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

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

32

33 **Abstract**

34

35 The efficiency of osmotic backwashing cleaning to remove bacteria from forward osmosis membranes
36 was systematically studied for the first time under different attachment and osmotic backwashing
37 conditions. It is hypothesised that biofouling is preventable when tackling initial adhesion, i.e. during
38 the reversible stage. Cell removal from the membrane was dependent on both adhesion and
39 backwashing conditions: tests were performed for backwashing solutions of different concentrations
40 and salt type, as well as different filtration durations and Ca^{2+} concentrations in the feed solution.
41 Following adhesion of *P. putida*, a backwashing draw solution (DS_{obw}) of 3 M NaCl was the most
42 efficient, removing 93% of the adhered cells after 1 minute of backwashing. All adhered cells left on
43 the membrane were dead/injured due to osmotic shock. To optimise the cleaning regime, the
44 maximum filtration time for which backwashing is efficient must be determined. This was determined
45 to be 30 minutes, after which backwashing became inefficient, only removing 78% of cells. The
46 addition of 5 mM Ca^{2+} to the feed caused a 50% increase in cell surface coverage compared to
47 adhesion without Ca^{2+}. This increase in adhesion rendered backwashing inefficient, as cell removal
48 was only 60%. To increase backwashing efficiency by increasing the backwashing flux, DS_{obw} with $CaCl_2$
49 were used. However, this was inefficient due to interactions between Ca^{2+} in the DS_{obw} and the
50 adhered cells, even for just 1 minute: for a 55.8 $L.h^{-1}m^{-2}$ flux, 39% of removal was obtained for a 3 M
51 $CaCl_2$ DS_{obw} when compared to 93% removal for 3 M NaCl for a 36 $L.h^{-1}m^{-2}$ flux. Therefore, both
52 adhesion and backwashing conditions are important for cleaning of FO membranes.

53

54

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

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

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

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

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

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

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

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

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

158

159 ## 2. Materials and Methods
160

161 *2.1 Forward Osmosis Membrane*

162 For all experiments, a commercial aquaporin based membrane was used (Aquaporin Inside^{TM},
163 Denmark). This is a thin-film composite membrane consisting of a polyamide active layer containing
164 aquaporin proteins, an intermediary polyester layer, and a non-woven polyester support layer.
165 Aquaporin proteins are water channel forming proteins added to the membrane to enhance its flux
166 [60]. The membrane is approximately 110 µm thick. The membrane was stored wet in MilliQwater
167 (Avidity, UK) at 4°C, and before use, it was gently washed with MilliQ water and cut to fit the
168 membrane crossflow cell.

169

170 *2.2 Model Bacteria Strain and Media*

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

178

179 *2.3 Fouling Solution*

180 The fouling feed solution consisted of *P. putida* in a background electrolyte solution of 0.1 M
181 NaCl (Fisher Scientific, UK). This solution was used in order to maintain the cells' viability and protect
182 them from osmotic stress [61]. Concentrations of $CaCl_2$ (Sigma-Aldrich, UK) ranging from 0 to 5 mM
183 were also added to the fouling solution for some experiments to see the effect Ca^{2+} ions have on
184 adhesion and osmotic backwashing efficiency. The 200 ml bacterial culture volume was added to 1 L
185 of the fouling feed solution, where the final concentration of *P. putida* in the FS_f was 10^7 $cells.ml^{-1}$. For
186 every experiment, an initial volume of 1 L DS of 0.7 M NaCl was used during the adhesion stage, to
187 mimic seawater [5, 11, 62].

188

189 *2.4 Forward Osmosis Bench Scale Crossflow System*

190 Experiments were carried out in a custom built forward osmosis bench scale rig as shown in
191 Figure S1 in the Supporting Information. Briefly, two variable speed gear pumps delivered pulse free
192 flow to two custom built Perspex membrane cells placed in parallel. Each membrane cell had an
193 effective membrane area of 0.0048 m^2 (width 25 mm, length 191 mm) and the membrane was placed
194 between two channels of 3 mm height each. Through a system of valves, the membrane cells were
195 either fed by two reservoirs containing the fouling feed solution (FS_f) and the fouling draw solution
196 (DS_f) during fouling, or fed by the osmotic backwashing feed solution (FS_{obw}) and the osmotic
197 backwashing draw solution (DS_{obw}) during osmotic backwashing. Both DS_f and DS_{obw} were positioned
198 on a balance (Ohaus, US), and the weight change was used to calculate the flux through the FO
199 membrane.

200

201 *2.4 Bacterial Adhesion Fouling protocol*

202 The membranes were secured in their respective cells using double sided tape to guarantee
203 they did not move in the cell during manipulation. For each bacterial adhesion fouling experiment, the
204 active layer faced the FS_f and the support layer faced the DS_f, i.e. AL-FS mode. Firstly, the pure water
205 flux (PWF) of the membranes was measured for 30 minutes for cell 1, followed by 30 minutes for cell
206 2, and then both cells in parallel. PWF testing was carried out with deionised water as the FS_f and 0.7
207 M NaCl as the DS_f.

