PUBLISHABLE SUMMARY

Project objectives

The main goal of this project was to test if we can visualise microbes directly, inside rock pores, at nanometre scale, to see how they are trapped or mobilised, and watch their activity by applying advanced X-ray nanotomography.

The three main objectives were:
1) To develop X-ray tomography methods capable of imaging microbial cells and their by-products inside rock pores
2) To elucidate the petrophysical and geochemical parameters that control microbial trapping and hence transport inside rock matrices
3) To advance the understanding of how microbial mineralisation impacts rock porosity, reactivity and permeability, and thus rock stability and fluid flow pathways

These project objectives were addressed by 4 work packages: WP1 (Months 1-4): Experimental and computational tools for 4D visualisation of microbes in rock, WP2 (Months 5-13): Parameters governing trapping of microbes in rock pores, WP2 (Months 14-22): Microbial mineralisation in pores and its impact on rock properties and fluid flow pathways, WP4 (Months 22-24): Publication of remaining results and submission of new research proposals

Work performed since the beginning of the project

During these two years, I have deepened my knowledge and skills in X-ray tomography and have gained experience working with the data. Multiple discussions with experts in the Nanogeoscience group were key to finding the most suitable approach to process and interpret the data I collected during my synchrotron measurements on microbes in rock. In addition, I also have acquired new technical skills in AFM tip modification and measurements, TIRFM measurements, and several microbiological techniques. Indeed working for 4 months in labs dedicated to molecular biology and bio-inspired nanomaterials at Lawrence Berkley National Laboratory, USA, greatly widened my scientific horizon. In fact, having had to explain my research to scientists completely outside my field was at times challenging and it has taught me great new skills in communicating my research more effectively to a wider audience.

My research outputs in these 2 years can be summarised as:
- Visualisation of single bacterial cells inside the strong X-ray adsorbing matrix of geologic porous materials using synchrotron based 3D-holotomography.
- Extensive set of experiments to probe key parameters affecting microbial attachment to different minerals in batch experiments and under flow conditions.
- Probing the kinetics of microbial mineralisation and determining its effect on mineral type, size and morphology, and hence impact on fluid flow properties in porous matrices
- Furthermore, collaborative projects on biomineralisation, particularly on $CaCO_3$ biomineral growth. These resulted from my interactions with colleagues and students in meetings, seminars and during laboratory work at Nanogeoscience.

Main results achieved so far

Microbial cells visualised inside soil and rock pores using X-rays nanotomography

3D-holotomography of a sandstone and a packed silica beads column (300 – 550 um in diameter), amended with fixed *M. gryphiswaldenseis* cells, were performed at beamline ID16A-NI at ESRF (France). *M. gryphiswaldenseis*, a magnetotactic bacterium that produces nanoscale magnetites (Fe_3O_4) inside their cells (Figure left), was fixed in 2% glutaraldehyde and dehydrated using ethanol. Sample preparation and acquisition parameters were tested by placing the fixed bacteria on a silicon nitride membrane and then imaged. As shown in Figure 1A, the bacteria can be clearly seen in 2D. In 3D samples, the cells are more

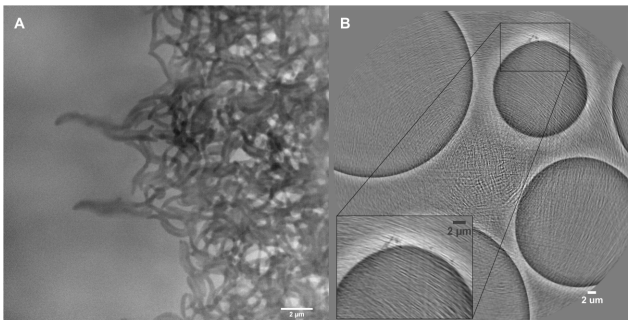

Figure 1: A) 2D tomographic image of M. gryphiswaldenseis, deposited on silicon nitride window (voxel size of 10 nm). B) Slice from 3D holotomography showing M. gryphiswaldenseis attached to pore surface in the beads column (inset shows higher magnification, voxel size of 40 nm).

difficult to spot. Figure 1B shows a 2D slice of the reconstructed 3D holotomogram obtained from the beads column. The cells are visible on top of the beads (magnified in the inset of Fig. 1B). The data quality could be improved using an in-house denoising code (Fig. 2), however the subtle differences in contrast between the bead and the bacteria, make segmentation challenging (work in progress). In sandstone samples, the pores were much smaller and the matrix more heterogeneous, resulting in much stronger X-ray absorption and thus little hope to identify the weakly absorbing cells (work in progress).

