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Disinfection of a Polyamide Nanofiltration Membrane using Ethanol

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Abstract

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

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1.   Introduction

Nanofiltration (NF) is becoming a prevalent process in the production of drinking water [1],

in water recycling [2] and may play an increasing role in water desalination [3].

Nanofiltration research in these areas has focused on two main objectives: 1) maximising

permeation while achieving a high level of rejection, and 2) maintaining the longevity of

filtration properties, primarily through the mitigation of fouling [4, 5].

Of the several types of fouling that occur, the adhesion and proliferation of microorganisms

on the membrane known as biofouling is particularly problematic. It is of utmost importance

that fundamental research in which bacterial pure cultures are used, for adhesion and

biofouling experiments, be conducted on sterile surfaces. Biofouling experiments performed

on non-sterile surfaces are at risk of experimental bias, resulting in unspecified multispecies

biofilms. Virgin membranes received from manufacturers may be initially clean but are non-

sterile, representing a potential contamination source for controlled adhesion and biofouling

studies in which pure cultures are utilised.

Sterilisation can be achieved by chemical, thermal, and irradiation means. There is a major

emphasis on sterility in microbiological research and yet there is little research into

sterilisation techniques for NF and reverse osmosis (RO) membranes. Although a number of

different research groups have reported the use of thermal [6, 7] and chemical [8-11]

sterilization procedures on membranes, few have considered the damaging effects that such

treatments could potentially have. This comes at notable risk considering the available

information showing the thermal [12, 13] and chemical sensitivity [14-16] of filtration

membranes. These studies show changes to the flux, active layer stability, pore size, pore

density and chemical composition of the membrane, all of which could be detrimental to a

study involving membrane permeation.

Alcohols are commonly noted to have biocidal properties. They act as cell membrane

disruptors and denature proteins, inhibiting growth [17, 18]. With their short carbon chains,

ethanol and isopropanol are the most widely used alcohols for this purpose. They are

commonly used in the food industry, as a preservative, and in the healthcare sector, where

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 $\log_{10}$ (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 $\log_{10}$

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 Casteele 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

2.1. **Chemicals**

The water used throughout this study was Grade 1 pure water (18.2 MΩcm^{-1}) obtained from an Elga Process Water System (Biopure 15 and Purelab flex 2, Veolia, Ireland), hereafter referred to as MilliQ water.

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

2.2. **Bacteria**

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

2.3. **Disinfection**

2.3.1. *Disinfection of cells in suspension*

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

2.3.2. *Disinfection of spiked membranes*

To test the inhibitory action of a selected range of ethanol concentrations on spiked membranes, a modified version of a disinfection surface test was used [35, 36]. Membrane samples were autoclaved and sterilized at 121°C for 15 minutes. The samples were spiked with 30 ml of an overnight bacterial culture (OD$_{600} \approx 2.7$) for 15 minutes and subsequently dried under laminar flow for one hour. The membrane was cut into 1cm^{2} sections and treated with 5 ml of a 0, 10, 20, 30 or 40 % ethanol solution for 1.5 hours. Disinfection experiments of spiked membranes were initially checked against non-autoclaved 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 ($CaCl_2.2H_2O$; 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 (γ^{LW}), electron-donor (γ^-) and electron-acceptor (γ^+) surface

247   tension components of dehydrated treated NF90 membrane samples (S) were determined by

248   measuring contact angles using the following expression:

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

249

250   Contact angles (θ) and surface energy measurements (γ^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:

$$\gamma_S^{AB} = 2\sqrt{\left(\gamma_S^+\gamma_S^-\right)} \qquad (2)$$

255

256   And the total surface energy was defined by:

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

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^2 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 $N.m^{-1}$ 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_{rms} 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_{rms}) 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_{rms} values were calculated for each

294 membrane sample and the average taken.

