Environmental Microbiology (2004) **6**(2), 183–187 doi:10.1046/j.1462-2920.2003.00546.x

Brief report

A novel approach to investigate biofilm accumulation and bacterial transport in porous matrices

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Summary

Knowledge of bacterial transport through, and biofilm growth in, porous media is vitally important in numerous natural and engineered environments. Despite this, porous media systems are generally oversimplified and the local complexity of cell transport, biofilm formation and the effect of biofilm accumulation on flow patterns is lost. In this study, cells of the sulphate-reducing bacterium, *Desulfovibrio* sp. EX265, accumulated primarily on the leading faces of obstructions and developed into biofilm, which grew to narrow and block pore throats (at a rate of 12 µm h^{-1} in one instance). This pore blocking corresponded to a decrease in permeability from 9.9 to 4.9 Darcy. Biofilm processes were observed in detail and quantitative data were used to describe the rate of biofilm accumulation temporally and spatially. Accumulation in the inlet zone of the micromodel was 10% higher than in the outlet zone and a mean biofilm height of 28.4 µm was measured in a micromodel with an average pore height of 34.9 µm. Backflow (flow reversal) of fluid was implemented on micromodels blocked with biofilm growth. Although biofilm surface area cover did immediately decrease (~5%), the biofilm quickly re-established and permeability was not significantly affected (9.4 Darcy). These results demonstrate that the glass micromodel used here is an effective tool for *in situ* analysis and quantification of bacteria in porous media.

Fields which depend on accurate knowledge of bacterial transport and biofilm growth in porous media include: pathogen migration to groundwater (Abuashour *et al.*, 1994); subsurface bioremediation using emplacement or enhancement of indigenous bacteria (Gross and Logan, 1995) and; prediction of the location of populations of microorganisms in oil reservoirs. Biofilm growth can block oil-bearing rock pores reducing permeability and water and oil flow (MacLeod *et al.*, 1988; Bass and Lappin-Scott, 1997) or contaminate crude oil with hydrogen sulphide (Ligthelm *et al.*, 1991; Sunde *et al.*, 1993). Often, excessive growth can be removed by reversing the flow of injection water. Conversely, it is possible to engineer spatial distribution of bacteria using nutrient application in a saturated porous medium (Thullner *et al.* 2002). Despite the numerous applications there is a lack of directly measured information on bacterial cell transport and biofilm processes in porous matrices. This has led to insufficient appropriate terms to account for complex microbial behaviour in most bacterial transport models (Lawrence and Hendry, 1996).

In triplicate experiments, biofilm covered approximately 18% (SE 3.6%) of the available surface area after 25 h of media flow (Fig. 1). Where the fluid flow through the micromodel was diverted by an obstruction (See A, Fig. 2 and 14 h), there was an accumulation of biofilm on the leading face of that obstruction. This suggests that a combination of collision and sedimentation is responsible for the accumulation of cells from the bulk fluid onto surfaces (Raiders *et al.*, 1989). This accumulation of bacteria was observed and captured using time-lapse photomicrography, allowing pore throat narrowing to be quantified over time (Fig. 1). The average rate of pore throat narrowing in this case was 12 µm h^{-1}. The average stack height of the biofilm clusters in the field of view was 28.4 µm (± 4.4) in a micromodel with an average pore height of 34.9 µm, and biofilm accumulation completely filled the lumen of some pore throats. The vast majority of observed pore throats narrowed causing the fluid flow rate to decrease. This pore blocking corresponded to a decrease in permeability from 9.9 to 4.9 Darcy. The rate at which pore throats narrow and block is particularly significant to microbial enhanced oil recovery strategies (Paulsen *et al.*, 1997).

Bacterial cells and aggregates accumulated on surfaces over time and commenced growth and division, resulting in the formation of a biofilm. Many dynamic bio-

