Novel experimental methods for the identification of anoxic micro-niches in porous media

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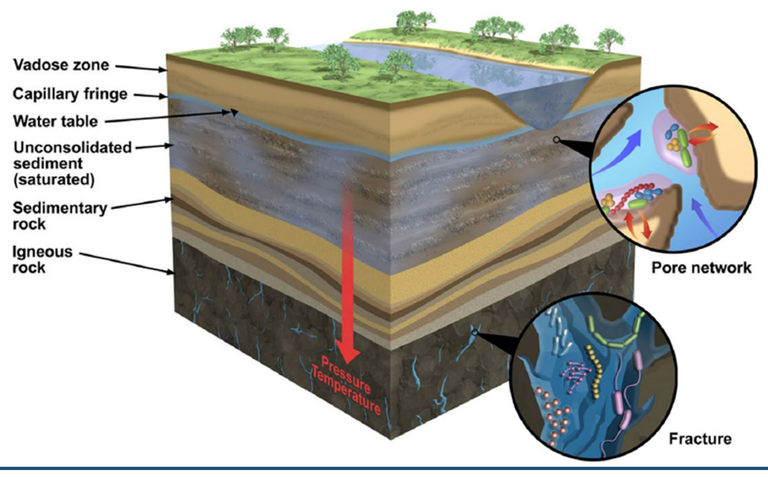
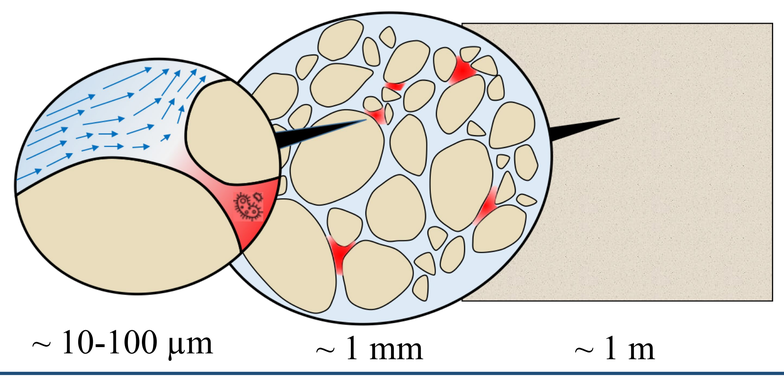
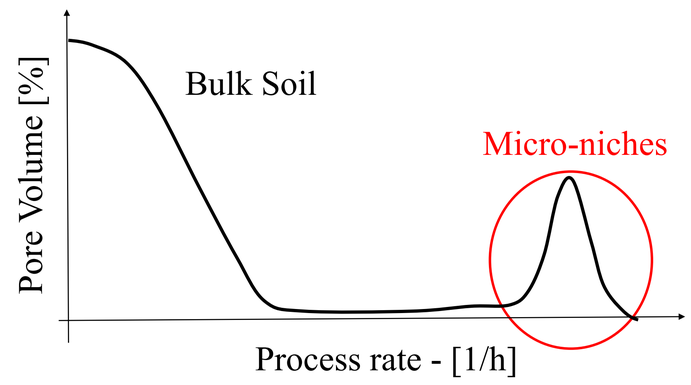
Introduction: Subsurface is an heterogeneous environment where soil, fluids, rocks and living organisms interact within it.



Multi-scale coupling of bacterial activity and water flow[1] may lead to the formation of persistent anoxic micro-niches in averagely well-oxygenated media.



Here, some communities might find a favorable habitat which boosts their activity[2] and thus leading to detectable macroscopic effects[3].



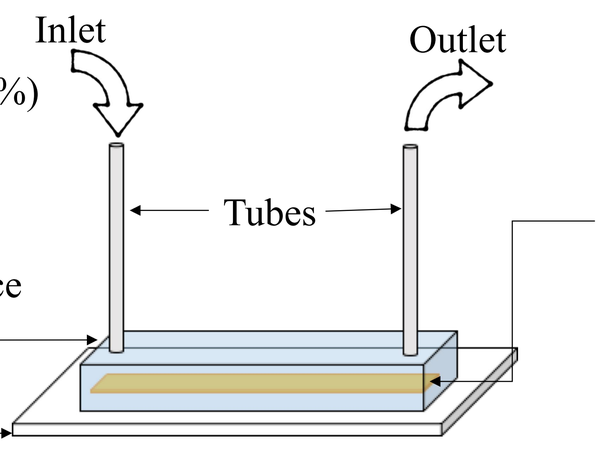
Objective: Developing an experimental method combining microfluidics devices and transparent optical sensors for real-time observing and understanding anoxic micro-niches formation and dynamics across different spatial scales (for micron to meter scale).

