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1 The importance of laboratory water quality for studying initial bacterial
2 adhesion during NF/RO filtration processes

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19

20 Abstract

21 Biofouling of nanofiltration (NF) and reverse osmosis (RO) membranes for water treatment
22 has been the subject of increased research effort in recent years. A prerequisite for
23 undertaking fundamental experimental investigation on NF and RO processes is a procedure
24 called compaction. This involves an initial phase of clean water permeation at high pressures
25 until a stable permeate flux is reached. However water quality used during the compaction
26 process may vary from one laboratory to another. The aim of this study was to investigate
27 the impact of laboratory water quality during compaction of NF membranes. A second
28 objective was to investigate if the water quality used during compaction influences initial
29 bacterial adhesion.

30 Experiments were undertaken with NF270 membranes at 15 bar for permeate volumes of
31 0.5L, 2L, and 5L using MilliQ, deionized or tap water. Membrane autopsies were performed
32 at each permeation point for membrane surface characterisation by contact angle
33 measurements, profilometry, and scanning electron microscopy. The biological content of
34 compacted membranes was assessed by direct epi-fluorescence observation following
35 nucleic acid staining. The compacted membranes were also employed as substrata for
36 monitoring the initial adhesion of *Ps. fluorescens* under dynamic flow conditions for 30
37 minutes at 5 minutes intervals.

38 Compared to MilliQ water, membrane compaction using deionized and tap water led to
39 decreases in permeate flux, increases in surface hydrophobicity and led to significant build-
40 up of a homogenous fouling layer composed of a both living and dead organisms ($>10^6$
41 cells.cm⁻²). Subsequent measurements of bacterial adhesion resulted in cell loadings of
42 0.2×10^5 , 1.0×10^5 cells \times cm⁻² and 2.6×10^5 cells.cm⁻² for deionized, tap water and MilliQ water

43 respectively. These differences in initial cell adhesion rates demonstrate that choice of
44 laboratory water can significantly impact the results of bacterial adhesion on NF
45 membranes. Standardized protocols are therefore needed for the fundamental studies of
46 bacterial adhesion and biofouling formation on NF and RO membrane. This can be
47 implemented by first employing pure water during all membrane compaction
48 procedures and for the modelled feed solutions used in the experiment
49

50 **1 Introduction**

51 Nanofiltration (NF) and reverse osmosis (RO) membranes are commonly used for the
52 removal of organic matter and trace contaminants, such as pesticides, during water
53 treatment processes (Cyna et al. 2002). The efficiency of NF and RO processes is however
54 adversely affected by membrane biofouling (Flemming 1997, Ivnitsky et al. 2007), principally
55 due to the formation of biofilms (Flemming 2002). These ecosystems are usually made up of
56 a community of dead and living microorganisms held together by a matrix of
57 polysaccharides, lipids, proteins, organic matter, amongst other components (Flemming
58 2002). Biofilms are ubiquitous in NF and RO membrane plants (Houari et al. 2009,
59 Vrouwenvelder et al. 1998, Vrouwenvelder et al. 2008, Khan et al. 2013) and are the Achilles
60 heel of NF and RO processes (Flemming et al. 1997) as they are difficult to remove (Hijnen et
61 al. 2012). Biofouling increases pressure drop along the membrane module (Vrouwenvelder
62 et al. 2009a, Hijnen et al. 2009), leading to increased costs associated with energy
63 consumption. The presence of biofilms on the membrane surface has also been shown to
64 significantly affect permeate flux, and solute retention (Ivnitsky et al. 2005, Huertas et al.

65 2008). The decrease in solute retention and permeate flux has been attributed to enhanced
66 concentration polarisation caused by the biofilms (Herzberg and Elimelech 2007). It has
67 been shown that the concentration polarization also maintains the presence of biofilms by
68 concentrating nutrients from the bulk environment (Chong et al. 2008, Vrouwenvelder et al.
69 2009b).

70 Biofilm formation is initiated by the irreversible adhesion of bacterial cells onto the
71 membrane's surface, which is influenced by a number of factors. Firstly, the cell properties
72 such as hydrophobicity (Ridgway et al. 1985) and cell surface 76 charge (Subramani and
73 Hoek 2008) have been found to affect adhesion. Secondly, the membrane physicochemical
74 properties (roughness, charge and hydrophobicity) have been shown to impact the degree
75 of adhesion. In general, the rougher and the more hydrophobic the membrane is, the more
76 cells will adhere to the surface (Subramani and Hoek 2008, Myint et al. 2010, Khan et al.
77 2011). Finally, the presence of a conditioning layer on the membrane also affects bacterial
78 adhesion (Subramani et al. 2009). A recent study has shown that a conditioning layer of salts
79 and organic carbon promoted a homogeneous biofilm, whilst the absence of a conditioning
80 layer resulted in a scattered and thin biofilm (Baek et al. 2011).

81 The intractable nature of the biofouling problem has led to a significant increase in research
82 in this area in recent years (Herzberg and Elimelech 2007, Chong et al. 2008, Subramani and
83 Hoek 2008, Baek et al. 2011, Fonseca et al. 2007). These studies range from the effects of
84 biofilms on process performance (Ivnitsky et al. 2005, Huertas et al. 2008) to biofouling control
85 through the design of antifouling membranes (Miller et al. 2012, Bernstein et al. 2011).

86 Although membrane biofouling research methodologies differ from one research laboratory
87 to another, they generally share a common pre-treatment procedure involving the

88 compaction of the studied membrane prior to biofouling experiments. To accurately
89 monitor flux changes and solute retention during NF and RO experiments caused by osmotic
90 pressure or membrane fouling, membranes are purposely compacted to prevent changes
91 due to the effect of pressure during the experiment. The compaction of NF and RO
92 membranes is carried out under different filtration conditions depending on the laboratory
93 they are carried out. The compaction is typically undertaken at a pressure between 6 and 25
94 bar and up to 18 hours in duration (Herzberg and Elimelech 2007, Baek et al. 2011, Fonseca
95 et al. 2007, Suwarno et al. 2012). This translates into a 99 typical water permeation volume
96 between 2 L (membrane flux=50 L.h⁻¹.m⁻², time=18 hours and 22.44 cm² 100 membrane
97 area) and 15 L (membrane flux=65 L.h⁻¹.m⁻², time=12 hours and 186 cm² 101 of membrane
98 area) (Baek et al. 2011, Suwarno et al. 2012) calculated as: $V(L)=Flux(L/h.m^2)\times time(h)$
99 $\times Membrane\ Area(m^2)$.

100 Although membrane compaction is a prerequisite to most NF and RO experimental studies,
101 including bioadhesion/biofouling, the type of water used to compact the membrane may
102 vary considerably from one laboratory to another. The water used in recent published
103 studies on initial adhesion and biofouling experiments spans from non-sterilised tap water
104 (Hijnen et al. 2009, Khan et al. 2011, Vrouwenvelder et al. 2009c, Vrouwenvelder et al.
105 2007, Botton et al. 2012, Khan et al. 2010), DI water (Huertas et al. 2008, Herzberg and
106 Elimelech 2007, Myint et al. 2010, Baek et al. 2011, Lee et al. 2010) and MilliQ water (Chong
107 et al. 2008, 2007, Pang et al. 2005). Tap water and DI water will vary in quality depending on
108 the water source and the yearly season (Gibbs et al. 1993). In essence, the total carbon,
109 biological and endotoxin contents will differ from one water type to another, whether the
110 water is sterilized or not. Moreover, when considering filtration aspects, all insoluble water
111 constituents will most certainly be deposited on the membrane surface during the

112 compaction, thus altering the membrane surface from its original state. The conditioning
113 layer formed during the compaction pre-treatment of NF/RO is likely to result in altered
114 surface characteristics thereby affecting subsequent biofouling experiments.

115 The objective of this study was to first demonstrate the impact of the choice of water used
116 during compaction of NF membranes in terms of membrane performance, surface
117 characterisation and secondly, to investigate whether the 121 water used during membrane
118 compaction also affects bioadhesion outcomes.

119

120

121 **2 Materials and Method**

122 2.1 Water source and characterisation

123 Three different water grades were used in our study: tap water provided by south Dublin
124 water municipality, deionized water obtained by a purifying water system (Elgastat B124,
125 Veolia, Ireland) and Grade 1 pure water ($18.2 \text{ M}\Omega\cdot\text{cm}^{-1}$) obtained by an Elga Process Water
126 System (Biopure 15 and Purelab flex 2, Veolia, Ireland), hereafter referred to as MilliQ
127 water. Conductivity and pH measurements were performed on all water samples at room
128 temperature (20°C) and total organic carbon of all water samples was determined using a
129 total organic carbon analyser (TOC-VCSH, Shimadzu, Ireland) in the NPOC mode, equipped
130 with an automatic sample injector and an NDIR detector. Calibration standards were made
131 using potassium hydrogen phthalate at different concentrations between 0 and 10 mgC/L.

132 The water samples were collected in new 50 mL sterile Eppendorf tubes, filtered twice with

133 polyethersulphone 0.2 μm filters (Corning Incorporated, VWR Ireland), put in TOC vials that
134 had been soaked overnight in 0.5 M NaOH in MilliQ water and left to dry at 30°C upside
135 down to avoid any contamination from the air followed by a thorough rinsing with MilliQ
136 water and analysed straight away. Measurement results are presented in Table 1. The
137 measurements were carried out from samples taken between October and November 2012.
138 In the particular case of MilliQ water, the samples have a measured TOC concentration that
139 varied from 0.00 to 0.24 $\text{mgC}\cdot\text{L}^{-1}$. The average value measured for 10 samples were
140 $0.13\pm 0.06 \text{ mgC}\cdot\text{L}^{-1}$ (Table 1).