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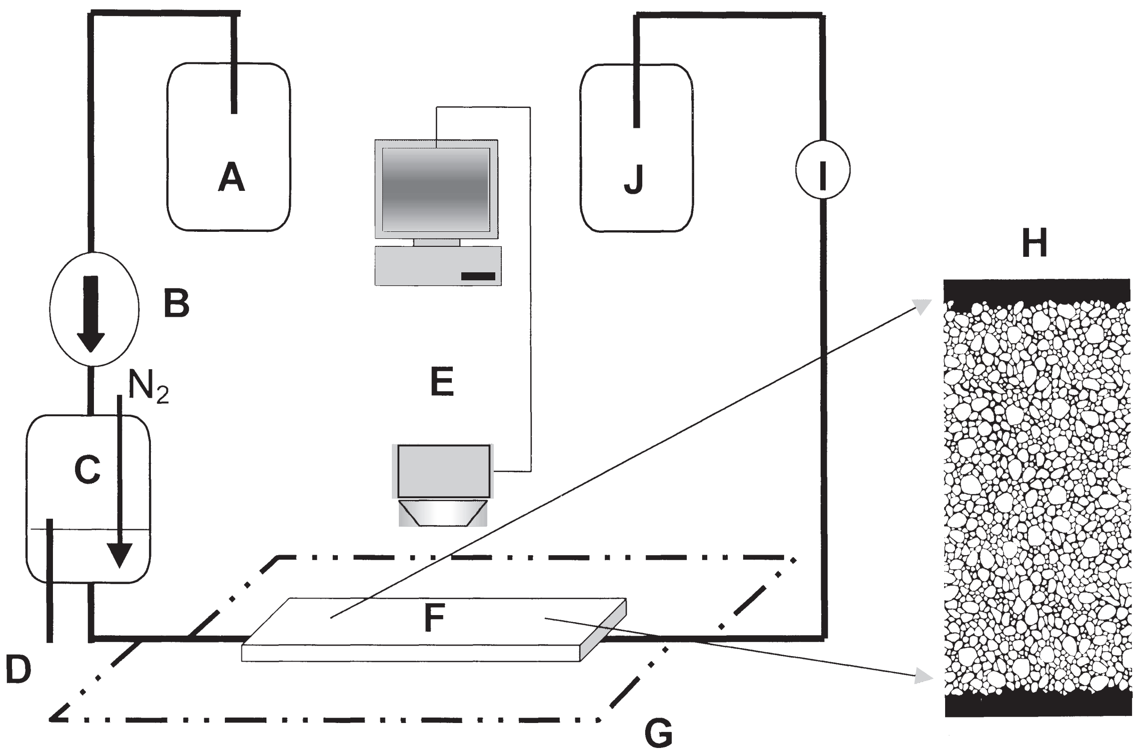

Fig. 1. Fluid flow apparatus. Fluid flow (A) was established at 4 ml h $^{-1}$ by means of a pressure head system to eliminate the 'pulsed flow' associated with slow running peristaltic pumps. This flow rate is estimated for some oil-bearing reservoirs and is realistic for subsurface studies (Lawrence and Hendry, 1996). No attempt was made to maintain the initial 4 ml h^{-1} throughput, which reduced to 2 ml h^{-1} over 24 h as the pore throats were constricted by accumulated and growing biofilm. A peristaltic pump (B) controlled the flow of media [anaerobic formation water medium (Bass *et al*., 1998) with 0.07 g l^{-1} sodium acetate and 0.6 g l^{-1} sodium pyruvate] into the (prefilled) header reservoir (C) and the fluid passed due to gravity through a (presaturated) micromodel (F) to the lower effluent vessel (J). This porous glass micromodel was specially designed and manufactured (see 'Micromodel design') and mounted horizontally on a heated microscope stage with a hole cut to allow light to pass from condenser to lens allowing examination of biofilm accumulation and transport (E). The volume of fluid in the header reservoir was maintained at 60 ml by a constant volume weir (D). Three replicate experiments were undertaken all inoculated with *Desulfovibrio* sp. EX265 (Dunsmore *et al*. 2002). A 1% inoculum of culture (10^9 cells ml^{-1} by total cell count) was injected directly into the header reservoir after vigorous agitation of the culture using a vortex for 2 min to disperse cell clusters, giving an initial concentration of cells in the reactor vessel of 10^7 ml^{-1}. The temperature of the flow system and micromodel were maintained at 30°C throughout all experiments and the mean redox potential (Eh) of the effluents was −179 mV (± 18).
Micromodel design. The micromodels consisted of two glass plates, one with an etched pattern, annealed together. The pattern used here was originally hand drawn from a thin section photomicrograph of sandstone rock (Dawe and Grattoni, 1998), such sandstone is a common reservoir rock (North, 1985). The manufacture method presented is an adaptation of that devised by Dawe and Grattoni (1998). Standard mirror pieces (800 × 400 × 40 mm) had their plastic backing removed using acetone and were coated on this metal side with RS photoresist spray (RS electronics). The pore pattern had previously been transformed into a high-resolution, lithium negative and this design was developed and etched into the metal surface of the mirror according to manufacturers instructions (RS electronics). Subsequently, the mirror piece was coated in bee's wax, leaving only the pattern exposed and left to harden. The mirror was then placed face up in 40% hydrofluoric acid for approximately five minutes. Neutralization in sodium bicarbonate ($NaHCO_3$) followed before rinsing in deionised water and removal of the wax. Residual photoresist and metal was removed using sequential rinses of acetone and 10% nitric acid. The model was finally neutralised in sodium bicarbonate. A fine cover glass (1 mm) was annealed over the etched pattern on the mirror glass in an oven with a temperature cycle reaching a maximum of 700°C.

film processes could be observed within the porous matrix. Bacterial attachment, biofilm growth and detachment of cells, and aggregates of cells, from the biofilm and surfaces were observed (Fig. 2). In addition, movement of biofilm units without detachment to the bulk fluid occurred (Fig. 3). This has previously been defined as 'creep' (Stoodley *et al*., 1999a).

