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Influence of Extracellular Polymeric Substances on Biogenic $CaCO_3$ Polymorph Selection under Different Substrate Conditions

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The formation of carbonate minerals in nature, such as beachrocks and coral reefs has attracted significant attention due to its emerging potential in engineered applications[1]. Microbially induced calcium carbonate precipitation (MICP) process has been mimicked in laboratory conditions using various microbial metabolic pathways[2]. However, despite extensive research, the factors influencing the polymorph selection in MICP remain unclear due to the complex biogeochemistry and interactions with the underlying substrate. We investigate the role of bacterial metabolic activities and EPS in carbonate precipitation over two different substrates (calcite and apatite) at a molecular level in this study. The analytical techniques used in the study included Scanning electron microscopy (SEM), Energy dispersive spectroscopy (EDS), Raman spectroscopy, Fourier transform infrared spectroscopy (FTIR) and Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS). Two different bacterial cultures (*Sporosarcina pasteurii (SP)* and *Bacillus subtilis (BS)*) with varying ureolytic activities, EPS potential and carbonate precipitation abilities were used. The EPS macromolecular composition along with active functional groups were quantified and precipitated calcium carbonate polymorphs were analysed. Experimental findings revealed that the highly ureolytic SP generates distinct regular rhombohedral crystals of sizes restricted to 40 microns, regardless of the substrate mineralogy. The precipitated crystals with SP are illustrated in Fig. 1 (a). High EPS producing culture BS precipitated much larger crystals upto 100 microns size as shown in Fig. 1 (b). The large crystals were found to be coupled closely with the microbially generated EPS for all the substrates. Microbial activity proved to be the dominant factor in polymorph selection over substrate. Raman results confirm the precipitation of calcite with low EPS producing microbe SP and precipitation of vaterite with high EPS producing microbe BS. With ToF-SIMS, we report that microbial influence dominates the polymorph selection over the influence of substrate mineralogy, and EPS plays a critical role in stabilising vaterite crystals. Further studies are being done to understand the reactions at nano to molecular scales to unpin the fundamental questions on the intertwined bio-physico-chemical factors driving the biomineralisation process.

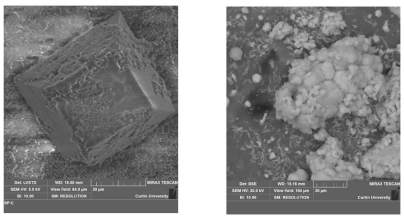

Fig. 1 (a) Rhombohedral calcite with SP;
(b) Coupling of vaterite precipitates in EPS with BS.

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