295

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

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 $\log_{10}$ 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 $\log_{10}$ 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 $\log_{10}$ 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 $\log_{10}$ and 6.5 $\log_{10}$ 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 $\log_{10}$

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.
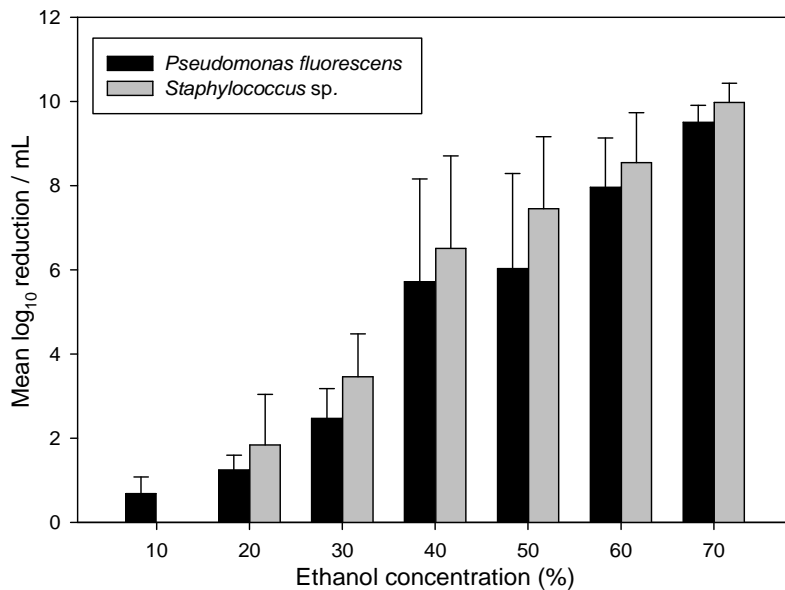
335

336 *Figure 1: The inihibitory 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 *Staphyloccus* 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 $\log_{10}$ reduction. *Staphylococcus* sp. spiked membranes

354   revealed the highest log reduction with values exceeding 5 $\log_{10}$ 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
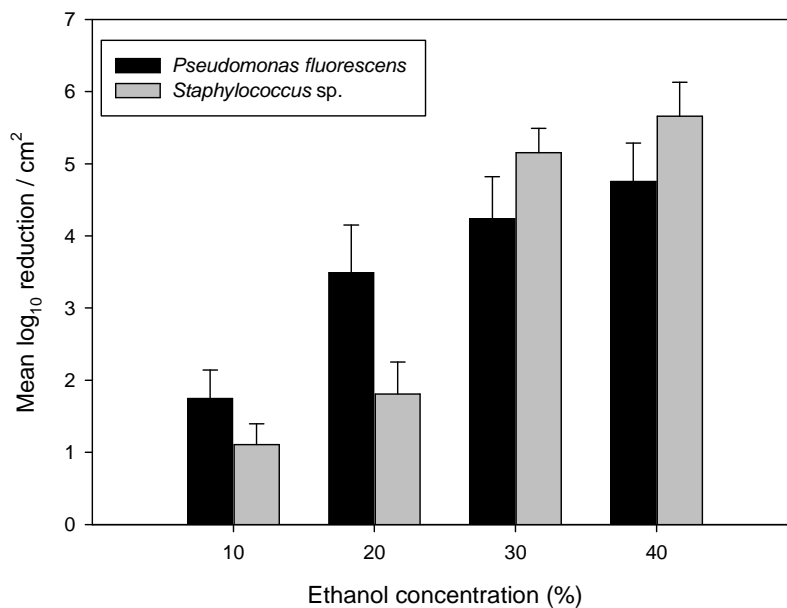
367

368   *Figure 2: The inihibitory 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 4$\log_{10}$ 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

