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On-line Biofilm Strength Detection in Cross-flow Membrane Filtration Systems

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ABSTRACT

A fluid dynamic gauging (FDG) technique was used for on-line and in-situ measurements of *Pseudomonas aeruginosa* PAO1 biofilm thickness and strength on flat sheet polyethersulfone membranes. The measurements are the first to be successfully conducted in a membrane cross-flow filtration system under constant permeation. In addition, FDG was used to demonstrate the removal behaviour of biofilms through local biofilm strength and removal energy estimation, which other conventional measurements such as flux and TMP cannot provide. The findings suggest that FDG can provide valuable additional information related to biofilm properties that have not been measured by other monitoring methods.

Keywords: Fluid dynamic gauging (FDG), biofilm strength, biofilm thickness, membrane biofouling

32 **Introduction**

33 Biofouling in membrane processes is a long-standing problem and biofilm development on
34 and/or within membrane surfaces can cause lower product water quality, increased energy
35 requirement and higher overall costs. Although biofouling predominantly occurs in high
36 pressure systems such as reverse osmosis (RO) and nanofiltration (NF) (Baker and Dudley
37 1998, Flemming et al. 1997), this problem may also affect other membrane systems including
38 low pressure microfiltration (MF) and ultrafiltration (UF) (Pontié et al. 2007), membrane
39 bioreactors (MBR) (Le-Clech et al. 2006), and other novel membrane systems (eg membrane
40 distillation, pressure retarded osmosis, etc.) (Bar-Zeev et al. 2015, Goh et al. 2013).

41 It has been understood that complete elimination of biofouling is almost impossible (Flemming
42 et al. 1997). Current pretreatment technologies mainly focus on the reduction of
43 microorganisms in the source water, which may not provide effective biofouling control since
44 biofilm development relies heavily on the availability of biodegradable nutrients (Chen et al.
45 2013, Jamaly et al. 2014, Nguyen et al. 2012). Despite the effort to lower biocide usage, it is
46 currently still the most commonly used method for membrane cleaning. While biocide does
47 kill bacteria, the dead cells are not totally removed but instead become a nutrient source for
48 surviving bacteria (Murthy and Venkatesan 2009). Therefore, a reliable monitoring method
49 which provides insights to biofilm removal under stress conditions is crucial for the
50 development of effective membrane cleaning protocols (Nguyen et al. 2012).

51 Traditionally, flux decline or transmembrane pressure (TMP) rise have been used to determine
52 and infer the occurrence and extent of membrane fouling because they can be measured readily
53 in the laboratory and industrial settings. However, these two parameters, though intuitive, are
54 indirect indicators of the properties of the fouling layer, which may not provide information
55 regarding the actual condition of membrane foulant thus causing ineffective membrane

56 cleaning. Moreover, flux and TMP are normally time, spatial or volume averaged
57 measurements. Therefore, direct and local information of the deposition and removal behavior
58 of foulant, by measuring the thickness and strength of the foulant, can assist the optimization
59 of the cleaning regimes, operating protocols and module design of membrane systems (Chavez
60 et al. 2016). Most existing on-line monitoring techniques including (i) microscopic (confocal
61 laser scanning microscopy) (Mukherjee et al. 2016), (ii) spectroscopic [infrared, nuclear
62 magnetic resonance spectroscopy (NMR) and Raman] (Graf von der Schulenburg et al. 2008,
63 Kögler et al. 2016), (iii) ultrasonic time-domain reflectometry (UTDR) (Sim et al. 2013), and
64 (iv) optical coherence tomography (OCT) (Chew et al. 2004b, Linares et al. 2016a), mostly
65 focus on the detection of foulant thickness or flow distribution and are unable to provide
66 information on foulant strength or attachment behaviour which could be the relevant parameter
67 for membrane fouling. Atomic force microscopy (AFM) is probably the only technique that
68 allows the measurement of the physical adhesive forces of foulants to surfaces in-situ, which
69 may include bacteria and biofilm adhesion to membrane surfaces (Powell et al. 2017). In
70 addition, it is especially challenging to obtain reliable measurements in flow systems
71 commonly found in membrane operations.

