



21

22 **Abstract**

23 It is imperative that nanofiltration membranes are disinfected before they are used for  
24 laboratory-scale bacterial adhesion or biofouling experiments, yet currently no suitable  
25 disinfection protocol exists. This study aimed to determine if an ethanol treatment at a  
26 minimum inhibitory concentration (MIC) could be used to effectively disinfect nanofiltration  
27 membranes without altering membrane properties which could affect research. Two strains of  
28 bacteria, *Pseudomonas fluorescens* and *Staphylococcus* sp., were exposed to a range of  
29 ethanol concentrations to determine the MIC required for a 4log<sub>10</sub> reduction in bacteria. In  
30 parallel, ethanol's effects on the filtration, surface and mechanical properties of a Dow  
31 Filmtec NF90 membrane were analysed. A 1.5 hour treatment with 40% ethanol was shown  
32 to effectively disinfect the membrane without significantly affecting any of the membranes  
33 properties tested. This treatment protocol can now be safely used to disinfect the studied  
34 membrane prior to bacterial adhesion or biofouling experiments. This study also acts as a  
35 guideline for researchers using other membranes to determine a suitable disinfection protocol  
36 for their needs.

37

38

39

40

41

42

43

44

45

46 **Keywords**

47 Disinfection, ethanol, pre-treatment, MIC, nanofiltration

48

## 49 **1. Introduction**

50 Nanofiltration (NF) is becoming a prevalent process in the production of drinking water [1],  
51 in water recycling [2] and may play an increasing role in water desalination [3].

52 Nanofiltration research in these areas has focused on two main objectives: 1) maximising  
53 permeation while achieving a high level of rejection, and 2) maintaining the longevity of  
54 filtration properties, primarily through the mitigation of fouling [4, 5].

55

56 Of the several types of fouling that occur, the adhesion and proliferation of microorganisms  
57 on the membrane known as biofouling is particularly problematic. It is of utmost importance  
58 that fundamental research in which bacterial pure cultures are used, for adhesion and  
59 biofouling experiments, be conducted on sterile surfaces. Biofouling experiments performed  
60 on non-sterile surfaces are at risk of experimental bias, resulting in unspecified multispecies  
61 biofilms. Virgin membranes received from manufacturers may be initially clean but are non-  
62 sterile, representing a potential contamination source for controlled adhesion and biofouling  
63 studies in which pure cultures are utilised.

64

65 Sterilisation can be achieved by chemical, thermal, and irradiation means. There is a major  
66 emphasis on sterility in microbiological research and yet there is little research into  
67 sterilisation techniques for NF and reverse osmosis (RO) membranes. Although a number of  
68 different research groups have reported the use of thermal [6, 7] and chemical [8-11]  
69 sterilization procedures on membranes, few have considered the damaging effects that such  
70 treatments could potentially have. This comes at notable risk considering the available  
71 information showing the thermal [12, 13] and chemical sensitivity [14-16] of filtration  
72 membranes. These studies show changes to the flux, active layer stability, pore size, pore  
73 density and chemical composition of the membrane, all of which could be detrimental to a  
74 study involving membrane permeation.

75

76 Alcohols are commonly noted to have biocidal properties. They act as cell membrane  
77 disruptors and denature proteins, inhibiting growth [17, 18]. With their short carbon chains,  
78 ethanol and isopropanol are the most widely used alcohols for this purpose. They are  
79 commonly used in the food industry, as a preservative, and in the healthcare sector, where  
80 their low volatilities makes them useful for hand sanitizers. Studies in both fields have shown

81 ethanol to be most potent within a concentration range from 30% to 70% [19, 20]. Even in  
82 this range ethanol still remains ineffective against a wide range of fungal and bacterial spores,  
83 and so cannot be considered a means of sterilisation, rather as a means of disinfection.

84

85 It is important to acknowledge that there is no universally accepted definition of disinfection,  
86 an observation made in Seymour Block's "Disinfection, Sterilization and Preservation" [21].  
87 While sterilisation is defined as the removal of all forms of life, disinfection is considered to  
88 be the removal of infection, *i.e.* the killing of microorganisms but not necessarily the removal  
89 of spores. Accepted levels of microorganism removal vary from 3 to 6 log<sub>10</sub> (99.9% to  
90 99.9999%) to qualify as disinfection, with the concept largely dependent on the application.

91

92 For the purposes of membrane biofouling research, the application of disinfection is to bring  
93 the quantity of microorganisms on the virgin membrane to a level whereby it will have  
94 minimal effect on the experiment. According to the European surface test (EN 13697), an  
95 effective disinfectant is one capable of reducing the number of sessile cells by 4 log<sub>10</sub>  
96 (99.99% removal) or more [22]. It is therefore important that a minimum inhibitory  
97 concentration (MIC) of ethanol is determined to achieve such a reduction in a membrane  
98 application.

99

100 To date, the effects of ethanol on water filtration properties of thin film composite (TFC)  
101 Nanofiltration membranes are still poorly understood. The majority of research in this field  
102 analyses solvent permeability through the membrane rather than using the solvent as a pre-  
103 treatment [23, 24]. Shukla et al. [25] expressed their concern that membrane polymers would  
104 become damaged upon re-exposure to organic solvents which had previously been used in the  
105 membrane's manufacture process. Although membrane surface destabilization seems likely  
106 to occur due to the solubility parameters of polysulfone and ethanol, Lencki et al. [26]  
107 mentioned that the affinity of ethanol to hydrogen bonding reduces this risk. Earlier studies  
108 have shown that exposure of ultrafiltration (UF) and NF membranes to solvents such as  
109 ethanol resulted in the swelling of the membrane polyamide and polysulfone layers,  
110 subsequently leading to membrane curling [27, 28]. Moreover, a series of papers by Geens,  
111 Van der Bruggen and Van der Castele on the effects of solvents, including ethanol, on  
112 nanofiltration membranes showed polymer swelling to alter the pore size and pore density of  
113 the membrane, as membrane polymers stretch and pores were forced to contract [29-31].

114 Furthermore, they showed that solvents can also affect the hydrophobicity of a nanofiltration  
115 membrane, and highlight how each solvent reacts uniquely with each polymer.

116

117 With most studies focusing purely on solvent-membrane interactions in the context of solvent  
118 permeability applications, it is still unclear what bearing their results would have on water  
119 permeability. Only two studies were found detailing the interaction of an alcohol treatment on  
120 the pure-water flux of a TFC membrane: 1) In their 2006 study Jeżowska et al. compared the  
121 pure water flux of a Dow Filmtec NF90 membrane before and after treatment with  
122 isopropanol, a chemically similar alcohol to ethanol [32]. Although a small increase in pure  
123 water flux was observed, the increase shown is an average of three separate treatments  
124 (pressure, alkaline treatment and alcohol treatment). 2) Van der Bruggen et al. in 2002 looked  
125 at the effects of solvent exposure on a collection of solvent-stable nanofiltration membranes  
126 [33]. In this paper they observe that a hydrophobic membrane exposed to ethanol for 10 days  
127 experiences a significant increase in pure water flux despite incurring no mechanical damage  
128 visible by scanning electron microscopy. Neither study analysed the other possible membrane  
129 changes resulting from treatment such as: changes in membrane surface physico-chemical  
130 properties, charge and mechanical properties, all of which play important roles in bacterial  
131 adhesion on NF membranes [34]. Thus, it is impossible to conclude the full effect ethanol  
132 treatment has in this application.

133

134 Due to the lack of sterilising methods of NF membranes and the unclear effect of ethanol on  
135 polyamide based TFC NF membranes, this study sought to assess the suitability of ethanol as  
136 a means of disinfecting polyamide NF membranes. Our aim was to determine a minimal  
137 ethanol concentration and a treatment protocol that could expressly be implemented for  
138 bioadhesion and biofouling research on NF and RO membranes. In this study, model Gram-  
139 positive and Gram-negative bacterial strains were used to test the disinfection efficacy of  
140 different ethanol concentrations. In parallel the filtration, surface and mechanical properties  
141 of Dow Filmtec NF90 membranes were characterised following different ethanol treatment  
142 regimes.

143

144

## 145 **2. Materials and methods**

146

147 **2.1. Chemicals**

148 The water used throughout this study was Grade 1 pure water ( $18.2 \text{ M}\Omega\text{cm}^{-1}$ ) obtained from  
149 an Elga Process Water System (Biopure 15 and Purelab flex 2, Veolia, Ireland), hereafter  
150 referred to as MilliQ water.

151 Emsure® absolute ethanol (Merck, Ireland) was used in this study. All ethanol concentrations  
152 are given as % vol. /vol. based on the ratio of MilliQ water or Phosphate Buffer Solution  
153 (PBS; Sigma-Aldrich, Ireland) and ethanol volumes added together.

154 **2.2. Bacteria**

155 Two strains of bacteria were used: *Pseudomonas fluorescens* NCTC 10038 (Gram-negative)  
156 and *Staphylococcus* sp. (Gram-positive). Prior to disinfection experiments, *P. fluorescens* and  
157 *S. sp.* strains were separately grown in Tryptic Soy Broth (TSB; Oxoid, Ireland) medium at  
158  $30^\circ\text{C}$  and 200 r.p.m. overnight, reaching cell densities of  $\text{OD}_{600}$  2.8 and 2.7 respectively.

159  
160 **2.3. Disinfection**

161 **2.3.1. Disinfection of cells in suspension**

162 200  $\mu\text{l}$  sample suspensions of each bacterium (containing  $10 \log_{10}$  cells) were treated with 1.8  
163 ml of aqueous ethanol for 1.5 hours. Treatment concentrations of 0, 10, 20, 30, 40, 50, 60 and  
164 70 % ethanol in PBS were used. Following treatment the suspensions were centrifuged at  
165 7000 rpm for 10 minutes in an Eppendorf Centrifuge 5415C (Eppendorf, Germany), the  
166 supernatant discarded and the pellet re-suspended in PBS, this step was repeated twice. Serial  
167 dilutions (to  $10^{-10}$ ) were plated on Trypticase Soy Agar (TSA; Sigma Aldrich) plates. These  
168 were incubated for 18 hours at  $30^\circ\text{C}$ . Finally the colony forming units (CFU) were counted  
169 and the post-treatment CFU/ml determined.