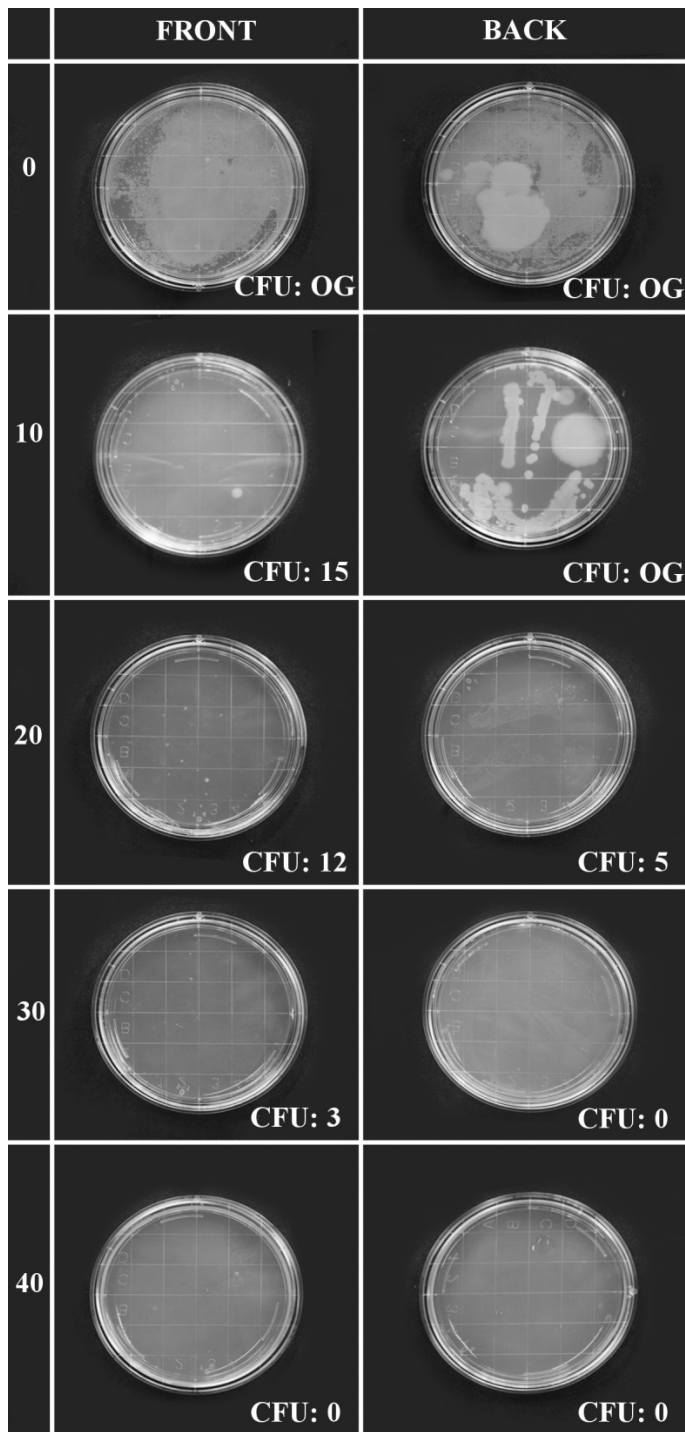379 amount of bacteria that had survived the treatment (Figure 3).

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).

398

	FRONT	BACK
0	CFU: OG	CFU: OG
10	CFU: 15	CFU: OG
20	CFU: 12	CFU: 5
30	CFU: 3	CFU: 0
40	CFU: 0	CFU: 0

399

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

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 $\log_{10}$ 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 ± 0.95 L/ hr bar m^2).

