

# Oxidation Kinetics of Manganese (II) by Spore Coats of Marine *Bacillus* sp. SG-1

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In aquatic environment, manganese is found in the dissolved form of Mn (II) or insoluble particulate oxides of Mn (III & IV). Mn oxides are the strongest oxidants among common chemical species except oxygen in the environment and promote the oxidation of a variety of inorganic species. And also, they serve as electron acceptors for anaerobic bacteria and scavenge a variety of other metals on their highly charged surfaces and then play an important role in the environment. The predominant production of Mn oxides in most environments is microbially mediated. Mn (II) oxidation rates increase by at least 3-5 orders of magnitude in the presence of Mn (II) oxidizing bacteria than abiotic Mn oxidation. Their activities have a tremendous impact on the geochemical cycling of Mn. Recent investigations have suggested that ever specified Mn-oxidizing components, MnxG of *Bacillus* sp. SG-1, MofA of *Leptothrix*. discorphora SS-1, and cumA of *Pseudomonas.putida* GB-1& MnB1, were identified as multi-copper oxidases-like enzymes from phylogenetically distinct. Many bacteria that produce dormant spores which oxidize enzymatically soluble Mn(II) to insoluble Mn(IV) oxides were isolated from environment and spore-forming *Bacillus* species are a major component of the total colony-forming bacteria. Phylogenetic analysis demonstrated 15 different Mn(II)-oxidizing spore-forming bacillus revealed extensive diversity. The marine Mn(II)-oxidizing *Bacillus* species strain SG-1 was isolated over 20 years ago from an enrichment culture inoculated with sediments collected off Scripps pier. Dormant spores of this organism, not the vegetative cells, enzymatically catalyze the oxidation of soluble Mn(II) to highly insoluble Mn(III, IV) oxide precipitates on the spore.

Factors influencing bacterial Mn(II) oxidation by the multi-copper oxidase-like enzyme on the spore of the marine *Bacillus* sp. SG-1 are examined in time course experiments with initial Mn (II) concentration between 0.03mM and 0.05mM. Ca<sup>2+</sup> ions have a dramatic effect on the oxidation in artificial seawater, while Mg<sup>2+</sup>, Sr<sup>2+</sup>, K<sup>+</sup>, NO<sub>3</sub><sup>-</sup> have no effects. The rate at 10mM of Ca<sup>2+</sup> ions (seawater composition) was four or five times that with no Ca<sup>2+</sup> ions. The significant difference of pH optimum between them suggests that Ca<sup>2+</sup> ion is a cofactor of the enzyme. The relationship between Ca<sup>2+</sup> content and the rate imply that the equilibrium constant is small (order of 0.1) and binding coefficient is 0.5, but further work is required to the data. But, a possible alternative interpretation is that the acceleration of Mn precipitation rate is due to the difference of mineral species.

The study of the kinetic salt effect demonstrates the rate constant depends on the ionic strength (I). The logarithm of the rate constant was linear function of root I with slopes of -3 (I: more than 0.2) and -0.31 (I: less than 0.2) in the solution contains 10 mM of Ca<sup>2+</sup> ions. The slope of -3 at higher ionic strength suggests that the oxidation from Mn(III) to Mn(IV) is the rate-determining step at higher ionic strength such as natural seawater, as the existence of Mn(III) oxides as intermediates in Mn oxidation products by SG-1 has reported previously. The result indicates the possibility on the existence of primary Mn(III) oxide species in seawater, as well as the secondary inorganic product. While the slope of -0.31 at lower ionic strength indicates that the adsorption of Mn(II) to the spore surface and/or the reaction from Mn(II) to Mn(III) is rate-limiting step at diluted solution, and this is consistent with the previous reports for a one-step from Mn(II) to Mn(V) oxidation model in 50mM NaCl solution. The significant difference of the rate between natural seawater and artificial seawater, against the similarities of both ionic strength (I=0.7) and salt contents between them, is probably due to the inhibition by humic substances in natural seawater.