Mn oxides biomineralization monitored by *in situ* liquid Scanning Transmission Electron Microscopy

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Biofilms, the most common bacteria organization mode in nature, consist in a 3D organic framework composed of water, microbial cells and exopolymeric substances (EPS). Due to their high reactivity, biofilms are the loci of mineral precipitation including Mn oxide, a highly oxidizing mineral able to control the cycling of toxic redox-sensitive elements. The chemical and biological compositions of biofilms are likely to influence nucleation and mineral growth, but mechanisms at the molecular scale remain poorly known. To assess the role of the overall biofilm structure and the extracellular polymers (EPS) types for biomineralization, five *E. coli* mutants were selected that express different exopolymers (pili, N-acetyl glucosamine, cellulose), and were exposed to Mn(II) solutions for time ranging between 1 day and 1 month.

Mn oxides formation, resulting from biologically mediated Mn(II) oxidation, was investigated by Scanning transmission X-ray microscopy (STXM) to locate and identify Mn-bearing minerals. The different *E. coli* strains showed different nano-scale minerals precipitated depending on the EPS chemical nature. While *E. coli* wild type did not bear any mineral, Mn(III) precipitates covering all the organic material were observed for one mutant, and a mixture of Mn(III)/ Mn(II) for another mutant. These results were further confirmed by EELS measurements.

In situ studies of liquid samples by transmission electron microscopy (TEM) using specific liquid cells has caught growing interest in recent years. It allowed to monitor in real time, and in liquid environment, the processes of nucleation and mineralization at the molecular scale, while not requiring any specific sample preparation. There, the first nucleation steps for Mn oxides formation could be observed, mainly located at the bacteria cell poles. Comparison among the different strains suggested that Mn oxides precipitation dynamics was strongly dependent on the EPS type and density. Our results open new perspectives to study biomineralization processes and to document nucleation sites on bacterial walls and EPS using STEM in liquid cells.