Fluorescence enabled direct visual observation for diagnosis of ultrafiltration membrane fouling by bi-disperse submicron particle suspensions

O. Autin¹, H. Sakar² & E. J. McAdam ¹

¹Cranfield Water Science Institute, Vincent Building, Cranfield University, Bedfordshire, UK and ²Environmental Engineering Department, Gebze Technical University, Gebze, Turkey

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Abstract
Whilst direct observation (DO) methodologies can describe back-transport of supra-micron particles, present technologies are unable to discriminate submicron particles, which are primarily responsible for membrane fouling. In this study, we therefore introduce a fluorescence enabled direct visual observation (RLF-DVO) methodology to permit visual characterisation of submicron particle transport during cross-flow filtration. Particle discrimination was achievable for particle diameters exceeding 0.25 μm; however, this was dependent upon particle concentration and the cross-flow velocity employed. Nevertheless, this is considerably below the detection limit of current techniques (around 3 μm). During filtration of a binary dispersion comprised of submicron particles, deposition was observed before a change in transmembrane pressure was detected, which underpins the important role of DO for fouling diagnosis. Based on observations made during this study, recommendations are proposed that will further improve resolution. Importantly, this study demonstrates RLF-DVO can provide real-time description of submicron particle transport during cross-flow filtration.

Introduction
Membrane bioreactors (MBRs) are an advanced wastewater treatment technology that enables process intensification and as the ultrafiltration membrane can achieve excellent separation, MBRs are capable of meeting particularly challenging discharge standards (Judd 2011). Process intensification is fostered through using the membrane for enhanced particulate rejection, which allows solids retention time to be decoupled from hydraulic retention time. However, this promotes particle polarisation at the membrane wall which constrains hydraulic productivity (Jiang et al. 2007). Consequently, gas or liquid pumping is used to introduce shear at the membrane wall, to promote particle back-transport into the bulk, as a means to sustain productivity. This membrane ‘fouling’ phenomenon has been the focus of extensive research as the energy penalty incurred for introducing shear, coupled with the need to specify excess membrane area to compensate for the reduction in hydraulic productivity, imposes added cost that constitutes a significant barrier to the wider deployment of MBRs (Judd 2011).

The particle matrix is heterogeneous both in size distribution and origin but broadly contains soluble microbial products (SMP), which are around 0.1–1 μm in size, single cell bacteria (0.1–5 μm in size) and flocs which comprise of cells and cell fragments embedded in a polymeric network of extracellular polymeric substances (EPS), which are around 5–100 μm in size. Several studies have demonstrated that the colloidal (approximate classification, 1.5–0.45 μm) and soluble (often classified as <0.45 μm) fractions primarily contribute to fouling (Bouhabila et al. 2001; Itonaga et al. 2004; Bae & Tak 2005; Grelier et al. 2005). The principal constituents of these particle subgroups are high molecular weight protein and polysaccharide compounds that are commonly less than 1 μm in size (Fig. 1).

Whilst their contribution to membrane fouling is well recognised, the explicit mechanisms which govern membrane fouling by this discrete submicron particle group are less well understood. (Bae and Tak 2005; Jiang et al. 2007; McAdam et al., 2011). This can be accounted for by the complex particle-particle and particle-membrane interactions that occur within the polarised region adjacent to the membrane surface (Neemann et al. 2013). Furthermore, the combination of drag and convective forces imposed on the
particle size distribution of an anaerobic MBR used to evidence
microbial Toolbox provided within micron sized binary dispersions,
studied. This is important as the enhancement in mass trans-
port provides a valuable indication of the formation of reten-
tate (O. Autin et al. 2016). Within other disciplines, reflected light fluorescence (RLF) has been used to enhance particle recognition within binary distributions, undergoing filtration. Specifically, we will: (i) demonstrate RLF-DVO for the characterisation of submicron particles; (ii) use RLF-DVO to quantify particle back-transport of submicron and super-

micron particles; (iii) compare classical pressure based and deposition based methods for the determination of critical flux in binary dispersions comprised of submicron particles.

