Flocculation on a chip: a novel screening approach to determine floc growth rates and select flocculating agents

A. N. P. Radhakrishnan†, M. P. C. Marques, M. J. Davies, B. O’Sullivan, D. G. Bracewell, N. Szita

Department of Biochemical Engineering, University College London, Bernard Katz Building, Gordon Street, London WC1H 0AH, United Kingdom.

†Current affiliation: Department of Chemical Engineering, University College London, Torrington Place, London WC1E 7JE, United Kingdom.

A. Characterisation of Mixing

Mixing was characterised by analysing images recorded through an inverted microscope (Nikon Ti-E, Nikon UK Ltd., UK) equipped with a colour CCD camera (DS-Fi1, Nikon UK Ltd., UK) and a motorised stage. A solution of 4 mM Allura Red (Sigma Aldrich, UK) and Milli-Q® water (Millipore Ltd, UK) was injected into the focussing stream and sheath stream inlets, respectively; and vice versa.

Two images (M) were captured at specific locations along the channel and averaged:

\[
\frac{M_A + M_B}{2} = M_{Avg}
\]  

(Equation 1)

The images along the spiralling channel were rotated by an angle, \( \theta \), which corresponds to the angle of the spiral at the point of imaging (Figure S1). The averaged image (M_{Avg}) contained red, blue and green colour channels. These were extracted separately into a pixel array, G_{Avg}, with values between 0 and 255. Green and blue channels were chosen to compute mixing time. The intensity values, I_{Avg}, along the cross-section of the green and blue channels were overlayed with a row vector L_{(2560 x 1)}:

\[
I_{Avg} = L_{(2560 \times 1)} G_{Avg}
\]  

(Equation 2)

The resulting row vectors were converted into absorbance A_{Avg}, providing optical density (OD) profiles

\[
OD, A_{Avg} = -\log_{10} \left( \frac{I_{Avg}}{I_0} \right)
\]  

(Equation 3)

where \( I_0 \) is the maximum intensity within an image. The maximum intensity was approx. 246 and 250 for dye present in the sheath flow and focussed stream, respectively (Figure S2).
Figure S1 – Image processing of images taken at different locations of the device. The images were rotated by $\theta$ degrees to ensure that the flow direction was normal to the width of the image. The degree of rotation was obtained from the original microfluidic device 3D CAD SolidWorks file. The red lines in the frames correspond to the row vector $L_{(2560 \times 1)}$.

<table>
<thead>
<tr>
<th>Distance from channel inlet (mm)</th>
<th>Rotation degrees ($\theta$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>34.3</td>
<td>53.7°</td>
</tr>
<tr>
<td>49.3</td>
<td>93.9°</td>
</tr>
<tr>
<td>131.6</td>
<td>326.9°</td>
</tr>
</tbody>
</table>

Figure S2 – Absorbance profile of Allura Red solution passing through the microfluidic flocculation device ($\mu$FLOC) across the width of the device channel. The absorbance profile is given for two positions from the inlet, 0 and 80.3 mm. The flow rates used were 75 µl min$^{-1}$ and 85 µl min$^{-1}$ (total fluid velocity of 10.7 mm s$^{-1}$) for the centre inlet and the side inlets, respectively. The $S_{\text{left peak}}$ and $S_{\text{right peak}}$ represent the local absorbance minima and $C_{\text{peak}}$ corresponds to the maxima between these two points. The dotted vertical lines represent the position of the channel walls.

The mixing time can be calculated from the extent of mixing by

$$S_{\text{Avg peak}} = \frac{S_{\text{left peak}} + S_{\text{right peak}}}{2}$$  \hspace{1cm} (Equation 4)

$$\text{Extent of mixing, } \varepsilon = 1 - \left( \frac{C_{\text{peak}} - S_{\text{Avg peak}}}{C_{\text{peak}} + S_{\text{Avg peak}}} \right)$$  \hspace{1cm} (Equation 5)

Where, $S_{\text{left peak}}$ and $S_{\text{right peak}}$ are the local minima of the absorbance profile and $C_{\text{peak}}$ corresponds to the maxima between $S_{\text{left peak}}$ and $S_{\text{right peak}}$.

The mixing length ($z_{\text{mix}}$) was defined as the distance from the inlet where the extent of mixing, $\varepsilon$, reaches 95% homogeneity across the entire cross-section of the channel$^{1,2}$. For flocculation studies flow rates of 75 µl min$^{-1}$ and 85 µl min$^{-1}$ (total fluid velocity of 10.7 mm s$^{-1}$) were used for the centre inlet and the side inlets, respectively. The calculated mixing time ($t_{\text{mix}}$) and $z_{\text{mix}}$ were 7.5 s and 80.3 mm, respectively (Figure S3). These values are in agreement with theoretical mixing lengths and mixing times$^{3,4}$. 
Figure S3 – Extent of mixing in the μFLOC when flow rates of 75 µl min⁻¹ and 85 µl min⁻¹ (total fluid velocity of 10.7 mm s⁻¹) were used for the centre inlet and the side inlets, respectively. Under these conditions, a mixing time and mixing length of 7.5 s and 80.3 mm were obtained, respectively. The grey area denotes the ± 5% range of the stabilised extent of mixing values (95% homogeneity). The vertical dotted lines depict the theoretical mixing lengths under the studied conditions. Experiments were performed in triplicates. Error bars represent ± 1 standard deviation. The corresponding Péclet number (Pe) can be calculated from

\[ Pe = \frac{w_f V}{2D} \]  

(Equation 6)

Where, \(w_f\), \(V\), and \(D\) are the width of the cannel, fluid linear velocity and diffusion coefficient, respectively. For a \(w_f\) of 159 µm, a Allura Red AC diffusion coefficient of \(3.4 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}\) (Werts et al., 2012) and \(t_{mix}\) of 7.5 s, the Pe is approx. 2500. A Reynolds number (Re) of 5 (assuming kinematic viscosity of water, \(1 \times 10^{-6} \text{ m}^2 \text{ s}^{-1}\), at 20 °C) was obtained for the tested conditions.