72 Fluid dynamic gauging (FDG) is a relatively simple technique which was initially developed
73 to measure the thickness of deposits on solid surfaces in situ and on-line (Tuladhar et al. 2000).
74 It has been employed to investigate foulant thickness formed on heated surfaces such as heat-
75 exchangers used primarily in food processing, polymer manufacturing and crude oil industries
76 (Gu et al. 2009, Peck et al. 2015, Tuladhar et al. 2002). The FDG technique can measure (in a
77 destructive mode) local strength properties throughout the different layers of deposits (Chew
78 et al. 2004a). The ability of the FDG to be operated at elevated temperature and pressure (Ali
79 et al. 2013) has gained some interest for use in membrane filtration scenarios, where permeation
80 is involved (Chew et al. 2007, Jones et al. 2010, Lewis et al. 2016). However, these studies

81 were mainly performed using synthetic organics to simulate constant TMP filtration in food
82 industries. Here, FDG is applied to membrane processes to simulate water and wastewater
83 treatment operations under constant permeation.

84 The objective of this study was to investigate the feasibility of FDG technique for on-line
85 membrane biofouling detection by measuring both biofilm thickness and strength. This study
86 is the first attempt to apply FDG to measure biofilm thickness and strength in a membrane
87 cross-flow filtration system under constant permeation. This study also explored the impact of
88 biofilm desiccation which could happen due to flow disturbances or during cleaning (transition
89 from feed to cleaning formulations).

90 Experimental

91 *Biofouling experimental protocol*

92 The experimental set-up and protocols used for simulating biofouling in cross-flow filtration
93 were adapted from previous work (Figure 1A) (Sim et al. 2013). A rectangular flat-sheet cross-
94 flow cell that had a membrane area of 0.0126 m^2 (180 mm × 70 mm) and a channel height of
95 2.0 mm was used. Before installation, the low protein binding polyethersulfone (PES) flat sheet
96 membrane (PALL, 10K OMEGATM, MWCO 10 kDa) was cut and soaked in deionised water
97 (Milli-Q, Merck-Millipore) for 24 h. The feed water contained background salinity of 500 mg
98 L^{-1} NaCl (Merck) and 20 mg L^{-1} nutrient broth (Difco NB, BD Diagnostics) which provided
99 total organic carbon (TOC) of approximately 8 mg L^{-1}, similar to typical TOC in secondary
100 effluent water. Feed water was circulated via a gear pump (Cole-Palmer, Model 74013-45) in
101 a closed loop as shown in Figure 1A. Wild type *Pseudomonas aeruginosa* PAO1, a common
102 representative of wastewater bacteria, was chosen as model bacterium in this study (Hentzer et
103 al. 2002, Kim et al. 2015, O'Toole and Kolter 1998). A stock solution of PAO1 (cell counts
104 ~10^6 CFU mL^{-1}) was injected at a constant rate of 0.25 mL min^{-1} via an injection pump

105 (ELDEX, model 5979-OptosPump 2HM). The preparation of bacteria stock solution can be
106 found elsewhere (Suwarno et al. 2012). The temperature of the feed was kept at 25°C by using
107 a continuous flow chiller (PolyScience 9706A, USA). A microfilter (0.2 µm pore size, Karei
108 Filtration) was installed at the retentate line to prevent bacteria from entering the feed tank.
109 Additionally, the feed solution was replenished within every 24 h to further ensure a controlled
110 feed condition throughout the whole experiment duration.

111 In this study biofouling experiments were conducted at constant feed pressure (P1) (80 kPa)
112 and cross-flow (0.95 cm s^{-1}) and flux (10 LMH) for durations of 2, 4, and 6 days in duplicates.
113 FDG analysis was conducted on-line (under same operating conditions) at the end of every
114 biofouling experiment. The experiments are identified as 2-day, 4-day and 6-day, respectively.

115 Apart from the biofouling experiment at varying durations, an additional experiment was
116 conducted by performing a 2-day biofouling experiment under the same operating conditions,
117 followed by 24-h desiccation under no cross-flow and no nutrient supply, followed by a 2-day
118 biofouling experiment. This experiment was aimed at investigating the impact of flow cessation
119 due to possible process interruption in a large-scale process. The above experiment is identified
120 as 4*-day.

