THE UNIVERSITY *of* EDINBURGH

Edinburgh Research Explorer

Osmotic backwashing of forward osmosis membranes to detach adhered bacteria and mitigate biofouling

Citation for published version:

Daly, S, Casey, E & Correia Semiao, A 2020, 'Osmotic backwashing of forward osmosis membranes to detach adhered bacteria and mitigate biofouling', *Journal of Membrane Science*.
<https://doi.org/10.1016/j.memsci.2020.118838>

Digital Object Identifier (DOI):

[10.1016/j.memsci.2020.118838](https://doi.org/10.1016/j.memsci.2020.118838)

Link:

[Link to publication record in Edinburgh Research Explorer](#)

Document Version:

Peer reviewed version

Published In:

Journal of Membrane Science

General rights

Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy

The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.

**Osmotic backwashing of forward osmosis membranes to detach
adhered bacteria and mitigate biofouling**

Sorcha Daly^a, Eoin Casey^c, Andrea JC Semião^{b*}

Journal of Membrane Science

^a Formerly School of Engineering, University of Edinburgh. Currently School of Chemical and
Bioprocess Engineering, University College Dublin (UCD), Belfield, Dublin 4, Ireland

^b School of Engineering, University of Edinburgh, Edinburgh EH9 3JL, UK

^c School of Chemical and Bioprocess Engineering, University College Dublin (UCD), Belfield, Dublin
4, Ireland

*corresponding author: Andrea.Semiao@ed.c.uk

Keywords: bacterial adhesion, forward osmosis, osmotic backwashing flux, calcium

Abstract

The efficiency of osmotic backwashing cleaning to remove bacteria from forward osmosis membranes was systematically studied for the first time under different attachment and osmotic backwashing conditions. It is hypothesised that biofouling is preventable when tackling initial adhesion, i.e. during the reversible stage. Cell removal from the membrane was dependent on both adhesion and backwashing conditions: tests were performed for backwashing solutions of different concentrations and salt type, as well as different filtration durations and Ca^{2+} concentrations in the feed solution. Following adhesion of *P. putida*, a backwashing draw solution (DS_{obw}) of 3 M NaCl was the most efficient, removing 93% of the adhered cells after 1 minute of backwashing. All adhered cells left on the membrane were dead/injured due to osmotic shock. To optimise the cleaning regime, the maximum filtration time for which backwashing is efficient must be determined. This was determined to be 30 minutes, after which backwashing became inefficient, only removing 78% of cells. The addition of 5 mM Ca^{2+} to the feed caused a 50% increase in cell surface coverage compared to adhesion without Ca^{2+} . This increase in adhesion rendered backwashing inefficient, as cell removal was only 60%. To increase backwashing efficiency by increasing the backwashing flux, DS_{obw} with CaCl_2 were used. However, this was inefficient due to interactions between Ca^{2+} in the DS_{obw} and the adhered cells, even for just 1 minute: for a $55.8 \text{ L.h}^{-1}\text{m}^{-2}$ flux, 39% of removal was obtained for a 3 M CaCl_2 DS_{obw} when compared to 93% removal for 3 M NaCl for a $36 \text{ L.h}^{-1}\text{m}^{-2}$ flux. Therefore, both adhesion and backwashing conditions are important for cleaning of FO membranes.

1. Introduction

Water shortages across the world have left 30% of the population without basic drinking water [1], and by 2025, 60% of the population is predicted to live in water stressed areas [2]. Membrane technology is used to successfully tackle this problem, and indirect desalination has been recently proposed as a low energy process through the coupling of forward osmosis (FO) and reverse osmosis (RO) processes [3, 4]. In indirect desalination, FO membranes are used for wastewater reclamation, where clean water permeates from a wastewater feed side (FS) of low osmolarity, to a seawater draw side (DS) of high osmolarity, using the natural process of osmosis. This leads to a diluted seawater DS, which can be subsequently desalinated by a low-pressure RO step [5-8]. Encouraging results have been reported in the literature in the application of FO for wastewater reclamation [9-12].

However, membrane processes, such as FO, have been shown to suffer from fouling, including biofouling [13-17]. Studies on biofouling and biofouling mitigation on FO membranes are very scarce, with most studies focusing on RO membranes. Biofouling formation on membranes and surfaces, in general, occurs in two stages [18, 19]: firstly bacteria adhere to a surface in what is considered a reversible process [20, 21], followed by biofilm development, where the community of adhered cells become enclosed in a 3D matrix of excreted exopolymeric substances (EPS) made up of polysaccharides, proteins and other compounds [22]. The biofilm, which is considered to be an irreversible phase, protects the microorganisms from the surrounding environment [20]. Biofilms coat membrane surfaces, resulting in increased flux resistance and reduced permeate flux and permeate quality [18], which are exacerbated by biofilm enhanced concentration polarisation [16, 23]. Kwan et al. [16] and Herzberg and Elimelech [23], reported a 30% and 80% decrease in RO membrane flux due to biofouling, respectively, and Kwan et al. [16] and Yoon et al. [17] reported up to 20% flux decline for biofouled FO membranes. Furthermore, a 4-fold increase in pressure drop along an RO membrane has been measured due to biofouling formation [24]. This increases energy demand and eventually damages the membranes and the membrane modules beyond usability. The fight against biofouling of RO membranes in a water purification plant in the US has been estimated to be 30% of the total operating costs [25]. Due to the issues caused by biofouling, efficient mitigation strategies need to be researched for membrane processes.

Several strategies have been attempted at dealing with biofouling, which include addition of bubbly flow to the feed solution [26], limiting phosphate in the feed solution [27], membrane modification [28-32], and chemical and physical cleaning [17, 33]. Kim et al. [27] studied limitation of phosphate in FS to reduce biofouling in FO. This resulted in a decrease in biofouling, illustrated by flux measurements. However, the flux still decreased, indicating that biofouling still occurs, albeit to a

lesser extent. The method of membrane modification to reduce biofouling has been recently explored for FO membranes [28, 32]. Perreault et al. [32] used graphene oxide functionalised TFC membranes to control biofouling. The membranes were effective in reducing attachment of *P. aeruginosa* by 36% and cell viability by 30% compared to unmodified membranes, through an increase in membrane surface hydrophilicity. Similarly, Faria et al. [28] studied biofouling control of TFC FO membranes functionalised with graphene oxide-silver nanocomposites. Biofouling of the unmodified membrane with *P. aeruginosa* resulted in a 50% reduction in flux, as opposed to membranes modified with graphene oxide-silver nanocomposites, which had less than 10% flux reduction. Furthermore, the average biofilm thickness was decreased by 45% in the membranes modified with the nanocomposites. These results are encouraging, however, even with membrane surface modification, biofouling cannot be completely eliminated, [31, 34] and therefore the need for an efficient cleaning method is necessary via chemical or physical cleaning, in order to manage biofilm formation.

Li et al. [35] studied chlorine cleaning of aquaporin based FO membranes, using NaOCl, which caused amide CN bond hydrolysis, making the membrane more hydrophilic. This has the potential to reduce cell adhesion as the more hydrophilic the membrane is, the lower the bacterial adhesion can be expected [36]. Although cleaning with chlorine could potentially be an effective way to mitigate biofouling, as it also kills bacteria, chlorine has been shown to damage the polyamide selective layer of FO and RO membranes, therefore affecting membrane performance and reducing its life [37, 38]. Chlorine is hence not an optional cleaning agent for polyamide based FO membranes. A recent study looking at chlorine resistant RO membranes showed that chlorination did not actually prevent biofouling formation [33]. Valladares et al. [37] used an Alconox and ETDA mixture to increase fouling reversibility up to 93.6% in FO for treatment of secondary wastewater. However, Wang et al. [39] obtained an increase in water flux and solute flux when cleaning FO membranes with NaOH, SDS and/or Alconox, where the latter caused a solute flux increase by a factor of 3. Furthermore, these chemicals are damaging to the environment as they are toxic to aquatic life [40], hence other cleaning processes should be explored.

Physical cleaning to remove biofouling from membranes has also been attempted. Zhang et al. [41] obtained > 45% flux decline due to biofouling formation on FO membranes in a bioreactor. Tap water rinsing at 15 minute intervals, which created shear stress across the membrane surface, was applied. However, after approximately 70 hours, this method was no longer effective at removing the biofilm.

Osmotic backwashing has been examined as a promising cleaning method for RO [42-49] and FO membranes [10, 11, 17, 50, 51]. Osmotic backwashing in RO and FO involves reversing the flow of

water through the membrane by replacing the feed solution with a solution of high ionic strength, for example seawater or RO brine, and replacing the permeate or draw solution with a solution of low ionic strength. Bar-Zeev and Elimelech [52] applied osmotic backwashing to RO biofouled membranes with a 60 second pulse of 1.5 M NaCl, which restored up to 70% of the initial flux. It also reduced the number of viable microorganisms by osmotic shock in reaction to the hypersaline solution [53]. However, due to its structural complexity, only 74% of the biofilm was removed. Yoon et al. [17] used increased crossflow rate from 4 to 33 cm.s⁻¹ and induced osmotic backwashing by replacing the FS with 4 M NaCl and the DS with deionised water in biofouled FO membranes, but neither of these methods restored the flux, showing that biofilm is very difficult to remove. This is even the case for FO, where organic fouling and biofouling have been shown to be less irreversible when compared to RO processes, due to the lack of hydraulic pressure applied [16, 54]. Lee et al. [54] describe the FO fouling layer as less compact and cohesive compared to the layer formed during RO. Kwan et al. [16] described biofouling layers in FO as loosely formed compared to RO biofouling layers which they describe as being “tightly organized” due to the applied pressure.

