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Biofouling phenomena in membrane distillation: mechanisms and mitigation strategies

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Membrane distillation (MD) is envisaged as a cost-effective water desalination technology. When operated by low-grade energy, MD surpasses the cost challenges faced by other water desalination technologies. Although MD operates under conditions that minimize the survival of biofilm causing microorganisms, their development and succession is being increasingly reported. This is believed to be caused by the presence of halophiles and thermophiles in the feed solution, inducing significant efficiency losses. Therefore, biofouling mitigation remains crucial. This study reports current developments toward MD biofouling and mitigation strategies. Also, effects of membrane biofouling on process performance are briefly highlighted. This provides an in-depth understanding of measures required to minimize biofouling of MD systems.

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Environmental significance

While membrane distillation (MD) is a promising technology in wastewater desalination, its commercial growth is harnessed by various factors including membrane fouling. To address this problem, various studies have extensively reported on organic and inorganic fouling. Biofouling was rarely reported on the basis that MD operational conditions prevent growth of microorganisms on membrane surfaces. However, this problem persists. The current study provides comprehensive and yet concise biofouling phenomenon in MD, its formation, mechanisms, specific microorganisms responsible for membrane fouling and control measures. Deposition and growth of these microorganisms on membrane surfaces is influenced by membrane properties, hydrodynamic conditions, feed solution properties and membrane module designs. To promote its industrial realization, MD requires significant experimental work to mitigate the existing fouling challenges.

Introduction

The unprecedented rise in climate change and an increase in population growth have resulted in significant global water shortages.^{1,2} Over the last century, water consumption has increased by approximately 600%, largely due to urbanization.³ Furthermore, based on scholarly reports, nearly 1.8 billion people are projected to live in water stressed areas by 2025.⁴ These alarming statistics and the rise in demand for potable water have stimulated the need to explore freshwater supply alternatives. Currently, scientists and engineers are developing sustainable solutions through research to address existing water shortages.

Membrane-based separation technologies have emerged as promising approaches to supply water to coastal areas. These include reverse osmosis (RO), forward osmosis (FO), nano-filtration (NF) and membrane distillation (MD).⁵ Although RO is the leading water desalination technology, its high energy consumption and extreme operating pressure increases

operational costs. These costs are significantly high compared to the volume of water produced. Therefore, alternative membrane technologies of low-cost should be considered. MD is envisaged as a cost-effective membrane technology, not only because of its ability to exploit solar thermal energy but its capacity to operate under moderate conditions, requiring inexpensive equipment.⁶ The driving force behind MD is the vapor pressure difference existing across the membrane, induced by differences in temperature (Fig. 1a).⁷ As illustrated in Fig. 1b, both mass (vapor) and heat are transferred through the membrane. The mechanism of mass transport is well explained by Poiseuille flow, molecular diffusion, and Knudsen diffusion, conditional to the existence of trapped air in the pores and the membrane pore size.⁸ Mass transport depends on the type of membrane used, its properties, the concentration of the feed solution, and the mass transfer coefficient. Further advantages of MD include high salt rejection ($\geq 99\%$), minimal sensitivity to salinity of the feed solution, low hydrostatic temperature and pressure requirements and the feasible utilization of renewable energy and low-grade/residual energy.⁹ Schwantes *et al.* (2013)¹⁰ used the waste heat generated from a diesel power station to drive a pilot-scale MD system. The waste heat supplied feed temperatures of 70–80 °C, enabling water production of 4 m³ per day. Although MD displays

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Fig. 1 Mechanism of operation of MD: (a) process mechanism and (b) heat and mass transfer, where T_F , T_D , T_1 , T_2 , P_F , P_D , Δp , dQ represent feed, distillate, evaporation and condensation temperatures, feed and distillate vapour pressure, vapor partial pressure difference and change in energy respectively.^{13–15}

attractive features, its industrial application has been hindered by low performance module design, production of low water fluxes (compared to current technologies), and membrane fouling.^{11,12}

Membrane fouling refers to the deposition and growth of inorganic, organic, colloidal and biological substances on the membrane surface.¹⁴ Inorganic fouling involves the deposition of mineral salts on the membrane surface following precipitation whereas organic fouling refers to the deposition of organic matter on the membrane surface.¹⁶ Membrane fouling is reported to increase membrane wetting, causing a reduction in distillate quality.¹⁷ Moreover, membrane fouling introduces regular membrane cleaning and frequent membrane replacements, further increasing costs of water production.¹⁸ Current reported studies mostly focus on inorganic and organic fouling, with limited information on the dynamics and succession of biofouling.¹⁹ By definition, biofouling refers to the deposition and accumulation of microorganisms on the surface of the membrane and within its pores, leading to the formation of a biofilm.²⁰ The biofilm consists of microbial cells and extracellular polymeric substances (EPS), covering the surface of the membrane. Biofouling of MD systems was overlooked with the perceptions that its operating conditions would minimize biofilm development. However, an in-depth study of fouling layers has revealed the increased presence of biofilms.²¹ Formation of biofilms induces membrane wetting²² and vapor pressure decline²³ causing decay in salt rejection²² and water flux.^{21,24–26} Krivorot *et al.*, (2011)²⁴ studied biofouling occurrence in MD, using coastal seawater as the feed. Reportedly, microbial attachment increased quantitatively as a function of time, thus affecting process performance. A 34% reduction in water flux was recorded, markedly due to membrane pore blockage caused

by biofilm formation. Furthermore, flux decline was caused by the growth of crystal salts on the membrane surface. Another phenomenon contributing to biofouling in MD is temperature polarization (TP), referring to the difference in temperature between the bulk feed stream and the membrane surface.⁶ The TP stimulates microbial growth at the cooler membrane interface.²⁴ Furthermore, TP decreases the driving force in MD.²⁷ A detailed review of TP and its holistic effects on MD is reported elsewhere.²⁸ Membrane wetting and a subsequent reduction in salt rejection induced by microbial accumulates on the membrane surface was reported by Bogler and Bar-Zeev (2018).²² Membrane wetting was clearly observed at feed temperature of 65 °C using optical coherence tomography (OCT) and facilitated penetration of bacteria and endospores through the membrane towards the distillate. Microbial deposition was dependent on the composition of feed solution and nutrient availability.²⁶ To establish high performing MD with excellent resistance to biofouling, elucidation of biofouling mechanism and its effects is essential. A recent review presenting biofilm development in MD systems was reported.⁶ However, specific illustration and collective reporting of microorganisms causing biofouling in MD was not highlighted. Therefore, the current review provides detailed insights of MD biofouling with particular focus on the survival of microbes under harsh MD operating conditions. Additionally, the mechanism of biofouling, its formation, causes, effects, and prevention for efficient MD operation are discussed.

Biofilm formation

Biofilm formation follows a pathway of successive steps including; (1) conditioning film formation with migration and



adhesion of bacterial cells to the membrane surface, (2) EPS secretion, growth and maturation of bacterial cells, and (3) proliferation and cell detachment for colonization of new areas.³⁰ For control and efficient mitigation of biofouling, insights into its mechanism must be expounded and understood.