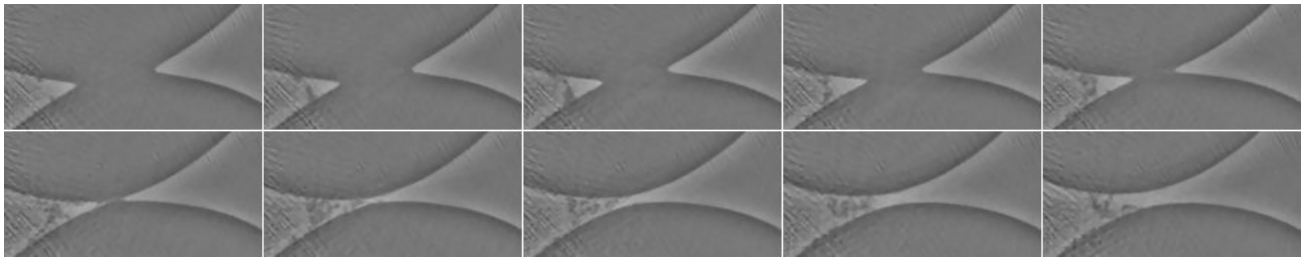

Figure 2: Sequence stacks of denoised 2D slices from 3D reconstruction of bead column amended with M. gryphiswaldenseis. A, B and C show consecutive slices at 3 different locations of the column indicating that the cells are preferably trapped in pore throats.

Attachment behaviour of *S. oneidensis* and *E. coli* to minerals under static and flow conditions

My work has shown that under static conditions *S. oneidensis* MR-1 and *E. coli* show substantially different attachment behaviour, with *E. coli* adsorbing considerably stronger to mineral surfaces, whether they were different iron oxides (ferrihydrate, goethite, hematite) or silicates (kaolinite, mica). The different behaviour can in parts be explained by the difference in microbial surface charge. This work is currently written up for publication.

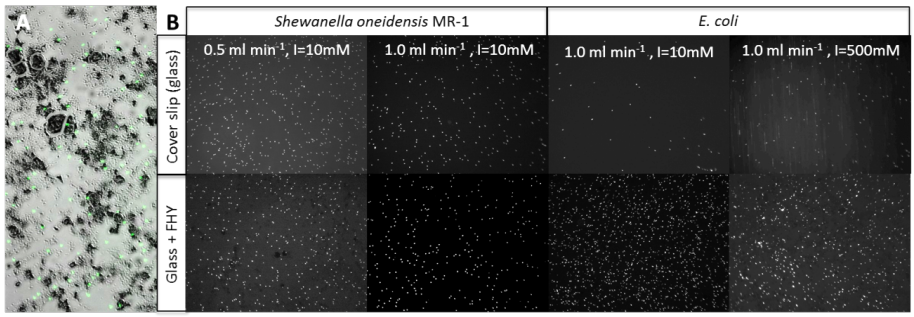

Figure 3: A) TIRFM of fluorescent *Shewanella oneidensis* MR-1 cells (green spots) on glass cover slip with deposited FHY (black particles). B) TIRF images showing how many *Shewanella oneidensis* MR-1 and *E.coli* cells (white spots) attached to the clean glass surface and the glass covered with FHY, at 0.5 and 1.0 ml/min pump rate (for *Shewanella*) and under varying ionic strength, I (for *E. coli*).

The attachment behaviour of these microbes was also investigated under flow, using a TIRF microscope and bacterial strains that express the green fluorescent protein. Experiments performed at varying flow rates and on surfaces with varying mineral coverage, showed that far fewer *E. coli* cells attached to a glass substrate compared to S. *oneidensis*. In contrast, when the glass was covered with ferrihydrate (FHY), *E. coli* attached far quicker than *Shewanella*. For *Shewanella*, little if any differences were observed in attachment behaviour, whether they were pumped at differing speed (0.5 vs 1.0 ml/min, but the same volume) or over different surfaces (glass vs FHY). Similarly, a change in ionic strength (I) from 10 to 500 mM did not affect attachment of *E. coli* cells as much.

Expected final results and their potential impact and use

There are two draft manuscripts of the work summarised above which will be submitted to high-impact, open-access journals. Several conference abstracts have resulted from this work, and I will present some of this work at conferences in 2018 (e.g., 33rd Nordic Geological Winter Meeting). Overall, this research has shown that nanotomography is a powerful technique that allows imaging of single microbial cells inside a simple bead column, however this seems almost impossible inside rock, where the porosity is lower and the mineral heterogeneity higher. Therefore, to get a mechanistic understanding on how microbes attach to rock pore surfaces and influence processes inside pores, other techniques such as magnetic resonance imaging may be more suitable. My work has further shown that combining techniques such as AFM and TIRFM with simple batch experiments provides key information on the mechanisms controlling microbial attachment to minerals. This understanding is important, as microbes are omnipresent in our environment, influencing all processes in our biospheres. Equally, microbes are excellent agents for pollutant remediation and clean up, thus knowing how they attach to surfaces is key for successful implementation.

Project website and publications: https://nanogeoscience.dk/projects/miro/