One of the potential reasons why the mentioned physical cleaning methods were inefficient was because they were applied when the biofilm was mature and irreversibly formed, as opposed to applying them during the reversible adhesion stages. Creber et al. [55] showed that the earlier the chemical cleaning was carried out in biofouled RO membranes, the more efficient the cleaning was. The evolution in adhesion stages has been measured with an Atomic Force Microscope: bacterial adhesion forces were found to increase with time from -1 nN to -5 nN [56, 57], showing a dynamic system in place, evolving from reversible to irreversible phases. Furthermore, the change between reversible bacterial adhesion to irreversible adhesion and early biofilm development has been determined to vary between 4 to 10 hours for RO membranes [58]. The more time the bacteria are allowed to adhere and the more mature the biofilm becomes, the more resilient it will be to cleaning. Hence, applying the cleaning method during the reversible bacterial adhesion stage and before the biofilm has a chance to develop, may effectively control the extent of biofouling development.

Of the cleaning methods studied in the literature, osmotic backwashing minimises operational downtime, provides shear force tangential and perpendicular to the membrane surface and does not require the use of potentially damaging chemicals, hence it has great promise. However, bacterial adhesion forces depend on bacteria-membrane interactions and filtration conditions adopted including membrane operational parameters, bacteria and membrane properties, and water quality such as the presence of dissolved organic matter on the feed water [21, 34, 59]. These parameters will influence bacterial adhesion, which will in turn influence osmotic backwashing efficiency. The aim of this study is hence to examine at a fundamental level the efficiency of osmotic backwashing as a

cleaning method in removing adhered bacteria from aquaporin based FO membranes, by studying these different parameters.

2. Materials and Methods

2.1 Forward Osmosis Membrane

For all experiments, a commercial aquaporin based membrane was used (Aquaporin Inside™, Denmark). This is a thin-film composite membrane consisting of a polyamide active layer containing aquaporin proteins, an intermediary polyester layer, and a non-woven polyester support layer. Aquaporin proteins are water channel forming proteins added to the membrane to enhance its flux [60]. The membrane is approximately 110 µm thick. The membrane was stored wet in MilliQwater (Avidity, UK) at 4°C, and before use, it was gently washed with MilliQ water and cut to fit the membrane crossflow cell.

2.2 Model Bacteria Strain and Media

Green fluorescent protein expressing *Pseudomonas putida* (PCL1482 eGFP) was the bacterial strain used for the membrane adhesion experiments. Cultures were obtained by inoculating 100 mL King's B broth supplemented with tetracycline at a final concentration of 10 µg.ml⁻¹ using single colonies grown on King B agar at 28 °C. The culture was incubated at 28 °C with shaking at 75 rpm and left to grow to mid exponential stage, corresponding to optical densities of 0.8. Next, the culture was centrifuged at 5000 rpm for 10 minutes and the bacteria were added to 200 ml of 0.1 M NaCl which was then added to the fouling solution.

2.3 Fouling Solution

The fouling feed solution consisted of *P. putida* in a background electrolyte solution of 0.1 M NaCl (Fisher Scientific, UK). This solution was used in order to maintain the cells' viability and protect them from osmotic stress [61]. Concentrations of CaCl₂ (Sigma-Aldrich, UK) ranging from 0 to 5 mM were also added to the fouling solution for some experiments to see the effect Ca²⁺ ions have on adhesion and osmotic backwashing efficiency. The 200 ml bacterial culture volume was added to 1 L of the fouling feed solution, where the final concentration of *P. putida* in the FS_f was 10⁷ cells.ml⁻¹. For every experiment, an initial volume of 1 L DS of 0.7 M NaCl was used during the adhesion stage, to mimic seawater [5, 11, 62].

2.4 Forward Osmosis Bench Scale Crossflow System

Experiments were carried out in a custom built forward osmosis bench scale rig as shown in Figure S1 in the Supporting Information. Briefly, two variable speed gear pumps delivered pulse free flow to two custom built Perspex membrane cells placed in parallel. Each membrane cell had an effective membrane area of 0.0048 m² (width 25 mm, length 191 mm) and the membrane was placed between two channels of 3 mm height each. Through a system of valves, the membrane cells were either fed by two reservoirs containing the fouling feed solution (FS_f) and the fouling draw solution (DS_f) during fouling, or fed by the osmotic backwashing feed solution (FS_{obw}) and the osmotic backwashing draw solution (DS_{obw}) during osmotic backwashing. Both DS_f and DS_{obw} were positioned on a balance (Ohaus, US), and the weight change was used to calculate the flux through the FO membrane.

2.4 Bacterial Adhesion Fouling protocol

The membranes were secured in their respective cells using double sided tape to guarantee they did not move in the cell during manipulation. For each bacterial adhesion fouling experiment, the active layer faced the FS_f and the support layer faced the DS_f, i.e. AL-FS mode. Firstly, the pure water flux (PWF) of the membranes was measured for 30 minutes for cell 1, followed by 30 minutes for cell 2, and then both cells in parallel. PWF testing was carried out with deionised water as the FS_f and 0.7 M NaCl as the DS_f.

Once the PWF was determined, the solutions in the FS_f and DS_f were substituted for the fouling ones, i.e. 0.1 M NaCl and 0.7 M NaCl, respectively. As previously mentioned, in some experiments, the FS_f was made up of 0.1 M NaCl and 2.5, 3.5, and 5 mM CaCl₂. The FS_f and DS_f were left recirculating in the crossflow system for 10 minutes at a crossflow rate of 1 L.min⁻¹ per membrane cell, in order to stabilize the system. Bacterial adhesion began when 200 ml of the bacteria culture was added to the FS_f tank. Unless otherwise stated, adhesion experiments lasted for 30 minutes.

Preliminary bacterial adhesion experiments were carried out on both membrane cells at the same time, to confirm the same surface coverage was obtained for both. This was observed to be the case, where the total surface coverage was 18.3% ± 4.7 and 20.7% ± 5.0 for cells 1 and 2, respectively.

2.5 Osmotic Backwashing Cleaning Protocol

Two membranes were fouled in parallel in AL-FS mode, but only one was osmotically backwashed in order to determine surface coverage before and after the cleaning method was applied. After fouling, the pumps were stopped, and the FS_f and DS_f reservoirs were replenished with 0.1 M NaCl to rinse the membrane cells and remove any unattached bacteria. Next, both gear pumps were stopped and the inlet and outlet valves of cell 2 (valves 1E, 1F, 2E, 2F in Figure S1) were closed to isolate the fouled membrane in cell 2. Osmotic backwashing was implemented for cell 1 immediately after bacterial adhesion, by switching the FS_f and DS_f reservoirs to the osmotic backwashing FS_{obw} and DS_{obw} reservoirs, using a series of valves as follows: closing valves 1A, 1B, 1G, 1H and opening valves 2A, 2B, 2G, 2H (Figure S1) and restarting the pumps at 0.5 L.min⁻¹ to feed membrane cell 1.

Osmotic backwashing was carried out in AL-DS mode. During osmotic backwashing, the DS_{obw} used ranged from 0.1 to 3 M NaCl or 0.5 to 3 M CaCl₂, depending on the experiment. A FS_{obw} of Milli Q water was used for every experiment. Osmotic backwashing was carried out for 1 minute, and the flux was measured by weighing the DS_{obw} tank (Ohaus, US). Although the higher range of DS_{obw} concentrations are potentially unfeasible for full-scale application, they were used to understand bacterial removal capacity at a more fundamental level and understand the potential of osmotic backwashing for cell removal from FO membranes.

After osmotic backwashing, the pumps were stopped, and the FS_f and DS_f reservoirs were replenished with 0.1 M NaCl to rinse cell 1 and remove any unattached bacteria. Subsequently, all valves to and from the membrane cells were closed and both membranes were removed from their cells and transferred to petri dishes, while completely submerged in 0.1 M NaCl. Each experiment was repeated at least twice, with the two most outermost data points (e.g. in Figure 2: osmotic backwashing with 0.1 M NaCl and 3 M NaCl) repeated at least 3 times.

After every experiment, the FO system was thoroughly rinsed with deionised water, followed by recirculation with 70% ethanol for 30 minutes and 0.1 M NaOH for another 30 minutes. Next, the system pH was neutralised by slowly adding 1 M HCl, measured using a pH probe (VWR, Germany). Finally, the system was thoroughly flushed with deionised water until the conductivity was < 1 µS.cm⁻¹.

2.6 Protocol for alginate detection

To detect the presence of EPS on the bacteria surface after osmotic backwashing with CaCl₂, experiments were performed with *P. putida* wild type in polystyrene 6 well plates. Cultures were obtained by inoculating 100 mL King's B broth supplemented using single colonies grown on King B

agar at 30 °C. The culture was incubated at 30 °C with shaking at 75 rpm and left to grow to mid exponential stage, corresponding to optical densities of 0.8. Next, the culture was centrifuged at 5000 rpm for 10 minutes and the bacteria were added to 200 ml of 0.1 M NaCl, which were then added to 1 L of 0.1 M NaCl solution. Next, 5 ml of bacterial solution was added to two 6 well plates. After 30 minutes of adhesion, the plates were rinsed with 0.1 M NaCl and then 0.1 M NaCl, 3 M NaCl or 3 M CaCl₂ were added to the well plate, depending on the experiment. After 20 minutes of contact time, the plates were again rinsed with 0.1 M NaCl before staining. For shorter contact times, EPS could not be detected.

2.7 Sample Staining

To visualise the dead/injured bacterial cells adhered to the membrane surface, the cells were stained with 4 ppm propidium iodide while submerged in petri dishes containing 0.1 M NaCl. They were then left to incubate for 15 minutes in the dark before the excess stain was removed by rinsing with 0.1 M NaCl, and the membrane samples were brought to the microscope. The membrane samples were submerged in a 0.1 M NaCl solution for the entire staining and imaging process.

To visualise EPS formed after contact with CaCl₂, the 6 well plates were stained with 20 µL of Calcofluor white stain and allowed to incubate in the dark for 15 minutes, after which excess stain was removed by rinsing with 0.1 M NaCl several times. Calcofluor white binds to β linkages of polysaccharides [63].