121 ***FDG System***

122 The schematic of the FDG system and experimental set-up is depicted in Figure 1B. The FDG
123 system was comprised of a stepper motor, linear slide with mount to provide vertical
124 movements, linear stainless steel FDG gauge, pressure transducer, and a motorized syringe
125 pump for a controlled suction speed. A desktop computer was connected with the stepper motor
126 and pressure transducer to record the gauge position and differential pressure (ΔP). The stepper
127 motor movement was controlled by a constant current drive (Nanotec, SMC42) in a
128 programmable circuit board (Arduino, ATmega2560). This circuit board also read voltage from

129 the linear potentiometer which provided an independent measurement of the position of the
130 gauge. A signal converter (RS Components, Solartron OD5) was used to transform the linear
131 variable differential transformer (LVDT) output into a steady ±10 V reading. A precision data
132 acquisition (DAQ) device (National Instruments, NI USB-6210) read both the LVDT and
133 pressure transducer signals. The programmable circuit board and DAQ device were configured
134 using LabVIEW^{TM} visual interface (VI) to perform control and data-logging activities.

135 The inset in Figure 1B shows the operation of FDG. The FDG gauge was constructed from a
136 stainless steel tube of a diameter (d) of 2.0 mm, connected to a tapered (45°) end with internal
137 nozzle diameter of d_t (0.5 mm). FDG is based in the principles of fluid dynamics to determine
138 the foulant thickness by reading the pressure difference ΔP (Lewis et al. 2016). A dimensionless
139 characteristic height – h/d_t, is uniquely correlated to ΔP in a calibration plot of ΔP vs. h/d_t, such
140 that the foulant thickness, δ, can be determined (Figure 2A). Principally, with a constant suction
141 mass flow rate (m_g = 0.2 g s^{-1}) controlled by the syringe pump, as the FDG gauge approaches
142 the biofilm surface (ie decreasing h/d_t), ΔP shall firstly be stable and then gradually increase,
143 thus a curve (ΔP vs. h/d_t) to indicate the position of biofilm surface could be generated. In non-
144 invasive mode, the biofilm is not disturbed by the suction flows as the FDG gauge approaches
145 the surface. Comparison of the biofilm surface and membrane surface curves in Figure 2A
146 allow biofilm thickness to be estimated (detailed calculation is described in Supporting
147 Information section 1-2).

148 In destructive mode, however, as the gauge approaches the biofilm surface, the suction flow
149 shall eventually cause removal of biofilm in the region directly underneath the gauge (Figure
150 2B).The gauge clearance from surface (h, as in Figure 1B) when removal of biofilm layer
151 occurs is recorded to estimate the strength (cohesive strength or adhesive strength) of biofilms.
152 The thickness of biofilm was estimated by comparing the biofilm surface and membrane
153 surface curves (Figure 2A), and strength of biofilm was calculated by

154 $$\tau_{w,max} = \frac{3\mu m_g}{\rho_L \pi h^2}\frac{1}{r} \qquad (1)$$

155 where μ is viscosity of water, m_g is the suction mass flow rate by syringe pump, ρ_L is density
156 of water, *h* is the clearance from surface when removal of biofilm layer occurs as indicated in
157 Figure 2A and r is $d_t/2$ (Chew et al. 2004a, Lewis et al. 2012). After destructive testing, the
158 energy required to remove the biofilm layers was also estimated (detailed calculation is
159 described in Supporting Information section 3). The fouled membrane was then carefully
160 removed from the test apparatus and immediately analysed using a confocal laser scanning
161 microscope (Figure 2B). Biofilm samples were maintained moist and stored in covered
162 containers during storage and transport to ensure minimum deformation and contamination.