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.
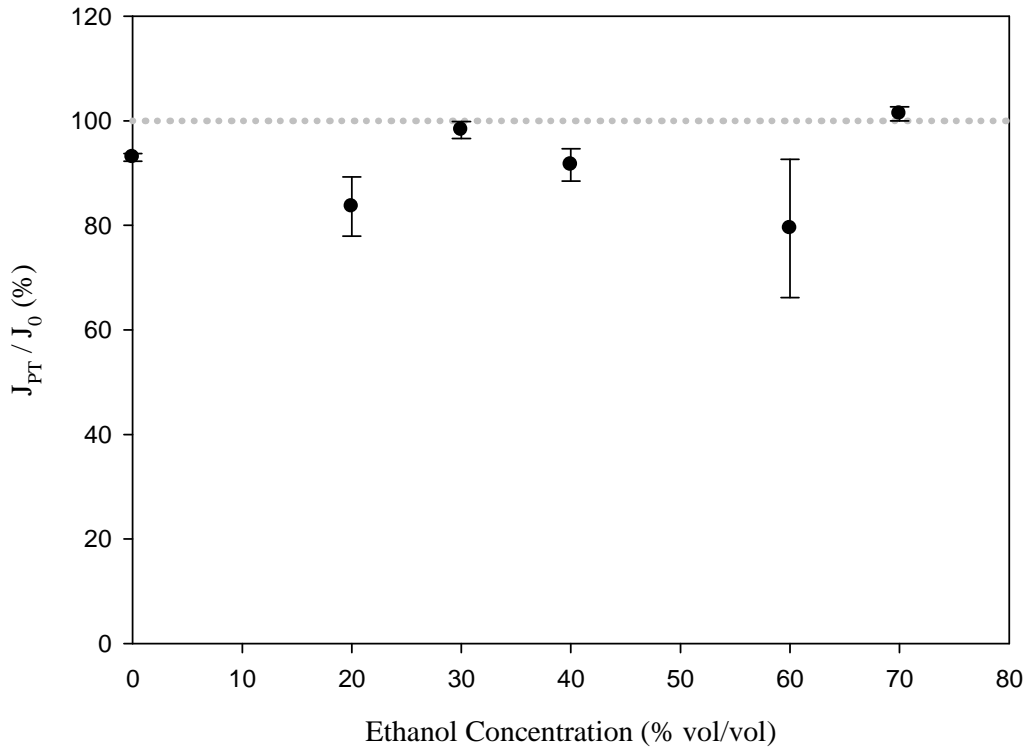
435

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$_2$ 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

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

449

450

(a)

(b)

451
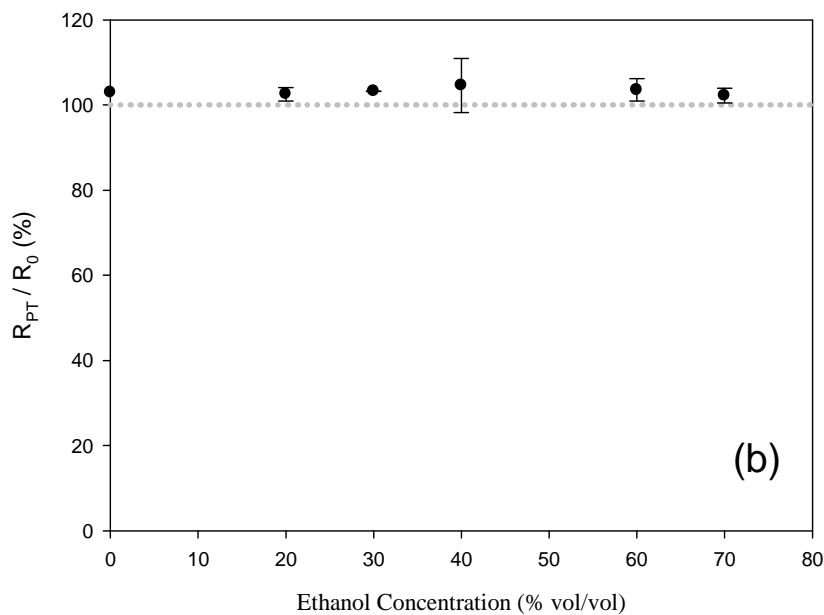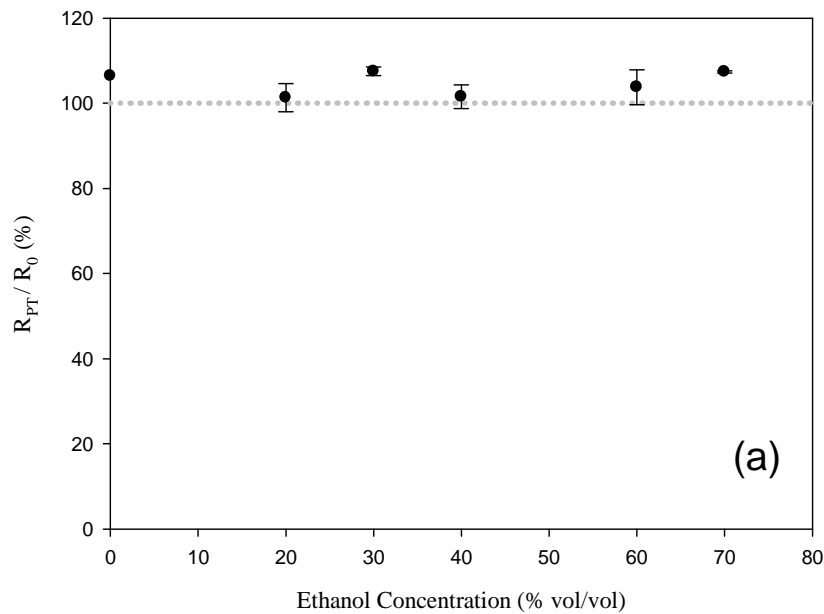
Figure 5: NaCl (10mM; a), and CaCl$_2$ (5mM; b) retentions of NF90 membrane samples after a 1.5 hour treatment (R_{PT}) with 0, 20, 30, 40, 60 or 70 % vol/vol ethanol in MilliQ water, as a percentage of initial salt retention (R_0). The dotted horizontal line represents no change in retention after treatment. Data points are averages of three experiments with error bars displaying standard deviations. Test conditions employed were: Temperature = 22°C, Pressure = 16 bar, and crossflow velocity = 2.2 m/min.

