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Short communication

Direct visualization of flowing biomass capture and release within a fibrous matrix

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Abstract

The way in which biomass can attach and detach to itself and other structures is of importance in a number of areas of bioscience and technology [J. Tampion, M.D. Tampion, Immobilized Cells: Principles and Applications, Cambridge Studies in Biotechnology, CUP, Cambridge, 1987; [1] M. Fletcher, The attachment of bacteria to surfaces in aquatic environments, in: D.C. Ellwood, J. Melling, P. Rutter (Eds.), Adhesion of Microorganisms to Surfaces, Academic Press, London, 1979, p. 87] [2]. Fluid flow will affect the situation [J.M. Lackie, Adhesion from flow, in: A.S.G. Curtis, J.M. Lackie (Eds.), Measuring Cell Adhesion, Wiley, Chichester, 1991, p. 41] [3] and this letter reports, for the first time, direct in situ observations of the way flowing biomass can deposit onto a fibrous structure and also how other flow conditions are able to re-suspend the biomass. In particular, fibre intersections are identified as regions of significant importance. The observations are of general interest in relation to biomass aggregation, growth and break-up and of specific relevance to filtration processes and immobilized bioreactor design.

Keywords: Porous fibrous network; Bioseparations; Wastewater treatment; Filtration; Immobilization

1. Introduction

Biocells can exist in a flowing fluid either as individual cells or as aggregates of cells [4] and the cell size distribution [5], concentration [6] and surface hydrophobicity [7] will influence whether or not aggregates can form. In addition, the physico-chemical nature of the flowing environment influences aggregation [8,9]. In general, slow flow conditions can enhance aggregation effects and strong flow conditions enhance break-up and the reformation of individual particles [10–12]. The introduction of an immobilized structure within a flowing biomass suspension further complicates the problem [13–16] and it is this situation that is considered in this short communication.

Laser scanning fluorescence confocal microscopy [17] (LSFCM) now enables in situ observations to be made of biomass deposition and removal from a support structure and this short communication reports observations that show the mechanism by which this can occur. A purpose-built micro flow cell (Fig. 1) was designed to operate in conjunction with the LSFCM. In order to produce high-resolution images of cells flowing within an immobilized structure, a high voidage network of polyethylene terephthalate (PET) fibres was introduced into a flow channel. In a previous work [18], this type of material has been shown to be effective in biomass capture.

2. Materials and methods

2.1. Biomass analysis

Effluent was collected from a conventional Trickling Filter used in domestic wastewater treatment at the AWG municipal wastewater treatment plant at Milton, Cambridge, UK. The suspended solids content of this biomass suspension was determined gravimetrically by filtering using a Buchner funnel using a pre-dried and pre-weighed 1.2 μm nominal pore size glass fibre filter (Whatman GF/C, Whatman, UK). The concentration of the biomass suspension used in this work was $60.0 \pm 0.4\,\mathrm{mg\,l^{-1}}$.

2.2. Flow cell construction

The micro flow cell shown in Fig. 1 was constructed from a PMMA block of dimensions 60 mm (width) ×

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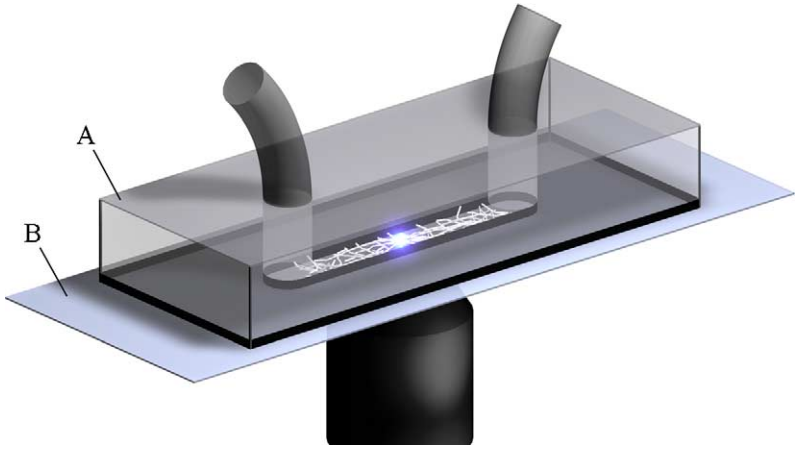

Fig. 1. Micro flow cell consisting of a PMMA block (A) with entry and exit holes and a channel milled at its base. Heat-bonded PET fibres at a bulk voidage of 95% are placed in the channel and a glass cover slip (B) is attached to the bottom of the PMMA block using double-sided sellotape. Biomass suspension is dispensed by a syringe pump and particle capture takes place in the flow cell fibre network. Biomass detachment is achieved using a fluid oscillator connected to the inlet of the flow cell. The laser scanning confocal fluorescence microscope, located beneath the flow cell, directly images both these processes.

