Advances in Water Resources 117 (2018) 1–13

Contents lists available at ScienceDirect

Advances in Water Resources

journal homepage: www.elsevier.com/locate/advwatres

Investigating the influence of flow rate on biofilm growth in three dimensions using microimaging

Sassan Ostvar[a], Gabriel Iltis[a], Yohan Davit[b], Steffen Schlüter[c], Linnéa Andersson[a], Brian D. Wood[a], Dorthe Wildenschild[a,*]

[a] School of Chemical, Biological and Environmental Engineering, Oregon State University, Corvallis, Oregon, USA
[b] Institut de Mécanique des Fluides de Toulouse (IMFT), Université de Toulouse, CNRS, INPT, UPS, Toulouse, France
[c] Department of Soil Physics, Helmholtz-Centre for Environmental Research - UFZ, Halle, Germany

ARTICLE INFO

Keywords:
Biofilms
Granular porous media
X-ray computed microtomography
Image processing
Statistical learning
Fluid phase topology

ABSTRACT

We explore how X-ray computed microtomography can be used to generate highly-resolved 3D biofilm datasets on length scales that span multiple pore bodies. The data is integrated into a study of the effects of flow rate on three-dimensional growth of biofilm in porous media. Three flow rates were investigated in model packed-bed columns. Biofilm growth was monitored during an 11-day growth period using a combination of differential pressure and effluent dissolved oxygen measurements. At the end of the growth period, all columns were scanned using X-ray computed microtomography and a barium sulfate-based contrast agent. The resulting images were prepared for quantitative analysis using a novel image processing workflow that was tailored to this specific system. The reduction in permeability due to biofilm growth was studied using both transducer-based pressure drop measurements and image-based calculations using the Kozeny–Carman model. In addition, a set of structural measures related to the spatial distribution of biofilms were computed and analyzed for the different flow rates. We generally observed 1 to 2 orders of magnitude decrease in permeability as a result of bioclogging for all columns (i.e, across flow rates). The greatest average permeability and porosity reduction was observed for the intermediate flow rate (4.5 ml/h). A combination of results from different measurements all suggest that biofilm growth was oxygen limited at the lowest flow rate, and affected by shear stresses at the highest flow rate. We hypothesize that the interplay between these two factors drives the spatial distribution and quantity of biofilm growth in the class of porous media studied here. Our approach opens the way to more systematic studies of the structure-function relationships involved in biofilm growth in porous media and the impact that such growth may have on physical properties such as hydraulic conductivity.

1. Introduction

The interaction of hydrodynamics and biofilm growth in media with tortuous geometries is of great scientific interest because of its prevalence in many natural and engineered systems (Drescher et al., 2013). As examples, biofilm growth in porous media has been both directly observed and indirectly hypothesized (with substantial supporting evidence) in a wide variety of natural and engineered systems. These include examples such as anaerobic reactors (e.g. Young and Dahab, 1983), microbially-enhanced oil recovery (e.g. Armstrong and Wildenschild, 2012; Sen, 2008), micromodel experiments (e.g. Kim and Fogler, 2000; Stewart and Fogler, 2001), laboratory porous media experiments (e.g. Vogt et al., 2013), and slow sand filter beds (e.g. Li et al., 2013). Although there are ongoing discussions regarding the ubiquity of biofilms (Baveye and Darnault, 2017; Coyte et al., 2017), these studies offer significant, and often direct, evidence that biofilm formation in porous media is an important component for many processes of relevance and interest.

In natural and synthetic porous media, biofilm growth at the pore scale affects various transport processes by altering the structure of interfaces, connectivity of the pore space, and bulk geometric properties of the medium (Baveye et al., 1998; Cunningham et al., 1991; Drescher et al., 2013; Rittmann, 1993). In turn, cellular growth and mesoscale structural evolution of biofilms are highly interconnected with various aspects of transport including shear and mass transfer. Non-destructive imaging of biofilms is essential to understanding the physics of these processes and their broader impacts on design, control, and prediction.

In disciplines that are concerned with natural porous media (e.g. hydrology, petroleum engineering), the ability to visualize biofilm growth under flow on the scale of millimeters to meters has been of great interest

* Corresponding author.
E-mail address: dorthe@engr.orst.edu (D. Wildenschild).

https://doi.org/10.1016/j.advwatres.2018.03.018
Received 24 February 2017; Received in revised form 21 March 2018; Accepted 27 March 2018
Available online 26 April 2018

Nomenclature

φ	porosity
ρ	fluid density
$v_s \equiv v_z$	(stream-wise) superficial (Darcy) velocity
D	pore radius ($\equiv$ characteristic length scale)
μ	fluid viscosity
Re	Reynolds number
Q	volumetric flow rate
A	cross-sectional area
κ_{zz}	stream-wise component of the permeability tensor
p	hydrodynamic pressure
g	acceleration due to gravity
K	column hydraulic conductivity
Φ	Hubbert's potential (hydraulic head)
L	column length
ΔP	net pressure difference across column
τ	tortuosity
S	interfacial area to volume ratio
β	constant in the Kozeny–Carman model
I	total number of evaluated images
N	total number of voxels evaluated at coordinate $\mathbf{x}$
Π	set of all pairs of neighboring voxels
$c(\mathbf{x})$	class label of voxel at point $\mathbf{x}$
$\mu_{c,\lambda}$	class mean in image λ
σ^2	class variance in image λ
$g_i(\mathbf{x})$	gray value of voxel $\mathbf{x}$ in image i
χ	Euler characteristic
$\mathcal{N}$	number of isolated objects in a material of interest
$\mathcal{L}$	number of redundant connections within all material clusters
$\mathcal{O}$	number of cavities in a material of interest
d_α	average pore diameter in phase α
e_α	average distance within phase α
CI	the connectivity index
PSI	the pore size index
SDI	the solid distance index

(e.g. Thullner, 2010; Yarwood et al., 2002). Visualization is gaining even more significance with the increasing complexity and fidelity of the mathematical and computational approaches to modeling biofilm growth in porous media (e.g. von der Schulenburg et al., 2009). The challenges associated with non-intrusive visualization of 3D systems often forces direct comparison between models and experiments to rely on bulk (aggregate) laboratory or field measurements; i.e. evaluations on scales where pore-scale (structural) information detrimental to transport is lost to the averaging inherent to measurements (Thullner, 2010).