163 ***Confocal Microscopy***

164 The thickness of biofilm formed on the membrane surface was also measured by observing the
165 fouled membrane via a confocal laser scanning microscope (CLSM, Zeiss, model LSM810).
166 Biofilm thickness measured by the CLSM and FDG were analysed statistically using the
167 Pearson's correlation analysis. Biofilms were prepared by staining with SYTO9 nucleic acid
168 fluorescent stain (Molecular Probes, S34854) in accordance with manufacturer's specifications.
169 Working solutions were prepared by mixing 1.5 µL SYTO9 in 10 mL phosphate buffered saline
170 (PBS) solution.

171 The flow cell was initially dismantled by removing the top-plate, followed by carefully
172 collecting the membrane samples by holding the two corners of the membranes with sterilized
173 forceps. Centre sections of the membrane samples (1.5 cm x 2.0 cm) were slowly cut and
174 separated from the rest of the membrane areas for CLSM analysis. CLSM samples were then
175 soaked in working solutions and incubated for 30 min in the dark at room temperature. After
176 the incubation the membrane samples were rinsed three times with sterile PBS before placing
177 on the glass slide. Each experimental variable (at different durations) was repeated in duplicate

178 and five replicates of CLSM three-dimensional (3D) images were constructed by stacking 2D
179 images of the biofilm at different thickness (Z-Stack mode).

180 **Results and Discussion**

181 ***Determination of Biofilm thickness by FDG***

182 Biofouling experiments were conducted at durations of 2, 4, and 6 days, and FDG analysis was
183 conducted at the end of every experiment. Typical biofilm and membrane surface curves from
184 FDG measurements are shown in Figure 2A which provides information of both biofilm
185 strength and thickness. The biofilm strength can be separated into cohesive and adhesive
186 strength. Cohesive strength is considered as the strength required to deform layers within the
187 biofilm, while the adhesive strength is the removal strength required to detach biofilms from
188 the membrane surface (FDG thickness = 0) (Peck et al. 2015). Biofilm thickness in this study
189 was measured by comparing the distance between before and after the FDG destructive mode
190 (i.e., cleaned membrane). The rationale behind this method is that the membrane reference
191 point was constantly changed and calibrated due to membrane compaction and possible
192 changes in hydrodynamic conditions caused by fouling. This method differed from previously
193 published literature in which the thickness was measured by taking a reference point at clean
194 condition before fouling (Chew et al. 2004b, Lewis et al. 2016, Peck et al. 2015).

195 The TMP rise (measured by the difference between P1 and P2 in Figure 1A), thickness
196 measured by FDG, and thickness measured by CLSM from different experimental durations
197 are summarized in Table 1. In general the results showed greater TMP rise and thickness
198 associated with more biofilm on the membrane surfaces at longer durations. This is consistent
199 with data reported in literature (Chen et al. 2013, Sim et al. 2013). Pearson correlation analysis
200 was conducted between FDG thickness and confocal thickness. The Pearson correlation
201 coefficient and significant correlation were 0.9733 and 0.0267 (< 0.05), respectively. The close

202 correlation between FDG thickness and confocal thickness shows that biofilm thickness can be

203 reliably determined by FDG.

204 Table 1. TMP rise and thickness of biofilm at different experiment durations.

Duration, d	TMP Rise, kPa	FDG Thickness, μm	Confocal Thickness, μm
2	7.7 (± 1.8)	19.4 (± 0.5)	18.0 (± 2.5)
4	11.0 (± 0.9)	27.9 (± 0.8)	28.0 (± 2.0)
6	13.9 (± 0.2)	43.1 (± 0.5)	45.0 (± 3.0)
4*	12.3 (± 0.4)	23.3 (± 2.3)	28.0 (± 3.0)

*) Special treated biofilm (4 days intermittent run).

205 ***Determination of biofilm strength by FDG and impact of biofilm desiccation***

206 The results for destructive strength testing at each time point are shown in Figure 3, in which

207 the biofilm thickness is plotted against the applied gauging shear stress (eq. 1) (Lewis et al.