170  
171 **2.3.2. Disinfection of spiked membranes**

172 To test the inhibitory action of a selected range of ethanol concentrations on spiked  
173 membranes, a modified version of a disinfection surface test was used [35, 36].  
174 Membrane samples were autoclaved and sterilized at  $121^\circ\text{C}$  for 15 minutes. The samples  
175 were spiked with 30 ml of an overnight bacterial culture ( $\text{OD}_{600} \approx 2.7$ ) for 15 minutes and  
176 subsequently dried under laminar flow for one hour. The membrane was cut into  $1\text{cm}^2$   
177 sections and treated with 5 ml of a 0, 10, 20, 30 or 40 % ethanol solution for 1.5 hours.  
178 Disinfection experiments of spiked membranes were initially checked against non-autoclaved  
179 membrane samples to ensure that autoclaving did not affect disinfection efficiency by the

180 infiltration of bacterial cells in structurally damaged sites on the membrane following  
181 sterilization. Each treatment was performed in duplicate in at least three independent  
182 replicates. The treated membranes were rinsed with PBS and sonicated at 44 kHz for 15  
183 minutes in an Ultrawave Ultrasonic bath. Serial dilutions (to  $10^{-10}$ ) were plated using the  
184 Miles & Misera method. These plates were then incubated for 18 hours at 30°C and the  
185 CFU/ml determined.

186

### 187 2.3.3. *Disinfection of virgin membranes*

188 Samples of membrane were cut and divided into two sections. One half was treated with an  
189 ethanol solution (0, 10, 20, 30 or 40% ethanol) while the other half was left untreated to  
190 ensure that all samples had a similar level of bacteria initially attached. TSA contact plates  
191 were pressed against the front and back of the membrane and incubated for 18 hours at 30°C.  
192 The number of post-treatment CFUs was counted. Experiments were performed in at least  
193 three independent replicates.

194

195

## 196 **2.4. Membrane, ethanol treatment and filtration protocol**

197 The NF90 nanofiltration membrane (Dow Filmtec) was used as a flat sheet in all the  
198 experiments. All membrane samples used were initially rinsed and soaked overnight at 4°C in  
199 MilliQ water to remove any preservatives from the surface.

200

201 Membrane pure-water flux and salt retention tests were performed in three cross-flow  
202 filtration cells operated in parallel in a closed-loop system driven by a high pressure pump  
203 (model P200, Hydra-Cell, UK). Details on the filtration cells and the system can be found in  
204 a previous work by the current authors [37].

205 The membrane samples were compacted in the cross flow system with MilliQ water for 22  
206 hours at 16 bar. When first exposed to high pressure these membranes underwent a period of  
207 compaction observed as a steady drop in pure-water flux. Six to ten hours of compaction  
208 resulted in an almost constant membrane flux. However it was noticed that if the pressure  
209 was alleviated and the membrane was left at atmospheric pressure for a few hours, restarting  
210 the compaction at 16 bar led to a higher pure water flux compared to the one obtained at the  
211 end of the compaction process. This was attributed to reversibility of the incomplete

212 compaction process [38]. It was hence determined that at least 18 hours of compaction were  
213 necessary to avoid reversibility of the compaction process.

214 Pure water flux was determined by measuring the mass of permeate over a two minute  
215 period. This was repeated three times at half hour intervals to ensure that a steady flux had  
216 been obtained.

217 Salt retention tests of the compacted membranes were performed by filtering a 10 mM  
218 Sodium Chloride (NaCl; Sigma-Aldrich Ireland) solution at 16 bar. Equilibrium was  
219 established after 15 minutes of filtration at which point the conductivities of the feed and  
220 permeate were measured for each cross-flow cell using an inoLab Cond Level 2 system with  
221 a Tetracon 325 probe (WTW, Germany). The system was rinsed out with MilliQ water and  
222 the salt retention of a 5 mM Calcium Chloride ( $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ ; Merck Ireland) solution was  
223 performed in the same conditions.

224

225 Ethanol treatment was then performed at atmospheric pressure with the cells disconnected  
226 from the system, with the membranes still sealed in place. The water on the feed side of the  
227 membranes was replaced with ethanol at the designated concentration and left for 1.5 hours.  
228 The ethanol concentrations used were 0, 20, 30, 40, 60 and 70 %. Final results are an average  
229 of three independent replicates for each concentration.

230

231 Once the treatment solution was rinsed from the cross-flow cells with MilliQ water, they  
232 were reconnected to the system and the membranes were compacted again for 22 hours in  
233 order to obtain a steady flux. Pure water flux and salt retention tests were performed again as  
234 previously described.

235

236

## 237 **2.5. Surface properties**

### 238 *2.5.1. Surface energy*

239 Membrane samples were pre-soaked in MilliQ water and then compacted at 15 bar for 22  
240 hours in the flow cell system. The flow cells were then disconnected from the system, filled  
241 with treatment solution (0% and 70% ethanol respectively) and left for 1.5 hours. The MFS-  
242 cells were rinsed with MilliQ water to remove the treatment solution. The membranes were  
243 removed from the cells and samples cut and affixed to a glass slide using double-sided tape.

244 These were left to dry fully. This process was repeated for another set of samples with the  
 245 addition of a second 22 hour compaction step at 15 bar immediately following treatment.  
 246 The Lifshitz-van der Waals ( $\gamma^{LW}$ ), electron-donor ( $\gamma^-$ ) and electron-acceptor ( $\gamma^+$ ) surface  
 247 tension components of dehydrated treated NF90 membrane samples (S) were determined by  
 248 measuring contact angles using the following expression:

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

249  
 250 Contact angles ( $\theta$ ) and surface energy measurements ( $\gamma^S$ ) of dehydrated compacted NF90  
 251 membrane were measured at room temperature using a goniometer (OCA 20 from  
 252 Dataphysics Instruments) with three static pure liquids (L): deionised water, diiodomethane  
 253 and ethylene glycol.

254 The Lewis acid-base component was deduced from:

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

255  
 256 And the total surface energy was defined by:

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

257  
 258 Contact angle values, and determined surface energy values, represent the mean of at least 6  
 259 to 10 measurements per compacted membrane sample.

260  
 261 *2.5.2. Bench treatment*

262 Membrane samples for surface property analysis were first soaked in MilliQ water and then  
 263 submerged in the designated treatment solution for 1.5 hours. After treatment they were then  
 264 rinsed again with MilliQ water to remove all traces of ethanol before experimentation.

265  
 266 *2.5.3. Fourier transform infrared (FTIR) spectroscopy*

267 Samples for FTIR spectroscopy were dried in air after bench treatment. Three absorption  
 268 spectrums were recorded for each membrane sample, using an Agilent Cary 670 FTIR air-  
 269 bearing spectrometer, and an average taken.

270

#### 271 2.5.4. *Zeta potential*

272 Zeta potential measurements were performed using a ZetaCAD® system (CAD Instruments,  
273 France). Bench treated samples were suspended in the buffer solution overnight (0.1M NaCl,  
274 pH 7) to equilibrate with the salt solution prior to analysis. Zeta potential values were  
275 determined by streaming the buffer solution across each sample, and measuring the resultant  
276 voltage difference. Measurements were taken for a range of flowrates, alternating flow  
277 direction between measurements, each sample was analysed three times.

278

#### 279 2.5.5. *Atomic force microscopy (AFM)*

280 Submerged contact mode AFM images were obtained for 10 x10 µm<sup>2</sup> scan areas, for  
281 membranes bench treated with 0, 30 and 70% ethanol, with a JPK AFM system (JPK  
282 Instruments, Germany) using a Silicone (DNP) cantilever manufactured by Bruker (UK).The  
283 cantilever spring constant was 0.32 N.m<sup>-1</sup> with a nominal tip radius of 10 nm and the line  
284 scan rate was 0.7 Hz. Prior to contact mode imaging of the treated membranes, it was  
285 demonstrated that no difference in R<sub>rms</sub> values was calculated for contact or tapping mode  
286 images for the NF90.

287

288 The scanned images were flattened using Gwyddion SPM image analysis software by fitting  
289 a second-order polynomial into the data in the scanned region and subtraction of the resulting  
290 best fit from the image. Flattening was done to remove curvature and slope from the images.  
291 After flattening, root-mean-squared roughness (R<sub>rms</sub>) was calculated using Equation 4, where  
292  $\bar{z}$  is the average of the z values within the given area,  $z_n$  is the current z value, and N is the  
293 number of data points within the given area. Three R<sub>rms</sub> values were calculated for each  
294 membrane sample and the average taken.

295

$$R_{rms} = \sqrt{\frac{\sum_{n=1}^N (z_n - \bar{z})^2}{N - 1}} \quad (4)$$

296

297

## 298 2.6. **Physical properties**

299

300 2.6.1. *Tension tests*

301 Samples for tension-failure analysis were cut using a dog-bone punch (3.3 mm wide in the  
302 testing region) prior to. Six samples were cut for each bench treatment (0, 30 and 70 %  
303 ethanol). The polyester support layer was removed from three of these, leaving only the  
304 polyamide and polysulfone layers. A Zwick/Roell tensile testing machine (Zwick, Germany)  
305 exerted an increasing tensile load on the samples while a VideoXtens camera unit (Zwick,  
306 Germany) recorded the extension of the testing region. Each sample was tested to failure.  
307

308 **3. Results and discussion**

309

310 **3.1. Disinfection efficacy of ethanol**

311 High concentrations of ethanol (60-70%) may be the most potent for disinfection but they are  
312 also more likely to damage a nanofiltration membrane. This study attempted to determine the  
313 minimum inhibitory concentration (MIC) required to achieve a 4 log<sub>10</sub> reduction of the  
314 chosen bacterial strains, based on European surface test (EN 13697). Preliminary tests (not  
315 shown) performed to determine a suitable exposure time, to achieve maximum efficacy with  
316 minimum concentration, showed insignificant difference in log<sub>10</sub> reduction between a 1.5  
317 hour and 24 hour treatment time.