Conditioning film formation and surface attachment

A conditioning film composed of macromolecules, organic matter, proteins, amino acids, and nucleic acids initially covers the surface of the membrane. Alongside, dead bacteria and soluble microbial products (SMP's) further adhere on the membrane. The presence of conditioning film alters the membrane surface, promoting better adhesion of cells. Planktonic (free-floating) cells migrate from the bulk solution to the membrane surface through the Brownian motion, where movement is induced by the collision of particles.¹⁵ However, attachment is not permanent, since planktonic cells preferentially select sites for adhesion through cell locomotion using flagella and pili.³¹ Cells exhibiting better motility and microbial activity attach first on membrane surfaces.³² Following attachment, cells undergo proliferation, where constant multiplication and division take place. Furthermore, transparent exopolymer substances (TEP) and gel-like polysaccharides (aggregates) adsorb on the membrane surface to influence fouling.³³ The TEPs generate a viscous surface, thus providing a transport for microbial cells to the membrane surface. However, due to their complex chemical composition, specific contribution of TEPs to fouling is not known. According to Zhang *et al.* 2018,³⁴ interaction between cells and the membrane (cell-to-surface) occur largely through electrostatic and hydrophobic-hydrophobic processes. Although positively charged bacterial cells adhere readily on membrane surfaces, negatively charged bacterial cells tend to adhere less, especially in cases where the membrane surface is predominantly negatively charged and the ionic strength of the feed solution is low.³⁵ Solutions of low ionic strength contain less ions to counter-act the electronegativity of bacterial cells, resulting in increased repulsion between the membrane and the cell. However, at high ionic strength, cells adhere irreversibly on membrane surfaces. Abu-Lail and Camesano (2003)³⁶ evaluated the bio-adhesion of *Pseudomonas putida* KT2442 and noted low adhesion rates at low ionic strength. This was attributed to the high energy barrier required for cell adhesion to take place.³⁷

Additionally, hydrophobic surfaces tend to adsorb hydrophobic microorganisms. Bacteria contain hydrophobins (known as adhesins), promoting adhesion and formation of conditioning layer. This layer initiates biofilm development.²⁷ Hori and Matsumoto (2010)³⁵ studied the binding mechanism of *Staphylococcus aureus* on hydrophilic and hydrophobic surfaces. Notably, macromolecules tethered more on hydrophobic surfaces than hydrophilic surfaces. This was attributed to cell-surface contact time and adhesion force. According to Fabre *et al.* (2018),³⁸ large quantities of protein adhered on hydrophobic surfaces compared to hydrophilic surfaces. This phenomenon was described by reduction in free energy and

a rise in entropy of the system.³⁹ However, full assurance of absolute adherence/non-adherence of cells is not well understood since bacteria exhibit endless mechanisms enabling their adaptation under different conditions.⁴⁰ For instance, hydrophilic bacteria adsorb on hydrophilic surfaces too, further promoting membrane fouling. Theoretically, polysaccharides (the major constituent of microbial cell exopolymer) favourably adhere on hydrophilic surfaces and form a gel-like firm matrix layer. Also, membrane roughness increases microbial attachment on membrane surfaces.⁴¹ Bernstein, Belfer, and Freger (2011)⁴² reported conflicting findings where fewer cells were deposited at high membrane surface roughness. In contrast, other studies have found no correlation between surface roughness and bacterial deposition. Hence, extensive research is required to ascertain these contradictions.

The presence of high organic material in the feed solution is reported to increase the deposition of microbial contaminants on surfaces (Bogler, Lin, and Bar-zeev 2017)²⁷ (Fig. 2). According to Chen *et al.*, (2021),²⁵ deposition of organic matter on membrane surface induced the formation of a conditioning layer, enabling more bacteria to adsorb on the surface. Other forces driving the adhesion of cells on membrane surfaces include thermodynamic and hydrodynamic interactions, polarity, oxygen content, bacteria abundance, process cross flow velocity and permeate flux. Furthermore, process parameters such as feed spacers and surface charge enhance biofilm development. Accumulation of microorganisms on feed spacers reduces the flow of the feed stream, thus affecting water flux.²⁷

Biofilm formation and EPS secretion

Following attachment, bacterial cells secrete EPS. The EPS and bacteria form a highly organised 3-dimensional structure known as a biofilm (Fig. 3). The biofilm forms between the solid and the liquid phase, where ultimate separation takes place. The EPS immobilizes and encapsulates bacterial cells, enhancing the firmness of the biofilm. Though biofilms vary in composition, they consist of bacteria (dead and alive), fungi, eukaryotic organisms, EPS, microalgae, and archaea.^{43,44} The biofilm structure contains interstitial water channels, facilitating the movement of oxygen, nutrients, and genetic material.²⁷ Bacteria are usually larger than other biofilm microorganisms and typically range from 0.5–2 μm .³⁵ Movement of bacteria through the biofilm results in the colonization of new areas. Also, EPS contains polysaccharides, lipoproteins, proteins, glycoproteins, and carbohydrates contributing towards bacteria resistance against inhibitors.^{45,46} Furthermore, EPS bind the cells to the surface and maintain a stable environment. The EPS enhances communication within cells and act as a source of nutrients for the bacteria.⁶ Bacteria are known to release significant EPS under thermal stress to overcome destruction by MD operational conditions.³² During membrane colonization, bacteria produce an exopolymer matrix, which grows and multiplies through the help of nutrients to adapt to the new environment (Fig. 3).

Notably, different biofilm layers contain different pore-size distributions. Goh *et al.*, (2013)²³ used evaporimetry to