208 2016). The scatter in the data points, especially for 4- and 6-day, reflect the dynamic nature of

209 the biofilm growth. The yield stress, characterised as that above which significant erosion of

210 the biofilm (due to suction flow from gauge), for biofilms developed over 2, 4 and 6 days were

211 estimated at 1165, 1600, and 1660 N m^{-2}, respectively (indicated by the vertical dotted lines on

212 Figure 3). These values were estimated from the average initial FDG strengths from duplicate

213 experiments. The dashed lines, obtained from the yield stress and the average adhesive

214 strengths, were drawn on the figure for each experiment duration to aid visualization. A general

215 negative trend was observed in all these results, showing that the layers closer to the membrane

216 surface were harder to remove than those at the top of the biofilm (ie the cohesive strength

217 increases as the biofilm gets thinner). The increased strength of the biofilm layers closer to the

218 membrane could be caused by the permeate flux through the membrane and/or the increase in

219 EPS concentration. It has been reported that permeate flux is a dominant factor in the

220 accumulation and compaction of EPS matrix within the biofilm which may further affect the
221 hydraulic resistance on membrane surfaces. The drag force caused by the permeate flux may
222 also lead to an increased number of binding points between EPS molecules, and thus, greater
223 cohesive and adhesive strengths (Dreszer et al. 2013).

224 It is clear from Figure 3 that the adhesion increased with the duration of biofouling experiments.
225 However, for 4- and 6-day experiments, the increase in adhesive strength was marginal. One
226 possible explanation could be reduced transfer of fresh nutrient to the bottom layers due to less
227 diffusion through the denser EPS layers (Oubekka et al. 2012). Hence, strengthening of the
228 layers closer to the membrane was marginal.

229 Another interesting observation was the degree of variation of biofilm strength at a particular
230 thickness at different experiment durations ie the gradient of the thickness versus strength curve
231 (Figure 3). There was an apparent increase of cohesive and adhesive strengths from the 2-day
232 biofilm to those of 4-day which resulted in a larger gradient, ie, -8.8×10^{-3} μm Pa^{-1} (2-day) vs.
233 -5.6×10^{-3} μm Pa^{-1} (4-day). However, the 6-day biofilm showed a slight increase in strength
234 with thickness ie -8.6×10^{-3} μm Pa^{-1} compared to that of 4-day.

235 Figure 4 shows that the average cohesive (more details provided in Supporting Information
236 section 3) and adhesive strengths for 2-day biofilms were lower than those for 4-day and 6-day.
237 This behaviour suggested that the biofilm developed its strength dramatically between 2 and 4
238 days. However, the increase in average cohesive and adhesive strengths from 4 days to 6 days
239 was marginal. The results in Figure 4 may further support the findings in Figure 3 which show
240 slower increase in biofilm strength with thickness at the 6-day duration.

241 Nevertheless, with the increasing thickness, the required removal energy was greater at longer
242 durations (see Figure 5). There was a good correlation between the removal energy (from FDG)
243 and the required energy to overcome fouling (as shown by the TMP rise). While the increasing

244 removal energy with longer duration and biofilm thickness is not counter-intuitive, this
245 information may be required in the consideration for membrane cleaning protocol, in contrast
246 to the traditional parameters of TMP rise or permeate quality.

247 It should be noted that the information of biofilm strength and biofilm removal energy proposed
248 in this study is not intended to be used independently for the consideration of membrane
249 cleaning. Instead, this additional biofilm characteristic may be used in conjunction with the
250 information of production energy (ie TMP) to provide the overall comparison between (1)
251 continuing production with presence of fouling, or (2) performing cleaning.

252 Both cohesive and adhesive strengths obtained from biofilms in the present study are
253 considerably higher than those of other FDG studies (Lewis et al. 2012, Mohle et al. 2007).
254 Mohle et. al (2007) used FDG to investigate the activated sludge forming biofilm grown on a
255 rotating disc biofilm reactor (rotation speed of less than 9 min^{-1} for 7 days) and found the
256 cohesive strength of the biofilm was only 6-7 N m^{-2}. Lewis et. al (2012) applied a cross-flow
257 system and formed biofilm by yeast suspension. Their experiment was conducted for 30 min
258 with a duct flow rate of 0.9 L min^{-1} under constant TMP of 3.5 kPa. The highest strength of
259 biofilm was around 55 N m^{-2}. In the present study, the operating conditions applied were
260 harsher and simulated the actual conditions of microfiltration for water treatment. Moreover
261 biofilms formed by *Pseudomonas aeruginosa* tend to have higher strength as evidenced by
262 other ex-situ methods (6,000-15,000 N m^{-2}) (Korstgens et al. 2001, Poppele and Hozalski 2003).