141 The total solids were measured by filling a glass vial with 40 mL of each water source and
142 allowing it to evaporate in an oven at 100°C. The vial weight was measured before and after
143 evaporation and the difference obtained in weight corresponded to the total solids weight.
144 A control sample was used, where an empty vial was placed in the oven to ensure no
145 floating particles present could affect the results.

146 The pH was measured with a HI1332 pH probe (Hannah, VWR, Ireland) and the conductivity
147 was measured with a TetraCon 325 conductivity probe (WTW, VWR, Ireland).

148 The total amount of cells in each water source was determined as follows: a volume of 100
149 mL of MilliQ and tap water and 80 ml of DI water were filtered with a 0.2 μm filter (GTBP-
150 25mm, Millipore, Ireland). The filter was then removed, placed in 3 mL of raw water without
151 carbon (as explained in section 2.3.1) and stained with SYTO[®] 9 and PI dyes. The filter was
152 then observed under an Epi-fluorescence microscope (Olympus BX 51) using a 40x objective.
153 Fluorescent organisms were observed using two filter cubes each exciting SYTO[®] 9 and PI
154 dyes at 450 nm and 550 nm respectively. At least ten micrographs were obtained at 5

155 random points on each compacted stained membrane sample. The number of fluorescent
156 organism were then counted using Image J® (NIH, Bethesda, MD, USA).

157

158

159 2.2 Compaction experiment

160 NF 270 membranes (Filmtec Corp. USA) were used as the reference nanofiltration
161 membrane in this study. The membranes were gently washed and left soaking overnight in
162 the fridge in the water source they were going to be exposed to during the experiment in
163 order to remove all the preservatives. The membrane compaction experiments were
164 performed in Membrane Fouling Simulators (Vrouwenvelder et al. (2006)) at 15 bar
165 pressure and a feed flow rate of 0.66L/min in each cell using one type of water grade for
166 each experimental run. This flow rate corresponds to a velocity of 0.35 m/s, a Re_{dh} of 579
167 and a shear rate of 2588 s^{-1} in each cell.

168 The cross-flow system was equipped with a 10 L autoclavable feed tank (Nalgene, VWR
169 Ireland) and a high pressure pump (P200 from Hydra-Cell, UK). The system was connected to
170 three MFS devices placed in parallel holding each NF 270 membranes on the experimental
171 rig at the start of compaction. The MFS cells are of the slit type channel height of 0.8 mm,
172 width of 40 mm and length of 255 mm. Each membrane cell holds a membrane of 102 cm^2 .
173 Separate experiments were carried out in the cross-flow system in order to confirm that the
174 feed flow rate was distributed evenly by the three cells. The pressure on the outlet side of
175 the slit feed channel of each of the 3 membrane cells was measured during operation at
176 different flow rates and pressures. The pressure between the different MFS cells did not
177 vary by more than 2% for the conditions tested, showing that the flow rate distributes

178 evenly in the system. Temperature was monitored in the feed tank with a temperature
179 indicator (Pt 100, Radionics, Ireland) and maintained at $20^{\circ}\text{C} \pm 1^{\circ}\text{C}$ with a coil inside the tank
180 connected to a temperature controlled MultiTemp III water bath (Pharmacia Biotech,
181 Ireland). A back pressure regulator (KPB1LOA415P20000, Swagelok, UK) allows the
182 pressurization of the system. The pressure was monitored in both feed and retentate side of
183 the membrane cells with two pressure transducers (PTX 7500, Druck, Radionics, Ireland).
184 The feed flow was measured using a flow meter (OG2, Nixon Flowmeters, UK). Datalogging
185 was set-up for monitoring inlet and outlet pressure, feed flow rate and temperature
186 (PicoLog 1000, PicoTechnology, Radionics, Ireland). The permeate volume collected was
187 measured using 1000 mL graduated bottle, where the permeate volume was not returned
188 to the feed tank. The permeate flux was determined by measuring a volume of permeate
189 with a balance HCB123 balance (Adams, Astech Ireland) with a stopper. The P&ID of the
190 crossflow filtration system is depicted in Figure 1.

191

192 Permeate flux and permeate conductivity measurements were performed throughout the
193 compaction experiment. Once permeate levels reached 0.5L for each MFS device, the
194 compaction was temporarily stopped to allow removal of one MFS device from the rig.
195 Compaction was thereafter continued for the two remaining MFS devices until permeate
196 levels reached 2L levels each, at which point the second MFS was removed from the system.
197 The last MFS was left compacting until 5L permeate was collected.

198 Once removed from the rig system, the MFS device containing a compacted NF270
199 membrane was opened while submerged under the corresponding water type. Membrane

200 samples were immediately cut to size for autopsy and dynamic adhesion experiments as
201 described in sections 2.2.1.4 and 2.3.2. The remainder of the membrane was left to dry in a
202 closed box at room temperature for at least 48 hours to ensure the membrane was dry.

203

204 **2.2.1 Surface characterisation assays of compacted NF270 membranes.**

205 **2.2.1.1 Profilometry**

206 Optical profilometry analysis was carried out to examine the morphology and to quantify
207 surface roughness. These measurements were carried out using a Wyko NT1100 optical
208 profilometer operating in vertical scanning interferometry (VSI) mode. The R_q (root mean
209 square roughness) was obtained on three different locations on each sample surface and an
210 average value obtained.

211

212 **2.2.1.2 Contact angle measurements**

213 The Lifshitz-van der Waals (γ^{LW}), electron-donor (γ^-) and electron-acceptor (γ^+) surface
214 tension components of dehydrated compacted NF 270 membrane samples (S) were
215 determined by measuring contact angles using the following expression:

$$216 \quad \cos\theta = -1 + 2 (\gamma_S^{LW} \gamma_L^{LW})^{\frac{1}{2}} / \gamma_L + 2 (\gamma_S^+ \gamma_L^-)^{\frac{1}{2}} / \gamma_L + 2 (\gamma_S^- \gamma_L^+)^{\frac{1}{2}} / \gamma_L \quad (1)$$

217 Contact angles (θ) and surface energy measurements (γ^S) of dehydrated compacted NF 270
218 membrane were measured at room temperature using a goniometer (OCA 20 from
219 Dataphysics Instruments) with three static pure liquids (L): deionised water, diiodomethane
220 and ethylene glycol.

221 The Lewis acid-base component was deduced from:

$$222 \quad \gamma_S^{AB} = 2\sqrt{(\gamma_S^+ \gamma_S^-)} \quad (2)$$

223 And the total surface energy was defined by:

$$224 \quad \gamma_S = \gamma^{AB} + \gamma^{LW} \quad (3)$$

225

226 Contact angle values and determined surface energies values presented in table 2
227 represent the mean of at least 10 measurements per compacted sample membrane.

228 Contact angle measurements were repeated in two independent replicates.

229

230 **2.2.1.3 Scanning electron microscopy**

231 For high resolution *ex-situ* observations of the membrane surface, the compacted NF 270
232 membranes were dehydrated by drying in air and then gold coated for 30 sec at x V 30 mA.

233 High magnification imaging of the membrane surfaces was performed under a Hitachi SEM
234 at the UCD Nano-imaging and Materials Analysis Centre.

235

236 **2.2.1.4 Biological assessment of NF 270 compacted membranes**

237 For assessing the biological presence on membranes samples, three regions of the
238 compacted NF 270 membranes were cut and placed in small petri dishes containing 3mL of

239 the water grade used during compaction. Membrane samples were then stained by adding

240 0.5 μ L of 3.34mM SYTO[®] 9 green-fluorescent nucleic acid staining solution and 0.5 μ L of 20

241 mM propidium iodide red-fluorescent nucleic stain. Stained membrane samples were

242 subsequently incubated in the dark for at least 15 minutes, after which the staining mix was
243 discarded from the petri dish and a cover slip placed on membrane surfaces. The stained
244 sample was then observed under an Epi-fluorescence microscope (Olympus Bx 51) using a
245 20x objective. Fluorescent organisms were observed using two filter cubes each exciting
246 SYTO[®] 9 and PI dyes at 450nm and 550nm respectively. Ten micrographs were obtained at 5
247 random points on each compacted stained membrane sample. The number of fluorescent
248 organism was then counted using Image J[®], an image quantification software.

249

250 2.3 Initial adhesion assay on NF270 compacted membranes

251 **2.3.1 Microbial strain and culture conditions**

252 The *Pseudomonas fluorescens* NCTC 10038 was selected for dynamic adhesion assays on
253 compacted NF 270 membrane. The cells were stored at -20°C with 20% volume glycerine as
254 a cryoprotectant. Prior to experiments, cells were spread on King B agar (Oxoid) and
255 incubated at 30°C overnight. Single colonies were then inoculated in were then cultured at
256 30°C and 150 rpm in Raw Water (RW) medium (tryptic soy broth 0.3 g.L⁻¹, sodium citrate
257 Na₃C₆H₅O₇ 0.26 g.L⁻¹, NaHCO₃ 0.042 g.L⁻¹, NaCl 0.12 g.L⁻¹, KH₂PO₄ 0.063 g.L⁻¹, MgSO₄ 0.15
258 g.L⁻¹, NH₄Cl 0.005 g.L⁻¹, CaCl₂ 0.076 g.L⁻¹). When cell density reached 0.270 at OD₆₀₀, 12mL
259 of the overnight culture were centrifuged at 7000 rpm for 10 minutes, before discarding
260 supernatant. The remaining pellet was suspended with Raw Water medium containing no C-
261 source (RW^C) to a final volume of 1 mL. The cells were then stained by adding 0.5 μL of
262 3.34mM SYTO[®] 9 and 0.5 μL of 20 mM propidium iodide, followed by a 15 minutes
263 incubation period at room temperature in the dark. The stained *Ps. fluorescens* cells were
264 then centrifuged at 7000 rpm for 10 min before discarding the supernatant. The remaining

265 pellet was then re-suspended in 24 mL RW^c medium prior to adhesion experiments order to
266 attain a final cell concentration of 10⁷ cells.mL⁻¹.

