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Atomic Force Microscopy study of the biofouling and mechanical properties of virgin and industrially fouled reverse osmosis membranes.

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1 **Abstract**

2

3 The mechanical properties of virgin and industrially fouled reverse osmosis membranes
4 (composite polyamide) used for the purification and desalination of seawater in desalination
5 processes were characterised using novel atomic force microscopy (AFM) methods.
6 Polymeric surface elasticity has previously been demonstrated to strongly affect the adhesion
7 of bacteria; hence the study examined membrane surface elasticity to demonstrate how AFM
8 can be used to assess the bio-fouling potential of membranes. An AFM colloid probe
9 technique was used to determine the mechanical properties of the membrane, the adhesion
10 forces and the work of adhesion at the membrane surfaces. The mean values of Young's
11 modulus for the virgin membrane decreased in magnitude with increasing pH values, where
12 these values were significantly different ($p < 0.017$) between both pH 3 (1450kPa), pH7
13 (1327kPa) and pH 9 (788kPa). These differences were attributed to differences in membrane
14 swelling and indicate possible control parameters that could be exploited to improve
15 membrane cleaning regimes. A membrane with a higher modulus will be stronger and
16 potentially more resistant to chemical and physical processes during operation and cleaning.
17 Significant differences ($p < 0.017$) in force measurements were also found between different
18 electrolytic conditions for each of the membranes, where for the virgin membrane the
19 adhesion force values were 6.00nN at pH 3, 1.77nN at pH 7 and 0.98N at pH 9, and also the
20 work of adhesion were 153.6nJ at pH 3, 22.8nJ at pH 7 and 9.9nJ at pH 9 in 0.6M NaCl.
21 These observations further confirm the importance of the electrolytic environment on the
22 nanoscale interactions of the membrane which should be considered to control fouling during
23 operation and cleaning cycles. AFM images and streaming potential measurements of virgin
24 and fouled membranes were also obtained to aid analysis of the industrial membrane system.

25 The novel application of AFM to membranes to measure Young's moduli and work of
26 adhesion is a new addition to the AFM tools that can be used to unravel separation processes
27 at the membrane surface. In addition, this study further demonstrates that AFM force
28 spectroscopy can be used as part of a sophisticated membrane autopsy procedure for the
29 elucidation of the mechanisms involved in membrane fouling.

30

31 Key Words: Membrane; Reverse Osmosis; Biofouling; Biofilm; Atomic Force Microscopy.

32

33 **1. Introduction**

34 Membrane separation is now an established technology that has been applied for the
35 production of ultra-pure water by the purification and desalination of seawater using reverse
36 osmosis (RO) membranes. However, membrane filtration processes are commonly impeded
37 by membrane fouling, which leads to considerable technical problems, such as reduction in
38 water product quality and requirement for higher pressure. The life span of the membrane is
39 also shortened by fouling and the subsequent need for cleaning processes [1,2]. A major
40 contributing factor to membrane fouling within industry is the formation of microbial
41 biofilms at the surface of the membrane [3,4]. Desalination plants can use a number of
42 strategies for the prevention and control of membrane fouling, which include the use of pre-
43 treatment units for the removal of organic and inorganic dissolved substances and the
44 cleaning of the membrane by either back-washing or chemical wash [5]. To augment these
45 strategies many studies have focussed on the membrane fabrication stage to reduce fouling
46 and optimise the membrane processes [6,7]. Thus, a greater understanding of the physical,
47 chemical and biological processes regulating biofilm formation and development is required
48 to aid in developing new strategies to inhibit or control the biofilm membrane fouling.

49 Atomic force microscopy (AFM) has become an essential tool for the membrane
50 technologist optimising separation processes through greater understanding of fouling
51 mechanisms [8-10]. This versatile instrument not only produces high resolution images of the
52 membrane surface in process relevant environments but can also be used to quantify the
53 forces acting at a membrane surface which govern fouling processes. Force is measured as a
54 function of distance when a probe attached to the apex of the AFM cantilever, approaches the
55 sample, makes contact and then retracts away from the sample. The displacement is varied
56 using the extension and retraction of a piezoelectric crystal. The deflection of the cantilever is
57 monitored and converted into values of force using Hooke's Law, calibration of the spring

58 constant and deflection sensitivity [11]. Force measurement data presented in the form of a
59 force-distance curve, as shown in Figure 1, can provide valuable information on interactions
60 forces and local material properties such as elasticity, hardness, adhesion and surface charge
61 densities. The local mechanical properties of a membrane or fouling layer indicate the
62 resilience of the material to chemical and physical processes. For polymer membranes a
63 harder surface may have reduced wear compared to softer membranes during operation and
64 cleaning cycles [12]. A foulant layer which has low mechanical robustness will be more
65 susceptible to hydrodynamic shear applied during cleaning procedures. The mechanical
66 properties of a membrane or a thin film can be studied by measuring force-distance curves
67 obtained by AFM and then analysing force data through a chosen mechanical theory which
68 considers the contact and indentation of two surfaces to obtain a value of Young's modulus.
69 AFM imaging can be used to identify changes in nanoscale morphology and surface
70 roughness when a foulant layer forms on a membrane surfaces. AFM force-distance curves
71 can be used to measure changes in the mechanical properties at the membrane surface when
72 the membrane-fouling layer system forms.

73 AFM imaging has been used to study the surface roughness and pore size distribution of
74 many types of membrane, the distribution of fouling materials at a membrane surface before
75 and after cleaning processes [13,14] and also to study the early bacterial colonisation events
76 that lead to biofilm formation at nanofiltration membranes [15]. The AFM force-
77 measurement capability has been used to study interactions that govern separation processes
78 including particle/bubble interactions [16] and particle/membrane interactions to provide an
79 assessment of the fouling potential of membranes [17,18]. The bulk mechanical properties of
80 membranes, such as polybenzimidazole (PBI), have been investigated for the determination
81 of tensile strength, storage modulus and Young's modulus, using techniques such as dynamic
82 mechanical analysis (DMA) [19,20]. However, the local mechanical properties of the

83 polymer, at the membrane surface, vary from the bulk of the polymer, and it is the local
84 properties that act at the commensurate size of microbes which will influence the microbial
85 attachment. The AFM nano-indentation technique allows for a simple, convenient
86 measurement of local Young's modulus of membranes. Franceschini and Corti [21]
87 determined the elastic moduli of nafion, PBI and poly [2, 5-benzimidazole] membranes, both
88 undoped and phosphoric acid doped, using AFM force spectroscopy. AFM nano-indentation
89 has also been used to measure the Young's modulus and hardness of various polymers, such
90 as polyethylene, polyvinyl alcohol, polyvinyl chloride, polycarbonate, Nylon6, poly(methyl
91 methacrylate), polystyrene and polyacrylic acid [22,23]. However, there is a limited
92 literature on the membrane elasticity of commercially available membranes, and it is only
93 recently that Chung et al. [24] reported the first measurement of the Young's modulus of the
94 active layer of RO membrane using a combined wrinkling-cracking methodology.

