



Magnetic resonance imaging of structure, diffusivity and copper immobilization in a phototrophic biofilm

V. Phoenix (1) and **W. Holmes** (2)

(1) Department of Geographical and Earth Sciences, University of Glasgow, UK.

(2) 7T MR Facility, Wellcome Surgical Institute, University of Glasgow, UK.

(Vernon.Phoenix@ges.gla.ac.uk)

The biofilm is an exceptionally common mode of life for bacteria. Significantly, the kinetics of biogeochemical processes within these complex three dimensional communities can be, unlike in planktonic systems, limited by mass transport rates throughout the biofilm matrix.

To improve our understanding of metal immobilization by biofilms, methods are needed which quantify both the mass transport and the immobilization of a metal, and moreover, correlate these factors with biofilm structure. Significantly, MRI has the potential to do this. MRI is a suitable technique for biofilm research as it is a non-invasive approach which allows analysis *in-vivo*, *in-situ* and in 3 dimensions. In this study, we utilize magnetic resonance imaging to shed light on biofilm heavy metal immobilization by quantifying biofilm structure, diffusivity and copper transport and fate in 3 dimensions and in real time.

Notably, MRI was able to resolve considerable structural heterogeneity, ranging from classic laminations $\sim 500 \mu\text{m}$ thick, to structures with no apparent ordering. Pulsed field gradient analysis (PFG) spatially resolved water diffusion coefficients which exhibited relatively little or no attenuation (diffusion coefficients ranged from 1.7 to $2.2 \times 10^{-9} \text{ m}^2\text{s}^{-1}$). The biofilm was then reacted with a $10 \text{ mg l}^{-1} \text{ Cu}^{2+}$ solution and T_2 parameter maps were used to spatially and temporally map copper immobilization within the biofilm. Significantly, a calibration protocol similar to that used in biomed-

ical research successfully quantified copper concentrations throughout the biofilm. Variations in Cu concentrations were controlled by biofilm structure; zones of high and low copper immobilization reflected the laminated structure. Copper immobilization was most rapid ($\sim 5 \text{ mg Cu l}^{-1} \text{ hr}^{-1}$) over the first 20-30 hours followed by much lower immobilization rates for the remaining 60 hours of the experiment. Furthermore, immobilization rates were, in general, slower deeper within the biofilm. The rates of metal immobilization and the change in these rates with depth are controlled by both diffusion and adsorption processes, which mediate the copper supply rate throughout the biofilm. Properties of the biofilm (diffusion coefficients and metal adsorption capacities) were input into a Bartlett and Gardner model to further examine combined diffusion-adsorption through a hypothetical biofilm. Results of the model are comparable with the experimental data, with adsorption constants (K) around 2×10^4 comparing favourably. Higher adsorption constants resulted in longer lag times until the onset of immobilization at depth, but higher actual adsorption rates. MRI and reaction-transport models are versatile tools which can significantly improve understanding of heavy metal immobilization in naturally occurring biofilms.