318

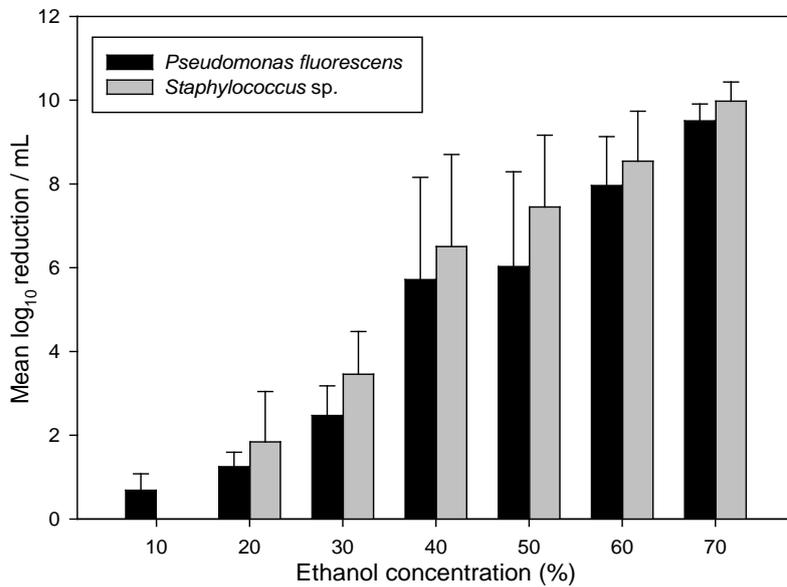
319 3.1.1. *The inhibitory action of different ethanol concentrations on planktonic cells*

320 The inhibitory action of different ethanol concentration was determined using planktonic  
321 Gram-positive and Gram-negative model bacterial cells. This study enabled to help define the  
322 minimum inhibitory ethanol concentration required to reduce the cultivable bacterial  
323 population in suspension by approximately 4 log<sub>10</sub> cfu/mL.

324

325 Results showed that following a treatment time of 1.5 hours, 40% ethanol was sufficient to  
326 reduce the bacterial population of planktonic *Pseudomonas fluorescens* and *Staphylococcus*  
327 sp. cells by 5.7 log<sub>10</sub> and 6.5 log<sub>10</sub> cfu/mL respectively (Figure 1). This MIC is within the  
328 effective range of ethanol (30 – 70%) on similar bacterial strains discussed by numerous  
329 sources [19-21]. Considering that disinfection efficacy assays are usually performed using a  
330 treatment period from 5 to 60 minutes [21], the long exposure time of 1.5 hours revealed that  
331 lower inhibitory concentrations can be employed to achieve acceptable levels of log<sub>10</sub>  
332 reductions. Lower concentrations of ethanol with the required inhibitory action are preferable

333 in this application as they reduce the risk of potentially damaging or altering the membranes  
334 properties.



335

336 *Figure 1: The inhibitory action following a 1.5 hour exposure period of ethanol at different*  
337 *concentrations (10-70% vol/vol ethanol in PBS) on Pseudomonas fluorescens and*  
338 *Staphylococcus sp. planktonic cell suspensions. Data points are averages of three*  
339 *independent replicates with error bars displaying standard deviations.*

340

### 341 3.1.2. Disinfection of spiked membranes

342 Based on the results obtained from the suspension test, the inhibitory action of ethanol on  
343 spiked membranes was investigated using 1.5 hour treatments with 10, 20, 30 and 40%  
344 ethanol. To ensure that membranes were free from organisms before spiking with either  
345 *Pseudomonas fluorescens* or *Staphylococcus sp.* model strains, membranes were autoclaved at  
346 121°C for 15 minutes. Although sterilising the membranes may have led to their physical  
347 damage, as described elsewhere [13], it did not affect the cell loading and susceptibility to  
348 ethanol exposure (results not shown).

349

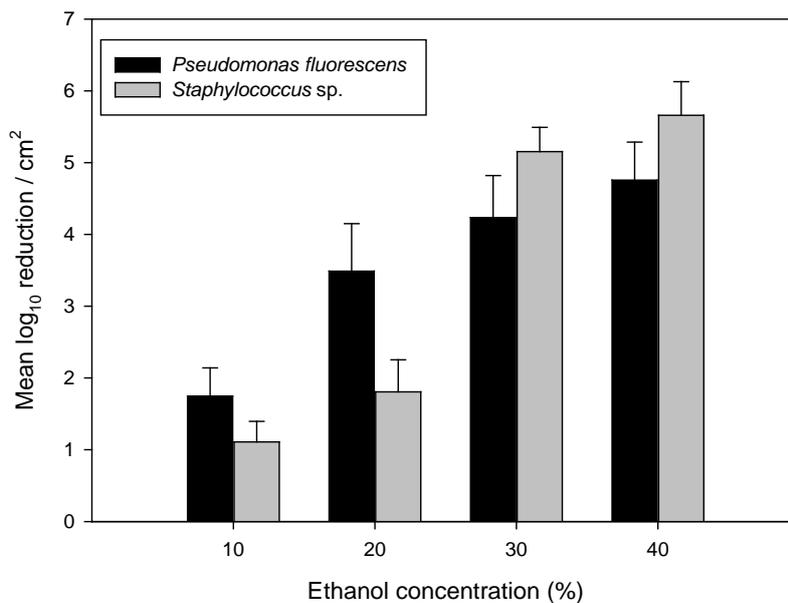
350 Membranes spiked with *Staphylococcus sp.* tended to be less susceptible following 1.5 hour  
351 exposure to low ethanol concentration 10% and 20% compared to *Pseudomonas fluorescens*  
352 spiked membranes (Figure 2). For both spiked membranes, 1.5 hour exposure to 30% and  
353 40% ethanol led to at least a 4 log<sub>10</sub> reduction. *Staphylococcus sp.* spiked membranes

354 revealed the highest log reduction with values exceeding 5 log<sub>10</sub> following exposure to 30%  
355 and 40% ethanol concentrations.

356

357 An equal MIC for adhered and planktonic cells for a specific exposure time is contrary to  
358 what is reported by Chambers et al. [20] who observed a rise in tolerance to 70% ethanol for  
359 plastic-adhered bacteria (including *Staphylococcus* sp.). They discussed that the material  
360 properties may influence the bacteria adhesion, and that the material may grant the cells  
361 protection by reducing the exposed cell surface area. The porosity of the membrane in our  
362 study however may not act in the same way as the plastics described, as the bacteria are in  
363 contact with the ethanol treatment solution absorbed within the membrane active layer. It is  
364 therefore understandable that an MIC for cells adhered to the membrane could equal to that  
365 of planktonic cells.

366



367

368 *Figure 2: The inhibitory action following 1.5 hour exposure to ethanol at different*  
369 *concentrations (10-40% vol/vol ethanol in PBS) on Pseudomonas fluorescens and*  
370 *Staphylococcus sp. spiked NF90 membranes. Data points are averages of three independent*  
371 *replicates with error bars displaying standard deviations.*

372

### 373 3.1.3. Disinfection of virgin membranes

374 In the previous sections it was shown that an MIC of 40% is necessary for a 4log<sub>10</sub> reduction  
375 of two sample strains. In practice the treatment will be applied to virgin membranes which

376 will host many wild strains of bacteria, each with a different tolerance to ethanol. To  
377 determine the efficacy of ethanol treatment on wild strains, samples of virgin membrane were  
378 treated with a range of ethanol concentrations and contacted with agar plates to quantify the  
379 amount of bacteria that had survived the treatment (Figure 3).

380

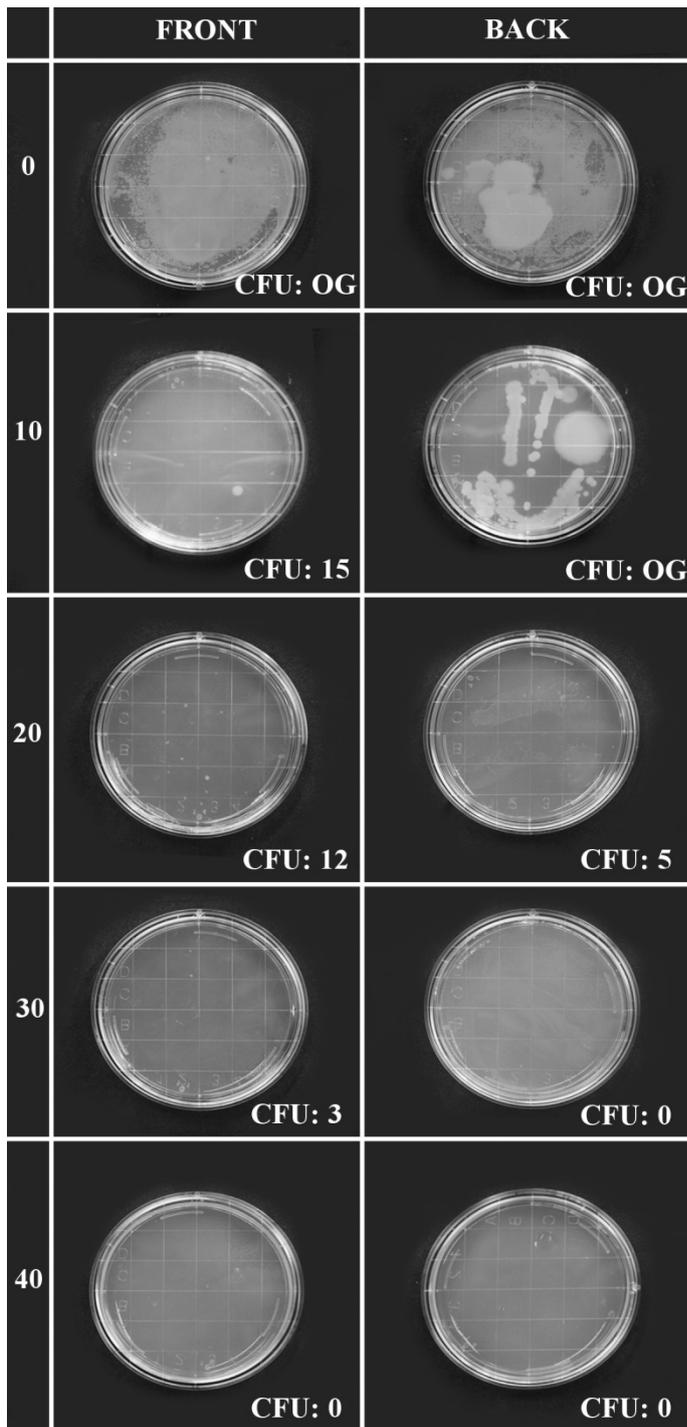
381 Each of the plates contacted with an untreated membrane showed excessive growth  
382 highlighting the importance of disinfecting membranes before conducting any form of  
383 bacterial adhesion of biofouling studies with them. There is a notable difference between the  
384 quantity and types of bacteria on the front (active layer) of the membrane and back (support  
385 layer) of the membrane. More growth was observed for plates which had contacted the back  
386 of the membrane, possibly due to the higher porosity and roughness of the support layer  
387 incurring a higher level of bacterial adhesion.