208 Once the PWF was determined, the solutions in the FS_f and DS_f were substituted for the fouling
209 ones, i.e. 0.1 M NaCl and 0.7 M NaCl, respectively. As previously mentioned, in some experiments, the
210 FS_f was made up of 0.1 M NaCl and 2.5, 3.5, and 5 mM $CaCl_2$. The FS_f and DS_f were left recirculating in
211 the crossflow system for 10 minutes at a crossflow rate of 1 $L.min^{-1}$ per membrane cell, in order to
212 stabilize the system. Bacterial adhesion began when 200 ml of the bacteria culture was added to the
213 FS_f tank. Unless otherwise stated, adhesion experiments lasted for 30 minutes.

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

217

218 *2.5 Osmotic Backwashing Cleaning Protocol*

219 Two membranes were fouled in parallel in AL-FS mode, but only one was osmotically 220 backwashed in order to determine surface coverage before and after the cleaning method was 221 applied. After fouling, the pumps were stopped, and the FS_f and DS_f reservoirs were replenished with 222 0.1 M NaCl to rinse the membrane cells and remove any unattached bacteria. Next, both gear pumps 223 were stopped and the inlet and outlet valves of cell 2 (valves 1E, 1F, 2E, 2F in Figure S1) were closed 224 to isolate the fouled membrane in cell 2. Osmotic backwashing was implemented for cell 1 225 immediately after bacterial adhesion, by switching the FS_f and DS_f reservoirs to the osmotic 226 backwashing FS_{obw} and DS_{obw} reservoirs, using a series of valves as follows: closing valves 1A, 1B, 1G, 227 1H and opening valves 2A, 2B, 2G, 2H (Figure S1) and restarting the pumps at 0.5 $L.min^{-1}$ to feed 228 membrane cell 1.

229 Osmotic backwashing was carried out in AL-DS mode. During osmotic backwashing, the DS_{obw} 230 used ranged from 0.1 to 3 M NaCl or 0.5 to 3 M $CaCl_2$, depending on the experiment. A FS_{obw} of Milli Q 231 water was used for every experiment. Osmotic backwashing was carried out for 1 minute, and the flux 232 was measured by weighing the DS_{obw} tank (Ohaus, US). Although the higher range of DS_{obw} 233 concentrations are potentially unfeasible for full-scale application, they were used to understand 234 bacterial removal capacity at a more fundamental level and understand the potential of osmotic 235 backwashing for cell removal from FO membranes.

236 After osmotic backwashing, the pumps were stopped, and the FS_f and DS_f reservoirs were 237 replenished with 0.1 M NaCl to rinse cell 1 and remove any unattached bacteria. Subsequently, all 238 valves to and from the membrane cells were closed and both membranes were removed from their 239 cells and transferred to petri dishes, while completely submerged in 0.1 M NaCl. Each experiment was 240 repeated at least twice, with the two most outermost data points (e.g. in Figure 2: osmotic 241 backwashing with 0.1 M NaCl and 3 M NaCl) repeated at least 3 times.

242 After every experiment, the FO system was thoroughly rinsed with deionised water, followed 243 by recirculation with 70% ethanol for 30 minutes and 0.1 M NaOH for another 30 minutes. Next, the 244 system pH was neutralised by slowly adding 1 M HCl, measured using a pH probe (VWR, Germany). 245 Finally, the system was thoroughly flushed with deionised water until the conductivity was < 1 $\mu S.cm^{-1}$ 246 .

247 *2.6 Protocol for alginate detection*

248 To detect the presence of EPS on the bacteria surface after osmotic backwashing with $CaCl_2$, 249 experiments were performed with *P. putida* wild type in polystyrene 6 well plates. Cultures were 250 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 $CaCl_2$ 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 $CaCl_2$, 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 florescence 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

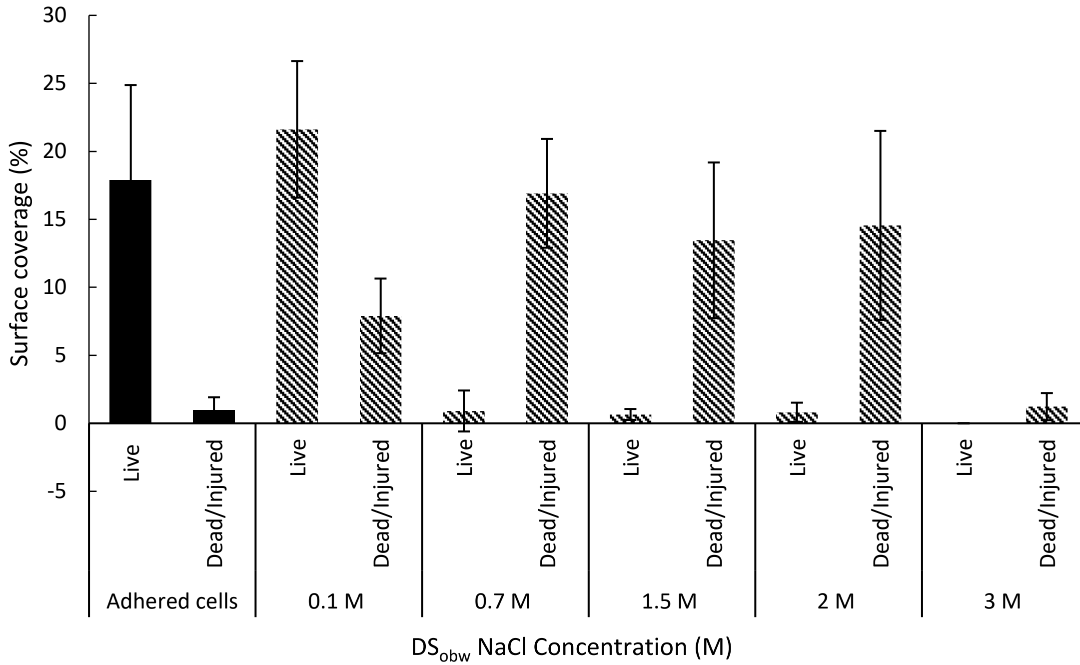
3.1 Effect of DS_{obw} Concentration on Bacterial Detachment