Magnetic Resonance Microscopy (MRM) and Nuclear Magnetic Resonance imaging (NMR) have been successfully applied to elucidate the global structure of biofilms in porous media and pore fluid velocities in media altered by microbial growth (Manz et al., 2003; Seymour et al., 2004; 2007), albeit with some resolution limitations. Confocal laser scanning microscopy (CLSM), and more recently optical coherence tomography (OCT), have been successfully applied to the visualization of the 3D structure of biofilms on smaller scales with higher resolutions (Davit et al., 2013; Dreszer et al., 2014; Neu and Lawrence, 2015; Wagner et al., 2010; Xi et al., 2006). Both methods enable temporal studies of growth in flow environments, and provide highly resolved reproductions of the internal structure of the biofilm matrix. A popular approach to direct visualization of biofilm structure in porous media at the pore scale has been to adopt these techniques to study growth in optically transparent (Leis et al., 2005), 2D and pseudo-3D micro-models (e.g. Beyenal et al., 2004; Kim and Fogler, 2000; Rodríguez and Bishop, 2007; Stoodley et al., 1999).

A limitation in extending this methodology to study biomass formation in 3D porous media is that experimental systems must be optically transparent, small enough to fit onto the microscope stage, and in the case of CLSM, thin enough to fit within the focal range of the device. Conversely, 3D porous systems are opaque and deep. Whether or not the conclusions drawn for 2D systems can be extended to 3D is an unanswered question. Topologically, the two are not equivalent (e.g., diffusions in 2- and 3-dimensions are fundamentally different), so one would not expect 2-dimensional experiments to capture the range of physical behaviors. Thus, the literature's prevailing answer to this question seems to be negative (e.g. see the discussion by Baveye, 2010; Thullner, 2010).

X-ray computed microtomography (CMT) is emerging as an alternative that enables visualization of biofilms in 3D in opaque media (Davit et al., 2011; Iltis et al., 2011; Wildenschild and Sheppard, 2013) on the pore scale. Though still in its infancy, the method was originally developed and explored using both polychromatic (Davit et al., 2011) and monochromatic synchrotron-based systems (Iltis, 2013; Iltis et al., 2011). Users can expect voxel resolutions on the order of 1–2 microns to be easily achieved. The technique relies on the use of contrast agents to facilitate the adsorption-based detection of different phases within an opaque sample using X-rays. Central to the method is creating a physical mechanism that, through the use of advanced image processing techniques, allow for differentiation between the fluid and biofilm phases, the natural attenuation (photon cross-section) of which are almost identical.

Use of different contrast agents, added to the aqueous phase, such as silver-coated hollow microspheres (Iltis et al., 2011), barium sulfate suspensions (Davit et al., 2011; Iltis, 2013), and 1-chloronaphtalene (Ivankovic et al., 2017; du Roscoat et al., 2014) have shown promise. Similarly, adding $FeSO_4$ as a contrast agent to the biofilm phase, and using the free space propagation of X-rays to bring out additional refractive effects, Carrel et al. (2017) were also able to image biofilms in opaque porous media. The latter study compared the $FeSO_4$ to the $BaSO_4$ method and found differences in the amount of biofilm imaged, with a significantly larger amount of biofilm identified using the $FeSO_4$ method. The authors pointed out three possible reasons for the discrepancy; (i) partial volume effects (eliminated from consideration); (ii) uncertainty related to the segmentation (caused by the significant heterogeneity of the biofilm phase and poor contrast of said phase); and (iii) interaction between the $BaSO_4$ and the biofilm (causing suspended biomass and loosely attached components of the biofilm to be washed out of the columns).

The potential for detachment of biofilm during addition of a denser and more viscous (aqueous phase) contrast agent has been discussed by Davit et al. (2011) and du Roscoat et al. (2014). In a thorough study of the problem, Ivankovic et al. (2017) found that this effect can be eliminated by using smaller beads (in their case, less than 2 mm) and by making measurements after longer periods of biofilm growth (>7 days) such that a more compact and tightly attached biofilm was formed. This allowed for reliable studies on biofilms that presented similar and reproducible spatial structure and allowed for quantitative evaluation of a number of different environmental variables. In their study, growth periods of less than 3 days, and larger pore sizes, resulted in less resilient biofilms (exhibiting streamers and other weakly attached components) that may be subject to detachment shear by a more viscous contrast agent (such as $BaSO_4$ or 1-chloronaphtalene). For the 1-chloronaphtalene (CN) an additional challenge was caused by the oily nature of the contrast agent, which caused pendular rings of CN to be left behind at bead contacts.

The exact growth period and pore size that will produce repeatable experiments will vary somewhat with organism, nutrient supply, flow rate (all factors contributing to the production of more or less dense biofilms), and the viscosity of the injected contrast agent (if relevant). The past studies using *aqueous* phase contrast agents serve as promising proof of the concept as long as the risk of viscous interaction with the biofilm is considered in the design of the experiments. While there