24 mm (depth) × 10 mm (height). A channel of dimensions 35 mm (width) × 5 mm (depth) × 1 mm (height) was milled into the base of this block, and holes drilled through the block at both ends of the channel. A known mass of heat-bonded PET fibre material was carefully placed in the channel and the channel base covered with a #1 cover slip (Agar, UK), attached using double-sided sellotape. The PET fibres were of order 20–40 μm in diameter and the voidage within the packing was of order 95%, as determined by the fibre weight and confirmed using image analysis (IDL software) of the obtained images of the fibrous network.

2.3. Microscope settings and deposition recording

An Olympus IX50 inverted fluorescence microscope was used with a 10× objective and a 488 nm (blue visible) laser in scanning mode. Good direct contrast of thin horizontal slices of the fibre network of size 1400 μm × 1400 μm and approximate thickness 1 μm could be obtained and amplified using a photomultiplier tube. Simultaneously, autofluorescence emission from the biomass particles induced by the same laser excitation could be directed to a different photomultiplier tube using a 515 nm dichroic mirror and a barrier filter with lower end cut-off of 660 nm. The two images were independently processed and subsequently superimposed. A 500-μm thick stack of planar slices could then be built to a three-dimensional image of the network system and the biomass capture process thus followed in situ. 3D image stacks were recorded at 5-min intervals during the deposition, and also before and after fluid oscillations. Volumetric throughflow of biomass suspension was controlled by a syringe pump and set to a superficial flow velocity equivalent to $4.4 \times 10^{-3}\,\mathrm{m\,s^{-1}}$. During release, a net flow of water with a flowrate $4.4 \times 10^{-3}\,\mathrm{m\,s^{-1}}$ was superimposed on sinusoidal fluid oscillations of frequency 4.4 Hz and amplitude 11 mm.

3. Results and discussion

At low flowrates, where the local Reynolds number *Re* of the biomass flow was of order 0.7 and the mean interstitial velocity $4.7\,\mathrm{mm\,s^{-1}}$, deposition of biomass was observed to occur ($Re = \rho u r_H/\mu$, where ρ is the liquid density, r_H the hydraulic radius of the channel [r_H = flow cross-sectional area/wetted perimeter of cross-section], u the superficial channel velocity, and μ the liquid viscosity).

Fig. 2a–d shows a time sequence of the progressive accumulation of biomass onto the fibrous filter. The mechanism of accumulation is clear. Small biomass aggregates deposit on fibre surfaces. Biomass is also trapped at fibre intersections and these grow in size to form aggregates as they capture further biomass that passes within the vicinity of the trapped particles. This direct, in situ observation appears similar to the 'ripening' effect reported in deep-bed filtration [19,20]. Additionally, the mechanism explains why for example we have found cell capture to be effective with other fibre supports but not effective with open foam structures that have a similar pore size but are without the fibre intersection geometry and associated topology.

In terms of deposition, we have explored a limited range of flowrates. If the deposition Reynolds number in the flow channel is below about 0.4, the biomass will sediment in the entry tubes before entering the test section. We have observed that if deposition times are extended further than that shown in Fig. 2d, a maximum loading of biomass is achieved. Beyond this point, 'breakthrough' is observed and there is no further net attachment of biomass. At this stage, the liquid voidage within the packing has decreased due to

