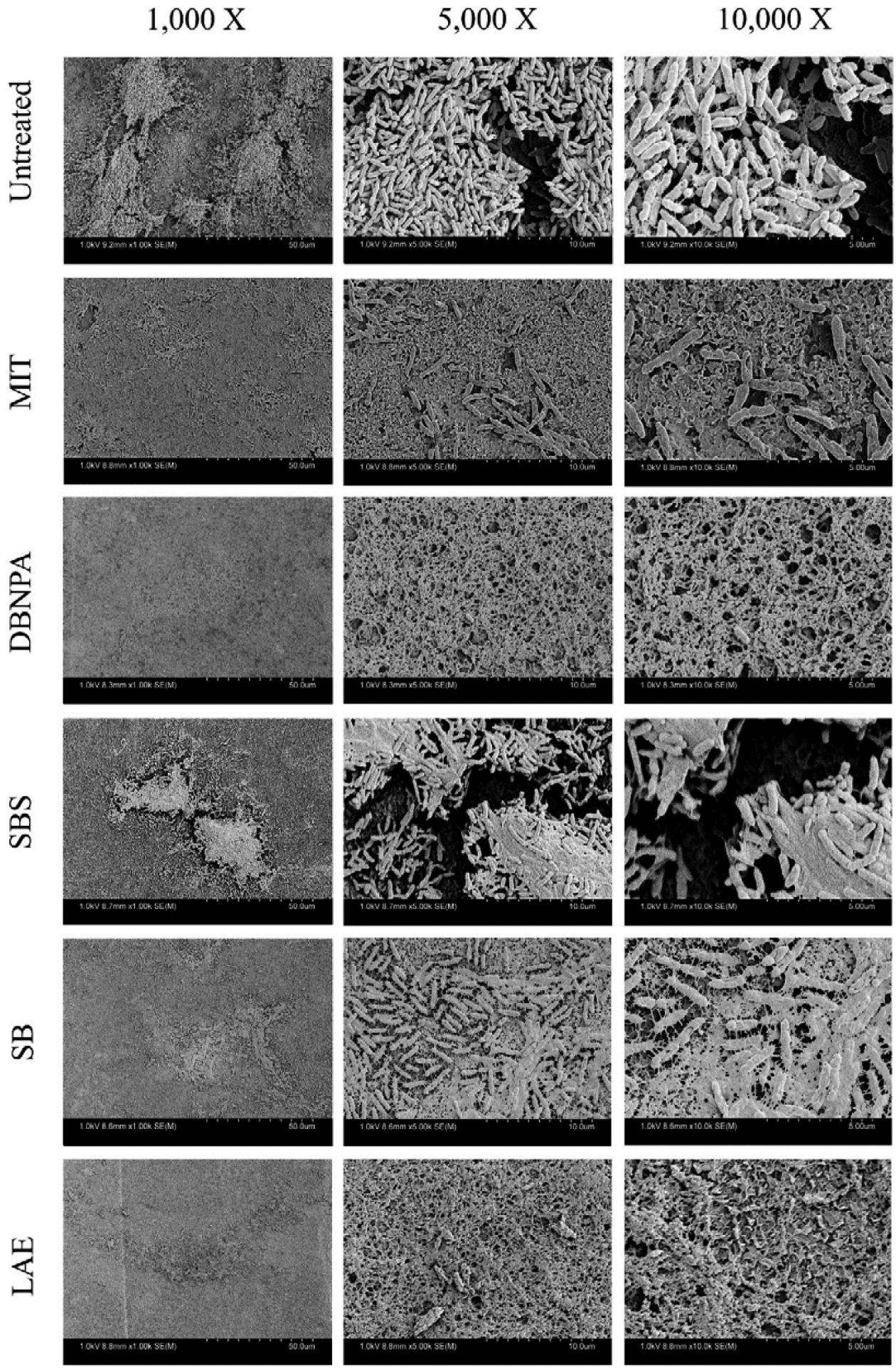

Figure 9 - Representative SEM images of 48-h pre-established *Pseudomonas aeruginosa* biofilms, treated with biocides at 2× MBEC values, on polyamide RO membranes (biofilm removal). Scales bars represent 50.0 μm (at 1,000×), 10.0 μm (at 5,000×), and 5.00 μm (at 10,000×).

According to Figure 9, biofilms treated with MIT and SB appeared to have a thin monolayer of EPS with a large number of damaged cells. In contrast, DBNPA presented few bacterial cells with a much thicker EPS layer. Previous biofilm studies indicated that DBNPA is not suitable for removing existing biofilms from RO applications because it cannot effectively disrupt the biofilm matrix[9,10,34]. However, our SEM images showed that when DBNPA is applied at a higher concentration than what is applied in full-scale RO installations (up to 30 mg/L), it has a significant effect on the treatment of existing biofilms[9,10]. Furthermore, SEM results confirmed that SBS was not efficacious at removing biofilms, since it was unable to disrupt the typical 3D structure of biofilms or reduce the number of healthy biofilm cells (Figure 9). Finally, the LAE treated biofilms presented a thin monolayer of EPS and an insignificant number of biofilm cells remaining after treatment. Therefore, the results of the SEM images suggest that LAE and MIT are the most efficacious biocides, with their treatment resulting in the highest removal of EPS and the lowest number of biofilm cells. Additionally, the SEM results also confirmed that treatment aimed at preventing biofilm formation (Figure 8) is more effective than treatment intended to remove existing biofilms (Figure 9). All these observations are consistent with the CLSM results. Furthermore, the results of the SEM analysis provided additional information on the morphological properties of the biofilms. Together, the CLSM and SEM analyses prove to be powerful tools to promote a comprehensive understanding of biocidal efficacy.

3.7 Conclusions and prospects

RO technology has the potential to end water shortages worldwide, as it can generate fresh water from different water sources. However, the efficiency and reliability of this membrane-based purification technique are threatened by biofouling since it (a) reduces permeate production; (b) increases energy demands; and (c) produces carcinogenic by-products consequent to feed water treatment with chlorine. In order to propose a safe solution for the reduction of detrimental effects of biofouling in RO systems, this study investigated the ability of five non-oxidizing biocides in preventing or removing biofilms from RO membranes. Biofouling tests were performed in a CDC biofilm reactor with biocidal concentrations estimated on 96-well microtiter plates. Generally, all biocides significantly impact biofilm health with removal and prevention efficacies ranging from 80 to 99%. Results also revealed that treatments aimed at preventing biofilms were more effective than those aimed at removing biofilms, which suggests that a continuous dose of biocidal is the best way to handle biofouling in the RO system. Specifically, the results from this study showed that:

- MIT and DBNPA (commercial biocides) were efficacious in removing and preventing biofilms from RO membranes, proving to be good models of anti-biofilm efficacy in biofouling studies. However, due to their toxicity to humans and the environment, they can only be applied in offline potable water applications.

- SBS was efficacious in preventing biofilm formation, but not in removing biofilms from RO membranes. Thus, SBS is not the optimal solution for biofouling control in RO systems.
- SB presented high–moderate efficacies in biofilm prevention and removal. However, the large concentration required to achieve those efficacies limits its application in RO drinking water applications.
- LAE effectively prevented and removed biofilms from RO membranes. Due to its high anti-biofilm efficacy and safety, LAE is an excellent candidate to control biofouling in RO drinking water applications.

Although LAE demonstrates great promise as an alternative biofilm treatment in RO systems, further research should be done before incorporating this chemical into biofouling control programs. Therefore, the following research studies are recommended: (a) determine LAE's anti-biofilm efficacy for different microorganisms common in RO units; (b) determine LAE's anti-biofilm efficacy against multi-species biofilms; (c) verify LAE's RO membrane-compatibility through short and long-term experiments; and (d) perform anti-biofouling tests on laboratory-, pilot-, and full-scale RO treatment systems.

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3.9 Author contributions

L.H.D.-S.-C. led the manuscript preparation; and performed the biofouling experiments in the CDC biofilm reactor with assistance from K.A., N.E.G., R.G., and N.R.. L.H.D.-S.-C and N.E.G. performed the 96-well plates experiments. L.H.D.-S.-C. performed the analysis and discussion of the data with assistance from K.A. and N.E.G. H.L.B. secured funding for this project and provided feedback and revised the manuscript.

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Chapter 4: Rapid polyamide membrane compatibility testing of potential anti-biofouling agents for reverse osmosis membrane systems

Luiz Henrique Da-Silva-Correa[a,d], Hayley A. Smith[a,d], Georgia Douglas[b,d], Danyka S. G. Thorburn[a,d], Rafaela Godoy[a,], Orielle-Floriane K. N. Henriquez[b,c], Nicole E. Gamm[a,d], and Heather L. Buckley[a,b,d*]

[a] Department of Civil Engineering. University of Victoria (UVic). Victoria, British Columbia (BC), Canada

[b] Department of Chemistry. UVic. Victoria - BC, Canada

[c] Department of Biochemistry and Microbiology. UVic. Victoria - BC, Canada

[d] Center for Advanced Materials and Related Technologies. UVic. Victoria - BC, Canada

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4.1 Preamble

In this chapter, the RO polyamide membrane compatibility of the selected biocides with unknown membrane compatibility (LAE, PE, and SB) was assessed via rapid membrane degradation tests. SBS was used as a control for membrane compatibility, given its application in RO systems for membrane storage. It is important to note that although PE showed indications of membrane damage during the antibiofouling experiments in the CDC biofilm reactor, membrane degradation tests needed to be performed to confirm PE's membrane incompatibility. These rapid membrane degradation tests were conducted under standard testing conditions (exposure time and biocide concentrations) commonly applied in membrane degradation studies in RO systems.

4.2 Abstract

The detrimental impacts of biofouling on reverse osmosis (RO) membrane (ROM) installations are one of the main technical barriers faced by RO technology to provide potable water. To unveil safer alternatives for biofouling prevention in ROM installations, this work assesses the ROM compatibility of three potential low-hazard anti-biofouling agents (LAE-lauroyl arginate ethyl, PE-phenoxyethanol, and SB-sodium benzoate) via a proposed rapid membrane degradation test. This study offers a cost-effective screening tool to select biocides for extensive compatibility studies. Attenuated Total Reflectance - Fourier Transform Infrared Spectroscopy (ATR-FTIR), Atomic Force Microscopy (AFM), and Scanning Electron Microscopy (SEM) assessed ROM surface damage due to biocide exposure. LAE did not show significant morphological or chemical membrane damage at any experimental conditions (Exposure time: 1h, 8h, 24h; pH: 4, 7, 9; Concentration: 100 mg/L, 50

g/L, 100 g/L, 150 g/L). However, results indicated that exposure to PE and SB led to membrane degradation. The proposed rapid membrane degradation test showed to be an excellent tool for improving current membrane compatibility testing practices. It identified two ROM-damaging biocides, SB and PE, streamlining large-scale testing efforts. Additionally, it identified a promising biocide, LAE, with potential to address biofouling in RO systems, prompting further long-term compatibility studies.

Keywords: Biocides; membrane compatibility testing; polyamide membrane; reverse osmosis.

4.3 Introduction

To address water scarcity challenges, new water treatment strategies, and technologies are needed to meet the global freshwater demand, including long-term potable water requirements[4,10,27,103,266]. Reverse osmosis (RO) membrane (ROM) systems have the potential to mitigate global water scarcity challenges by effectively producing freshwater from diverse water sources including seawater, brackish water, and wastewater[4,9,103,266]. One of the main technical challenges in potable water ROM applications is membrane fouling. It reduces system efficiency while also increasing the energy demands and environmental impacts associated with the technology[4,9,10,17–20,22,56]. Among the four types of fouling encountered in RO systems, namely inorganic fouling, biofouling, colloid fouling, and organic fouling, biofouling stands out as the most challenging one[4,9,22]. Biofouling consists of the unwanted adhesion, accumulation, and proliferation of microorganisms as biofilms on the surface of RO membranes[9,10]. Biofilms form readily on the membrane surface, leading to adverse effects such as depreciation of permeate flux, an increase in the operational pressure, and a decline in the permeate quality, as well as causing irreversible damage to the membrane, shortening its lifespan[4,9,10,266]. Thus, addressing the challenges posed by biofouling is crucial to promoting sustainable drinking water provision via RO technologies.

The current approach to mitigate membrane biofouling involves a two-step process consisting of feedwater pretreatment, followed by membrane cleaning (cleaning-in-place). However, this approach often results in damage to the ROM surface layer and leads to significant increases in operating costs[9,10,40]. Chlorine is the most widely used biocide for RO pretreatment due to its rapid and effective ability to eliminate a broad spectrum of microorganisms[9,10]. However, chlorine is incompatible with RO membranes as it causes oxidative degradation of the membrane polyamide surface layer[9,10,22]. Hence, the ROM vessels must be free of chlorine after treating the feedwater (dechlorination), commonly achieved via SBS dosing[9,10,22,40,103]. Additionally, feedwater pretreatment with chlorine needs proper risk management during handling and may lead to the generation of carcinogenic disinfection by-products (DBPs), such as haloacetic acids and trihalomethanes[9,10]. Along with feedwater pretreatment, the control of membrane biofouling is partly achieved through shock

treatments using hazardous chemicals, such as isothiazolones and 2,2-dibromo-3-nitrilopropionamide (DBNPA). These chemicals are only used in off-line RO applications, due to concerns regarding their toxicity and potential environmental impact[9,27].

Recent studies reveal that non-oxidizing biocides are an alternative for the prevention of biofouling in reverse osmosis systems, as these treatments can be used directly inside RO modules without the risk of degrading the RO polyamide membrane active surface layer[9,10,27,38]. In our previous review paper, we investigated the applicability of several non-oxidizing anti-biofouling agents to control/prevent biofouling in drinking water ROM systems[27]. After analyzing several attributes of various biocide candidates, including generation, mechanisms of action, capability to mitigate biofouling, possible risks to human and environmental health, membrane compatibility, and cost-effectiveness, the following three biocides were identified as potential low-hazard candidates for use in RO applications: 2-phenoxyethanol (PE), lauroyl arginate ethyl (LAE), and sodium benzoate (SB)[38,66,68,103].

PE and SB are common ingredients in cosmetics and personal care products, and are notable for their broad-spectrum antimicrobial efficacy and safety[68,114,165,179]. PE effectively kills bacteria, yeast, and moulds by damaging their cell membranes and inhibiting DNA and RNA synthesis[27,109,170,174]. SB demonstrates efficacy against fungi, gram-positive and gram-negative bacteria[188,194]. The primary mechanism of action of SB involves the overstimulation of ATP consumption, ultimately leading to cell death[27,194,195,197]. LAE effectively inactivates microorganisms through the modification of cell membranes and the disruption of membrane integrity[210]. Being a commercially approved food preservative, LAE has been applied to prevent microbial contamination in various food and beverage products, due to its high efficacy against a broad spectrum of microorganisms including yeast, fungi, algae, and bacteria[187,206,210].

Although these biocides exhibit high antimicrobial properties and potential safety for use in RO potable water applications, little is known about their RO membrane compatibility[27,38]. Assessing the efficacy of a biocide in the removal/prevention of biofilms from RO polyamide membranes is just as crucial as demonstrating its compatibility with RO membranes. This is because if a biocide is not membrane-compatible, it cannot be used inside RO membrane vessels. Recognizing the importance of both assessments when seeking safer chemical alternatives to control biofouling in ROM installations, in previous experimental work, we investigated the antibiofilm properties of the selected biocides[38], and in the present work, the polyamide membrane compatibility of these biocides is investigated. The present study builds upon our previous investigations on the anti-biofouling properties of the selected biocides by including RO membrane compatibility in the assessment of the biocides' applicability in biofouling prevention, addressing a significant research gap regarding these biocides[27,38].

Currently, the conventional methods to determine membrane compatibility of a biocide applied in the RO industry are resource-intensive, time-consuming, and expensive[9,51]. The methods of compatibility testing performed on large scales commonly involve biocide soak tests, which often take several months to a few years to complete, demanding significant amounts of water, energy, and biocides. Further, these larger-scale tests produce a significant amount of waste, adding pressure to concentrate disposal[9,17,18,51]. Therefore, there is a need for a screening tool to streamline the selection of biocides, allowing only the most promising candidates to proceed to further membrane compatibility testing on larger scales. This tool would reduce operational costs, minimize resource usage, and expedite the experimental process associated with the conventional membrane compatibility methods.

4.3.1 Aim and scope of the study

The goal of our present work is to introduce a screening tool to streamline the selection of RO membrane-compatible biocides, which ultimately optimizes conventional membrane compatibility testing practices. Furthermore, our work also aims to apply this tool to assess the membrane compatibility of three potential anti-biofouling agents (PE, SB, and LAE), which demonstrated significant promise in addressing biofouling in RO systems[27,38]. In this study, a series of rapid membrane degradation tests, designed to simulate prolonged continuous biocide dosing inside the RO membrane module, were performed to assess the biocides' ROM compatibility. These tests consisted of exposing the RO membrane to biocides at several biocide concentrations (100 mg/L, 50 g/L, 100 g/L, 150 g/L), and multiple pH levels (pH 4, pH 7, pH 9), for a short amount of time (1h, 8h, 24h)[9,10,40,103,265,267]. Following the rapid membrane degradation tests, an investigation of the chemical and morphological changes of the membrane was performed. SEM and AFM were applied to investigate morphological changes on the RO membrane surface layer. ATR-FTIR spectroscopy was applied to assess the oxidative damage of biocides to the polyamide membranes. The results were compared with a commercial membrane-compatible non-oxidizing agent (SBS; a biocide commonly used in RO membrane storage), and chlorine (oxidizing biocide). The proposed rapid membrane degradation testing method offers a cost-effective and efficient way to optimize conventional membrane compatibility tests by minimizing the allocation of resources, as well as the expense and time involved in assessing membrane-incompatible biocides. Therefore, given the current need to develop green, safer approaches to address biofouling in potable ROM installations, this investigation can benefit municipalities, industries, remote work sites, and water-stressed countries by the provision of a secure, safe, reliable, sustainable water supply via RO technologies.

4.4 Material and methods

4.4.1 Biocides and RO membranes

Rapid membrane compatibility tests were performed using commercial polyamide membranes (TriSep-YMACM34205). These RO membranes are applicable to surface water and brackish water applications presenting a surface polyamide selective layer[9,10]. The following selected biocides had their membrane compatibility assessed in this study: a) Lauroyl arginate ethyl - LAE (The Agricultural Research Service, the United States; CAS Number: 60372772), b) Sodium Benzoate - SB (Botanic Planet, Canada; CAS Number: 532321), and c) 2-Phenoxyethanol - PE (Thermo Fisher Scientific, the United States; CAS Number: 122996). Sodium hypochlorite (Sigma Aldrich, Canada; CAS Number: 7681529) was used as a positive control since it is a biocide well-known for its detrimental effects on RO polyamide membranes[9,10,40,103,268]. Sodium bisulfite - SBS (Sigma Aldrich, Canada; CAS Number: 7631905), and deionized (DI) water (Milli-Q water) were used as a negative control and an undamaged membrane reference, respectively[9,10,40].

4.4.2 Rapid membrane degradation testing

The rapid RO polyamide membrane degradation testing protocol was designed based on membrane degradation studies in ROM systems and membrane autopsy research[9,40,51,102,103,265,267–269]. The rapid membrane degradation tests proposed expedite the evaluation of membrane compatibility by accelerating membrane degradation, allowing RO membrane compatibility assessment within a shorter timeframe[9,40,103,265]. In summary, 12 mm diameter RO membrane samples were soaked in the identified biocides under 8 different experimental conditions as described in Table 9. All experimental runs were performed at 25°C in an incubator shaker (New Brunswick I26-Incubator-Shaker; 2.5 cm orbital-diameter) set to 100 rpm. After the soaking step, the incubator was adjusted to 140 rpm and 25°C and the samples were rinsed using DI water for 1 hour. Additionally, in order to remove any biocide residue, the samples were subjected to 3 DI water rinses. After rinsing, the membrane samples were dried in a desiccator for 3 days. Subsequently, the following analyses were performed i) SEM analysis to provide a qualitative assessment of the RO membranes' surface layer, ii) AFM analysis to provide a quantitative assessment of membrane surface roughness, and iii) ATR-FTIR spectroscopy to evaluate the oxidative degradation of polyamide membranes as described in Kucera, 2019, and Kwon & Leckie, 2006[10,268]. Experiments were carried out in triplicate during distinct weeks and the measurements were performed in three different random positions for each sample.