95 Many studies that have used AFM to characterise virgin and fouled membrane surfaces have
96 only measured adhesive forces and have been limited as they have used small numbers of
97 AFM force-distance measurements to characterise the surfaces. Recent developments in
98 AFM technology and its associated data capture now permit the measurement of multiple
99 force measurements across a surface and the creation of force measurement distributions.
100 This has enabled statistical analysis and improved comparison between different surface
101 systems including fouled membranes [13, 25].

102 AFM has been used extensively for the study of bacteria but only a few researchers have used
103 this technique to study biofilms [26,27]. AFM imaging has provided high resolution images
104 of the bacterial cell surface and AFM force measurement has been used to quantify bacterial
105 cell-surface adhesion. The AFM nanoindentation technique allows the measurement of
106 substrate elasticity and is being used to unravel the fundamental processes involved in the
107 attachment of cells and bacteria to elastic surfaces. Recently substrate elasticity has emerged

108 as an important physical factor in the response of many cell types to surfaces. Some research
109 groups consider that the elasticity of a substrate will have an effect on the cell rigidity;
110 therefore the stiffness of a substrate is intrinsic to the cells response of attachment and growth
111 [28-30]. A few studies were performed on the mechanical properties of bacterial cells grown
112 on soft elastic surfaces. Bakker et al. [28] investigated the deposition of three marine strains,
113 *Halomonas pacifica*, *Marinobacter hydrocarbonoclasticus* and *Psychrobacter* sp. onto
114 polyurethane coated glass with varying elastic modulus in a stagnation point flow chamber. It
115 was shown that the bacteria adhered in higher numbers to hard surfaces compared to that at
116 surface coatings of lower elastic moduli. Lichter et al. [30], engineered weak polyelectrolyte
117 multilayered (PEM) thin films within the elasticity range $1\text{MPa} < E < 100\text{MPa}$ to investigate
118 if surface elasticity affects bacterial adhesion. The adhesion of viable *Staphylococcus*
119 *epidermidis* and *Eschericia coli* was found to correlate positively with increasing elastic
120 modulus of PEM, independently of surface roughness, surface interaction energy and surface
121 charge density of the surface. These studies demonstrate that the stiffness of nanoscale
122 polymeric substrata can strongly affect the adhesion of bacteria from aqueous suspensions
123 and that the measurement of local mechanical properties of a substrate and bacterial cells are
124 required for the understanding of cell responses on surfaces [29]. Oh et al. [31] have also
125 observed that the formation of a bio-fouling layer (biofilm) is strongly dependent on the
126 mechanical characteristics of the solid substrate. Therefore, membrane elasticity is a key
127 factor in bacterial cell attachment and hence biofilm formation, where force measurements
128 could provide an assessment of membrane bio-fouling potential within process environments.
129 Therefore, in the present study a comprehensive AFM force measurement investigation was
130 performed on both virgin and industrially fouled commercial RO membrane surfaces; the
131 membrane samples were removed from a desalination plant, for pre-treated seawater, that
132 was experiencing fouling. This was to determine the micromechanical properties of the

133 systems as indicators of their propensity for biofouling and also for the development of
134 fouling control regimes for optimisation of membrane processes and water treatment. This
135 was achieved through obtaining Young's modulus values, adhesion force and work of
136 adhesion at different pH values and in media containing the salt content of seawater. Surface
137 charge measurements were also performed on the membranes surfaces to further examine the
138 processes of adhesion. It is the first time, to the author's knowledge, that AFM
139 nanoindentation experiments have been performed on RO membranes and on industrially
140 fouled membranes for the measurement of elasticity properties.

141 **2. Materials and Methods**

142 **2.1 Membrane preparation**

143 The membrane element, SWC3+, used was obtained from Hydranautics (Nitto Denko
144 company). The membrane polymer is composite polyamide and has a nominal salt rejection
145 of 99.8%. An industrially fouled section of the membrane element, SWC3+, was obtained
146 from the Fujairah Water and Power plant, located in the United Arab Emirates (UAE). The
147 fouling was known to be bacterial in origin; a culture independent method based on the 16S
148 rDNA sequence and constructed gene libraries for the identification of the microbial diversity
149 was used to identify the most significant bacteria responsible for biofilm formation and
150 biofouling at the industrial RO membrane system of the present study [3]. *Proteobacteria*
151 was determined to be the most abundant identified group, with *γ-Proteobacteria* being the
152 most predominant class within the phylum. The next most abundant grouping was the
153 *Bacterioidetes* and *Plantomycetes*. The fouled membrane section was stored at -20°C until
154 used. Storage may impact on the structure of the foulant layer, this is a problem that is innate
155 to all such studies which remove bio-fouled samples from a process environment to facilitate
156 membrane study. However, in this case the thin foulant film may be protected by the

157 underlying substrate membrane. Prior to AFM analysis, the virgin and industrially fouled
158 membrane samples were soaked in deionised water at 4°C for 24 hours, taking care not to
159 disrupt the foulant layer present on the industrially fouled membrane. In preparation for
160 imaging after 24 hours of initial soaking, the virgin and industrially fouled membrane
161 samples were left to dry then cut into small sections of 15 mm² within a sterile environment
162 and attached to one side of a glass cover slip using double sided adhesive tape.

163 In preparation for AFM force measurements, the virgin and industrially fouled membrane
164 samples were cut into small sections within a sterile environment and attached to one side of
165 a 25mm² circular glass cover slip using double sided adhesive tape. A circular 25mm² plastic
166 fluid cell was then placed over the cover slip and secured in place with silica gel. 3ml of the
167 liquid of varying pH and salt concentration was placed carefully into the fluid cell and left for
168 20 mins for the system to stabilise.

169 In preparation for streaming potential measurements, the virgin and industrially fouled
170 membrane samples were cut using provided templates (Anton Paar) into two rectangular
171 shaped pieces with specific holes for liquid flow, which would fit the rectangular fluid cell of
172 the instrument.