267

268 **2.3.2 Initial adhesion assay**

269 Initial adhesion assays were performed on freshly cut compacted membranes that were
270 placed on a support inserted in a flow cell (Model BST FC 81, Biosurface Technologies
271 Corporation, Bozeman, MT) with modified channel dimensions of 0.8 by 12.7 by 47.5 mm.
272 Compacted membranes were immobilized on the support using double sided tape, and
273 hydration was ensured by filling the flow cell chamber with RW^c prior to adhesion
274 experiments. The flow cells are small continuous-flow systems with a glass viewing port that
275 allowed *in situ* observation using an Epi-fluorescence microscope (Olympus BX 51) and a 20x
276 objective. After removing bubbles from the system, “zero point” images of the NF270
277 compacted surface were recorded using two filters with excitation wavelengths set at
278 λ_{ex} 450nm and λ_{ex} 550nm respectively. The freshly stained 24 mL *Ps. fluorescens* cells
279 suspension was then circulated in the system at a volumetric flow rate of 1.5 ml min⁻¹.
280 Adhesion time was recorded after 1, 5, 10, 15, 20, 25 and 30 minutes after the first observed
281 cell adhered on the surface. Two images were recorded at λ_{ex} 450 nm and λ_{ex} 550 nm for
282 each time point. Images of a size of 223 μ m x 1627 μ m were taken and analysed by counting
283 adhered stained *Ps. fluorescens* cells using Image J[®].

284 The initial adhesion kinetics of *Ps. fluorescens* on compacted membranes was established
285 for all water sources using the following equation:

$$286 \quad q(t) = q_{max} \cdot (1 - e^{-\beta t}) \quad (4)$$

287 where $q(t)$ is the bacterial loading as a function of time (t), the maximum cell loading q_{max}
288 and the accumulation factor β obtained by the exponential fit of the adhesion experimental
289 data. The linear region of the obtained curve was used to calculate the rate of adhesion by
290 using the following expression:

$$291 \quad k_d = \frac{\theta(t)}{\Delta t} \cdot \frac{1}{C_0} \quad (5)$$

292

293 where, k_d is the deposition rate of *Ps. fluorescens* on NF 270 membranes, $\vartheta(t)$ the number
294 of adhered cells over a time period (Δt) between two time points and C_0 the initial bacterial
295 suspension feed concentration.

296

297

298

299

300 **3 Results**

301 3.1 Water quality assessment

302 The different water qualities used for compacting NF 270 membranes used in this study
303 were characterized prior to pre-compaction experiments and are presented in Table 1.

304 No detectable solids were measured in MilliQ and deionized water samples used in this
305 study. MilliQ water had the lowest pH, total organic carbon, and conductivity values
306 compared to deionized and tap water, respectively. MilliQ water has a very low conductivity

307 of $0.4 \mu\text{S}\cdot\text{cm}^{-1}$ followed by DI water with a conductivity of $4 \mu\text{S}\cdot\text{cm}^{-1}$. The highest
308 conductivity obtained was for tap water with $168 \mu\text{S}\cdot\text{cm}^{-1}$.

309 No cultivable cells (determined by CFU) were found in MilliQ. However deionized water was
310 found to contain 170 times the number of cultivable organisms found in tap water. Direct
311 count analysis (counting both culturable and non-culturable cells) revealed the presence of
312 significant higher amounts of microorganisms in all tested water samples. Deionized water
313 was found to contain 1800 and 15 times more microorganisms than in MilliQ and tap water,
314 respectively. Moreover, deionized water also contained 850 and 30 times the amounts of
315 dead/injured microorganisms found in MilliQ and tap water, respectively.

316

317 3.2 Effects of different water grades on NF 270 membrane performance during
318 compaction.

319 In order to determine how each water source impacted the permeate flux during
320 compaction, the permeate flux was measured for 0.5, 2 and 5 L of permeated water in the
321 MFS cells. The results are presented in Figure 2. After a volume of 0.5 L of water permeated
322 through the membrane, membranes compacted with tap water showed the lowest
323 permeate flux of $195 \text{ L}\cdot\text{h}^{-1}\cdot\text{m}^{-2}\cdot\text{bar}^{-1}$ compared to membranes compacted with deionized and
324 MilliQ water, which had permeate fluxes of 283 and $339 \text{ L}\cdot\text{h}^{-1}\cdot\text{m}^{-2}\cdot\text{bar}^{-1}$ respectively.
325 Additionally, the use of tap water during compaction led to a constant decrease in permeate
326 flux from $195 \text{ L}\cdot\text{h}^{-1}\cdot\text{m}^{-2}\cdot\text{bar}^{-1}$ to $96 \text{ L}\cdot\text{h}^{-1}\cdot\text{m}^{-2}\cdot\text{bar}^{-1}$ with the NF 270 membrane, as permeate
327 volume increased from 0.5 L to 5 L. Visual inspection showed that the membrane surface
328 was light coloured at 0.5 L and gradually increased to a dark yellow colour at 5 L (not shown)
329 for tap water. In contrast, permeate flux stabilized after 0.5 L of water permeated through

330 the membrane for membranes compacted with deionized and MilliQ: from 2 L to 5 L the
331 permeate flux stabilised at 251 and 301 L.h⁻¹.m⁻².bar⁻¹, respectively.

332

333 3.3. Effect of different water grades on NF 270 membrane surface properties.

334 The physico-chemical properties of NF 270 compacted membranes were evaluated by
335 contact angle measurements and the associated van der Waals (γ^{LW}), Lewis Base (γ^-) and
336 Lewis Acid (γ^+) components are presented in Table 2. Membranes exhibited increased
337 hydrophobic character with increased permeate volumes. Membranes compacted with 5 L
338 of MilliQ water showed the lowest hydrophobic properties ($\theta_{water} = 49.7$), followed by
339 membranes compacted with tap water ($\theta_{water} = 56$), and deionized water ($\theta_{water} = 68.9$)
340 respectively, with the surface hydrophobicity varying in the following order: MilliQ < tap < DI
341 water. Increasing permeate volumes can be seen to have not affected the van der Waals
342 (γ^{LW}) character of the compacted membranes. However, membranes compacted with
343 deionized water showed lowest van der Waal surface properties with 37 mJ.m⁻² compared
344 to membranes compacted with MilliQ (42.3 mJ.m⁻²) and tap (45.5 mJ.m⁻²) water
345 respectively. A significant decrease in Lewis Base (γ^-) membrane character was noticeable
346 from 2L to 5L permeation using MilliQ water, whereas the same drop in Lewis base
347 properties occurred earlier from 0.5L to 2L in membranes compacted with deionized and
348 tap water. Membrane Lewis Acid (γ^+) character decreased by 2.5 fold from 0.5L and 2L
349 permeated volumes of MilliQ, and during 2L to 5 L permeated volumes of deionized water.
350 A 13.7 fold increase of Lewis Acid (γ^+) character was observed in compacted membrane
351 after permeation of 2L to 5L tap water.

352 Roughness measurements of the different compacted membranes measured by optical
353 profilometry are also presented in Table 2. No significant differences in surface roughness
354 were observed for compacted membranes after permeation of 0.5L and 2L MilliQ, deionized
355 water, and tap water. Although no differences in surface roughness were observed for
356 membranes compacted with MilliQ (511 ± 143 nm) and deionized water (562 ± 123 nm)
357 after 5 L permeation, membranes compacted with tap water showed highest roughness
358 with 1166 ± 147 nm. Height topography results presented in Figure 3 showed that the
359 surface of compacted membranes using MilliQ water (Figure 3 A) showed areas of very
360 smooth surface topology profiles and areas with irregular and heterogeneous surface
361 topology profiles, due to what looked like surface defects, presumably from the
362 manufacturing process. Topological profiles of membranes compacted with deionized
363 (Figure 3 C) and tap water (Figure 3 D) had a consistent and homogeneous roughness and
364 no surface defects were observed. The surface of NF270 membranes compacted with tap
365 water (Figure 3 D) had however frequent high narrow peaks compared to the smaller peaks
366 obtained with the DI water compaction.

367 Closer examination of the membrane surfaces using scanning electron microscopy revealed
368 distinct levels of deposition depending on water grade (Figure 4). The virgin NF270 surface
369 was relatively smooth with the presence of numerous large heterogeneities (Figure 4 A).
370 These structures were still visible after compaction with MilliQ water (Figure 4 B). Following
371 compaction with deionized, the membrane's surface was covered by what seemed to be a
372 matrix layer composed of microorganisms, and biological debris and possibly organic carbon
373 (Figure 4 C). When compacted with the DI water the large heterogeneities found on the
374 virgin membrane were not present, and the fouling layer caused by the DI water filtration,

375 although rough in the nanoscale, was homogeneous in the microscale. In this case a
376 distinction needs to be made: although Table 2 shows similar roughness values (R_q) for the
377 membranes compacted with the MilliQ water and the DI water, in the first case the
378 membrane is very smooth with a scattered distribution of imperfections generally with
379 valley widths between 20 to 50 μm (Figure 3 A) and in the second case, although the
380 membrane is rough, it is homogeneously rough (Figure 3 B).

381 Membrane compaction using tap water led significant membrane fouling also including the
382 presence of aquatic organisms such as diatoms, small microorganisms and a pronounced
383 amount of debris (*e.g.* organic carbon) (Figure 4 D). The level of membrane fouling is
384 apparent the degree of crack artefacts observed on the membrane's surface (Figure 4 C-D)
385 caused by dehydration, especially in the case of samples compacted with tap water.

