# Visualization and characterization of dynamic patterns of flow, growth and activity of biofilms growing in porous media

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**Abstract** Using a mesoscale porous media flat plate reactor we utilized a naturally bioluminescent biofilm (*V. fischeri)* and dye studies to obtain valuable information on the interactions between biofilms and reactive flow in porous media. The growth and development of the *V. fischeri* biofilm in a porous media geometry was studied using digital time lapse images of the bioluminescent signal given off by the developing biofilm. The effect of biofilm development on porous media hydrodynamics was examined using dye tracer studies and image analysis. The natural bioluminescence of the *V. fischeri* allowed real-time, in-situ study of biofilm development in porous media, without destruction of the biofilm. Dye studies and image analysis enabled the study of effects of biofilm accumulation on porous media hydraulics, with comparisons to plug flow and completely mixed systems with varying degrees of biofilm accumulation. The real-time nature of the study permitted us to visualize dynamic flow channel formation within the biofilm/porous media system. In addition, the sensitivity of the *V. fischeri* biofilm to dissolved oxygen allowed us to capture real-time images of reactive transport within the system. This work is the first meso-scale visualization of the interactions between biofilm and flow in porous media.

**Keywords** Biofilm; bioluminescence; hydrodynamics; meso-scale; porous media; reactive transport

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## Introduction

The growth of biofilms in porous media has applications and implications in the fields of water and wastewater treatment, microbial mining, groundwater recharge, enhanced oil recovery, and in-situ bioremediation (Bouwer *et al.*, 2000). All of these applications and processes involve the attachment and growth of bacterial populations within a porous media matrix via the production of exocellular polysaccharide (EPS). A thorough understanding of biofilm growth and accumulation within porous media and its effects on porous media mass transport characteristics is necessary to properly design, operate and control these systems for environmental and industrial applications.

The study of biofilm growth in porous media and its effects on hydraulic properties has been carried out by numerous researchers (Cunningham *et al.*, 1991, 1997; Sharp *et al.*, 1999). A multitude of model systems have been used including individual pore channel reactors, engineered porous media, glass beads and sand. The effects of biofilm growth on porous media hydraulic conductivity, pore velocity, permeability, porosity and hydrodynamic dispersivity have typically been demonstrated using standard soil methods, microscopy, dye tracer studies, numerical analysis, and transport models (Vandevivere *et al.*, 1995; Cunningham *et al.*, 1991, 1997; Thullner *et al.*, 2002; Sharp *et al.*, 1999;

Stoodley *et al.*, 1994). Sharp *et al.* (1999) and Thullner *et al.* (2002) developed non-destructive methods for evaluating biomass accumulation in biofilm. Sharp *et al.* (1999) used bulk dye tracer and image analysis methods to show flow channel development and localized dispersion effects in thin and thick biofilm systems in fine porous media. This work indicated that as biofilm accumulation increased, a primary porosity was established in the porous media, resulting in defined pore channels where advective flow and longitudinal dispersion dominate. Thullner *et al.* (2002) used direct visible light transmission through a two-dimensional flow cell and bulk fluid dye studies to show interactions between advective and dispersive flow in saturated porous media.

The use of bioluminescence to monitor bacterial growth has been used for some time. Bioluminescence has been used to study cell–cell communications in biofilms at the microscopic level and in the study of medical biofilms (Wilson and Hastings, 1998).

The goal of this study was to utilize natural bioluminescence to visualize and characterize biofilm accumulation in porous media and its effects on advection, dispersion and reaction within the biofilm/porous media system. The work combines hydrodynamics/hydraulic analysis with bioluminescent imaging to better understand the effects of biofilm accumulation on reactive transport in porous media at the meso-scale.

## Methods

### Model biofilm – *Vibrio fischeri* MJ1

The well characterized bioluminescent *Vibrio fischeri* MJ1 was used in these studies for its biofilm growth potential, high intensity bioluminescent signal, and relative ease of culturability. *V. fischeri*'s bioluminescence signal is produced by the lux autoregulator N-(3-oxohexanoyl) homoserine lactone. The intensity of this signal is sensitive to nutrient availability, dissolved oxygen, and population density (quorum type sensing). The cultures of *V. fischeri* were maintained on salt-water complete media (SWC) at 25 degrees Celsius. This same salt-rich nutrient was used to establish and maintain biofilm growth in the porous media reactor studies. To initiate biofilm growth in the porous media reactor, a single 1.0 ml inoculation of pure culture *Vibrio fischeri* MJ1 was introduced to the reactor via an injection port located in the influent media line. After inoculation, media was continuously pumped through the reactor at 0.25 ml/min. for 24 hours prior to initiating the dye tracer studies, image analysis and hydraulic testing.