173 **2.2 Membrane AFM images**

174 A Dimension 3100 AFM (Bruker) was used for membrane imaging. Non contact cantilevers
175 were used for imaging (OTESPA, Bruker). The membrane images were achieved using
176 tapping mode within an air environment of 21°C and a relative humidity of 40%. Tapping
177 mode AFM has been used extensively to image membranes and microbial surfaces [8, 9, 11].
178 Tapping mode in air was used and not imaging in liquid, the latter is experimentally
179 demanding and risks imaging artefacts by damaging diffuse foulant layers if present. A scan
180 rate of 0.4Hz was used for most images, however for the largest scan size of 100µm x

181 100 μ m, a scan rate of 0.2Hz was used to achieve a higher quality image. The images were
182 analysed through the AFM Nanoscope software to obtain peak to valley and surface
183 roughness measurements for both the virgin and fouled membranes at each scan size.

184 **2.3 Force spectrometry measurement of local elastic properties**

185 The NanoWizard II BioAFM with top view optics (JPK Instruments) was used for the force
186 measurements on all membrane surfaces. AFM colloid probes were made using a
187 micromanipulator (Singer Instruments). In this technique a silica sphere of 3 μ m radius was
188 attached to the apex of a tipless cantilever using glass bond glue. Only AFM colloid probes of
189 radius 3 μ m were used in experimentation to standardise the contact area between the probe
190 and the surface. The colloid probe's cantilever spring constant was measured via the thermal
191 tune method. The silica colloid probe was chosen for indentation studies of the membranes
192 due to its well defined shape and larger tip radius when compared to manufactured silicon
193 nitride sharp tips [32]. This means that on a soft surface, the silica colloid probe will more
194 likely compress the surface, unlike sharp tips which could puncture and disrupt the surface as
195 sharp tips induce local strains that can far exceed the linear material regime. 50 force curves
196 were measured across the membrane surfaces at randomly chosen locations in each
197 experimental system.

198 **2.4 Streaming Potential of the Membranes**

199 The Electro Kinetic Analyzer (Anton Paar) was used to determine the streaming potential of
200 virgin and industrial fouled membranes at 0.1M NaCl concentration and pH values 3 - 9. The
201 cut membrane sample pieces were clamped between two measuring cell parts separated by a
202 defined streaming channel within the rectangular fluid cell. Once the preliminary rinse cycles
203 were performed, the pH of the salt solution was adjusted and the zeta potential measurements
204 were repeated three times for each pH value in each direction of fluid flow. Zeta potential of

205 the membrane surfaces was calculated from the measured streaming potential using the
206 Helmholtz-Smoluchowski equation with the Fairbrother-Mastin approach.

207 **3. Data Analysis**

208 The AFM force data was analysed to determine values of maximum adhesion force, work of
209 adhesion and the Hertz model was used to calculate Young's Modulus values. The value of
210 force is calculated from the deflection of the cantilever using Hooke's law. The force as a
211 function of the piezo scanner displacement is sufficient to calculate the parameters and no
212 further manipulation of the piezo scanner displacement data is required. The contact region of
213 the approach curve is compared to that measured at a hard un-deformable surface to calculate
214 the indentation depth (h) when a force (F) was applied [27]. The value of adhesion is
215 determined from the minimum point of the retraction curve. The contact point was defined
216 as the height where the cantilever deflection begins to leave the horizontal axis on the
217 deflection vs. sample height curve [33]. The work of adhesion was determined from the area
218 between the retraction curve and the x axis (Figure 1) applying the trapezoidal rule to
219 integrate.

220 The equation widely used to calculate the force on the cantilever $F(h)$ by using Hertz
221 mechanisms is [11] :

$$222 \quad F(h) = \frac{4\sqrt{R}}{3} E^* h^{3/2}$$

223 The tip is approximated by a sphere with the radius R . E^* is known as the effective modulus
224 of a system tip-sample. The material of the tip is considerably harder than the sample, thus
225 the following equation was used:

$$226 \quad E^* \approx \frac{E_{sample}}{1 - \nu_{sample}^2}$$

227 Where E_{sample} and ν_{sample} are the denotations for the Young's Modulus and the Poisson ratio
228 for the materials of the sample, respectively. Through the substitution of the above equations
229 into the Hertz equation and analysis of the data from the force-distance curves, the Young's
230 Modulus of the sample was determined.

231 **3.1 Statistical Analysis**

232 All data are expressed as mean \pm standard deviation. The statistical analysis was performed
233 using MiniTab Software, where normal distribution of variables was assessed using the
234 Krustill Wallis test. The non-parametric Mann-Whitney U test for independent samples was
235 used to compare mean values of adhesion force, work of adhesion and Young's modulus
236 between virgin and fouled membranes. Due to multiple testing, a Bonferroni correction was
237 applied for each analysis.

238 **4. Results and Discussion**

239 **4.1 High resolution images**

240 Atomic force microscopy imaging elucidated the morphology of the fouling layers at the
241 industrial fouled RO membranes. Figure 2 presents high resolution $10 \times 10 \mu\text{m}$ and $100 \times$
242 $100 \mu\text{m}$ AFM topographical images of virgin and process fouled SWC 3+ RO membranes.
243 The virgin membrane images clearly show the structure of the RO membrane surface, where
244 the structural surface of the membrane is composed from peaks and valleys. A similar RO
245 surface topography was observed in an AFM and SEM study by Kwak and Ihm [34]. The
246 fouled membrane images clearly show the relatively non-uniform distribution of fouling
247 present on the RO surface, where the structure of the RO membrane can be seen in gaps
248 within the fouling layer (Figure 2B). The high resolution images of the fouled membranes
249 suggested the presence of a bacterial biofilm, with the presence of particles of commensurate

250 size of bacteria embedded in a loose film like structure. The presence of the bacterial biofilm
251 was further supported by previous work that used standard DNA techniques to identify the
252 key bacterial species involved in the industrial fouling of the membrane of this study [3].
253 AFM technology has previously been utilized for the low resolution imaging of foulant layers
254 formed on process membranes [35,36].