A small increase in the membrane's retention for each salt solution (maximum of 7.5% for NaCl and 4.6% for CaCl$_2$) 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 rentention.

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 Casteele 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

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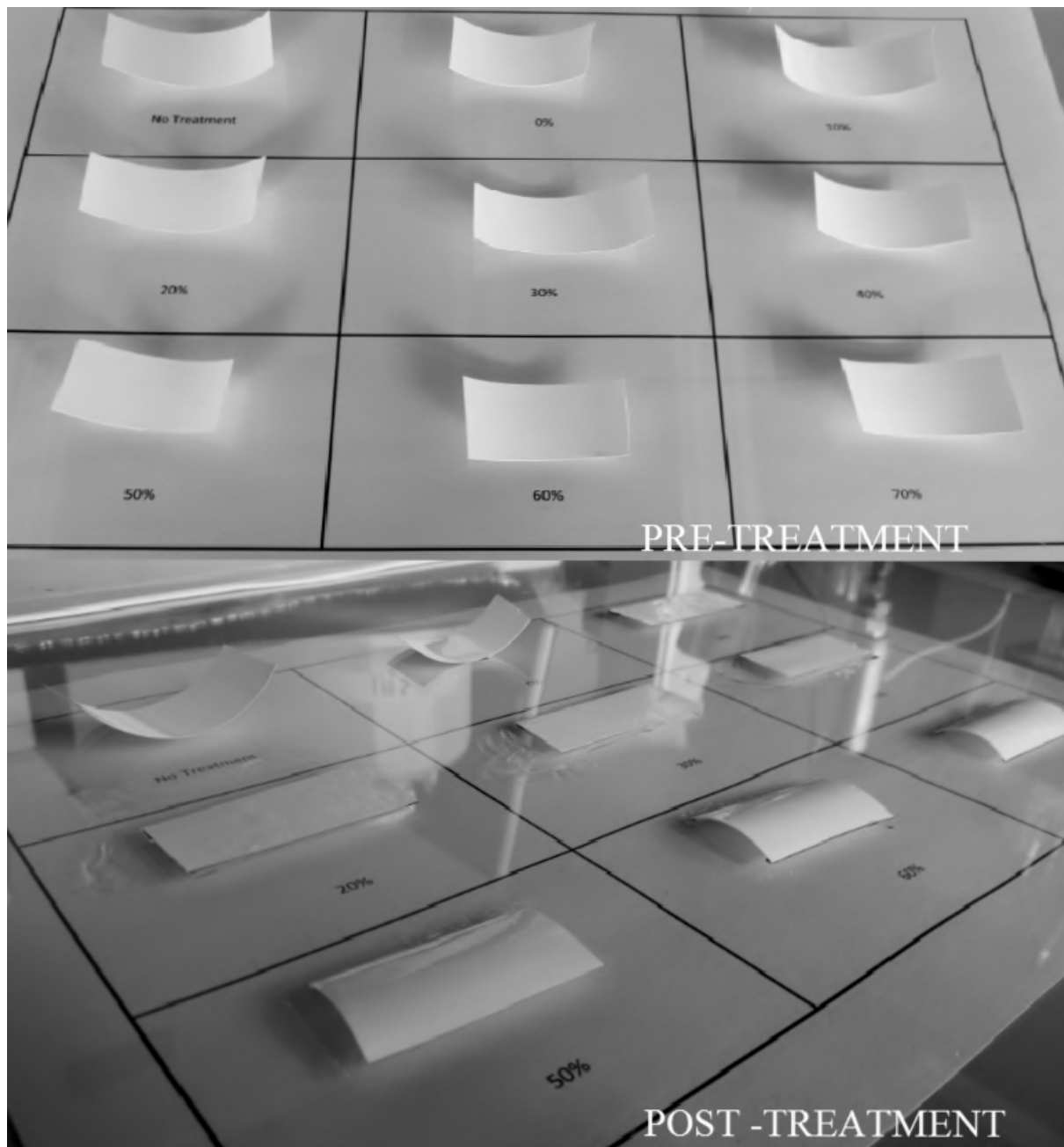
496