### Model porous media reactor

The model flow-through system consisted of a custom-made 12 cm × 8 cm × 1 cm flat plate reactor with fixed diamond elements serving as the porous media equivalent. The diamond elements were 1.0 cm deep, with 0.4 cm sides. A peristaltic pump provided a constant flow rate of approximately 1.0 ± 0.05 mL/min. The system had sampling ports and piezometers located at the influent and effluent to accommodate *V. fischeri* inoculation, nigrosine dye injection, effluent sampling and measurement of head loss across the system.

### Nigrosine dye tracer studies and hydraulic characterization

Standard nigrosine dye tracer studies were performed on the flat plate reactor to determine the hydraulic characteristics of the model system during varying degrees of biofilm accumulation. Analysis of the nigrosine dye was performed using an EL 808 Ultra Microplate Reader (Bio-Tek Instruments, Inc.) at 340 nm wavelength. Standard break-through curve (BTC) analysis was performed on the tracer data so comparisons could be made between ideal plug flow, clean porous media hydraulics, and porous media hydraulics

with varying degrees of biofilm growth. Hydraulic conductivity was measured using head loss and flow rate measurement.

#### Bulk fluid and biofilm accumulation imaging

All imaging was performed using a Nikon 950 digital camera and a stereoscope hooked up to a computer. The digital camera captured images of nigrosine dye flow through an empty reactor and at various degrees of biofilm accumulation within the reactor. Long exposure, time-lapse images of the bioluminescent biofilm were taken during different stages of biofilm accumulation and during nigrosine dye tracer studies. The images were used to show bulk fluid flow, biofilm accumulation, biofilm activity, and flow channel dynamics over time during biofilm accumulation.



## Results and discussion

As in past studies, a series of dye tracer experiments illustrated the hydraulic effects that biofilm accumulation has on flow though porous media (Sharp *et al.*, 2001). Table 1 shows the average results of results for two duplicate sets of tracer studies that were performed on a reactor with low and high level of biofilm accumulation. As biofilm initially starts to accumulate in the reactor the dispersion within the system increases substantially (T10/T90), and the hydraulic residence time decreases as expected. As the biofilm accumulation increases, the flow becomes more "plug-flow"-like with less dispersion and a more defined break-through. Figure 1 shows the progression toward plug flow when thick biofilm accumulation is present. For this study, a thick biofilm is one that fills the pore space in the porous media, which relates to a thickness of 200–250 microns. The thin biofilm in Figure 1 tends to disperse the flow throughout the biofilm, while the thick biofilm has well defined flow paths. The movement toward plug flow during thick biofilm accumulation is caused by defined pore channels that develop within the porous media. These pore channels develop and become more defined as the biofilm accumulation increases. The pore channels are dynamic, changing in size, number and location with time as shown in Figure 2.

The dynamic nature of these pore channels is caused by nutrient availability within the channel which encourages biofilm growth and the subsequent closing/clogging of the old channel and the requisite development of new channels where the biofilm/porous media matrix is weakest or has a higher permeability. These pore channels have been hypothesized and observed before, but their dynamic nature has not been well documented.

Digital images of the bioluminescent signal emitted by the biofilm are shown in Figure 3 for two different degrees of biofilm development. Again, the pore channels become more defined as the biofilm content increases. The bioluminescent signal indicates the presence of significant biofilm density, ample substrate and dissolved oxygen. In the thin biofilm, the intensity of the bioluminescence is relatively low, but is pervasive throughout much of the reactor. In the thick biofilm system, the bioluminescence is seen only along defined pore channels. The decrease in bioluminescence in the thin biofilm is

**Table 1** Breakthrough curve analysis results

| Parameter | Description | Plug flow | Empty reactor | Thin biofilm | Thick biofilm |
|---|---|---|---|---|---|
| Ti (min.) | Initial | Td | 3.5 | 1 | 2 |
| Ta (min.) | 50% pass | Td | 7.09 | 6.5 | 3.4 |
| Tp (min.) | Peak | Td | 4.75 | 4.5 | 2.75 |
| Tg (min.) | Centroid | Td | 4.77 | 4.16 | 2.07 |
| T90/T10 | Dispersion coef. | 1 | 2.98 | 8.44 | 4.27 |