255 The surface roughness and peak to valley measurements are shown in Table 1, where the root
256 mean square (RMS) surface roughness of the virgin RO membrane was 174 ± 26 nm and the
257 peak to valley measurement of 1975 ± 757 nm and the RMS surface roughness of the fouled
258 membrane was 297 ± 44 nm and peak to valley measurement of 3837 ± 1013 nm measured
259 from the $100 \times 100 \mu\text{m}$ images. The results also show that as the scan size increases from 1 to
260 $100 \mu\text{m}^2$, the values of surface roughness and peak to valley increase for both the virgin and
261 fouled membranes. The current results agree with the work of Boussu et al. [37] which found
262 that surface roughness increases with scan area from $0.5\mu\text{m}^2$ to $10\mu\text{m}^2$ with different NF
263 membranes, where an explanation is that when the scan size is changed, it is possible to get a
264 different surface topography, therefore resulting in a different roughness value [37]. From
265 Table 1, it can also be seen that the fouled membrane surface has the largest surface
266 roughness and peak to valley measurements for all scan sizes when compared to the virgin
267 membrane, apart from the surface roughness values measured from the $10 \times 10 \mu\text{m}$ image,
268 where the fouled membrane surface is smoother than the virgin membrane. The smoother
269 surface could be due to the presence of a cohesive fouling layer, which may have been
270 facilitated by bacterial fouling producing extracellular polymeric substance (EPS) within the
271 foulant layer. Previous research has found that surface roughness measurements of similar,
272 virgin RO membranes were 66nm [38] (from $4 \times 4 \mu\text{m}$) and 50nm [34] (from $10 \times 10 \mu\text{m}$)
273 and the corresponding peak to valley measurements were 560nm [38] and 400nm [34]. The
274 range of these values confirm with current findings of surface roughness and peak-to-valley

275 measurements of virgin RO membranes at small scan sizes (Table 1). The topography,
276 surface roughness and peak to valley measurements show that the virgin RO membranes
277 could be susceptible to biofouling, as the rough surface topography, with valleys of size
278 commensurate with that of bacteria, could facilitate attachment of bacterial cells and shield
279 the attached bacteria from the shear flow.

280 **4.2 Young's modulus measurement by force spectrometry analysis**

281 The mean Young's modulus values were determined from force measurements achieved at
282 virgin and fouled membranes in 0.6M NaCl at pH values of 3, 7 and 9 (Table 2). The
283 distribution of Young's modulus values from the virgin membrane and fouled membranes are
284 summarized using boxplots shown in Figure 3, where the distribution of all variables were
285 non-parametric. The mean values of Young's modulus for the virgin membrane decreased in
286 magnitude with increasing pH values, where these values were significantly different
287 ($p < 0.017$) between pH 3 (1.45MPa) and pH 9 (0.79MPa) and pH 7 (1.33MPa) and pH 9. The
288 change in Young's modulus values with pH could be due to polymer expansion. Elliott et al.
289 [39] has shown that expansion of the ionic polymer film was related to the solution pH. Yang
290 et al. [40] also demonstrated that the ionization of functional groups through pH change plays
291 a key role in the expansion of the membrane, where for certain membranes the higher the
292 pH above the IEP in terms of zeta potential can lead to favourable polar interactions with the
293 surrounding solution so that the foulant compounds are more extended. The IEP of the
294 polyamide membrane in this study is at low pH (section 4.5). Thus, the higher Young'
295 Modulus at lower pH coincides with a membrane that has a denser polymeric structure.
296 These results indicate that the electrolytic environment is a potential control parameter that
297 could be exploited to improve the mechanical robustness of a polymer membrane, where at
298 pH 3 the surface is at its most robust with the highest Young's modulus measured for all
299 electrolytic environments tested (1.45MPa). These results could provide the membrane

300 technologist with a method to improve the maintenance of membrane structure during the
301 chemical and physical challenges of operation and cleaning.

302 The mean values of Young's modulus for the fouled membrane increased from pH 3 to pH 7
303 and then decreased in magnitude from pH 7 to pH 9, where these values were only
304 significantly different ($p < 0.017$) from pH 7 (93.8kPa) to pH 9 (48.0kPa). The mechanical
305 properties of the fouling layer present on RO membrane surface will affect the shape and
306 stability of the layer and therefore can determine the failure and detachment of the fouling
307 layer in reaction to a physical force such as fluid-induced shear and also the accumulation of
308 such fouling layers in industrial environments [41]. The dependence of the membrane foulant
309 layer Young's modulus on pH could be due to the nature of the different foulant materials
310 within the biofilm and their difference response to the environmental conditions. Therefore
311 the elasticity of the foulant surface present on the membrane surface could vary with
312 industrial conditions. This suggests that optimum electrolytic conditions could be determined
313 where the elasticity of the foulant layer is at its lowest, rendering cleaning regimes, such as
314 cross-flow, more effective in removing and breaking up the foulant biofilm.

315 The comparison between the values of Young's modulus achieved from the virgin and fouled
316 membrane at each pH value showed that all the values of Young's modulus of the virgin were
317 significantly different from the Young's modulus of the fouled membrane ($p < 0.017$), where
318 the values of Young's modulus were greater in magnitude for the virgin membrane (1450kPa
319 at pH3) than that for the fouled membrane (70.9kPa at pH3). The measurement of Young's
320 modulus can be used to compare the fouling within different process conditions for
321 optimisation, in that lowering the mechanical robustness of the fouling layer and/or raising
322 that of the membrane surface will enable the cleaning regime to be more effective.

323 Franceschini and Corti [21] performed AFM force measurements to determine the elastic
324 moduli of non hydrated nafion, PBI and poly [2, 5-benzimidazole] membranes, using a sharp

325 contact tip in a N₂ environment, where the elasticity values measured for the different
326 membranes ranged from 0.104 ± 0.036 to 6.17 ± 0.93 GPa. Chung et al. [24] reported the first
327 measure of Young's modulus of the active layer of RO membrane, both in a dry (1.40 ± 0.53
328 GPa) and hydrated state (0.36 ± 0.14 GPa), using a combined wrinkling-cracking
329 methodology. The difference between these values and those measured in this study are due
330 to the environment in which the measurements were taken and the level of membrane
331 hydration. The indentation experiments in the present study are performed in a liquid
332 environment, as would be in the industrial process, and a colloid probe was used which could
333 also account for this difference in elasticity values; measurements made using a sharp AFM
334 tip will be based on material rupture during layer penetration rather than compression by the
335 colloid sphere. In addition, the studies performed here are industrially relevant with ionic
336 concentrations which would be present within the desalination process, unlike other studies.