386

387 3.3 Biological assessment of NF 270 membranes after compaction using different water 388 grades.

389 The biological characteristics of the compacted NF 270 membranes was assessed by nucleic
390 acid BakLight® staining and is presented in Figure 5. Compaction using deionized and tap
391 water led to a pronounced two log difference in the total presence of microorganisms (10^7
392 cells.cm^{-2}) on the membrane compared to membranes compacted with MilliQ water after 5L
393 volume permeation ($10^5 \text{ cells.cm}^{-2}$). A one log biological accumulation was noticeable from
394 0.5L to 5 L permeated volumes during compaction using all tested water qualities.
395 Compacted membranes using MilliQ water showed lowest counts of dead/injured
396 microorganisms throughout the compaction experiment with counts below $2 \times 10^4 \text{ cells.cm}^{-2}$.

397 A significant increase in dead-injured cell counts was noticeable for membranes compacted
398 with deionized and tap water only after 5L volume permeation.

399

400 3.4 Dynamic adhesion assay on compacted NF 270 membranes using different water
401 grades.

402 Dynamic adhesion assays were performed on compacted membranes to establish whether
403 permeation using different water qualities could affect the initial adhesion of *Ps. fluorescens*
404 in terms of amount deposited on membranes and their deposition rates. Adhesion results of
405 *Ps. fluorescens* cells conducted on compacted membranes which underwent 5 L permeation
406 volumes of different water qualities are presented in Figure 6 and Table 3.

407 Different adhesion profiles were observed for *Ps. fluorescens* cells on the different
408 compacted membranes during the 30 minutes period. Cell adhesion was highest on
409 membranes compacted with MilliQ water after 30 minutes with 2.6×10^5 cells.cm⁻² followed
410 by cell deposition on membranes compacted with tap water at 1.0×10^5 cells.cm⁻² (Figure 6).
411 Cell deposition was lowest on membranes compacted with deionized water at 0.2×10^5
412 cells.cm⁻². The order of total cells adhered on the different surfaces after 30 min showed the
413 following order: DI<tap<MilliQ water. The experimental data allowed maximum cell loadings
414 on the different membranes to be deduced based on kinetic model (cf. eq.2). Membranes
415 compacted with MilliQ water revealed highest maximum cell loadings at 2.6×10^5 cells×cm⁻²,
416 being 5 times higher than cell loadings on membranes compacted with deionized water
417 (Table 3). No maximum could be established after 30 minutes adhesion on membranes
418 compacted with tap water (Figure 6) since the adhesion was still in its linear phase after 30
419 minutes of the experiment. However, Figure 6 clearly shows that q_{max} on membranes

420 compacted with tap water is higher than the q_{max} value obtained for membranes compacted
421 with deionized water. Adhesion velocity was found to be slowest on membranes compacted
422 with deionized water at 1.06×10^{-5} cm.min⁻¹ and tap water at 3.13×10^{-5} cm.min⁻¹. *Ps.*
423 *fluorescens* cells expressed highest adhesion velocities on membranes compacted with
424 MilliQ water at 11.7×10^{-5} cm.min⁻¹ (Table 3).

425

426 **4 Discussion**

427

428 The aim of this study was to investigate the effects of laboratory water quality during pre-
429 compaction of nanofiltration membranes in terms of performance, surface property
430 changes as well as its influence on standard bio-adhesion assays.

431 Filtration performance together with the physicochemical, physical properties and biological
432 assessment of NF270 membrane surface were analysed at 0.5L, 2L and 5L set permeation
433 volumes during compaction with different water sources. The dynamic bioadhesion assays
434 were subsequently performed on the compacted NF 270 membranes using *Ps. fluorescens*
435 cells, and experimental data was used to calculate adhesion rates as well as estimate
436 maximum cell loadings on membranes. This allowed conclusions to be drawn about the
437 consequential effects of laboratory water quality in membrane compaction of nanofiltration
438 processes.

439 Results obtained in this study show that the water quality used during compaction of
440 membranes, a requisite in most membrane research laboratories, will most certainly affect
441 membrane surface physicochemical properties prior to performing key experiments

442 involving bacterial adhesion. Such changes on the membrane's surface due to membrane
443 pre-treatment might be the basis of experimental biases. Indeed, membrane compaction is
444 in itself a form of filtration whereby the elements found in the water will end up deposited
445 on the membrane's surface.

446 Membrane performance in terms of permeate flux is directly linked to water quality. The
447 observed higher decrease of the permeate flux with increased permeated volume of tap
448 water compared to DI and MilliQ water was caused by a higher concentration in the feed
449 solution of organic matter, ions and dissolved solids which, not only led to the formation of
450 a thicker fouling layer on the membrane surface, but also led to a higher osmotic pressure
451 difference between the feed and the permeate side. In this case, a combination 446 of the
452 cake build-up on the membrane surface and higher ionic concentration on the feed side can
453 aggravate the permeate flux decline due to cake enhanced concentration polarisation (Hoek
454 and Elimelech 2003). Moreover this fouling was also visible in the form of a coloration
455 gradient as the volume of water permeated through the membrane increased: the degree
456 of membrane coloration (yellow coloration) increased with increasing permeation volume
457 and decreasing water purity. A study by Van der Bruggen and Vandecasteele (2001) showed
458 that flux decline during nanofiltration was predominantly caused by adsorption of organic
459 compounds in aqueous solution onto the membrane, leading to the blocking of pores.

460 It might be surprising that the amount of microorganisms in DI water is higher compared to
461 tap water, as DI water is purified tap water. However, the ion exchange resin has been
462 found to be a good place for microorganisms to adhere onto and proliferate (Flemming
463 1987) for several reasons: (1) the negatively charged solutes such as TOC and other
464 nutrients such as nitrate are removed from the water by the ion exchange resin and

465 consumed by the microorganisms in the resin, (2) nutrients dissolved in tap water are used
466 by the microorganisms in the ion exchange resin as a food source and (3) the resin itself is a
467 possible food source for bacteria as it can leach solutes to the solution.

468 The deposition of solutes on the membrane surface will inevitably change the membrane
469 surface properties. The observed change in surface hydrophobicity in NF270 membranes
470 was dependent on the water used during compaction. The NF 270 membrane, known for its
471 hydrophilic properties (Boussu et al. (2006)), became more hydrophobic following
472 compaction with tap and deionized water respectively. In an earlier study, Her *et al.* ((2008))
473 demonstrated that the levels of hydrophobic and hydrophilic fractions in Natural Organic
474 Matter (NOM) found in water that deposited onto NF membranes determined the change in
475 surface hydrophobicity. Likewise, the observed difference in surface hydrophobicity
476 following compaction could have been attributed to the original fraction of hydrophilic
477 levels of NOM found in the tested water. Interestingly, membranes compacted with
478 deionized water having a factor 6 times less total organic carbon than tap water, were found
479 to be most hydrophobic. This may suggest that the deionized water in this study might have
480 contained a higher fraction of hydrophobic NOM, most likely caused by leachable residuals
481 from the ion exchange resin of the laboratory's water purifier. Surprisingly, the biological
482 content of the deionized water was also found to be prominent. The combination of high
483 TOC biological levels found in deionized water might be an indication of a possible polluted
484 and contaminated ion exchange resin. Accordingly, simple conductivity measurements
485 should not be the sole basis for verifying the purity of deionized water. Moreover, the use of
486 sterilized deionized water would only kill the microorganisms present, but not prevent their
487 deposition on the membrane surface after compaction (Figure 7).

488 The change in surface properties following membrane compaction was sufficient to
489 influence bacterial adhesion rates. Membranes compacted with MilliQ water attained the
490 highest bioadhesion and adhesion velocities followed by membranes compacted with tap
491 water and deionized respectively. Surprisingly, the extent of cell adhesion was not
492 proportional to hydrophobicity. Despite hydrophobicity being pinpointed as one of the
493 causes for higher adhesion onto surfaces (Lee et al. (2010), Myint et al. (2010), Subramani
494 and Hoek (2008)), it does not in itself explain the adhesion extent of the bacteria in this
495 study: the MilliQ water compacted membrane despite being more hydrophilic has a higher
496 cell adhesion than the more hydrophobic surfaces of the tap and DI water compacted
497 membranes. This indicates that surface hydrophobicity is not the sole determining factor in
498 cell adhesion in this study and factors other than surface hydrophobicity, such as surface
499 topology, could play more prominent role in bioadhesion. Membranes compacted with
500 deionized and tap water, whose surfaces were covered by a fouling layer, were showed to
501 have an unaltered surface topology compared to MilliQ water compacted membranes. The
502 surface heterogeneities found on membranes compacted with MilliQ water might explain
503 the observed higher bacterial adhesion compared to the smoother and more homogenous
504 membranes following deionized and tap water compactations. A study conducted on NF 270
505 membranes, linked bacterial adhesion to surface heterogeneities (Subramani and Hoek
506 2008). Another similar study showed that surface roughness, or more specifically surface
507 topography capable of accommodating bacterial cells, was particularly favourable for
508 bacterial adhesion compared to other types of surface (Medilanski et al. 2002). In general,
509 surface roughness can create conditions for the favourable initial adhesion of a single
510 bacterium, possibly in a topological feature and this in turn forms the seed for the
511 subsequent growth of a micro-colony. The roughness values obtained in this study were

512 performed under dehydrated compacted membranes, giving rise to high roughness readings
513 and artefacts in the form of surface cracks. However, when a specific area of 20 µm by 20
514 µm without cracks was analysed with the profilometer software, surface roughness was still
515 higher for the tap water compacted membrane (425.2 nm ± 152.9) compared to the
516 roughness obtained for DI water (164 nm ± 51.7) and MilliQ water (60.5 nm ± 17.2)
517 compacted membranes.