388

389 There is an expected decreasing trend of bacterial growth with increasing ethanol  
390 concentration, with almost no colonies detected on samples treated with concentrations of  
391 30% or higher. Spore growth could be seen after incubation, however, on plates treated with  
392 concentrations as high as 70% ethanol (not shown) concurrent with the understanding that  
393 ethanol can be used for disinfection but not sterilisation [21].

394

395 The MIC of 40% discussed previously was high enough to remove almost all microorganisms  
396 from the three membranes tested at this concentration (7 CFU were counted on a single plate  
397 contacted with the back of one of the membranes).



399

400 *Figure 3: TSA plates, contacted with the front (active layer) or back (support layer) of an*  
 401 *NF90 membrane pre-treated with 0, 10, 20, 30 or 40% ethanol after 18 hours of incubation.*  
 402 *Plates with a cell density too high to be counted are marked as having overgrowth (OG).*  
 403 *Images shown are representative of the three repetitions.*

404

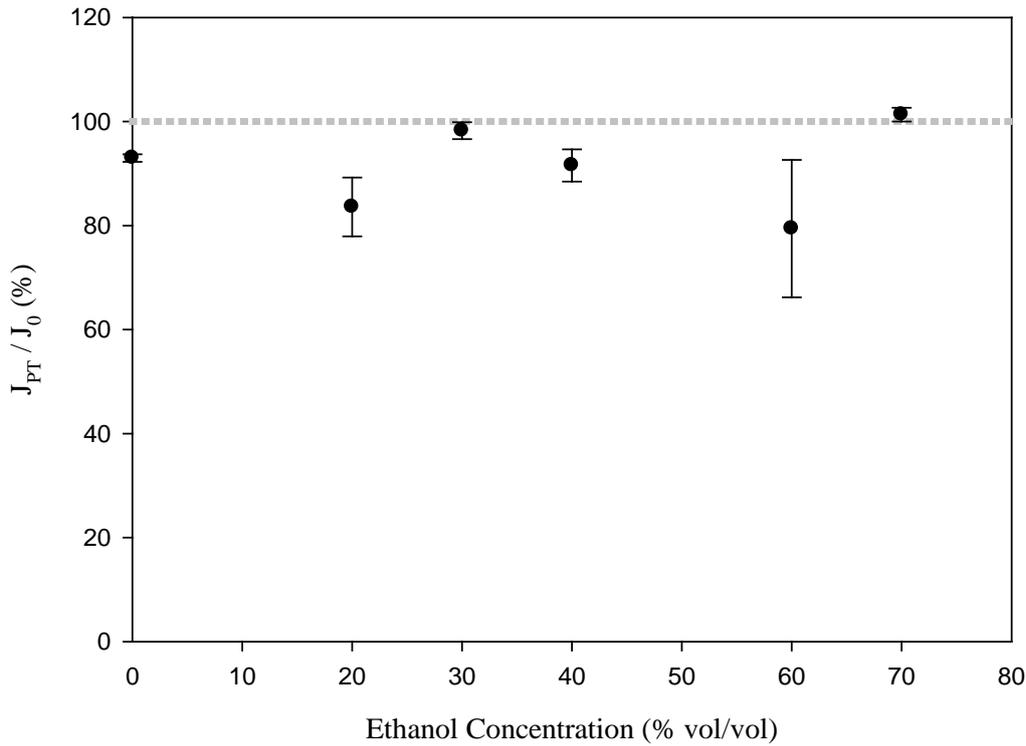
### 405 3.2. Ethanol's effects on membrane performance

406 It has been shown in Section 3.1 that ethanol can be used as a very effective means of  
407 membrane disinfection, capable of at least a 4 log<sub>10</sub> reduction of both planktonic and sessile  
408 bacterial populations. Before it can be considered a suitable disinfection method for  
409 polyamide NF membranes however, it is vital to understand how ethanol affects membrane  
410 performance as well as its physical and chemical properties.

411 Two parameters, pure-water flux and salt retention, were considered in order to quantify  
412 ethanol's effects on the NF membrane's filtration performance. The change in pure water flux  
413 before and after ethanol exposure was initially used to assess if ethanol had any effect in the  
414 membrane structure, such as pore size or porosity.

415  
416 No significant correlation between increasing ethanol concentration and flux change was  
417 observed (Figure 4). As pure water flux is predominantly dependant on pore size and porosity  
418 (when all filtration conditions are the same) it appears that ethanol exposure within the tested  
419 range followed by MilliQ water compaction did not significantly damage the NF90  
420 membrane structure. The observed variance in water flux values over the range of ethanol  
421 treatment concentrations is more likely attributable to the heterogeneity of the membrane  
422 sheet. Membrane samples, although cut from the same sheet, had a range of initial pure-water  
423 fluxes ( $5.03 \pm 0.95$  L/ hr bar m<sup>2</sup>).

424  
425 A small decrease in flux after treatment was seen for the majority of samples measured. Since  
426 this decrease is also present for each of the samples treated with 0% ethanol, the experimental  
427 process rather than exposure to ethanol was concluded to be the cause. Despite the steady  
428 flux achieved during the compaction period, a rise in pure-water flux (up to 28% of the initial  
429 flux) was measured immediately after treatment: evidence that the membrane expanded  
430 during this time. Over the second compaction period the flux decreased once again to a  
431 steady state, lower than the initial steady state in most cases. The decrease is therefore  
432 determined to be associated with slight changes in the polymer configuration following the  
433 expansion and re-compaction of the membrane due to the changing pressure conditions over  
434 the course of the experiment.



435

436 *Figure 4: Pure water flux of NF90 membrane samples, after a 1.5 hour treatment ( $J_{PT}$ ) with*  
 437 *0, 20, 30, 40, 60 or 70 % vol/vol ethanol in MilliQ water, as a percentage of initial pure*  
 438 *water flux ( $J_0$ ). The dotted horizontal line represents no change in flux after treatment. Data*  
 439 *points are averages of three independent replicates with error bars displaying standard*  
 440 *deviations. Test conditions employed were: Temperature = 22°C, Pressure = 16 bar, and*  
 441 *crossflow velocity = 2.2 L/min.*

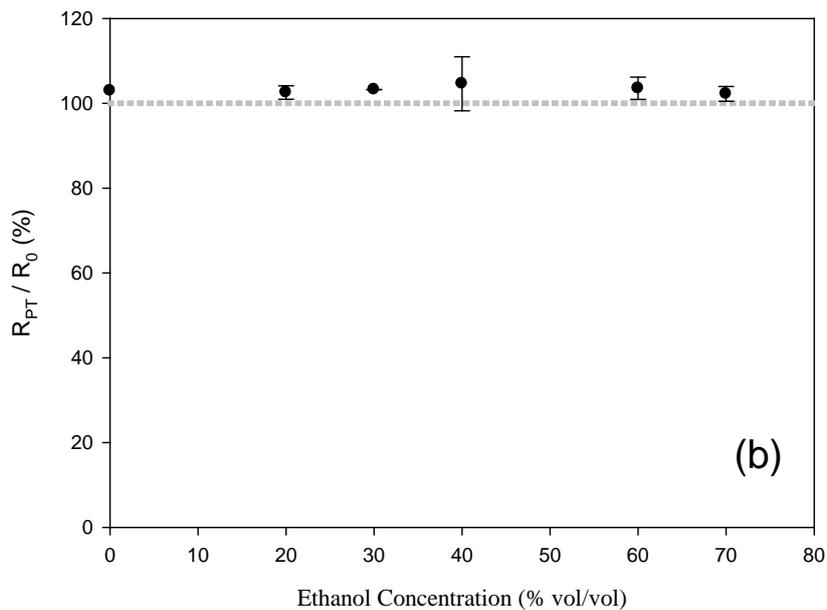
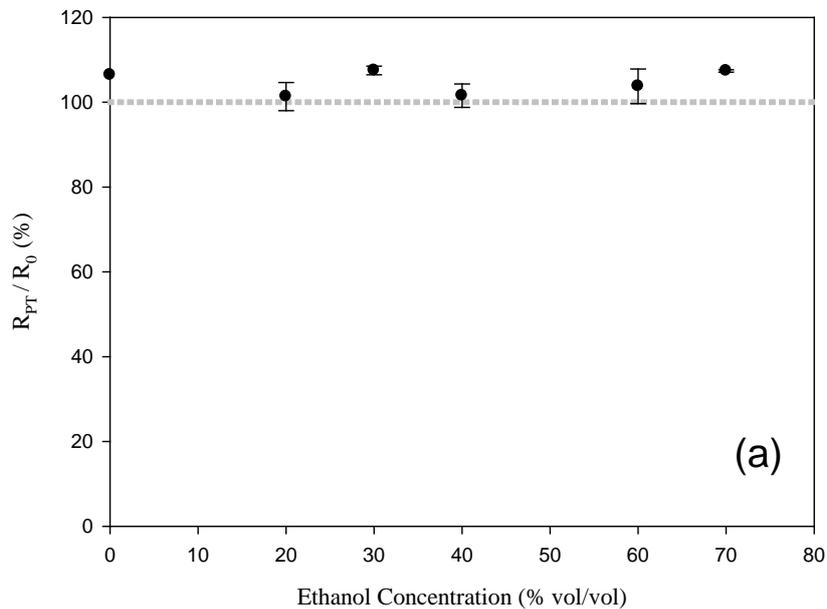
442