337

338 **4.3 Adhesion Force**

339 The mean adhesion force values were determined from force measurements achieved at
340 virgin and fouled membranes in 0.6M NaCl at pH 3, 7 and 9, where the data is shown in
341 Table 2. NaCl was chosen for the electrolytic media to keep the experimental environment
342 simple, however the lack of divalent and trivalent ions, which can influence colloidal
343 interaction, may limit extension of the results to seawater. The distribution of adhesion force
344 values from virgin and fouled membranes are summarized using boxplots shown in Figure 4,
345 where the distribution of all variables were non-parametric. The mean values of adhesion
346 force for the virgin membrane revealed a high adhesion force of 6.0nN at pH 3, where the
347 values of adhesion force decreased with increasing pH, as measurements of 1.8Nn at pH 7
348 and 1.0nN at pH 9 were obtained. The mean adhesion force values were significantly
349 different ($p < 0.017$) between all pH values. The differences in adhesion force values could be

350 due to the charge held by the membrane surface in various electrolytic environments (see
351 section 4.5). The material that will foul a reverse osmosis membrane will be highly
352 heterogeneous containing materials that are biological in origin including humic acids (a
353 naturally occurring breakdown product of organic matter) and biofilm materials such as xanthan,
354 hyaluronan and dextran. Thus, there will be a range of interactions occurring between fouling
355 material, membrane and the silica probe, including electrostatic, van der Waals and hydrophobic. The
356 amount of information that can be inferred from the measurement of adhesion of an inorganic
357 silica particle at a membrane surface is limited as reverse osmosis membrane fouling is a
358 multi-component process, however the interaction of the colloid probe can be used as an
359 indicator of the degree of particle interaction with that surface. This will be more relevant to
360 the surface interaction with inorganic particles rather than biological particles where specific
361 macromolecular interactions may dominate. It should be noted that many bacteria have a
362 negative charge similar to that of a silica colloid. Thus, the significant differences ($p < 0.017$)
363 in adhesion at the virgin membrane surface as the pH is changed demonstrates that some
364 fouling could be reduced at this membrane surface by controlling the pH. However, this
365 should be considered with reference to pH tolerance of the membrane material and how the
366 electrolytic environment affects the mechanical properties of the membrane (as discussed in
367 section 4.2) as previous research has observed the bacteria adhere to harder surfaces [28].

368 The average values of adhesion force for the fouled membrane showed very little variation
369 between the different pH values, where the adhesion force value at pH 3 was 0.7nN. The
370 mean values were not significantly different ($p > 0.017$) between all pH values, so it was
371 concluded that pH did not have a measurable influence on the values of adhesion force. This
372 was expected, the chemical heterogeneity of the fouling material will mean that different
373 chemical groups will be ionised to different degrees within the different pH environments,
374 thus there will be no net change in the interactivity of the fouling film. The extent of

375 electrostatic interactions will be reduced within the high ionic strength environment of the
376 present research, thus another explanation for the consistency of adhesive force measured at
377 the different pHs could be the dominance of hydrophobicity on the interaction of the colloid
378 probe and the fouled surface.

379 The comparison between the values of adhesion force achieved from the virgin and fouled
380 membrane at each pH value revealed that the values of adhesion force of the virgin
381 membrane at pH 3 (6.0nN) and pH 7 (1.8nN) were significantly different from the adhesion
382 measured at the fouled membrane at pH 3 (0.7nN) and 7 (0.9nN) ($p < 0.017$), apart from the
383 adhesion force values at pH 9 which were not significantly different ($p > 0.017$). The values of
384 adhesion force achieved from the virgin membrane at pH values of 3 and 7 were greater in
385 magnitude than that obtained for the fouled membrane. The differences in adhesion forces
386 observed between the fouled and virgin membrane indicate that the surfaces are chemically
387 different and confirm that the presence of a fouling layer will alter the nanoscale interactions
388 of the membrane surface with potential compromise of the separation process.

389 Previous adhesion force measurement studies were performed by Bowen and Doneva [38] on
390 a virgin RO membrane using silica colloid probes with a maximum loading force of 120nN –
391 140nN, where force measurements were performed in 10^{-1} M and at pH 9. The force
392 measurements performed on peaks of the membrane revealed an adhesion of 2.3 ± 0.48
393 mN/m and those performed within the membrane valleys, the adhesion was 8.7 ± 4.0 mN/m.
394 The results obtained at 0.6M NaCl at pH 9 in the present study revealed adhesion force
395 values of 0.98 nN, which when normalized for colloid radius of $3\mu\text{m}$ the adhesion value is
396 0.33mN/m, where the differences between the adhesion forces measured by the studies could
397 be due to the very high loading force used by Bowen and Doneva [38]. The colloid would
398 have indented the sample to a greater extent than the present study, hence more surface area
399 contact would have occurred between the sample and the colloid. In addition, the

400 measurements in the present study were at random positions across the membrane surface,
401 with no separation of measurements from peaks and valleys..

402 **4.4 Work of Adhesion**

403 The measurement of adhesion from the retraction force curve minima does not account for
404 the mechanisms involved in adhesion (Figure 1). It is the shape of the force curve adhesion
405 component, which can be described by the work of adhesion, that provides information on
406 interaction mechanisms such as ligand-receptor peeling. The work of adhesion is the work
407 which must be done to separate two adjacent phases, liquid-liquid or liquid-solid phase as in
408 the case here. The mean work of adhesion values were determined from force measurements
409 at virgin and fouled membranes in 0.6M NaCl at pH 3, 7 and 9, where the data is shown in
410 Table 2. The distribution of work of adhesion values from the virgin and fouled membranes
411 were summarized using boxplots shown in Figure 5, where the distribution of all variables
412 were non-parametric. The average values of work of adhesion from the virgin membrane
413 revealed a high adhesion energy at pH 3 (153.6nJ), which decreased in magnitude at pH 7
414 (22.8nJ) and then again at pH 9 (9.9nJ). The mean work of adhesion values were significantly
415 different ($p < 0.017$) between all pH values. The differences in work of adhesion values could
416 be due to the charge held by the membrane surface in various electrolytic environments (see
417 section 4.5). The average values of work of adhesion for the fouled membrane showed very
418 little variation between the different pH values, for example at pH 3 the work of adhesion
419 was 16.2nJ. The mean values were not significantly difference ($p < 0.017$), so it was
420 concluded that pH did not have a measurable influence on the values of work of adhesion
421 obtained from the fouled membrane.

422 The comparison between the values of work of adhesion achieved from the virgin and fouled
423 membrane at each pH value showed that the values of work of adhesion of the virgin

424 membrane at pH 3 and 7 were significantly different and greater in magnitude from that for
425 the fouled membrane ($p < 0.017$). The work of adhesion values at pH 9 were not significantly
426 different ($p > 0.017$).

427 The work of adhesion and the adhesive force determined from a force measurement are
428 closely linked. However, the adhesive force gives no indication as to how the AFM probe
429 attaches and detaches from a surface. This can be dramatically different when the minimum
430 force recorded in force curves, the adhesive force, is the same. If deformation occurs when
431 the AFM probe interacts with the surface, during the approach and retraction phases of the
432 force measurement, then the work of adhesion will be greater. In addition, another
433 contribution to an increase in the work of adhesion could be an increase in the recruitment of
434 ligands to the interactions, so that the probe has to peel away from the surface and break more
435 bonds. In the present study the high work of adhesion measured at the virgin membrane at
436 pH 3 could be due to the deformation of the surface and/or the increased number of bonds
437 involved in the interaction. This argument demonstrates the potential that AFM force
438 measurement offers the membrane technologist to unravel the processes operating during the
439 formation of fouling layers. In the past this has been restricted by using only AFM adhesive
440 force measurements.