**B. Determination of the Floc Size by Image analysis**

The floc diameter was determined by an image-analysis routine developed in Python 3.2.5 (Figure S4), with NumPy⁷, SciPy⁸ and PyGame modules installed. Briefly, the grayscale images captured (512 × 512 pixels) by a high-speed camera (MC-1, Photron USA Inc., California, USA) were binarised by applying a threshold value, chosen after visual inspection of the resulting binary images. The binary images were converted to a Boolean array where the flocs were considered to be objects (pixel value 1) and the background pixels carrying a value 0. As an additional noise filtering step, along with the binarisation process, reference images filled with water (and no flocs) were subtracted from the sample images to remove artefacts like the channel walls. The end result of the pre-processing is thus a sequence of binary arrays, one for each image frame, containing...
the pixel indices of the flocs in each frame. Following pre-processing of the images, different morphological operations (close-open filter followed by a median filter) were applied to smooth the perimeter of each particle and also to remove noise (pixel islands from the channel bed). Afterwards, the different flocs within a single frame were identified and labeled by using a connected-component labeling (CCL) step. To account for flocs present in multiple frames, binary masks in a particular frame were compared against masks in preceding frames. This overlapping is given by

\[
\text{Overlap} = \sum \left( \text{indices of labels}_n \cap \text{indices of labels}_{n+1} \right)
\]

Where, \( n \) corresponds to the image frames. The label of a given particle in frame \( n \), was passed on to a label in frame \( n+1 \), if their centres of mass were the closest amongst all labels in frame \( n \). The pixel area of each label was calculated and stored in a separate array, thereby being able to trace the floc flowing past the field-of-view, including the frame numbers where they enter and leave the field-of-view. When a particle is fully-in-focus, the pixel area value reached a maximum, which was stored in a separate array as the area of the particle with a unique label. The area was then computed into an equivalent circular diameter by assuming that the particles are circular. By measuring the magnification ratio, square pixels were converted to \( \mu m^2 \). This ratio was validated using a cell-counter grid of known size. The horizontal and vertical spans of the flocs were then calculated from the width and height of the array carrying the pixel area information. The particle traces and morphology measurements were then saved into comma-separated values (CSV) file.

Figure S4 – Flow diagram of the image-analysis routine developed in Python 3.2.5. The images taken by the high-speed camera underwent three separate processing steps: pre-processing, processing and post-processing step. The routine output a .csv file containing a trace record of individual particles across the entire video stream file and the particle characteristics, namely the length, width, area and equivalent circular diameter.

The data was then post-processed to check whether the particles were constrained by the width of the channels. If the width of the flocs were almost equal to the width of the channel, these flocs were filtered out. Additionally, to account for flocs going out-of-focus, the labels were checked whether they touch both the bottom and the top edges of the frame.
C. Particle Size Distribution

Figure S5 – Comparison of the particle size distribution (PSD) of the flocs obtained in the μFLOC (open circles) and bench-scale experiments (closed circles) at different flocculant, PEI (MW 50-100 kDa) concentrations at pH 7.4 and 25% solids yeast homogenate. (a) 5 g kg\(^{-1}\)\(\text{yeast}\) (b) 10 g kg\(^{-1}\)\(\text{yeast}\) (c) 15 g kg\(^{-1}\)\(\text{yeast}\) (d) 25 g kg\(^{-1}\)\(\text{yeast}\).

D. Characterisation of Shear Stress Levels

A laminar flow module in COMSOL Multiphysics 4.4 (COMSOL, Cambridge, UK) to simulate the μFLOC was used to extract the shear rate values (Figure S6). Results showed that under laminar flow (Re < 5 for operation conditions of the μFLOC) and a 30 μm structured mesh, a shear gradient was obtained across the channel width. A maximum shear rate of 207 s\(^{-1}\) was obtained at the channel's outer-wall, while at the channel's inner wall the value was approx. 152 s\(^{-1}\) due to the curvilinear geometry of the channel. A shear rate of 12 and 80 s\(^{-1}\) was obtained at the centre of the channel and at 125 μm from either side of the walls, respectively.
Figure S6 – Linear velocity and shear rate profiles extracted from the laminar flow module in COMSOL Multiphysics 4.4 of the μFLOC. The profiles were extracted across the channel at a depth of 250 μm and 131 mm from the device inlet. The background image of a floc was obtained with 25 g PEI kg⁻¹ yeast at pH 7.4 at 140 mm from the device inlet. The floc has a diameter of 209.1 μm.

**E. Comparison of PSDs of flocs in the μFLOC**

Figure S7: PSDs of *S. cerevisiae* flocs with 5 (green), 15 (purple) and 25 (red) g kg⁻¹ packed yeast of PEI (MW 50-100 kDa) along the length of the μFLOC (x = 8.03, 25.94, and 45.42 cm, respectively), measured via in situ image analysis. The black lines denote a Gaussian fit of the raw data for ease of comparison with the PSD obtained from the Mastersizer (Figure 3C). Gaussian fitting was performed using OriginPro 8.6 software (OriginLab Corporation, Massachusetts, USA) with the lowest $R^2 > 0.60$. 

![Figure S6](image1.png)

![Figure S7](image2.png)
References