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Sub-Micron Scale Chemical and Mineralogical Analyses on Microbially Induced Calcium Carbonate Precipitates

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Microbially induced calcium carbonate precipitation, or MICP, is a process by which bacterial metabolic processes lead to the formation of calcium carbonate ($CaCO_3$). Metabolic processes that can raise the pH and alkalinity of the surrounding media can facilitate MICP in the presence of calcium ions. Hydrolysis of urea (ureolysis) is one such metabolic process. MICP has numerous environmental and engineering applications, especially in porous media [1]. Precipitates formed by MICP can be used as biological cement to alter porous media properties such as permeability, allowing for strengthening of subsurface media and sealing of leakage pathways of geologically stored carbon dioxide. MICP is also being studied as a biotechnology to reduce the spread of contaminants by trapping them in the precipitates.

Although its applications are macro-scale, MICP is heavily impacted by the micro-scale relationships between the bacteria and the formed minerals. For example, organics produced by bacteria can influence crystallinity and polymorphism in the precipitates [2] which in turn has implications in mineral stability. To study polymorph selection and the role of organics therein, it is necessary to visualize this process and generate samples for mineralogical and organics analyses at an appropriately micro-scale.

Our previous work that utilized a droplet-based microfluidics platform for MICP enables us to produce single micro-precipitates inside droplets [3]. After collecting and drying the micro-precipitates, we image and analyze them using a suite of correlative microscopy and microanalysis techniques. In our current work, we employed the droplet platform to study MICP by two distinct bacterial strains. Protocols were developed to ensure that the resulting MICP occurred within the droplets despite the bacterial capacity for rapid ureolysis. Single micro-precipitates successfully formed in the droplets and were then extracted and dried. Scanning Electron Microscopy coupled with Focused Ion Beam milling was employed to study the morphology of the precipitates and to test the hypothesis that the bacterial strain, specifically organic secretions by bacteria, controls precipitate morphology.

For more in-depth characterization, diffraction analyses were performed on single micro-precipitates. The major challenge for diffraction analyses on samples was to optimize their thickness for transmission-based measurements. The small size (<5 μm diameter) and rounded nature of the precipitates presented a challenge for FIB-milling. Once lamellae were obtained with our optimized approach, regions of interest were further thinned, making them amenable to transmission-based methods. Samples prepared in this way were analyzed for biogenic signatures by Near Edge X-ray Absorbance Fine Structure spectroscopy using Scanning Transmission Electron Microscopy.

Our current work includes characterizing the distribution of $CaCO_3$ polymorphs within a single micro-precipitate based on our earlier finding of potential co-presence of two polymorphs.[3] To determine whether the precipitates contained calcite or vaterite, Selected Area Electron Diffraction (SAED) was performed using a Themis Scanning Transmission Electron Microscope. Our studies apply a correlative microscopic and microanalytical approach with a unique micro-scale system to study biologically

precipitated minerals. The resulting chemical and mineralogical findings contribute to understanding the influence of organics on calcium carbonate (bio)mineral formation [4].

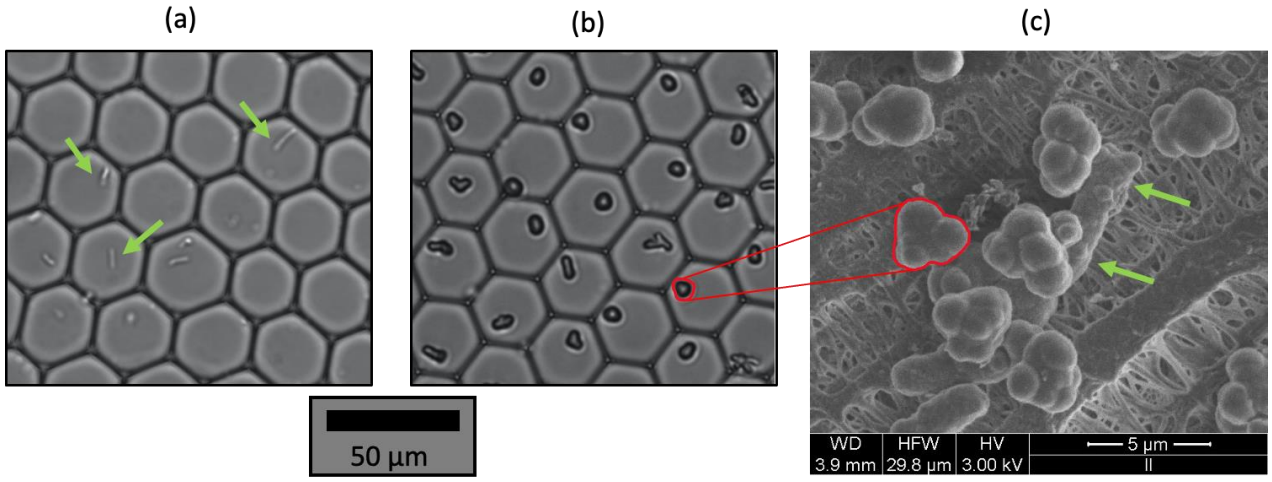

Figure 1. (a) Droplets at time of formation. Green arrows point to single bacterial cells trapped within droplets. (b) Droplets at day 7 of incubation with developed micro-precipitates via ureolysis-driven MICP. Red lines show an individual micro-precipitate in a droplet in (b) and a representative magnified micro-precipitate in the SEM image in panel (c). The green arrows in (c) show a bacterial cell-like object encrusted in precipitate.

References:

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