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 ± 7% live cells and 0.98 ± 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 ± 5.5° [69], when compared to other FO membranes such as the cellulose triacetate membrane by Hydration Technology Innovations with a contact angle of 62 ± 7.2° [69].

The surface coverage obtained during bacterial adhesion did not affect the flux, which remained at 16.2 ± 1.8 $L.h^{-1}.m^{2}$ 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].

312



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

319

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

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

Concentration (M NaCl)	Osmotic Backwashing flux (L. h^{-1}. 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

326

327 As shown in Figure 1, as the adhered cells were exposed to DS_{obw} concentrations varying
328 between 0.1 and 1.5 M NaCl, the surface coverage of total adhered cells remained relatively
329 unchanged at 22% ± 6%, showing that the osmotic backwashing fluxes obtained up to 31 $L.h^{-1}.m^{-2}$ (see
330 Table 1) were not sufficient to detach the bacteria (please see Figure S3 in the Supporting Information
331 and related text for statistical analysis of adhered bacteria vs osmotic backwashing with 0.1 M NaCl).
332 There was, however, a dramatic effect on the surface coverages of live *vs* dead/injured cells: whilst
333 live cell surface coverage reduced from 21% down to < 1%, the dead/injured cells surface coverage
334 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
335 osmotic backwashing had a severe effect on the viability of the adhered bacteria, which became more
336 compromised for higher concentrations of DS_{obw}, or for higher osmotic backwashing fluxes. This could
337 be caused by two factors. Firstly, bacteria that experience a spike of high salinity undergo osmotic
338 stress [52], as a response to adapt to changes to their external environment in order to continue to
339 function [70]. Katebian and Jiang [53] used 0.55 M of NaCl to induce hyperosmotic stress on
340 *Shewanella sp.* biofilm producing bacteria adhered to filter cartridges used in desalination, resulting
341 in greater than 99.5% mortality rate.

342 The other factor that could have contributed to affecting bacterial viability was the reversal
343 of flux direction from the fouling stage to the osmotic backwashing stage. Habimana et al. [36] showed
344 that shear stress through increase in permeate flux can have a significant effect on the viability of
345 bacterial cells adhered to NF and RO membranes. The increase in flux reversal due to an increase in
346 DS_{obw} could also have contributed to the increased surface coverage of dead/injured cells from 7.9%
347 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
348 from the membrane surface, their compromise in viability translates to biofouling development being
349 avoided, as dead bacteria cannot produce EPS [23]. The results are potentially the combination of both
350 osmotic shock and shear stress.

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

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

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

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

364

365 *3.2 Effect of Ca^{2+} in the FS_f on adhesion and osmotic backwashing efficiency*