443 Changes to the Donnan charge and steric exclusion of the membrane can be quantified by  
 444 analysing the rejection ability of the membrane to charged particles of different sizes such as  
 445 monovalent and divalent ions, obtained from the dissolution of NaCl and CaCl<sub>2</sub> respectively.  
 446 Salt retention was determined by measuring the conductivity of the feed ( $C_f$ ) and permeate  
 447 ( $C_p$ ) samples and employing Equation 5.

448

$$R = 100 * \left( 1 - \frac{C_p}{C_f} \right) \quad (5)$$

449



451

452 *Figure 5: NaCl (10mM; a), and CaCl<sub>2</sub> (5mM; b) retentions of NF90 membrane samples after*  
 453 *a 1.5 hour treatment ( $R_{PT}$ ) with 0, 20, 30, 40, 60 or 70 % vol/vol ethanol in MilliQ water, as a*  
 454 *percentage of initial salt retention ( $R_0$ ). The dotted horizontal line represents no change in*  
 455 *retention after treatment. Data points are averages of three experiments with error bars*  
 456 *displaying standard deviations. Test conditions employed were: Temperature = 22°C,*  
 457 *Pressure = 16 bar, and crossflow velocity = 2.2 m/min.*

458 A small increase in the membrane's retention for each salt solution (maximum of 7.5% for  
 459 NaCl and 4.6% for CaCl<sub>2</sub>) was observed after all treatments, even for samples treated with

460 0% ethanol. This could be associated with the minor drop in post-treatment flux seen  
461 previously in Figure 4. A reduction in porosity of the membrane's active layer due to a  
462 polymer rearrangement during the membrane expansion and recompaction explains both the  
463 membranes drop in pure water flux and this increased retention.

464 Ethanol exposure did not affect the membrane performance in terms of salt retention (Figure  
465 5) and pure water flux (Figure 4), and therefore appears not to have affected the membrane's  
466 pore-size or charge exclusion capacity. These results are contrary to those found by Geens,  
467 Van der Bruggen and Van der Castele who showed that membrane swelling in ethanol lead  
468 to a higher pure-water flux for a hydrophobic membrane [33]. The differences in results most  
469 probably arise from the different active layer polymers of the membranes studied, and the  
470 different treatment times used: 10 days (to see the maximum damage wrought by the solvent)  
471 versus 1.5 hours (to incur a minimal amount of damage by the solvent).

472

### 473 3.3. Ethanol's effects on membrane surface properties

474 Filtration performance is not the only factor that could be affected by exposure to ethanol. An  
475 important prerequisite for undertaking membrane fouling and biofouling research is the  
476 characterisation of the membrane surface properties including hydrophobicity, surface  
477 chemical groups and roughness [39]. Alterations to membrane surface physic-chemical  
478 properties could potentially lead to experimental biases during dynamic biofouling studies. It  
479 was therefore necessary to assess the membrane surface properties following ethanol  
480 treatment.

#### 481 3.3.1. Visual observations

482 During the treatment process some changes in the physical shape and colour of the membrane  
483 were observed. Exposure to ethanol solutions of 20-70% led to a deviation from the  
484 membranes natural curl (originating from the flat sheet having been rolled up by the  
485 supplier). Figure 6 shows how increasing ethanol concentration causes the membrane  
486 samples to become flat and then to bend against the natural curl. Once rinsed with MilliQ  
487 water, these membranes reverted to their original shape.

488

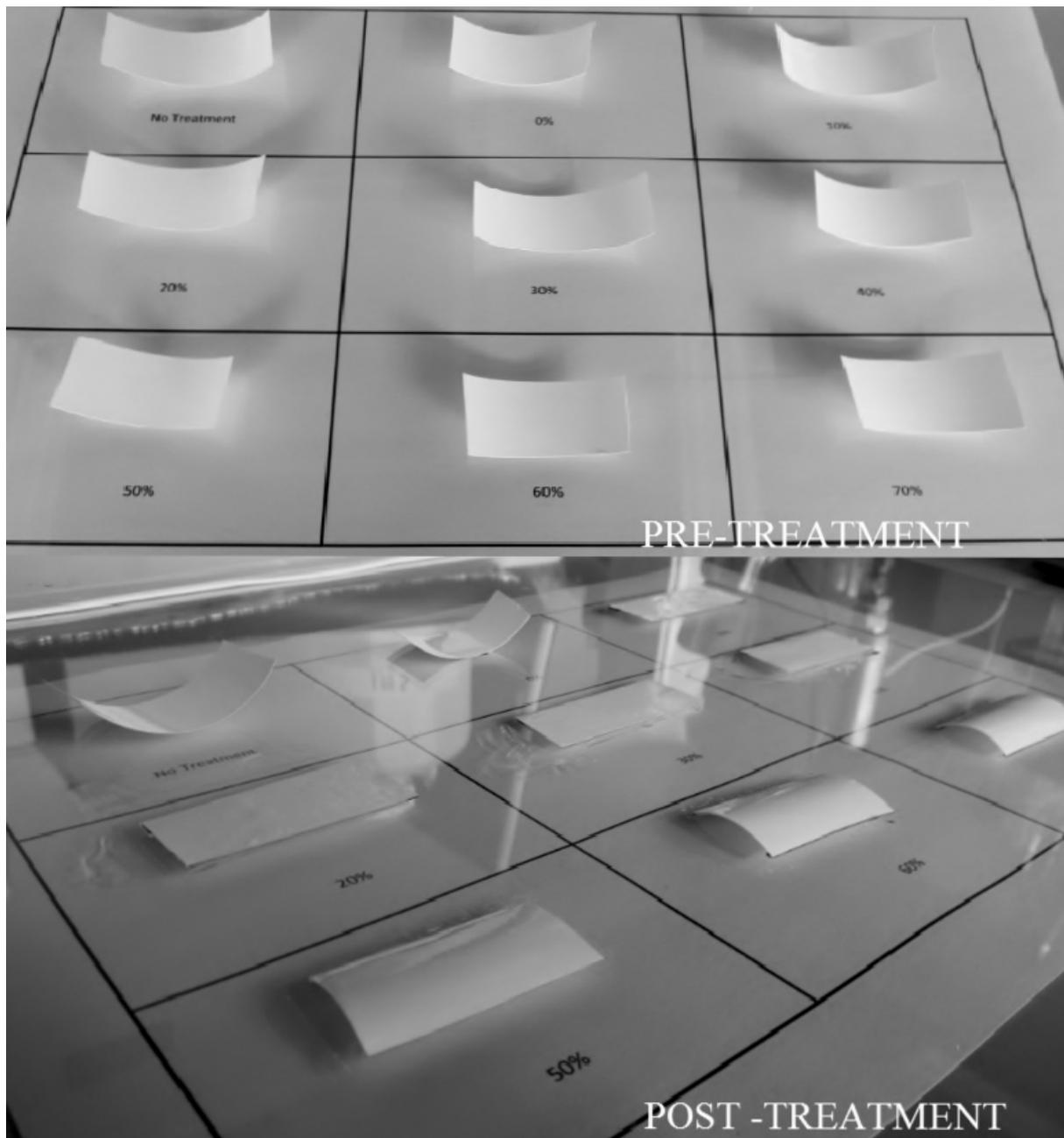
489 Likewise, a visible loss in opacity was observed on membranes treated with high ethanol  
490 concentrations. The NF90 membrane has a natural cream colour which becomes more

491 transparent after exposure to higher ethanol concentrations (>30%). This, however, does not  
492 revert to its original state after rinsing. These results have been combined in Table 1.

493 *Table 1: Visual effects of ethanol treatment on the NF90 membrane samples.*

Ethanol (% vol/vol)	0	10	20	30	40	50	60	70
Curvature	Roll curve	Flat				Polyamide expanding (Curling)		
Colour	No Colour Change				Discolouring/transparency			

494



496

497 *Figure 6: NF90 membrane samples before and after treatment. Treatments shown: No*  
 498 *treatment, 0%, 10%, 20%, 30%, 40%, 50%, 60%, 70% ethanol. Rinsing with MilliQ water*  
 499 *returned the samples to their original shape.*

500

501 Membrane curvature and transparency changes have been reported in previous studies  
 502 involving similar membranes and solvents [27, 28]. Water molecules linked to the hydrogen  
 503 bonds within the polyamide structure are reportedly replaced by ethanol molecules for the  
 504 higher concentration treatments. This causes the polyamide layer to swell slightly stretching

505 the top layer, causing the membrane to curl. When the membrane is immersed in water this  
506 effect is reversed due to water's higher affinity to hydrogen bonding.