2.8 Microscopy Imaging

A Widefield Nikon TE2000 fluorescence microscope was used to image the membranes. Live cells were imaged in the green FITC filter while dead/injured cells were imaged in the Texas red filter. The magnification used was x40. Threshold analysis using Image J software was used to determine the total area of live and dead cells. At least 10 images of both live and dead cells were taken to determine the average surface layer coverage. Representative images of membranes containing live and dead/injured cells are shown in Figure S2. An Olympus BX51 microscope was used to image plates to detect the presence of alginate. Images were taken using a UV filter at x10 magnification and 10 images of each surface was taken.

3. Results and Discussion

3.1 Effect of DS_{obw} Concentration on Bacterial Detachment

The influence of the DS_{obw} concentration, hence osmotic backwashing flux, on the removal efficiency of adhered bacteria to FO membranes was studied. It is anticipated that flux reversal during osmotic backwashing will detach adhered bacteria from the membrane surface, thus preventing further biofouling from developing. Hydraulic backwashing is a known cleaning protocol applied for the removal of bacteria and biofouling from membrane bio-reactors [64, 65].

As can be seen in Figure 1, a 30 minute adhesion period resulted in an average membrane surface coverage of $17.9 \pm 7\%$ live cells and $0.98 \pm 0.94\%$ dead/injured cells. Reports into initial bioadhesion in FO membranes are very limited, with most studies focusing on anti-biofouling FO membranes [66, 67]. The high surface coverage shown in Figure 1 is not surprising due to the slight hydrophobic nature of the Aquaporin Inside™ membrane surface. Li and Logan [68] showed that a decrease in surface contact angle will result in a more hydrophilic surface and a decrease in cell adhesion. Habimana et al. [21] also showed in their review that increased hydrophobicity of NF and RO membrane surfaces generally increase bacterial adhesion. The active layer of the Aquaporin Inside™ FO membrane is borderline hydrophobic, with a contact angle of $96.2 \pm 5.5^\circ$ [69], when compared to other FO membranes such as the cellulose triacetate membrane by Hydration Technology Innovations with a contact angle of $62 \pm 7.2^\circ$ [69].

The surface coverage obtained during bacterial adhesion did not affect the flux, which remained at $16.2 \pm 1.8 \text{ L.h}^{-1}.\text{m}^2$ throughout the adhesion stage. A 30 minute filtration has no significant effect on the flux, since the fouling is not severe enough and the biofilm has not developed during this short time. Yoon et al. [17] biofouled FO membranes with *P. aeruginosa* and reported a negligible flux decline only after approximately 2 hours of biofouling. Similarly, Semião et al. [34] reported that 30 minutes of initial bacterial adhesion on NF and RO membranes had no effect on the permeate flux. This shows that initial adhesion of cells has no effect on FO flux during fouling, as the adhered bacteria offer very little resistance to flux and do not cause biofilm-enhanced concentration polarization [16, 23].

Figure 1 - Surface coverage (%) of live and dead/injured cells before and after osmotic backwashing for different DS_{ow} NaCl concentrations (fouling conditions: $DS_f = 0.7$ M NaCl, $FS_f = 0.1$ M NaCl containing 10^7 cells. ml^{-1} *P. putida* in 0.1 M NaCl, adhesion duration = 30 minutes; osmotic backwashing conditions: duration = 1 minute, FS_{ow} = deionised water; error bars show standard deviation for repeated experiments where the count was determined from 10 areas on the membrane surface)

In order to determine an optimal osmotic backwashing flux for the efficient removal of adhered bacteria from FO membranes, a DS_{ow} of NaCl concentrations varying between 0.1 and 3 M were tested. Increasing DS_{ow} concentrations resulted in increasing osmotic backwashing fluxes during cleaning, as shown in Table 1: fluxes varied between $8.7 \text{ L.h}^{-1}.\text{m}^{-2}$ for 0.1 M NaCl and $36 \text{ L.h}^{-1}.\text{m}^{-2}$ for 3 M NaCl.

Table 1 - Osmotic backwashing fluxes for DS_{ow} of varying NaCl concentrations

Concentration (M NaCl)	Osmotic Backwashing flux ($\text{L. h}^{-1}.\text{m}^{-2}$)
0.1	8.7 ± 1.6
0.7	20.2 ± 7.7
1.5	31.0 ± 2.1
2	30.3 ± 2.7
3	36.0 ± 5.0

As shown in Figure 1, as the adhered cells were exposed to DS_{obw} concentrations varying between 0.1 and 1.5 M NaCl, the surface coverage of total adhered cells remained relatively unchanged at $22\% \pm 6\%$, showing that the osmotic backwashing fluxes obtained up to $31 \text{ L.h}^{-1}.\text{m}^{-2}$ (see Table 1) were not sufficient to detach the bacteria (please see Figure S3 in the Supporting Information and related text for statistical analysis of adhered bacteria vs osmotic backwashing with 0.1 M NaCl). There was, however, a dramatic effect on the surface coverages of live vs dead/injured cells: whilst live cell surface coverage reduced from 21% down to $< 1\%$, the dead/injured cells surface coverage increased from 7.9% for a DS_{obw} of 0.1 M NaCl to 19.2% for a DS_{obw} of 1.5 M NaCl. This shows that osmotic backwashing had a severe effect on the viability of the adhered bacteria, which became more compromised for higher concentrations of DS_{obw} , or for higher osmotic backwashing fluxes. This could be caused by two factors. Firstly, bacteria that experience a spike of high salinity undergo osmotic stress [52], as a response to adapt to changes to their external environment in order to continue to function [70]. Katebian and Jiang [53] used 0.55 M of NaCl to induce hyperosmotic stress on *Shewanella sp.* biofilm producing bacteria adhered to filter cartridges used in desalination, resulting in greater than 99.5% mortality rate.

The other factor that could have contributed to affecting bacterial viability was the reversal of flux direction from the fouling stage to the osmotic backwashing stage. Habimana et al. [36] showed that shear stress through increase in permeate flux can have a significant effect on the viability of bacterial cells adhered to NF and RO membranes. The increase in flux reversal due to an increase in DS_{obw} could also have contributed to the increased surface coverage of dead/injured cells from 7.9% for a DS_{obw} of 0.1 M NaCl to 19.2% for a DS_{obw} of 1.5 M NaCl. Although the bacteria are not detached from the membrane surface, their compromise in viability translates to biofouling development being avoided, as dead bacteria cannot produce EPS [23]. The results are potentially the combination of both osmotic shock and shear stress.

The remaining dead/injured cells may, however, still have negative consequences during subsequent biofouling cycles, by working as a scaffold for further bacteria to adhere. Furthermore, Kwan et al. [16] showed that, albeit to a lesser extent than with RO, biofouling enhances concentration polarisation and resistance to permeation in FO, resulting in a reduction of flux. Therefore, an efficient osmotic backwashing method should ideally aim at removing all adhered cells from the membrane surface.

As the DS_{obw} concentration increased from 1.5 M to 3 M NaCl, the osmotic backwashing flux increased from 31 to $36 \text{ L.h}^{-1}.\text{m}^{-2}$ (see Table 1). This resulted in increased removal rates of 0% and 93%,

respectively. The higher permeate drag force for higher osmotic backwashing fluxes, combined with compromise of bacteria viability, contributed to detaching the bacteria from the membrane surface.

From Figure 1, osmotic backwashing with a DS_{obw} of 3 M NaCl, generating $36 \text{ L.h}^{-1}.\text{m}^{-2}$ flux, was deemed the most efficient cleaning method, with a 93% removal of adhered bacteria from the FO membrane surface. Therefore this osmotic backwashing solution was used for further experiments.

3.2 Effect of Ca^{2+} in the FS_f on adhesion and osmotic backwashing efficiency

Feed characteristics have an important effect on the physical, chemical and biological factors affecting bacterial adhesion [71]. Divalent ions, such as Ca^{2+} ions, are known to increase adhesion due to both compression of the electric double layer and ion bridging between the cell and membrane surface [72]. Therefore, varying Ca^{2+} concentration in FS_f is expected to affect bacterial adhesion and hence affect osmotic backwashing efficiency.

Bacterial adhesion was carried out for 30 min with varying concentrations of Ca^{2+} in the FS_f . As the Ca^{2+} concentration in the FS_f increased from 0 to 5 mM, the total surface coverage increased by 33%: the addition of 5 mM Ca^{2+} resulted in an increase in total surface coverage from 18.9% to 28.2%, when compared to adhesion in the absence of Ca^{2+} . Of this total cell surface coverage, a high proportion, 17.9% and 26.8%, respectively, were live cells (see Figure 2). Statistical significance of differences between values in Figure 3 were tested using 2-sample t tests in MINITAB software version 18. All tests were performed at 5% significance level. The difference in values obtained for feed solutions containing 3 mM and 5 mM NaCl are not statistically significant ($P = 0.106, 0.975, 0.399$ for adhered live, adhered dead and backwashed dead values).

The different factors affecting cell adhesion on the membrane surface in this case are the type of salt (monovalent or divalent) and the specific influence of Ca^{2+} on bacterial adhesion. Despite increasing the ionic strength of the feed solution has been shown to increase cell adhesion due to compaction of the electric double layer bringing the cells and the membrane surface into closer contact [72], this effect is considered negligible in this case, as CaCl_2 contributes with 15 mM in ionic strength vs 100 mM contribution for NaCl.

