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Using bacterial bioluminescence to evaluate the impact of biofilm on porous media hydraulic properties

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ABSTRACT

Biofilm formation in natural and engineered porous systems can significantly impact hydrodynamics by reducing porosity and permeability. To better understand and characterize how biofilms influence hydrodynamic properties in porous systems, the genetically engineered bioluminescent bacterial strain *Pseudomonas fluorescens* HK44 was used to quantify microbial population characteristics and biofilm properties in a translucent porous medium. Power law relationships were found to exist between bacterial bioluminescence and cell density, fraction of void space occupied by biofilm (i.e. biofilm saturation), and hydraulic conductivity. The simultaneous evaluation of biofilm saturation and porous medium hydraulic conductivity in real time using a non-destructive approach enabled the construction of relative hydraulic conductivity curves. Such information can facilitate simulation studies related to biological activity in porous structures, and support the development of new models to describe the dynamic behavior of biofilm and fluid flow in porous media. The bioluminescence based approach described here will allow for improved understanding and control of industrially relevant processes such as biofiltration and bioremediation.

1. Introduction

Biofilms are structured communities of microorganisms embedded in a self-produced organic matrix of extracellular polymeric substances (EPS). In porous structures, which inherently have high surface area to volume ratios in comparison to non-porous structures, microbes can quickly colonize pore surfaces and form biofilms rather than remaining in a planktonic state (van Loosdrecht et al., 1990; Bouwer et al., 2000). With sufficient nutrient supply and metabolic waste removal, biofilms can progressively accumulate within a pore space making it increasingly difficult for fluids to flow through the porous structure (i.e. bioclogging) (Taylor and Jaffé, 1990; Cunningham et al., 1991; Thullner et al., 2002; Bozorg et al., 2011, 2012). In general, past studies have revealed that the permeability and porosity spatial distributions of a porous medium are related to the rate at which biofilms grow and spread within that medium.

Biofilms are often considered to be undesirable as they can negatively impact industrial processes that rely on the flow of fluids in porous media. However, management of biofilm growth in porous media can improve the performance of those industrial and environmental processes that rely on biofilms to achieve a process goal, such as in situ bioremediation (Thomas and Ward, 1989; Madsen, 1991; Singh et al., 2006), biobarrier containment (Kim et al., 2006), wastewater treatment (Nicolella et al., 2000), enhanced oil recovery (Lappin-Scott et al., 1988), and carbon sequestration (Mitchell et al., 2009). Development of robust methods to engineer biofilms in porous structures requires comprehensive knowledge of the processes that affect their spatiotemporal development under different flow conditions. However, due to an inadequate understanding of the interactions between porosity and permeability, biofilm growth kinetics, multiphase flow effects, spatial variations of cell nutrients, and impact of medium heterogeneity, field scale applications of biofilm based processes are still unpredictable (Gerlach and Cunningham, 2010).

Different models have been used to simulate biofilm growth in porous media (Baveye and Valocchi, 1989; Vandevivere et al., 1995; Clement et al., 1996; Thullner et al., 2004; Kim and Whittle, 2006; Bozorg et al., 2011). The general approach used in these models has been to incorporate relationships that link porosity to hydraulic conductivity by treating biofilm as an emerging solid phase that changes the intrinsic porosity and permeability of a medium. Whereas such models can qualitatively reproduce experimental results, they are limited due to the lack of reliable correlations between porosity and permeability (Baveye and Valocchi, 1989; Clement et al., 1996; Thullner et al., 2002; Bozorg et al., 2011). Recently, Bozorg et al. (2011) introduced a new macroscopic approach to model biofilm spatiotemporal development in porous media by treating biofilm as a high viscosity liquid phase that shares pore space with a low viscosity aqueous phase. In that study, efforts were made to quantify effective conductivities of water and biofilm phases via relative permeability curves based on biofilm saturation (i.e. fracture of pore space occupied by biofilm). However, calibration of the parameters used in this approach is challenging since it

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requires simultaneous evaluation of biofilm evolution and hydraulic conductivity.

A majority of studies on biofilm growth in porous media involve destructive sampling, i.e. removing biofilm from an experimental apparatus for characterization purposes (Taylor and Jaffé, 1990; Cunningham et al., 1991; Seki et al., 2006). Evaluation typically occurs only at the end of an experiment, which is not conducive to studying how biofilms evolve and affect fluid flow in real time. Real-time quantification of biofilm characteristics and porosity and permeability have been a subject of interest in porous media based processes for many years (Oostrom et al., 1998; Niemet and Selker, 2001; Seymour et al., 2004; Bozorg et al., 2012). Recently, new approaches have been developed that use bioluminescence emitted by certain natural and engineered microorganisms to monitor microbial processes in natural and engineered environments (Burlage et al., 1990; Shaw et al., 1992; Ripp et al., 2000; Uesugi et al., 2001; Sharp et al., 2005; Bozorg et al., 2012). For instance, Sharp et al. (2005) used naturally luminescent bacteria in a flat-plate flow chamber to study biofilm growth under flow; whereas bacterial bioluminescence was used to track biofilm development, no quantification was made of the detected bioluminescence intensity (BI). In another experimental study, Bozorg et al. (2012) used a CCD camera to monitor growth of biofilm forming bioluminescent bacteria in a translucent porous medium. They demonstrated that it may be possible to use inducible bacterial bioluminescence to nondestructively evaluate cell density and hydraulic properties in porous media.

The objective of the research that will be described here was to develop an approach to quantify, nondestructively and in real-time, porous medium hydraulic conductivity and biofilm saturation, and to then use these parameters to develop relative hydraulic conductivity curves for the flowing aqueous phase. The ability to determine such information will enable better understanding of interactions between biofilm growth and fluid flow in porous structures.