263 Comparison of 4*-day with 4-day tests shows that biofilm desiccation did not significantly
264 impact the overall TMP and thickness (see Table 1). There was around 8% increase of TMP
265 and 8% decrease of FDG thickness, and the CLSM measurement did not show any thickness
266 change. Interestingly, the strength observation by the FDG showed significant increase in both
267 adhesive and cohesive strength of around 101.5% and 85.6% respectively (see Figure 4). The

268 apparent changes of biofilm condition were also shown by the slope strength at different
269 biofilm layers (Figure 6). Therefore, although the thickness and TMP rise were similar between
270 4-day and 4*-day, the latter showed significant increase of biofilm strength and resulted in an
271 increase of required removal energy (see Figure 5). An interruption to a biofilm development
272 process may cause undesired impact (eg accelerated attachment process) which affect biofilm
273 growth (Murthy and Venkatesan 2009, Timoner et al. 2012) and it is possible that desiccated
274 biofilm may produce an additional evaporation barrier and denser EPS, which may result in a
275 stronger biofilm (Flemming et al. 2016). These results may indicate that the FDG strength
276 analysis was able to provide additional information related to biofilm structural properties
277 which could not be reflected by TMP rise and biofilm thickness.

278 ***FDG as an aid for biofouling detection and cleaning in membrane systems***

279 There have been previous studies related to biofilm properties and biofouling. In general, these
280 studies can be grouped into three main areas: biofilm surface characteristics, biofilm structure
281 and thickness, and biofilm adhesion to surface (see Table 2). Apart from these studies, there
282 have also been some interests on the impact of biofilm development toward flow channel
283 constriction and localized channeling (Graf von der Schulenburg et al. 2008).

284 In this study, the FDG technique provided unique additional information related to biofilm
285 strength for both biofilm-biofilm (cohesive) and biofilm-surface (adhesive) through an on-line
286 and simple method. This information is unique and can be correlated to the requirements of
287 foulant removal energy due to biofilm development on membrane surfaces. This study also
288 presented comparisons between the energy for maintaining permeate production rate and the
289 required energy for foulant removal (see Figure 5).

290 Biofouling is still a major fouling problem in membrane operations and the most common
291 indicator for exercising the cleaning-in-place is pressure drop (TMP). FDG showed different

292 levels of cohesive and adhesive strength, while the TMP and thickness did not show significant
293 differences. The results in this study may provide an avenue for more developments on the use
294 of FDG in future studies related to membrane biofouling. Several areas that can be considered
295 for future research include impact of different operating conditions and validation of the FDG
296 strength information in a large-scale plant.

297 Table 2. Biofilm characteristic studies in literature.

298

Biofilm properties	Detailed characteristics	Literature	Note
Surface characteristics	Hydrophobicity	(van Oss 1997)	Surface energy measurements using contact angle technique.
	Surface charge	(He et al. 2015, Ikuma et al. 2014)	Surface zeta-potential measurements of biofilm coated or EPS surfaces.
	Viscoelastic	(Ferrando et al. 2017, Kundukad et al. 2016)	Surface viscoelastic determination including modulus and biofilm viscosity.
Biofilm structure	Porosity	(Chew et al. 2014, Goh et al. 2013)	Biofilm porosity distribution determination.
	Rheological	(Körstgens et al. 2001, Linares et al. 2016b)	Compressibility of biofilm, including impact of membrane permeations.
	Thickness	(Linares et al. 2016a, Mukherjee et al. 2016, Sim et al. 2013)	Most techniques are able to provide accurate thickness prediction of biofilm both on-line and off-line.
Adhesion	Surface adhesion	(Habimana et al. 2014, Huang et al. 2015, Suwarno et al. 2016)	Most studies focus on bacterial attachment to surfaces including impact of initial conditioning layers.
	Cohesive strength	(Mohle et al. 2007)	Measurement of cohesive strength through an offline FDG method.

299

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