Materials and methods

Experimental set-up

The microscope was equipped with RLF detection to provide emission at specific wavelengths (DM5500B, Leica Microsystems, Milton Keynes, UK). A digital high speed camera (DFC365 FX, Leica Microsystems, Milton Keynes, UK) was mounted on the microscope for image collection and analysed using Leica Application Suite software and VideoStudio software for particle tracking (Ulead, Malavida). A cross-flow filtration cell was mounted onto the microscope stage; a viewing window was routed into the top of the cell to enable imaging. A PVDF hollow fibre membrane with fitted within the channel and operated in an ‘out-to-in’ filtration mode. The ultrafiltration (0.04 m) hollow fibre membrane had an outside diameter of 0.0019 m, and an active surface area of 0.00125 m² (Zeeweed, GE Power and Water, Ontario, CA, USA). The ‘fluid-gap’ between the outer fibre wall and the viewing window was around 2.5 mm. The filtration cell was fitted with pressure transducers with a reported sensi-
tivity of <0.25% of range, on the retentate (0–1 barg) and per-
meate (+0.5 to −0.5 barg) channel to measure transmembrane pressure (TMP). Critical flux (Jc) was experimen-
tally determined by assuming TMP of the suspension remain equal to the TMP of clean water at the corresponding flow pro-
vided there is no deposition (Field et al. 1995). Particle polarisa-
tion will occur at the membrane wall once the convective force (applied by the permeate pump) exceeds the force applied by diffusive back-transport. Back-transport is described by Brown-
ian diffusion (Eq. (1)), shear-induced diffusion (Eq. (2)), which refers to particle motion induced by particle-particle interaction in a shear flow (Tardieu et al. 1998, Rusconi & Stone 2008), and inertial lift (Eq. (3)), which describes convective interaction

![Fig. 1. Particle size distribution of an anaerobic MBR used to evidence bimodal distribution, with a peak in the submicron range. This is compared to the model polysaccharide Sodium Alginate, often used as a surrogate of SMP, whose particle size is within the submicron range.](image-url)
between particles and the surrounding undisturbed flow field (Tardieu et al. 1998; Li et al. 2000):

\[ J = 1.3 \gamma^{1/3} \sigma^{-2/3} D_{B0}^{2/3} \left( \frac{\phi_W}{\phi_B} \right)^{1/3} L^{-1/3} \]  
(1)

\[ J = 0.078 \gamma^{4/3} L^{-1/3} \ln \left( \frac{\phi_W}{\phi_B} \right) \]  
(2)

\[ J = 0.036 \left( \frac{\rho \gamma^{3/2}}{\eta} \right) \]  
(3)

Numerous models have been derived to predict packing efficiency in binary dispersions. In the Cavern model, there is an assumption that small particles fill the cavities remaining in a packed bed constructed by large particles:

\[ \epsilon_{L,av} = \frac{\phi_L - 1 + \epsilon_L}{\phi_L} \]  
(4)

where \( \phi \) is the volume fraction of particles, and the subscript \( L \) represents large particles. An alternate proposition is that some small particles are replaced by several large particles in a packed bed constructed mostly of small particles. The packing density can also be estimated by material balance (German 1989; Hwang & Lin 2016):

\[ \epsilon_{L,av} = 1 - \frac{1 - \epsilon_s}{1 - \epsilon_s \phi_L} \]  
(5)

where the subscript \( s \) represents small particles. As both cake porosity and particle volume fraction are smaller than one, the cakes formed by dual-size particles have smaller porosity than that of a uniform-sized mono-dispersion. The lowest porosity can be given by the intersection point of the curves plotted using Eqs (4) and (5):

\[ \epsilon_{av} = \epsilon_s \epsilon_L \]  
(6)

The final average volume fraction, \( \phi_{av} \), can then be estimated by \( 1 - \epsilon_{av} \) and can be applied to estimate \( m_i \) in a binary particle suspension.
Fluorochrome enabled latex microspheres were used as candidate particles (from 0.25 to 10 µm), which are hard dyed to limit bleeding (Firefli™, Thermo Fisher Scientific, USA). Green microspheres were used for mono-dispersion testing which have an excitation/emission spectrum of 468/508 nm. Red 3 µm microspheres were used for binary testing which exhibit excitation/emission at 542/612 nm. Model solutions were prepared from microsphere concentrates containing 1% solids and diluted to the desired concentration in ultrapure water (Purelab Option – S7/15, 18.2 MΩ cm² and TOC < 3 ppb) to which 20 mM sodium chloride and 1 mM sodium hydrogen carbonate were added to increase ionic strength and buffering capacity of the water (Fisher Scientific, Loughborough, UK). Unless otherwise stated, experiments were run for 3h using a flux ($J$) of 100 L m⁻² h⁻¹, and an initial particle concentration of 25 mg L⁻¹ at a cross-flow velocity ($V_L$) of 11 mm s⁻¹ (Fig. 2).

