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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

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

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

Experimental

Biofouling experimental protocol

The experimental set-up and protocols used for simulating biofouling in cross-flow filtration were adapted from previous work (Figure 1A) (Sim et al. 2013). A rectangular flat-sheet cross-flow cell that had a membrane area of 0.0126 m² (180 mm × 70 mm) and a channel height of 2.0 mm was used. Before installation, the low protein binding polyethersulfone (PES) flat sheet membrane (PALL, 10K OMEGATM, MWCO 10 kDa) was cut and soaked in deionised water (Milli-Q, Merck-Millipore) for 24 h. The feed water contained background salinity of 500 mg L⁻¹ NaCl (Merck) and 20 mg L⁻¹ nutrient broth (Difco NB, BD Diagnostics) which provided total organic carbon (TOC) of approximately 8 mg L⁻¹, similar to typical TOC in secondary effluent water. Feed water was circulated via a gear pump (Cole-Palmer, Model 74013-45) in a closed loop as shown in Figure 1A. Wild type *Pseudomonas aeruginosa* PAO1, a common representative of wastewater bacteria, was chosen as model bacterium in this study (Hentzer et al. 2002, Kim et al. 2015, O'Toole and Kolter 1998). A stock solution of PAO1 (cell counts ~10⁶ CFU mL⁻¹) was injected at a constant rate of 0.25 mL min⁻¹ via an injection pump

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

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

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

FDG System

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

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

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

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

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

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

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

Results and Discussion

Determination of Biofilm thickness by FDG

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

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

correlation between FDG thickness and confocal thickness shows that biofilm thickness can be reliably determined by FDG.

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 ($\pm$ 1.8)	19.4 ($\pm$ 0.5)	18.0 ($\pm$ 2.5)
4	11.0 ($\pm$ 0.9)	27.9 ($\pm$ 0.8)	28.0 ($\pm$ 2.0)
6	13.9 ($\pm$ 0.2)	43.1 ($\pm$ 0.5)	45.0 ($\pm$ 3.0)
4*	12.3 ($\pm$ 0.4)	23.3 ($\pm$ 2.3)	28.0 ($\pm$ 3.0)

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

Determination of biofilm strength by FDG and impact of biofilm desiccation

The results for destructive strength testing at each time point are shown in Figure 3, in which the biofilm thickness is plotted against the applied gauging shear stress (eq. 1) (Lewis et al. 2016). The scatter in the data points, especially for 4- and 6-day, reflect the dynamic nature of the biofilm growth. The yield stress, characterised as that above which significant erosion of the biofilm (due to suction flow from gauge), for biofilms developed over 2, 4 and 6 days were estimated at 1165, 1600, and 1660 N m^{-2} , respectively (indicated by the vertical dotted lines on Figure 3). These values were estimated from the average initial FDG strengths from duplicate experiments. The dashed lines, obtained from the yield stress and the average adhesive strengths, were drawn on the figure for each experiment duration to aid visualization. A general negative trend was observed in all these results, showing that the layers closer to the membrane surface were harder to remove than those at the top of the biofilm (ie the cohesive strength increases as the biofilm gets thinner). The increased strength of the biofilm layers closer to the membrane could be caused by the permeate flux through the membrane and/or the increase in EPS concentration. It has been reported that permeate flux is a dominant factor in the

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

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

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

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

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

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

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

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

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

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

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

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

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

Biofouling is still a major fouling problem in membrane operations and the most common 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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