Table 9 - Rapid Membrane Degradation Experimental Conditions

Experimental Run	Biocide concentration (g/L)	pH	Duration (h)
1	0.1	7	24
2	50	7	1
3	100	7	1
4	100	4	1
5	100	9	1
6	100	7	8
7	100	7	24
8	150	7	1

4.4.3 Characterization of the surface morphology of polyamide reverse osmosis membranes: AFM and SEM

SEM and AFM were applied to analyze variations in the surface morphology of the samples treated with biocides[9,40,51,103,265]. AFM images were obtained with an AFM Agilent microscope (Non-tapping mode AFM; Model: 5500 AFM) with an approaching force of 48 N/m, scanning speed of 0.6 lines/s, and scan area of 10µm x 10µm from 3 random locations per sample[40,103,265]. The Silicon AFM probes (TAP190-G-10; TED PELLA, Canada) had a <10 nm nominal tip radius and a resonance frequency of 190 kHz. Average root mean square roughness (RMS) and membrane surface roughness (Sa) were calculated from the AFM measurements using the Gwyddion 2.61 Software[269]. For a qualitative analysis of changes in the surface layer of polyamide membranes and validation of AFM results, SEM images were taken at three different, random positions for each membrane sample with a magnification of 10,000x and a voltage of 1.5kV in a SEM Hitachi S-4800 microscope[38,40,103,265]. Before imaging, the membrane coupons were coated with gold for 180 seconds with an Anatech Hummer VI Sputter Coater[38,40,265].

4.4.4 ATR-FTIR spectroscopy

ATR-FTIR spectroscopy was employed to analyze the biocides' oxidative damage to the RO polyamide membranes[40,265]. The ATR-FTIR spectra of the treated and untreated membrane coupons were obtained with a Spectrum Two Fourier-Transform infrared spectrometer (PerkinElmer Spectrum Two UATR 100150). Each ATR-FTIR spectrum was recorded with a minimum of 10 scans (1 cm^{-1} resolution) and a wave-number range of 800 - 2,200 cm^{-1} [40,103,265]. All readings were taken at room temperature.

4.4.5 Statistical analysis

The statistical differences in all experiments were evaluated through Student's t-test and one-way ANOVA. Statistical significance was attributed to p-values lower than 0.05.

4.5 Results and discussion

The membrane compatibility analysis discussed herein was based on the comparison between membranes exposed to biocides with unknown membrane compatibility (LAE, PE, and SB) to membranes exposed to DI water (undamaged membrane reference), SBS (negative control; standard commercial biocide used in membrane storage), and chlorine (positive control; chemical known to degrade RO membranes)[9,10,40,267,268]. The results of the biocides under investigation (LAE, PE, SB) were exclusively compared to the results of the controls (DI Water, SBS, NaOCl) within the same experimental run. Appendix C, Figures 29-33 present images for all control experimental runs.

4.5.1 Effects of Biocides on the Morphology of Reverse Osmosis Polyamide Membranes

SEM and AFM were applied to investigate changes in the morphology of the surface layer of RO polyamide membrane coupons exposed to the biocides. RO polyamide membranes typically present a ridge-and-valley structure when undamaged[10,40,103,268,270]. The primary method for evaluating the impacts of chemicals on the surface layer of polyamide membranes is by examining changes in membrane morphology and membrane chemical structure via membrane autopsies[9,40,51,268]. Typically, RO membranes present a smooth surface layer when damaged by oxidizing biocides and a swollen membrane surface layer when damaged by non-oxidizing biocides[9,268,271–274]. Morphological changes on RO membranes' surface layer are also often characterized by the estimation of RMS and Sa values. Sa values consist of the average deviation of the lowest points (valleys) and the highest points (peaks) of the membrane surface layer from its center plane, and RMS values correspond to standard deviations of these peaks and valleys. Considering the typical ridge-and-valley structure observed for undamaged RO membranes, damages to RO membranes by oxidizing biocides are characterized by a decrease in Sa and RMS values, and membrane damages due to the exposure of non-oxidizing biocides are characterized by an increase of Sa and RMS values, all in comparison to typical Sa and RMS values[9,10,40,103,265,268,271]. Figure 10 displays representative AFM 3D images of the RO membrane coupons' active surface layer after exposure to LAE (and representative controls) as well as their respective Sa and RMS values. Figure 11 displays representative AFM 3D images of the RO membrane coupons' active surface layer after exposure to PE, and Figure 12 presents the representative AFM images of the membranes after exposure to SB. Appendix C, Figures 29-31 present AFM images for all control experimental runs.

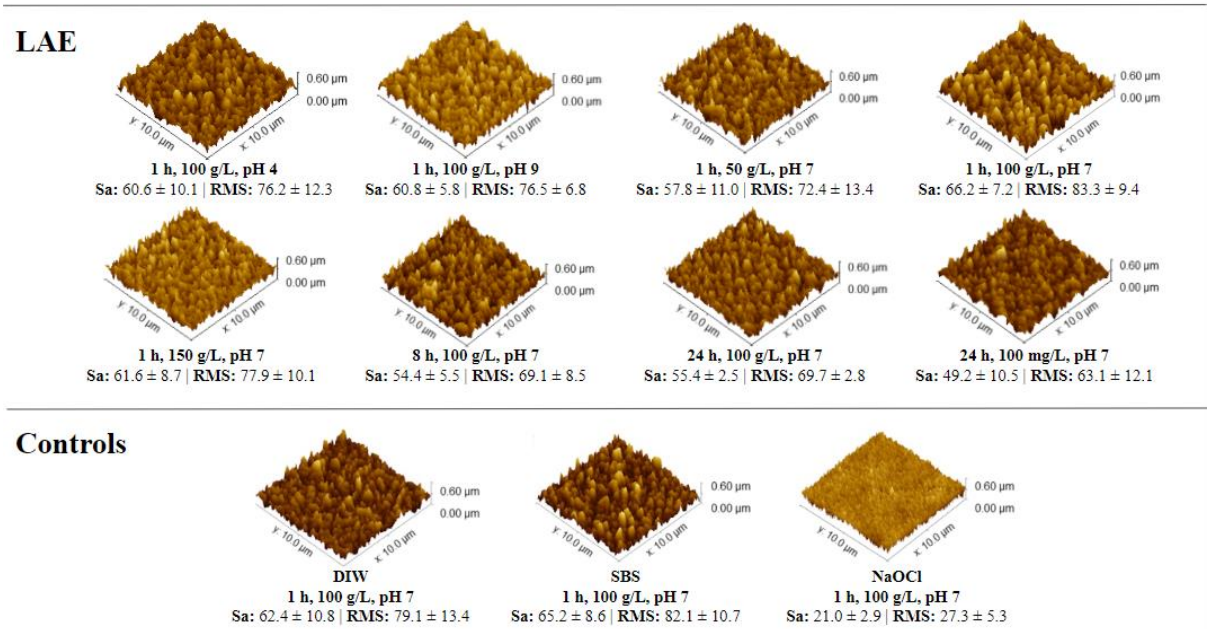

Figure 10 - Representative AFM images and Sa and RMS values of polyamide membrane coupons exposed to LAE at different experimental conditions. Sa and RMS values are displayed as mean ± standard deviation.

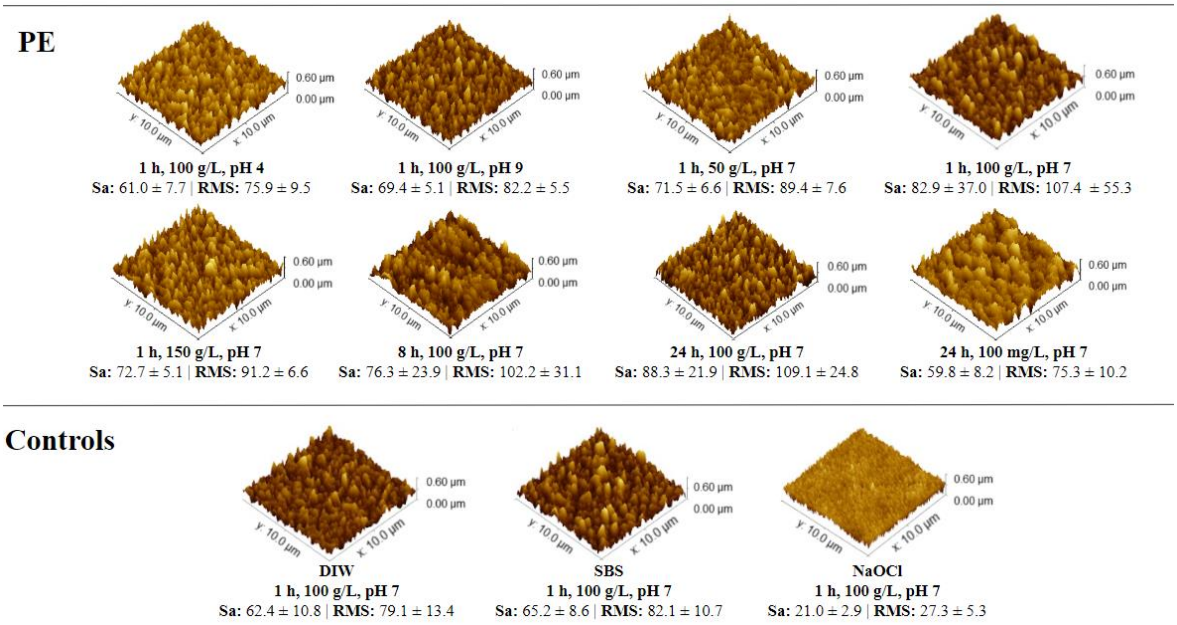

Figure 11 - Representative AFM images and Sa and RMS values of polyamide membrane coupons exposed to PE at different experimental conditions. Sa and RMS values are displayed as mean ± standard deviation.

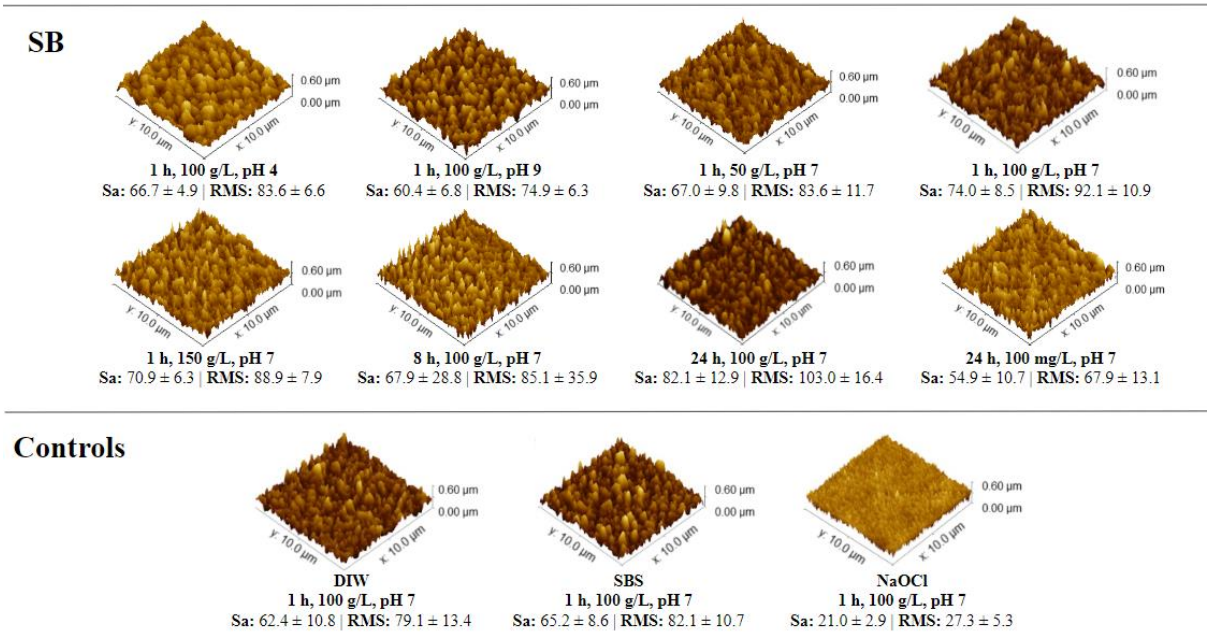

Figure 12 - Representative AFM images and Sa and RMS values of polyamide membrane coupons exposed to SB at different experimental conditions. Sa and RMS values are displayed as mean ± standard deviation.

The 3D topographies of the polyamide membranes' active layer displayed in Figures 10-12 and Figures 29-31 reveal that as expected the membrane coupons exposed to water and SBS presented a ridge-and-valley structure, typical of undamaged membranes. Membrane coupons exposed to DI water presented Sa values ranging from 51.5 ± 5.2 nm to 62.4 ± 10.8 nm and RMS values ranging from 62.9 ± 17.3 nm to 79.1 ± 13.4 nm (Figure 29), while the membranes exposed to the negative control (SBS) presented Sa and RMS values ranging from 49.0 ± 5.6 nm to 69.7 ± 3.2 nm and from 62.2 ± 7.3 nm to 87.0 ± 4.3 nm (Figure 30), respectively. Statistical analysis indicated that the Sa and RMS for these runs were not significantly different from each other under all experimental conditions (Student's t-test; $p>0.05$). Similar results were found by Kwon & Leckie, who studied the morphological changes on RO membrane surface layers due to membrane degradation and observed that virgin RO membranes presented Sa and RMS values of 58.1 ± 2.9 nm and 73.3 ± 3.8 nm, respectively[268]. In contrast, the membrane coupons exposed to chlorine (positive control) presented a smooth surface layer with Sa and RMS values ranging from 7.1 ± 2.2 nm to 41.4 ± 6.4 nm and from 9.5 ± 3.6 nm to 53.3 ± 8.4 nm (Figure 31), respectively, which are attributed to the oxidative damage of the RO membrane coupons from the chlorine exposure[9,40]. Thus, not surprisingly the values RMS and Sa values of polyamide membranes exposed to chlorine were significantly decreased in comparison to Sa and RMS values of undamaged membranes (Student's t-test; $p<0.05$) (Figures 10-12 and Figures 29-30), confirming the consistency of these results.

As depicted in Figure 11 and 12, RO membrane coupons exposed to PE (Sa values ranging from 59.8 ± 8.2 nm to 88.3 ± 21.9 nm and RMS values ranging from 75.3 ± 10.2 nm to 109.1 ± 24.8 nm) and SB (Sa values ranging from 54.9 ± 10.7 nm to 82.1 ± 12.9 nm and RMS values ranging from 67.9

± 13.1 nm to 103.0 ± 16.4 nm) presented, in general, Sa and RMS values greater than the values observed for membranes exposed to DI water and SBS (undamaged membranes) under all experimental conditions (Table 9; Figures 11 and 12). The observed increase in Sa and RMS values due to exposure to PE and SB indicates membrane degradation via membrane swelling[9,71,271–273]. The swelling of RO polyamide membranes resulting from exposure to non-oxidizing biocides such as PE and SB primarily occurs due to the ionization of functional groups (carboxylic and amide groups) present in the polyamide membranes[9,71,273,275,276]. Statistical analysis indicated that the increase in the Sa and RMS values observed for PE in experimental run 4 (pH 4, 100 g/L, 1h) and SB in experimental run 5 (pH 9, 100 g/L, 1h) were not significantly different from the controls associated with undamaged membranes (DI water and SBS controls) (Student's t-test; $p>0.05$). The observed difference in the Sa and RMS values at different pH levels is expected, given that the interaction between biocides and the functional groups present in the polyamide membranes is pH-dependent[9,273]. However, despite PE not showing significant membrane damage at lower pH levels (experimental run 4) and SB at higher pH levels (experimental run 5), both are still considered unsuitable for RO potable water membrane applications. This is because both biocides caused significant membrane damage in the experimental runs conducted at pH 7 (Figures 10-12 and 29-31), the typical operational pH found inside membrane vessels in RO potable water applications[9].

Additionally, although both non-oxidizing biocides seemed to swell the RO membrane coupons, PE showed a higher membrane degradative effect when compared to SB. This is because, during the experiments, it was observed that the membrane coupons' active layer exposed to PE detached from the membranes' microporous support layer (irreversible membrane damage). Severe damage caused by PE was evident, not only from the Sa and RMS values obtained via AFM, but also visually, as all membrane coupons treated with PE exhibited a change in colour and curling of the membrane edges. Therefore, due to the incompatibility with polyamide membranes, PE and SB are not recommended for direct dosing into RO membrane vessels.

In contrast, the AFM results indicated that LAE did not change the membrane morphology of RO polyamide membranes, as evidenced by the Sa and RMS values of RO membrane coupons soaked with LAE (Sa values ranging from 49.2 ± 10.5 nm to 66.2 ± 7.2 nm and RMS values ranging from 63.1 ± 12.1 nm to 83.3 ± 9.4 nm) that were not significantly different from the typical Sa and RMS values of the undamaged RO membranes in all experimental runs ($p>0.05$; t-test) (Figure 10; Figures 29 and 30). In general, it was observed that the biocides which caused damage to RO polyamide membranes such as PE and SB (and chlorine), had their damaging effect, in regards to Sa and RMS values, intensified by increasing the biocide concentration and exposure time (Figures 10-12 and 29-31). Biocides that did not cause damage to RO membranes, such as LAE and SBS, did not show any impact on the typical Sa and RMS values when concentration and exposure time were increased nor when pH conditions were changed (Figures 10-12 and 30-31). Therefore, the AFM results suggest

that LAE is a promising membrane compatible biocide applicable to ROM water treatment applications.

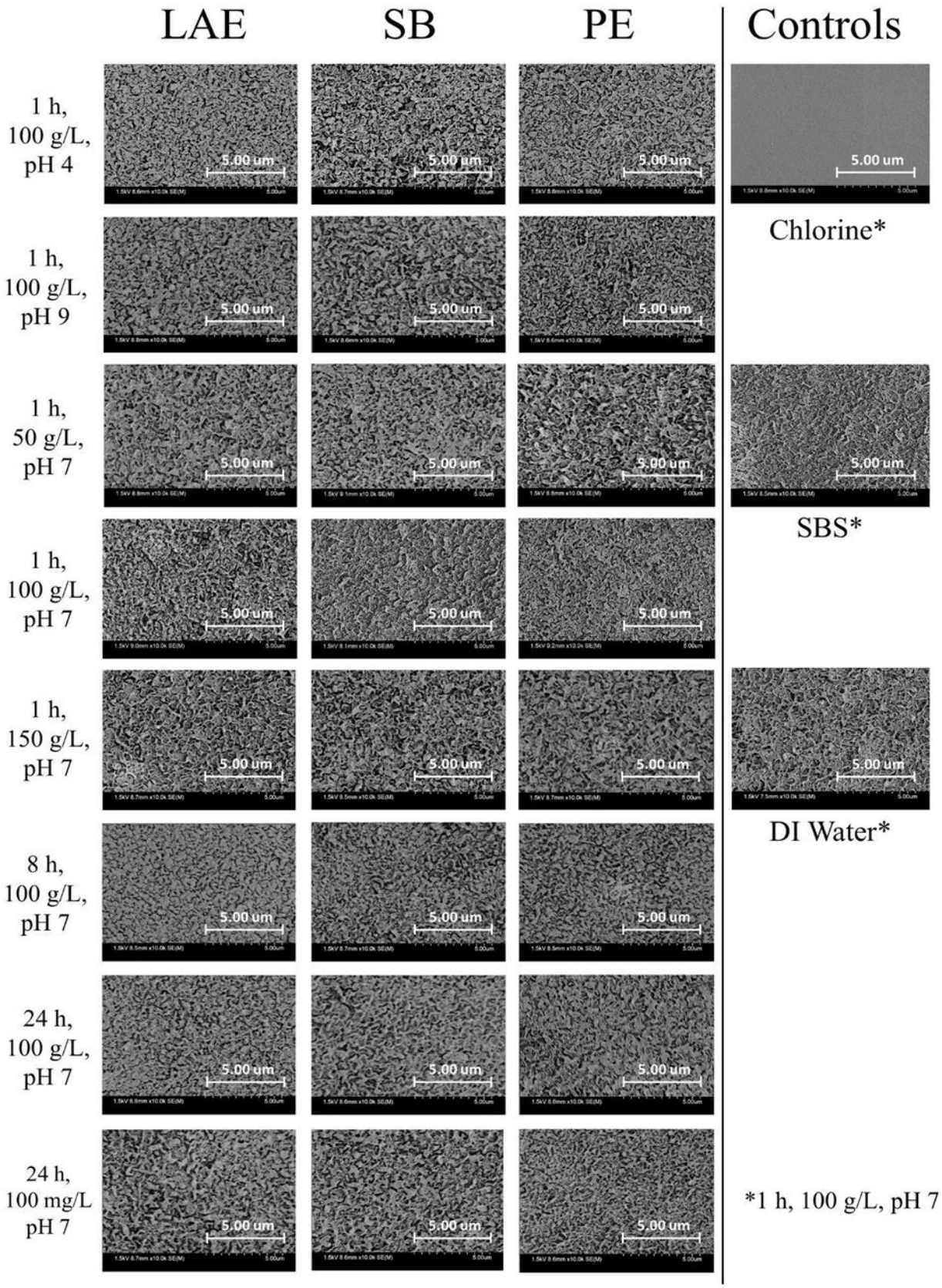

Figure 13 - Representative SEM images of RO polyamide membrane coupons exposed to the selected biocides (SB, PE, and LAE) at different experimental conditions. Scale bars represent 5.00 μm (at 10,000x). The representative SEM images of the controls (SBS, chlorine, or DI water) for all experimental runs are displayed in Figure 32 in Appendix C.