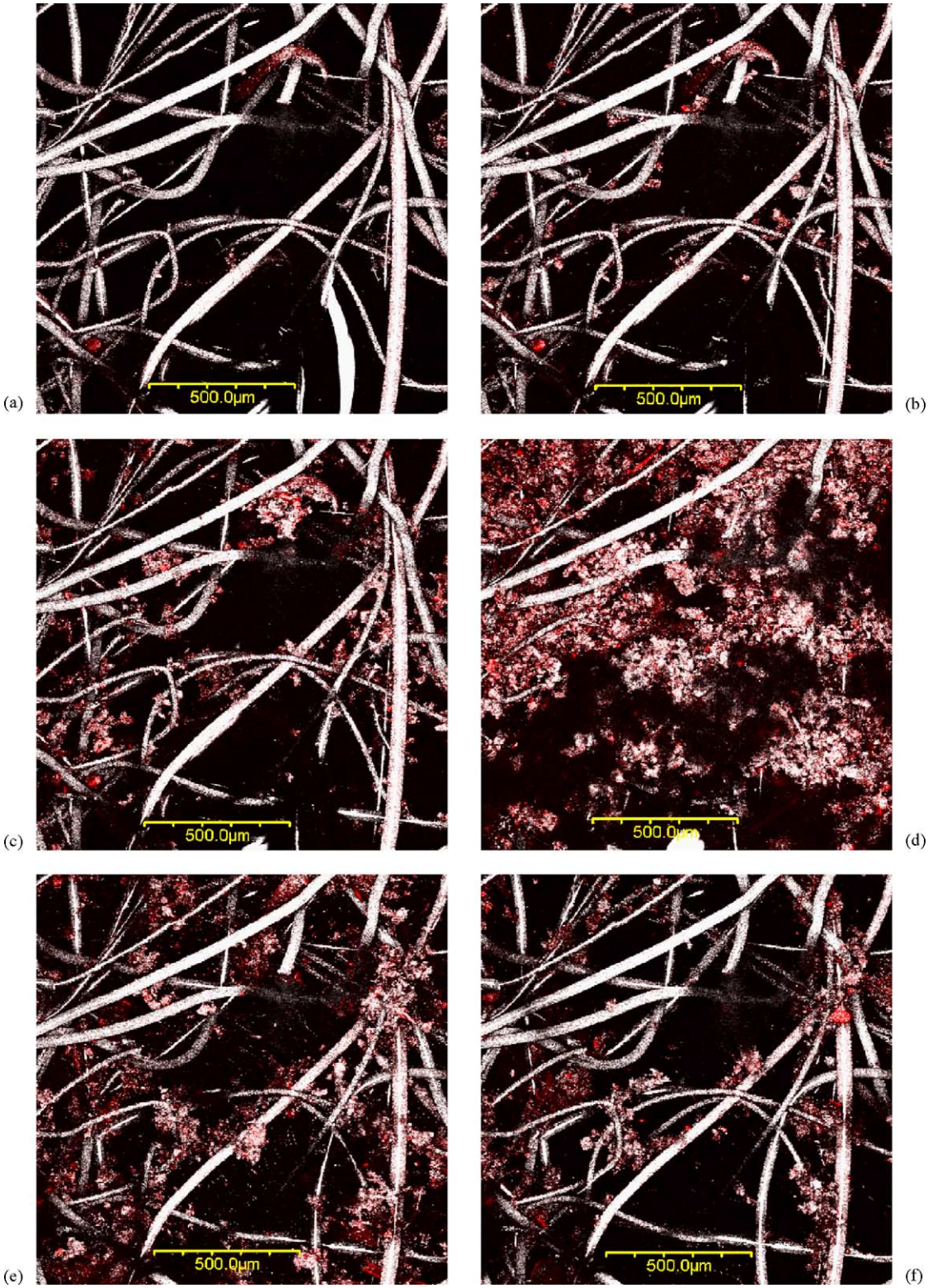

Fig. 2. Capture and release of biomass on PET fibre network, with a net flow *Re* of 0.7 and feed biomass concentration of 60 mg l^{-1}. (a) Clean filter section, imaged to 500 μm depth. (b) Filter section 15 min after start of capture. Small nuclei of deposits are evident, predominantly in the fibre intersections. (c) Twenty-five minutes after start of capture. Clusters of deposit are forming at nucleation sites. (d) Sixty minutes after start of capture. Clusters have developed into a semi-continuous network. (e) Image taken after 1 min of fluid oscillations at Re_o of 44 with superimposed flow at *Re* of 0.7, showing substantial release and removal of deposited biomass. (f) Image taken after 10 min of oscillation. Most of the deposit is removed and only core clusters remain.

the cell deposition and consequently the interstitial flow velocities apparently become sufficiently high to prevent further biomass attachment.

We have discovered an effective way of removing the accumulated biocells from the support. If the fluid is intensely oscillated at an oscillatory Reynolds number Re_o of order 45 with an oscillation cycle peak interstitial velocity of 330 mm s^{-1} (where $Re_o = 2\pi fa\rho r_H/\mu$ and $\{f, a\}$ are the oscillation frequency and center-to-peak amplitude, respectively), and a net flow of clean water is simultaneously imposed with Reynolds number of order 0.7, we have found that most of the biomass can be re-suspended and removed from the test cell. The fibre network and remaining deposit obtained after oscillation at $Re_o = 44$ with superimposed net flow with $Re = 0.6$ for 1 min is shown in Fig. 2e. The effectiveness of this fibre network regeneration procedure is strongly dependent on oscillation times and conditions. If the oscillation time is extended up to 10 min (Fig. 2f), most of the deposit is removed from the network.

4. Conclusions

The results show that LFSCM can successfully be used to monitor biomass deposition and release and we have identified different flow conditions when this can occur. A key finding of this short communication is the importance of the network microstructure geometry. Initial deposition of biomass was observed to occur at apparently arbitrary positions on the fibres, however formation and accumulation of aggregates concentrated at the fibre intersections. This demonstrates the importance of the local nature of the surface geometry, and the result could be of importance in a number of biological systems.

At this stage, it is not clear whether the flow conditions for deposition and removal are best described by a Reynolds number (using the hydraulic radius as the characteristic dimension) or in terms of an interstitial velocity. Given that biocells and void spaces in the fibre network have characteristic dimensions of microns, it would appear likely that attachment and detachment conditions will depend on local velocities in the vicinity of the cells rather than the overall Reynolds number of the flow. Notwithstanding this issue, the technique described in this letter can clearly be extended to follow the evolution of growing cell aggregates, with and without external flow of different magnitudes and types.

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