### 507 3.3.2. *Surface energy*

508 Surface energy plays a leading role in bacterial adhesion, as shown in a study by Lee et al.  
509 whereby an increasing concentration of adhered bacteria was linearly correlated to increasing  
510 hydrophobicity [39]. Changes to membrane surface energy would therefore negatively impact  
511 on biofouling fundamental research.

512

513 Surface energy experiments were performed on membranes that had been compacted once  
514 (prior to treatment with 0 and 70% ethanol) with MilliQ water, and on membranes that had  
515 been compacted twice (before and after treatment with 0 and 70% ethanol). In this way the  
516 effects of both compaction and ethanol on membrane hydrophobicity could be analysed in  
517 parallel. The results are presented in Table 2.

518

519 The results show membranes that had been compacted a single time to have a hydrophobic  
520 tendency with a contact angle ( $\theta$ ) in the range 106-110° while those that had been compacted  
521 twice had a hydrophilic nature with a contact angle in the range 80-86°. Contact angle  
522 measurements of membranes are often difficult to perform as the porous surface of the active  
523 layer absorbs the droplets: the contact angle has been found to vary with time [40]. This issue  
524 was alleviated somewhat for the compacted membranes: the droplets retained their shape for  
525 longer and the results were more reproducible for each sample than ones measured previously  
526 with non-compacted membranes (results not shown). While ethanol had no significant effect  
527 on the membrane's surface energy, compaction seems to have affected its hydrophobicity,  
528 possibly linked to the decrease in porosity of the active layer shown previously in section 3.2,  
529 hence the differences in absorption of the water droplets on the membrane surface. This could  
530 be an interesting area for future research.

531

532 The values obtained for the other components of surface energy show a large variance for the  
533 membrane samples studied probably linked to the heterogeneous nature of the membrane's  
534 active layer.

535

536 *Table 2: Surface energy components of NF90 membrane samples treated with 0 and 70%*  
 537 *ethanol: contact angle ( $\theta$ ), electron-donor ( $\gamma^-$ ), electron-acceptor ( $\gamma^+$ ), Lewis acid-base ( $\gamma^{AB}$ ),*  
 538 *Lifshitz-van der Waals ( $\gamma^{LW}$ ), total surface free energy ( $\gamma^S$ ).*

Sample Treatment	$\theta$	$\gamma^-$	$\gamma^+$	$\gamma^{LW}$	$\gamma^{AB}$	$\gamma^S$
0% 1 compaction	106.9 ± 0.69	40.15 ± 0.28	0.67 ± 0.14	2.42 ± 0.38	10.17 ± 1.2	12.60 ± 1.49
70% 1 compaction	108.3 ± 1.23	41.80 ± 0.36	0.99 ± 0.35	4.06 ± 0.969	12.10 ± 2.26	16.16 ± 3.08
0% 2 compactations	81.28 ± 1.41	37.60 ± 0.33	1.39 ± 0.68	14.19 ± 3.58	12.01 ± 4.13	26.21 ± 7.65
70% 2 compactations	85.74 ± 2.19	41.02 ± 0.18	0.47 ± 0.27	6.17 ± 2.61	7.16 ± 2.57	13.34 ± 5.1

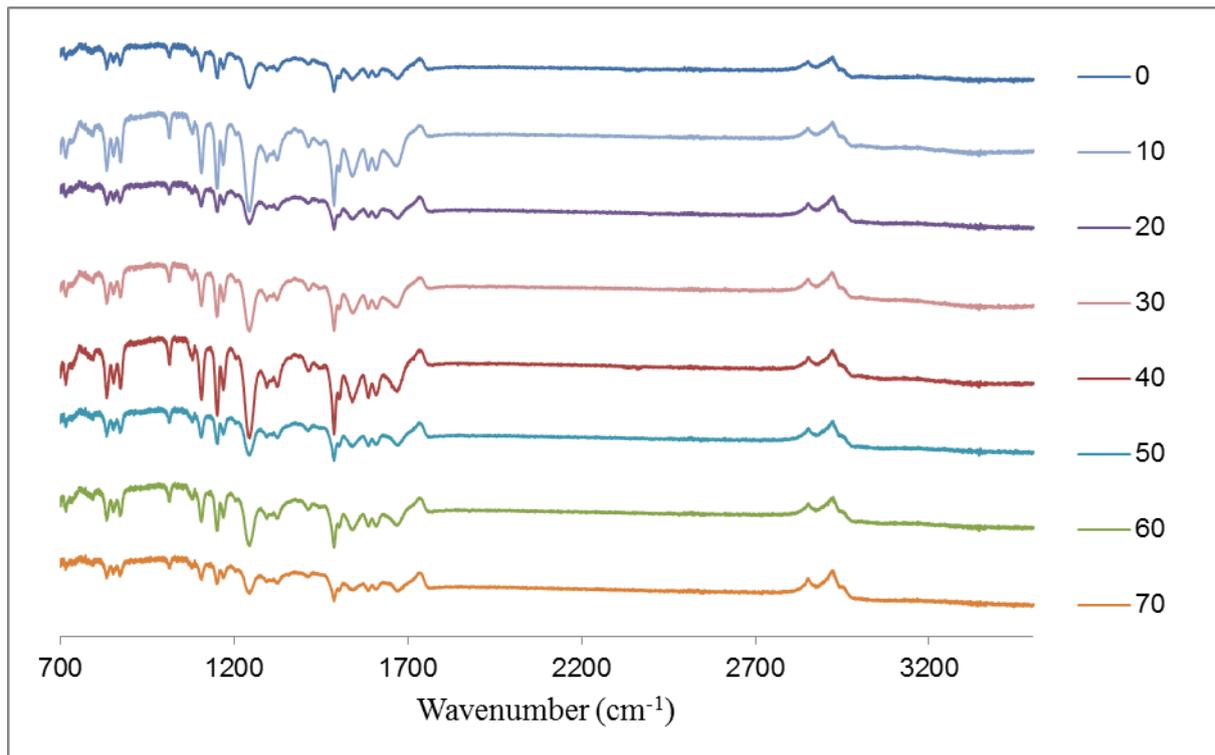
539

540

### 541 3.3.3. *Surface chemistry*

542 FTIR spectrums of the active layer chemistry of membrane samples treated with various  
 543 ethanol concentrations are shown in Figure 7. The results show no peak straying and minimal  
 544 change in peak area. The largest changes in peak area were around wavenumbers 800, 1100,  
 545 1220 and 1480. The largest deviations in each case were for 10 and 40 % ethanol while those  
 546 treated with intermittent concentrations remained unaffected. Furthermore, these  
 547 wavenumbers are commonly associated to ‘C-H bend’ and ‘C-O stretch’ molecular motions;  
 548 these bonds are abundant in the polyamide structure. As there is no peak change around  
 549 wavenumbers 3300-3400 (associated with ‘O-H stretch’ motions) it is unlikely that these  
 550 peaks are associated to any alterations of the membrane’s surface chemistry by ethanol.

551 These results show that ethanol was not responsible for the observed surface chemistry  
 552 variations. They are merely further evidence of the heterogeneous nature of the polyamide  
 553 structure and surface chemistry of the membrane.



554

555 *Figure 7: FTIR spectrums of NF90 membrane samples treated with 0, 10, 20, 30, 40, 50, 60,*  
 556 *or 70% vol/vol ethanol. Each spectrum shown is an average of readings done in triplicate.*

557

#### 558 3.3.4. Zeta potential

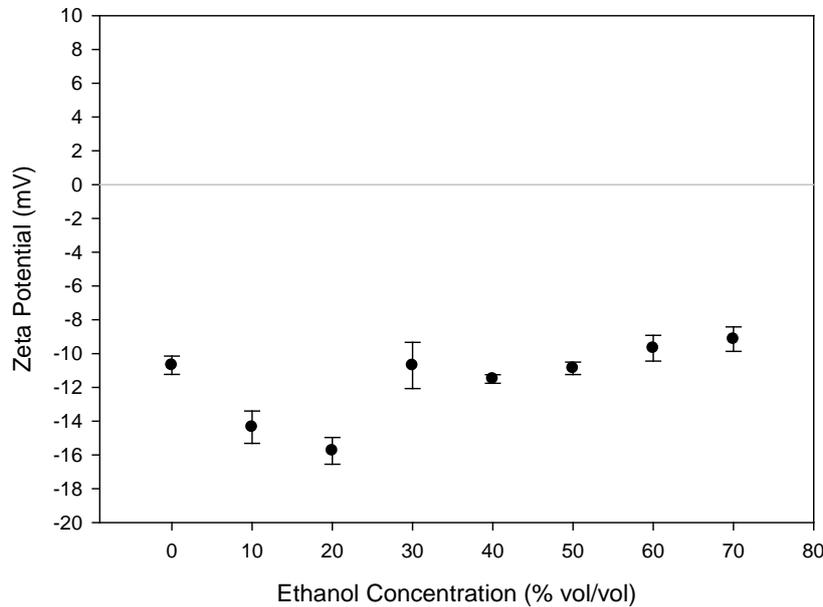
559 Zeta potential is another factor which is important to characterise due to the role it plays in  
 560 colloidal deposition and bio-adhesion on NF membranes [41]. If ethanol exposure has had a  
 561 significant effect on the membrane's surface charge it could affect the membrane's biofouling  
 562 outcome.

563 The results of the study performed for the range of ethanol treatments can be seen in Figure 8.

564 The zeta potential of the membrane samples was relatively constant ( $-10 \pm 1.5\text{mV}$ ) for the  
 565 majority of the ethanol concentrations tested, concurring with the previous salt retention  
 566 analysis (*Figure 5*). The samples which were exposed to 10% and 20% ethanol however each  
 567 had a notably lower zeta potential,  $-14.4\text{mV}$  and  $-15.7\text{mV}$  respectively. No correlation could  
 568 be found for these two concentrations with the salt retention results in Section 3.2.