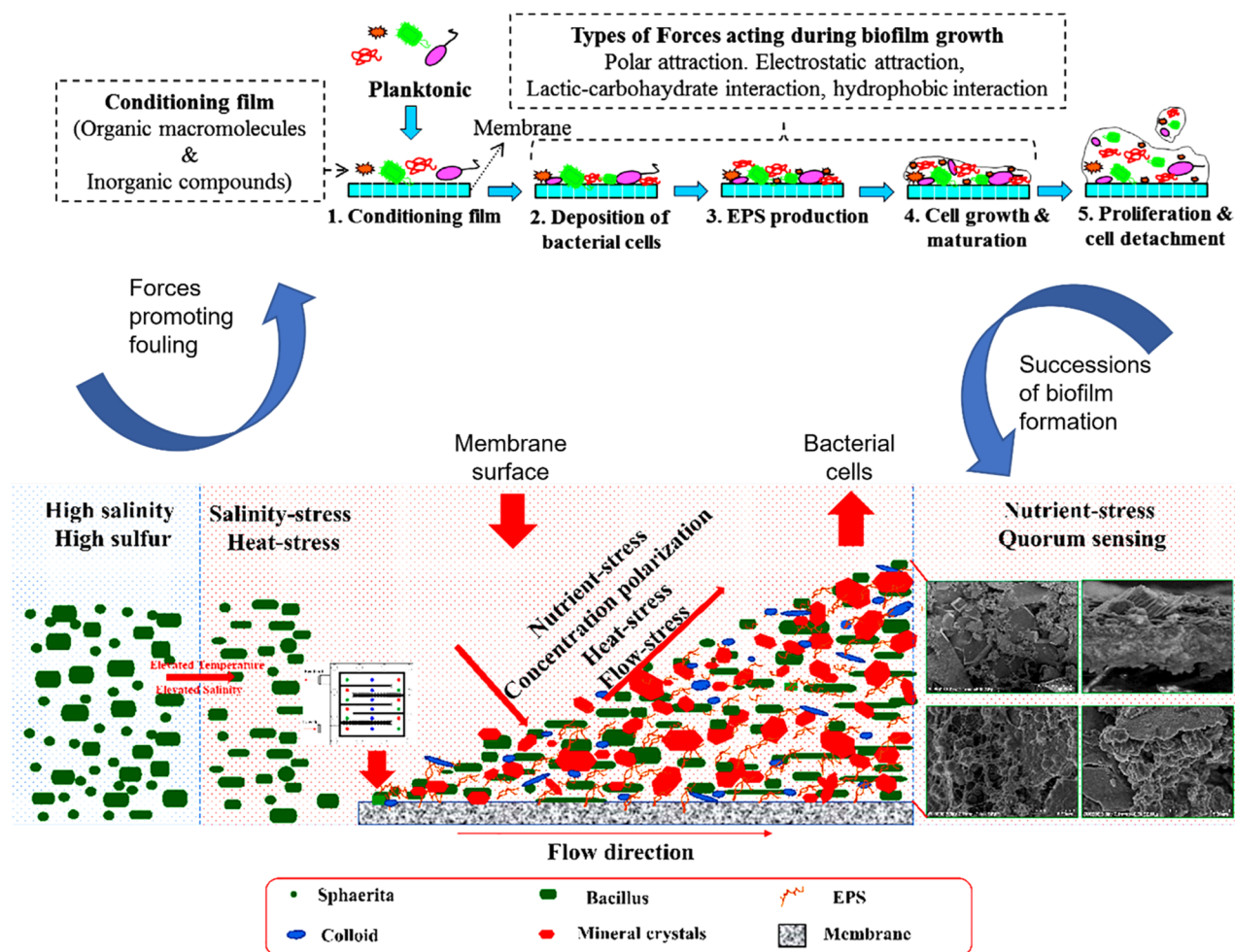


Fig. 2 Mechanistic relationship of the salinity, heat, nutrient, and flow stresses on the distribution of bacteria on membrane surfaces during MD operation.^{30,32}

study the particle-size distribution of the biofilm layer caused by two sludge solutions of different hydrophobicities. Reportedly, the hydrophilic sludge containing smaller pores was

responsible for vapor pressure reduction. Moreover, smaller pores depressed the vapor flux, owing to the Kelvin effect rather than the effect of an increase in hydraulic resistance. During

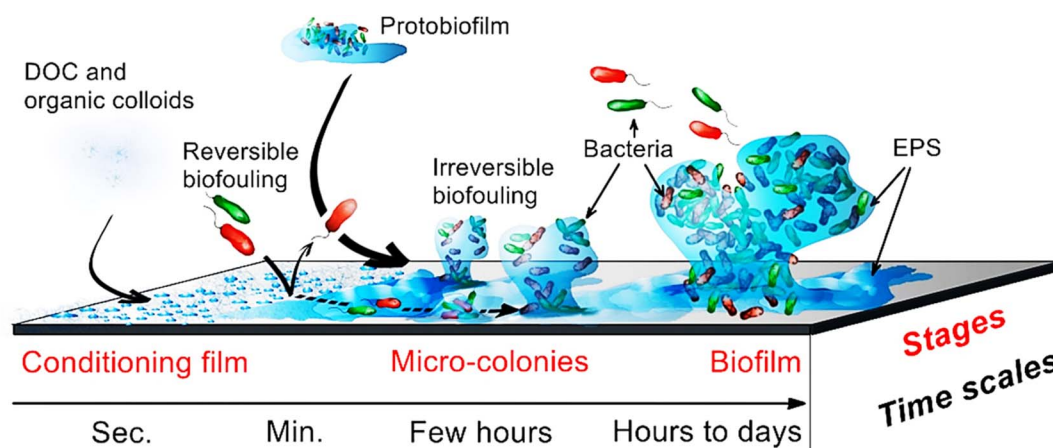


Fig. 3 Biofilm progression on membrane surfaces. Organic matter forms a conditioning layer enabling attachment of bacterial cells. The EPS are subsequently secreted to further promote attachment of bacterial cells and their colonies. During this process, the biofilm forms thus promoting dispersion and continuous growth of bacterial clusters.^{15,27}



biofilm development, bacteria senses each other within the vicinity, a process known as quorum sensing (QS). Bacteria secrete autoinducers during QS development, enhancing their communication.⁴⁷ The QS further promotes microbial social activities and enhance community behaviour through the expression of certain genes.⁴⁸ The dispersion of the biofilm is augmented by QS.⁴⁹ Thus, QS further encourages biofilm development. According to Zheng *et al.*, (2022),³² the development and succession of the microbial community is exerted by QS, amongst other factors. The obstinacy of biofilm development and EPS secretion have deemed membrane biofouling a key research concern.

Enumeration and identification of microbial cells on the membrane surface

Biofilm identification on the membrane surface is evaluated using a variety of techniques including scanning electron microscopy coupled with energy dispersive spectroscopy (SEM-EDS), optical coherence tomography (OCT) and confocal laser scanning microscopy (CLSM).^{50,51} Zodrow *et al.*, (2014)⁵² evaluated membrane biofilm formation using CLSM, assessing the architecture, heterogeneity and biovolume of the microbial community. The biofilms were heterogeneous and contained several colonies, with a plethora of Burkholderiales, Rhodobacterales, and Flavobacteriales. Bogler & Bar-Zeev, (2018)²² identified the presence of polysaccharides and detected dead and live bacterial cells using CLSM, with a further evaluation of the average biofilm thickness and total biovolume. Overall, feed operating temperatures of 55 °C led to a slightly thicker biovolume compared to 47 °C. This was attributed to provision of the optimum growth temperature of *Anoxybacillus* sp. Krivorot *et al.*, (2011)²⁴ evaluated the progression of biofilm development on the membrane surface using SEM. The experiment was conducted over a period of 19 days. Reportedly, deposition of microbial cells increased qualitatively as a function of time. Initial bacterial attachment was detected after 28 h, with the conditioning film seen only after 20 h of operation. From 48 h towards 19 days, the biofilm formed, thus covering the membrane surface. This later caused a reduction in process performance.

Physiochemical characteristics and viscoelastic properties of the biofilm are largely determined *via* UV/vis spectrometry, flow cytometry, atomic force microscopy (AFM) and Fourier Transform Infrared Spectroscopy (FTIR).²⁷ Phattaranawik *et al.*, (2009)²¹ utilized UV/vis spectrometry to quantify the concentration and composition of the deposited biofilm on the surface of polyvinylidene fluoride (PVDF) and polytetrafluoroethylene (PTFE) membranes. Reportedly, the fouling layer was composed of protein, polysaccharides, and EPS. Since the concentration of polysaccharides on PVDF was higher than PTFE, it was envisaged that polysaccharides played a crucial role in flux decay. The flux of PVDF decreased considerably while that of PTFE remained stable. In another study, Krivorot *et al.*, (2011)²⁴ determined the protein content of the biofilm developed on the membrane surface using the Lowry protein assay. Notably, the protein concentration deposited on the membrane gave an

estimation of the biofilm thickness on the surface, further confirming SEM results. In another study, Goh *et al.*, (2013)²³ employed FTIR to study and confirm the presence of polysaccharides and proteins, indicating the presence of bacteria. Specifically, peaks at 1500–1700 cm⁻¹, 950–1170 cm⁻¹ and 2800–3000 cm⁻¹ were attributed to proteins, polysaccharides, and fatty acids, respectively. The use of highly specialized sequencing techniques for identification of bacterial species within the biofilm structure has gained significant attraction. For instance, Goh *et al.*, (2013),²³ identified bacterial species on fouled membranes through DNA amplification using polymerase chain reaction (PCR). Bacterial organisms were predominantly hydrophilic, thermophilic, and halotolerant species. These included *Rubrobacter taiwanensis*, *Caldalkalibacillus uzonensis*, *Caldalkalibacillus uzonensis*, *Tepidimonas* sp, and *Meiothermus hypogaeus*. More recently, specialized techniques such as 16S rDNA and gene sequencing have gained research consideration. With these, bacterial species, growth patterns and behavioural changes of specific microorganisms are identified. Liu *et al.*, (2020a)⁵⁵ studied the bacterial composition of the biofilm on the membrane surface using 16S rRNA and gene sequencing following DNA extraction. The abundance of live bacteria was higher during initial biofilm development stage which sharply declined as a function of time. This decrease was associated with an increase in salt crystal deposition on the membrane. A further increase in organic and inorganic substances contributed to the remarkable succession and evolution of biofilm bacterial community.