Experimental Setup:

Sterile well-oxygenated water + LB (20%)
$Q = 0.2\ \mu L\ min^{-1}$ for 72 hours

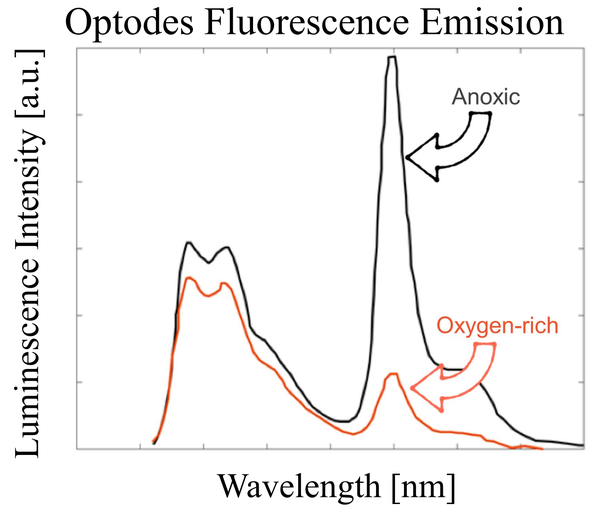
PDMS transparent microfluidics device contaminated by P. Putida GB1

Glass slide for microscopy

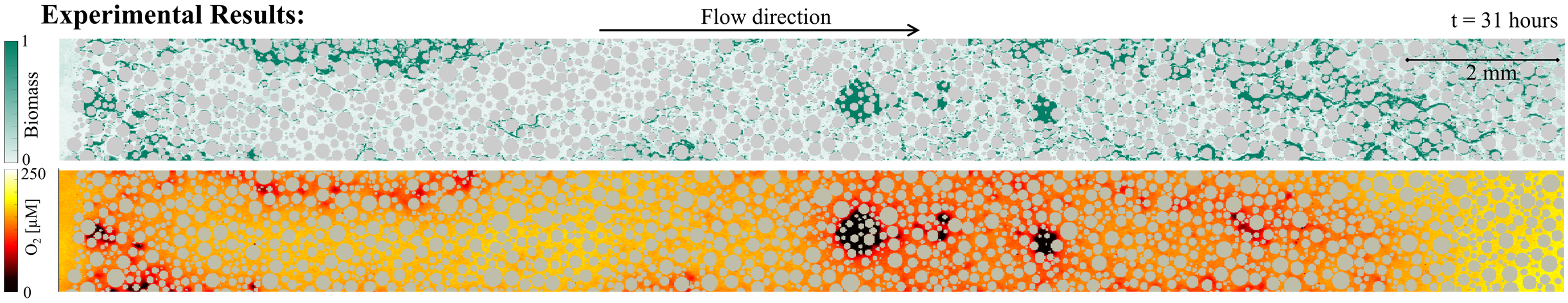


Transparent planar optodes[4] composed of two luminescent dyes embedded in polymer (polystyrene) that is spread on the glass slide with screen printing technique. The luminescence intensity of one of the dyes is quenched proportionally to the concentration of oxygen in the sample.

Optodes Fluorescence Emission

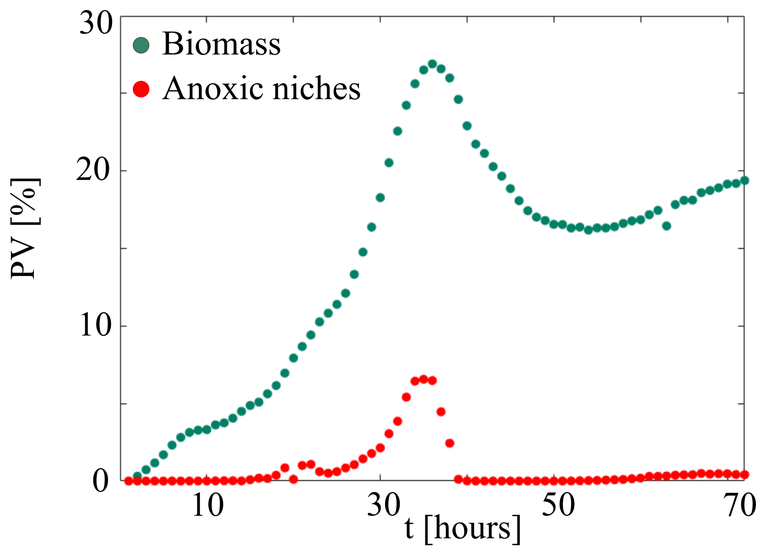


Experimental Results:

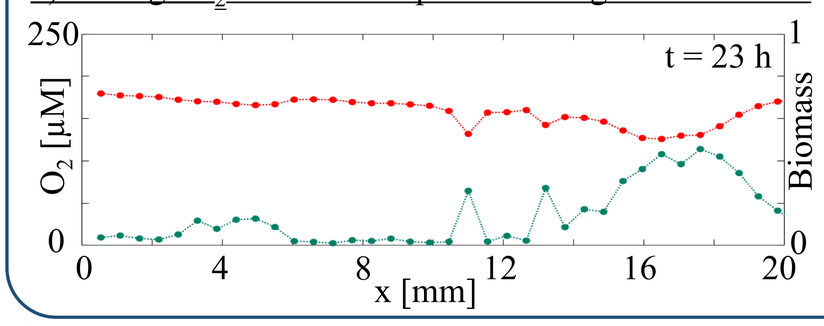


Quantitative Analyses:

a) Pore Volume (PV) occupied by niches and biomass



b) Average O_2 and biomass profiles along flow direction



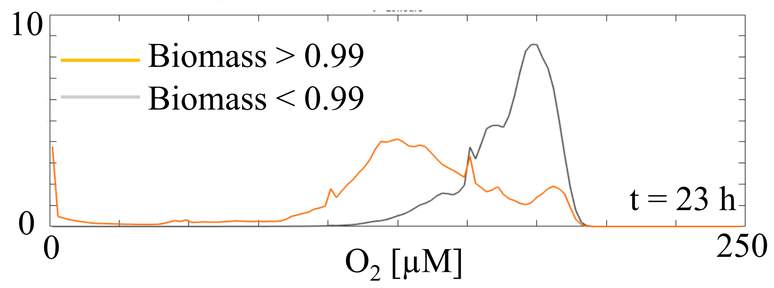
a) Biomass and oxygen temporal correlation:
The PV amount occupied by micro-niches evolves in time as a function of the biomass growth..

b) Biomass and oxygen spatial correlation:
Profiles of O_2 and biomass computed along flow direction show lower oxygen for colonized sections of the medium.

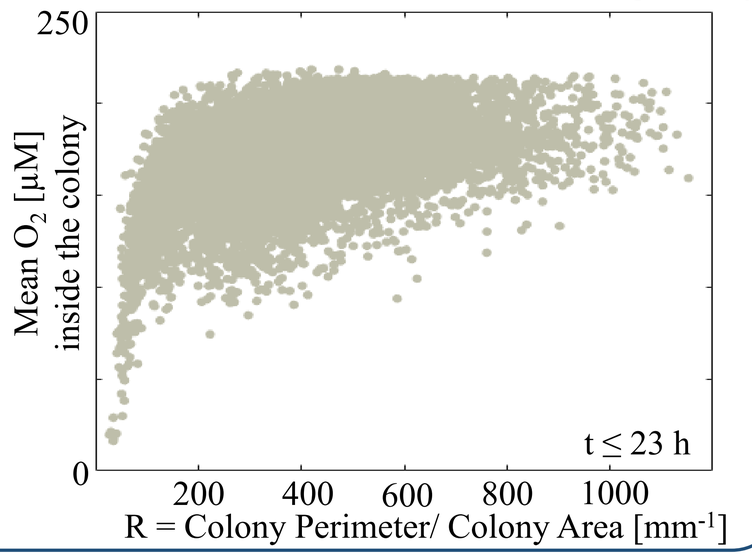
c) Denser colonies form anoxic micro-niches:
O_2 *pdf* computed for densely populated locations indicates that bacterial population density is an important factor controlling anoxic micro-niches formation.

d) Micro-niches formation links to colonies shape:
In addition to density, the geometry of the colony is clearly correlated to the formation of niches. More rounded colonies (low R ratio values) are associated with lower oxygen values within the colony than more elongated or indented colonies (high R).

c) *Pdfs* of O_2 conditional to biomass concentration



d) Anoxic micro-niches formation and colonies geometry



1 Or et al. (2007). DOI: 0.1016/j.advwatres.2006.05.025
2 Kuzyakov et al. (2015). DOI: 10.1016/j.soilbio.2015.01.025
3 Kravchenko et al. (2017). DOI: 10.1038/NGEO2963
4 Borisov (2018) 10.1039/9781788013451-00001