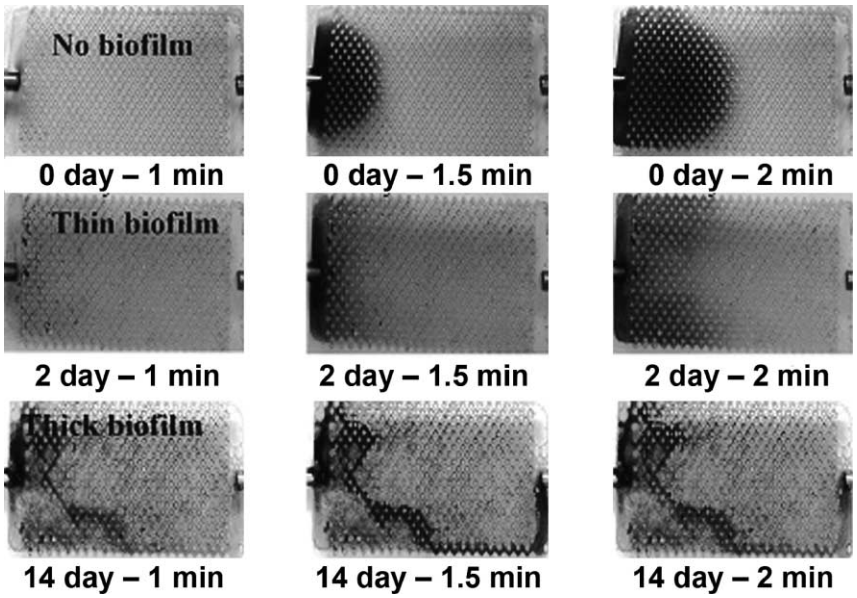


**Figure 1** Nigrosine dye injection shows flow paths in thin and thick biofilm systems. The thick biofilm results in highly defined flow channels, while thin biofilm results in more dispersed, undefined flow channels

caused by both a decrease in biofilm density in the downstream half of the reactor and the loss of oxygen or nutrient due to reaction. In the thick biofilm system, the fading of the bioluminescent signal was caused by lack of dissolved oxygen. Nigrosine dye images of the thick biofilm condition show the same pore channel network as the bioluminescent images. Figure 4 shows a composite over-lay image of the biofilm with nigrosine dye and the bioluminescent image. The bioluminescence overlays the nigrosine dye filled flow channels. The arrow depicts a possible flow path through the biofilm/porous media matrix following the bioluminescence and nigrosine dye. As can be seen the bioluminescent signal fades in the direction of flow due to utilization and limitations of dissolved oxygen along the flow path.

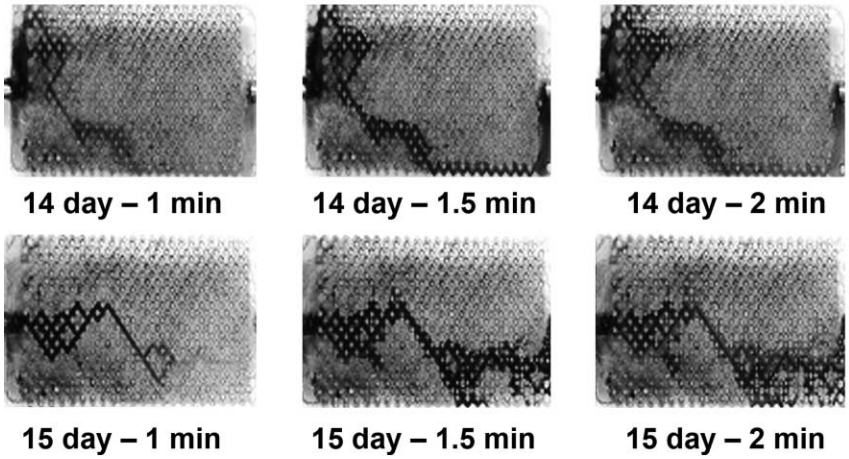


**Figure 2** Flow channel dynamics in thick biofilm growth/porous media system. The flow channels change size, shape and location as nutrient feeds the biofilm, plugging preferred path and forcing flow to find a new path of least resistance