441

442 **4.5 Streaming Potential**

443 The results of zeta potential versus pH for the virgin and process fouled RO membrane at
444 0.1M NaCl concentrations are shown in Figure 6. The streaming potential equipment was
445 only designed to be operated at low salt concentrations therefore a maximum salt
446 concentration of 0.1M was used to provide an indication of the surface charge of the
447 membrane. The virgin membrane was positively charged or had no charge at low pH (pH 4, 0

448 $\pm 2\text{mV}$) and negatively charged at high pH (pH 9, $-5.8 \pm 3.5\text{mV}$), where the IEP was
449 determined to be pH 4.0 for 0.1M NaCl. The fouled membrane was entirely negatively
450 charged over the pH range, where at pH 3 the zeta potential was $-2.3 \pm 1.7\text{mV}$ and at pH 9
451 was $-11.3 \pm 3.5\text{mV}$ and therefore no IEP could be determined.

452 This differences in AFM force measurements obtained using AFM as a function of pH could
453 be attributed to the change in the ionization state of the membrane surface. The present study
454 measured greatest adhesion and work of adhesion at pH3, 6.0nN and153.6nJ respectively at
455 0.6M NaCl, however there is a suggested agreement with the streaming potential study that
456 indicated that a positive or neutral charge was measured at lower pH. Thus, at pH 3 the
457 membrane would be exhibiting a charge that would not tend to repel the AFM colloid probe
458 and so adhesion parameters would be at their highest. At low pH, the surface charge of the
459 membrane may be positively charged at 0.6M NaCl and close to the IEP of the membrane
460 where the electric double layer is relatively thin, as shown in Figure 6 for the virgin
461 membrane at 0.1M NaCl [42]. As the pH is increased, the membrane may become negatively
462 charged, where the silica colloid is also negatively charged at high pH values [42,43] so as
463 the colloid approaches the membrane surface in these conditions, the colloid could
464 experience increasing electrostatic double-layer repulsive force which opposes the motion of
465 the colloid. Due to the repulsive force, the particle may be prevented from coming into
466 intimate contact with the membrane surface, which leads to a lower adhesion force and work
467 of adhesion

468 For the fouled membrane, the adhesion force and work of adhesion values revealed no
469 significant differences as a function of pH. The reason could be the surface charge of the
470 membrane being negative over all pH values (Figure 6) which means that the colloid
471 experiences a repulsive force over all pH values which could explain why there was no
472 significant difference of adhesion force and work of adhesion between pH values [44].

473 Previous research by Al-Amoudi et al. [45] investigated the zeta potential of three virgin and
474 one fouled NF membrane at different pH values and the results agree with our current
475 findings for RO membranes, as the study concluded that the fouled DK (GE Osmonics)
476 membrane were almost negatively charged with no IEP over a range of pH values when
477 compared to a virgin DK membrane.

478 **5. Conclusions**

479 Mechanical measurements obtained from AFM force-distance measurements can provide an
480 assessment of the fouling potential of the membranes by the elucidation of mechanical factors
481 that affect membrane fouling, which has the potential to reduce commissioning studies and
482 optimise process operation. In this study a novel and comprehensive AFM characterisation
483 of the mechanical properties of virgin and industrially fouled membranes was achieved,
484 which detected differences between the virgin and fouled membranes in different electrolyte
485 conditions. The results of the paper suggest that pH control could be investigated to
486 strengthen membranes against chemical and physical challenges, where the increased
487 Young's modulus measured at pH 3 of the virgin membrane indicate, for example, that
488 cleaning at a low pH may be advantageous for the protection of the membrane. A careful
489 balance could be considered by the membrane technologist, which during a cleaning process
490 uses an electrolytic environment that renders the membrane at its strongest, while the
491 biofouling layer could be at its weakest, with the caveat that disruption of the chemical
492 properties of the membrane material are kept to a minimum.

493 Recent studies have indicated that the mechanical properties of nanoscale polymeric substrata
494 can strongly influence the adhesion of bacteria in aqueous suspensions [30]. However, there
495 are limited examples within the literature on the use of nano-indentation and measurement
496 of the mechanical properties of commercially available membranes and biofilm. Therefore,

497 the mechanical measurements at virgin and biofouled membranes of the present study are
498 extremely timely and such AFM characterisation is a novel technique, when applied to RO
499 membranes, which holds promise for further elucidation of the mechanisms involved in
500 membrane fouling. The present study on industrially fouled membranes has shown that the
501 analysis of AFM force-distance data can be extended beyond simple adhesion measurements
502 to aid diagnosis of processes problems such as fouling, as part of industrial membrane
503 autopsies. It is hoped that this encompassing research study within an industrial context will
504 aid in developing a rational strategy for the prevention of biofouling and biofilm formation,
505 with economic and effective cleaning within desalination processes, which will maintain
506 efficient membrane operation and prolong membrane life.

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513

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List of Figures and Legends

Figure 1. Typical AFM Force curve

Figure 2. Tapping mode AFM topographic images of virgin and fouled SWC 3+ RO Membrane. A and C images show the topography of virgin SWC 3+ membrane at 10 μ m and 100 μ m respectively, while B and D images show the topography of fouled SWC 3+ membrane at 10 μ m and 100 μ m respectively . Z range in all images is 2.0 μ m.

Figure 3. Variation of Young's modulus of (a) virgin and (b) fouled membranes between different pH values shown in box plot forms.

Figure 4. Variation of Adhesion force of (a) virgin and (b) fouled membranes between different pH values shown in box plot forms.

Figure 5. Variation of work of adhesion for (a) virgin and (b) fouled membranes between different pH values shown in box plot t forms.

Figure 6: Zeta potential measurements of virgin and fouled RO membrane at 0.1M NaCl

Table 1. Surface Roughness measurements from the virgin and process fouled membrane.