518 The presence of microorganisms on RO/NF membranes following compaction could lead to
519 significant degrees of bias when performing adhesion and biofouling assays. This is
520 especially important when studying biofouling using a monoculture 515 system. The
521 presence of viable organisms on freshly compacted membrane would most certainly lead to
522 the development of unanticipated outcomes.

523

524 **5 Conclusion**

525 The impact of laboratory water quality was assessed following compaction of the NF 270
526 membrane by analysing the membrane performance and surface characteristics, as well as
527 the adhesion characteristics of *P. fluorescens*. Tap and DI water compaction resulted in a
528 cake layer on the membrane surface consisting of living and dead bacteria and diatoms,
529 organic matter, dissolved solids and other components, as these were present in the water
530 used for compaction. There was a clear difference in the performance characteristics of the
531 different membranes following compaction with different water types. Compacting with DI
532 and tap water resulted in a lower permeate flux and cell adhesion rate compared to MilliQ
533 water. In contrast, compaction with MilliQ water generated the highest fluxes through the
534 membrane and a significantly higher initial adhesion of *P. fluorescens*. The reasons for the

535 different cell adhesion rates is difficult to elucidate due to the complexity of the tap and DI
536 water. However, there seems to be a correlation with the topography of the surface: large
537 heterogeneities on the surface seem to enhance *P. fluorescens* adhesion.

538 Overall, these results illustrate the importance of laboratory water quality in the
539 compaction stage of NF/RO experiments and the consequent impact it has when
540 undertaking bacterial adhesion studies. It needs to be noted while tap and DI water quality
541 will vary significantly from laboratory to laboratory, these differences in quality can make it
542 difficult to compare results of adhesion studies from different 537 research groups. The
543 present study identifies the need for standardized protocols for studying membrane
544 biofouling in laboratory conditions, particularly with respect to the water quality during
545 membrane compaction procedures and for the feed solutions in subsequent experiments.

546

547

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558 **References**

559 Cyna, B., Chagneau, G., Bablon, G. and Tanghe, N. (2002) Two years of nanofiltration at the Méry554
560 sur-Oise plant, France. *Desalination* 147(1–3), 69-75.

561 Flemming, H.C. (1997) Reverse osmosis membrane biofouling. *Experimental Thermal and Fluid*
562 *Science* 14(4), 382-391.

563 Ivnitsky, H., Katz, I., Minz, D., Volvovic, G., Shimoni, E., Kesselman, E., Semiat, R. and Dosoretz, C.G.
564 (2007) Bacterial community composition and structure of biofilms developing on nanofiltration
565 membranes applied to wastewater treatment. *Water Research* 41(17), 3924-3935.

566 Flemming, H.C. (2002) Biofouling in water systems – cases, causes and countermeasures. *Applied*
567 *Microbiology and Biotechnology* 59(6), 629-640.

568 Houari, A., Seyer, D., Couquard, F., Kecili, K., Démocrate, C., Heim, V. and Martino, P.D. (2009)
569 Characterization of the biofouling and cleaning efficiency of nanofiltration membranes. *Biofouling*
570 26(1), 15-21.

571 Vrouwenvelder, H.S., van Paassen, J.A.M., Folmer, H.C., Hofman, J.A.M.H., Nederlof, M.M. and van
572 der Kooij, D. (1998) Biofouling of membranes for drinking water production. *Desalination* 118(1–3),
573 157-166.

574 Vrouwenvelder, J.S., Manolarakis, S.A., van der Hoek, J.P., van Paassen, J.A.M., van der Meer, W.G.J.,
575 van Agtmaal, J.M.C., Prummel, H.D.M., Kruithof, J.C. and van Loosdrecht, M.C.M. (2008)
576 Quantitative
577 biofouling diagnosis in full scale nanofiltration and reverse osmosis installations. *Water Research*
578 42(19), 4856-4868.

579 Khan, M.T., Manes, C.-L.d.O., Aubry, C. and Croué, J.-P. (2013) Source water quality shaping
580 different
581 fouling scenarios in a full-scale desalination plant at the Red Sea. *Water Research* 47(2), 558-568.

582 Flemming, H.C., Schaule, G., Griebe, T., Schmitt, J. and Tamachkiarowa, A. (1997) Biofouling—the
583 Achilles heel of membrane processes. *Desalination* 113(2–3), 215-225.

584 Hijnen, W.A.M., Castillo, C., Brouwer-Hanzens, A.H., Harmsen, D.J.H., Cornelissen, E.R. and van der
585 Kooij, D. (2012) Quantitative assessment of the efficacy of spiral-wound membrane cleaning
586 procedures to remove biofilms. *Water Research* 46(19), 6369-6381.

587 Vrouwenvelder, J.S., Hinrichs, C., Van der Meer, W.G.J., Van Loosdrecht, M.C.M. and Kruithof, J.C.
588 (2009a) Pressure drop increase by biofilm accumulation in spiral wound RO and NF membrane
589 systems: role of substrate concentration, flow velocity, substrate load and flow direction. *Biofouling*
590 25(6), 543-555.

591 Hijnen, W.A.M., Biraud, D., Cornelissen, E.R. and van der Kooij, D. (2009) Threshold Concentration of
592 Easily Assimilable Organic Carbon in Feedwater for Biofouling of Spiral-Wound Membranes.
593 *Environmental Science & Technology* 43(13), 4890-4895.

594 Ivnitsky, H., Katz, I., Minz, D., Shimoni, E., Chen, Y., Tarchitzky, J., Semiat, R. and Dosoretz, C.G.
595 (2005) Characterization of membrane biofouling in nanofiltration processes of wastewater
596 treatment. *Desalination* 185(1–3), 255-268.

597 Huertas, E., Herzberg, M., Oron, G. and Elimelech, M. (2008) Influence of biofouling on boron
598 removal by nanofiltration and reverse osmosis membranes. *Journal of Membrane Science* 318(1–2),
599 264-270.

600 Herzberg, M. and Elimelech, M. (2007) Biofouling of reverse osmosis membranes: Role of biofilm593
601 enhanced osmotic pressure. *Journal of Membrane Science* 295(1–2), 11-20.

602 Chong, T.H., Wong, F.S. and Fane, A.G. (2008) The effect of imposed flux on biofouling in reverse
603 osmosis: Role of concentration polarisation and biofilm enhanced osmotic pressure phenomena.
604 *Journal of Membrane Science* 325(2), 840-850.

605 Vrouwenvelder, J.S., Graf von der Schulenburg, D.A., Kruithof, J. 597 C., Johns, M.L. and van
606 Loosdrecht,

607 M.C.M. (2009b) Biofouling of spiral-wound nanofiltration and reverse osmosis membranes: A feed
608 spacer problem. *Water Research* 43(3), 583-594.

609 Ridgway, H.F., Rigby, M.G. and Argo, D.G. (1985) Bacterial Adhesion and Fouling of Reverse Osmosis
610 Membranes. *Journal of American Water Works Association* 77(7), 97-106.

611 Subramani, A. and Hoek, E.M.V. (2008) Direct observation of initial microbial deposition onto
612 reverse
613 osmosis and nanofiltration membranes. *Journal of Membrane Science* 319(1-2), 111-125.

614 Myint, A.A., Lee, W., Mun, S., Ahn, C.H., Lee, S. and Yoon, J. (2010) Influence of membrane surface
615 properties on the behavior of initial bacterial adhesion and biofilm development onto nanofiltration
616 membranes. *Biofouling* 26(3), 313-321.

617 Khan, M.M.T., Stewart, P.S., Moll, D.J., Mickols, W.E., Nelson, S.E. and Camper, A.K. (2011)
618 Characterization and effect of biofouling on polyamide reverse osmosis and nanofiltration
619 membrane surfaces. *Biofouling* 27(2), 173-183.

620 Subramani, A., Huang, X. and Hoek, E.M.V. (2009) Direct observation of bacterial deposition onto
621 clean and organic-fouled polyamide membranes. *Journal of Colloid and Interface Science* 336(1), 13-
622 20.

623 Baek, Y., Yu, J., Kim, S.-H., Lee, S. and Yoon, J. (2011) Effect of surface properties of reverse osmosis
624 membranes on biofouling occurrence under filtration conditions. *Journal of Membrane Science*
625 382(1-2), 91-99.

626 Fonseca, A.C., Summers, R.S., Greenberg, A.R. and Hernandez, M.T. (2007) Extra-Cellular
627 Polysaccharides, Soluble Microbial Products, and Natural Organic Matter Impact on Nanofiltration
628 Membranes Flux Decline. *Environmental Science & Technology* 41(7), 2491-2497.

629 Miller, D.J., Araújo, P.A., Correia, P.B., Ramsey, M.M., Kruithof, J.C., van Loosdrecht, M.C.M.,
630 Freeman, B.D., Paul, D.R., Whiteley, M. and Vrouwenvelder, J.S. (2012) Short-term adhesion and
631 long-term biofouling testing of polydopamine and poly(ethylene glycol) surface modifications of
632 membranes and feed spacers for biofouling control. *Water Research* 46(12), 3737-3753.

633 Bernstein, R., Belfer, S. and Freger, V. (2011) Bacterial Attachment to RO Membranes Surface-
634 Modified by Concentration-Polarization-Enhanced Graft Polymerization. *Environmental Science &*
635 *Technology* 45(14), 5973-5980.

636 Suwarno, S.R., Chen, X., Chong, T.H., Puspitasari, V.L., McDougald, D., Cohen, Y., Rice, S.A. and Fane,
637 A.G. (2012) The impact of flux and spacers on biofilm development on reverse osmosis membranes.
638 *Journal of Membrane Science* 405-406(0), 219-232.