Figure 2 - Surface coverage (%) of live and dead/injured cells before and after osmotic backwashing for different Ca^{2+} concentrations in the FS_f (Fouling conditions: $\text{DS}_f = 0.7 \text{ M NaCl}$, $\text{FS}_f = 200 \text{ ml}$ of media containing 10^7 cells/ml *P. putida* in 0.1 M NaCl and different Ca^{2+} concentrations, adhesion duration 30 minutes; osmotic backwashing conditions: $\text{DS}_{\text{obw}} = 3 \text{ M NaCl}$, $\text{FS}_{\text{obw}} = \text{deionised water}$, duration = 1 minute; error bars show standard deviation of repeated experiments where the count was determined from 10 areas on the membrane surface

Divalent ions such as Ca^{2+} and Mg^{2+} promote adhesion by ion-bridging between anionic groups on the membrane surface and on the bacterial cell surface [73-75]. Surface adhesion of various types of bacteria cells have been shown to increase with Ca^{2+} [71, 76, 77]: Safari et al. [77], for example, showed that increasing the Ca^{2+} concentration from 0 to 15 mM increased the biofouling surface coverage from 29.3% to 47.8% due to an increase in initial adhesion. They also showed that the presence of Ca^{2+} leads to an increase in EPS and higher adhesion forces due to crosslinking between Ca^{2+} ions and alginate, a major component of the EPS [63]. Xie et al. [78] showed that biofouling in FO is enhanced by complexation between Ca^{2+} ions and the EPS produced by bacteria. Biofouling was enhanced by DS_f containing CaCl_2 when compared to DS_f with NaCl due to reverse diffusion of the salts during

fouling from the DS_f side to the FS_f side. The complexation and bridging of Ca²⁺ and EPS caused a thick, dense, and stable biofilm.

Furthermore, Ca²⁺ has been shown to bind with the protein LapF, a key protein for biofilm development of *P. putida*, and therefore form large aggregates in biofouling [76]. They reported an 11% increase in attached biomass on an LB agar plate containing *P. putida* after 2 hours of contact with 10 mM CaCl₂. This occurred as Ca²⁺ promotes the interaction between the LapF molecules in adjacent bacteria and therefore cell to cell interactions. This increase in cell interaction promotes microcolony formation leading to an increase in biomass. This occurrence could also explain the increase in surface coverage upon addition of Ca²⁺ to the FS_f.

The impact of Ca²⁺ concentration in the FS_f on osmotic backwashing efficiency was assessed. The increase in Ca²⁺ concentration in the FS_f from 0 mM to 5 mM led to a decrease in osmotic backwashing efficiency from 93% to just 60%, respectively (see Figure 2). The osmotic backwashing flux, however, showed no trend with increased Ca²⁺ concentration in the FS_f, varying between 31.94 ± 3.34 L.m⁻².h⁻¹. As the adhesion forces between the bacterial cells and the membrane surface become stronger with increasing Ca²⁺ concentration, the osmotic backwashing efficiency in detaching these reduces. de Kerchove and Elimelech [79] demonstrated an increase in attachment efficiency of *P. aeruginosa* onto clean and conditioned surfaces in the presence of divalent cations such as Ca²⁺. Safari et al. [77] also demonstrated higher adhesion of biofilms formed in the presence of Ca²⁺ using AFM: adhesion forces increased from 1.61 ± 0.56 nN to 2.06 ± 1.03 nN as the Ca²⁺ concentration was increased from 0 to 15 mM. As the adhesion increases with increasing Ca²⁺ concentration, the osmotic backwashing method becomes inefficient as it needs to overcome stronger interaction forces between the cells and the membrane surface. Hence higher osmotic backwashing fluxes might be required for more challenging cases.

3.3 Effect of filtration time on osmotic backwashing efficiency

Membrane cleaning results in both energy losses and downtime. Therefore, the frequency at which cleaning is carried out should be minimised, whilst maintaining cleaning efficiency at a maximum. Cleaning too sporadically may lead to a reduction in cleaning efficiency as biofouling becomes irreversible, determined to vary between 4 to 10 hours for RO membranes [58]. Bar-Zeev and Elimelech [52] osmotically backwashed RO membranes subjected to 15 hours of biofouling but only restored up to 70% of the permeate flux. Performing osmotic backwashing before irreversible

adhesion occurs could improve this. Different filtration times were tested to see how cleaning efficiency was impacted.

As can be seen in Figure 3, longer filtration times resulted in higher bacteria surface coverage on the FO membrane: surface coverage increased from 14% to >55% as the filtration time increased from 15 to 60 minutes, respectively. Ridgway et al. [80] studied adhesion of *Mycobacterium sp.* to cellulose diacetate RO membranes and found that adhesion increased with time, until reaching a plateau due to a finite number of adhesive sites on the membrane surface becoming occupied.

Figure 3 – Surface coverage (%) of live and dead/injured cells before and after osmotic backwashing for different filtration times (Fouling conditions: $DS_f = 0.7$ M NaCl, $FS_f = 200$ ml of media containing 10^7 cells/ml *P. putida* in 0.1 M NaCl; osmotic backwashing conditions: $DS_{obw} = 3$ M NaCl, $FS_{obw} =$ deionised water, duration = 1 minute; error bars show standard deviation of repeated experiments where the count was determined from 10 areas on the membrane surface)

In the same way the membrane flux during adhesion was unaffected by the adhered bacteria, remaining at 16.7 ± 4.0 L.h⁻¹.m², the osmotic backwashing flux variation showed no trend by increasing

filtration time, varying between $37.1 \pm 4.7 \text{ L.h}^{-1}.\text{m}^2$ for filtration times between 15 and 60 min. The adhered bacterial cells did not cause issues of biofilm enhanced concentration polarisation [23].

As the filtration duration increased and more cells adhered to the membrane surface, the cells became more difficult to detach, with osmotic backwashing efficiency in bacterial removal decreasing from 98% to 78% for filtration times of 15 and 60 minutes, respectively (see Figure 3). This is because the adhesion force between the bacterial cell and the membrane surface increases with contact time. Evidence for this is provided by several studies using AFM: adhesion forces between bacterial cells and surfaces, measured by the pull off force during retraction of the tip from the surface, increased with time [14, 81, 82]. Vadillo-Rodriguez [56] determined that bond strengthening occurred between the AFM tip and a *Streptococcus thermophiles* cell within just 100 seconds of contact. BinAhmed et al. [83] showed that *P. fluorescens* cells exhibited higher adhesion forces to ultrafiltration membranes, from 0.4 nN to over 0.5 nN, when cell-surface contact time was increased from 2 to 5 seconds. Higher increases in adhesion forces with time, from around -1.6 nN to -3.5 nN, have been measured for *S. thermophilus* in 200 seconds [56, 57]. Also Harimawan et al. used AFM to show that the adhesion force for *P. aeruginosa* increased from 3.84 nN to 8.53 nN when the contact duration between a bacteria and a stainless steel surface increased from 0 to 60 seconds [57]. Therefore, in this study, it is likely that as the filtration time was increased from 30 minutes to 60 minutes, the number of adhesive sites between bacteria and membrane surface increased, hence increasing adhesion forces [84]. For this reason, the cells became more difficult to remove for longer filtration times with this cleaning method. However, for the cases of 45 and 60 minutes of filtration time, 11% and 12.7% of the surface remained covered by dead/injured cells after osmotic backwashing, as opposed to < 1.5% coverage in live cells. Although the osmotic backwashing method is not 100% efficient in detaching the bacterial cells from the membrane surface, it is efficient in killing or injuring the bacteria, hence preventing biofilm development on the membrane surface.

In order to remove the remaining cells, it is postulated that a higher osmotic backwashing flux would be required to overcome the stronger adhesion forces developed for longer filtration times. In order to do this, other ionic salts, such as Ca^{2+} can be tested for that purpose, as they impart a higher osmotic pressure difference, hence a higher osmotic backwashing flux than NaCl [85].

3.4 Use of Ca^{2+} as DS_{obw} for bacteria detachment in osmotic backwashing

The results presented in Figures 1 to 3 show that as the adhesion conditions become more severe, i.e. Ca^{2+} concentrations >2.5 mM and filtration durations >30 minutes, the osmotic

backwashing method with 3 M NaCl, i.e. an osmotic backwashing flux of $36 \text{ L.h}^{-1}\text{.m}^2$, is no longer effective. One way to increase the cleaning efficiency is to increase the osmotic backwashing flux by increasing the driving force and hence use a DS_{obw} of higher osmotic pressure. A DS_{obw} with CaCl_2 can be used to achieve this, as a solution of CaCl_2 has a much higher osmotic pressure than a NaCl solution of the same osmolarity, and hence a higher osmotic backwashing flux is achieved (see Table 2). However, osmotic backwashing with CaCl_2 may have drawbacks. The presence of Ca^{2+} ions in the FS_f during adhesion was showed to enhance *P. putida* adhesion for 30 min, which made it more challenging to detach (see Figure 2). Hence, the presence of Ca^{2+} in the DS_{obw} might increase adhesion forces between the already adhered bacteria and the membrane surface, rather than detach the bacteria, even with a higher osmotic backwashing flux. However, the impact of Ca^{2+} on already adhered bacteria is unknown, and as osmotic backwashing occurs for 1 minute only, it is hypothesized this would be too short a time for Ca^{2+} to have a significant impact on bacterial adhesion forces onto the FO membrane surfaces, and a higher backwashing flux would be effective in detaching adhered bacteria. In a previous study on alginate acid fouling control in FO with osmotic backwashing, it was demonstrated that 1 minute of osmotic backwashing with CaCl_2 was enough time for the Ca^{2+} ions to influence the fouling layer by cross linking with carboxyl groups in the alginate layer. This hindered backwashing, rendering it inefficient [86]. However, de Kerchove and Elimelech [79] showed that the impact of Ca^{2+} and Mg^{2+} on *P. aeruginosa* attachment efficiency was similar for these two cations, but alginates responded differently when exposed to Ca^{2+} vs Mg^{2+} : this is potentially due to the difference between bacterial EPS and alginate. The questions that now arise are: (1) what is the impact of Ca^{2+} in the DS_{obw} on already adhered bacteria, and (2) will the increase in backwashing flux with a CaCl_2 DS_{obw} be enough to overcome any potential interaction between Ca^{2+} in the DS_{obw} and the adhered bacterial cells. This will be investigated in this section, where FO membranes were fouled for 30 minutes, and DS_{obw} of varying CaCl_2 concentrations were tested in order to compare cleaning efficiency of NaCl vs CaCl_2 DS_{obw} solutions.