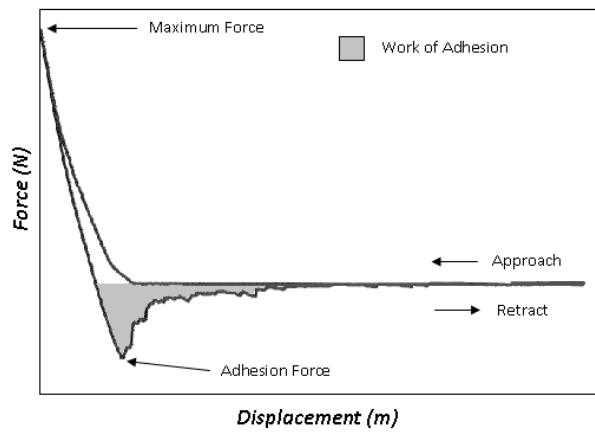
Table 2. Mean values of Young's modulus, adhesion force and work of adhesion achieved from both virgin and fouled membranes at various pH values.

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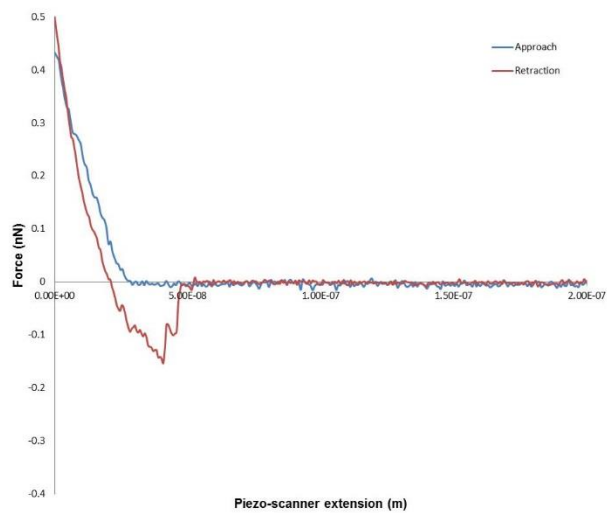
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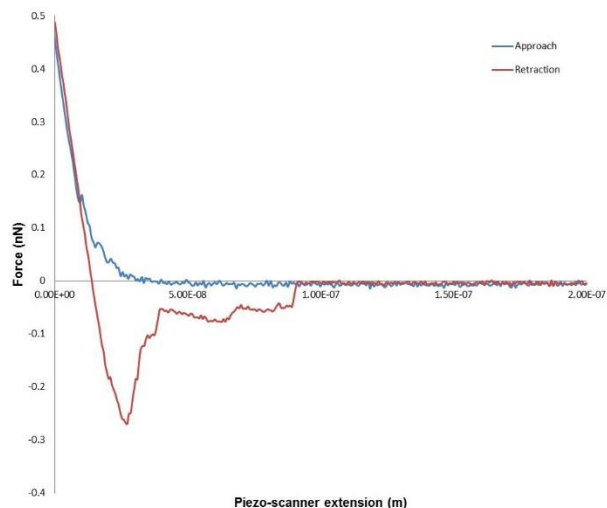
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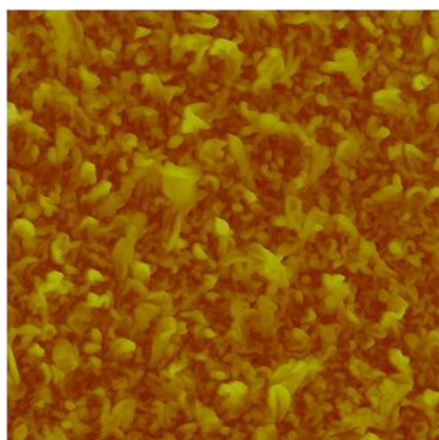
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681 **Figure 1** Force-distance measurement curves: (a) Annotated AFM force curve typically
 682 measured over 1 μm (b) Typical AFM force curve measured at a virgin membrane (c) Typical
 683 AFM force curve measured at a fouled membrane.

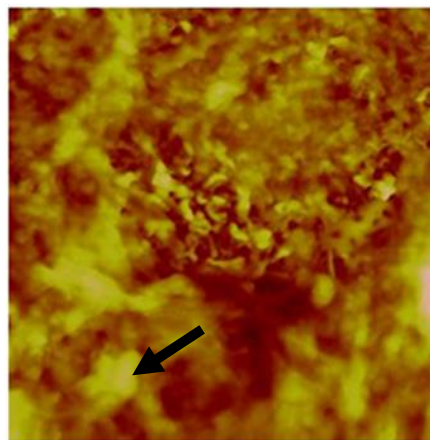
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686 A.



B.



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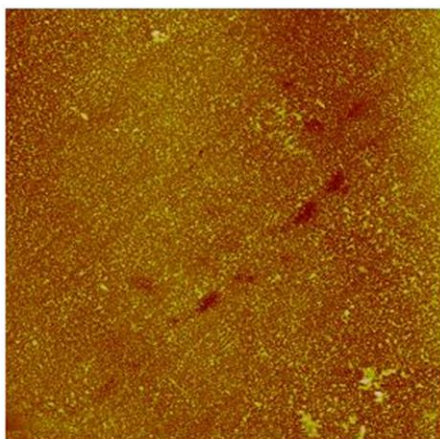
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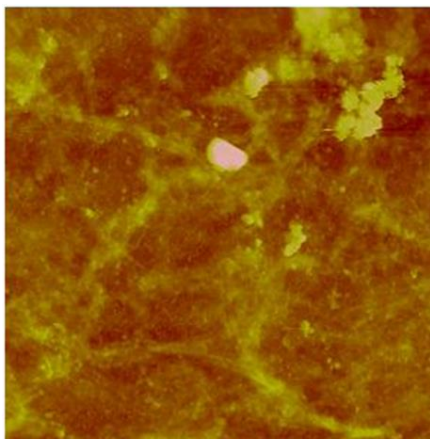
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699 **Figure 2.** Tapping mode AFM topographic images of virgin and fouled SWC 3+ RO
 700 Membrane. A and C images show the topography of virgin SWC 3+ membrane at 10 μm x
 701 10 μm and 100 μm x 100 μm , respectively, while B and D images show the topography of
 702 fouled SWC 3+ membrane at 10 μm x 10 μm and 100 μm x 100 μm respectively. The arrow
 703 in image B is suggested to be a cluster of bacterial cells. Z range in all images is 2.0 μm .

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Membrane Size (μm)	RMS (nm)		Peak to Valley (nm)	
	Virgin	Process Fouled	Virgin	Process Fouled
1 x 1	69.7 \pm 13.7	87.5 \pm 11.2	444.3 \pm 112.1	490.2 \pm 109.6
10 x 10	107.9 \pm 9.68	99.6 \pm 14.3	786.0 \pm 116.6	824.3 \pm 112.5
100 x100	173.7 \pm 25.9	297.7 \pm 44.2	1974.7 \pm 756.8	3837.0 \pm 1013.3

712

713 **Table 1.** Surface Roughness measurements from the virgin and process fouled membrane.