After 24 h biofilm covered 21% (±7.5%) of the total pore space available in the 'inlet' zone of the micromodel, whereas biofilm in the middle zone covered 13% (±4%) and biofilm in the 'outlet' zone covered less than 7% (± 3%) of the available surface for colonisation. This finding is generally predicted by most bacterial transport models which are based on filtration, advection-dispersion or a combination of the two processes (Mills, 1997). This is despite recent evidence suggesting that in practice complex bacterial processes confuse this simple theory (Tufenkji *et al*., 2003). The flow direction through all micromodels was subsequently reversed. An initial decrease of approximately 5% biofilm cover was observed across each micro-

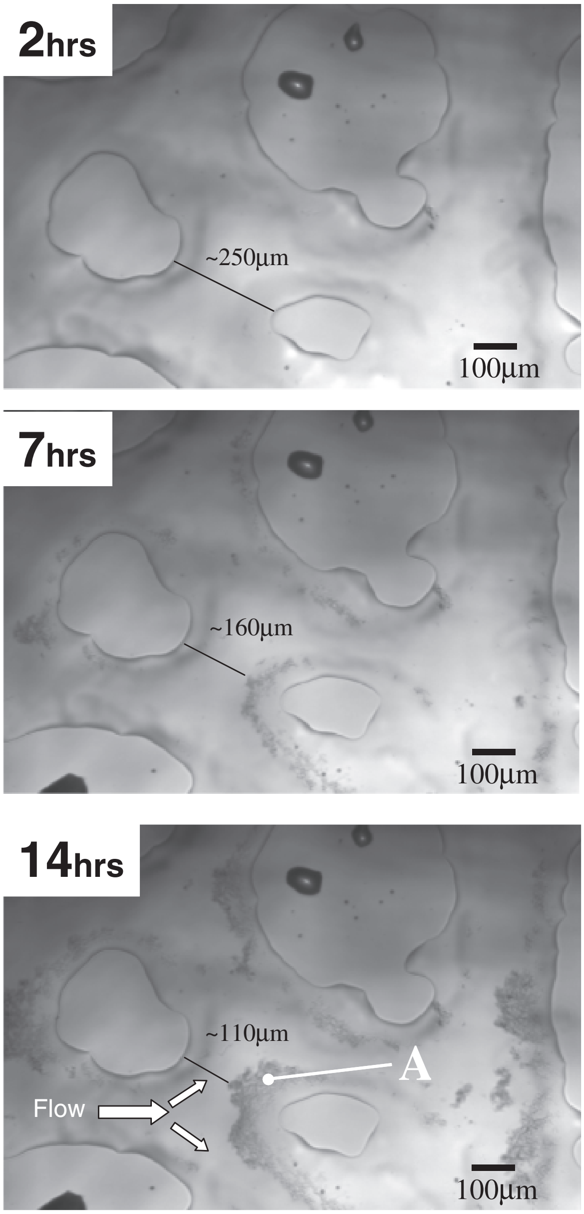

Fig. 2. *Desulfovibrio* sp. EX265 biofilm accumulation occurs in the pore spaces of a glass micromodel over time. Distances shown describe the narrowing of the pore lumen with subsequent biofilm development. Fluid flow path (⇒); biofilm accumulation (A). Image analysis was carried out on a Leica microscope using a digital camera linked to a Power Macintosh 7200/90 equipped with Scion Image 1.62a (NIH Image modified for windows by Scion, MA 21703, USA). Biofilm accumulation is defined as cell/cluster attachment plus growth minus cell/cluster detachment, and was measured here using image analysis to calculate as surface area covered by biofilm. Percent surface area cover was measured using a ×5 objective according to an adaptation of the method presented by Stoodley *et al*. (1999b) devised by Bass (2000). Three images from each of three zones, inlet, middle and outlet of the micromodel were routinely sampled at random while certain fields of view were concentrated upon for time-lapse image capture, having first been chosen at random by computer. Biofilm stack heights were measured microscopically by calibrating the micrometer as described by Bakke and Olsson, 1986). A 1-mm graticule with 10 µm divisions (CS990; Graticules) was used for microscope calibration allowing pore throat narrowing to be quantified.