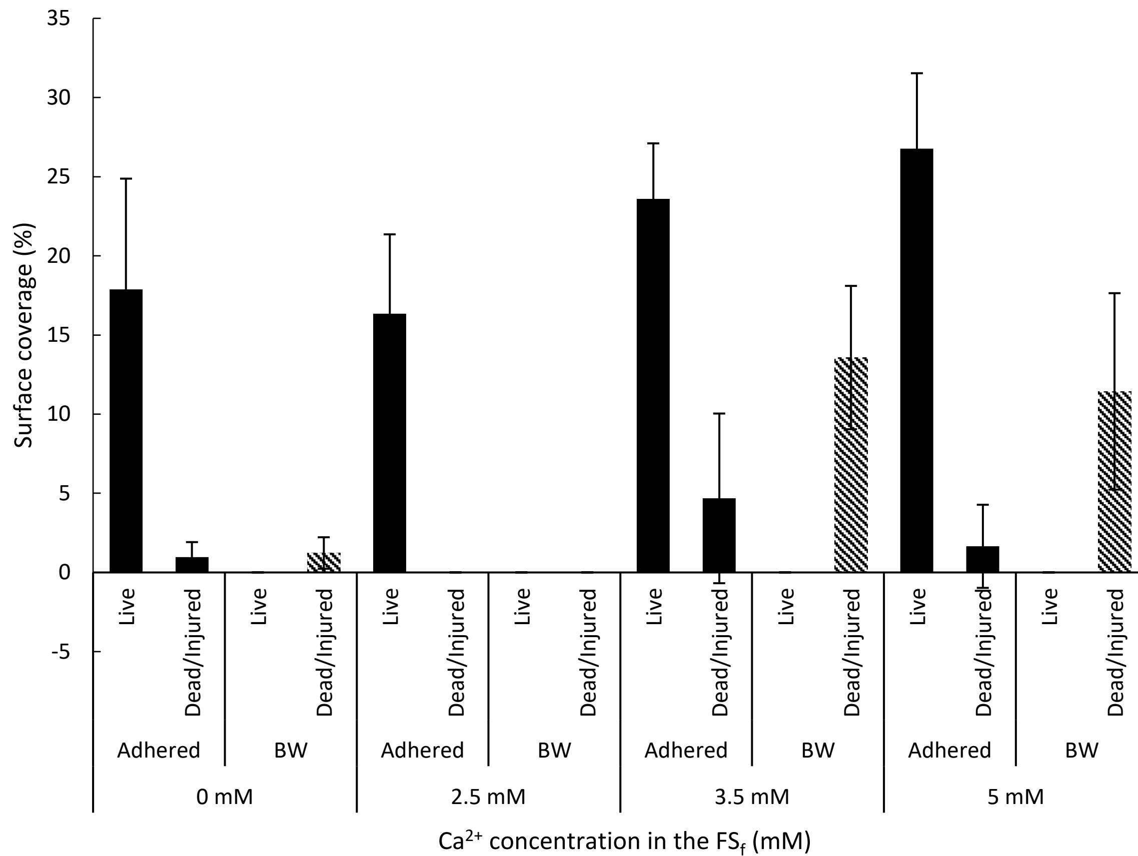
366 Feed characteristics have an important effect on the physical, chemical and biological factors
367 affecting bacterial adhesion [71]. Divalent ions, such as Ca^{2+} ions, are known to increase adhesion due
368 to both compression of the electric double layer and ion bridging between the cell and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

371 Bacterial adhesion was carried out for 30 min with varying concentrations of Ca^{2+} in the FS_f.
372 As the Ca^{2+} concentration in the FS_f increased from 0 to 5 mM, the total surface coverage increased by
373 33%: the addition of 5 mM Ca^{2+} resulted in an increase in total surface coverage from 18.9% to 28.2%,
374 when compared to adhesion in the absence of Ca^{2+}. Of this total cell surface coverage, a high
375 proportion, 17.9% and 26.8%, respectively, were live cells (see Figure 2). Statistical significance of
376 differences between values in Figure 3 were tested using 2-sample t tests in MINITAB software version
377 18. All tests were performed at 5% significance level. The difference in values obtained for feed
378 solutions containing 3 mm and 5 mM NaCl are not statistically significant ($P = 0.106, 0.975, 0.399$ for
379 adhered live, adhered dead and backwashed dead values).

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

386

387



388

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

395

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

405 fouling from the DS_f side to the FS_f side. The complexation and bridging of Ca^{2+} and EPS caused a thick,
406 dense, and stable biofilm.

407 Furthermore, Ca^{2+} has been shown to bind with the protein LapF, a key protein for biofilm
408 development of *P. putida*, and therefore form large aggregates in biofouling [76]. They reported an
409 11% increase in attached biomass on an LB agar plate containing *P. putida* after 2 hours of contact
410 with 10 mM $CaCl_2$. This occurred as Ca^{2+} promotes the interaction between the LapF molecules in
411 adjacent bacteria and therefore cell to cell interactions. This increase in cell interaction promotes
412 microcolony formation leading to an increase in biomass. This occurrence could also explain the
413 increase in surface coverage upon addition of Ca^{2+} to the FS_f.

414 The impact of Ca^{2+} concentration in the FS_f on osmotic backwashing efficiency was assessed.
415 The increase in Ca^{2+} concentration in the FS_f from 0 mM to 5 mM led to a decrease in osmotic
416 backwashing efficiency from 93% to just 60%, respectively (see Figure 2). The osmotic backwashing
417 flux, however, showed no trend with increased Ca^{2+} concentration in the FS_f, varying between 31.94
418 ± 3.34 $L.m^{-2}.h^{-1}$. As the adhesion forces between the bacterial cells and the membrane surface become
419 stronger with increasing Ca^{2+} concentration, the osmotic backwashing efficiency in detaching these
420 reduces. de Kerchove and Elimelech [79] demonstrated an increase in attachment efficiency of *P.*
421 *aeruginosa* onto clean and conditioned surfaces in the presence of divalent cations such as Ca^{2+}. Safari
422 et al. [77] also demonstrated higher adhesion of biofilms formed in the presence of Ca^{2+} using AFM:
423 adhesion forces increased from 1.61 ± 0.56 nN to 2.06 ± 1.03 nN as the Ca^{2+} concentration was
424 increased from 0 to 15 mM. As the adhesion increases with increasing Ca^{2+} concentration, the osmotic
425 backwashing method becomes inefficient as it needs to overcome stronger interaction forces between
426 the cells and the membrane surface. Hence higher osmotic backwashing fluxes might be required for
427 more challenging cases.