### Results

**Verification of particle identification and fouling characterisation in mono-dispersions**

Using RLF-DVO, particle visualisation was initially undertaken to define the operating conditions at which discrete particles of fixed diameters could be determined (Table 1). Individual particle determination of 0.1 µm particles, could only be determined when cross-flow velocity approached stagnation (0 m s⁻¹). For particles diameters between 0.25 and 1 µm, particle discretisation was similarly dependent upon cross-flow velocity but also particle concentration. Discrimination of 3 µm particles was independent of concentration or cross-flow velocity. For subsequent experiments, $V_L$ was fixed to 0.011 m s⁻¹ which ensured good resolution for particle diameters exceeding 0.5 µm across a range on solution concentrations and is analogous to that successfully applied in earlier experiments (Autin & McAdam 2015; Autin et al. 2016).

Fouling assessment was undertaken for mono-dispersions with particle diameters 0.25, 0.5, 1, 3, 5, 10 µm (Fig. 3). For the smallest particle sizes, starting at a particle diameter of 0.25 µm, $dP/dt$ was observed to increase as particle diameter increased. Conversely, for the large particle sizes, $dP/dt$ was observed to increase as particle size reduced. The highest fouling rate was recorded for the mono-dispersion comprised of 1 µm diameter particles. Fouling data was compared to back-transport velocity modelling which evidenced a minimum back-transport velocity at 1 µm, which is coincident with measurement of the highest $dP/dt$. Particle back-transport velocity for 0.5 and 3 µm mono-dispersions were measured between 10 and 150 µm from the membrane surface (Fig. 4). Higher particle velocities were determined further from the membrane surface. Back-transport velocities appeared similar for both 0.5 and 3 µm mono-dispersions, and were considerably below the cross-flow velocity of 11 000 µm s⁻¹.

**Fouling characterisation in binary dispersions**

Critical flux ($J_c$) assessment was undertaken on mono-dispersions and bi-dispersions, using pressure as a

### Table 1

| Cross-flow velocity (m s⁻¹) | Particle size under testing (µm) | Particle concentration (mg L⁻¹) |
|----------------------------|---------------------------------|--------------------------------|
|                            | 0.1                             | 0.25                            |
|                            | 0.001                           | 0.005                           |
|                            | 0.011                           | 0.05                            |
|                            | 0.1                             |                                 |

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**Fouling characterisation in binary dispersions**

Critical flux ($J_c$) assessment was undertaken on mono-dispersions and bi-dispersions, using pressure as a
surrogate determination of fouling (Fig. 5). For the 3 μm mono-dispersion, increasing bulk concentration reduced \( J_c \) considerably. Lower \( J_c \) was attained with the 0.25, 0.5 and 1 μm mono-dispersions. Critical flux measured within the binary dispersion, was similar to that of the 0.25 μm mono-dispersion. Assessment of the binary dispersion comprising 0.5 and 3 μm diameter particles, evidenced a reduction in \( J_c \). In contrast, for the binary dispersion comprised of 1 and 3 μm diameter particles, \( J_c \) increased and was increasingly evident at higher particle concentrations. Particle deposition of the 0.5 and 3 μm bi-dispersion was evaluated during critical flux analysis through quantitation of cake height (\( H_c \)) (Fig. 6). Deposition was evidenced from the outset of filtration.

