Brief report

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

Braden C. Dunsmore,1* Catherine J. Bass2 and Hilary M. Lappin-Scott2
1Oil Plus Ltd, Hambridge Road, Newbury, Berkshire, RG14 5TR, UK.
2Environmental Microbiology Research Group,1 University of Exeter, Devon, UK.

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 \( \mu \text{m h}^{-1} \)) 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 \( \mu \text{m} \) was measured in a micromodel with an average pore height of 34.9 \( \mu \text{m} \). Backflow (flow reversal) of fluid was implemented on micromodels blocked with biofilm growth. Although biofilm surface area coverage 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 \( \mu \text{m h}^{-1} \). The average stack height of the biofilm clusters in the field of view was 28.4 \( \mu \text{m} \) in a micromodel with an average pore height of 34.9 \( \mu \text{m} \), 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-
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-
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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.

References


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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.
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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