428

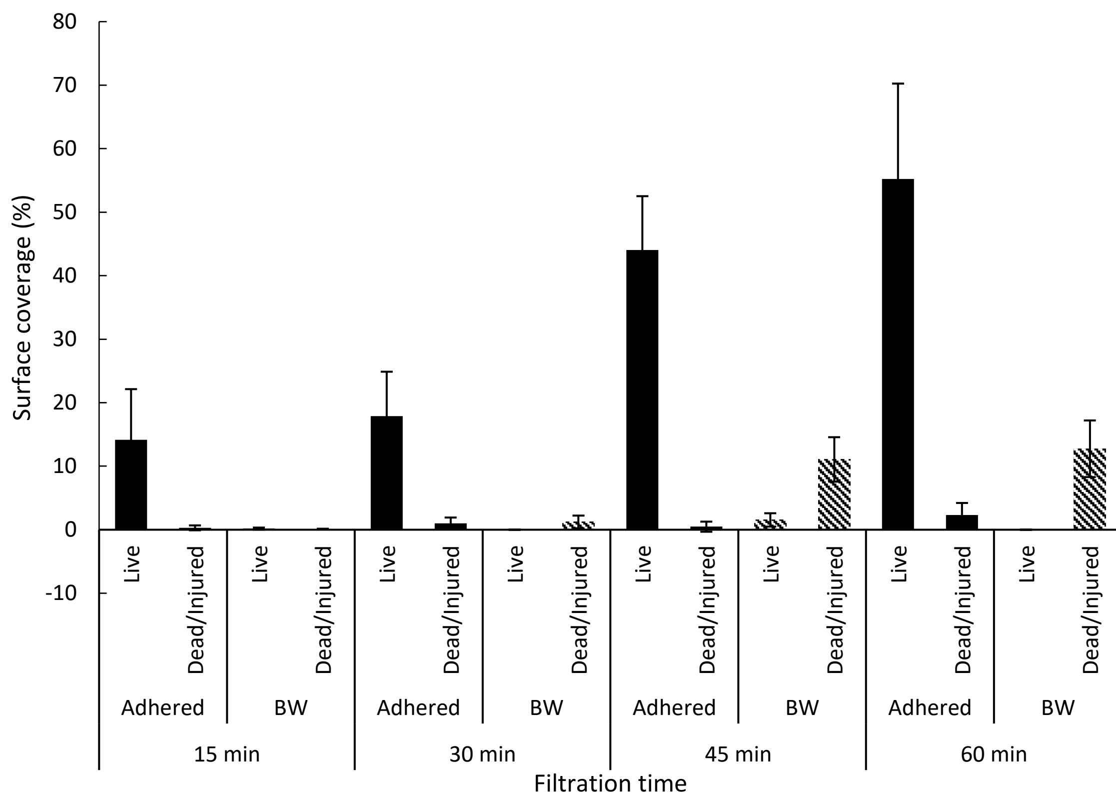
429 *3.3 Effect of filtration time on osmotic backwashing efficiency*

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

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

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

443



444

445 Figure 3 – Surface coverage (%) of live and dead/injured cells before and after osmotic backwashing
446 for different filtration times (Fouling conditions: DS_f = 0.7 M NaCl, FS_f = 200 ml of media containing
447 10^7 cells/ml *P. putida* in 0.1 M NaCl; osmotic backwashing conditions: DS_{obw} = 3 M NaCl, FS_{obw} =
448 deionised water, duration = 1 minute; error bars show standard deviation of repeated experiments
449 where the count was determined from 10 areas on the membrane surface)

450

451 In the same way the membrane flux during adhesion was unaffected by the adhered bacteria,
452 remaining at 16.7 ± 4.0 $L.h^{-1}.m^2$, the osmotic backwashing flux variation showed no trend by increasing

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

455 As the filtration duration increased and more cells adhered to the membrane surface, the cells
456 became more difficult to detach, with osmotic backwashing efficiency in bacterial removal decreasing
457 from 98% to 78% for filtration times of 15 and 60 minutes, respectively (see Figure 3). This is because
458 the adhesion force between the bacterial cell and the membrane surface increases with contact time.
459 Evidence for this is provided by several studies using AFM: adhesion forces between bacterial cells
460 and surfaces, measured by the pull off force during retraction of the tip from the surface, increased
461 with time [14, 81, 82]. Vadillo-Rodrigues [56] determined that bond strengthening occurred between
462 the AFM tip and a *Streptococcus thermophiles* cell within just 100 seconds of contact. BinAhmed et al.
463 [83] showed that *P. fluorescens* cells exhibited higher adhesion forces to ultrafiltration membranes,
464 from 0.4 nN to over 0.5 nN, when cell-surface contact time was increased from 2 to 5 seconds. Higher
465 increases in adhesion forces with time, from around -1.6 nN to -3.5 nN, have been measured for *S.*
466 *thermophilus* in 200 seconds [56, 57]. Also Harimawan et al. used AFM to show that the adhesion force
467 for *P. aerugin*osa increased from 3.84 nN to 8.53 nN when the contact duration between a bacteria
468 and a stainless steel surface increased from 0 to 60 seconds [57]. Therefore, in this study, it is likely
469 that as the filtration time was increased from 30 minutes to 60 minutes, the number of adhesive sites
470 between bacteria and membrane surface increased, hence increasing adhesion forces [84]. For this
471 reason, the cells became more difficult to remove for longer filtration times with this cleaning method.
472 However, for the cases of 45 and 60 minutes of filtration time, 11% and 12.7% of the surface remained
473 covered by dead/injured cells after osmotic backwashing, as opposed to $< 1.5\%$ coverage in live cells.
474 Although the osmotic backwashing method is not 100% efficient in detaching the bacterial cells from
475 the membrane surface, it is efficient in killing or injuring the bacteria, hence preventing biofilm
476 development on the membrane surface.