Table 2 – Osmotic backwashing fluxes for increasing DS_{obw} concentrations of NaCl or CaCl_2

Concentration (M)	Backwashing flux ($\text{L.h}^{-1}\text{.m}^{-2}$)	
	NaCl	CaCl_2
0.1	8.7 ± 1.6	-
0.5	-	23.7 ± 0.6
0.7	20.2 ± 7.7	34.0 ± 2.0
2	30.3 ± 2.7	47.5 ± 2.0
3	36.0 ± 5.0	55.8 ± 1.7

Each membrane in Figure 2 and Figure 4 was subjected to identical adhesion conditions, resulting in an average surface coverage of 17.9% live cells and 1.0% dead/injured cells. An increase in NaCl concentration of the DS_{obw} was shown to cause higher osmotic backwashing fluxes, which was accompanied by a higher cleaning efficiency (see Figure 1). As can be seen in Table 2, an increase in CaCl₂ concentration of the DS_{obw} between 0.5 M and 3 M also resulted in increased osmotic backwashing fluxes during cleaning, ranging between 23.7 L.h⁻¹m⁻² and 55.8 L.h⁻¹m⁻², respectively.

Figure 4 - Surface coverage (%) of live and dead/injured cells before and after osmotic backwashing with DS_{obw} of different CaCl₂ concentrations (Fouling conditions: DS_f = 0.7 M NaCl, FS_f = 200 ml of media containing 10⁷ cells/ml *P. putida* in 0.1 M NaCl; osmotic backwashing conditions: DS_{obw} = varying CaCl₂ concentrations, FS_{obw} = deionised water, duration = 1 minute; ; error bars show standard deviation of repeated experiments where the count was determined from 10 areas on the membrane surface)

As opposed to NaCl, a reduced cleaning efficiency was obtained with an increased DS_{obw} CaCl₂ concentration: the total bacterial removal rates were 58% for concentrations up to 0.7 M CaCl₂, reducing to 22% and 39% for 2 M and 3 M CaCl₂ concentrations, respectively. There is quite a contrast in the results obtained when using NaCl or CaCl₂ as a DS_{obw}: 2 M and 3 M CaCl₂ concentrations originated osmotic backwashing fluxes of 47.5 L.h⁻¹m⁻² and 55.8 L.h⁻¹m⁻², which are higher than 36 L.h⁻¹m⁻².

1m^{-2} using a DS_{obw} of 3 M NaCl. As seen in Figure 2, a DS_{obw} of 3 M NaCl removed 93% of the total adhered bacteria, as opposed to 22% and 39% for a DS_{obw} of 2 and 3 M CaCl_2 , respectively. Osmotic backwashing with CaCl_2 hence becomes less efficient as the DS_{obw} concentration increases despite the fact that the osmotic backwashing flux is increasing, indicating an interaction between the adhered bacterial cells and the DS_{obw} , even if just for 1 minute. This immediate influence of Ca^{2+} was also demonstrated in previous studies with alginate fouling on FO and RO membranes [86, 87].

It has been reported that *P. putida* produce alginate in response to stress [63, 88], and *P. aeruginosa* have been shown to increase their EPS production, including alginate, when Ca^{2+} ions increased in the surrounding environment [89]. Although these studies were done for longer time scales, up to 72 hours, the results in Figure 4 suggest that interaction between Ca^{2+} and bacterial EPS might already be occurring for 30 min filtration times and 1 min osmotic backwashing, which is rather surprising at these short times. It has been shown that *P. aeruginosa* demonstrate up-expression of the alginate biosynthesis gene *algC* immediately after attachment [90], hence the same is likely to occur with *P. putida*, where EPS is formed when subjected to high Ca^{2+} concentrations. The production of alginate in the presence of Ca^{2+} will therefore protect the cells and increase cell adhesion forces [77, 79], therefore making their detachment more difficult. Xie et al. [78] also demonstrated how Ca^{2+} ions increased the hydrodynamic radius of bacterial EPS produced by *Pseudomonas aeruginosa* due to ion complexation and bridging. Bacterial detachment is hence more difficult, even at higher osmotic backwashing fluxes of $55.8\text{ L}\cdot\text{h}^{-1}\text{m}^{-2}$ achieved in the present study. Removal does, however, increase from 2 M to 3 M, from 22% to 39%, respectively, indicating that an osmotic backwashing flux of $55.8\text{ L}\cdot\text{h}^{-1}\text{m}^{-2}$ was high enough to remove more bacteria.

Experiments were performed on *P. putida* adhered onto well plates and subsequently exposed to 0.1 M NaCl, 3 M NaCl and 3 M CaCl_2 to mimic osmotic backwashing conditions, to detect EPS formation. The results are shown below in Figure 5 (more representative images can be found in the SI in Figure S4-S6).

A – *P. Putida* cells after 30 minutes adhesion followed by 20 minutes contact with 0.1 M NaCl

B - *P. Putida* cells after 30 minutes adhesion followed by 20 minutes contact with 0.1 M NaCl

C – *P. Putida* cells after 30 minutes adhesion followed by 20 minutes contact with 3 M NaCl

D – *P. Putida* cells after 30 minutes adhesion followed by 20 minutes contact with 3 M NaCl

E – *P. Putida* cells after 30 minutes adhesion followed by 20 minutes contact with 3 M CaCl_2

F – *P. Putida* cells after 30 minutes adhesion followed by 20 minutes contact with 3 M CaCl_2

559

560

561

562

563

Figure 5 - Microscopy representative images of 30 min *P. putida* adhesion onto well plates, followed by exposure to 0.1 M NaCl, 3 M NaCl and 3 M CaCl_2 . Blue areas represent EPS. Figures 5B, 5D and 5F were converted to black and thresholded using ImageJ software to improve the illustration of the blue areas.

564

565 The images shown in Figure 5, clearly confirm the presence of polysaccharides after attached *P. putida*
566 were exposed to CaCl_2 for 20 minutes (Figure 5 E and F). In contrast, after exposing the adhered
567 bacteria to 0.1 M NaCl and 3 M NaCl for 20 minutes, the production of EPS was barely noticeable
568 (Figure 5 A, B, C and D). This confirmed that Ca^{2+} has indeed an effect on bacterial production of EPS,
569 which does not occur for 0.1 M NaCl, and not to the same extent with 3 M NaCl.

570 As the DS_{obw} CaCl_2 concentration increased from 0.5 M to 3 M, the dead/injured cell coverage
571 increased from 0% to 6%, whilst the live cell coverage remained relatively constant at $7.3\% \pm 1.5\%$
572 (see Figure 5). The increase in dead/injured cells is due to the increasing osmotic pressure for higher
573 CaCl_2 concentrations, which causes osmotic shock on the bacterial cells, similar to what occurred with
574 NaCl in Figure 2. It is surprising that dead/injured cells with CaCl_2 in Figure 5, which originates higher
575 osmotic pressures than NaCl for the same osmolality, seems to cause lower dead/injured cells when
576 compared to NaCl in Figure 2, e.g. for 0.7 M and 2 M. Chang et al. [63] showed that *P. putida* produces
577 alginate to create a hydrated microenvironment that increases cell stress tolerance, hence the
578 production of alginate in the presence of CaCl_2 will protect the cells and increase adhesion forces [77],
579 therefore making them more difficult to remove and kill/damage. It is however important to bear in
580 mind, that some adhered cells were removed with DS_{obw} made up of CaCl_2 , which could include
581 dead/injured cells.

582 It is noteworthy to compare NaCl and CaCl_2 DS_{obw} with similar osmotic backwashing fluxes.
583 Although DS_{obw} of 0.7 M NaCl and 0.5 M CaCl_2 offered similar osmotic backwashing fluxes of $20.17 \text{ L.h}^{-1}\text{m}^{-2}$
584 and $23.7 \text{ L.h}^{-1}\text{m}^{-2}$, respectively (see Table 2), very different results are obtained. For the 0.7 M
585 NaCl solution, most of the adhered cells died and the total surface coverage was reduced by only 5%
586 after osmotic backwashing (Figure 2). For the 0.5 M CaCl_2 solution, the total surface coverage is
587 reduced by 58% and no dead/injured cells were detected. This result suggests that the CaCl_2 DS_{obw} is
588 less efficient at killing/damaging the cells than the NaCl solution because 16.9% of dead/injured cell
589 surface coverage and 0.9% live surface coverage remains on the membrane surface after cleaning with
590 0.7 M NaCl (see Figure 2) when compared to 7.89% of live cells surface coverage remaining after
591 cleaning with 0.5 M CaCl_2 (see Figure 3). This further supports the theory that the production of EPS
592 in the presence of CaCl_2 protects the cells.

593 The second observation is the cells' response to osmotic stress during osmotic backwashing
594 with NaCl and CaCl_2 DS_{obw} concentrations which originate the same osmotic pressure: DS_{obw} of 3 M
595 NaCl and 2 M CaCl_2 have osmotic pressures of 143.2 atm [85] [92]. Cleaning with 3 M NaCl results in
596 almost complete removal of adhered bacteria, with 1.2% surface coverage of dead/injured cells

remaining on the membrane surface for a flux of $36 \text{ L.h}^{-1}\text{m}^{-2}$. When cleaning with 2 M CaCl_2 , 8.9% surface coverage of live cells and 5.7% surface coverage of dead/injured cells remain on the membrane surface, despite the higher osmotic backwashing flux of $47.5 \text{ L.h}^{-1}\text{m}^{-2}$ (see Table 2). Again, this is due to the stress response of the bacterial cells resulting in the production of alginate. In response to the osmotic stress to CaCl_2 the cells produce alginate, which is hygroscopic [93], meaning it can hold several times its weight in water and potentially loses water slowly. This alginate layer can therefore keep the cells protected long enough for them to make metabolic adjustments to osmotic shock in order to increase survival [63].