**Discussion**

Within this study, the application of fluorescence enabled DVO has been evidenced to permit the quantification of particle back-transport for dispersions comprised of submicron particles. There have previously been very few studies of binary dispersions comprised of submicron particles, due to method detection limits of existing methodologies (Le-Clech et al. 2007). Particle back-transport velocities of 0.5 and 3 μm particles were within the range reported by Marselina et al. (2009) for bentonite particles exposed to a CFV of 15 mm s\(^{-1}\). The authors similarly noted particle velocity to increase further from the membrane (below the nominal CFV), which was accounted for by the heterogeneous distribution of fluid velocity within the channel. In this study, particle velocity for 0.5 and 3 μm particles was similar. Caldwell (2000) described an analogous separation problem in which small and large particles ‘co-eluted’ during field-flow fractionation (FFF), where a flat-sheet cross-flow filtration cell is used to replace packed columns for the chromatographic separation of particles. Submicron particles are driven by the field toward the membrane, increasing the particle wall concentration, which increases the opposing diffusive flux proportionately until an equilibrium is reached with field-driven transport toward the wall (Giddings 2000). The result is a particle cloud which comprises of an equilibrium distribution whose concentration declines exponentially with distance from the membrane wall (Giddings 2000). Consequently, whilst 0.5 and 3 μm particle velocities are quantitatively similar within this hydrodynamic range, the different primary back-transport mechanisms impose contrasting particle distributions within the flow. This was visually illustrated when operated at analogous particle volume fractions (Fig. 7). For dilute suspensions of large particles exposed to high shear, preferential segregation from the membrane surface is promoted (Krompcamp et al. 2006). In this study, wall shear rate was constrained to ensure visibility of individual particles. It is proposed that limited accumulation of 3 μm particles at the wall could have also been promoted by the wide ‘fluid gap’ adopted (2.5 mm) which reduced the probability for particles migrating to the wall from the faster stream-lines in the centre of the channel. In FFF, a 250 μm channel height is used to ensure particle migration is controlled through both convective and diffusive forces (Giddings 2000). The recommended breadth to thickness aspect ratio (b/w) for FFF is \( \sim 100 \) (Barman et al. 1989). For comparison, the present b/w is around 2. Decreasing channel depth will increase b/w to 24, which will extend the parabolic flow profile in the radial direction, thereby improving particle distribution. The consequential impact will be the improved control over particle migration, enabling more refined governance of particle deposition, providing improved and significant insight into particle-particle interaction and particle deposition of the submicron particle fraction, which is the critical particle fraction to influence fouling in cross-flow filtration.

Analysis of mono-dispersions indicated 1 μm diameter particles presented the highest fouling rate. This was...
coincident with the onset of the minimum back-transport velocity and is analogous to earlier description of ultrafiltration for silica particle suspensions between 0.025 and 20 \( \mu \text{m} \) (Fane 1984). Evaluation of particle deposition during critical flux determination of binary dispersions, demonstrated particle deposition was noted by RLF-DVO in advance of TMP detection (Fig. 6). Such discontinuity has been previously observed in the study of super-micron suspensions. The authors ascribed the TMP ‘lag time’ to low pressure transducer sensitivity, insufficient particle accumulation to impose a detectable increase in pressure, and the low specific resistance provided by the foulant (Zhang et al. 2006). Importantly, this evidences the powerful resolution of this RLF-DVO methodology, compared to conventional methods, in enabling the characterisation of submicron particle deposition. Krompcamp et al. (2006) reported that only small particles in super-micron bi-dispersions under cross-flow were deposited at the membrane, as these have lower critical fluxes (Li et al. 1998) and proposed that \( J_c \) of the small particles could be provided by:

\[
J_c = 0.072(\frac{D_{\text{inh}}}{D_L})^{1/3}
\]  

(7)

For the specified shear rate, their model estimates \( J_c \) of 0.5, 1.4 and 3.4 \( \text{L m}^{-2} \text{h}^{-1} \) for 0.25, 0.5 and 1 \( \mu \text{m} \) particles respectively which provides supportive explanation for early deposition in this study (Fig. 6). Higher particle backtransport could have been achieved by increasing shear rate, particularly for binary dispersions comprised of 1 \( \mu \text{m} \) particles. However, RLF-DVO is currently limited to shear rates \(<50 \text{ s}^{-1}\) to ensure measurement of particles as individual entities. The proposed changes to the aspect ratio, specifically reducing the distance between the outer fibre wall and viewing window, will also reduce the overall distance to the microscope objective. Whilst the current set-up dimensions were within the working distance of the lens (11 mm; Autin et al. 2016), this will inevitably improve resolution, enabling individual particle identification at higher velocities and across a broader range of concentration and particle size ranges than presently possible (Table 1), helping to further advance understanding of submicron particle interactions in complex dispersions.