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

481

482 *3.4 Use of Ca^{2+} as DS_{obw} for bacteria detachment in osmotic backwashing*

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

485 backwashing method with 3 M NaCl, i.e. an osmotic backwashing flux of 36 $L.h^{-1}.m^2$, is no longer
486 effective. One way to increase the cleaning efficiency is to increase the osmotic backwashing flux by
487 increasing the driving force and hence use a DS_{obw} of higher osmotic pressure. A DS_{obw} with $CaCl_2$ can
488 be used to achieve this, as a solution of $CaCl_2$ has a much higher osmotic pressure than a NaCl solution
489 of the same osmolarity, and hence a higher osmotic backwashing flux is achieved (see Table 2).
490 However, osmotic backwashing with $CaCl_2$ may have drawbacks. The presence of Ca^{2+} ions in the FS_f
491 during adhesion was showed to enhance *P. putida* adhesion for 30 min, which made it more
492 challenging to detach (see Figure 2). Hence, the presence of Ca^{2+} in the DS_{obw} might increase adhesion
493 forces between the already adhered bacteria and the membrane surface, rather than detach the
494 bacteria, even with a higher osmotic backwashing flux. However, the impact of Ca^{2+} on already
495 adhered bacteria is unknown, and as osmotic backwashing occurs for 1 minute only, it is hypothesized
496 this would be too short a time for Ca^{2+} to have a significant impact on bacterial adhesion forces onto
497 the FO membrane surfaces, and a higher backwashing flux would be effective in detaching adhered
498 bacteria. In a previous study on alginic acid fouling control in FO with osmotic backwashing, it was
499 demonstrated that 1 minute of osmotic backwashing with $CaCl_2$ was enough time for the Ca^{2+} ions to
500 influence the fouling layer by cross linking with carboxyl groups in the alginate layer. This hindered
501 backwashing, rendering it inefficient [86]. However, de Kerchove and Elimelech [79] showed that the
502 impact of Ca^{2+} and Mg^{2+} on P. *aeruginosa* attachment efficiency was similar for these two cations, but
503 alginates responded differently when exposed to Ca^{2+} vs Mg^{2+}: this is potentially due to the difference
504 between bacterial EPS and alginate. The questions that now arise are: (1) what is the impact of Ca^{2+} in
505 the DS_{obw} on already adhered bacteria, and (2) will the increase in backwashing flux with a $CaCl_2$ DS_{obw}
506 be enough to overcome any potential interaction between Ca^{2+} in the DS_{obw} and the adhered bacterial
507 cells. This will be investigated in this section, where FO membranes were fouled for 30 minutes, and
508 DS_{obw} of varying $CaCl_2$ concentrations were tested in order to compare cleaning efficiency of NaCl *vs*
509 $CaCl_2$ DS_{obw} solutions.

510

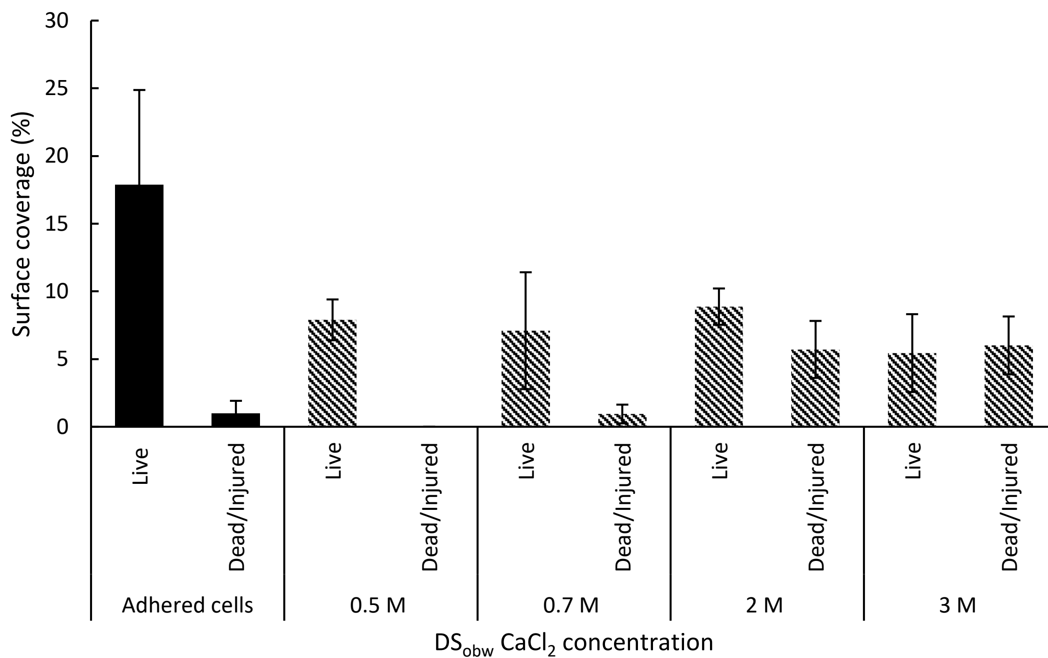
511 Table 2 – Osmotic backwashing fluxes for increasing DS_{obw} concentrations of NaCl or $CaCl_2$