Microorganisms responsible for membrane biofouling in MD

Biofouling in MD is predominantly exacerbated by thermophilic, mesophilic, and halophilic bacteria present in the feed solution. Since feed solutions sourced from different environmental locations contain microorganisms of different identity, analytical assessment of feed solution composition is imperative. For example, feed solutions sourced from marine environments cause biofouling through deposition of phyla Firmicutes, Bacteroidetes and Proteobacteria, largely at high temperatures and water salinity.⁵³ From a general perspective, bacteria enhancing biofilm development in MD include genera *Mycobacterium*, *Bacillus*, *Lactobacillus*, *Cytophaga*, and *Flavobacterium*.⁴⁵ Notably, not all microorganisms present in the feed solution cause biofouling in MD. This uncertainty of events emphasises the importance of extensive biofouling assessment in MD systems.^{26,29} Gryta (2002)²⁶ evaluated biofouling occurrence in MD. Prior to MD operation, the feed solution was characterized by genera *Pseudomonas*, *Penicillium* bacteria, *Aspergillus* fungi and species *S. faecalis*. Evaluation of biofilm on membrane surface displayed the presence of *S. faecalis* and *Aspergillus* fungi only. Genera *Pseudomonas* growth was hindered by oxygen deficiency and high-water salinity. However, *S. faecalis* presented resistance to process conditions, thus promoting membrane fouling. In another study, Zheng *et al.* (2022)³² reported a change of plump sphere or short rod to lankier rhabditiform with a microbial community transformation from *Algoriphagus*, *Marinobater*, *Sulfurihydrogenibium*



to *Chelativorans*, *Acinetobacter*, *Idiomarina*. Change in microbial community was attributed to elevated temperatures and high saline conditions. Moreover, the latter strains notably survived due to their high motility, good quorum sensing effect and EPS secretion. Microbial succession is detrimental to MD as it leads to production of more EPS, thus decreasing membrane life span and process performance. In certain instances, microbial communities present in the feed solution do not evolve, but deposit on the membrane surface. Zdrov *et al.*, (2014)⁵² evaluated the biofouling impact of seawater, predominantly characterized by *Octadecabacter*, *Sediminicola*, *Loktanella*, and *Pelagibacteraceae*. These strains were detected on the membrane surface, although in varying concentrations. The most abundant strain was mesophilic *Octadecabacter*, due to its high temperature and salinity resistance. Other strains detected included thermophilic Bacillales and spore self-protective *Ralstonia*. Additional microbes identified from the biofilm are presented in Table 1.

Different modes of MD operations (closed and open loop) affect biofilm development differently.^{19,32} Liu *et al.*, (2020a)⁵⁵

used lake water (Xuanwu Lake) characterized by Proteobacteria, Actinobacteria, Bacteroidetes, and Cyanobacteria to assess biofilm development in MD. Early biofilm development consisted of genera *Acidovorax* and *Acetobacteraceae*, which was replaced by thermophilic *Methyloversatilis* at stage 2 of flux decay. Only Gammaproteobacteria and *Deinococcus-Thermus* were detected from the biofilm under closed loop operation. The viability of these strains was explained by their halotolerant mechanism induced by a change in morphology to withstand heat.³² Under open loop operation, the membrane biofilm was dominated by *Anoxybacillus*, *Meiothermus*, *Schlegelella*, *Tepidimonas*, and *Vulcaniibacterium*. These examples show the dependence of microorganisms on process parameters. The impact of feed salinity was further evaluated by Chen *et al.*, (2021),²⁵ where the membrane biofilm was composed of Proteobacteria, Bacteroidetes, *Deinococcus-Thermus*, *Tepidimonas*, *Meiothermus*, *OLB14_norank*, *Env.OPS 17_norank* and *Schlegelella*. The increase in feed concentration induced by continuous MD operation inhibited the growth of *OLB14_norank*, *Schlegelella*, and *Tepidimonas*. Evidently, MD conditions affect

Table 1 Biofouling-causing microbial organisms in MD^a

Feed source	Operating conditions	Microorganism	Ref.
Saline wastewater (from animal intestines)	Temp: $T_F - 80$ °C $T_P - 25$ °C CV: 0.367 m s ⁻¹	<i>S. faecalis</i> (S)	26
Seawater (Long Island Sound)	Temp: $T_F - 50.4$ °C $T_P - 18.1$ °C CV: 4.3 cm s ⁻¹	<i>Ralstonia</i> (G), <i>Octadecabacter</i> (G), <i>Pelagibacteraceae</i> (F), <i>Loktanella</i> (G), <i>Sediminicola</i> (G), <i>Vibrionaceae</i> (F), <i>Rhodobacteraceae</i> (F), <i>Cryomorphaceae</i> (F), <i>Flavobacteriaceae</i> (F), <i>Bacillales</i> (O)	52
Xuanwu Lake (China)	Temp: $T_F - 60$ °C $T_P - 10$ °C CV: —	<i>Anoxybacillus</i> (G), <i>Meiothermus</i> (G), <i>Schlegelella</i> (G), <i>Tepidimona</i> (G), <i>Vulcaniibacterium</i> (G), <i>Proteobacteria</i> (P), <i>Deinococcus-Thermus</i> (P)	55
Xuanwu Lake, Nan Lake and Qinhuai, River (Nanjing, China)	Temp: $T_F - 60$ °C $T_P - 15$ °C CV: 10.5 mm s ⁻¹	<i>Tepidimonas</i> (G), <i>Meiothermus</i> (G), <i>OLB14_norank</i> (G), <i>Schlegelella</i> (G), <i>Hydrogenophilaceae</i> (F), <i>Env.OPS 17_norank</i> (G), <i>Armatimonadetes_norank</i> (G)	25
Wastewater from power plant	Temp: $T_F - 55$ °C $T_P - 25$ °C CV: —	<i>Idiomarina</i> (G), <i>Chelativorans</i> (G), <i>Phenylobacterium</i> (G), <i>Methyloversatilis</i> (G), <i>Schlegelella</i> (G), <i>Aeribacillus</i> (G), <i>Bacillus</i> (G), <i>Actinobacteria</i> (P), <i>Chloroflexi</i> (P), <i>Microgenomates</i> (P)	32
Xuanwu Lake (China)	Temp: $T_F - 60$ °C $T_P - 10$ °C CV: -10.5 mm s ⁻¹	<i>Tepidimonas</i> (G), <i>Meiothermus</i> (G), <i>Sphingobium</i> (G), <i>Env.OPS 17_norank</i> (G), <i>Curvibacter</i> (G), <i>OLB14_norank</i> (G), <i>Pelomonas</i> (G), <i>Novosphingobium</i> (G), <i>Sphingomonas</i> (G), <i>Bradyrhizobium</i> (G), <i>Chelatococcus</i> (G), <i>Geobacillus</i> (G)	55
Artificial sterile wastewater	Temp: $T_F - 55$ °C T_P — CV: —	<i>Anoxybacillus</i> sp (G)	22