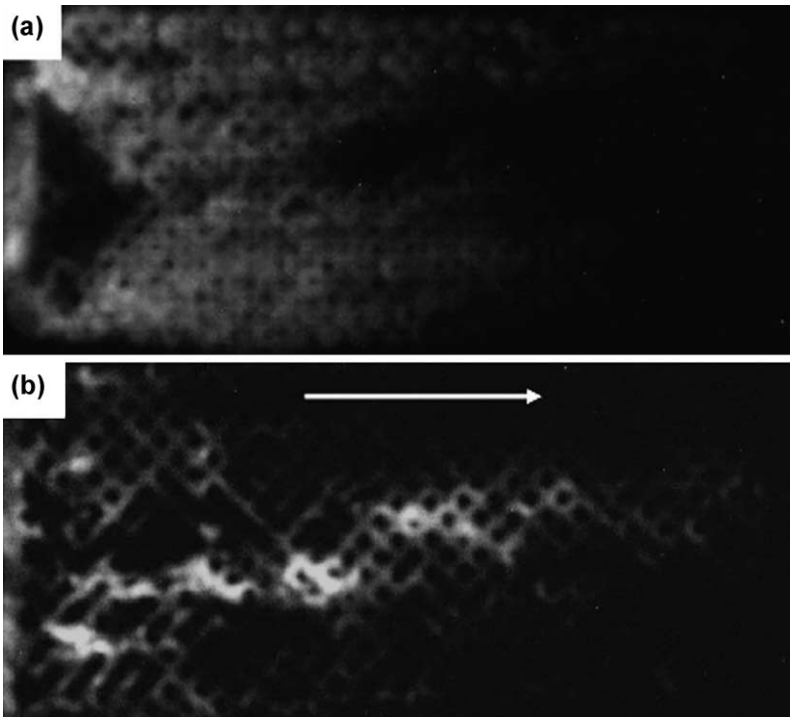


**Figure 3** Bioluminescent biofilm: (a) thin biofilm, (b) thick biofilm with defined flow channel and high activity

## Conclusions

The imaging of a bioluminescent biofilm allowed for the visualization of not only the dynamic development and change in pore channels in the biofilm/porous media system, but also relative degree of reaction by comparing the intensity of bioluminescence along

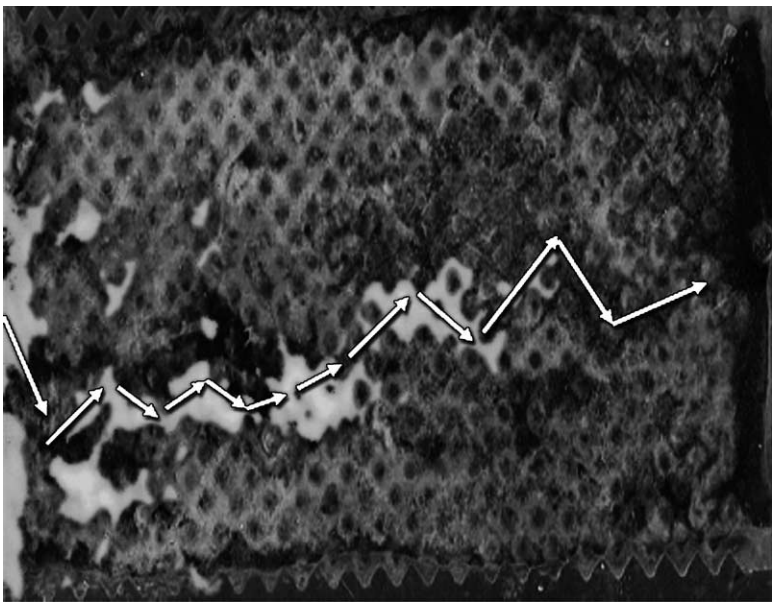

**Figure 4** Composite photo of bioluminescent image over-laying biofilm/nigrosine dye image. Bioluminescence shows primary flow channel within porous media and biological activity (respiration) resulting from oxygen and nutrient availability

the flow channel. The bioluminescent images supported the dye tracer images and hydraulic analysis. As expected the hydraulic conductivity was severely reduced by as much as four orders of magnitude when thick biofilm was allowed to accumulate. However, a well defined, dynamic flow channel did develop allowing media and oxygen to support growth and activity along the flow paths. The bioluminescent images showed the location of oxygen limitations and varying degrees of microbial activity along the dynamic flow channels. This work indicates that flow patterns in porous media colonized by biofilm are very dynamic due to cycles of increased growth in the flow channels, which leads to plugging and redirection of flow. Hydraulic analysis supports previous findings that as the thick biofilm develops, the flow of the biofilm/porous media system begins to have plug flow characteristics, reducing dispersion and dilution. Bioluminescence can be used to study biofilm reactions in porous media in real time, without the need for destructive sampling. Ongoing studies are attempting to correlate bioluminescent intensity and location with degradative activity.



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