Concentration (M)	Backwashing flux ($L.h^{-1}m^{-2}$)	
	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

512

513 Each membrane in Figure 2 and Figure 4 was subjected to identical adhesion conditions, 514 resulting in an average surface coverage of 17.9% live cells and 1.0% dead/injured cells. An increase 515 in NaCl concentration of the DS_{obw} was shown to cause higher osmotic backwashing fluxes, which was 516 accompanied by a higher cleaning efficiency (see Figure 1). As can be seen in Table 2, an increase in 517 $CaCl_2$ concentration of the DS_{obw} between 0.5 M and 3 M also resulted in increased osmotic 518 backwashing fluxes during cleaning, ranging between 23.7 $L.h^{-1}m^{-2}$ and 55.8 $L.h^{-1}m^{-2}$, respectively.

519



520

521 Figure 4 - Surface coverage (%) of live and dead/injured cells before and after osmotic backwashing 522 with DS_{obw} of different $CaCl_2$ concentrations (Fouling conditions: DS_f = 0.7 M NaCl, FS_f = 200 ml of 523 media containing 10^7 cells/ml *P. putida* in 0.1 M NaCl; osmotic backwashing conditions: DS_{obw} = 524 varying $CaCl_2$ concentrations, FS_{obw} = deionised water, duration = 1 minute; ; error bars show 525 standard deviation of repeated experiments where the count was determined from 10 areas on the 526 membrane surface)

527

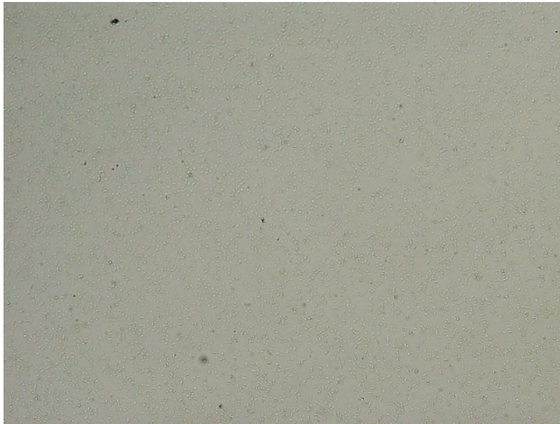
528 As opposed to NaCl, a reduced cleaning efficiency was obtained with an increased DS_{obw} $CaCl_2$ 529 concentration: the total bacterial removal rates were 58% for concentrations up to 0.7 M $CaCl_2$, 530 reducing to 22% and 39% for 2 M and 3 M $CaCl_2$ concentrations, respectively. There is quite a contrast 531 in the results obtained when using NaCl or $CaCl_2$ as a DS_{obw}: 2 M and 3 M $CaCl_2$ concentrations 532 originated osmotic backwashing fluxes of 47.5 $L.h^{-1}m^{-2}$ and 55.8 $L.h^{-1}m^{-2}$, which are higher than 36 $L.h^{-}$

533 $^{1}m^{-2}$ 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
534 adhered bacteria, as opposed to 22% and 39% for a DS_{obw} of 2 and 3 M $CaCl_2$, respectively. Osmotic
535 backwashing with $CaCl_2$ hence becomes less efficient as the DS_{obw} concentration increases despite the
536 fact that the osmotic backwashing flux is increasing, indicating an interaction between the adhered
537 bacterial cells and the DS_{obw}, even if just for 1 minute. This immediate influence of Ca^{2+} was also
538 demonstrated in previous studies with alginic acid fouling on FO and RO membranes [86, 87].

539 It has been reported that *P. putida* produce alginate in response to stress [63, 88], and *P.*
540 *aeruginosa* have been shown to increase their EPS production, including alginate, when Ca^{2+} ions
541 increased in the surrounding environment [89]. Although these studies were done for longer time
542 scales, up to 72 hours, the results in Figure 4 suggest that interaction between Ca^{2+} and bacterial EPS
543 might already be occurring for 30 min filtration times and 1 min osmotic backwashing, which is rather
544 surprising at these short times. It has been shown that P. *aeruginosa* demonstrate up-expression of
545 the alginate biosynthesis gene algC immediately after attachment [90], hence the same is likely to
546 occur with P. *putida*, where EPS is formed when subjected to high Ca^{2+} concentrations. The production
547 of alginate in the presence of Ca^{2+} will therefore protect the cells and increase cell adhesion forces
548 [77, 79], therefore making their detachment more difficult. Xie et al. [78] also demonstrated how Ca^{2+}
549 ions increased the hydrodynamic radius of bacterial EPS produced by Pseudomonas aeruginosa due
550 to ion complexation and bridging. Bacterial detachment is hence more difficult, even at higher osmotic
551 backwashing fluxes of 55.8 $L.h^{-1}m^{-2}$ achieved in the present study. Removal does, however, increase
552 from 2 M to 3 M, from 22% to 39%, respectively, indicating that an osmotic backwashing flux of 55.8
553 $L.h^{-1}m^{-2}$ was high enough to remove more bacteria.