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 (θ) in the range $106\text{-}110^{o}$ while those that had been compacted
521 twice had a hydrophilic nature with a contact angle in the range $80\text{-}86^{o}$. 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 (θ), electron-donor (γ⁻), electron-acceptor (γ⁺), Lewis acid-base (γ^{AB}),*

538 *Lifshitz-van der Waals (γ^{LW}), total surface free energy (γ^{S}).*

Sample Treatment	θ	γ^-	γ^+	γ^{LW}	γ^{AB}	γ^{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 compactions	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 compactions	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
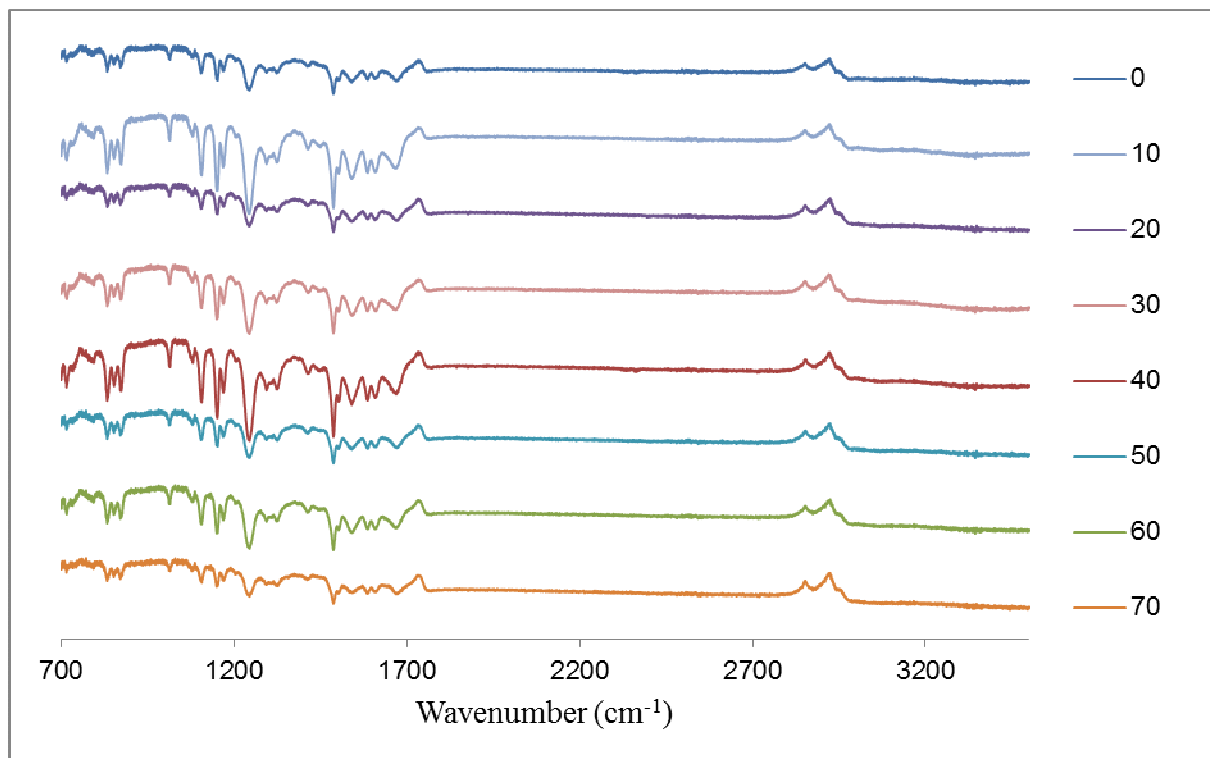
Figure 7: FTIR spectrums of NF90 membrane samples treated with 0, 10, 20, 30, 40, 50, 60,
or 70% vol/vol ethanol. Each spectrum shown is an average of readings done in triplicate.
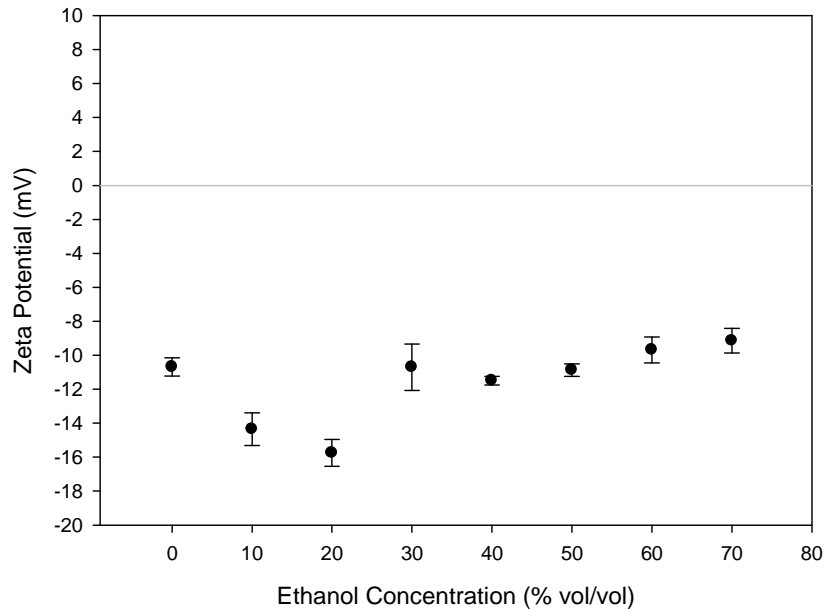
3.3.4. *Zeta potential*

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

The results of the study performed for the range of ethanol treatments can be seen in Figure 8.
The zeta potential of the membrane samples was relatively constant (-10 ± 1.5mV) for the
majority of the ethanol concentrations tested, concurring with the previous salt retention
analysis (*Figure 5*). The samples which were exposed to 10% and 20% ethanol however each
had a notably lower zeta potential, -14.4mV and -15.7mV respectively. No correlation could
be found for these two concentrations with the salt retention results in Section 3.2.

Pasmore et al. [41] showed in their experiments on the role of zeta potential in bacterial
adhesion that a positive (greater than 0) or highly negative (lower than -20) zeta potential can
have a significant effect on bacterial adhesion. In this context the range of results obtained in
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 = $20^{o}C$, 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 10x10μm^{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)	**Average Roughness (R_{rms}) (nm)**
0%	90.96	99.52	100.00	**96.83 ± 5.09**
30%	91.64	83.70	83.47	**86.27 ± 4.65**
70%	96.35	103.00	89.97	**96.44 ± 6.52**

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 ± 25 MPa), and those without (76.4 ± 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

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 Fungicidall 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. Klingeren, 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.

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