Subject offered for a contract starting october 2015

SUBJECT TITTLE: Biomineralization in bacterial biofilms

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Financing: Doctoral contract with or without assignment

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Context: Bacteria constitute a major phylogenetic group present in almost all surface environments, with a total biomass equivalent to plants on Earth. Because of their high reactivity and their metabolic activities, they are also considered a major control for various biogeochemical processes. Bacteria are usually organized in communities called biofilms, described as gel-like structures where the diffusion of chemical elements is limited. Consequently, because of their metabolic activities, microorganisms within these structures create and maintain micro-environments that are radically different from the surrounding bulk solution (pH, Eh, pO_2…), thus creating concentration gradients for organic or inorganic compounds and physico-chemical parameters. Hence, the particular biofilm gel-like structure is able to induce formation of biominerals, as well as sequestration of chemical elements; and the existence of such biofilm-induced precipitates has been extensively reported in the literature. It is now accepted that biominerals formation and micro-environments exert a major control on various environmental processes, and the elucidation of mechanisms driven by biofilms is a central question common to different geochemical, environmental, and even operational (depollution) domains. As a result, high quality studies have been conducted in order to better define the processes linked to biominerals and micro-environments formation in biofilm structures. Some of these studies underline the importance of the submicrometer spatial scale information to understand metal(loid)s dynamics during biomineralization, and important information has been gathered using high-resolution techniques. From the literature, three different mineralization mechanisms are proposed: i) intracellular biologically-controlled precipitation, ii) biologically-induced biomineralization resulting from modification of chemical conditions by metabolic activities (pH and/or Eh change with photosynthesis, metals reduction, oxidative or reductive processes, inorganic phosphate production with phosphatase activity, etc.), and iii) biologically-influenced biomineralization considered as a passive precipitation on nucleation sites from organic polymers (cell surfaces or EPS), with in this case an important role of the 3D polymer template structure.

Given the difficulties associated to identify these processes that necessarily encompass chemical, mineralogical and biological approaches, many questions relative to biomineralization mechanisms and kinetics in biofilms remain open. In order to better constraint these processes, we think necessary to link local sub-nanometer scale information to a global description of the physico-chemical parameters evolution at the whole biofilm scale. This is the approach proposed in this PhD study, and which benefits from the recent development of state-of-the-art analytical tools.

Objectives: Due to the large diversity of metabolic reactions that bacteria can carry out, biofilms can induce the formation of various minerals which results in the trapping of metal(loid)s, hence controlling their environmental toxicity. For instance, bacteria can produce inorganic phosphates that can be trapped into the biofilm structure and generate metal-bearing phosphate precipitation: pyromorphite ($Pb_5(PO_4)_3(OH,Cl,F)$), HUO_2PO_4, and Fe(III) and Cr(III)-phosphates have all been reported. However, although several studies are dedicated to bacterial biomineralization, many questions remain regarding the associated processes in realistic biofilm structures. **We propose in this PhD study i) to identify the scale and nature of physico-chemical gradients existing in bacterial biofilms, and ii) to determine the processes controlling the formation of metal-bearing precipitates within biofilms.** To do so, we selected Fe(III), an ubiquitous element in most surface environments, as well as three toxic elements for which associations to bacterial mats have been reported (Pb(II), U(VI), Cr(VI)).

Methodology: Our knowledge on the biofilm-driven mineralization processes remains limited, mainly because of the challenging manipulations and observation limitations attached to these 3D biological structures when trying to investigate their interaction with metals (e.g.: drying steps prior to SEM/TEM observation, microscopes spatial resolution, microscopy probes sensitivity…). As a result, only few characterization techniques are adapted to biofilms exposed to metals, and most of them generate potential artifacts. To circumvent many of the analytical limitations attached to biofilms analysis, this innovative project is centered on a combination of state-of-the-art techniques: confocal laser scanning microscopy, SEM/TEM observations, synchrotron related techniques (Grazing incidence EXAFS, X-ray Standing Waves), and ultra high-resolution observations using aberration-corrected TEM (superTEM platform-MPQ) coupled with TEM analysis in liquid cell. Thus, this study will benefit from the collaboration of two scientific domains (earth sciences and material physics), and from their recent analytical development. We postulate that this combined approach will strongly constraint our study, and thus allow significant progresses.