4. Conclusion

Membrane cleaning at the initial cell adhesion stage is necessary to avoid the irreversible effects of biofouling. This study has shown that osmotic backwashing is potentially an effective cleaning method for bioadhesion, although efficiency is dependent on conditions tested. Detachment is more efficient with higher osmotic backwashing fluxes using a NaCl solution, due to the increased perpendicular drag force through the membrane. An osmotic backwashing solution of 3 M of NaCl, with a $36 \text{ L.h}^{-1}\text{m}^{-2}$ osmotic backwashing flux, was efficient for 30 minutes of adhesion, removing 93% of adhered cells. However, longer filtration durations caused increased cell surface coverage on the membrane, which osmotic backwashing could not overcome as efficiently. The bacterial cells that were left adhered on the membrane surface after osmotic backwashing with 3 M NaCl were all dead/injured due to osmotic shock, which has the positive outcome of preventing the formation of biofouling. When Ca^{2+} was added to the feed solution during bacterial adhesion the presence of divalent cations and the specific influence of Ca^{2+} on bacteria cells' EPS, increased cell adhesion and adhesion forces, therefore making bacterial detachment more difficult. Performing osmotic backwashing for longer durations or at more frequent intervals may overcome this increase in adhesion.

This study further showed the Ca^{2+} ions influence in adhesion during osmotic backwashing. With the aim to increase osmotic backwashing flux, osmotic backwashing draw solutions using CaCl_2 instead of NaCl were applied. It was shown that osmotic backwashing for 1 minute is sufficient for the Ca^{2+} ions in the osmotic backwashing solution to have a significant effect on bacterial adhesion and detachment. This was shown by the contrasting results presented between osmotic backwashing solutions of NaCl and CaCl_2 . This contrast is due to the different physicochemical and possible physiological influences of the Ca^{2+} ions and the Na^+ ions on the bacteria. The Ca^{2+} ions can enhance

adhesion by ion-bridging, by causing a stress response in the cells making them produce EPS which protects the cells, and by interacting specifically with the EPS produced by the bacteria: during osmotic backwashing with CaCl_2 , high concentrations of Ca^{2+} are in contact with the adhered cells and increase adhesion forces onto the membrane surface, making osmotic backwashing less efficient, even at high osmotic backwashing fluxes of 47.5 and 55.8 $\text{L}\cdot\text{h}^{-1}\cdot\text{m}^{-2}$, where a maximum removal of 39% was obtained.

In real operations, osmotic backwashing with a 3 M NaCl draw solution every 30 minutes is unfeasible and uneconomical, however the results show that, for the first time in such a system, removal of adhered cells via osmotic backwashing is possible. The results obtained with osmotic backwashing, invite further research on draw solutions that do not interact with the bacterial cells, but which produce high osmotic backwashing fluxes. The impact this might have on the integrity of the active layer, should however be considered. Furthermore, longer osmotic backwashing durations, more frequent cleaning, or a combination of cleaning techniques may also result in more effective cleaning.

Acknowledgements

The authors would like to thank the School of Engineering at the University of Edinburgh for the PhD studentship awarded to Dr. Sorchá Daly and the start-up funds awarded to Dr. Andrea Semião, as well as thank EPSRC funding (EP/P021646/1). The authors would like to acknowledge the invaluable help and input from the School of Engineering Workshop in the design and construction of the custom made FO membrane cells. The authors would like to thank Dr Ashley Allen (EP/P021646/1) for her help and training with initial bacteria growth and bacterial stock preparation. The authors would like to thank the School of Biological Sciences at the University of Edinburgh for access to the ultracentrifuge, and to Dr. David Kelly from the Centre Optical Instrumentation Laboratory (COIL) for all his help with the fluorescent microscopy. We would like to thank Dishon Hiebner for his advice and help with EPS staining and imaging in UCD. We especially thank Dr. Ellen L. Lagendijk and Dr. Mark Arentshorst, Institute of Biology Leiden, The Netherlands, for the gift of the green fluorescent protein expressing *Pseudomonas putida* (PCL1482 eGFP strain).

References

1. WHO. *Basic and safely managed drinking water services. Data by WHO region*. 2015; Available from: <http://www.who.int/health-statistics>.
2. Service, R.F., *Desalination Freshens Up*. Science, 2006. **313**(5790): p. 1088-1090.DOI: 10.1126/science.313.5790.1088.
3. Bamaga, O.A., et al., *Hybrid FO/RO desalination system: Preliminary assessment of osmotic energy recovery and designs of new FO membrane module configurations*. Desalination, 2011. **268**(1): p. 163-169.DOI: <https://doi.org/10.1016/j.desal.2010.10.013>.
4. Yangali-Quintanilla, V., et al., *Indirect desalination of Red Sea water with forward osmosis and low pressure reverse osmosis for water reuse*. Desalination, 2011. **280**(1): p. 160-166.DOI: <https://doi.org/10.1016/j.desal.2011.06.066>.
5. Cath, T.Y., et al., *A multi-barrier osmotic dilution process for simultaneous desalination and purification of impaired water*. Journal of Membrane Science, 2010. **362**(1–2): p. 417-426.DOI: <http://dx.doi.org/10.1016/j.memsci.2010.06.056>.
6. Hoover, L.A., et al., *Forward with Osmosis: Emerging Applications for Greater Sustainability*. Environmental Science & Technology, 2011. **45**(23): p. 9824-9830.DOI: 10.1021/es202576h.
7. Valladares Linares, R., et al., *Forward osmosis niches in seawater desalination and wastewater reuse*. Water Research, 2014. **66**: p. 122-139.DOI: <https://doi.org/10.1016/j.watres.2014.08.021>.
8. Van der Bruggen, B. and P. Luis, *Forward osmosis: understanding the hype*, in *Reviews in Chemical Engineering*. 2015. p. 1.
9. Gebreyohannes, A.Y., et al., *Treatment of Olive Mill Wastewater by Forward Osmosis*. Separation and Purification Technology, 2015. **147**(0): p. 292-302.DOI: <http://dx.doi.org/10.1016/j.seppur.2015.04.021>.
10. Hickenbottom, K.L., et al., *Forward osmosis treatment of drilling mud and fracturing wastewater from oil and gas operations*. Desalination, 2013. **312**(0): p. 60-66.DOI: <http://dx.doi.org/10.1016/j.desal.2012.05.037>.
11. Valladares Linares, R., et al., *Water harvesting from municipal wastewater via osmotic gradient: An evaluation of process performance*. Journal of Membrane Science, 2013. **447**(0): p. 50-56.DOI: <http://dx.doi.org/10.1016/j.memsci.2013.07.018>.
12. Wu, C.-Y., et al., *Removal of trace-amount mercury from wastewater by forward osmosis*. Journal of Water Process Engineering, 2016. **14**: p. 108-116.DOI: <https://doi.org/10.1016/j.jwpe.2016.10.010>.
13. Bogler, A., S. Lin, and E. Bar-Zeev, *Biofouling of membrane distillation, forward osmosis and pressure retarded osmosis: Principles, impacts and future directions*. Journal of Membrane Science, 2017. **542**: p. 378-398.DOI: <https://doi.org/10.1016/j.memsci.2017.08.001>.
14. Boks, N.P., et al., *Forces involved in bacterial adhesion to hydrophilic and hydrophobic surfaces*. Microbiology, 2008. **154**(10): p. 3122-3133.DOI: <https://doi.org/10.1099/mic.0.2008/018622-0>.
15. Hancock, N.T., et al., *Towards direct potable reuse with forward osmosis: Technical assessment of long-term process performance at the pilot scale*. Journal of Membrane Science, 2013. **445**: p. 34-46.DOI: <https://doi.org/10.1016/j.memsci.2013.04.056>.
16. Kwan, S.E., E. Bar-Zeev, and M. Elimelech, *Biofouling in forward osmosis and reverse osmosis: Measurements and mechanisms*. Journal of Membrane Science, 2015. **493**: p. 703-708.DOI: <https://doi.org/10.1016/j.memsci.2015.07.027>.
17. Yoon, H., et al., *Biofouling occurrence process and its control in the forward osmosis*. Desalination, 2013. **325**(0): p. 30-36.DOI: <http://dx.doi.org/10.1016/j.desal.2013.06.018>.
18. Flemming, H.-C., *Reverse osmosis membrane biofouling*. Experimental Thermal and Fluid Science, 1997. **14**(4): p. 382-391.DOI: [https://doi.org/10.1016/S0894-1777\(96\)00140-9](https://doi.org/10.1016/S0894-1777(96)00140-9).
19. O'Toole, G., H.B. Kaplan, and R. Kolter, *Biofilm Formation as Microbial Development*. Annual Review of Microbiology, 2000. **54**(1): p. 49-79.DOI: 10.1146/annurev.micro.54.1.49.