Figure 13 displays the SEM images of the RO membrane coupons' polyamide layer exposed to the selected biocides. According to Figure 13, all tested biocides presented membrane surface layer morphology similar to the untreated and SBS-treated membranes when compared to the membrane coupons treated with chlorine under all experimental conditions. Further, no significant morphological differences on the membrane surface layer were observed between the reference, negative control, and the three low-hazard biocides (PE, LAE, and SB). This suggests that while SEM (Figure 13) allows for the visualization of severe damage to RO membranes, the subtle differences in membrane surface morphology noticed in the AFM quantitative analysis (Figures 10-12 and Figures 29 - 31) could not be visualized in SEM (Figure 13). However, it is important to note that the AFM quantitative analysis was conducted on a limited area of the membrane surface while the SEM images have a much larger area of view. The SEM images showed that the membrane surfaces had consistent morphological characteristics for each biocide in each respective experimental run. This indicates that the AFM results can be considered representative of the overall membrane surface, and the morphological differences detected by the AFM quantitative analysis may be extrapolated to the entire membrane surface[9,40,51,268]. This shines light on the importance of combining SEM and AFM techniques to perform membrane autopsies, as these methods were shown to be complementary[9,40,51,268].

4.5.2 Effects of Biocide on the Chemical Structure of Reverse Osmosis Polyamide Membranes

Following morphological analysis of the active layer of the polyamide membrane coupons through AFM and SEM, ATR-FTIR was used to assess the effect of the biocides on the chemical structure of the polyamide membranes. Figure 14 shows the three characteristic peaks (1609 cm^{-1}; 1665 cm^{-1}; 1542 cm^{-1}) selected to assess the potential oxidative degradation of the biocides to the RO membrane chemical structure[40,103,265,268]. Amide I and amide II bands correspond to the peaks 1665 cm^{-1} and 1542 cm^{-1}, respectively[40,103,265,268]. The 1665 cm^{-1} peak is derived from the C-C-N deformation vibration of the secondary amide group, C-N stretching, and C=O stretching[40,103,265,268]. The 1609 cm^{-1} peak is derived from C=C ring stretching and finally, N-H in-plane bending leads to the appearance of the 1542 cm^{-1} peak.

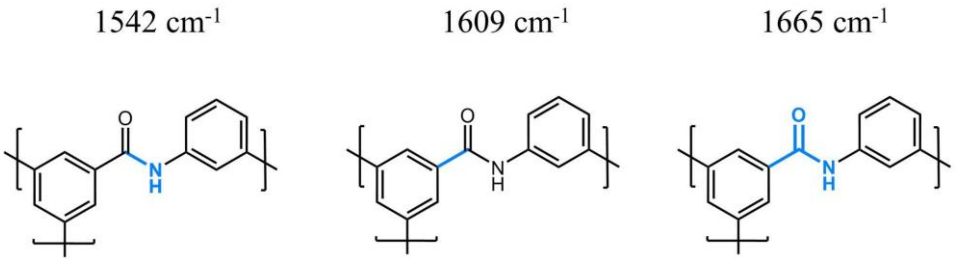

Figure 14 - Characteristic absorption peaks and chemical structure of RO polyamide membranes.

Figure 15 displays the ATR-FTIR spectra of RO polyamide membrane coupons after exposure to the selected biocides (PE, SB, and LAE), alongside representative ATR-FTIR spectra of the controls (SBS, DI Water, and Chlorine). Appendix C, Figure 33 displays ATR-FTIR spectra images for all control experimental runs. In the ATR-FTIR spectra corresponding to the runs with chlorine, all three characteristic peaks were absent under all experimental conditions or significantly decreased, confirming its known oxidative damage to polyamide membranes[9,40,103,265,268]. On the other hand, comparing RO membrane coupons soaked in SBS to those soaked in DI water, there is no reduction in the three peaks, or at any other wavenumber in the spectrum, confirming that SBS does not damage polyamide membranes[40,103,265,268]. The ATR-FTIR spectra of membranes exposed to three non-oxidizing biocides (SB, LAE, and PE) did not present any significant difference compared to the spectra of undamaged membranes (DI water and SBS). Additionally, it was observed that the three characteristic peaks were significantly different from the characteristic peaks observed in membranes soaked in chlorine, further validating the results. Therefore, the ATR-FTIR results indicated that the three selected biocides (SB, LAE, and PE) showed no oxidative damage to RO membranes.

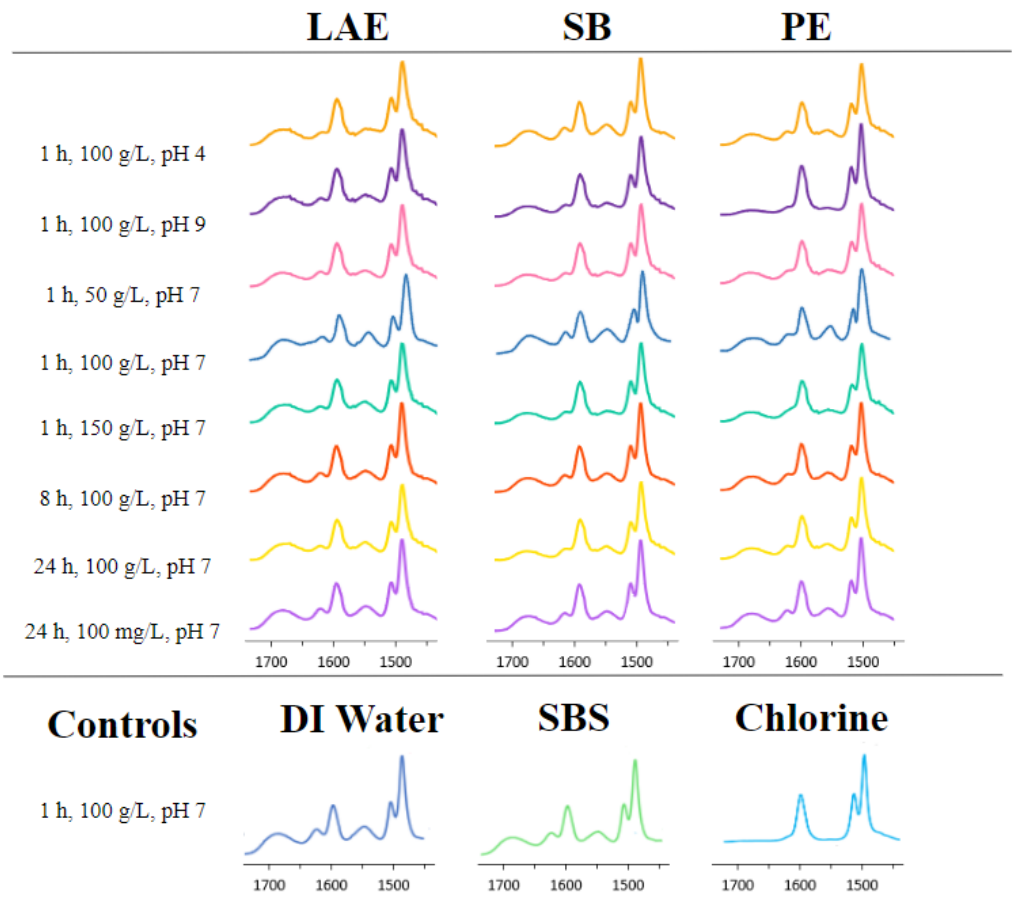

Figure 15 - ATR-FTIR Spectra of RO polyamide membrane coupons exposed to the selected biocides (SB, LAE and PE) for all experimental conditions. The ATR-FTIR Spectra for the controls (Chlorine, SBS, DI Water) are displayed in Figure 33 in Appendix C.

4.5.3 Rapid membrane degradation testing as a screening tool in RO membrane compatibility studies

The rapid membrane degradation testing applied herein has shown to be a powerful screening tool in selecting biocides for more extensive RO membrane compatibility studies. Our study enabled the rapid screening of multiple candidate anti-biofouling agents in regards to RO polyamide membrane compatibility within a short time frame at a low cost, which enhances its feasibility and relevance for widespread adoption within the RO industry. The proposed test revealed that LAE exhibited no apparent damage to RO polyamide membranes, unlike PE and SB (Figures 10-13 and 15). The application of the proposed rapid membrane degradation testing eliminated the need for more thorough, resource-intensive membrane compatibility testing in large volumes of PE and SB, saving resources that can be applied to the testing of more promising biocides[9,40,51,103,265]. Therefore, this study offers an efficient screening method for identifying promising anti-biofouling agents in RO polyamide membrane compatibility investigations. Additionally, the present work promotes sustainable practices within the RO industry as it ultimately improves the traditional membrane compatibility approach by reducing resource-intensive testing of biocides that are quickly recognized as incompatible with polyamide membranes.

4.6 Conclusions and future prospects

Biofouling is the main obstacle to the efficiency of RO technology in delivering potable water. It leads to reduced permeate production and RO membrane damage, which results in the need for the development of effective approaches to prevent biofouling in ROM installations. To propose both an environmentally friendly solution for biofouling, and a screening protocol for ROM compatibility testing, this study developed and applied an effective screening tool to investigate the ROM compatibility of three low-hazard anti-biofouling agents (LAE, PE, and SB), across various experimental conditions. RO polyamide membrane compatibility was assessed using AFM, SEM, and ATR-FTIR techniques through rapid membrane tests. The results suggested that LAE demonstrates compatibility with RO membranes, as it did not cause significant morphological or chemical damage to RO polyamide membrane coupons. ATR-FTIR analysis showed no reduction in the peaks attributed to undamaged RO membranes, and AFM and SEM images indicated no changes in the RO membrane morphology. On the other hand, PE and SB appeared to be incompatible with RO membranes, as exposure to these biocides resulted in changes to RO membrane morphology, suggesting membrane degradation via membrane swelling, a common degradative effect observed in non-oxidizing biocides. The results demonstrated the effectiveness of the proposed screening tool. It successfully identified two biocides that caused damage to the membranes (PE and SB), along with one biocide candidate (LAE) that did not exhibit observable damage to RO membranes. Therefore, as LAE demonstrated no significant morphological or chemical damage to the RO polyamide membranes among the three tested biocides, LAE is the only biocide recommended for further extensive RO membrane compatibility studies. Therefore, the following future studies are recommended:

- the conduction of extensive RO membrane compatibility from laboratory to full-scale ROM systems with LAE, including a comparison of the RO membrane performance in terms of salt rejection, trans-membrane pressure, and water flux before and after biocide treatments.

- the conduction of an in-depth cost-effectiveness analysis of LAE in comparison to conventional strategies applied to address biofouling in RO systems.

4.7 Author contributions

The manuscript preparation was led by Luiz Henrique Da Silva Correa. Luiz Henrique Da Silva Correa and Hayley A. Smith contributed to the experimental design of the study. Luiz Henrique Da Silva Correa, Hayley Smith, Georgia Douglas, Danyka Thorburn, Rafaela Godoy, Orielle-Floriane Henriquez, and Nicole Gamm performed membrane compatibility experiments and data analysis in this study. Heather Buckley ensured project funding and provided feedback on the paper.

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Chapter 5: The application of ethyl lauryl arginate to prevent biofouling in reverse osmosis polyamide membrane systems: A benchtop study

Luiz Henrique Da Silva Correa[a,d], Hayley Alexandra Smith[a,d], Danyka Shelagh Gabrielle Thorburn[a,d], Orielle-Floriane Kaneza Nduwimana Henriquez[b,d], Heather Louisa Buckley[a,c,d]*

a - Department of Civil Engineering. University of Victoria (UVic). Victoria, British Columbia (BC), V8P 5C2, Canada.

b - Department of Biochemistry and Microbiology. UVic. Victoria - BC, Canada

c - Department of Chemistry. UVic. Victoria - BC, Canada

d - Center for Advanced Materials and Related Technologies & Institute for Integrated Energy System. UVic. Victoria - BC, Canada

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5.1 Preamble

In this chapter, a methodology for testing and analyzing the anti-biofouling efficacy and membrane compatibility of the most effective antifoulant candidate (LAE) identified in the screening protocol (Validation Protocol) is presented. Due to several delays beyond my control and time constraints, the RO benchtop was not constructed in time to perform experiments as part of my PhD work. However, I have contributed to the design of the RO benchtop in collaboration with staff, students, and alumni from the University of Victoria. Additionally, I have developed the validation protocol that will guide the use of the RO benchtop. Consequently, the design of the RO benchtop and the validation protocol are integral components of my PhD work. Therefore, As advised by my PhD committee members and PhD Supervisor, this chapter exclusively details the design of the RO benchtop and the experimental approach in a manuscript-based format, as this work is being prepared for submission to Environmental Science & Technology (ES&T) – ACS Journal. The experiments in the RO benchtop with LAE will be conducted during my post-doctoral research in the Green Safe Water Lab (Dr. Buckley's Research Group), given the unforeseen delays in the RO benchtop construction.

5.2 Introduction

Water scarcity is the inability to meet current local water demand due to the insufficiency of available freshwater. This crisis is further aggravated by several factors such as water contamination, climate change, and increasing agricultural and industrial water demand[1,2,4,7,277]. Water contamination due to anthropogenic activities, severe droughts caused by climate change, and a continued increase in global population, which further augments potable water demand, are all factors that compromise the availability of freshwater[1,4,7,41,277]. As reported by the United Nations, 2023, freshwater shortages are a global issue[5]. Nearly one third of the global population is living in water-stressed countries, and one seventh of the world is living without access to clean water[5]. Additionally, the prediction model created by Kuzma et al., 2023 indicates that by the year 2050, nearly two thirds of the global population will experience water stress[6]. The application of clean water technologies such as desalination techniques can be used to address challenges in potable water supply[2,7,277]. Desalination techniques such as reverse osmosis (RO) membrane systems possess the ability to produce freshwater from wastewater, brackish water, and seawater, which allows a variety of water sources to be utilized to meet potable water demands[2,4,7,9,10,51]. Polyamide membrane systems are the most common desalination membrane technology used in RO water treatment applications as polyamide membranes have wider pH ranges, lower operating pressures, and a higher rejection in comparison to other types of membranes such as cellulose acetate membranes[9,10,278]. RO polyamide membrane systems were developed in the early 1980s, and as of 2020, a total capacity of around 67 million m³ of freshwater was produced daily from RO plants, mainly consisting of polyamide membrane systems, with that capacity set to increase by 2025[4,227,279]. This current water production capacity (67 million m³) is sufficient to meet the needs of 700 million to 1.3 billion people, considering the WHO standard of 50-100 liters per capita per day for the full right to water[11]. This demonstrates that desalination technology is a possible solution for overcoming freshwater scarcity challenges[2,4,7,9,10]. The production of freshwater from various water sources offers an opportunity to decrease the effects of water scarcity around the world[2,7,9], and presents RO polyamide membrane technology as a solution to tackle the ongoing water crisis.

Although RO water treatment systems hold great promise in addressing global water scarcity, biofouling poses a major obstacle to promote water supply via RO technology as it escalates operational and maintenance costs, energy consumption, and adverse environmental impacts associated with the technology[4,7,10,17–20,22,23,36]. Biofouling is characterized by the build up of microorganisms embedded in extracellular polymeric substances (EPS) (biofilm matrix) on membrane surfaces[10,28,29,32,32,245]. Membrane biofouling leads to more frequent membrane replacements and greater chemical usage, exacerbating challenges and environmental risks associated with concentrate disposal, and overall cost[2,7,9,17–19]. Despite efforts to enhance energy efficiency in RO systems, such as incorporating energy recovery devices and utilizing renewable energy sources,

biofouling continues to pose as a challenge for achieving energy efficiency in RO applications as it increases operational pressure and overall energy consumption in RO plants[2,7,9,20,23,36]. Biofouling is imperfectly addressed by control programs which primarily consists of feed water pretreatment with chlorine, followed by membrane cleaning[4,9,22]. The disadvantages of this approach are numerous. Firstly, chlorine is incompatible with polyamide membranes, necessitating its removal from the system after feed water pretreatment (dechlorination)[4,9,10,22]. Secondly, chlorine reacts with organics in the feed water, leading to the formation of carcinogenic by-products such as trihalomethanes and haloacetic acids[4,9,10,22]. Furthermore, although current membrane cleaning is partially achieved via the usage of non-oxidizing biocides such as 2,2-dibromo-3-nitropropionamide (DBNPA) and isothiazolones, the application of these biocides is prohibited for inline potable water use due to their potential hazards to both humans and the environment[4,9,10,22,27]. The existing methods to control biofouling fail to provide long-term sustainable solutions, merely offering temporary relief of biofouling without addressing the root of the problem[4,9,22,27]. Biofouling prevention emerges as a more sustainable approach, mitigating strain on RO systems, preserving membrane integrity, reducing associated environmental impacts, and presenting a cost-effective alternative to mitigation strategies[4,10,10,20,22,27,36]. Thus, developing effective methods to prevent biofouling in RO polyamide membrane systems is crucial for ensuring a reliable supply of potable water via RO technologies, given the challenges in current biofouling control methods and the benefits of biofouling prevention strategies[4,7,9,22,27].

In our previous work, we performed a series of preliminary studies aimed to develop biofouling prevention strategies applicable to potable water RO polyamide membrane systems[27,38,280]. Our studies revealed that LAE exhibits several characteristics attributed to an ideal biocide as described in Bates, 1998, particularly in the context of preventing biofouling in drinking water membrane applications[27,38,39,280]. LAE is characterized by a hydrophobic lauric acid tail and a positively charged L-arginine head[103,206,264,265,281–283]. LAE is effective against several microorganisms common in RO membrane systems such as *Pseudomonas aeruginosa* and *Staphylococcus aureus* (biofilm-forming microorganisms)[38,206,264,265,281–283]. It has several mechanisms of action, and a fast inactivation time (2-6 minutes), primarily killing microorganisms by disrupting cell membranes[38,103,206,265,281,282,284]. Our preliminary antibiofouling studies have demonstrated that LAE effectively inhibits biofilm formation and removes mature *Pseudomonas aeruginosa* biofilms from RO membranes, with observed biofilm prevention and removal efficacy values of 98.9 ± 0.3% and 99.6 ± 0.2%, respectively[38]. Additionally, LAE exhibited low effective inhibitory doses, with a minimum biofilm inhibitory concentration (MBIC) of 0.06 g/L and a minimum biofilm eradication concentration (MBEC) of 5 g/L[38] (Da-Silva-Correa et al., 2023). Moreover, our preliminary polyamide membrane study revealed that LAE does not cause morphological changes, nor damage via oxidation, in RO polyamide membranes[280].

Considering human health implications, LAE has shown low toxicity when used during in vivo studies[206,281,284,285]. Furthermore, it has been approved by the US Food and Drug Administration as a food preservative since 2005[187,206,281,284,285]. Studies in humans have shown that low doses of LAE are fully metabolized within 24 h, while larger doses are completely hydrolyzed into amino acids over a more extended period and expelled as urine, faeces, or expired air[206,281,284,285]. Additionally, the chemical properties of LAE allow it to be suitable for implementation into RO membrane systems[38,103,280,281,284,286]. This is because, LAE maintains its antimicrobial properties over a broad temperature range (2 - 180°C) and broad pH level range (optimal pH 2-8)[281,283,287]. Further, LAE presents high solubility in water (247 g/L), high biodegradability, and has a long shelf life (2 years)[281,283,284,287]. Although LAE holds great potential to be applied in RO potable water applications, further research is required to fully assess the capability of LAE to address biofouling in RO water treatment systems[27,38,280].

5.2.1 Aim of study and scope

Our current study aims to assess the antibiofouling properties and polyamide membrane compatibility of LAE for potential application in potable RO water treatment systems. The efficacy and membrane compatibility were assessed by experiments conducted in a benchtop RO system to provide a more realistic context of the applicability of LAE on a larger scale, as compared to our previous studies[27,38,280]. In our current work, we assessed the capability of LAE for biofilm prevention and removal from RO membranes (antibiofouling testing) in the RO benchtop system. In addition, we determined the polyamide membrane compatibility of LAE by exposing RO membranes to a high dosage of the chemical for a 12-month period, and then testing the membrane in the benchtop system (polyamide membrane compatibility testing). To assess the performance of the RO benchtop system, the normalized rates of salt rejection, water flux, and trans-membrane pressures within the system were observed and measured to examine the effects of biofouling on the system. This is an established method in the industry and is applied in previous membrane studies for assessing biofouling and membrane degradation in RO systems[9,10,22,103,252,255]. Any changes in the normalized metrics that vary 15% from the expected values (as per industry standard assessment practice) were considered detrimental to RO performance, and deemed incompatible[9,10]. Subsequent to the experiments conducted in the RO benchtop, membrane autopsies were performed to validate the results, and to further evaluate the antibiofouling properties and membrane compatibility of LAE[9,10,32,38,40,103,247,265,280]. The application of confocal scanning laser microscopy (CLSM) and scanning electron microscopy (SEM) for quantitative and qualitative analysis were used to evaluate the antibiofilm efficacy of LAE[10,32,38,247]. We utilized attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) to assess the impact of any oxidative damages from LAE on the polyamide layer of the membrane surface[9,40,103,265,280]. Additionally, atomic force microscopy (AFM) and SEM were applied to examine any morphological changes to the membrane from exposure to

LAE[9,40,103,265,280]. The results from this study are crucial for finding safe, green solutions to mitigate the detrimental effects of biofouling in RO potable water membrane applications, and to offer a pathway for addressing the challenges of water scarcity.