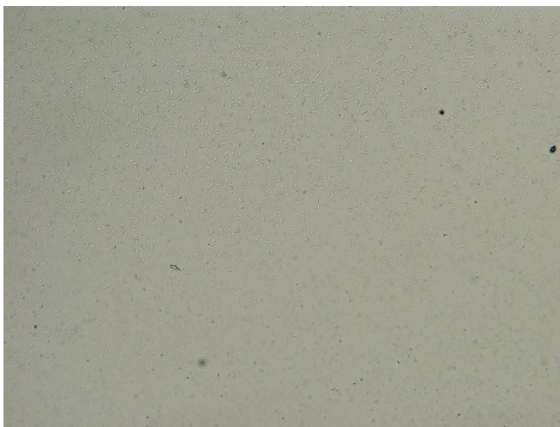
554 Experiments were performed on P. putida adhered onto well plates and subsequently
555 exposed to 0.1 M NaCl, 3 M NaCl and 3 M $CaCl_2$ to mimic osmotic backwashing conditions, to detect
556 EPS formation. The results are shown below in Figure 5 (more representative images can be found in
557 the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

558

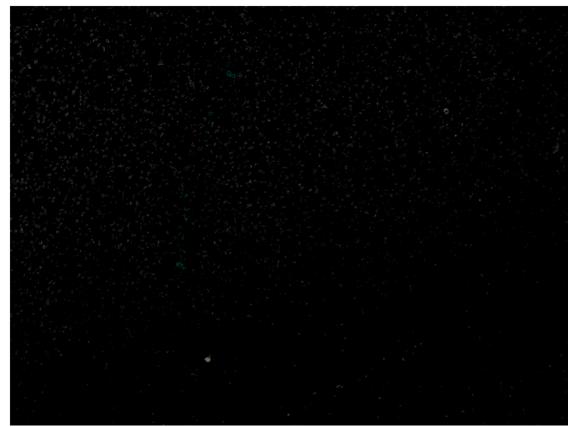


A – P. Putida cells after 30 minutes adhesion followed by 20 minutes contact with 0.1 M NaCl

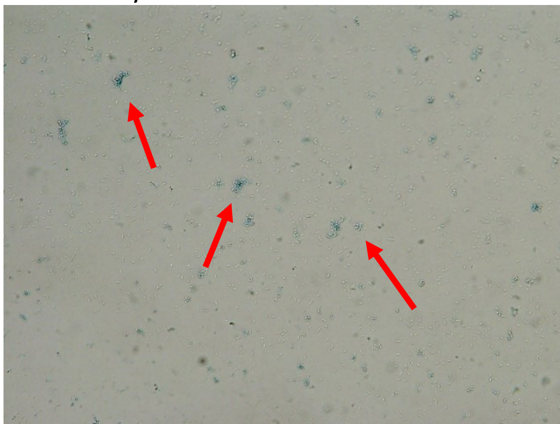
B - P. Putida cells after 30 minutes adhesion followed by 20 minutes contact with 0.1 M NaCl



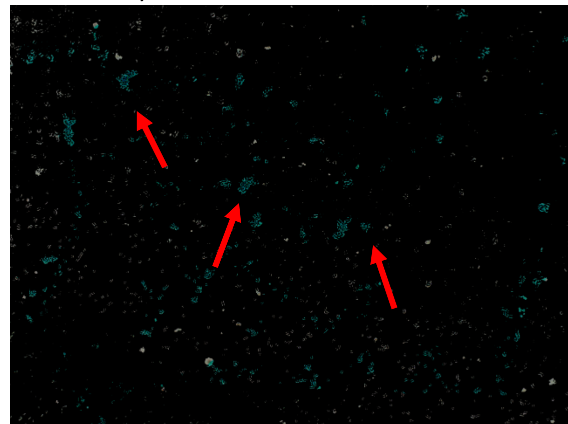
C – P. Putida cells after 30 minutes adhesion followed by 20 minutes contact with 3 M NaCl



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 Figure 5 - Microscopy representative images of 30 min P. *putid*a adhesion onto well plates, followed
561 by exposure to 0.1 M NaCl, 3 M NaCl and 3 M $CaCl_2$. Blue areas represent EPS. Figures 5B, 5D and 5F
562 were converted to black and thresholded using ImageJ software to improve the illustration of the
563 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% ± 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 osmolarity, 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 $L.h^{-}$
584 $^{1}m^{-2}$ and 23.7 $L.h^{-1}m^{-2}$, 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

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

605

606 ## 4. Conclusion
607

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

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

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

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

644

645

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659

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661

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