20. Dunne, W.M., *Bacterial Adhesion: Seen Any Good Biofilms Lately?* Clinical Microbiology Reviews, 2002. **15**(2): p. 155-166.DOI: 10.1128/cmr.15.2.155-166.2002.
21. Habimana, O., A.J.C. Semião, and E. Casey, *The role of cell-surface interactions in bacterial initial adhesion and consequent biofilm formation on nanofiltration/reverse osmosis membranes*. Journal of Membrane Science, 2014. **454**: p. 82-96.DOI: <https://doi.org/10.1016/j.memsci.2013.11.043>.
22. Wingender, J., T.R. Neu, and H.-C. Flemming, *What are Bacterial Extracellular Polymeric Substances?*, in *Microbial Extracellular Polymeric Substances: Characterization, Structure and Function*, J. Wingender, T.R. Neu, and H.-C. Flemming, Editors. 1999, Springer Berlin Heidelberg: Berlin, Heidelberg. p. 1-19.
23. Herzberg, M. and M. Elimelech, *Biofouling of reverse osmosis membranes: Role of biofilm-enhanced osmotic pressure*. Journal of Membrane Science, 2007. **295**(1): p. 11-20.DOI: <https://doi.org/10.1016/j.memsci.2007.02.024>.
24. Vrouwenvelder, J.S., et al., *Pressure drop increase by biofilm accumulation in spiral wound RO and NF membrane systems: role of substrate concentration, flow velocity, substrate load and flow direction*. Biofouling, 2009. **25**(6): p. 543-555.DOI: 10.1080/08927010902972225.
25. Flemming, H.-C., et al., *Effects and extent of biofilm accumulation in membrane systems*, in *Biofouling and Biocorrosion in Industrial Water Systems*, G.G. Geesey, Z. Lewandowski, and H.-C. Flemming, Editors. 1994, CRC Press, USA. p. 63-105.
26. Du, X., et al., *Impact of bubbly flow in feed channel of forward osmosis for wastewater treatment: Flux performance and biofouling*. Chemical Engineering Journal, 2017. **316**: p. 1047-1058.DOI: <https://doi.org/10.1016/j.cej.2017.02.031>.
27. Kim, C.-M., et al., *Effects of phosphate limitation in feed water on biofouling in forward osmosis (FO) process*. Desalination, 2014. **349**: p. 51-59.DOI: <https://doi.org/10.1016/j.desal.2014.06.013>.
28. Faria, A.F., et al., *Thin-film composite forward osmosis membranes functionalized with graphene oxide–silver nanocomposites for biofouling control*. Journal of Membrane Science, 2017. **525**: p. 146-156.DOI: <https://doi.org/10.1016/j.memsci.2016.10.040>.
29. Hegab, H.M., et al., *Effective in-situ chemical surface modification of forward osmosis membranes with polydopamine-induced graphene oxide for biofouling mitigation*. Desalination, 2016. **385**: p. 126-137.DOI: <https://doi.org/10.1016/j.desal.2016.02.021>.
30. Liu, Z. and Y. Hu, *Sustainable Antibiofouling Properties of Thin Film Composite Forward Osmosis Membrane with Rechargeable Silver Nanoparticles Loading*. ACS Applied Materials & Interfaces, 2016. **8**(33): p. 21666-21673.DOI: 10.1021/acsami.6b06727.
31. Miller, D.J., et al., *Short-term adhesion and long-term biofouling testing of polydopamine and poly(ethylene glycol) surface modifications of membranes and feed spacers for biofouling control*. Water Research, 2012. **46**(12): p. 3737-3753.DOI: <https://doi.org/10.1016/j.watres.2012.03.058>.
32. Perreault, F., et al., *Biofouling Mitigation in Forward Osmosis Using Graphene Oxide Functionalized Thin-Film Composite Membranes*. Environmental Science & Technology, 2016. **50**(11): p. 5840-5848.DOI: 10.1021/acs.est.5b06364.
33. Khan, M.T., et al., *Does chlorination of seawater reverse osmosis membranes control biofouling?* Water Research, 2015. **78**: p. 84-97.DOI: <https://doi.org/10.1016/j.watres.2015.03.029>.
34. Semião, A.J.C., O. Habimana, and E. Casey, *Bacterial adhesion onto nanofiltration and reverse osmosis membranes: Effect of permeate flux*. Water Research, 2014. **63**: p. 296-305.DOI: <https://doi.org/10.1016/j.watres.2014.06.031>.
35. Li, Z., et al., *Aquaporin based biomimetic membrane in forward osmosis: Chemical cleaning resistance and practical operation*. Desalination, 2017. **420**: p. 208-215.DOI: <https://doi.org/10.1016/j.desal.2017.07.015>.

- 762 36. Habimana, O., A.J.C. Semião, and E. Casey, *Upon Impact: The Fate of Adhering Pseudomonas*
763 *fluorescens* Cells during Nanofiltration. *Environmental Science & Technology*, 2014. **48**(16):
764 p. 9641-9650.DOI: 10.1021/es500585e.
- 765 37. Valladares Linares, R., et al., *Cleaning protocol for a FO membrane fouled in wastewater*
766 *reuse*. *Desalination and Water Treatment*, 2013. **51**(25-27): p. 4821-4824.DOI:
767 10.1080/19443994.2013.795345.
- 768 38. Valladares Linares, R., et al., *NOM and TEP fouling of a forward osmosis (FO) membrane:*
769 *Foulant identification and cleaning*. *Journal of Membrane Science*, 2012. **421–422**(0): p. 217-
770 224.DOI: <http://dx.doi.org/10.1016/j.memsci.2012.07.019>.
- 771 39. Wang, Z., et al., *Chemical cleaning protocols for thin film composite (TFC) polyamide forward*
772 *osmosis membranes used for municipal wastewater treatment*. *Journal of Membrane*
773 *Science*, 2015. **475**: p. 184-192.DOI: <https://doi.org/10.1016/j.memsci.2014.10.032>.
- 774 40. Lattemann, S. and T. Höpner, *Environmental impact and impact assessment of seawater*
775 *desalination*. *Desalination*, 2008. **220**(1–3): p. 1-15.DOI:
776 <http://dx.doi.org/10.1016/j.desal.2007.03.009>.
- 777 41. Zhang, J., et al., *Membrane biofouling and scaling in forward osmosis membrane bioreactor*.
778 *Journal of Membrane Science*, 2012. **403–404**: p. 8-14.DOI:
779 <https://doi.org/10.1016/j.memsci.2012.01.032>.
- 780 42. Dana, A., S. Hadas, and G.Z. Ramon, *Potential application of osmotic backwashing to*
781 *brackish water desalination membranes*. *Desalination*, 2019. **468**: p. 114029.DOI:
782 <https://doi.org/10.1016/j.desal.2019.05.012>.
- 783 43. Qin, J.-J., et al., *Development of novel backwash cleaning technique for reverse osmosis in*
784 *reclamation of secondary effluent*. *Journal of Membrane Science*, 2010. **346**(1): p. 8-14.DOI:
785 <http://dx.doi.org/10.1016/j.memsci.2009.08.011>.
- 786 44. Ramon, G., Y. Agnon, and C. Dosoretz, *Dynamics of an osmotic backwash cycle*. *Journal of*
787 *Membrane Science*, 2010. **364**(1–2): p. 157-166.DOI:
788 <http://dx.doi.org/10.1016/j.memsci.2010.08.008>.
- 789 45. Ramon, G.Z., T.-V. Nguyen, and E.M.V. Hoek, *Osmosis-assisted cleaning of organic-fouled*
790 *seawater RO membranes*. *Chemical Engineering Journal*, 2013. **218**(0): p. 173-182.DOI:
791 <http://dx.doi.org/10.1016/j.cej.2012.12.006>.
- 792 46. Sagiv, A., et al., *Osmotic backwash mechanism of reverse osmosis membranes*. *Journal of*
793 *Membrane Science*, 2008. **322**(1): p. 225-233.DOI:
794 <http://dx.doi.org/10.1016/j.memsci.2008.05.055>.
- 795 47. Sagiv, A. and R. Semiat, *Backwash of RO spiral wound membranes*. *Desalination*, 2005.
796 **179**(1–3): p. 1-9.DOI: <http://dx.doi.org/10.1016/j.desal.2004.11.050>.
- 797 48. Sagiv, A. and R. Semiat, *Parameters affecting backwash variables of RO membranes*.
798 *Desalination*, 2010. **261**(3): p. 347-353.DOI: <http://dx.doi.org/10.1016/j.desal.2010.04.012>.
- 799 49. Spiegler, K.S. and J.H. Macleish, *Molecular (osmotic and electro-osmotic) backwash of*
800 *cellulose acetate hyperfiltration membranes*. *Journal of Membrane Science*, 1981. **8**(2): p.
801 173-192.DOI: [http://dx.doi.org/10.1016/S0376-7388\(00\)82089-X](http://dx.doi.org/10.1016/S0376-7388(00)82089-X).
- 802 50. Holloway, R.W., et al., *Forward osmosis for concentration of anaerobic digester centrate*.
803 *Water Research*, 2007. **41**(17): p. 4005-4014.DOI:
804 <http://dx.doi.org/10.1016/j.watres.2007.05.054>.
- 805 51. Motsa, M.M., et al., *Organic fouling in forward osmosis membranes: The role of feed solution*
806 *chemistry and membrane structural properties*. *Journal of Membrane Science*, 2014. **460**(0):
807 p. 99-109.DOI: <http://dx.doi.org/10.1016/j.memsci.2014.02.035>.
- 808 52. Bar-Zeev, E. and M. Elimelech, *Reverse Osmosis Biofilm Dispersal by Osmotic Back-Flushing:*
809 *Cleaning via Substratum Perforation*. *Environmental Science & Technology Letters*, 2014.
810 **1**(2): p. 162-166.DOI: 10.1021/ez400183d.
- 811 53. Katebian, L. and S.C. Jiang, *Marine bacterial biofilm formation and its responses to periodic*
812 *hyperosmotic stress on a flat sheet membrane for seawater desalination pretreatment*.