^a T_F and T_P are the feed and permeate temperatures, CV is the crossflow velocity, and the letters G, F, O, P and S represent the taxonomic classification of the microorganisms namely: G = genus, F = family, O = order, P = phylum, and S = species, respectively.



microbial diversity, mobility and activity, leading to a biological selection of cells.⁵⁴

Effects of biofouling on process performance

Microbial accumulation on membrane surface produces undesirable effects such as permeate flux decays, salt rejection decays and a reduction in membrane lifespan. These effects are largely caused by vapor pressure depression/pore blockage, membrane wetting, and the accumulation of EPS and protein. Microbial accumulates further adsorb on feed spacers, thus exacerbating fouling. However, it is worth noting that decay in MD process performance is complex and may not be attributed to biofouling alone. Table 2 summarizes the impact of biofouling on water flux and salt rejection.

Permeate flux decay

Membrane biofilm development notably reduces water flux. The water flux decreases due to: (1) membrane pore-blockage, (2) vapor pressure reduction, and (3) temperature and concentration polarization effects (Fig. 4). Commonly, permeate flux decay is caused by membrane pore blockage induced by the biofouling layer.^{17,52} However, degree of pore blockage determines the extent at which vapour pressure decays, hence flux decline. Goh *et al.*, (2013)²³ noted a 60% decrease in water flux caused by the accumulation of microbial contaminants on the surface of the membrane. These contaminants essentially caused a 32% decrease in the vapor pressure, thus reducing the rate of water recovery. This was ascribed to an increase in heat and mass transfer resistance across the boundary layer. Resistance to heat transfer minimizes water vaporization, thus reducing process performance.⁶ Liu *et al.*, (2020a)⁵⁵ evaluated the occurrence of biofouling on a closed loop direct contact

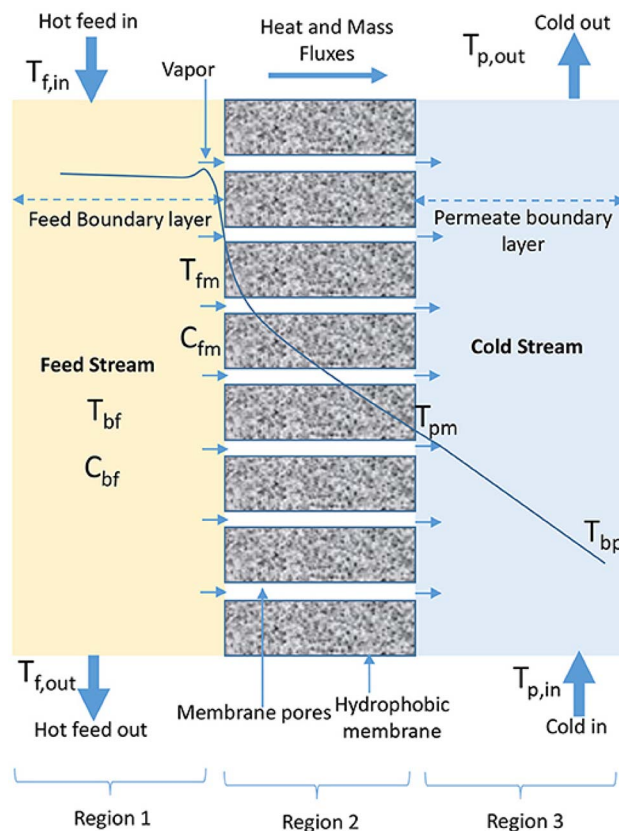


Fig. 4 Temperature polarization phenomenon in MD. $T_{f,in}$, T_{fm} , T_{pm} and $T_{p,out}$ represent temperatures in the bulk feed solution, near the membrane surface (feed side), near the membrane surface (permeate side) and in the bulk permeate, respectively.⁵⁸

membrane distillation (DCMD). Reportedly, the flux decreased by 55.79% over time due to the increased deposition of foulants.

Permeate flux decay of MD systems vary depending on feed temperature. This phenomenon was reported by Bogler & Bar-

Table 2 A summarized impact of biofouling on MD efficiency^a

Configuration of MD	Type of membrane	Feed type	Temperature (°C)	Flux decay ($L m^{-2} h^{-1}$)	Salt rejection decay	Ref.
DCMD	PP	Coastal seawater	$T_F - 40$ $T_P - 20$	$J_I - 3.85$ $J_F - 2.55$	No effect on salt rejection efficiency	24
MDBR	PVDF	Sludge suspension	$T_F - 55$ $T_P - 19.5$	$J_I - 8.42$ $J_F - 3.36$	$k_I - 217 \mu S cm^{-1}$ $k_F - <600 \mu S cm^{-1}$	23
DCMD	—	Artificial sterile wastewater	$T_F - 65$ $T_P -$	$J_I - 23.0$ $J_F - 15.6$	$k_I - 1000 \mu S cm^{-1}$ $k_F - 90\,000 \mu S cm^{-1}$	22
MDBR	PVDF/PTFE	Sludge	$T_F - 56$ $T_P - 25$	$J_I - 12.7$ $J_F - 1.90$	$k_I - 1.6 g L^{-1}$ $k_F -$	21
DCMD	PTFE	Estuarine water	$T_F - 50.4$ $T_P - 18.1$	$J_I - 20.0$ $J_F - 10.0$	No effect on salt rejection	52
DCMD	PTFE	Lake water	$T_F - 60$ $T_P - 10$	$J_I - 9.67$ $J_F - 4.28$	$k_I - 2.33 \mu S cm^{-1}$ $k_F - <12.7 \mu S cm^{-1}$	19
DCMD	PTFE	Qinhuai River	$T_F - 60$ $T_P - 15$	$J_I - 8.10$ $J_F - 4.30$	$k_I - 324.7 \mu S cm^{-1}$ $k_F - <23.0 \mu S cm^{-1}$	25
DCMD	PVDF	Effluent water	$T_F - 60$ $T_P - 20$	$J_I - 40$ $J_F - 21$	$k_I - 4.5 \mu S cm^{-1}$ $k_F - 5.8 \mu S cm^{-1}$	56

^a Where T_F , T_P , J_I , J_F , k_I and k_F represent the feed and permeate temperature, initial and final water flux, initial and final permeate conductivity respectively.



