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

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20   **ABSTRACT**

21   A fluid dynamic gauging (FDG) technique was used for on-line and in-situ measurements of

22   *Pseudomonas aeruginosa* PAO1 biofilm thickness and strength on flat sheet polyethersulfone

23   membranes. The measurements are the first to be successfully conducted in a membrane cross-

24   flow filtration system under constant permeation. In addition, FDG was used to demonstrate

25   the removal behaviour of biofilms through local biofilm strength and removal energy

26   estimation, which other conventional measurements such as flux and TMP cannot provide. The

27   findings suggest that FDG can provide valuable additional information related to biofilm

28   properties that have not been measured by other monitoring methods.

29

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

31   biofouling

Introduction

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

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

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

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

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

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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   $\sim 10^6$ 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

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

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

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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.

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