5.3 Materials and methods

5.3.1 Biocides, synthetic feed water, membranes, and bacteria

Commercial RO polyamide membranes (TriSep and YMACM34205) were used in the anti-biofouling and polyamide membrane compatibility testing. All membranes were obtained from the same lot. The formulation of the synthetic feed water for both experiments carried out on the benchtop system was designed to simulate the water characteristics found inside an RO membrane vessel in RO potable water applications with brackish water as water source, following similar formulation from several RO studies found in the literature[9,10,27,38,103,249,255]. The experiments were performed following the standard temperature, pressure, and pH (25°C, 225 psi, pH 7.0) used in RO performance testing in the industry[9,10,51]. Table 10 summarizes the synthetic feed water composition used for the anti-biofouling efficacy and polyamide membrane compatibility experiments. In brief, the nutrient concentration was set to 20 mg/L of Tryptic Soy Broth (Thermo Fisher Scientific, MA, USA; 6 mg/L equivalent Total Organic Carbon) and the background salinity was set to 500 mg/L (NaCl; 7647-14-5; Thermo Fisher Scientific, USA)[249,252,255]. NaCl was used to trace salinity rejection in both biofouling and membrane degradation experiments in the RO system. LAE (CAS-Number: 60372-77-2; The US Agriculture Research Service, CA, USA) was the selected biocide to be evaluated on its membrane degradation and anti-biofouling ability. MIT (CAS-Number: 2682-20-4, Sigma-Aldrich, Canada) was used as a control of anti-biofouling efficacy in biofouling experiments[9,38]. SBS (CAS-Number: 7631-90-5; Sigma-Aldrich, Canada) was used as a control for membrane compatibility in the membrane degradation experiments[9,38]. Polyamide membrane compatibility was assessed with 10 x MBEC through membrane soaking[51]. In the anti-biofouling experiment, the biocides were continually dosed at either 2x MBIC (prevention protocol) or MBEC (removal protocol) into the RO benchtop system[9,38,254]. Being a well-known biofilm-forming pioneer in RO systems, the bacterial strain *P. aeruginosa* ATCC 10145^{TM} was employed to investigate biofouling in RO polyamide membrane systems[4,9,10,22]. The preparation of the *P. aeruginosa* inoculum involved streaking a single isolated colony from a TSA plate stored at 4°C[38,91,250]. The plates were derived from *P. aeruginosa* stocks preserved at -80°C in glycerol[38,91,250]. Seed cultures were prepared by inoculating four 50 mL Falcon tubes of TSB with a single bacterial colony each. The Falcon tubes were then transferred to an incubator shaker (VWR 1575 Incubator Shaker, orbital diameter of 1.9 cm) set to 37°C, 200 rpm for 18 h, allowing the cells to reach the exponential growth phase (OD = 1.0 [600; WPA CO 8000 cell density meter])[38,91,249,250,255]. Then, the overnight cultures underwent centrifugation (3,000 rpm, 22 °C, 10 min, centrifuge model: Allegra X-12R) and were subsequently

resuspended in 500 mg/L NaCl solution (four 50 mL Falcon tubes)[38,91,249,252,255,277]. Then, a 10 L bacterial stock solution (in NaCl) was prepared as described in Suwarno, 2012, to achieve a 10^5 CFU/mL concentration in the RO system by employing a dilution factor of 1:1000 and an injection flow rate of 1 mL/min[249,252,255,255,288–291].

Table 10 - Synthetic feed water formulation for anti-biofouling efficacy and polyamide membrane compatibility testing

Parameters	**Unit**	**Antibiofouling testing**	**Polyamide membrane compatibility testing**
Non-oxidizing biocides (Prevention)	-	2 x MBIC	10 x MBIC
Non-oxidizing biocides (Removal)	-	2 x MBEC	10 x MBEC
P. aeruginosa	CFU/ml	10^5	-
pH	-	7	7
Background salinity	mg/L	500	500
TSB (Nutrient)	mg/L	20	-

Note: LAE (tested biocide) - MBIC: 63 mg/L & MBEC: 5000 mg/L. MIT (control for antibiofouling efficacy)- MBIC: 22.5 mg/L & MBEC: 78 mg/L. SBS (control for membrane compatibility) - MBIC: 1,667 mg/L & MBEC: 2,500 mg/L. The membrane soaking performed in the polyamide membrane compatibility testing was done with non-oxidizing biocides concentration of 10 x MBEC.

5.3.2 RO Benchtop system

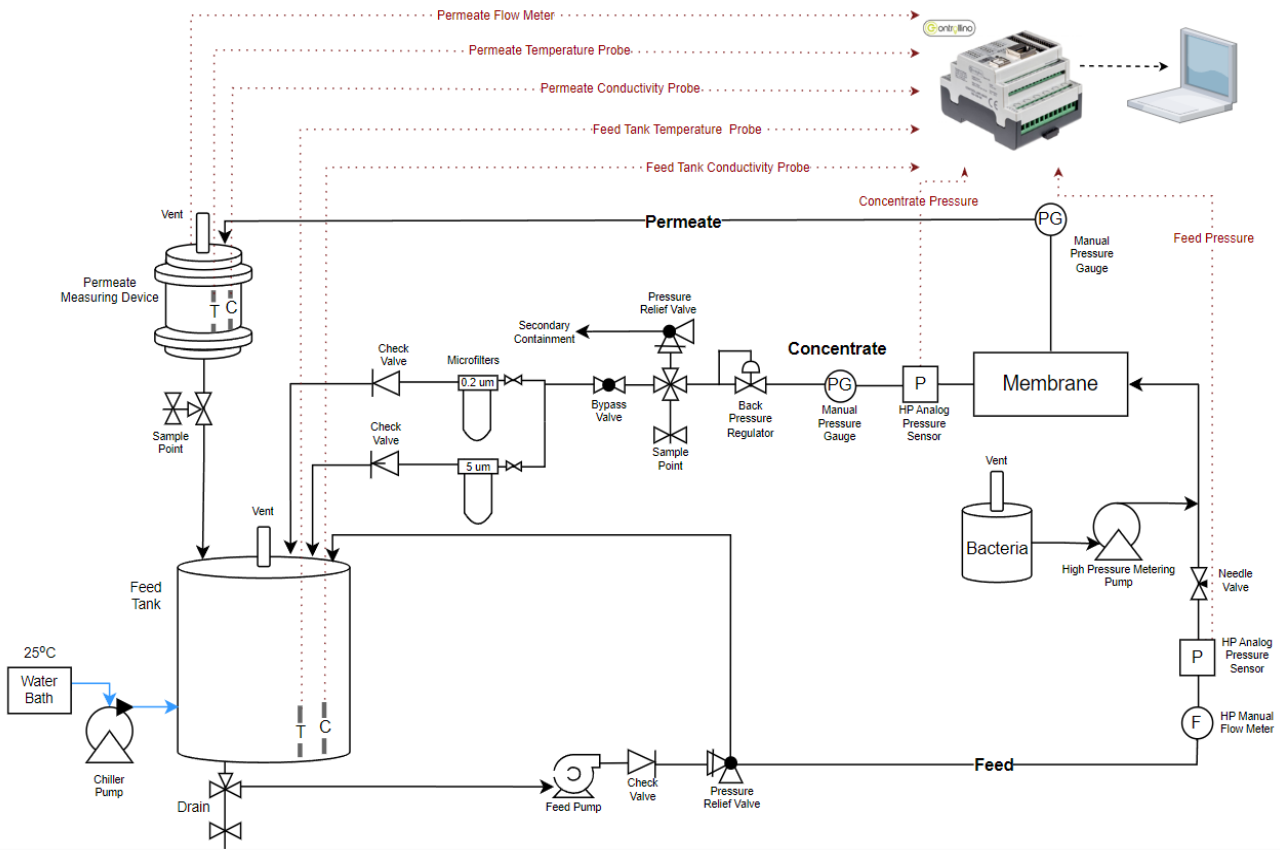

Figure 16 - RO benchtop system schematic diagram

Our benchtop RO system was designed based on previous biofouling/membrane degradation studies in RO systems, particularly the work of Suwarno et al., 2012[10,34,103,249,252,255]. The schematic of the RO benchtop system is shown in Figure 16. A temperature probe (Atlas Scientific) was placed inside the feed tank and was used to monitor the feed solution's temperature. The feed solution was constantly mixed and replenished every 24 h[249,252,255]. The benchtop's crossflow membrane module (Sterlitech, CF042SS Cell) with an active membrane area of 42 cm², separated the permeate and concentrate into different lines. Both the concentrate and permeate were recycled into the feed tank. The permeate line flow rate was monitored by a customized device composed of a displacement piston and a peristaltic pump (Anko 24VDC Peristaltic Pump). The permeate measuring device included a temperature probe (Atlas Scientific), and a permeate conductivity probe to monitor salt rejection (Atlas Scientific). A manual flow meter (McMaster-Carr model 41995K61) was used to monitor the feed line flow rate. A feed pump (Hydra Cell model P200-991-2400C) was used to pump the feed solution throughout the system. A second conductivity probe was situated near the outlet of the feed tank to measure the conductivity of the feed water. The bacterial solution was injected into the RO system by a high-pressure metering pump (Eldex - Model 2HM) directly before the feed solution entered the membrane vessel. Microfilters (Durapore multilayer cartridge filters, 0.2 µm and 5 µm) were installed in parallel in the concentrate line to prevent the feed tank from turning into a bioreactor[249,252,255]. A pressure regulator (Swagelok - model KVV11DI1) followed by a manual

pressure gauge (McMaster-Carr model 3795K13) were installed in the concentrate line to monitor the pressure within the system. An analog pressure sensor (Kavlico Industrial model P528-500-S-F4C) was placed on each line (concentrate, permeate, and feed lines) to determine the trans-membrane pressure (TMP) drops across the membrane cell. The microcontroller (Controllino Maxi) continuously recorded the normalized pressure, flow, and conductivity values observed in the RO system as described in the Appendix C.

5.3.3 Anti-biofouling efficacy testing

The biofouling experiments, as well as the polyamide membrane compatibility experiments, were carried out following an adapted experimental procedure performed by Suwarno et al., 2012, 2014, 2018; Kim & Park, 2016; and DuPont, 2021[51,103,249,252,255]. The biofouling experiments were carried out following two distinct approaches: a biofouling prevention protocol and a biofouling removal protocol. These protocols aimed to evaluate the performance of the benchtop RO system by assessing normalized permeate flux, trans-membrane pressure, and salt rejection under experimental conditions designed to simulate the conditions found inside RO membrane vessels in potable water applications with brackish water as the water source[9,10]. The biofouling prevention protocol consisted of the assessment of RO performance as a 48-hours single-species biofilm developed in the presence of the identified biocides. The biofouling removal protocol consisted of the development of a 48-hours biofilm on the RO membrane and subsequent treatment with the identified biocides, as well as an RO performance assessment for an additional 24 h[249,252,255,264]. The biocides were determined to be efficacious when normalized permeate flux, trans-membrane pressure, and salt rejection values did not exceed a 15% deviation from the expected values (baseline scenario)[9,10,38].

Initially, the polyamide membranes and support layers were soaked in deionized (DI) water for 12 h[249,252,255]. Subsequently, the membranes and support layers were soaked in 70% ethanol for 1.5 h[249,252,255]. Post-sterilization, the membranes were rinsed with sterile DI water and placed in the membrane vessel[249,252,255]. The prepared membranes were compacted for 12 h by operating the RO system at 1 L/min and 225 psi at room temperature as per the manufacturer's instructions[51,249,252,255]. After membrane compaction, NaCl was added to the feed tank to the desired background salinity concentration (500 mg/L) and the system was operated for 1.5 h to allow mixing[9,51,255]. Then, nutrients (TSB) were added, followed by another 1.5 h of mixing[249,252,255]. *P. aeruginosa* was continuously injected from the bacterial stock solution into the system in order to yield a concentration of 10^5 CFU/mL *P. aeruginosa* in the system, initiating the biofouling experiment[9,10,249,252,255]. Biocides were continuously injected into the system during (biofouling prevention protocol) or after the development of the 48-hours biofilms (biofouling removal protocol)[9,38,249,252,255]. RO performance was assessed by measuring and analyzing normalized permeate flux, trans-membrane pressure, and salt rejection[9,10,103,249,252,255]. After the completion of

each experimental run, the polyamide membranes were taken out of the membrane vessels for membrane autopsy (CLSM and SEM) and the RO benchtop was cleaned with an acidic solution (HCl; pH 2) and then a caustic solution (NaOH; pH 13) for 1.5 h each at 35°C and 60 psi, removing any accumulated inorganic compounds and organics in the system[9,51].

5.3.4 Polyamide membrane compatibility testing

The polyamide membrane compatibility experiments were conducted under the same operational conditions as the biofouling experiments, also following experimental procedures that are described in Suwarno et al., 2012, 2014, Kim & Park, 2016, and DuPont, 2021[51,103,249,252,255]. In contrast to the biofouling experiments, the membrane compatibility tests did not involve the injection of bacteria. Prior to the membrane compatibility experiments, the polyamide membranes were soaked with the selected biocides for several months. Then, the membranes were tested on the benchtop to assess membrane damage[51,102]. Initially, membranes were soaked with biocides at a concentration ten times higher than their respective MBEC values at pH 7 for 12 months, inside an orbital incubator shaker set to 100 rpm at room temperature[38,40,51,102,103,265]. The 100 mL soaking solution was replenished monthly. After soaking, the prepared membranes were rinsed, sterilized, and compacted as described in the biofouling experiments. To initiate membrane compatibility testing, NaCl was added to the feed tank to achieve a background salinity concentration of 500 mg/L[9,51,249,252,255]. Then, the system was operated for 1.5 h to allow mixing. Subsequently, the normalized permeate flux, trans-membrane pressure, and salt rejection were measured for 48 h[9,51,249,252,255]. After each experimental run, polyamide membranes were subjected to membrane autopsies (SEM, AFM, ATR-FTIR spectroscopy), and the RO benchtop was cleaned as previously described. To be considered compatible with polyamide membranes, the biocides must not affect the membranes' performance negatively; changes of greater than 15% vs. expected values are considered to be significant[9,51].

5.3.5 Membrane autopsies

Membrane autopsies were performed on the RO membranes to further assess the anti-biofouling efficacy and membrane compatibility of the selected biocides and to validate the results from the benchtop experiments. CLSM and SEM were applied to provide a quantitative and qualitative analysis of the biofilm prevention and removal from the RO membranes due to the biocide treatments in the biofouling experiments, respectively[32,38,103,249,252,255]. ATR-FTIR, AFM, and SEM were applied to further assess membrane degradation in the polyamide membrane compatibility experiments[9,40,103,265,280]. ATR-FTIR was used to evaluate the impacts of the selected biocides on the oxidative damages to the surface polyamide layer of membranes, while AFM and SEM were used to assess the changes in the polyamide membrane morphology[9,40,103,265,280].

The membrane autopsy procedure used in the anti-biofouling testing followed an adapted methodology from Shatila et al., 2020, Nagaraja et al., 2017, Hazrin-Chong & Manefield, 2012, Heydorn et al., 2000, Donlan, 2002, and Lade & Paul, 2015[28,32,238,247,251,260]. A detailed description of the membrane autopsy methodology applied for the biofouling experiments can be found in our previous study, Da Silva Correa et al., 2024[280]. In summary, after each biofouling experiment, the RO membrane was taken out of the membrane vessel, rinsed three times with DI water to remove planktonic bacteria, then cut into membrane coupon discs with 12 mm of diameter[38,91,238,245]. After that, biofilms on the membrane coupons were stained with dyes from LIVE/DEAD BacLight viability kit (LN 7007) at room temperature as per the manufacturer's specifications. The SYTO 9 dye (5 μM) was used to visualize alive biomass, and PI dye (30 μM) was used to visualize the dead biomass[32,38,91,247,249,282]. After staining, the RO membrane coupons were rinsed with DI water three times to remove residual dyes then wet-mounted on a glass slide[32,38,91,252,264]. Subsequently, the biofilms on the RO membrane coupons were visualized in a Zeiss LSM 880 CLSM. Three representative 3D CLSM stack images were taken from each membrane coupon at a magnification of 20x, 16-bit resolution with an area of 531.4 μm × 531.4 μm[32,247]. The Bitplane software (version 9.8.0) was used to quantify biofilm thickness, biofilm volume, biofilm appearance, and live-to-dead bacterial ratio from the CLSM stack images following the same methodology described in Nagaraja et al., 2017, Heydorn et al., 2000, Lade & Paul, 2015, and Dolan, 2002[28,32,238,247]. For further assessment of antibiofilm efficacies of the selected biocides, the biofilms developed on the RO membrane coupons were visualized in SEM Hitachi S-4800. Prior to performing SEM imaging, the membrane coupons were prepared according to the procedure outlined in Da Silva Correa, 2023[38]. In brief, the preparation for SEM imaging of the membrane coupons included the following steps: a rinse with DI water, bacteria fixation with 2.5% glutaraldehyde at 4°C for 4 h, followed by a second rinse with DI water, dehydration using increasing ethanol concentrations (30, 50, 70, 80, 96, and 100%; 5 min each), and drying with hexamethyldisilazane (50 and 100%; 30 min each) at room temperature[38,251,260,261,265]. After SEM membrane coupon preparation, the RO membranes were coated with gold via sputter deposition for 150s in the Anatech Hummer VI Sputter Coater[38,251,260,261]. The SEM images were taken at magnifications of 1,000x, 5,000x, and 10,000x at a voltage of 1 kV[38,251,261,265].

The membrane autopsy procedure applied in the polyamide membrane compatibility testing is an adaptation of the experimental procedure described in Kim & Park, 2015, 2016, Yadhuraj et al., 2016, and Kim et al., 2018[40,103,265,269]. A detailed description of the membrane autopsy methodology applied in the polyamide membrane degradation experiments can be found in our previous paper, Da Silva Correa et al., 2024[280]. In brief, after each membrane compatibility study, the membranes were taken out of the membrane vessel and rinsed with DI water three times to remove chemical residuals from the RO membranes. Next, the membranes were cut in 12 mm membrane coupons. After rinsing, the membranes were left in a desiccator to dry for three days[40,103,265,280]. AFM Agilent microscope

(Model: 5500 AFM; Non-tapping mode) was used to obtain the AFM images. The AFM images were obtained from three different locations per sample[40,103,265,280]. AFM imaging was performed using a scanning area of 10µm x 10µm, an approaching force of 48 N/m, and a scanning speed of 0.6 lines per second[40,103,265,269,280]. Additionally, the resonance frequency used in the AFM imaging was 190 kHz and the AFM probes (TED PELLA; TAP190-G-10) presented a nominal tip radius less than 10 nm[40,103,280]. The Gwydion 2.61 software was used to estimate the membrane surface roughness (Sa) and average root mean square roughness (RMS) of the polyamide membrane samples as described in Yadhuraj et al., 2016[269]. SEM images were obtained from an SEM Hitachi S-4800 microscope at a magnification of 10,000x and a voltage of 1.5kV[40,103,265,280]. Gold sputtering of the polyamide membranes was done as described in the biofouling experiments. ATR-FTIR spectra of the membrane treated with the selected biocides and untreated membranes were obtained using the following spectrometer: PerkinElmer Spectrum Two UATR 100150. Each ATR-FTIR spectrum was obtained with a resolution of 1 cm^{-1}, wave-number ranges of 800 to 2,200 cm^{-1}, with a minimum of 10 scans[40,103,265,280].

5.3.6 Statistical analysis

All experiments, measurements, and microscopies detailed in this study were conducted in triplicate and in independent weeks. Statistical differences were assessed via Student's t-test and one-way ANOVA (Excel) with significance being attributed to p-values below 0.05.

