Imaging nitrate-dependent Fe(II) oxidation by a newly isolated As(III)-oxidising bacterium

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Arsenic is a naturally occurring toxic element affecting the health of more than 150 million people worldwide due to its presence in soil and groundwater [1]. Some bacteria couple the reduction of nitrate to the oxidation of As(III) and Fe(II) and may therefore promote the immobilisation of As via the formation of As(V)/Fe(III) mineral assemblages [2]. The mechanism of Fe(II) oxidation remains unclear; both direct enzymatic and indirect (nitrite-mediated) mechanisms have been proposed. In this work we used NanoSIMS to study the active fraction of As(III)-oxidiser bacteria ($^{13}C/^{12}C > 1.11$ %) with complimentary SEM, XRD, S/TEM and EDS analyses. *Acidovorax* sp. strain ST3 [3] was cultured in anaerobic medium with $NaNO_3$, Fe(II), As(III) and ^{13}C-labelled acetate. SEM-NanoSIMS samples were prepared on silicon wafers to collect biofilm cells and Fe/As biominerals and analysed in a CAMECA NanoSIMS 50L with a 16 KeV Cs^+ primary ion beam. A FEI Titan G2 80-200 S/TEM ChemiSTEM was used at 200 kV to analyse planktonic cells and any biominerals formed.

Biominerals with different morphologies were observed extracellularly and on the cell surfaces. The cells showed variable Fe encrustation/biomineralisation on or near the cell membrane as observed in TEM, and this encrustation was more pronounced at the cell poles. NanoSIMS revealed a heterogeneous accumulation of ^{13}C labelling in cells and a threshold beyond which biomineralised cells no longer accumulate ^{13}C was proposed. Fe biominerals were also observed adjoining inactive cells, consistent with indirect mechanisms, rather than direct enzymatic Fe(II) oxidation during Fe(III) biomineral formation. NanoSIMS analysis suggested that As did not co-localise strongly with Fe in the biominerals or biomineralised cells, although EDS in S/TEM revealed ratios as high as 3:1 Fe:As. Lepidocrocite [$Fe^{3+}O(OH)$] was identified as an Fe(II) biooxidation product by bulk XRD analyses, and TEM/EDS diffraction patterns are being used alongisde XPS to further characterise the end point mineralogy in this system.

[1] Sarkar, A. & Paul, B. (2016). *Chemosphere*, 158, pp.37-49. [2] Hedrich, S., et al. (2011) *Microbiology*, 157, 1551-64 [3] Zhang, J., et al. (2017) *Environ Sci Technol*, 51, 4377-4386.