

Microbial and geochemical synergy in manganese oxide formation

COLLEEN M. HANSEL^{1*}, DERIC R. LEARMAN², CAROLYN A. ZEINER¹, CARA M. SANTELLI³ AND SAM M. WEBB⁴

¹Harvard University, Cambridge, MA, USA, hansel@seas.harvard.edu (*presenting author)

²Central Michigan University, Mount Pleasant, MI, USA, deric.learman@cmich.edu

³Smithsonian Institution, Washington, DC, USA, santellic@si.edu

⁴Stanford Synchrotron Radiation Lightsource, Menlo Park, CA, USA, samwebb@slac.stanford.edu

Manganese (Mn) oxides are among the strongest sorbents and oxidants within the environment, controlling the fate and transport of numerous elements and the degradation of recalcitrant carbon. Both bacteria and fungi mediate the oxidation of Mn(II) to Mn(III/IV) oxides but the genetic and biochemical mechanisms responsible remain poorly understood. Furthermore, the physiological basis for microbial Mn(II) oxidation remains an enigma.

We have recently reported that a common marine bacterium (*Roseobacter* sp. AzwK-3b) oxidizes Mn(II) via reaction with extracellular superoxide (O₂⁻) produced during exponential growth [1]. Here we expand this superoxide-mediated Mn(II) oxidation pathway to fungi, introducing a surprising homology between prokaryotic and eukaryotic metal redox processes. For instance, *Stilbella aciculosa*, a common soil Ascomycete filamentous fungus, precipitates Mn oxides at the base of asexual reproductive structures. This distribution is a consequence of localized production of superoxide by the well-known NADPH oxidase enzymes, leading to abiotic oxidation of Mn(II) by superoxide. Disruption of NADPH oxidase activity using the common oxidoreductase inhibitor DPI leads to diminished cell differentiation and subsequent Mn(II) oxidation inhibition.

We also show here that similar to fungi extracellular superoxide production is widespread throughout the bacterial domain. Yet, superoxide production does not, in fact, confer the ability to produce Mn oxides. This is a consequence of a backreaction between the products (Mn(III) and hydrogen peroxide) formed upon Mn(II) and superoxide reaction, leading to the regeneration of Mn(II). Indeed, we show that superoxide-mediated Mn oxide formation is reliant upon the removal of the hydrogen peroxide product. Thus, the formation of Mn oxides by the *Roseobacter* and *Stilbella* spp. requires both the production of superoxide and consumption of hydrogen peroxide. We believe that this need for hydrogen peroxide consumption hints to the role of heme peroxidases (recently implicated Mn oxidases) in bacterial Mn oxidation.

Taking into consideration that superoxide is a strong and versatile redox reactant serving as both an oxidant (e.g., Mn) and reductant (e.g., Fe, Cu, Hg) of metals, biological superoxide production may have profound influences on metal biogeochemistry beyond Mn.

[1] Learman et al. (2011) *Nature Geo.* **4**, 95-98.

Structural constraints on Mn(II) oxidation by biogenic Mn oxides

COLLEEN M. HANSEL^{1*}, DERIC R. LEARMAN², ANDY S. MADDEN³, SCOTT D. WANKEL¹ AND SAM M. WEBB⁴

¹Harvard University, Cambridge, MA, USA, hansel@seas.harvard.edu (*presenting author)

²Central Michigan University, Mount Pleasant, MI, USA, deric.learman@cmich.edu

³University of Oklahoma, Norman, OK, USA, amadden@ou.edu

⁴Stanford Synchrotron Radiation Lightsource, Menlo Park, CA, USA, samwebb@slac.stanford.edu

Manganese (Mn) oxides are vital components of environmental systems, controlling the fate and transport of contaminants, bioavailability of nutrients, degradation of recalcitrant carbon, and respiratory capacity of microbial populations. The oxidation of Mn(II) to Mn(III/IV) oxides has been primarily attributed to biological processes, due in part to the faster rates of bacterial Mn(II) oxidation compared to observed mineral-induced and other abiotic rates.

Here we reveal the reactivity of biogenic Mn oxides formed by a common marine bacterium (*Roseobacter* sp. AzwK-3b), which has been previously shown to oxidize Mn(II) via the production of extracellular superoxide [1]. The reactivity of the biogenic Mn oxides was explored by harvesting and characterizing Mn oxides formed within *Roseobacter* AzwK-3b filtrate at various time points followed by reaction with Mn(II) under an array of aqueous conditions [2]. Oxidation of Mn(II) by extracellular superoxide within *Roseobacter* filtrate results in the formation of colloidal (~20-40 nm in diameter) Mn oxides. These oxides are most similar to δ -MnO₂ – a highly disordered birnessite phase with turbostratic stacking and hexagonal symmetry. These colloidal Mn oxides are extremely reactive and induce further Mn(II) oxidation and Mn oxide formation. Interestingly, the oxidation of Mn(II) mediated by the colloidal Mn oxides includes a number of reaction pathways, involving the formation of organic and oxygen radicals at the oxide surface.

The reactivity of these Mn oxide minerals, however, is short-lived due to the rapid evolution of the initial hexagonal birnessite phase to a non-reactive triclinic birnessite phase. Thus, this structural evolution imposes the need for continuous production of new colloidal hexagonal particles for Mn(II) oxidation to be sustained, illustrating an intimate dependency of enzymatic and mineral-based reactions in Mn(II) oxidation. Further, the coupled enzymatic and mineral-induced pathways are linked such that enzymatic formation of Mn oxide is requisite for the mineral-induced pathway to occur. These findings highlight the important (yet frequently overlooked) role that reactive metabolites and biominerals play in metal redox biogeochemistry.

[1] Learman, et al. (2011) *Nature Geo.* **4**, 95-98. [2] Learman, et al. (2011) *Geochim. Cosmochim. Acta* **75**, 6048-6063.