5.4 Author contributions

Luiz Henrique Da Silva Correa led the preparation of the manuscript and designed the experiments performed in this study. Luiz Henrique Da Silva Correa and Hayley Alexandra Smith contributed to the design of the RO benchtop. Luiz Henrique Da Silva Correa conducted the biofouling and membrane compatibility experiments, with assistance from Danyka Shelagh Gabrielle Thorburn, Orielle Kaneza Henriquez, and Hayley Alexandra Smith. Luiz Henrique Da Silva Correa performed the discussion and data analysis in the present study. Heather Louisa Buckley provided guidance throughout the design and experimental process, feedback on the manuscript, and secured funding that facilitated the completion of the work.

5.5 Funding sources

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5.6 Supplementary data

Normalization equations can be found in the Appendix D

5.7 Acknowledgment

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Chapter 6: Overall discussion and prospects

6.1 Chapter synthesis

To address the adverse impacts of biofouling on RO polyamide membrane systems in potable water applications, this PhD research introduces a standardized protocol aimed at identifying greener and safer chemical solutions to prevent biofouling in RO systems. This protocol may be used as a guideline or standard protocol by industries and municipalities to find new anti-biofouling agents that promote sustainable water use via RO technologies. Figure 17 provides an overview of my PhD work, along with details on the proposed subsequent phases of my research (post-doctoral research) and the contributions of my research project. Chapters 2 - 4 consist of the screening protocol, while Chapter 5 outlines the validation protocol. Both the screening and validation protocols are components of my PhD work (Figure 17). The next stages of my research project such as pilot experiments and experiments in the RO benchtop (with LAE) will be continued during my postdoctoral research (Figure 17).

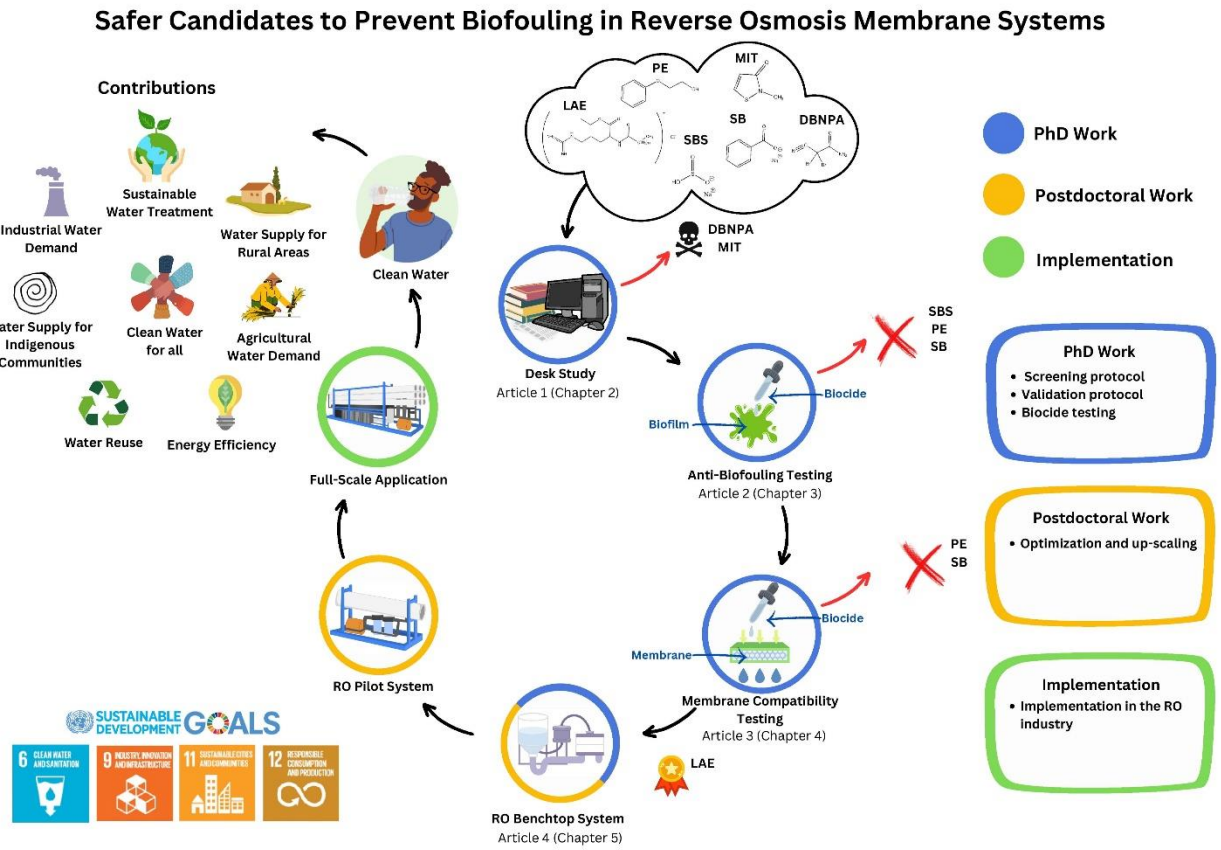

Figure 17 – Chapter synthesis & contributions of PhD work

In Chapter 2, a review study was conducted to assess the applicability of several anti-biofouling candidates in preventing biofouling in RO potable water polyamide membrane applications based on attributes identified by Bates (1998)[39]. A total of 9 chemicals (MIT, DBNPA, SBS, SB, PE, LAE, PHMGH, BDMDAC, SNP) were examined[27]. From these, 6 biocides (MIT, DBNPA, SBS, SB, PE, LAE) were deemed relevant for subsequent biofouling prevention studies[27]. DBNPA and MIT were

considered excellent antibiofilm efficacy models for prevention studies; however, safety concerns have led to the conclusion that they are not suitable as a long-term solution for mitigating biofouling in RO potable water applications. It is important to note that safety for humans and the environment should be prioritized over all other attributes of an ideal biocide identified by Bates (1998)[39]. From a green chemistry perspective, even if a biocide is degradable or highly effective at preventing and removing biofilms, it should not be considered a safer alternative to address membrane biofouling if it is not safe for both humans and the environment[27,38,66,73]. SBS was identified as excellent model for membrane compatibility studies. However, SBS's low antimicrobial efficiency and pH dependency raised concerns for its applications as a potential anti-biofouling agent in RO systems. PE and SB were considered excellent low-hazard biocide models, but their relatively high required doses raised concerns for inline RO membrane applications. Thus, the first phase of the screening protocol indicated that DBNPA and MIT were not recommended as full-scale solutions for biofouling prevention, while SBS, PE, and SB were deemed worthy of progressing to the following phases of the screening protocol with flagged concerns. BDMDAC was not recommended for application as a preventative biocide in RO systems due to its inefficacy against removing biofilms and low efficacy against common biofilm forming microorganisms found in RO systems. Limited information was found on PHMGH and SNP in the literature due to their early stage of development. Consequently, the lack of available data led to the conclusion that further research, outside the scope of this PhD, needs to be performed to assess the applicability these biocides in RO potable water applications. Finally, LAE was identified as a promising biocide to move forward in the screening process due to its safety, antimicrobial properties, and potential membrane compatibility.

In Chapter 3, experiments were performed in a CDC biofilm reactor, with MBIC and MBEC determined in microtiter plates, to investigate the anti-biofouling efficacy of the selected biocides from the first phase of the screening protocol (SBS, SB, LAE, PE) to prevent and remove *P. aeruginosa* biofilms from RO polyamide membranes[38]. As expected, results revealed that biofilm prevention treatments proved to be more efficient and required lower concentrations of chemicals/biocides than biofilm removal treatments[38]. Among the tested biocides (SBS, SB, LAE), only LAE effectively prevented and removed biofilms from RO membranes. Hence, only LAE passed the antibiofouling screening phase. SBS was not considered to be a biocide option for biofouling control in RO systems due to its inefficiency in removing existing biofilms from RO membranes. Similarly, SB was not recommended as a full-scale solution to address membrane biofouling because it demonstrated moderate effectiveness in both biofilm prevention and removal with high biocide dosages. While PE was initially included in the experiments performed in the CDC biofilm reactor, antibiofouling efficacy testing could not be performed with this biocide. This is because when exposed to PE, the active membrane layer detached from the microporous membrane support layer due to irreversible membrane damage.

Recognizing that an anti-biofouling biocide applicable to potable RO water treatment must also be membrane-compatible, Chapter 5 assessed the RO polyamide membrane compatibility of LAE, PE, and SB through a rapid membrane degradation test[280]. These tests were conducted at high biocide concentrations over short exposure times, under standard testing conditions (exposure time and biocide concentrations) commonly applied in membrane degradation studies in RO systems[40,141,150,265,268,292]. This approach is commonly used because the actual dosing concentrations of biocides in full-scale RO installations are significantly lower than the required biocide doses[141,150]. Additionally, the MBIC and MBEC values for the selected biocides are much lower than the concentrations used in rapid membrane degradation tests (e.g., 100 g/L)[38]. Thus, applying these higher concentrations helps expedite the degradation of the RO membrane, simulating a long-term continuous biocide dosing scenario inside the RO membrane module[40,141,292,292]. The membrane autopsy results indicated that PE and SB damaged RO membranes through membrane swelling, rendering them unsuitable for potable water inline applications[280]. This result confirmed the membrane degradation by PE exposure observed in the antibiofouling testing. In contrast, LAE caused no morphological damage or oxidative damage on RO polyamide membranes. Therefore, PE and SB did not pass the membrane compatibility screening phase. LAE was the only biocide that passed all phases of the proposed screening protocol, showcasing several attributes that make it a promising candidate for biofouling prevention in RO potable water membrane applications[27,38,280].

To facilitate the transition from the screening protocol to the upscaling and optimization of the most effective antifoulant candidate identified in the screening protocol (LAE), Chapter 5 proposes a methodology for testing and analyzing the anti-biofouling efficacy and membrane compatibility of LAE in a RO benchtop system. This same methodology should be applied to any biocides that pass the screening protocol. This step is crucial, as the screening protocol acts as a proof-of-concept. Thus, the validation protocol plays an important role the upscaling and optimization of LAE's application as a safer and greener solution to prevent biofouling in RO polyamide membrane water treatment systems.

6.2 PhD research contributions

This PhD work contributes to several of the United Nations (UN) Sustainable Development Goals (SDG): Goal 6 (Clean water and sanitation), Goal 9 (Industry, innovation, and infrastructure), Goal 11 (Sustainable cities and communities), and Goal 12 (Responsible consumption and production) (Figure 17). Overcoming the existing challenge of biofouling in RO systems is fundamental to promoting several positive social, economic, and environmental impacts. RO polyamide membrane systems hold great potential to end water shortage problems throughout Canada and the world due to their capability of producing fresh water from different types of water sources such as wastewater,

seawater, and brackish water[2,4,9,22,277]. Given the current need to develop safer alternative strategies to prevent and control biofouling in RO systems, this PhD project directly benefits membrane manufacturers and desalination companies and ultimately benefits municipalities, industry, marginalized communities, remote work sites, and Indigenous communities by the provision of a secure, safe, reliable water supply via RO technologies. This is because the present PhD project is the first step towards an innovative green solution to address the main technical barrier (membrane biofouling) that prevents the dissemination of the application of RO polyamide membrane technology for potable water production (SDG Goal 6: Assurance of safe drinking water availability)[7,10,20]. The mitigation of biofouling is key to optimizing RO polyamide membrane systems, reducing costs, and ultimately enhancing affordability for various applications (SDG Goal 9: Promotion of innovative resilient infrastructure) [7,10,20,27]. Overall, the socio-economic impacts of my research include increased accessibility of clean and affordable water to Indigenous and remote communities (SDG Goal 6: Assurance of safe drinking water availability) as well as resilience of local water supplies against large-scale infrastructure damage (flooding and forest fires) (SDG Goal 11: Making the access to drinking water safe, resilient, and sustainable in cities and communities)[8,16]. Additional benefits include the production of reliable freshwater for agricultural activities, the application of RO technology for water reuse, and the promotion of reliable sustainable water supply for domestic and industrial purposes via RO technologies (SDG Goal 9: Promotion of innovative resilient infrastructure; SDG Goal 12: Promotion of sustainable water consumption and production)[2,4,7,14,16]. Another significant socio-economic impact of my PhD research is the reduction of energy requirements and operational costs of RO technologies (SDG Goal 9: Promotion of innovative resilient infrastructure)[36,228]. Furthermore, a positive environmental impact would be the reduction of hazards to humans and the environment compared to the current chemistries applied to control biofouling in RO systems in potable applications (SDG Goal 6: Assurance of safe drinking water availability; SDG Goal 11: Making the access to drinking water safe, resilient, and sustainable in cities and communities)[10,17–20,27].

6.3 Limitations

6.3.1 Single-species biofilms as a model for anti-biofouling experiments

In this project, we used single-species *P. aeruginosa* biofilms to perform antibiofouling testing (Chapter 3 and Chapter 5). *P. aeruginosa*, a well-known biofilm-forming pioneer organism commonly found in RO systems, is a excellent model for studies that focused on biofilm prevention applicable to RO systems[9,26,38,91,103,252]. Additionally, the biofilm matrix of mature biofilms found in RO systems is mainly produced by *P. aeruginosa*, making single species *P. aeruginosa* biofilms a great model for biofilm removal studies in the context of RO systems[9,27,38]. While single-species *P. aeruginosa* biofilms are commonly applied to assess the antibiofouling efficacy of biocides applicable

to RO systems, it is important to recognize the limitations associated with their use in antibiofouling studies[9,26,27,38,91,103,265,293]. The main limitation of using single-species *P. aeruginosa* biofilms in antibiofouling studies lies in the potential underestimation of antibiofilm efficacy when compared to the more complex biofilms found in real-world RO applications. The underestimation of antibiofilm efficacy is a limitation in the context of a screening process because it may lead to increased costs associated with biocide research and development, potentially decreasing the attractiveness of the biocide for the industry. Studies in the literature indicate that treating single-species *P. aeruginosa* biofilms often requires higher biocide concentrations compared to other single-species biofilms or multi-species biofilms due to their resilient nature and multi-drug resistance[9,103,127,239,248,265,293]. Several mechanisms contribute to the challenge of treating single-species *P. aeruginosa* biofilms. These include the production of diverse enzymes that deactivate antimicrobials, the formation of a biofilm matrix that substantially reduces the diffusion of different types of biocides within the biofilm, a high presence of "persister" biofilm cells in the biofilm community, elevated expression of genes responsible for multidrug efflux systems, and alterations in the cell membrane[9,28,127,239,248,282,293]. Despite this limitation, the difficulty in treating single-species *P. aeruginosa* biofilms offers an advantage for prevention studies. The underestimation of antibiofilm efficacy facilitates relatively translatable findings to more complex biofilms found in real-world applications, aligning recommended biocide dosing with those applicable to multi-species biofilms[9,26,27,34,91,103,248,264,265,293].

6.3.2 Quantifying anti-biofilm efficacy through the application of fluorescence stains

The LIVE/DEAD Baclight viability kit used in this project is commonly used in biofilm studies for the visualization and quantification of biofilms as well as determination of minimum biofilm inhibitory concentrations[32,91,94,245,252,255]. The working principle of the LIVE/DEAD Baclight viability kit consists in the use of fluorescent stains that binds to the DNA of microorganisms differentiating dead cells (ruptured cells) from live cells (intact cells)[32,91,94,245,252,255]. The LIVE stain (SYTO 9; 5 μM) permeates easily through the membrane cell and binds to the DNA. The DEAD stain (propidium iodide; 30 μM) also binds to DNA, but can only penetrate damaged membrane cells[38,91,94]. The DEAD stain has a higher affinity to DNA than the LIVE stain[32,38,91,94]. Therefore, both stains must be used together as without them the number of live cells would be overestimated[32,38,91,94]. Both stains are excited by the same excitation wavelength (488nm); however, the DEAD stain fluoresces red (emission wavelength: 560 – 650 nm) and the LIVE stain fluoresces green (emission wavelength: 490 - 560 nm) allowing the quantification of live and dead bacterial cells as well as total biomass[32,91,245,252,255]. Since polysaccharides are also a significant component of the biofilm matrix, the Concanavalin A stain (50 μM), which binds to polysaccharides and like the LIVE stain is also excited by a 488 nm wavelength excitation light and fluoresces green (emission wavelength: 460 to 560 nm), was also used to quantify and visualize biofilm matrix[9,28,29,32,38,94,251]. This is because there

is not a stain that binds to all the main components of the biofilm matrix (DNA, polysaccharides, and proteins)[26,32].

In the experiments performed in the CDC biofilm reactor (chapter 3), it was observed that there was no significant difference between the total biofilm biomass estimated only with the LIVE/DEAD stain and the total biofilm biomass calculated using both the LIVE/DEAD stain and the Concanavalin A stain [38]. This observation was particularly prominent in the treated biofilms, where the number of live bacterial cells was significantly lower than the number of dead bacterial cells. Additionally, while there were slightly higher differences in the total biomass between untreated biofilms calculated with only the LIVE/DEAD stain and those calculated with both the LIVE/DEAD stain and the Concanavalin A stain, these differences were still not significant. These observations suggest a limitation of the Concanavalin A stain in the quantification of biofilm biomass. Other studies in the literature also reported a similar limitation for the Concanavalin A[26,32,294–298]. Due to its limited impact on the quantification of total biomass and high cost, the inclusion of both stains did not contribute substantially to the quantitative analysis of biofilm biomass. Therefore, for the continued application of the screening protocol, it is recommended to use either the LIVE/DEAD stain alone or a combination of LIVE/DEAD stain with other polysaccharide stains in antibiofilm studies.

6.3.3 Limitations of the proposed screening protocol

The current PhD project proposes a standardized screening protocol to select greener safer chemical solutions to prevent biofouling in RO systems (chapters 2-4), saving both resources and time that would otherwise be expended on testing biocides that are easily identifiable as unsuitable for potable RO membrane applications[9,51]. However, it is important to understand the limitations of the proposed screening protocol to ensure its optimal application. One of the main limitations of the proposed screening protocol is the necessity for more extensive studies to scale up and optimize biocides[9,51]. This is because biocides selected through the proposed screening process cannot be directly applied in full-scale applications. To address this limitation, a validation protocol was proposed in Chapter 5. Another limitation involves the potential need for collaboration between research institutes and companies to implement the screening protocol, especially for smaller desalination companies that lack the necessary resources such as microbial growth and microscopy facilities to conduct the experiments outlined in the screening protocol. The practical application of the screening protocol is exemplified in this PhD project, where membrane autopsies and experimental work were conducted in partnership with the Green Safe Water Lab at the University of Victoria and a desalination manufacturing company, BI Pure Water INC (Vancouver, BC - Canada).

6.4 Directions for future work

This section addresses the primary limitations of my research project and explores how these limitations present opportunities for future research.

6.4.1 Exploring the antibiofilm efficacy of biocides against different single-species biofilms and multi-species biofilm communities

The screening protocol serves as a proof-of-concept methodology designed to select greener and safer antibiofouling candidates for preventing biofouling in RO systems. As previously discussed, the use of single-species *P. aeruginosa* biofilms as a model in the screening process is justified by their multi-drug resistance and the fact that *P. aeruginosa* is a biofilm-forming pioneer organism commonly found in RO systems, contributing to the formation of the biofilm matrix observed in mature biofilms within RO systems[9,27,248,293]. However, it is also important to investigate the antibiofilm efficacy of the biocides that pass the screening process using different single-species biofilms and multi-species biofilm communities. This is because the complexity of biocide efficacy in treating biofilms involves factors such as microorganism type, biofilm matrix composition, biocide type, feed water quality, and environmental conditions[9,22,27–29]. According to the literature, *S. aureus* is the second most common biofilm former in RO systems, following *P. aeruginosa*[9,26,27]. Moreover, several RO membrane autopsy studies indicate that *S. paucimobilis* is one of the most common microorganisms found in mature biofilms in RO systems[9,10,299]. Therefore, it is recommended to conduct future research focusing on evaluating the antibiofilm efficacy of the screened biocides on single-species biofilms containing these microorganisms. Furthermore, considering the potential impact of microorganism diversity in a biofilm community on antibiofilm efficacy, it is also recommended to investigate the effectiveness of the screened biocides against multi-species biofilms containing these microorganisms (*P. aeruginosa*, *S. aureus*, and *S. paucimobilis*).

Specifically, additional studies should involve determining the MBIC and MBEC values of LAE against single-species *S. aureus* and/or *S. paucimobilis* biofilms, as well as a multi-species biofilm composed of *S. paucimobilis*, *P. aeruginosa*, and *S. aureus*. These studies should follow the experimental procedure outlined in Chapter 3. Furthermore, it is recommended to conduct biofouling experiments in both the CDC biofilm reactor and the RO benchtop system to assess the antibiofilm efficacy of LAE against single-species *S. aureus* and/or *S. paucimobilis* biofilms, as well as a multi-species biofilm composed of *S. paucimobilis*, *P. aeruginosa*, and *S. aureus*. The experimental procedures for these investigations are detailed in both Chapter 3 and Chapter 5. These studies will provide additional insight into the antibiofilm efficacy of LAE, facilitating the optimization of recommended biocide dosing of this biocide.

