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Impact of microbial biofilms on Mn(II) oxidation dynamics

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Manganese is considered a central element in biogeochemical cycling since Mn oxides are among the strongest oxidizers at the Earth's surface, thus controlling the fate of various redox-sensitive metals such as Cr or U. In nature, most of Mn(II) oxidation is thought to be a biologically-driven process, involving catalysis by a multicopper oxidase (MCO) enzyme in the case of some bacterial strains [1], and by reactive oxygen species (ROS) [2]. In natural environments, most microorganisms are organized as complex biofilms, exhibiting gel-like structures with microenvironments of specific physico-chemical conditions imposing a strong control on redox properties at local scale [3]. Biofilms are then likely to partly control the global cycling of redox-sensitive elements, such as Mn, but an accurate quantification of their environmental impact remains poorly estimated. In that regard, the goal of this study is to define how these specific biofilm structures are able to trigger Mn oxidation. To do so, biofilms of *Escherichia coli K12,* a model bacteria deprived of MCO related to Mn(II) oxidation, were grown and exposed to Mn(II). After one week, Mn total sequestration within biofilms was monitored by ICP-MS measurements. This Mn immobilization can be described by a Freundlich-type isotherm, as the sum of multiple processes including adsorption onto bacteria cell walls and exopolymeric substances, internalization inside the cells, and Mn oxidation and formation of secondary bearing minerals. Simultaneously, total Mn oxidation was quantified by using the leucoberbelin blue dye. Interestingly, *E. coli* biofilms were shown to oxidize Mn(II), but only at cell densities higher than 10^9 cells/mL. Also, no oxidation occurred in planktonic conditions. This suggests a direct relationship between biofilm structure and its oxidation capacity. Moreover, STEM analysis coupled with diffraction of nanocrystals allow us to better define the manganese oxide minerals formed in our biofilms. This study thus provides critical information on the microenvironments' properties in biofilms and contributes to better define metal(loid) cycling in the critical zone.

[1] Tebo BM et al., *Trends Microbiol*., 2005

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