model. However, this decrease was temporary; accumulated biofilm subsequently increased in all cases, returning to similar levels within 12 h. In addition, permeability did not alter significantly (mean permeability = 9.4 Darcy). This is in agreement with results from a rock core system where backflow without prior biocide treatment only led to a transient increase in the permeability (Cusack *et al*., 1998). Hence, our micromodel appears to behave similarly here to actual reservoir rock. There is scope to relate these micro and mesoscale results to actual macroscopic situations (Cunningham *et al*., 1991) suggesting that micromodels could effectively be used to improve predictive models and assess biofilm removal methods.

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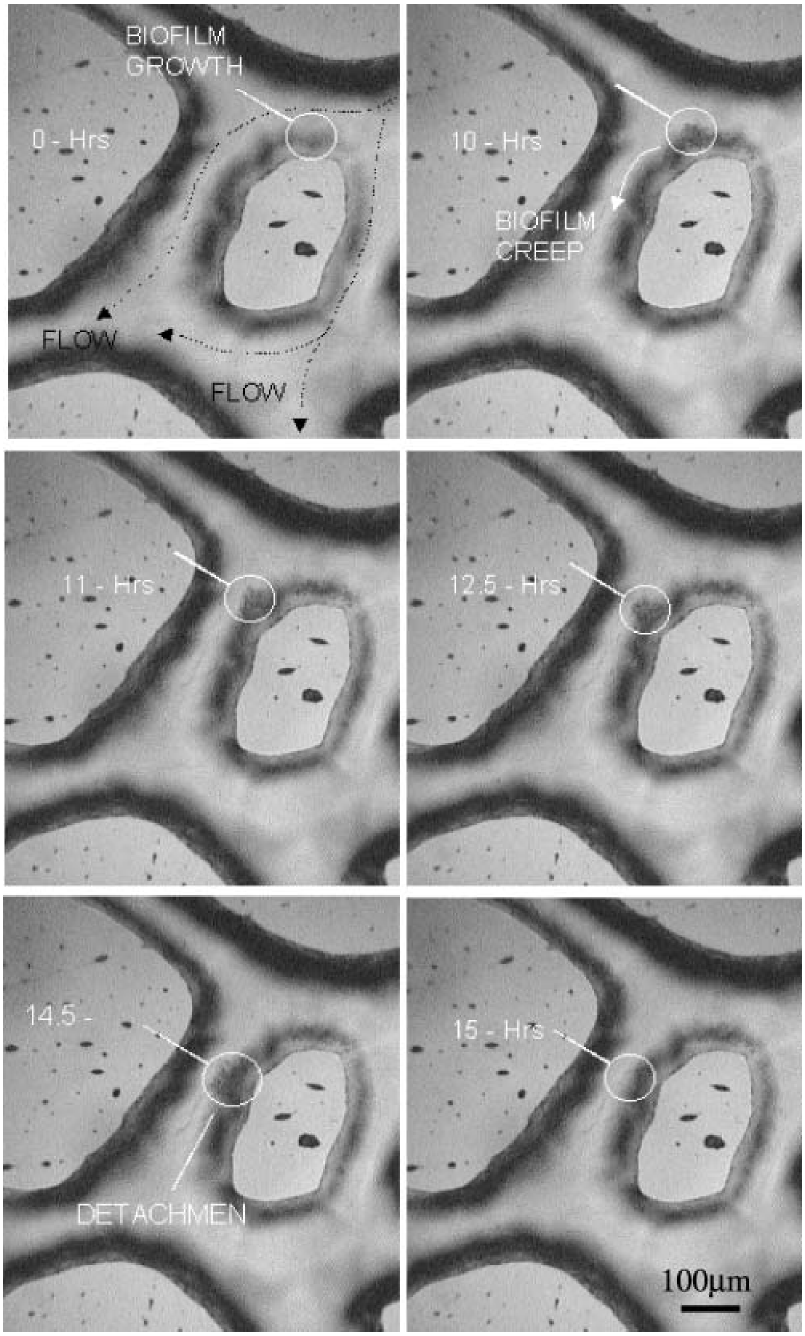

Fig. 3. A highlighted *Desulfovibrio* sp. EX265 biofilm cluster exhibits growth, creep and detachment in the pore channel of a micro-model over 15 h. The fluid flow rate was approximately 4 ml h^{-1} from top right to bottom left of all images.

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