639 Vrouwenvelder, J.S., van Paassen, J.A.M., van Agtmaal, J.M.C., van Loosdrecht, M.C.M. and Kruithof,
640 J.C. (2009c) A critical flux to avoid biofouling of spiral wound nanofiltration and reverse osmosis
641 membranes: Fact or fiction? *Journal of Membrane Science* 326(1), 36-44.

642 Vrouwenvelder, J.S., Bakker, S.M., Wessels, L.P. and van Paassen, J.A.M. (2007) The Membrane
643 Fouling Simulator as a new tool for biofouling control of spiral-wound membranes. *Desalination*
644 204(1-3), 170-174.

645 Botton, S., Verliefde, A.R.D., Quach, N.T. and Cornelissen, E.R. (2012) Influence of biofouling on
646 pharmaceuticals rejection in NF membrane filtration. *Water Research* (0).

647 Khan, M.M.T., Stewart, P.S., Moll, D.J., Mickols, W.E., Burr, M.D., Nelson, S.E. and Camper, A.K.
648 (2010) Assessing biofouling on polyamide reverse osmosis (RO) membrane surfaces in a laboratory
649 system. *Journal of Membrane Science* 349(1-2), 429-437.

650 Lee, W., Ahn, C.H., Hong, S., Kim, S., Lee, S., Baek, Y. and Yoon, J. (2010) Evaluation of surface
651 properties of reverse osmosis membranes on the initial biofouling stages under no filtration
652 condition. *Journal of Membrane Science* 351(1-2), 112-122.

653 Chong, T.H., Wong, F.S. and Fane, A.G. (2007) Enhanced concentration polarization by unstirred
654 fouling layers in reverse osmosis: Detection by sodium chloride tracer response technique. *Journal*
655 *of Membrane Science* 287(2), 198-210.

656 Pang, C.M., Hong, P., Guo, H. and Liu, W.-T. (2005) Biofilm 646 Formation Characteristics of Bacterial
657 Isolates Retrieved from a Reverse Osmosis Membrane. *Environmental Science & Technology* 39(19),

658 7541-7550.
659 Gibbs, R.A., Scutt, J.E. and Croll, B.T. (1993) Assimilable Organic Carbon Concentrations and Bacterial
660 Numbers in a Water Distribution System. *Water Science & Technology* 27(3-4), 159-166.
661 Vrouwenvelder, J.S., van Paassen, J.A.M., Wessels, L.P., van Dam, A.F. and Bakker, S.M. (2006) The
662 Membrane Fouling Simulator: A practical tool for fouling prediction and control. *Journal of*
663 *Membrane Science* 281(1-2), 316-324.
664 Hoek, E.M.V. and Elimelech, M. (2003) Cake-Enhanced Concentration Polarization: A New Fouling
665 Mechanism for Salt-Rejecting Membranes. *Environmental Science & Technology* 37(24), 5581-5588.
666 Van der Bruggen, B. and Vandecasteele, C. (2001) Flux Decline during Nanofiltration of Organic
667 Components in Aqueous Solution. *Environmental Science & Technology* 35(17), 3535-3540.
668 Flemming, H.-C. (1987) Microbial growth on ion exchangers. *Water Research* 21(7), 745-756.
669 Boussu, K., Zhang, Y., Cocquyt, J., Van der Meeren, P., Volodin, A., Van Haesendonck, C., Martens,
670 J.A. and Van der Bruggen, B. (2006) Characterization of polymeric nanofiltration membranes for
671 systematic analysis of membrane performance. *Journal of Membrane Science* 278(1-2), 418-427.
672 Her, N., Amy, G., Chung, J., Yoon, J. and Yoon, Y. (2008) Characterizing dissolved organic matter and
673 evaluating associated nanofiltration membrane fouling. *Chemosphere* 70(3), 495-502.
674 Medilanski, E., Kaufmann, K., Wick, L.Y., Wanner, O. and Harms, H. (2002) Influence of the Surface
675 Topography of Stainless Steel on Bacterial Adhesion. *Biofouling* 18(3), 193-203.

676

677

678

679

680 **List of Tables**

681 Table 1 Chemical and biological characterisation of the different water qualities used during
682 the membrane compaction study.

683

684 Table 2 NF 270 membrane surface properties after compaction with different water sources
685 at different permeate volumes.

686

687 Table 3: Estimated maximum cell loading and adhesion velocity values of *Ps. fluorescens*
688 cells on compacted NF270 membranes with different water qualities. Error is represented as
689 standard error.

690

691

692 **List of Figures**

693 Figure 1 MFS Cross-Flow system.

694

695 Figure 2 Flux decline of the NF 270 membrane during compaction with different water
696 sources (0.5, 2 and 5 L of source water filtered, 0.66 L.min⁻¹ 690 in each MFS, 15 bar and
697 21°C). Each experiment was repeated twice. The error bar represents the variability of the
698 flux obtained for all the membranes used in duplicate exposed to that particular permeate
699 volume (0.5, 2 and 5 L).

700

701 Figure 3 Height topography of a compacted membrane with MilliQ (A), DI (B) and tap
702 water(C) were obtained by optical profilometry (223×293 μm). The 2D images associated
703 to each profile are shown, all X and Y axes are in microns.

704

705 Figure 4 Scanning electron micrographs of non-compacted NF 270 membranes (A) and
706 compacted NF270 membranes after 5L permeated volumes of Milli Q water (B), deionized
707 water (C), and tap water (D) at 0.66 L•min⁻¹, 15 bar and 21°C .

708

709 Figure 5 Number of accumulated microorganisms on NF270 membranes following
710 compaction with increasing permeated volumes of different water qualities. Error bars
711 represent standard error.

712

713 Figure 6 Dynamic adhesion of *Ps. fluorescens* cells on compacted NF-270 membranes after
714 5L permeated volumes of different water qualities.

715

716 Figure 7 Fluorescence micrographs showing membrane samples following membrane
717 compaction with autoclaved DI water at 15 bar stained with (A) Syto 9 and (B) Propidium
718 Iodide and membrane compaction with non-autoclaved DI water at 15 bar stained with (C)
719 Syto 9 and (D) Propidium Iodide

720

721 Table 1

Water Quality	MilliQ Water	Deionized Water	Tap Water
Total Solids (mg•L ⁻¹)	ND*	ND*	109.17 ± 5.2
pH	6.01 ± 0.11	6.31 ± 0.29	7.44 ± 0.07
TOC (mg•C•L ⁻¹)	0.04 ± 0.04	1.6 ± 0.7	9.6 ± 0.8
Conductivity (μS•cm ⁻¹)	0.4 ± 0.1	4 ± 2	168 ± 7
Culturable counts (Cells•mL ⁻¹)	ND*	239	1.4
Total cell counts (10 ³ Cells•mL ⁻¹)	1 ± 1	1472 ± 421	123 ± 47
Total dead/injured counts (10 ³ Cells•mL ⁻¹)	3 ± 2	2020 ± 482	82 ± 44

722 *ND: not detected

723

724

725

726

1

2 Table 2

3

	Permeated volume	Compacted NF 270 membranes								
		MilliQ water			Deionized water			Tap water		
		0.5L	2L	5L	0.5L	2L	5L	0.5L	2L	5L
Contact angle	θ_{water}	40.5 ± 1.11	45.5 ± 0.89	49.7 ± 0.58	55.7 ± 0.34	64.3 ± 0.45	68.9 ± 0.63	45.8 ± 0.9	53.4 ± 0.6	56.0 ± 3.9
	$\theta_{\text{diiodomethane}}$	36.6 ± 0.41	36.9 ± 0.88	34.4 ± 0.67	45.3 ± 0.56	46.8 ± 0.88	44.6 ± 0.97	38.6 ± 0.7	36.4 ± 1.6	28.7 ± 6.7
	$\theta_{\text{ethylene glycol}}$	28.9 ± 0.98	25.8 ± 0.73	26.6 ± 0.42	34.6 ± 0.64	41.5 ± 0.81	47.8 ± 0.59	32.9 ± 0.9	36.7 ± 0.7	40.6 ± 5.6
Surface tension (mJ·m ⁻²)	γ^{LW}	41.4 ± 0.18	41.3 ± 0.41	42.3 ± 0.33	36.9 ± 0.31	35.7 ± 0.51	37.0 ± 0.56	40.3 ± 0.4	41.9 ± 0.7	45.5 ± 0.5
	γ^-	49.1 ± 1.83	44.8 ± 1.4	33.7 ± 0.99	28.5 ± 0.55	19.9 ± 0.85	17.8 ± 1.24	44.9 ± 1.4	35.5 ± 1.2	33.4 ± 1.2
	γ^+	0.1 ± 0.04	0.04 ± 0.01	0.03 ± 0.01	0.1 ± 0.02	0.2 ± 0.04	0.08 ± 0.02	0.15 ± 0.05	0.19 ± 0.04	0.55 ± 0.1
	γ^{AB}	4.06 ± 0.6	2.22 ± 0.4	1.86 ± 0.32	3.9 ± 0.4	4.9 ± 0.6	2.77 ± 0.4	3.94 ± 0.73	4.80 ± 0.8	9.1 ± 0.9
	γ^{S}	53.2 ± 2.3	47.0 ± 1.65	35.6 ± 1.03	32.5 ± 0.4	24.8 ± 0.7	20.6 ± 1.32	48.9 ± 1.9	40.3 ± 1.8	42.6 ± 2.04
Surface roughness (RMS) (nm)	200 μm x 200 μm surface area *	468 ± 142	417 ± 121	511 ± 143	452 ± 177	592 ± 144	562 ± 123	521 ± 160	695 ± 251	1166 ± 147

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5 * Roughness deduced from surface profilometry.