- Journal of Membrane Science, 2013. **425–426**: p. 182-189.DOI: <http://dx.doi.org/10.1016/j.memsci.2012.08.027>.
54. Lee, S., et al., *Comparison of fouling behavior in forward osmosis (FO) and reverse osmosis (RO)*. Journal of Membrane Science, 2010. **365**(1–2): p. 34-39.DOI: <http://dx.doi.org/10.1016/j.memsci.2010.08.036>.
 55. Creber, S.A., et al., *Chemical cleaning of biofouling in reverse osmosis membranes evaluated using magnetic resonance imaging*. Journal of Membrane Science, 2010. **362**(1): p. 202-210.DOI: <https://doi.org/10.1016/j.memsci.2010.06.052>.
 56. Vadillo-Rodríguez, V., et al., *Atomic force microscopic corroboration of bond aging for adhesion of Streptococcus thermophilus to solid substrata*. Journal of Colloid and Interface Science, 2004. **278**(1): p. 251-254.DOI: <https://doi.org/10.1016/j.jcis.2004.05.045>.
 57. Harimawan, A., A. Rajasekar, and Y.-P. Ting, *Bacteria attachment to surfaces – AFM force spectroscopy and physicochemical analyses*. Journal of Colloid and Interface Science, 2011. **364**(1): p. 213-218.DOI: <https://doi.org/10.1016/j.jcis.2011.08.021>.
 58. Lutskiy, M.-Y., et al., *A microbiology-based assay for quantification of bacterial early stage biofilm formation on reverse-osmosis and nanofiltration membranes*. Separation and Purification Technology, 2015. **141**: p. 214-220.DOI: <http://dx.doi.org/10.1016/j.seppur.2014.12.003>.
 59. Subramani, A. and E.M.V. Hoek, *Direct observation of initial microbial deposition onto reverse osmosis and nanofiltration membranes*. Journal of Membrane Science, 2008. **319**(1–2): p. 111-125.DOI: 10.1016/j.memsci.2008.03.025.
 60. de Groot, B.L. and H. Grubmüller, *Water Permeation Across Biological Membranes: Mechanism and Dynamics of Aquaporin-1 and GlpF*. Science, 2001. **294**(5550): p. 2353-2357.DOI: 10.1126/science.1066115.
 61. Colombo, G., S.J. Marrink, and A.E. Mark, *Simulation of MscL Gating in a Bilayer under Stress*. Biophysical Journal, 2003. **84**(4): p. 2331-2337.DOI: [https://doi.org/10.1016/S0006-3495\(03\)75038-3](https://doi.org/10.1016/S0006-3495(03)75038-3).
 62. Valladares Linares, R., et al., *Impact of spacer thickness on biofouling in forward osmosis*. Water Research, 2014. **57**: p. 223-233.DOI: <https://doi.org/10.1016/j.watres.2014.03.046>.
 63. Chang, W.-S., et al., *Alginate Production by Pseudomonas putida Creates a Hydrated Microenvironment and Contributes to Biofilm Architecture and Stress Tolerance under Water-Limiting Conditions*. Journal of Bacteriology, 2007. **189**(22): p. 8290-8299.DOI: 10.1128/jb.00727-07.
 64. Achilli, A., et al., *The forward osmosis membrane bioreactor: A low fouling alternative to MBR processes*. Desalination, 2009. **239**(1–3): p. 10-21.DOI: <http://dx.doi.org/10.1016/j.desal.2008.02.022>.
 65. Zhou, Z., et al., *Simultaneous alkali supplementation and fouling mitigation in membrane bioreactors by on-line NaOH backwashing*. Journal of Membrane Science, 2014. **457**: p. 120-127.DOI: <https://doi.org/10.1016/j.memsci.2014.01.008>.
 66. Liu, X., et al., *Synthesis and characterization of novel antibacterial silver nanocomposite nanofiltration and forward osmosis membranes based on layer-by-layer assembly*. Water Research, 2013. **47**(9): p. 3081-3092.DOI: <https://doi.org/10.1016/j.watres.2013.03.018>.
 67. Soroush, A., et al., *In Situ Silver Decoration on Graphene Oxide-Treated Thin Film Composite Forward Osmosis Membranes: Biocidal Properties and Regeneration Potential*. Environmental Science & Technology Letters, 2016. **3**(1): p. 13-18.DOI: 10.1021/acs.estlett.5b00304.
 68. Li, B. and B.E. Logan, *Bacterial adhesion to glass and metal-oxide surfaces*. Colloids and Surfaces B: Biointerfaces, 2004. **36**(2): p. 81-90.DOI: <https://doi.org/10.1016/j.colsurfb.2004.05.006>.

69. Xia, L., et al., *Novel Commercial Aquaporin Flat-Sheet Membrane for Forward Osmosis*. Industrial & Engineering Chemistry Research, 2017. **56**(41): p. 11919-11925.DOI: 10.1021/acs.iecr.7b02368.
70. Kültz, D., *Chapter 12 - Osmotic regulation of DNA activity and the cell cycle*, in *Cell and Molecular Response to Stress*, K.B. Storey and J.M. Storey, Editors. 2000, Elsevier. p. 157-179.
71. Fletcher, M., *Attachment of Pseudomonas fluorescens to glass and influence of electrolytes on bacterium-substratum separation distance*. Journal of Bacteriology, 1988. **170**(5): p. 2027-2030.
72. Sadr Ghayeni, S.B., et al., *Adhesion of waste water bacteria to reverse osmosis membranes*. Journal of Membrane Science, 1998. **138**(1): p. 29-42.DOI: [https://doi.org/10.1016/S0376-7388\(97\)00196-8](https://doi.org/10.1016/S0376-7388(97)00196-8).
73. Gingell, D., D.R. Garrod, and J.F. Palmer, *Divalent Cations and Cell Adhesion*, in *A Symposium on Calcium and Cellular Function*, A.W. Cuthbert, Editor. 1970, Palgrave Macmillan UK: London. p. 59-64.
74. McEldowney, S. and M. Fletcher, *Variability of the influence of physicochemical factors affecting bacterial adhesion to polystyrene substrata*. Applied and environmental microbiology, 1986. **52**(3): p. 460-465.
75. Sobek, D.C. and M.J. Higgins, *Examination of three theories for mechanisms of cation-induced bioflocculation*. Water Research, 2002. **36**(3): p. 527-538.DOI: [https://doi.org/10.1016/S0043-1354\(01\)00254-8](https://doi.org/10.1016/S0043-1354(01)00254-8).
76. Martínez-Gil, M., et al., *Calcium Causes Multimerization of the Large Adhesin LapF and Modulates Biofilm Formation by Pseudomonas putida*. Journal of Bacteriology, 2012. **194**(24): p. 6782-6789.DOI: 10.1128/jb.01094-12.
77. Safari, A., et al., *The significance of calcium ions on Pseudomonas fluorescens biofilms – a structural and mechanical study*. Biofouling, 2014. **30**(7): p. 859-869.DOI: 10.1080/08927014.2014.938648.
78. Xie, M., et al., *Role of Reverse Divalent Cation Diffusion in Forward Osmosis Biofouling*. Environmental Science & Technology, 2015. **49**(22): p. 13222-13229.DOI: 10.1021/acs.est.5b02728.
79. de Kerchove, A.J. and M. Elimelech, *Calcium and Magnesium Cations Enhance the Adhesion of Motile and Nonmotile Pseudomonas aeruginosa on Alginate Films*. Langmuir, 2008. **24**(7): p. 3392-3399.DOI: 10.1021/la7036229.
80. Ridgway, H.F., M.G. Rigby, and D.G. Argo, *Adhesion of a Mycobacterium sp. to cellulose diacetate membranes used in reverse osmosis*. Applied and environmental microbiology, 1984. **47**(1): p. 61-67.
81. Thwala, J.M., et al., *Bacteria–Polymeric Membrane Interactions: Atomic Force Microscopy and XDLVO Predictions*. Langmuir, 2013. **29**(45): p. 13773-13782.DOI: 10.1021/la402749y.
82. Xu, L.-C., V. Vadiello-Rodriguez, and B.E. Logan, *Residence Time, Loading Force, pH, and Ionic Strength Affect Adhesion Forces between Colloids and Biopolymer-Coated Surfaces*. Langmuir, 2005. **21**(16): p. 7491-7500.DOI: 10.1021/la0509091.
83. BinAhmed, S., et al., *Bacterial Adhesion to Ultrafiltration Membranes: Role of Hydrophilicity, Natural Organic Matter, and Cell-Surface Macromolecules*. Environmental Science & Technology, 2018. **52**(1): p. 162-172.DOI: 10.1021/acs.est.7b03682.
84. Vissers, T., et al., *Bacteria as living patchy colloids: Phenotypic heterogeneity in surface adhesion*. Science Advances, 2018. **4**(4): p. eaao1170.DOI: 10.1126/sciadv.aao1170.
85. Cath, T.Y., A.E. Childress, and M. Elimelech, *Forward osmosis: Principles, applications, and recent developments*. Journal of Membrane Science, 2006. **281**(1–2): p. 70-87.DOI: <http://dx.doi.org/10.1016/j.memsci.2006.05.048>.

86. Daly, S. and A.J.C. Semiao, *Mechanisms Involved in Osmotic Backwashing of Fouled Forward Osmosis (FO) Membranes*. Journal of Membrane Science and Research, 2020. **6**(2): p. 158-167.DOI: 10.22079/jmsr.2020.118843.1315.
87. Daly, S., et al., *Influence of organic fouling layer characteristics and osmotic backwashing conditions on cleaning efficiency of RO membranes*. Journal of Membrane Science, 2020: p. 118604.DOI: <https://doi.org/10.1016/j.memsci.2020.118604>.
88. Sandhya, V. and S.Z. Ali, *The production of exopolysaccharide by Pseudomonas putida GAP-P45 under various abiotic stress conditions and its role in soil aggregation*. Microbiology, 2015. **84**(4): p. 512-519.DOI: 10.1134/s0026261715040153.
89. Sarkisova, S., et al., *Calcium-induced virulence factors associated with the extracellular matrix of mucoid Pseudomonas aeruginosa biofilms*. Journal of bacteriology, 2005. **187**(13): p. 4327-4337.DOI: 10.1128/JB.187.13.4327-4337.2005.
90. Davies, D.G. and G.G. Geesey, *Regulation of the alginate biosynthesis gene algC in Pseudomonas aeruginosa during biofilm development in continuous culture*. Applied and environmental microbiology, 1995. **61**(3): p. 860-867.DOI: 10.1128/AEM.61.3.860-867.1995.
91. Achilli, A., T.Y. Cath, and A.E. Childress, *Selection of inorganic-based draw solutions for forward osmosis applications*. Journal of Membrane Science, 2010. **364**(1): p. 233-241.DOI: <https://doi.org/10.1016/j.memsci.2010.08.010>.
92. Hamdan, M., et al., *Draw solutions for Forward Osmosis process: Osmotic pressure of binary and ternary aqueous solutions of magnesium chloride, sodium chloride, sucrose and maltose*. Journal of Food Engineering, 2015. **155**: p. 10-15.DOI: <https://doi.org/10.1016/j.jfoodeng.2015.01.010>.
93. Kallenberger, P.A. and M. Fröba, *Water harvesting from air with a hygroscopic salt in a hydrogel-derived matrix*. Communications Chemistry, 2018. **1**(1): p. 28.DOI: 10.1038/s42004-018-0028-9.