6.4.2 Upscaling and optimization of selected anti-biofouling candidates

Although anti-biofouling candidates that have passed the screening protocol show great promise as an alternative biofilm treatment in RO systems, further research is necessary before incorporating these chemicals into biofouling prevention programs in RO installations. Chapter 5 proposes a methodology for testing and analyzing the anti-biofouling efficacy and membrane compatibility of the most effective antifoulant candidates identified in the screening protocol (validation protocol). Section 6.4.1 proposes studies aimed at optimizing biocide dosage and gaining more insight into the antibiofilm efficacy of successful anti-biofouling candidates through further experiments on a CDC biofilm reactor (RO membrane active area: 1 cm^2; flow rate: 11.7 mL/min) and RO benchtop system (RO membrane active area: 42 cm^2; flow rate: 1 L/min). Expanding future research recommendations on a laboratory scale, it is recommended to assess the anti-biofouling efficacy and membrane compatibility of the most effective antifoulant candidates identified in the screening protocol under different experimental conditions, including different biocide concentrations, exposure times, pH levels, and temperatures. This is important because these parameters may also influence anti-biofouling efficacy and membrane compatibility.

Once optimal conditions (optimal pH, biocide concentration, exposure time, and temperature) for biocide applications are determined through laboratory work, more extensive experiments on a pilot scale can be conducted (upscaling) (RO membrane active area: 5 m^2; flow rate: 35 L/min; Sterlitech Pilot System). The pilot-scale experiments should include comprehensive antibiofouling and membrane compatibility studies under experimental conditions (such as feedwater composition, pressure, flow, temperature, and microbial composition) closer to those found in full-scale applications. In the pilot-scale studies, a comparison of RO membrane performance in terms of salt rejection, trans-membrane pressure, and water flux before and after biocide treatments (optimal dosage) followed by membrane autopsies should be conducted. In this phase, the optimal conditions for biocide application can be reassessed, further refined, and utilized to design a biofouling prevention program. Additionally, a reduction of exposure times and doses used in membrane compatibility testing can be proposed, tested, and validated to optimize current industry practices. The subsequent studies discussed herein will be performed with LAE during my post-doctoral research. After pilot experiments, the biocides can undergo final trial runs in full-scale RO installations.

6.4.3 Assessing the market readiness of LAE in the RO industry

The protocol proposed in this project offers a screening approach to select antibiofouling candidates for preventing biofouling in potable RO applications through a technical lens. To replace established biocides like chlorine in the RO industry, it is essential to assess the market readiness of the biocide

candidates that pass the screening protocol through a market analysis study[9,300]. This assessment would provide a business perspective in the decision-making process[300]. Alongside efficacy, factors such as cost, compliance with environmental regulations, and commercial viability play a significant role in introducing new biocides to the RO industry[9,300]. Therefore, conducting a business plan for the biocides that pass the proposed screening protocol is recommended. Specifically, it is recommended to conduct a business plan to assess the market potential of LAE in the RO industry. Additionally, conducting an in-depth cost-effectiveness analysis of LAE compared to conventional strategies used to address biofouling in RO systems is also recommended as a future study. Further, considering that chlorine is the most predominant biocide currently used in the RO industry to control biofouling in potable water treatment applications, a market analysis study should be performed to compare costs, manufacturing, and widespread distribution between chlorine and LAE in the RO industry[4,9,10,22,300].

6.4.4 Incorporating biocidal functional groups into RO membrane chemical structure

The screening protocol proposed in this study can be extended to aid in selecting safer and greener chemicals for other techniques aimed at preventing biofouling in RO systems. For instance, membrane modification techniques, though in early stages of development, show great promise as a method to prevent membrane biofouling[9,239]. Modifications to polyamide membranes may involve coating them with antimicrobial chemicals or incorporating nanoparticles into the membrane itself[9,239]. Since the protocol proposed in this study selects green, safe chemical candidates to address biofouling, a potential future study could investigate integrating the functional groups of the biocide candidates that pass the screening protocol into RO membranes (Chapters 2 - 4). The validation protocol outlined in Chapter 5 could be used to assess the effects of coated membranes on RO performance, antibiofouling efficacy, and membrane compatibility. Thus, the investigation of the effectiveness of polyamide membranes coated with LAE in addressing biofouling in RO systems presents a promising opportunity for future research. Therefore, the versatility and applicability of the protocol proposed in this PhD work further support its use as a method for addressing membrane biofouling in RO systems.

Conclusion

Biofouling is the main obstacle to the widespread use of RO technology for addressing global water scarcity, as it reduces permeate production, increases energy requirements, and aggravates the environmental impacts associated with the technology. To overcome these challenges, this PhD project proposed a platform to select and test safe and green anti-biofouling agents to prevent biofouling in drinking water reverse osmosis systems applications. The platform consisted of a screening protocol followed by a validation protocol. In this project, the proposed platform was applied to assess the applicability of nine chemicals (MIT, DBNPA, SBS, SB, PE, LAE, PHMGH, BDMDAC, SNP) for preventing membrane biofouling in RO potable water applications. The screening protocol involved three phases: a comprehensive review (Objective 1), antibiofouling testing on CDC biofilm reactors with minimum concentrations determined in microtiter plates (Objective 2), and polyamide membranes compatibility testing via rapid membrane degradation tests (Objective 3). Among the examined chemicals, LAE was the only biocide to successfully pass all phases of the proposed screening protocol, emerging as a promising biocide with potential applicability in preventing biofouling in RO systems. Consequently, it was the only biocide recommended to advance to further experiments outlined in the validation protocol (Objective 4). Therefore, considering the current need for the development of green, safe alternative methods and practices for the prevention and control of biofouling in RO systems, this PhD project holds the potential to ultimately provide reliable, secure, and safe water supply to municipalities, industry, marginalized communities, remote work sites, and Indigenous communities via the optimization of RO polyamide membrane technologies. This was achieved through the development of a platform to select anti-biofouling candidates for preventing biofouling in RO potable water applications, along with the selection of a promising biocide like LAE to address membrane biofouling in RO potable water applications.

Appendix A: The application of non-oxidizing biocides to prevent biofouling in reverse osmosis polyamide membrane systems - A review - Supplementary material (Chapter 2)

Study Approach

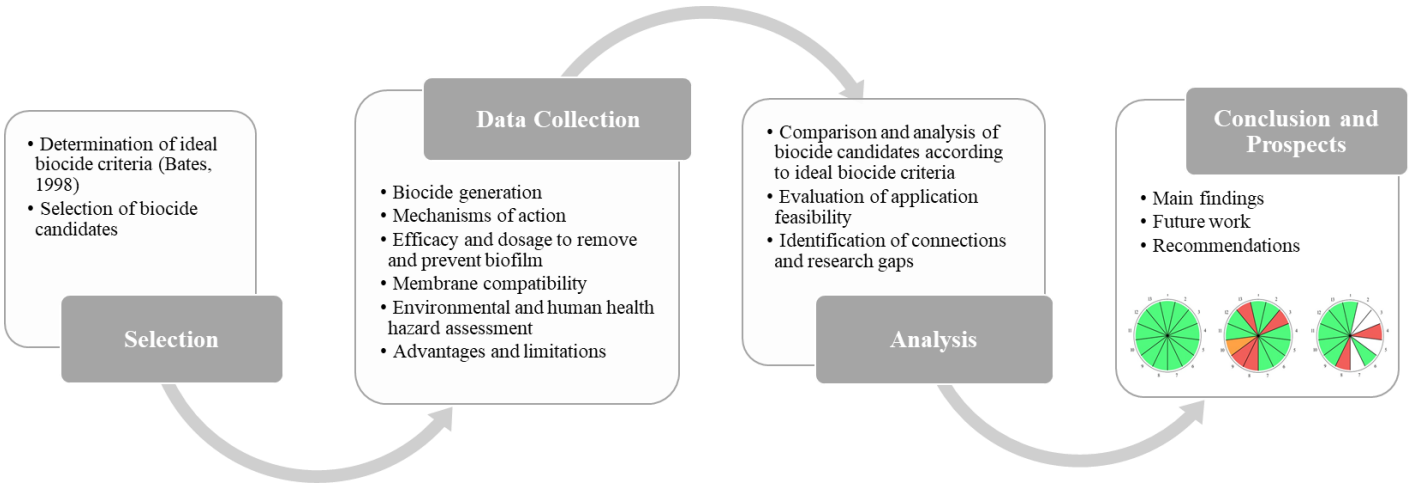

Figure 18 - Study Approach

Human and environmental health hazards

Table 11 - Human hazard levels of non-oxidizing biocides

Biocides	Byprodutcs	Carcinogenicity	Neurotoxicity Single Exposure	Neurotoxicity Repeated Exposure	Skin Sensitization	Respiratory Sensitization	Eye Irritation	Systemic Toxicity Single Exposure	Systemic Toxicity Repeated Exposure	Endocrine Activity
DBNPA	Dibromoacetonitrile	High[1]	-	-	-	-	-	-	-	Moderate-High[2]
DBNPA	Dibromoacetamide	High[3]	-	-	-	-	-	-	-	Moderate-High[4]
DBNPA	2-Bromoacetamide	-	-	-	-	-	-	-	-	-
MIT	Malonamic acid	-	-	-	-	-	-	-	-	-
MIT	Malonic acid	-	-	-	-	-	-	-	-	-
MIT	Acetic acid	Low[5]	Low[6]	Low[6]	Moderate[5,6]	Moderate[6]	Very high[6]	Moderate[6]	Low[5]	-
MIT	Formic acid	Low[7]	Very high[8]	High[8]	Moderate[7]	Moderate[7]	Very high[7]	Very high[9]	Low[9]	-
SB	Benzoic acid	-	-	-	High[10]	-	Very high[10]	-	High[11]	-
PE	-	-	-	-	-	-	-	-	-	-
SBS	Sulfur dioxide	Low[12]	High[13]	High[13]	Very high[14]	Very High[14, 15]	Very high[14]	-	High[12]	-

Hazard levels: very low, low, moderate, high, very high. "-": data gap. Source (1 - NTP 2010[301]; 2 - Poon *et al.* 2003[302]; 3 - NTP 2007[303]; 4 - Linder 1997[304]; 5 -Api *et al*. 2019[305]; 6 - EFSA 2013[306]; 7 - Nishikawa *et al*. 2021[307]; 8 - Kapur *et al*. 2007[308]; 9 - Gibson 1969[309]; 10 - WHO 2000[121]; 11 - ECHA 2021[310]; 12 - CCOHS 2017[311]; 13 - Sang *et al.* 2011[312]; 14 - Khan *et al.* 2014[313]; 15 - Petruzzi *et al*. 1994[314]).

Table 12 - Environmental health hazard levels of non-oxidizing biocides

Biocides	Byprodutcs	Acute Mammalian Toxicity	Acute Aquatic Toxicity	Terrestrial Ecotoxicity	Bioaccumulation	Chronic Aquatic Toxicity	Persistence
DBNPA	Dibromoacetonitrile	-	-	-	-	-	-
DBNPA	Dibromoacetamide	-	-	-	-	-	-
DBNPA	2-Bromoacetamide	-	-	-	-	-	-
MIT	Malonamic acid	-	-	-	-	-	-
MIT	Malonic acid	-	-	-	-	-	-
MIT	Acetic acid	Low[1]	Low[1]	-	Very low[1]	Low[1]	Very low[1]
MIT	Formic Acid	High[2]	Moderate[2]	-	Very low[3]	Moderate[3]	Very low[3]
SB	Benzoic acid	Low[4]	Low[4]	-	Low[4]	Low[4]	Low[4]
PE	-	-	-	-	-	-	-
SBS	Sulfur dioxide	High[5]	Moderate[6]	Moderate[7]	Low[8]	-	High[9]

Hazard levels: very low, low, moderate, high, very high. "-": data gap. Source (1 - Api *et al*. 2019[305]; 2 - Thompson 1992[315]; 3 - OECD 2008[316]; 4 - WHO 2000[121]; 5 - Petruzzi *et al*. 1994[314]; 6 - Driscoll *et al*. 2003[317]; 7 - Kirchhübel & Fantke 2019[318]; 8 - U.S. DHHS 1998[319]; 9 - Solarin *et al* 2021[320]).

Appendix B: Efficacy testing of non-oxidizing biocides for polyamide membrane biofouling prevention using a modified CDC biofilm reactor - Supplementary material (Chapter 3)

96 well plates arrangement for the determination of MBICS and MBECS

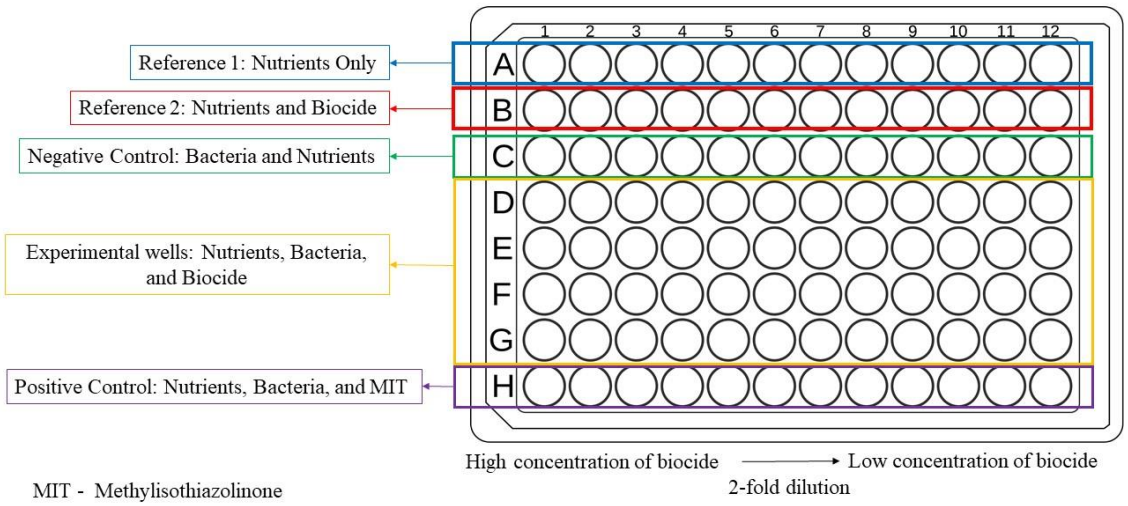

Figure 19 - The 96 well plates arrangement for the determination of MBICs and MBECs.

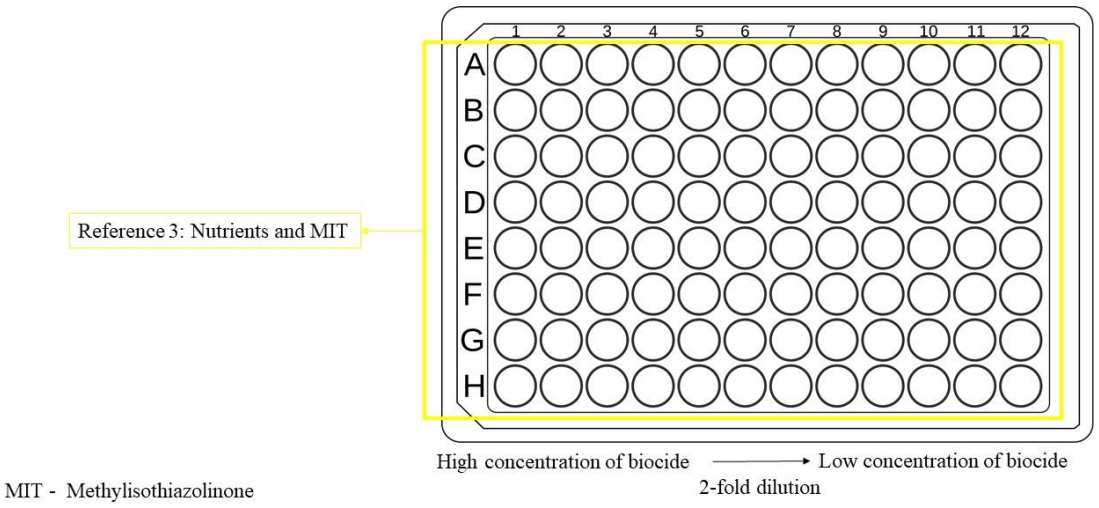

Figure 20 - The 96 well plates arrangement for the determination of the background fluorescence of MIT (nutrients and biocide).

Minimum biofilm inhibitory concentrations (MBICs)

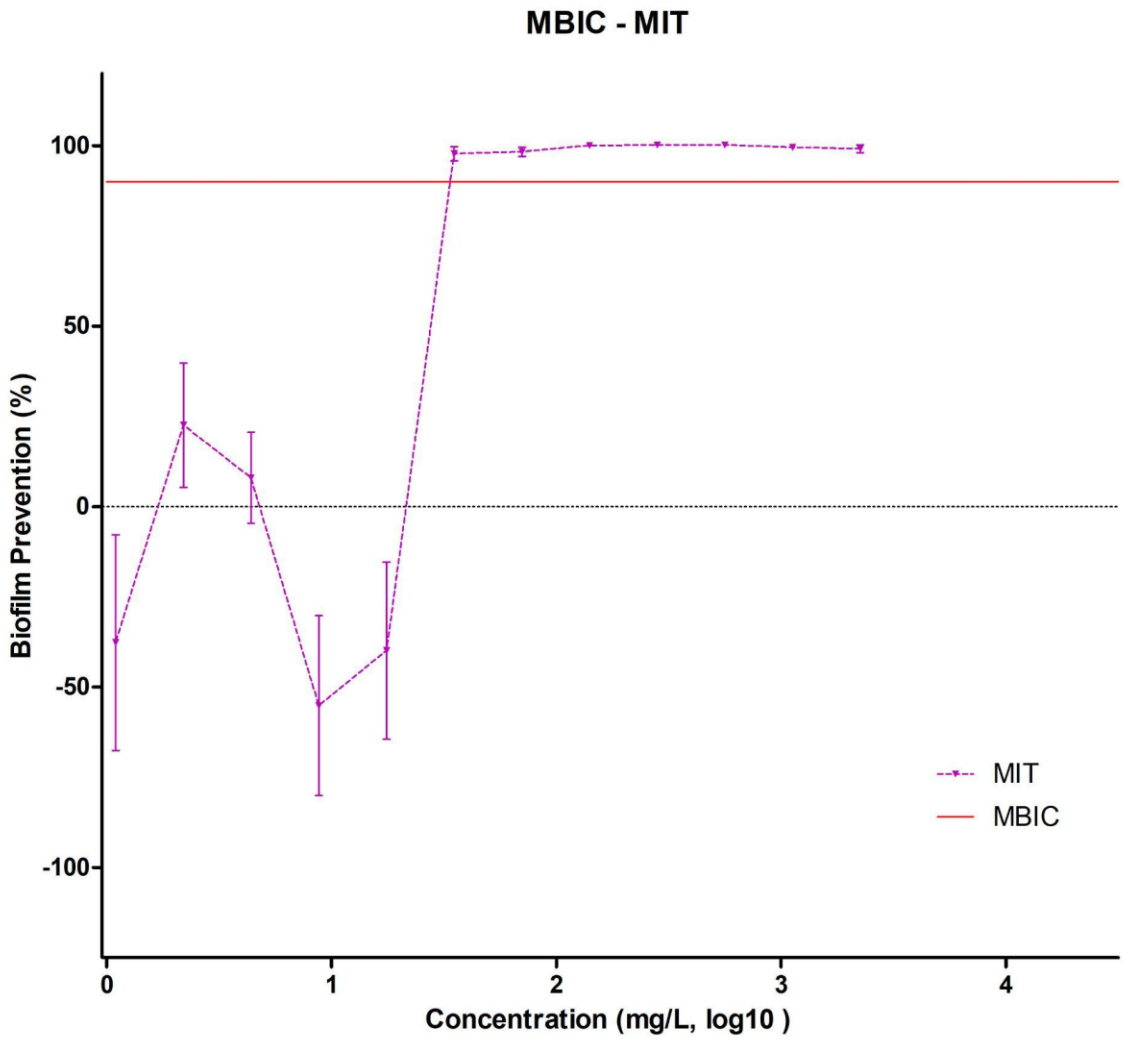

Figure 21 - Minimum biofilm inhibitory concentration of MIT for *Pseudomonas aeruginosa* (positive control). The MBIC line is only a guide for the eye.