569 Pasmore et al. [41] showed in their experiments on the role of zeta potential in bacterial  
 570 adhesion that a positive (greater than 0) or highly negative (lower than  $-20$ ) zeta potential can  
 571 have a significant effect on bacterial adhesion. In this context the range of results obtained in  
 572 this experiment is not enough to impact on the bacterial adhesion potential of the NF90

573 membrane. Therefore ethanol's effects on this membrane's zeta potential can be concluded to  
574 be insignificant.



575  
576 *Figure 8: Zeta Potential of NF90 samples after bench treatment with 0,10,20,30,40,50,60, or*  
577 *70% ethanol. Data points are averages of three experiments with error bars displaying*  
578 *standard deviations. Test conditions employed were: Temperature = 20°C, Test solution =*  
579 *0.1M NaCl and PH = 7.*

580

### 581 3.3.5. Membrane roughness

582 Membrane roughness has been linked in previous studies as a leading factor in the adhesion  
583 of bacteria onto a membrane [42]. The roughness values presented in Table 3 show no  
584 correlating effect with increasing ethanol concentration on the roughness of the NF90  
585 membrane. There is however a minor decrease (11%) in average roughness for the  
586 membranes treated with 30% ethanol. When seen in the context of Subramani's analysis of  
587 bacterial adhesion onto membranes with a range of roughness averages (4-108 nm) [34],  
588 however, it is obvious that this minor difference would not significantly affect bacterial  
589 adhesion experiments. This decrease in roughness is most likely an error due to the small  
590 sample size (3 images) taken, and the heterogeneity of the membrane.

591 *Table 3: Roughness values for three NF90 membrane samples treated with 0, 30 or 70%*  
 592 *ethanol.  $R_{rms}$  values shown are an average three  $10 \times 10 \mu\text{m}^2$  images for each sample; the*  
 593 *standard deviation of these values is shown.*

Ethanol Concentration (vol/vol)	$R_{rms}$ Image 1 (nm)	$R_{rms}$ Image 2 (nm)	$R_{rms}$ Image 3 (nm)	<b>Average Roughness (<math>R_{rms}</math>) (nm)</b>
0%	90.96	99.52	100.00	<b><math>96.83 \pm 5.09</math></b>
30%	91.64	83.70	83.47	<b><math>86.27 \pm 4.65</math></b>
70%	96.35	103.00	89.97	<b><math>96.44 \pm 6.52</math></b>

594

595

### 596 **3.4. Ethanol’s effects on membrane mechanical properties**

597

#### 598 3.4.1. *Tensile strength*

599 The polyamide layer of the membrane consists of many long-chain polymers which may be  
 600 arranged in a crystalline or amorphous way. If ethanol has affected this structure physically,  
 601 through the swelling behaviour described previously in the visual results or otherwise, it  
 602 could lead to a weakening of the active layer that may be undetectable via the methods  
 603 described previously. As these membranes operate at high pressure, changes in strength may  
 604 elevate the risk of membrane failure.

605

606 Table 4 shows the tensile strength properties that were determined by testing samples of the  
 607 NF90 membrane, with and without the polyester support layer, to failure. The role of the  
 608 support layer is obvious from the large difference in tensile strength between the membrane  
 609 samples with ( $226 \pm 25$  MPa), and those without ( $76.4 \pm 17$  MPa) the support layer. For each  
 610 case the membrane samples exhibited characteristic stress/strain curves with similar slopes  
 611 (Young’s modulus), yield stresses and sharp declines upon failure indicative of the brittle  
 612 nature of the polymer material. These values are comparable to those found by Chung et  
 613 al.[43] in their 2011 study of chlorine-induced mechanical deterioration of a polyamide RO  
 614 membrane; their results showed the polyamide layer to have a Young’s modulus in the range  
 615 of 1GPa with an ultimate tensile strength of 67MPa. Furthermore, their study showed no  
 616 significant increase in Young’s modulus after a short exposure to chlorine but a four times  
 617 increase in Young’s modulus after a long exposure time.

618

619 Ethanol treatment for such a short exposure time had no significant effect on tensile strength,  
620 with results from samples treated with each concentration within the margin of error. The  
621 error in this experiment stemmed from the tiny cross-sectional area due to the membrane’s  
622 ultrathin nature.

623 *Table 4: Tensile strength and elastic properties of an NF90 membrane after treatment with*  
624 *various concentrations of ethanol.*

Ethanol Concentration (% vol/vol)	Entire Membrane			Polyamide/Polysulfone		
	Young’s Modulus (GPa)	Yield Stress (MPa)	Ultimate Tensile Strength (MPa)	Young’s Modulus (GPa)	Yield Stress (MPa)	Ultimate Tensile Strength (MPa)
0%	8.14±0.36	62.4±0.37	236±16	3.1±0.20	33.7±3.4	74.1±24
30%	7.76±0.68	58.9±1.19	209±39	3.26±0.13	31.8±8.0	83.3±17
70%	8.26±0.97	66.6±1.11	232±12	3.27±0.58	30.9±3.6	71.8±13

625

626

627 **4. Conclusion**

628 This study has shown a 1.5 hour treatment of 40% ethanol to be a suitable and effective  
629 method of disinfecting Dow Filmtec NF90 membranes. Researchers conducting bacterial  
630 adhesion and biofouling studies with NF90 membranes can now use this treatment to remove  
631 competing microorganisms from their samples without affecting membrane properties.

632

633 It is important to note that each membrane has a unique active-layer polymeric structure, and  
634 thus has a specific chemical interaction with ethanol. While this treatment has been shown to  
635 be suitable for an NF90 membrane this may not be true for all other polyamide RO and NF  
636 membranes. It is therefore imperative that researchers are aware of the chemical sensitivity of  
637 these membranes and that they evaluate a disinfectant’s effects on their studied membrane  
638 prior to experimental use. Further investigation is required to assess the suitability of this  
639 disinfection protocol for other polyamide membranes.

640

641 While this study focused on the application of ethanol as a means of disinfecting a membrane  
642 prior to biological fouling, the results (showing bacterial removal without damaging  
643 membrane properties) may be of interest as a means of removing biological fouling. For this  
644 purpose further study would also be required on the repeated treatment of these membranes  
645 with ethanol.

646

647 The effects of membrane compaction and swelling were seen in a number of the experiments  
648 in this study. Further research in this area is required to fully understand reversible  
649 compaction and the way in which the polyamide layer restructures itself upon re-compaction.

650

## 651 5. Acknowledgments

652 This research was supported by the European Research Council (ERC), project 278530,  
653 funded under the EU Framework Programme 7 and also with the financial support of Science  
654 Foundation Ireland under Grant Number “SFI 11/RFP.1/ENM/3145. The authors would like  
655 to thank Dr. Dennis Dowling and the Surface Engineering research group at UCD, and Prof.  
656 Suzi Jarvis and the Nanoscale Function Group at UCD. We thank Mr. Pat O'Halloran for his  
657 invaluable technical assistance, and Mr. Liam Morris for the construction of the MFS  
658 devices.

659

660

## 661 6. References

- 662 1. Cyna, B., G. Chagneau, G. Bablon, and N. Tanghe, *Two years of nanofiltration at the Méry-*  
663 *sur-Oise plant, France*. Desalination, 2002. **147**(1–3): p. 69-75.
- 664 2. Nghiem, L.D. and A.I. Schäfer, *Critical risk points of nanofiltration and reverse osmosis*  
665 *processes in water recycling applications*. Desalination, 2006. **187**(1–3): p. 303-312.
- 666 3. Pontié, M., H. Dach, J. Leparc, M. Hafsi, and A. Lhassani, *Novel approach combining physico-*  
667 *chemical characterizations and mass transfer modelling of nanofiltration and low pressure*  
668 *reverse osmosis membranes for brackish water desalination intensification*. Desalination,  
669 2008. **221**(1–3): p. 174-191.
- 670 4. Ba, C., D.A. Ladner, and J. Economy, *Using polyelectrolyte coatings to improve fouling*  
671 *resistance of a positively charged nanofiltration membrane*. Journal of Membrane Science,  
672 2010. **347**(1–2): p. 250-259.
- 673 5. Vrouwenvelder, J.S., S.A. Manolarakis, J.P. van der Hoek, J.A.M. van Paassen, W.G.J. van der  
674 Meer, J.M.C. van Agtmaal, H.D.M. Prummel, J.C. Kruithof, and M.C.M. van Loosdrecht,  
675 *Quantitative biofouling diagnosis in full scale nanofiltration and reverse osmosis*  
676 *installations*. Water Research, 2008. **42**(19): p. 4856-4868.
- 677 6. Khan, M.M.T., P.S. Stewart, D.J. Moll, W.E. Mickols, M.D. Burr, S.E. Nelson, and A.K. Camper,  
678 *Assessing biofouling on polyamide reverse osmosis (RO) membrane surfaces in a laboratory*  
679 *system*. Journal of Membrane Science, 2010. **349**(1–2): p. 429-437.

