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The Role of Functionalized Organic Surfaces in Metal Biomineralization: Insights from Liquid-Cell STEM Experiments

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Assessing the impact exerted by bacteria on metals cycling in the environment is fundamental for questions related to chemical weathering at the Earth's surface, and for those related to pollution. Particularly, understanding how bacteria can mineralize metals such as manganese, by forming Mn oxides that are among the most powerful oxidants on Earth, is essential.

Recent direct observations of mineral growth on bacteria under liquid conditions highlight the critical role of chemical functions carried by cell surfaces and exopolymers during metal biomineralization [1]. In this first study, the capabilities of liquid-cell scanning transmission electron microscopy (LC-STEM) were developed by using the incident electron beam in interaction with water. Consequently, this radiolysis results in the production of reactive oxygen species, which in turn trigger the precipitation of Mn-bearing minerals. When tested on two distinct *Escherichia coli* strains, differences in the morphology and distribution of Mn precipitates were observed. Specific nucleation site densities, as well as Mn-accessibility to bacteria cell surfaces and their associated exopolymers, were proposed to explain these differences.

In order to better define the role of the various chemical groups present on microbial surfaces, we used here functionalized polystyrene beads (1 µm diameter) as ideal analogues of bacteria cells during Mn mineralization. Ten representative types of functionalization were selected to mimic the chemical groups and exopolymers diversity borne by bacteria cell walls: one without any functionalization called plain, simple ones like carboxylic acids (–COOH), amine groups ($–NH_2$), or sulfonic acids ($–SO_3H$), sugar such as chitosan, chelating agents like nitrilotriacetic acid (NTA), and protein compounds like streptavidin, protein A and collagen. This allowed to better constrain the specific impact of individual chemical functions taken independently during LC-STEM experiments.

Manganese mineralization occurs for all functionalized beads. In order to quantify mineralization rates, image analyses are performed to isolate the bead and the precipitated features from the surrounding background. To do so, a suite of treatment steps is conducted on each series, among which smoothing, contrast adjustment, and the most critical, local thresholding to overcome liquid thickness differences. After this treatment, we are able to accurately measure surface area and convexity of the mineralized bead evolutions as a function of time. Consequently, significant differences in growth rates and mineralization patterns are observed for each bead type. As illustrated in Figure 1, at the same electron dose rate, Mn precipitation for COOH-bearing beads is much faster (Figure 1(b)) than non-functionalized beads (Figure 1(a)). COOH-bearing beads also exhibit massive dendritic precipitates at their surface after exposure while non-functionalized beads only developed small structures. Electrophoretic mobility measurements are used to tentatively correlate surface charge of each bead type to Mn mineral growth rate. However, bulk

surface charge does only partially explain Mn mineralization properties at the beads surface, and other parameters like steric effect thus need to be explored [2].

References:

[1] T Couasnon *et al*, Sci. Adv. **6** (2020), eaaz3125.
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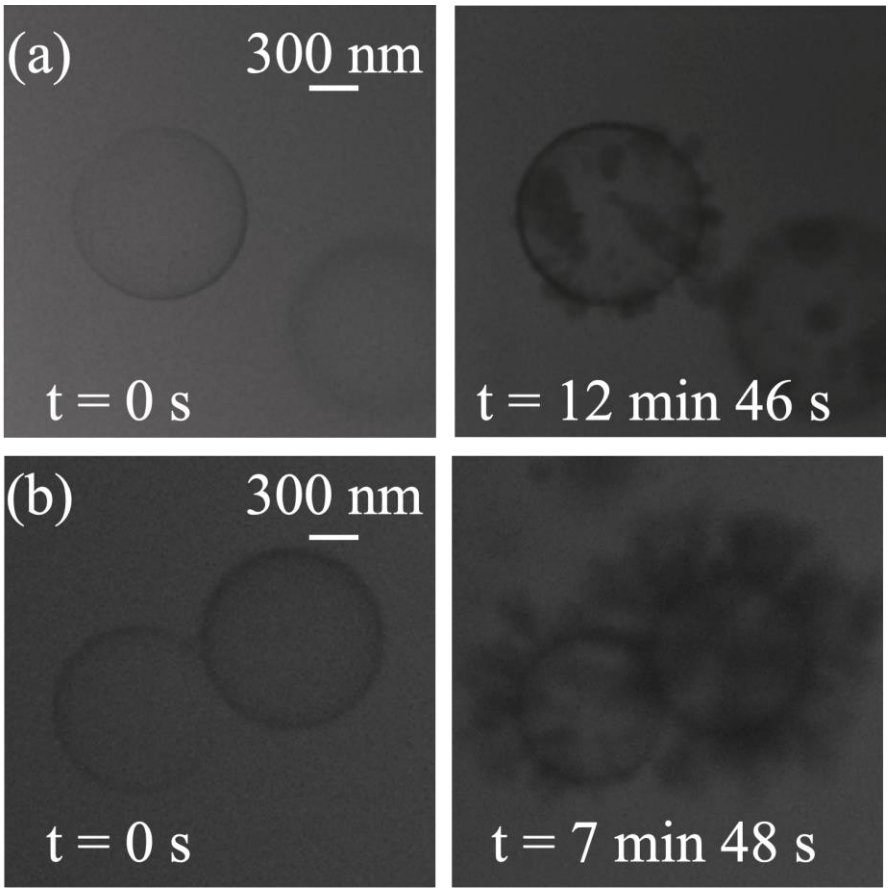

Figure 1. LC-STEM images of 1 µm diameter polystyrene beads in a Mn^{2+} solution exposed to the same electron dose rate. (a) is a non-functionalized bead showing a slow mineralization, and (b) is a COOH-functionalized bead showing a fast dendritic mineralization.

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