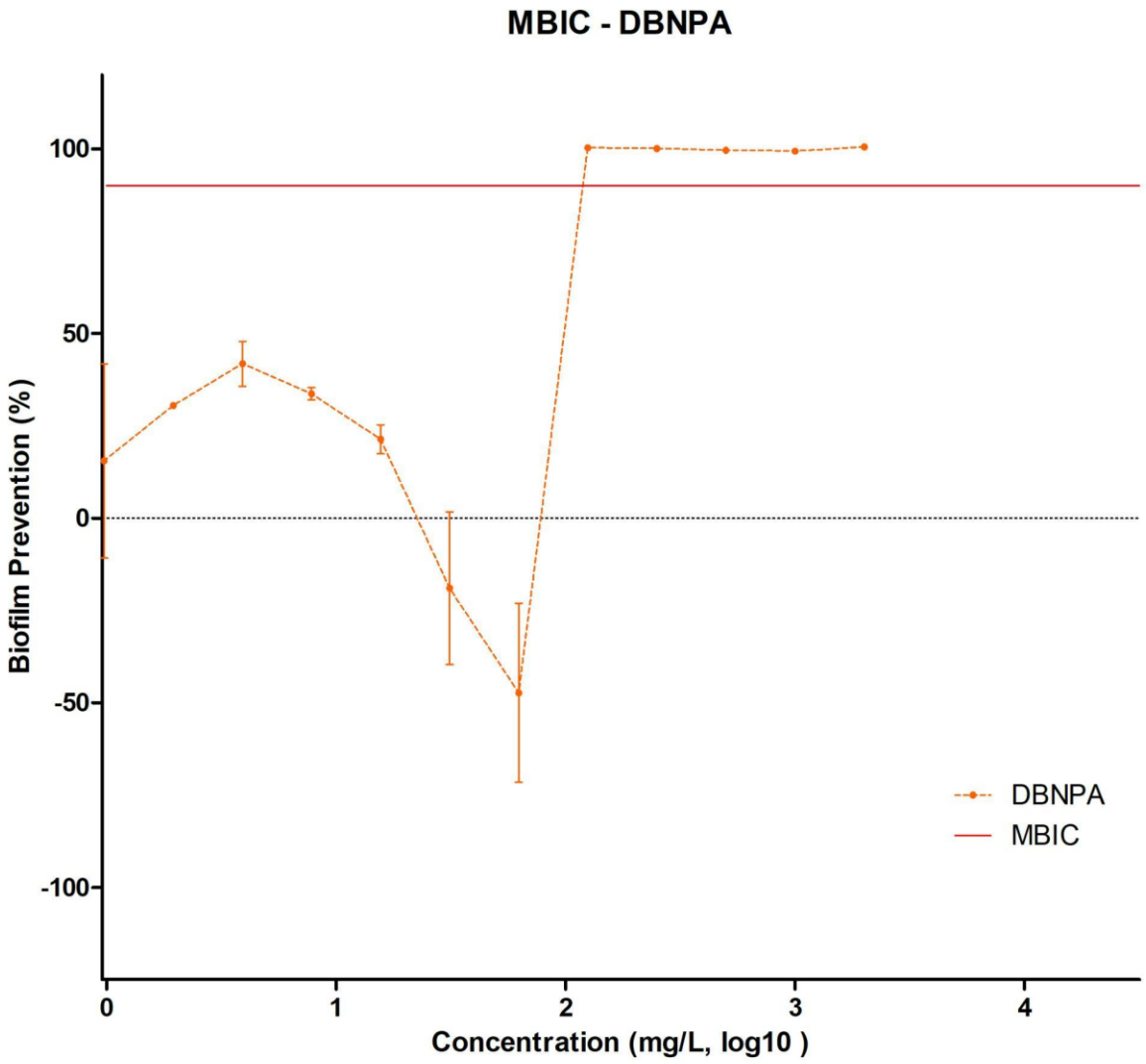

Figure 22 - Minimum biofilm inhibitory concentration of DBNPA for *Pseudomonas aeruginosa.* The MBIC line is only a guide for the eye.

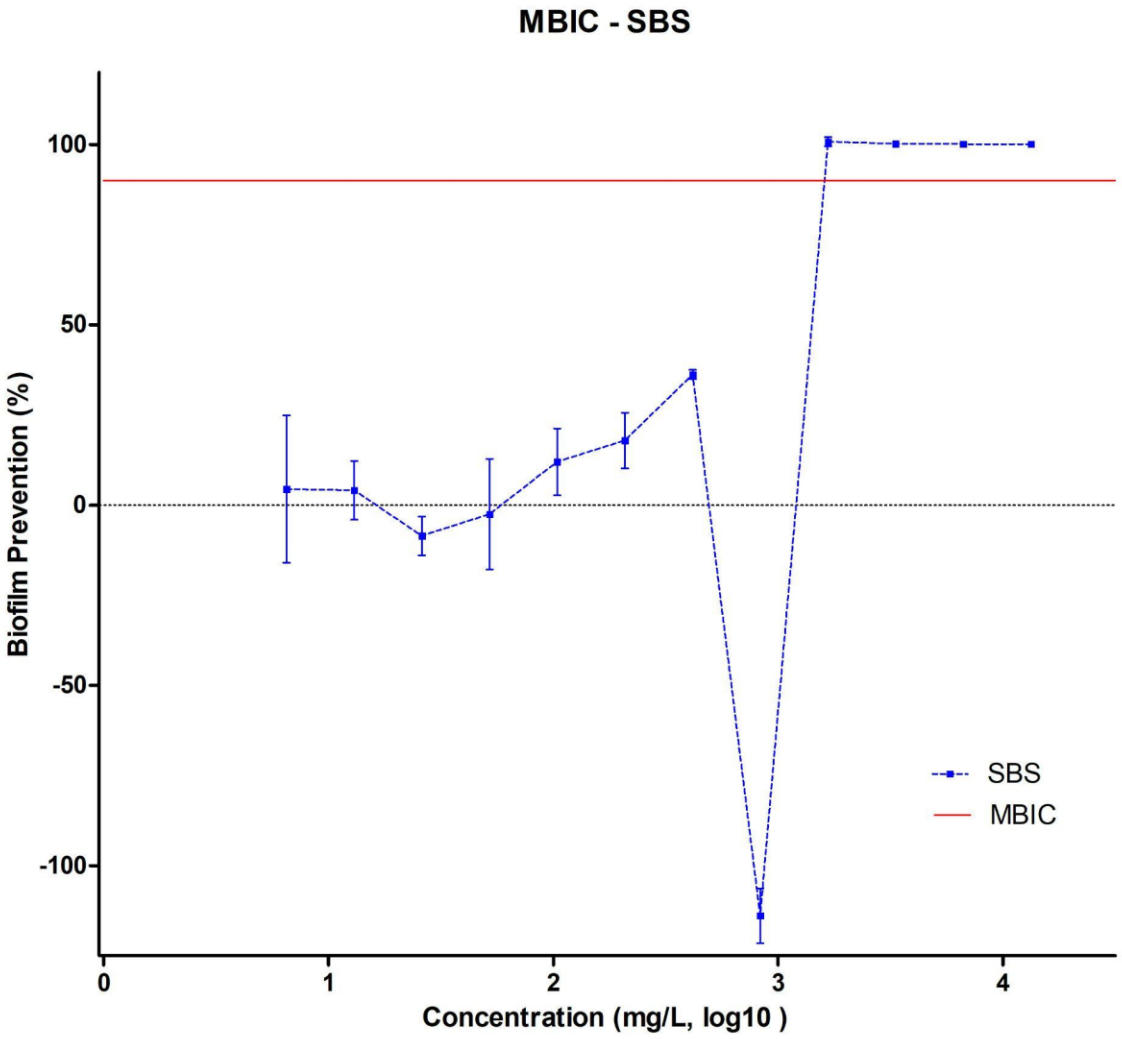

Figure 23 - Minimum biofilm inhibitory concentration of SBS for *Pseudomonas aeruginosa.* The MBIC line is only a guide for the eye.

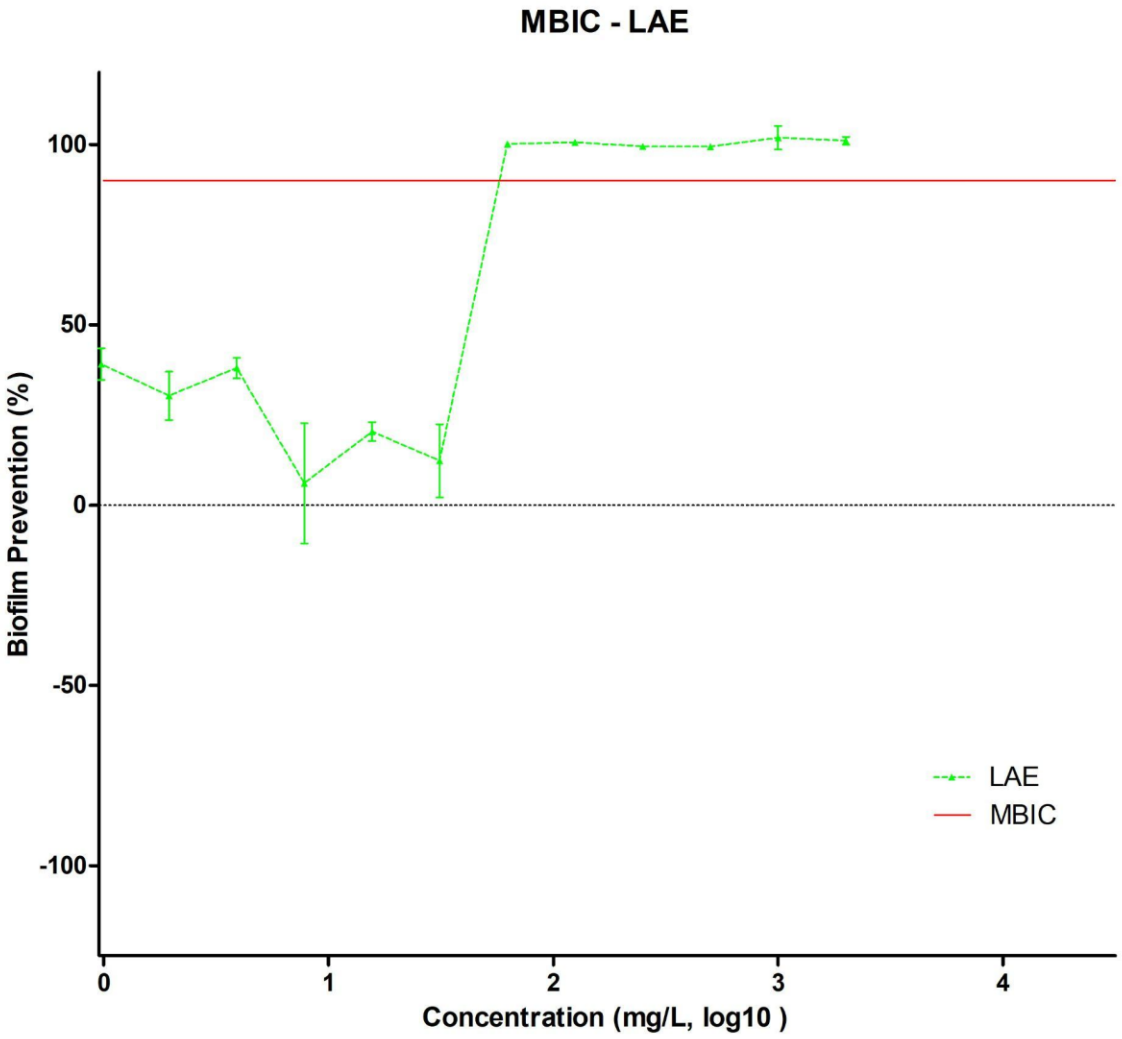

Figure 24- Minimum biofilm inhibitory concentration of LAE for *Pseudomonas aeruginosa.* The MBIC line is only a guide for the eye.

Minimum biofilm eradication concentrations (MBECs)

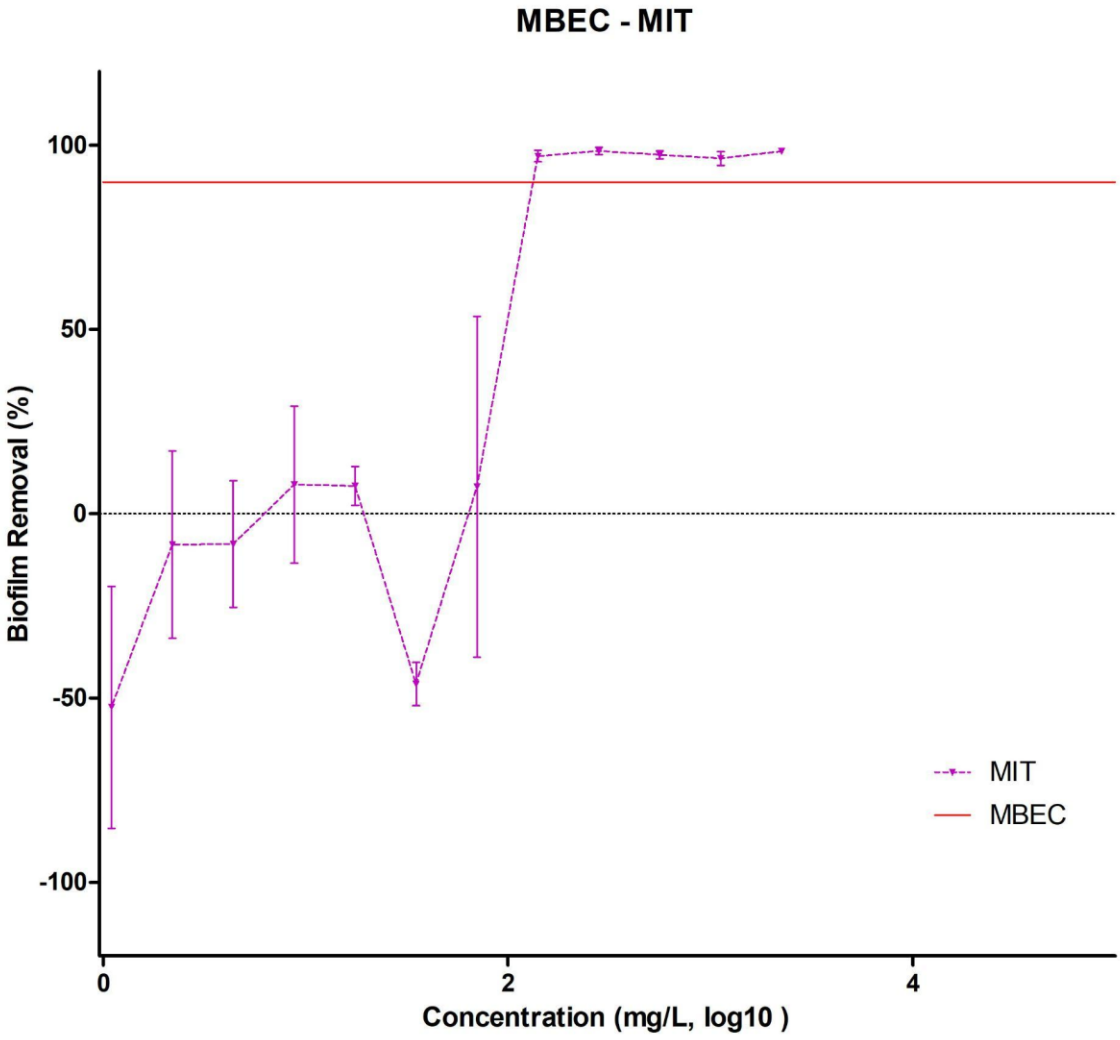

Figure 25 - Minimum biofilm eradication concentration of MIT (positive control) for *Pseudomonas aeruginosa.* The MBEC line is only a guide for the eye.

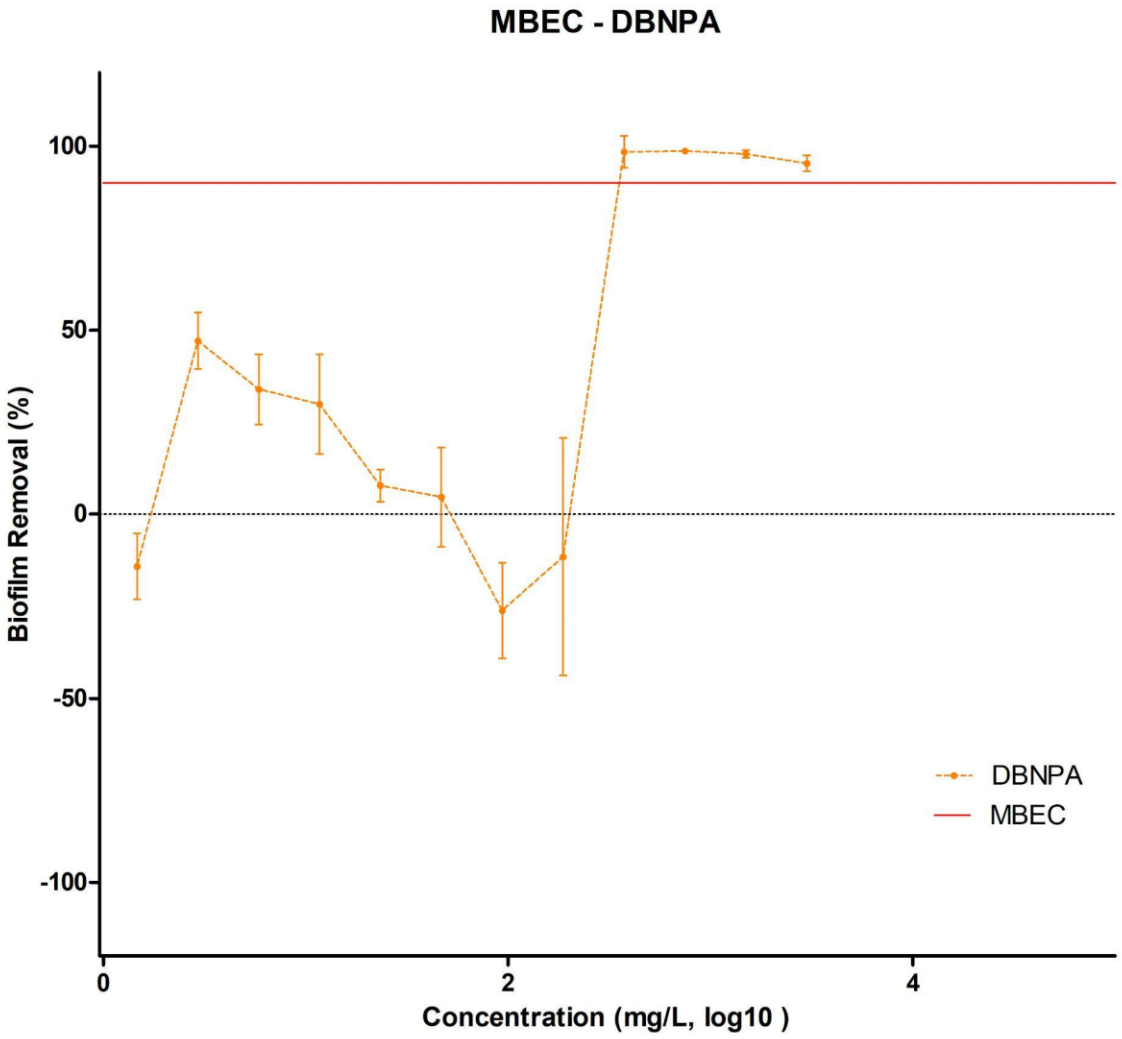

Figure 26 - Minimum biofilm eradication concentration of DBNPA for *Pseudomonas aeruginosa.* The MBEC line is only a guide for the eye.

