A novel platform for monitoring and imaging bacterial biofilm growth in complex structures

C. Papadopoulos[1*], L. Malaquin[2], J. Foncy[2], Y. Davit[1]

[1] Institut de Mécanique des Fluides de Toulouse (IMFT), Université de Toulouse, CNRS, 31400 Toulouse, France
[2] LAAS-CNRS, Université de Toulouse, CNRS, 31400 Toulouse, France
* Corresponding author: christos.papadopoulos@imft.fr

Biofilms are complex microbial communities that grow primarily on solid surfaces where the microorganisms are nested in a self-secreted polymer matrix. Recent experimental studies have aimed at understanding the mechanisms that control the development of biofilms in porous media, including architectural plasticity [1], ecological interactions in connected structures [2] or bio-clogging [3]. These mechanisms cannot be easily studied due to the structural complexity and the opacity of the 3D porous structure but also due to the multiplicity of coupled factors that control biofilm, such as nutrient/oxygen availability, flow rate, communications and shear stress [4]. Improved quantification of the physical aspects of biofilm growth is essential in unravelling the mechanisms of biofilm formation in porous materials and progress towards new biotechnologies.

In order to get an insight into how fluid flow, transport phenomena and biofilms interact within heterogeneous structures, we have devised a versatile, micro-scale, 3D-printed micro-bioreactor for the precise measurement of several parameters of such systems that would allow for controlled and reproducible studies of biofilms in 3D porous systems. We have also developed a novel approach to 3D imaging of biofilms in such systems using X-ray micro-tomography using functionalized gold nanoparticles as a contrast agent.

Preliminary results of P. aeruginosa biofilm development at room temperature show a permanent regime after about 3 days with persistent temporal fluctuations in the pressure drop and oxygen consumption. This suggests that biofilm growth in porous media under flow is a dynamic process resulting from an equilibrium between competing mechanisms such as bacterial growth and biofilm detachment due to hydrodynamic stresses. This is confirmed by direct optical absorption measurements of the biofilm detachment events at the outlet of the bioreactor. Complementary to the biofilm development measurements, the x-ray tomography scans showed that the spatial distribution of biofilm within the porous medium is heterogeneous, with most of the biomass being concentrated at the inlet of the bioreactor and regions within the porous medium that are completely inaccessible to the main flow due to pore clogging.

Our experimental setup, with the 3D-printed micro-bioreactor being in the heart of it, provides an adjustable, versatile experimental workbench for the study of the growth and detachment dynamics of biofilms in porous media under controlled conditions over long periods of time. The combination with X-ray tomography imaging provided an insight on how physical (flowrate, shear stress) and chemical (oxygen, nutrient availability) parameters of the system affect the biofilm's spatial distribution. A better understanding of biofilm growth dynamics in the mesoscale could potentially unlock novel biotechnologies [5,6].

(1) Coyte, K. Z.; Tabuteau, H.; Gaffney, E. A.; Foster, K. R.; Durham, W. M. Microbial Competition in Porous Environments Can Select against Rapid Biofilm Growth. *Proc. Natl. Acad. Sci.* **2017**, *114* (2), E161–E170. https://doi.org/10.1073/pnas.1525228113.

(2) Scheidweiler, D.; Peter, H.; Pramateftaki, P.; Anna, P. de; Battin, T. J. Unraveling the Biophysical Underpinnings to the Success of Multispecies Biofilms in Porous Environments. *ISME J.* **2019**, *13* (7), 1700–1710. https://doi.org/10.1038/s41396-019-0381-4.

(3) Zhang, Y.; Xu, A.; Lv, X.; Wang, Q.; Feng, C.; Lin, J. Non-Invasive Measurement, Mathematical Simulation and In Situ Detection of Biofilm Evolution in Porous Media: A Review. *Appl. Sci.* **2021**, *11* (4), 1391. https://doi.org/10.3390/app11041391.

(4) Rittmann, B. E. The Significance of Biofilms in Porous Media. *Water Resour. Res.* **1993**, *29* (7), 2195–2202. https://doi.org/10.1029/93WR00611.

(5) Dhami, N.; Reddy, S. M.; Mukherjee, A. Biofilm and Microbial Applications in Biomineralized Concrete. **2012**. https://doi.org/10.5772/31124.

(6) Jiang, Y.; Liu, Y.; Zhang, X.; Gao, H.; Mou, L.; Wu, M.; Zhang, W.; Xin, F.; Jiang, M. Biofilm Application in the Microbial Biochemicals Production Process. *Biotechnol. Adv.* **2021**, *48*, 107724. https://doi.org/10.1016/j.biotechadv.2021.107724.