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	Mean Young's modulus (kPa)		Mean Adhesion Force (nN)		Mean Work of Adhesion (nJ)	
	Virgin	Fouled	Virgin	Fouled	Virgin	Fouled
pH 3	1450 \pm 986	70.9 \pm 36.3	6.00 \pm 4.02	0.73 \pm 0.90	153.6 \pm 89.8	16.2 \pm 48.4

pH 7	1327 ± 947	93.8 ± 55.1	1.77 ± 1.14	0.85 ± 0.96	22.8 ± 28.6	13.4 ± 23.9
pH 9	788 ± 432	48.1 ± 26.4	0.98 ± 0.72	0.84 ± 0.85	9.9 ± 10.2	12.9 ± 21.9

716

717 **Table 2.** Mean values of Young’s modulus, adhesion force and work of adhesion achieved
718 from both virgin and fouled membranes at various pH values.

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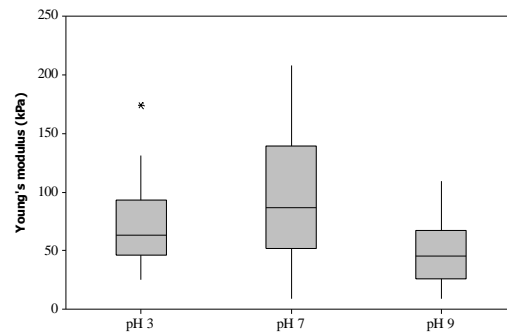
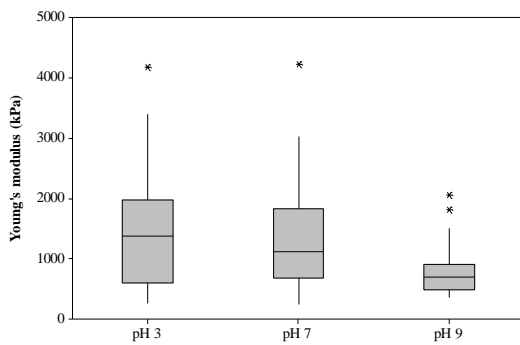
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726 (a)

(b)

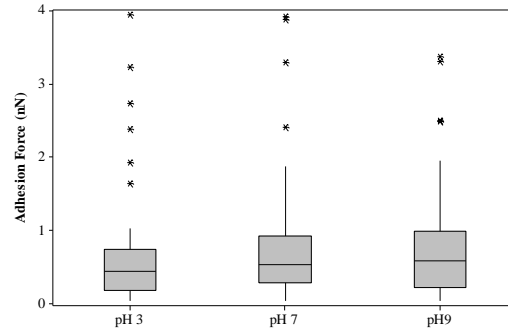
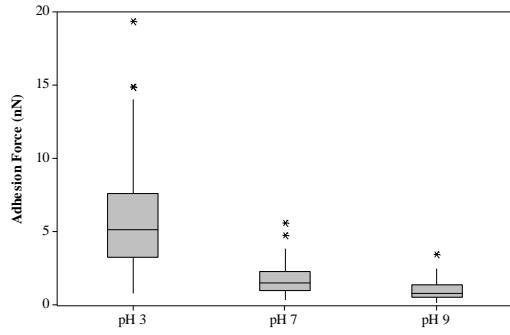


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728 **Figure 3.** Variation of Young’s modulus of (a) virgin and (b) fouled membranes between
729 different pH values shown in boxplot forms.

730 (a)

(b)



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733 **Figure 4.** Variation of Adhesion force of (a) virgin and (b) fouled membranes between
 734 different pH values shown in boxplot forms.

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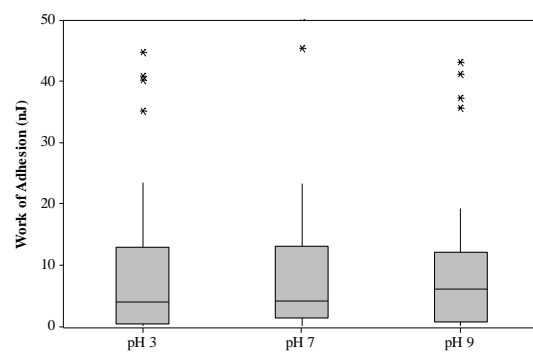
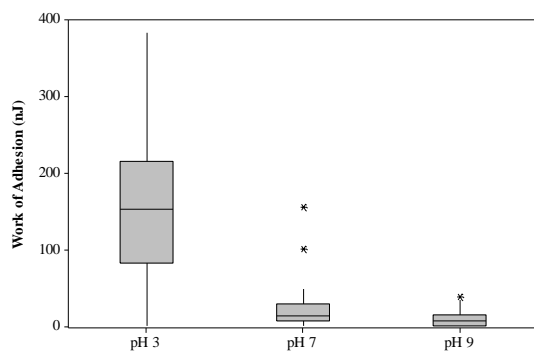
740

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743 (a)

(b)

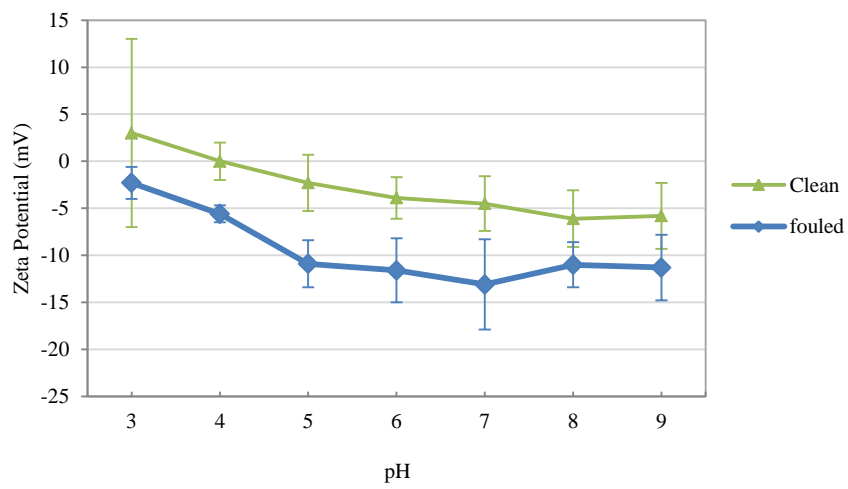


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746 **Figure 5.** Variation of work of adhesion for (a) virgin and (b) fouled membranes between
 747 different pH values shown in boxplot forms.

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750 **Figure 6:** Zeta potential measurements of virgin and fouled RO membrane at 0.1M NaCl

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