2. Materials and methods

The bioluminescent bacterial strain, porous medium, imaging system, and fluid application system have been described in detail elsewhere (Bozorg et al., 2012), and so will only be described here briefly.

2.1. Bacterial strain and culturing conditions

This study used the bioluminescent reporter strain *Pseudomonas fluorescens* HK44 (hereafter referred to as HK44) obtained from the University of Tennessee Center for Environmental Technology (University of Tennessee, Knoxville, TN). The strain harbor had been genetically modified previously by transposon insertion of the salicylate-inducible *luxCDABE* gene cassette and a tetracycline resistance marker, thereby enabling it to produce luciferase when salicylate is present, which in turn causes the cells to luminesce (King et al., 1990). HK44 was grown for 18 h on an orbital shaker (Heidolph Unimax 2010, Germany) at 150 rpm and 25 °C in an oxygen-saturated, nitrate-free growth medium ($pH = 7.20 \pm 0.05$) consisting of $MgSO_4$, 0.4 g/L; $CaCl_2 \cdot 2H_2O$, 0.1 g/L; NH_4Cl, 0.4 g/L, NaCl, 8 g/L; KCl, 0.2 g/L; NaH_2PO_4, 1.15 g/L; K_2HPO_4, 0.26 g/L; HCl, 0.00366 g/L; $FeSO_4 \cdot 7H_2O$, 0.021 g/L; H_3BO_3, 0.0003 g/L; $MnCl_2 \cdot 4H_2O$, 0.001 g/L; $CoCl_2 \cdot 6H_2O$, 0.0019 g/L; $NiCl_2 \cdot 6H_2O$, 0.00024 g/L; $CuCl_2 \cdot 2H_2O$, 0.00002 g/L; $Na_2EDTA \cdot 2H_2O$, 0.01 g/L; $ZnSO_4 \cdot 7H_2O$, 0.00144 g/L; and $Na_2MoO_4 \cdot 2H_2O$, 0.00036 g/L. Glucose was added to a final concentration of 1.0 g/L as the main carbon source. After being examined for bacterial bioluminescence, actively growing cultures were concentrated by centrifugation (Beckman Coulter®, X-22R), suspended in a glycerol stock culture, and stored at −80 °C in 1 mL aliquots. All experiments were inoculated directly using cells from the frozen stock.

To induce luminescence, an induction medium was prepared by removing all phosphate sources from the growth medium, and adding 0.1 g/L (final concentration) of salicylate (Bozorg et al., 2012). Also, all media used in this study were supplemented with 30 mg/L tetracycline (EMD Chemicals, OmniPur® EM-8990) to ensure plasmid maintenance.

2.2. Porous media

In this study, translucent acid-washed glass beads with diameters ranging between 425 and 600 μm (Sigma-Aldrich, G8772) were used as porous medium. The porosity of the bead pack was determined gravimetrically using the mass of water within the pores (Bozorg et al., 2012). Prior to use, the glass beads were washed twice with distilled water to remove fines, and autoclaved at 121 °C for 20 min.

2.3. Dissolved oxygen measurement

An oxygen microelectrode (MI-730, Microelectrodes Inc.) was used to monitor oxygen concentration. In the flow chamber, an inline microelectrode was placed at the chamber outlet for real-time evaluation of oxygen concentration during biofilm growth and bacterial bioluminescence under flow conditions. The electrode was connected via an amplifier to an eDAQ Data Acquisition System (eDAQ PTY LTD, Australia) interfaced to a computer through a USB-port. Two separate media with 0% and 100% oxygen saturation, respectively, were used to calibrate the electrode.

2.4. Imaging

A 14-bit digital charged-coupled device (CCD) camera (Progres MFcool, Jenoptik, Germany) equipped with a Computar Megapixel lens with 35 mm focal length and f/1.4 focal ratio was used to capture grayscale bioluminescence images. The CCD camera was positioned 40 cm above the media. All bioluminescence experiments were conducted with the equipment in a dark box to eliminate the effects of extraneous light. Throughout the experiments, all images were acquired with a 5 minute exposure time with a fully open aperture. The imaging process, and method to evaluate BI were previously validated and are described in detail in Bozorg et al. (2012).

2.5. Experimental procedures

To accomplish the goals of this project using imaging technology, it was necessary to correlate bacterial bioluminescence to bacterial cell density and biofilm saturation. As bioluminescence has been reported to be a function of oxygen concentration, we evaluated the impact of oxygen on detected bioluminescence in our samples under induction conditions and used this information to ensure that O_2 was not limiting our observations. We then determined the impact of the packing material on the observed bioluminescence from both planktonic bacteria as well as bacteria within biofilms, and subsequently used this knowledge to study how biofilms affect fluid flow in porous media. We finally used established correlations to nondestructively evaluate biofilm saturation under flow conditions. The methods used to carry out these studies are described here.

2.5.1. Effect of oxygen on bacterial bioluminescence

To evaluate the effect of oxygen concentration on the bioluminescence intensity of HK44, liquid batch cultures of these cells were grown on a rotary shaker at 150 rpm and room temperature. After 18 h, bacteria were harvested by centrifugation at 5000 relative centrifugal force (rcf) for 20 min and washed twice with phosphate buffered saline (PBS). Known quantities of the harvested cells were then redistributed in batch cultures containing induction medium, and subsequently, the oxygen concentration and bioluminescence intensity of induced cells were monitored. All cell densities were evaluated by measuring light absorbance at 550 nm in a spectrophotometer (DU 730, UV/Vis Spectrophotometer, Beckman Coulter®) which had been calibrated