Zeev, (2018).²² The feed stream temperatures were controlled at 47 °C, 55 °C and 65 °C with corresponding flux declines of 30%, 78% and 32%, respectively. Flux decay was attributed to; (1) rapid growth of *Anoxybacillus* sp at the operating temperatures, (2), membrane pore wetting, (3) TP and CP, and (4) vapor pressure depression and hydraulic resistance. Though temperature may be increased beyond bacterial optimum growth temperature, cell adaptability at new operating conditions continue to threaten MD performance. For these reasons, the growth of biofilms increase at higher operating temperatures (Elcik *et al.*, 2022).⁵⁷

Salt rejection decline

Membrane wetting is a fundamental aspect requiring significant attention in MD systems. Wetting promotes the passage of feed in liquid form, thus reducing salt rejection.^{5,55,59} Deposition of hydrophilic biofilms causes membrane wetting. Bogler & Bar-Zeev, (2018)²² reported a reduced salt rejection caused by EPS-induced membrane wetting. A significant difference in salt rejection was obtained for different feed operating temperatures. 30-fold and 90-fold increase in distillate salinity was recorded at 55 °C and 65 °C respectively. 90-fold increase was attributed to the improved bacterial growth conditions causing rapid biofilm formation. Evidently, salt rejection and water flux declined upon inoculation of feed water with bacteria (*Anoxybacillus* sp.). However, bacterial film conditioning occurring on membrane surface may not affect process performance, especially in cases where hydrophobicity of membrane pores is not altered. This phenomenon was reported by Zodrow *et al.*, (2014)⁵². Although the contact angle of the hydrophobic membrane decreased from $134 \pm 4^\circ$ to $32 \pm 6^\circ$ due to deposition of foulant layer, no membrane wetting occurred. The hydrophilic layer grew on the membrane surface without changing pore hydrophobicity.

Frequency of membrane replacement

A decrease in membrane lifespan caused by the accumulation and persistence of biofilm development leads to frequent membrane replacements. This causes significant increases in operational costs.⁵ The frequency of membrane replacement largely depends on the type of feed characteristics and hydrodynamic conditions.⁶⁰ In desalination plants, membrane replacement occurs within 4–5 years whereas industries treating dairy products replace membranes within 3 years.⁶⁰ In general, membrane replacements are not recommended due to interruptions in the process and significant amount of labour requirements.⁶¹

Mitigation of biofouling in MD

Major strategies used to mitigate biofouling in MD include pre-treatment of the feed solution, membrane modification and cleaning (Fig. 5). Detailed impact of these strategies towards water flux and salt rejection stabilities is presented in Table 3.

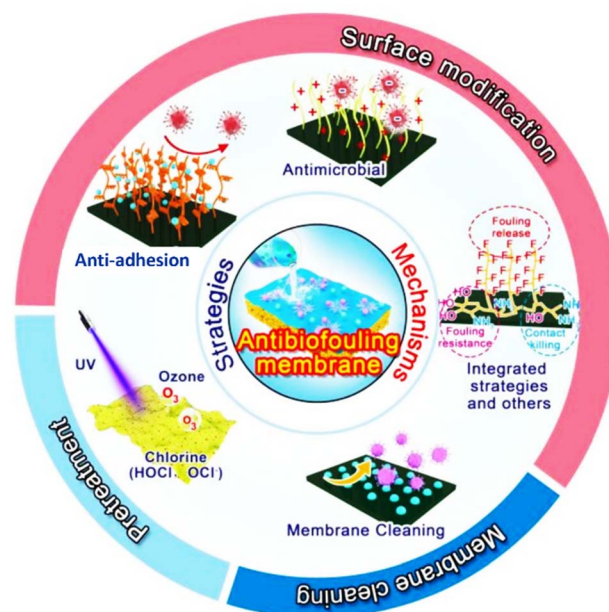


Fig. 5 Latest developments of biofouling control strategies in MD.⁶²

Pre-treatment of the feed solution

Pre-treatment of the feed solution is a useful tool to minimize biofouling. This involves removal of potential foulants from the feed solution before MD processing. Pre-treated feed improves resistance to flux decay and reduces frequency of membrane cleaning.^{36,62} Pre-treatment methods are largely dependent on the composition of the feed solution.⁶⁴ There are three types of pre-treatment methods widely applied in membrane-based technologies. These are physical, chemical and hybrid pre-treatment processes.

Physicochemical pre-treatment processes. Physical and chemical pre-treatment processes include filtration (ultra/micro), sonication, coagulation, and chlorination, amongst others. Ultrafiltration (UF) and microfiltration (MF) remove foulants from the feed solution using size-exclusion mechanism. The MF and UF minimize nutrients required for microbial growth. The UF was reported to effectively remove algal cells (96%) and non-algal cells (98%) from the feed solution in a seawater reverse osmosis (SWRO) plant in Saudi Arabia.⁶⁵ It is worth noting that planktonic cells passing through UF reproduce, thus enhancing biofilm development.⁶⁶ For example, Zodrow *et al.*, (2014)⁵² evaluated MF pre-treatment of RO and MD processes. Notably, inefficient removal of organic and biological matter from the feed stream caused blockage of membrane pores, thus a decline in water flux within 12 h of operation. Pre-treatment processes are currently evaluated for MD systems with limited literature reported.⁵⁶

Alternatively, sonication (with ultrasound frequencies of ≥ 18 kHz) is strategically used to manage bacterial growth.⁶⁷ Fouling is controlled through cavitation, where production of strong convective currents is triggered.⁶⁸ However, this pre-treatment process is rarely reported. Mathieu *et al.* (2019)⁶⁹ applied ultrasound (frequency of 46 kHz) to inhibit bacterial



Table 3 Mitigation strategies of biofouling in MD^a

Membrane configuration	Mitigation strategy	Mitigation process	Feed solution	Process duration	Impact on water flux and salt rejection	Change in flux and salt rejection	Ref.
DCMD	Pre-treatment	Chlorination (addition of HCl in feed stream)	Tap-water	58 days	Stable water flux and salt rejection	$J_I = 19.8 \text{ L m}^{-2} \text{ h}^{-1}$ $J_F = 19.5 \text{ L m}^{-2} \text{ h}^{-1}$ $k_I = 3 \mu\text{S cm}^{-1}$ $k_F = 10 \mu\text{S cm}^{-1}$	26
DCMD	Pre-treatment and membrane cleaning	Magnetic coagulation and HCl cleaning	Wastewater	65 days	97% flux and salt rejection were restored after cleaning (at high HCl concentration)	$J_I = 19.2 \text{ L m}^{-2} \text{ h}^{-1}$ $J_F = 17.7 \text{ L m}^{-2} \text{ h}^{-1}$ $R_I = 99.5\%$ $R_F = 98\%$	32
DCMD	Pre-treatment	Microfiltration	Estuarine water	4 days	50% decline in flux	$J_I = 20 \text{ L m}^{-2} \text{ h}^{-1}$ $J_F = 11 \text{ L m}^{-2} \text{ h}^{-1}$	52
DCMD	Membrane cleaning	NaOH, distilled water, 70% ethanol cleaning	Coastal seawater	14 days	Original flux was restored with minimal increase in distillate conductivity	$J_I = 3.9 \text{ L m}^{-2} \text{ h}^{-1}$ $J_F = 3.8 \text{ L m}^{-2} \text{ h}^{-1}$ $k_I = 6 \mu\text{S cm}^{-1}$ $k_F = 34 \mu\text{S cm}^{-1}$	24
AGMD	Backwash	Reversion of direction of flow	Pond water	91 days	Original flux was restored	$J_I = 0.8 \text{ kg m}^{-2} \text{ h}^{-1}$ $J_F = 0.7 \text{ kg m}^{-2} \text{ h}^{-1}$	97
DCMD	Membrane modification	Coating membrane with hydrophilic active layer	Effluent water	2.5 days	47% flux decline and slight changes in salt rejection	$J_I = 13.1 \text{ L m}^{-2} \text{ h}^{-1}$ $J_F = 6.8 \text{ L m}^{-2} \text{ h}^{-1}$ $R_I = 99.8\%$ $R_F = 97.7\%$	56
DCMD	Membrane modification	Membrane modification (f-MWCNTs and AgNPs)	Scheldt estuary water	2 days	20.8% flux decline with minimal salt rejection decay	$J_I = 37.1 \text{ L m}^{-2} \text{ h}^{-1}$ $J_F = 95.4 \text{ L m}^{-2} \text{ h}^{-1}$ $R_I = 99.99\%$ $R_F = 95.4\%$	88
DCMD	Membrane modification	Membrane modification (f-MWCNTs and AgNPs)	Effluent water	2 days	Stable flux and salt rejection	$J_I = 16.7 \text{ L m}^{-2} \text{ h}^{-1}$ $J_F = 15.2 \text{ L m}^{-2} \text{ h}^{-1}$ $R_I = 94.8\%$ $R_F = 94.6\%$	85