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2 **Table 3**

	Compacted NF 270 membranes			3
	MilliQ water	Deionized water	Tap water	4
Estimated maximum cell loading q_{\max} (10^4 Cells \cdot cm $^{-2}$)	26 ± 2.5	5.2 ± 1.3	ND	5
Adhesion velocity k_d (10^{-5} cm \cdot min $^{-1}$)	11.7 ± 2.4	1.06 ± 0.09	3.13 ± 0.3	6 7

8

9 *ND: not determined.

10

11

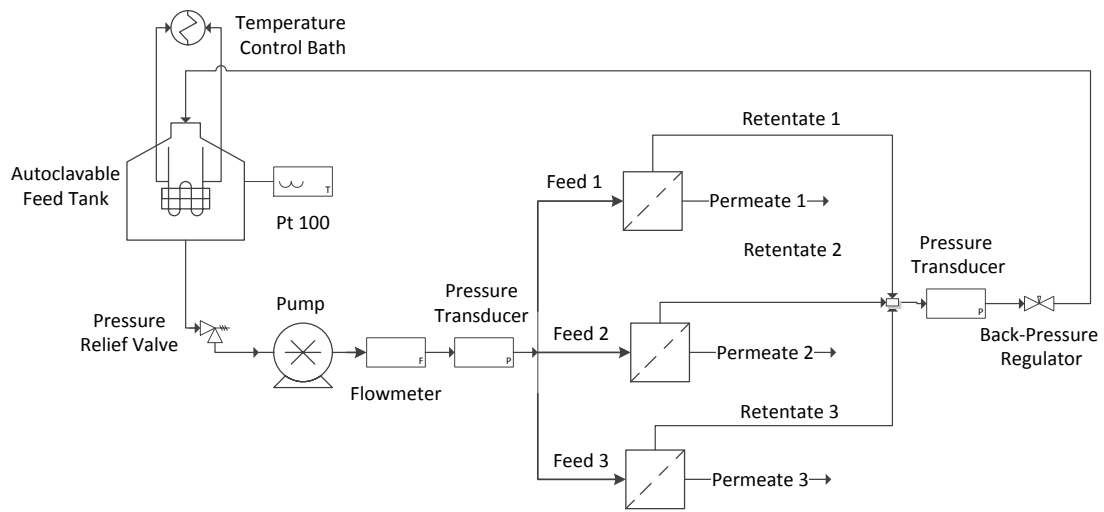
12

13

14

1 Figure 1

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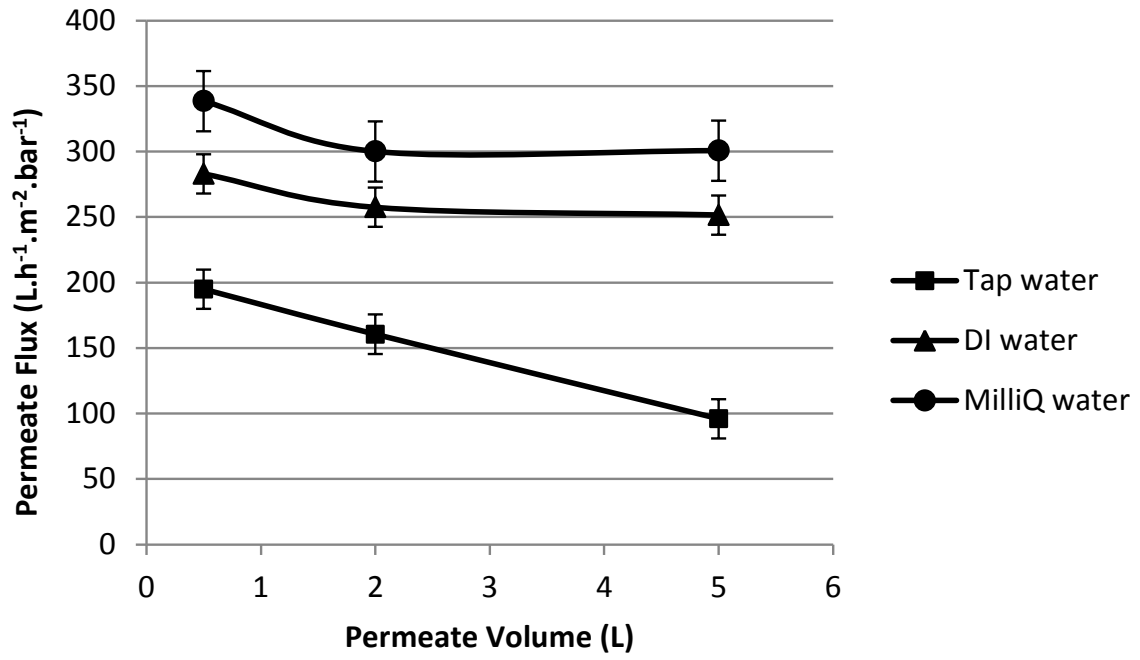
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4

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3 Figure 2



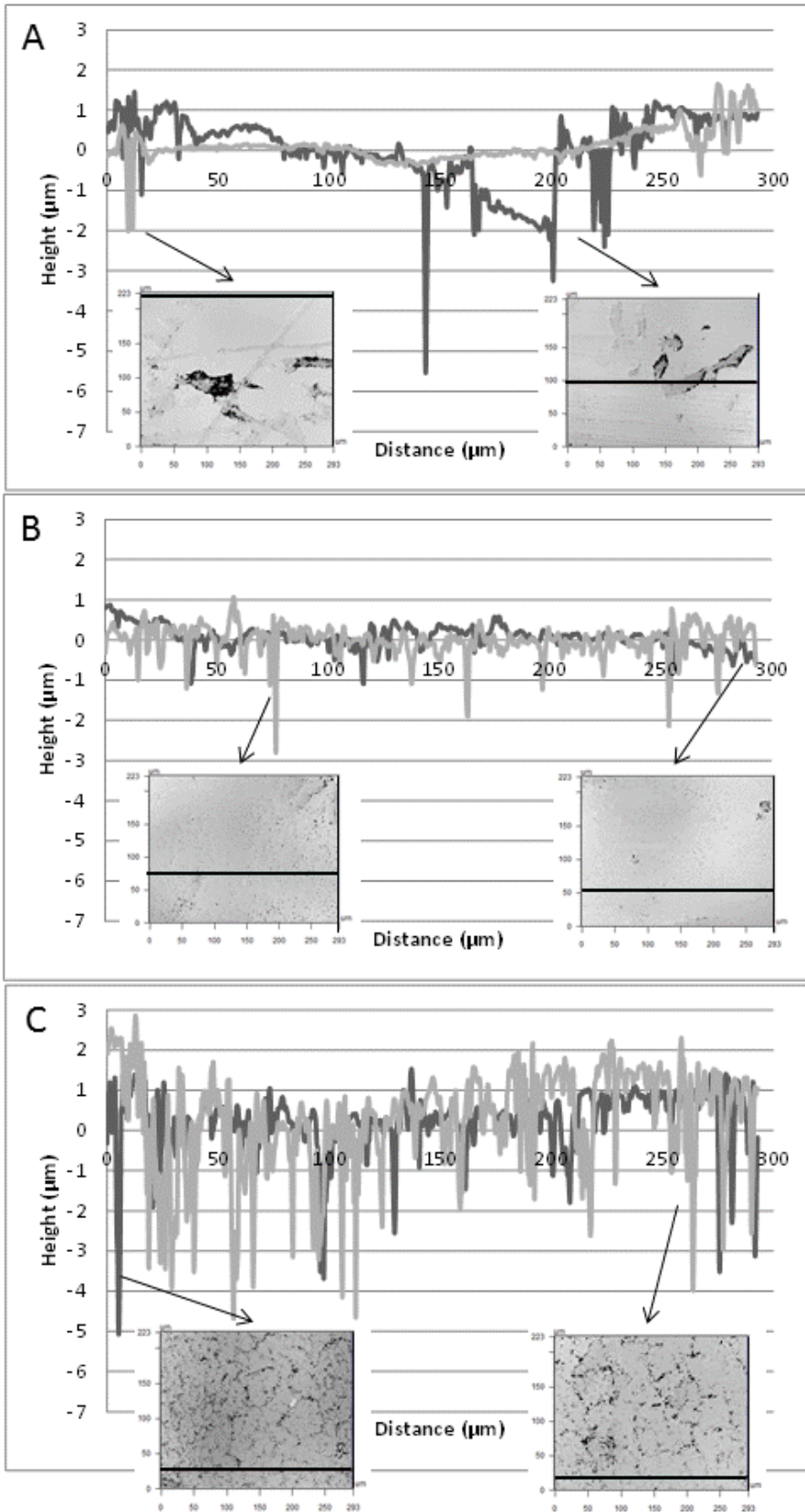
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5