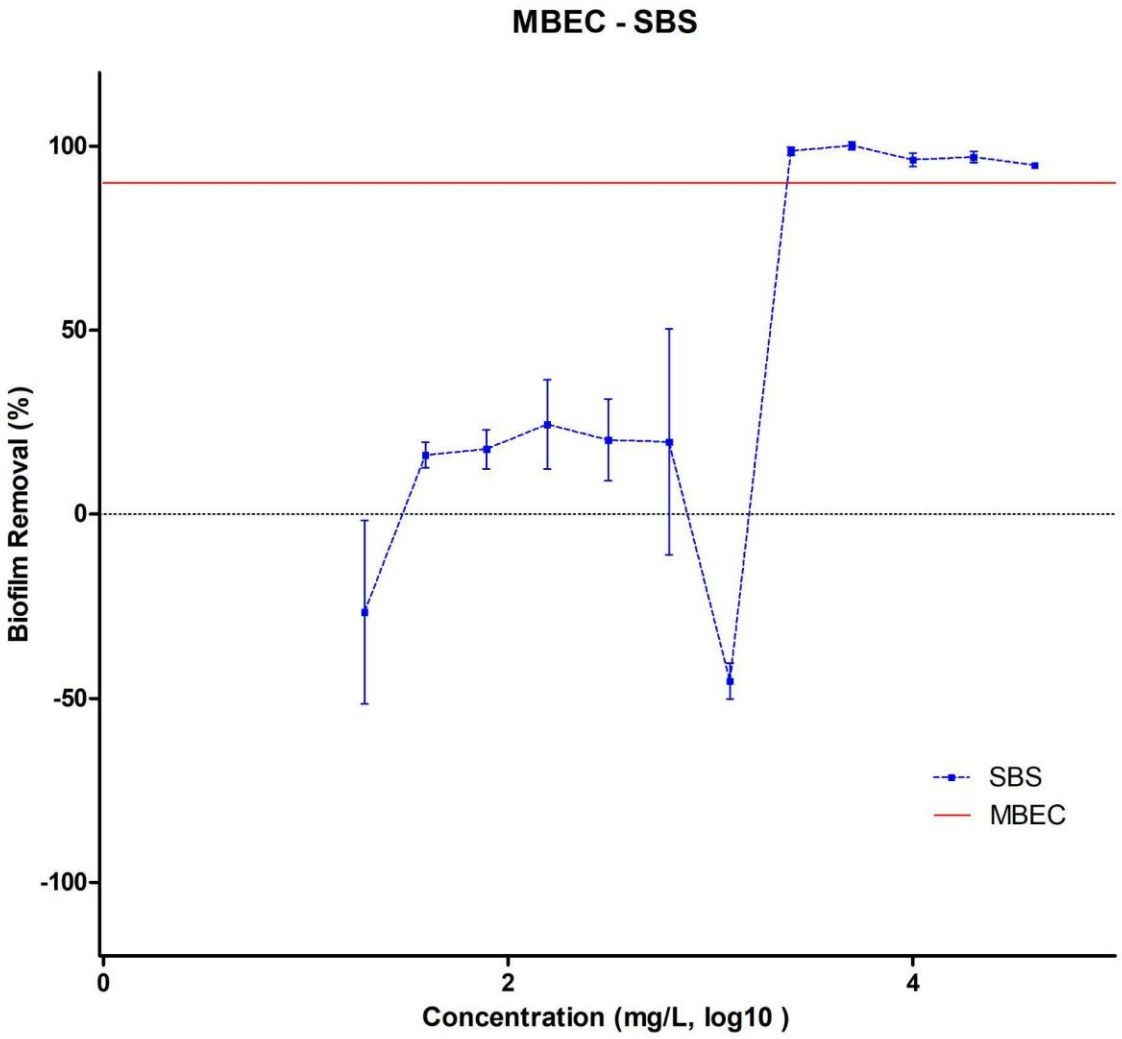

Figure 27 - Minimum biofilm eradication concentration of SBS for *Pseudomonas aeruginosa.* The MBEC line is only a guide for the eye.

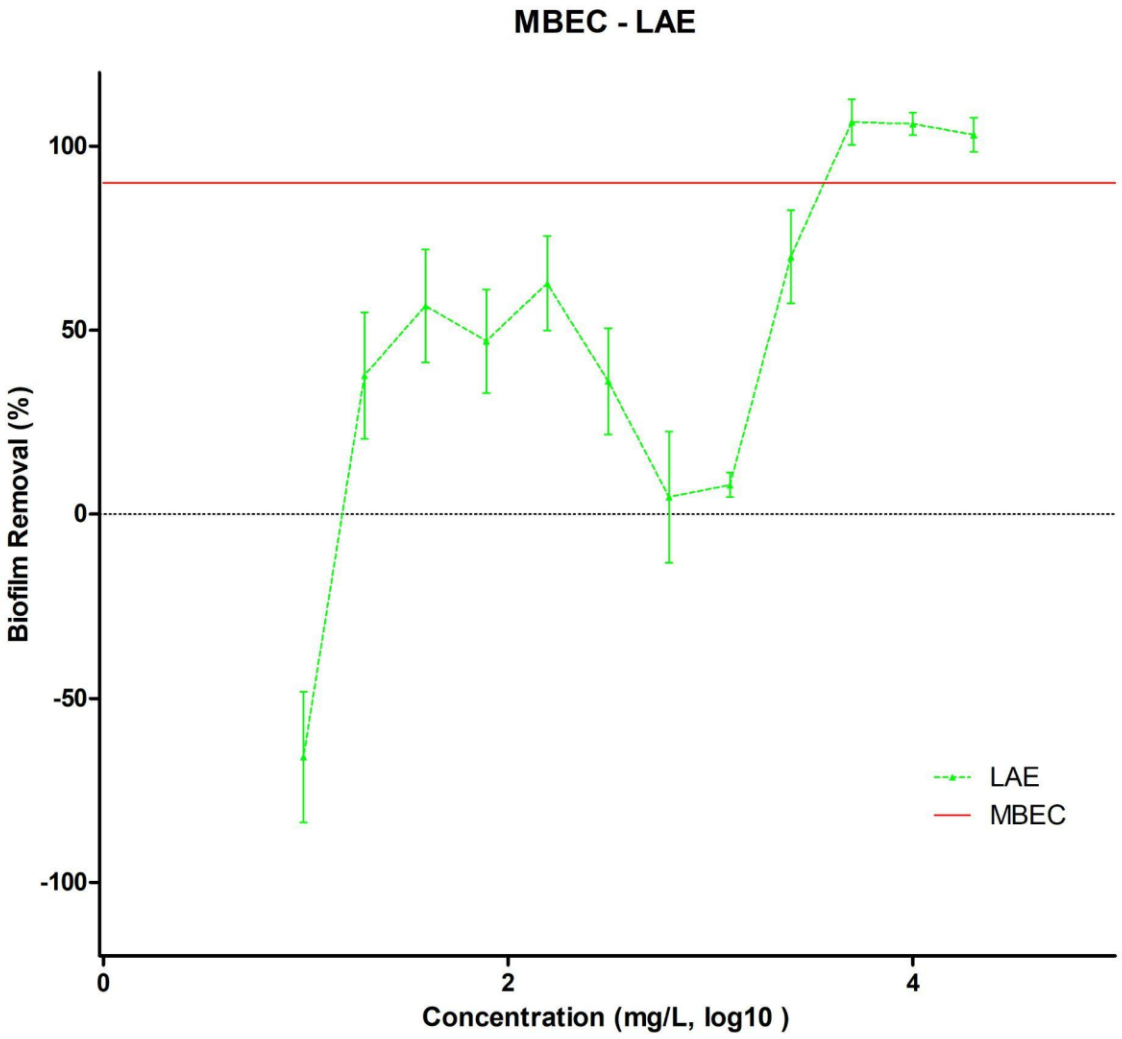

Figure 28 - Minimum biofilm eradication concentration of LAE for *Pseudomonas aeruginosa.* The MBEC line is only a guide for the eye.

Appendix C: Rapid Polyamide Membrane Compatibility Testing of Potential Anti-Biofouling Agents for Reverse Osmosis Membrane Systems - Supplementary material (Chapter 4)

AFM Controls

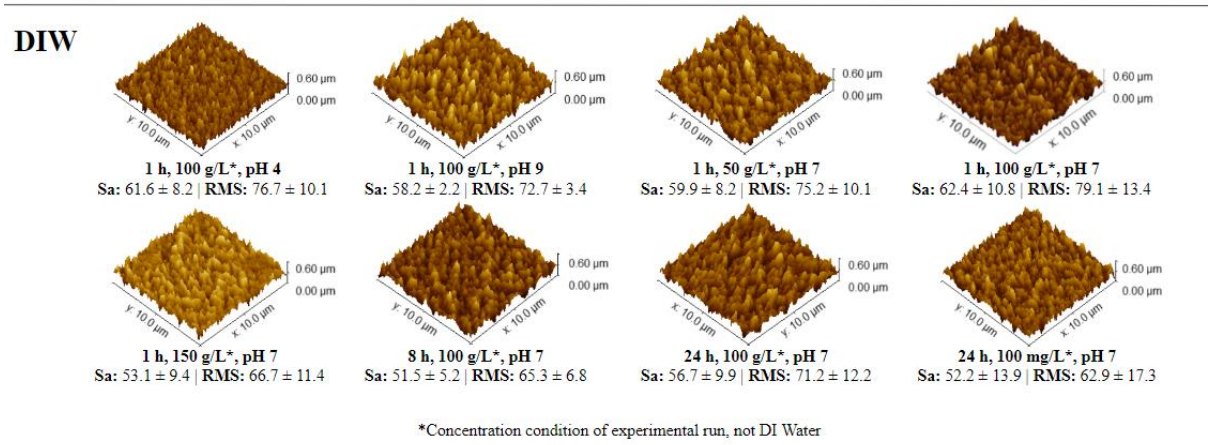

Figure 29 - Representative AFM images and Sa and RMS values of polyamide membrane coupons exposed to DI Water at different experimental conditions. Sa and RMS values are displayed as mean ± standard deviation.

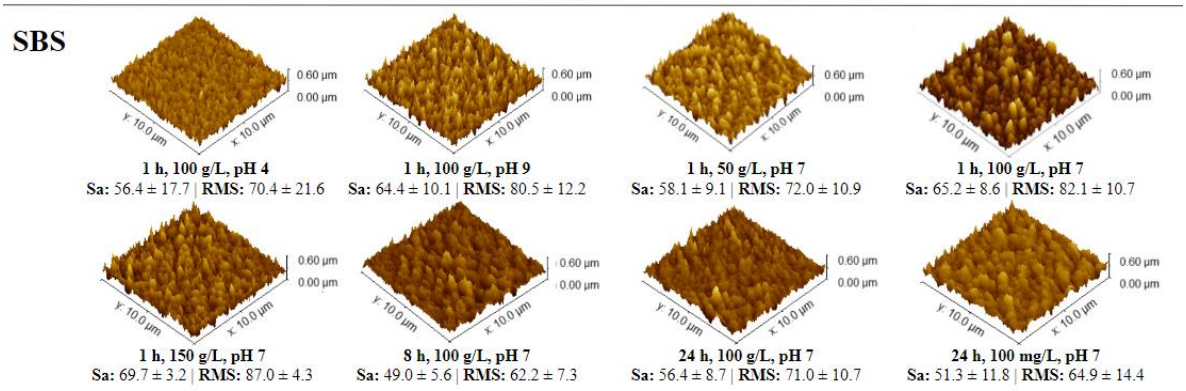

Figure 30 - Representative AFM images and Sa and RMS values of polyamide membrane coupons exposed to SBS at different experimental conditions. Sa and RMS values are displayed as mean ± standard deviation.

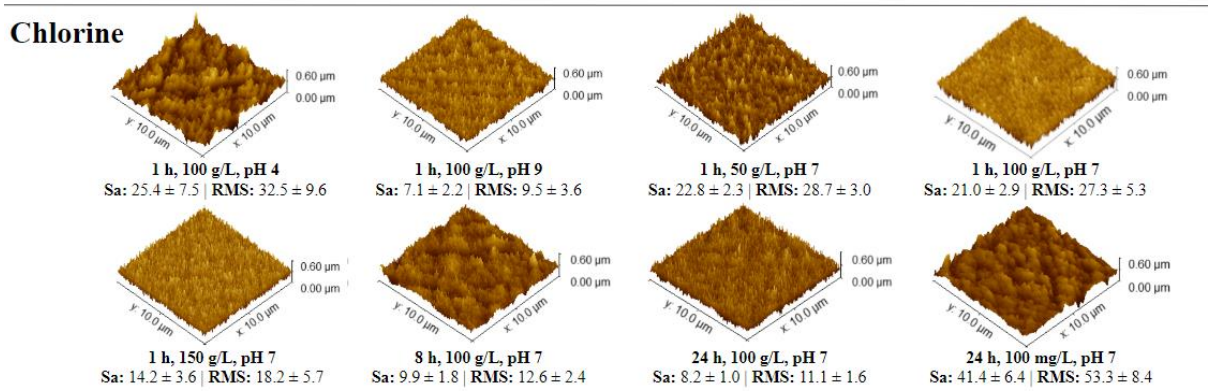

Figure 31 - Representative AFM images and Sa and RMS values of polyamide membrane coupons exposed to Chlorine at different experimental conditions. Sa and RMS values are displayed as mean ± standard deviation.

SEM Controls

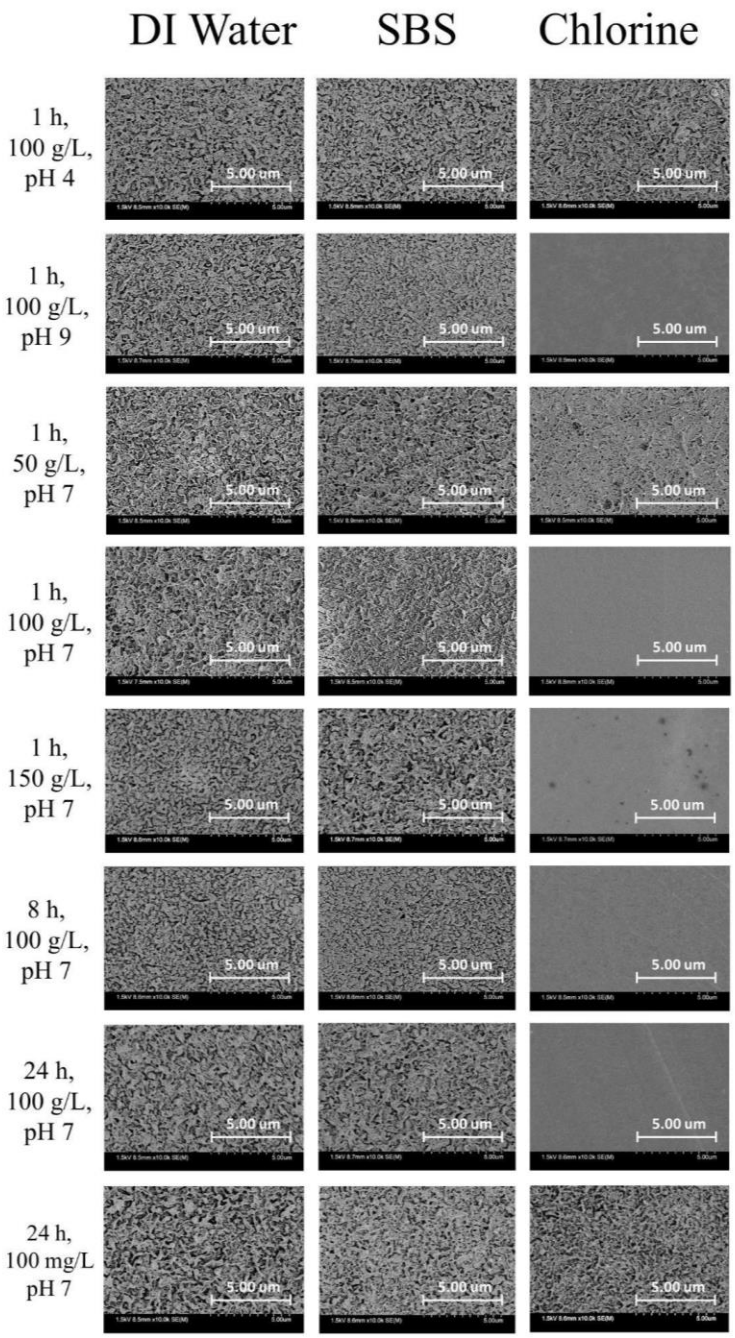

Figure 32 - Representative SEM images of RO polyamide membrane coupons exposed to the selected biocide controls (DI Water, SBS, and Chlorine) at different experimental conditions. Scale bars represent 5.00 μm (at 10,000x).

ATR - FTIR Controls

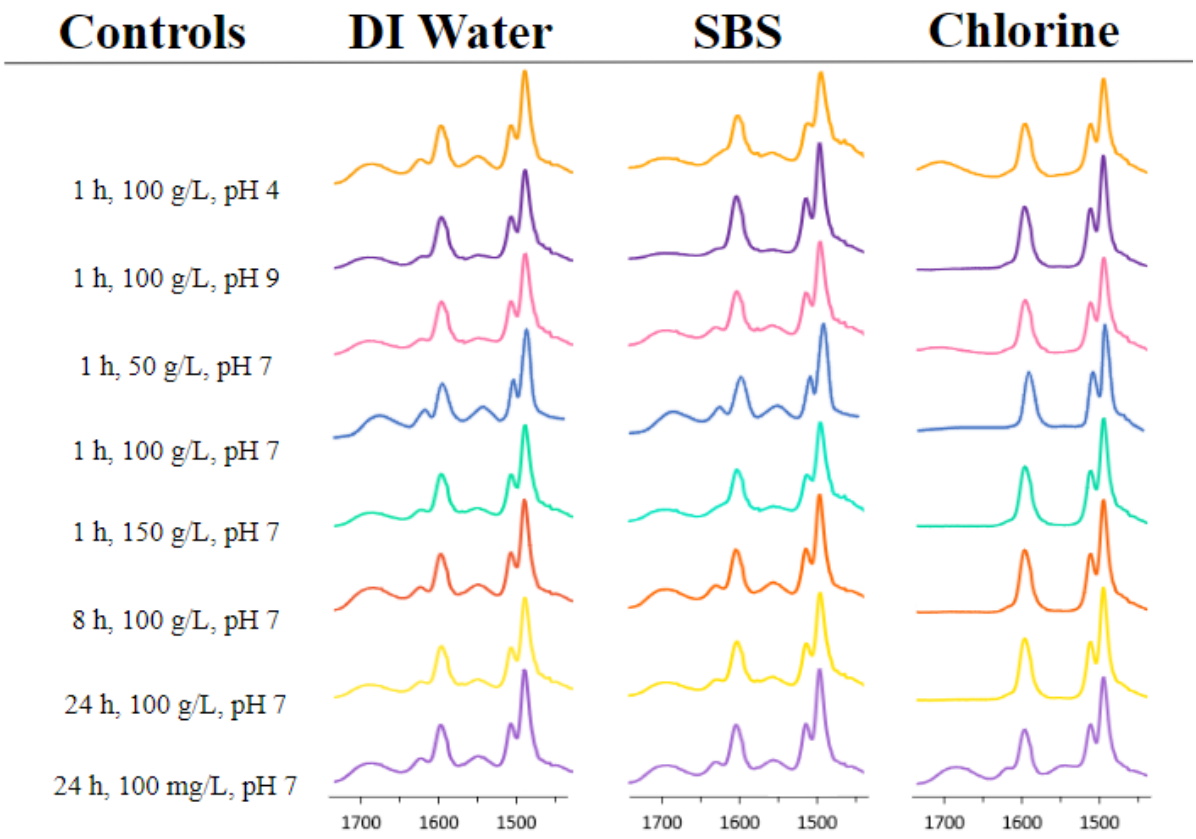

Figure 33 - ATR-FTIR Spectra of RO polyamide membrane coupons exposed to the selected biocide controls (DI Water, SBS, and Chlorine) for all experimental conditions.

Appendix D: The application of ethyl lauryl arginate to prevent biofouling in reverse osmosis polyamide membrane systems: A benchtop study - Supplementary material (Chapter 5)

Normalized permeate flux (J_n)

$$J_n = \frac{J_p}{J_0} \quad \text{(Eq. 1)}^9$$

Where,

J_p - Actual permeate flux [$Lm^{-2}h^{-1}$]

J_0 - Initial permeate flux [$Lm^{-2}h^{-1}$]

Where,

$$J_p = \frac{F_p}{A_m} \quad \text{(Eq. 2)}^9$$

F_p - Actual permeate flow [Lh^{-1}]

A_m - Membrane active area [m^2]

Where,

$$J_0 = \frac{F_0}{A_m} \quad \text{(Eq. 3)}^9$$

F_0 - Initial permeate flow [Lh^{-1}]

A_m - Membrane active area [m^2]

Normalized salt rejection (R_n)

$$R_n = \frac{R_a}{R_0} \quad \text{(Eq. 4)}^9$$

Where,

R_a - Actual salt rejection [%]

R_0 - Initial salt rejection [%]

Where,

$$R_a = \left(1 - \frac{C_p}{Cf}\right) \times 100\% \quad \text{(Eq. 5)}^9$$

C_p - Actual permeate conductivity [µS/cm]

C_f - Actual feed conductivity [µS/cm]

Where,

$$R_0 = \left(1 - \frac{C_{p0}}{Cf_0}\right) \times 100\% \quad \text{(Eq. 6)}^9$$

C_{p0} - Initial permeate conductivity [µS/cm]

C_{f0} - Initial feed conductivity [µS/cm]

Normalized trans-membrane pressure (TMP_n)

$$TMP_n = \frac{TMP_a}{TMP_0} \quad \text{(Eq. 7)}^9$$

Where,

TMP_a = Actual trans-membrane pressure [psi]

TMP_0 = Initial trans-membrane pressure [psi]

Where,

$$TMP_a = P_{fa} - P_{ca} \quad \text{(Eq. 8)}^9$$

P_{fa} - Actual pressure in the feed line [psi]

P_{ca} - Actual pressure in the concentrate line [psi]

Where,

$$TMP_0 = P_{f0} - P_{c0} \quad \text{(Eq. 9)}^9$$

P_{f0} - Initial pressure in the feed line [psi]

P_{c0}- Initial pressure in the concentrate line [psi]

Trans-membrane pressure rise (TMP_{rise})

$$TMP_{rise} = TMP_a - TMP_0 \quad \text{(Eq. 10)}^9$$

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