The particle deposition visually observed in binary dispersions, indicates that by definition, the critical flux was exceeded near the onset of filtration. Consequently, the TMP

![Fig. 5. Critical flux (\( J_c \)) determination for mono-dispersions and binary dispersions using the flux-step method. \( C_b = 0.01 \text{ kg m}^{-3}; V_L = 0.011 \text{ m s}^{-1} \).](image)

![Fig. 6. Particle deposition determined for binary dispersions of 0.5 + 3 \( \mu \text{m} \) and 1 + 3 \( \mu \text{m} \), when using the flux-step method. \( C_b = 0.01 \text{ kg m}^{-3}; V_L = 0.011 \text{ m s}^{-1} \).](image)
data provides indicative information of cake formation in the ‘super-critical’ state (i.e. post-particle deposition) rather than a definitive force-balance determination (Fig. 5). For 0.25 μm particles, $J_c$ was ostensibly similar between the mono-dispersion and bi-dispersion. It is proposed that the preferential deposition of 0.25 μm particles in the bi-dispersion resulted in the formation of a cake with similar characteristics (Krompcamp et al. 2006). Increasing submicron particle diameter from 0.25 to 0.5 μm reduced $J_c$ of the bi-dispersion. Madaeni (1998) similarly identified that $J_c$ of a bi-dispersion comprising submicron particles was below $J_c$ of the mono-dispersion (50 nm gold-sol, 1 μm latex). The authors used invasive imaging to evidence that large particles within the cake increased packing density and hence deposition resistance; this phenomenon being dependent upon the particle fraction. In the transition from 0.25 to 0.5 μm, submicron particle number in the bulk decreased by almost an order of magnitude and the relative proportion of 3 μm particles increased from 0.058 to 0.46%. This particle number fraction is the same order as Madaeni (1998). The estimated porosity for the 0.5 μm mono-dispersion was 0.33, compared to only 0.23 for the bi-dispersion, which is similar to cakes produced from bi-dispersions with particle size ratios of 0.01–0.06 (Madaeni 1998). The $J_c$ of 1 and 3 μm bi-dispersion was above the $J_c$ of the mono-dispersion (Fig. 5) and is analogous to observations by Zhang et al. (2006) for super-micron binary dispersions in which SiD dominated back-transport (ranging 3–10 μm). Whilst the specific mechanisms require elucidation, it is apparent that in the super-critical state (i.e. post-particle deposition), the impact of bigger particles is dependent upon the relative particle number fraction in the cake.

**Conclusions**

Within this study, fluorescence enabled DVO has been evidenced to permit direct measurement of submicron particles in the binary dispersion. A limiting particle diameter of 0.1 μm was identified at which individual particles could not be discriminated in cross-flow. For particles exceeding 0.25 μm, resolution was determined to be a function of concentration and cross-flow velocity. It is asserted that enhanced control of particle migration can be facilitated through selection of a shallower channel depth. Hydrodynamic conditions were selected to enable evaluation of an example binary dispersion composed of 0.5 and 3 micron particles, in which submicron particle back-transport was measured through DO for the first time. Through comparison of mono-dispersion and bi-dispersion data, it is suggested that particle deposition at low fluxes is primarily by submicron particles due to their lower particle critical flux. Higher shear rates could have improved back-transport but RLF-DVO is currently limited to wall shear rates <50 s$^{-1}$ to ensure single particle discrimination. We suggest the shallow depth channel will increase microscope resolution by reducing overall distance between microscope objective and membrane wall, which will enable higher fluid velocities to be evaluated without influencing image quality. Because RLF-DVO does not rely on natural light for luminescence, we consider that the methodology is equally valid for application to flat-sheet DOTM methods, whose membrane selection has been previously limited to those with a capillary pore structure as they enable light transmission. The methodology enabled determination of the early onset of particle deposition, before pressure measurement determined the critical flux which supports earlier observations of methods capable of determining super-micron bi-dispersions, underpinning the importance of DO methodologies for diagnostic investigation of fouling. Importantly, this study demonstrates fluorescence enabled DO can provide new insight into particle-particle and particle-membrane interactions for submicron particle transport during cross-flow filtration, which has been seldom described due to the limitations of previous techniques.

*Fig. 7. Raw images captured in real-time during ultrafiltration of mono-dispersions undergoing ultrafiltration: $C_0$, 10 mg L$^{-1}$; CFV, 0.011 m s$^{-1}$. Magnification: 10x (3 μm); 20x (0.5 μm). [Colour figure can be viewed at wileyonlinelibrary.com]*
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