6

1

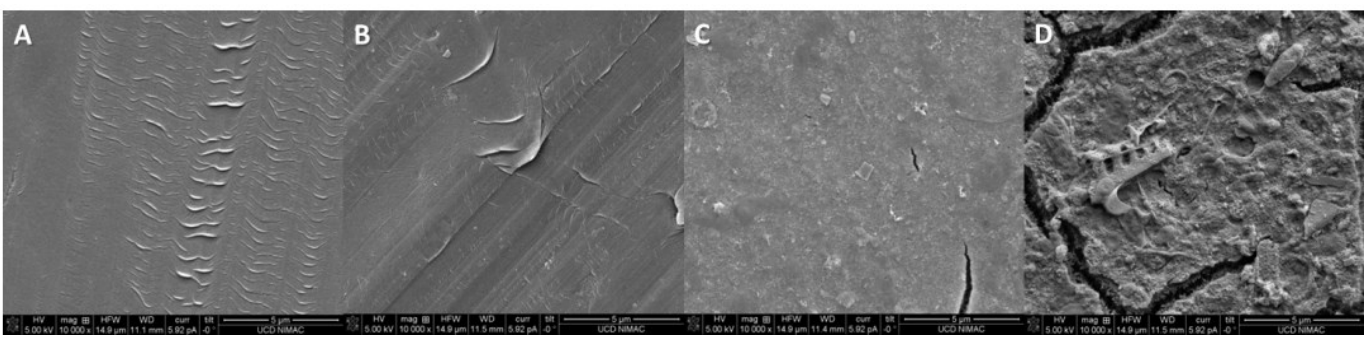
2 **Figure 3**



3

1

2 Figure 4



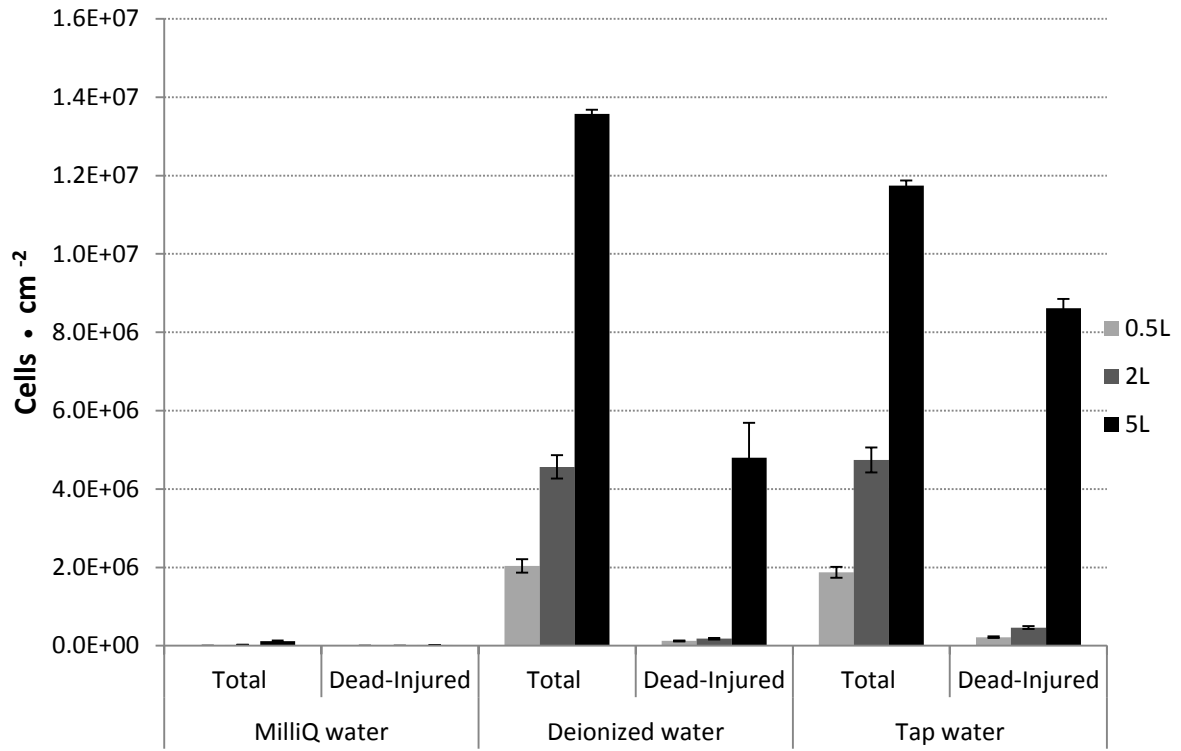
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4

5

1

2 Figure 5



3

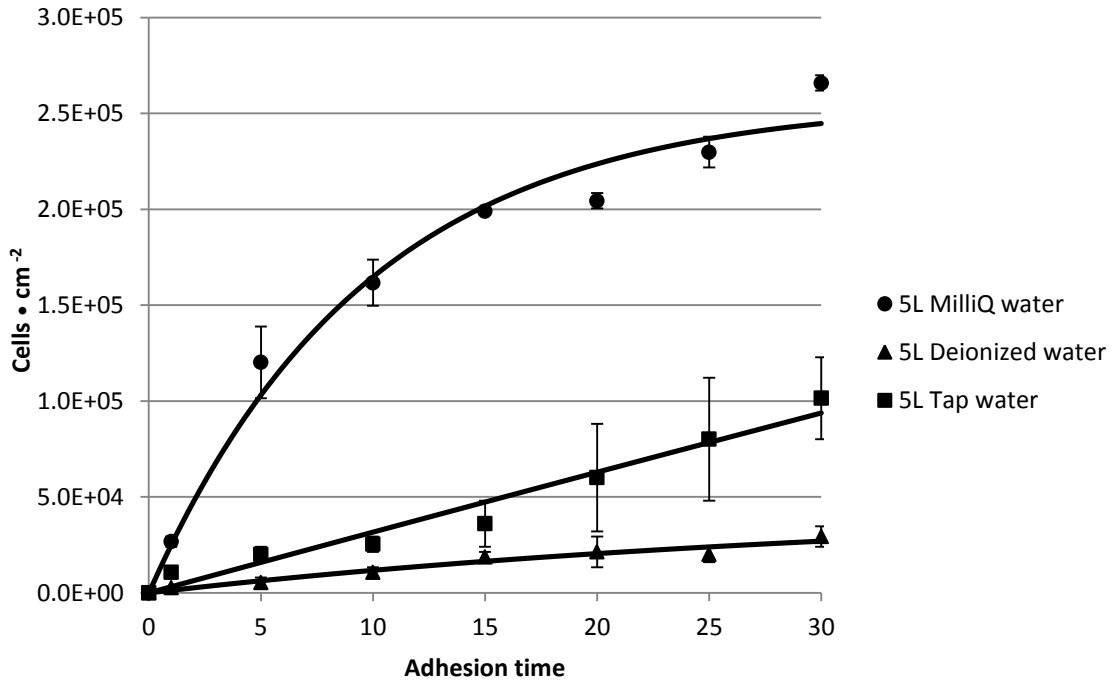
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3 **Figure 6**

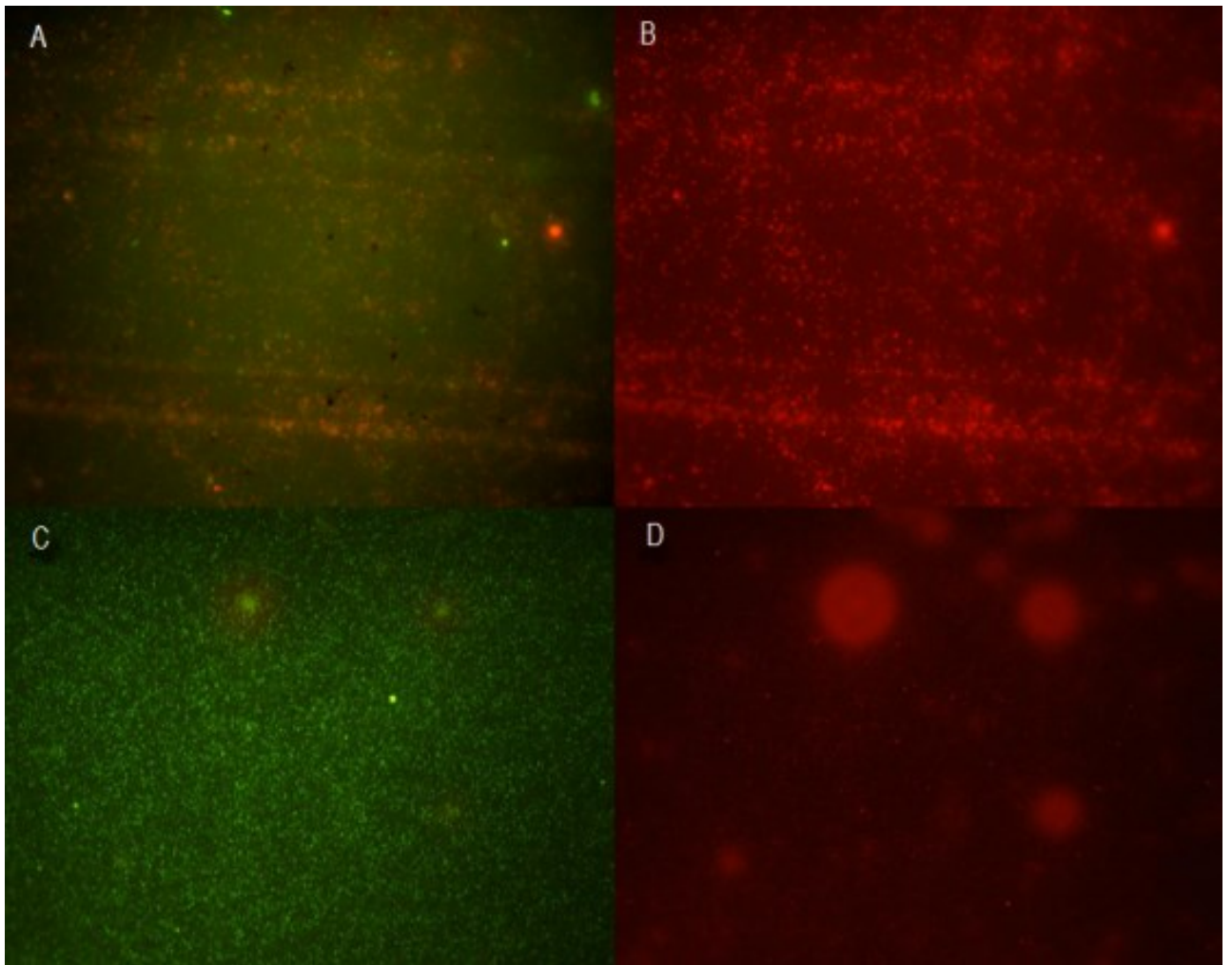


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1 Figure 7



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