^a J_I and J_F are initial and final water flux, k_I and k_F are initial and final permeate conductivity, R_I and R_F are initial and final salt rejection respectively.

growth in bulk drinking water "N" spiked with 100 mg L^{-1} of Ca(OH)_2 . Evidently, a 7-fold reduction in bacterial growth was reported. Ultraviolet (UV) treatment is another alternative, where hydroxyl radicals are produced to inhibit bacterial growth.⁴⁴ During UV pre-treatment, bacterial DNA is broken down while proteins are effectively denatured.⁴⁴ However, UV is costly, thus limiting its application in MD water purification.⁷⁹ Due to prevention of UV rays through the feed solution by contaminants, UV treatment is limited to feed turbidity. Generally, wavelength of 200–400 nm is required to initiate cell death.⁶ To minimize reactivation of microbial cells, UV is applied in conjunction with other mitigation strategies.

Coagulation, chlorine and ozone injection are some of the chemical processes used during pre-treatment. Coagulation is a cost-effective and most convenient process, involving stabilization of particulates through the addition of a coagulant.⁷¹ Inorganic coagulants include ferric chloride (FeCl_3), aluminium chloride (AlCl_3) and polymeric aluminium chloride (PAC) whereas organic coagulants include polyacrylamide (PAM), polydimethyl diallyl ammonium chloride (PDMDAAC) and microbial flocculants.^{72,73} Since higher doses of inorganic flocculants are known to cause secondary pollution due to the presence of residual metal ions, a hybrid system of organic and inorganic coagulants is used to improve process performance. Zhang *et al.* (2022)⁶³ used a hybrid system to evaluate the performance of microbial flocculants modified with PAC (MMF/PAC) to

minimize membrane fouling. Cake layer formation on the membrane surface was effectively minimized. Management of biofilm development has been predominantly realized through chlorine dosing.⁶ Although chlorination of the feed stream effectively prevents biofilm development, it produces harmful mutagenic and carcinogenic by-products.⁷⁴ Moreover, chlorination damages polymeric membranes due to its high oxidizing ability.⁶³ Spore-forming microbes such as *Bacillus* are reported to resist chlorination, thus making pre-treatment ineffective.⁴⁴ Also, ozone (O_3) is used to control the growth of bacterial cells in MD systems. Owing to its high oxidizing properties, ozone inactivates viruses, bacteria and organic contaminants.⁴⁴ Yong Zhang *et al.* (2016)⁷⁵ coupled a DCMD system with ozone injection to treat organic pollutants from wastewater. 49% flux recovery was attained. Although ozonation minimizes membrane fouling, its application is limited to process costs.⁶ Similarly, ozone is insoluble at high temperature, thus making it unsuitable for MD applications. Other oxidative chemicals used to reduce biofouling include peracetic acid and hydrogen peroxide.

Hybrid pre-treatment processes. The complete removal of microorganisms require hybrid rather than a standalone process. Therefore, a combination of these processes can be chosen from a wide range including ozonation, chlorination and UV irradiation. Stand-alone processes portray a certain level of limitation, hence the need for hybridization. Chen *et al.*



(2022)⁷⁶ evaluated a combination of UV and chlorine to mitigate the growth of *Staphylococcus aureus*. Notably, the generation of free radicals such as OH[•] and Cl[•] minimized bacterial growth and inactivated the photo-reactivation of the bacteria. In another study, O₃, UV/O₃ and UV-assisted peroxidation (UV/H₂O₂) were used to pre-treat the feed solution (wastewater) in DCMD systems (Kumar *et al.* 2020).⁷⁷ A combination of UV/O₃ and UV/H₂O₂ achieved a 99% and 53% bacterial removal efficiency, respectively. Improved performance of UV/H₂O₂ relative to stand-alone UV was attributed to the cleavage of H₂O₂ under UV irradiation to produce hydroxyl radicals responsible for destruction of bacterial cells. Notably, UV/H₂O₂ is recommended for pre-treatment of feed solutions characterized by low dissolved organic carbon (DOC).

Membrane cleaning

Membrane cleaning involves removal of microbial accumulates from membrane surfaces. The concept of cleaning was introduced to restore the original performance of the fouled membranes.⁶ Furthermore, membrane cleaning reduces frequency of membrane replacements.⁷⁸ There are two types of cleaning methods widely applied in membrane-based technologies: (1) physical cleaning and (2) chemical cleaning.

Physical cleaning. Physical cleaning involves the use of deionized water during MD operation to facilitate foulant detachment.¹⁵ The use of water guarantees minimal damage to the membrane structure. During cleaning, the feed solution is pumped into the module, followed by deionised water through the system at higher flowrates. These flowrates ensure increased shear forces to enable detachment of foulants from the membrane surface and spacers.²⁷ In other instances, high pressure water is utilized.⁶⁷ Lyly *et al.* (2021)⁷⁹ used deionised water to restore the performance of PTFE membranes during treatment of synthetic seawater spiked with BSA and microalgae (*Cylindrotheca fusiformis*). The following steps were implemented for physical cleaning: (1) flow of pure cold-water (at 25 °C) for 10 min, followed by (2) hot water (at 60 °C) for 10 min and finally (3) cold-water for 5 min. Reportedly, 62.69% of water flux was restored, displaying promising results for future applications.

Chemical cleaning. Chemical cleaning involves the use of chemicals to continuously remove foulants from the membrane surface. To minimize membrane damage, frequency and concentration of the cleaning agent should be optimized.⁶⁴ Dominating chemical cleaning involves removal of inorganic and organic foulants using acidic and basic solutions respectively with limited information on biofouling treatment.⁸⁰ Zheng *et al.*, (2022)³² used HCl and water to remove microbial accumulates from the membrane surface. The process ensured complete removal of Euryarchaeota. However, full restoration of the flux was not achieved, largely due to *Idiomarina* cells' affinity for the membrane surface. Krivorot *et al.*, (2011)²⁴ investigated the removal of biofoulants from the membrane surface through flushing with a series of steps: (1) NaOH (pH 12 at 40 °C), (2) distilled water, and (3) 70% ethanol. Notably, the original flux of the membrane was fully restored. To minimize cleaning

requirements, modification of the membranes is recommended to render them resistant to biofouling (Bogler *et al.*, 2017).²⁷ This approach ensures minimal waste disposal, thus ensuring environmental safety.⁸¹