- 680 7. Khan, M.M.T., P.S. Stewart, D.J. Moll, W.E. Mickols, S.E. Nelson, and A.K. Camper,  
681 *Characterization and effect of biofouling on polyamide reverse osmosis and nanofiltration*  
682 *membrane surfaces*. *Biofouling*, 2011. **27**(2): p. 173-183.
- 683 8. Ridgway, H.F., M.G. Rigby, and D.G. Argo, *Adhesion of a Mycobacterium sp. to cellulose*  
684 *diacetate membranes used in reverse osmosis*. *Applied and Environmental Microbiology*,  
685 1984. **47**(1): p. 61-67.
- 686 9. Miller, D.J., P.A. Araújo, P.B. Correia, M.M. Ramsey, J.C. Kruithof, M.C.M. van Loosdrecht,  
687 B.D. Freeman, D.R. Paul, M. Whiteley, and J.S. Vrouwenvelder, *Short-term adhesion and*  
688 *long-term biofouling testing of polydopamine and poly(ethylene glycol) surface modifications*  
689 *of membranes and feed spacers for biofouling control*. *Water Research*, 2012. **46**(12): p.  
690 3737-3753.
- 691 10. Suwarno, S.R., X. Chen, T.H. Chong, V.L. Puspitasari, D. McDougald, Y. Cohen, S.A. Rice, and  
692 A.G. Fane, *The impact of flux and spacers on biofilm development on reverse osmosis*  
693 *membranes*. *Journal of Membrane Science*, 2012. **405–406**(0): p. 219-232.
- 694 11. Vanysacker, L., P. Declerck, and I. Vankelecom, *Development of a high throughput cross-flow*  
695 *filtration system for detailed investigation of fouling processes*. *Journal of Membrane*  
696 *Science*, 2013. **442**(0): p. 168-176.
- 697 12. Nilsson, M., G. Trägårdh, and K. Östergren, *The influence of sodium chloride on mass transfer*  
698 *in a polyamide nanofiltration membrane at elevated temperatures*. *Journal of Membrane*  
699 *Science*, 2006. **280**(1–2): p. 928-936.
- 700 13. Sharma, R.R. and S. Chellam, *Temperature Effects on the Morphology of Porous Thin Film*  
701 *Composite Nanofiltration Membranes*. *Environmental Science & Technology*, 2005. **39**(13):  
702 p. 5022-5030.
- 703 14. Nilsson, M., G. Trägårdh, and K. Östergren, *The influence of different kinds of pre-treatment*  
704 *on the performance of a polyamide nanofiltration membrane*. *Desalination*, 2006. **195**(1–3):  
705 p. 160-168.
- 706 15. Antony, A., R. Fudianto, S. Cox, and G. Leslie, *Assessing the oxidative degradation of*  
707 *polyamide reverse osmosis membrane—Accelerated ageing with hypochlorite exposure*.  
708 *Journal of Membrane Science*, 2010. **347**(1–2): p. 159-164.
- 709 16. Benavente, J. and M.I. Vázquez, *Effect of age and chemical treatments on characteristic*  
710 *parameters for active and porous sublayers of polymeric composite membranes*. *Journal of*  
711 *Colloid and Interface Science*, 2004. **273**(2): p. 547-555.
- 712 17. Ortega Morente, E., M.A. Fernández-Fuentes, M.J. Grande Burgos, H. Abriouel, R. Pérez  
713 Pulido, and A. Gálvez, *Biocide tolerance in bacteria*. *International Journal of Food*  
714 *Microbiology*, 2013. **162**(1): p. 13-25.
- 715 18. Kalathenos, P. and N.J. Russell, *Ethanol as a food preservative*, in *Food Preservatives*, N.J.  
716 Russell and G. Gould, Editors. 2003, Springer US. p. 196-217.
- 717 19. Penna, T.C., P.G. Mazzola, and A.M.S. Martins, *The efficacy of chemical agents in cleaning*  
718 *and disinfection programs*. *BMC Infectious Diseases*, 2001. **1**(1): p. 16.
- 719 20. Chambers, S.T., B. Peddie, and A. Pithie, *Ethanol disinfection of plastic-adherent micro-*  
720 *organisms*. *Journal of Hospital Infection*, 2006. **63**(2): p. 193-196.
- 721 21. Block, S.S., *Disinfection, Sterilization, and Preservation, 5e*2001: Lippincott Williams &  
722 Wilkins.
- 723 22. C.E.N., *NS-EN 136697: Quantitative Non-porous Surface Test for the Evaluation of*  
724 *Bacteriocidal and /or Fungicidal Activity of Chemical Disinfectants Used in Food, industrial,*  
725 *Domestic and Institutional Areas. Test Method and Requirements with Mechanical Action.* ,  
726 2001: Brussels, Belgium.
- 727 23. Machado, D.o.R., D. Hasson, and R. Semiat, *Effect of solvent properties on permeate flow*  
728 *through nanofiltration membranes. Part I: investigation of parameters affecting solvent flux*.  
729 *Journal of Membrane Science*, 1999. **163**(1): p. 93-102.

- 730 24. Van der Bruggen, B., J. Geens, and C. Vandecasteele, *Fluxes and rejections for nanofiltration*  
731 *with solvent stable polymeric membranes in water, ethanol and n-hexane*. Chemical  
732 Engineering Science, 2002. **57**(13): p. 2511-2518.
- 733 25. Shukla, R. and M. Cheryan, *Performance of ultrafiltration membranes in ethanol–water*  
734 *solutions: effect of membrane conditioning*. Journal of Membrane Science, 2002. **198**(1): p.  
735 75-85.
- 736 26. Lencki, R.W. and S. Williams, *Effect of nonaqueous solvents on the flux behavior of*  
737 *ultrafiltration membranes*. Journal of Membrane Science, 1995. **101**(1–2): p. 43-51.
- 738 27. Kwiatkowski, J. and M. Cheryan, *Performance of Nanofiltration Membranes in Ethanol*.  
739 Separation Science and Technology, 2005. **40**(13): p. 2651-2662.
- 740 28. Yang, X.J., A.G. Livingston, and L. Freitas dos Santos, *Experimental observations of*  
741 *nanofiltration with organic solvents*. Journal of Membrane Science, 2001. **190**(1): p. 45-55.
- 742 29. Geens, J., K. Peeters, B. Van der Bruggen, and C. Vandecasteele, *Polymeric nanofiltration of*  
743 *binary water–alcohol mixtures: Influence of feed composition and membrane properties on*  
744 *permeability and rejection*. Journal of Membrane Science, 2005. **255**(1–2): p. 255-264.
- 745 30. Geens, J., B. Van der Bruggen, and C. Vandecasteele, *Characterisation of the solvent stability*  
746 *of polymeric nanofiltration membranes by measurement of contact angles and swelling*.  
747 Chemical Engineering Science, 2004. **59**(5): p. 1161-1164.
- 748 31. Geens, J., K. Boussu, C. Vandecasteele, and B. Van der Bruggen, *Modelling of solute transport*  
749 *in non-aqueous nanofiltration*. Journal of Membrane Science, 2006. **281**(1–2): p. 139-148.
- 750 32. Jeżowska, A., T. Schipolowski, and G. Wozny, *Influence of simple pre-treatment methods on*  
751 *properties of membrane material*. Desalination, 2006. **189**(1–3): p. 43-52.
- 752 33. Van der Bruggen, B., J. Geens, and C. Vandecasteele, *Influence of organic solvents on the*  
753 *performance of polymeric nanofiltration membranes*. Separation Science and Technology,  
754 2002. **37**(4): p. 783-797.
- 755 34. Subramani, A. and E.M.V. Hoek, *Direct observation of initial microbial deposition onto*  
756 *reverse osmosis and nanofiltration membranes*. Journal of Membrane Science, 2008. **319**(1–  
757 2): p. 111-125.
- 758 35. Bloomfield, S.F., M. Arthur, K. Begun, and H. Patel, *Comparative testing of disinfectants using*  
759 *proposed European surface test methods*. Letters in Applied Microbiology, 1993. **17**(3): p.  
760 119-125.
- 761 36. Bloomfield, S.F., M. Arthur, B.v. Klingerren, W. Pullen, J. Holah, and R. Elton, *An evaluation of*  
762 *the repeatability and reproducibility of a surface test for the activity of disinfectants*. Journal  
763 of Applied Microbiology, 1994. **76**(1): p. 86-94.
- 764 37. Semiao, A.J.C., O. Habimana, H. Cao, R. Heffernan, A. Safari, and E. Casey, *The importance of*  
765 *laboratory water quality for studying initial bacterial adhesion during NF filtration processes*.  
766 Water Research. **47**(8): p. 2909-2920.
- 767 38. Košutić, K., L. Kaštelan-Kunst, and B. Kunst, *Porosity of some commercial reverse osmosis and*  
768 *nanofiltration polyamide thin-film composite membranes*. Journal of Membrane Science,  
769 2000. **168**(1–2): p. 101-108.
- 770 39. Lee, W., C.H. Ahn, S. Hong, S. Kim, S. Lee, Y. Baek, and J. Yoon, *Evaluation of surface*  
771 *properties of reverse osmosis membranes on the initial biofouling stages under no filtration*  
772 *condition*. Journal of Membrane Science, 2010. **351**(1–2): p. 112-122.
- 773 40. Verliefde, A.R.D., E.R. Cornelissen, S.G.J. Heijman, I. Petrinic, T. Luxbacher, G.L. Amy, B. Van  
774 der Bruggen, and J.C. van Dijk, *Influence of membrane fouling by (pretreated) surface water*  
775 *on rejection of pharmaceutically active compounds (PhACs) by nanofiltration membranes*.  
776 Journal of Membrane Science, 2009. **330**(1–2): p. 90-103.
- 777 41. Pasmore, M., P. Todd, S. Smith, D. Baker, J. Silverstein, D. Coons, and C.N. Bowman, *Effects*  
778 *of ultrafiltration membrane surface properties on Pseudomonas aeruginosa biofilm initiation*  
779 *for the purpose of reducing biofouling*. Journal of Membrane Science, 2001. **194**(1): p. 15-32.

- 780 42. Vrijenhoek, E.M., S. Hong, and M. Elimelech, *Influence of membrane surface properties on*  
781 *initial rate of colloidal fouling of reverse osmosis and nanofiltration membranes*. Journal of  
782 Membrane Science, 2001. **188**(1): p. 115-128.
- 783 43. Chung, J.Y., J.-H. Lee, K.L. Beers, and C.M. Stafford, *Stiffness, strength, and ductility of*  
784 *nanoscale thin films and membranes: a combined wrinkling–cracking methodology*. Nano  
785 letters, 2011. **11**(8): p. 3361-3365.

786

787