Membrane modification

Modification involves the alteration of membrane properties to render them resistant to biofouling. Membranes are modified through various approaches; including incorporation of metal nanoparticles (MNPs) (Fig. 6) and alteration of membrane surface properties such as surface roughness, membrane hydrophobicity and surface charge.^{59,82}

Incorporation of MNPs. Attachment and proliferation of microbial cells on membrane surfaces is largely minimized through the incorporation of biocidal MNPs. Upon oxidation, ionic counterparts of biocidal MNPs are released to react with microbial cells. Biocidal MNPs capable of inactivating bacterial cells include Ag, Ti, Zn and Fe.^{15,83} Inactivation occurs due to the electrostatic interaction between positively charged metal ions and negatively charged thiol groups on bacterial DNA.⁸⁴ Nthunya *et al.*, (2020)⁸⁵ modified polyvinylidene fluoride (PVDF) membrane using silver nanoparticles (AgNPs) and functionalized carbon nanotubes (f-MWCNTs), achieving successful biofouling, colloidal and organic fouling control in MD. Although fouling mitigation is evident, biocidal MNPs tend to leach from the membrane surface, thus reducing fouling control.²⁷ In addition, leaching of MNPs reduces the quality of produced water. Hence, ensuring the stability of these MNPs on membrane surfaces is crucial.

Alteration of membrane surface properties. Rendering MD membranes superhydrophobic is a useful approach to mitigate biofouling, where the surface energy of the membrane is reduced.⁸⁵ Ideally, superhydrophobic membranes are characterized by contact angles $\geq 150^\circ$. Teoh *et al.* (2022)⁸⁶ enhanced the surface hydrophobicity of PVDF membranes using nylon taffeta substrate for MD treatment of aquaculture wastewater. Self-cleaning microtextured membrane was characterized by water contact angle of 153° and a sliding angle of 8.9° . Notably, the water flux ($30 \text{ kg m}^{-2} \text{ h}^{-1}$) remained stable. Also, hydrophilic coating of hydrophobic membranes is reported to minimize biofouling. This is achieved through fabrication of Janus membranes. During the process, membranes of asymmetric wettabilities, *i.e.* hydrophobic/hydrophilic combinations are produced.²⁷ Yang *et al.* (2010)⁸⁷ grafted zwitterionic poly-sulfobetaine methacrylate (polySBMA) to polypropylene (PP) membrane. Water contact angle of the active site was reduced from 145° to 15° . Reportedly, grafting density of $560 \mu\text{g cm}^{-2}$ provided resistance to bacterial adhesion. This was attributed to a decrease in interaction between hydrophilic bacteria (*E. coli*) and membrane active surface. Furthermore, hydrophobic membranes are coated with a thin layer containing hydrophilic MWCNTs and AgNPs to reduce combined fouling of organic, inorganic and biofoulants. While uncoated membranes face flux decays of approximately 90% and salt rejection decays of 6%, hydrophilic coating of membranes minimized flux and salt rejection decays to 24% and 0.75% respectively. Therefore,





Fig. 6 Membrane modification using MNPs.

hydrophilic modification of membranes maintains process resistance to fouling.⁸⁸

Other biofouling mitigation strategies

Other techniques used to control biofouling of MD systems include air-sparging (bubbling), quorum quenching (QQ), nitric oxide (NO_x) injection and alteration of hydrodynamic conditions. The NO_x enhances biofilm dispersal by decreasing 60% of membrane biofilm coverage.⁸⁹ However, due to NO_x instability and insolubility, their application in water treatment is minimal. The QQ involves the use of quorum quenching enzymes to deactivate signalling molecules required for biofilm development.⁶ The quorum sensing system regulates the communication and aggregation of bacterial species. Its suppression assists in managing biofouling in membrane systems. Gram-negative and Gram-positive bacteria use signalling molecules such as *N*-acyl-homoserine lactones (AHLs) and autoinducing peptides (AIPs) to promote biofilm development and successions.⁹⁰ To minimise these developments, QQ enzymes such as AHLs lactases damage the lactone rings of the cells. This reduces effective communication between cells, thus slowing biofilm progression.^{47,91,92} Air-sparging involves the bubbling of air in the feed stream to increase flow rate, shear stress and turbulence, thus preventing membrane clogging.²⁷ Chang *et al.* (2021)⁹³ studied air bubbling to minimize biofilm development. Reportedly, membrane deposition of inorganic, organic and microalgae was significantly reduced leading to a recovery of 16.95% water flux. This was attributed to an increase in turbulence flow at the feed-membrane interface. Comparatively, shear turbulence of gas bubbling removes foulants better than shear turbulence of feed flowrates.⁹⁴ However, the increase in air-bubbling beyond a certain threshold reduces the permeate flux.⁹⁵ Therefore, optimization of air-water ratio is imperative.²⁷ Lastly, hydrodynamic conditions affect MD process performance. Silva *et al.* (2018)⁹⁶ evaluated different operating conditions such as solute concentration, flowrates, and different membrane modules on process performance.

Reportedly, water flux was higher in a perpendicular flow (W-cell) of the feed to the membrane compared to a parallel flow (H-cell). This was ascribed to a decrease in temperature and concentration polarization. Remarkably, feed flowrate should be optimised to maintain high water flux while simultaneously preventing biofouling. According to Zheng *et al.* (2022)³² bacterial cells got thinner and longer as feed flow passed through the membrane. The inlet was less fouled by *Chelativorans*, *Acinetobater*, and *Sphingobium*. On one hand, temperature tolerant bacteria such as *Idiomarina* and *Phenylobacterium* were detected in abundance at the inlet where process velocity is high. Although a high feed flowrate is desired to minimize the feed boundary layer through high shear stress,⁹⁶ caution must be exercised to minimize bacterial succession and abundance.

Conclusions and future outlook

MD remains a promising technology to purify high saline wastewater and seawater. However, hydrophobic membranes used in MD are susceptible to biofouling, a process involving the deposition and accumulation of microorganisms. Requiring nutrient availability to mature into a biofilm, these microorganisms largely originate from the feed solution. Several factors affecting microbial deposition include membrane properties, hydrodynamic conditions, feed solution properties and membrane module designs. Mitigation of biofouling is essential as it affects membrane lifespan, increases operational costs, and diminishes permeate water quality. Moreover, large scale application of MD has been hindered by fouling, amongst other factors. Various approaches minimizing biofouling are proposed. These include pre-treatment of the feed solution, membrane cleaning, and metal nanoparticle and surface alteration-based modification. Current reported studies largely focus on the characterization of the microbial community within the biofilm structure. Although this is essential, research work focusing on different approaches to mitigate biofouling is essential. Moreover, various module designs need to be



explored. To establish the sustainable application of MD systems at pilot scale, fouling experiments should be carried over long operating periods (600 h and more). Elucidating cell-to-surface interactions using various mathematical models to simulate real operating conditions requires experimental attention. Directing efforts toward lessening attachment will ensure minimal EPS secretion and hinder biofilm progression. Optimization of MNPs concentration on membranes to determine a balance between optimum activity over long periods and the rate of leachability is required. Indeed, membrane biofouling in MD requires significant experimental work to mitigate the existing challenges.

Author contributions

Tshepiso Mpala investigated, wrote the manuscript draft, and analysed, Lebea Nthunya, Anita Etale, Heidi Richards conceptualized, validated manuscript and curated data as well as supervised the study.

Conflicts of interest

Authors declare no conflict of interest.

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