The molecular biogeochemistry of manganese(II) oxidation

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Abstract

Micro-organisms capable of oxidizing the redox-active transition metal manganese play an important role in the biogeochemical cycle of manganese. In the present mini-review, we focus specifically on Mn(II)-oxidizing bacteria. The mechanisms by which bacteria oxidize Mn(II) include a two-electron oxidation reaction catalysed by a novel multicopper oxidase that produces Mn(IV) oxides as the primary product. Bacteria also produce organic ligands, such as siderophores, that bind to and stabilize Mn(III). The realization that this stabilized Mn(III) is present in many environments and can affect the redox cycles of other elements such as sulfur has made it clear that manganese and the bacteria that oxidize it profoundly affect the Earth's biogeochemistry.

Geochemical cycling of manganese

The carbon and nitrogen cycles may get most of the attention in high school science class, but the manganese cycle also has a tremendous impact on the Earth's geochemistry. Manganese is essential for life as a cofactor in enzymes such as Photosystem II and manganese superoxide dismutase and is one of the most abundant redox-active transition metals in the Earth's crust [1]. In Nature, manganese is commonly found in three oxidation states: Mn(II), Mn(III) and Mn(IV) [2]. The reduced form, Mn(II), is generally soluble and stable in the absence of oxygen. Mn(IV), the most oxidized form, is insoluble, forming oxides which are strong oxidants, capable of oxidizing inorganic and organic compounds, for example, Fe(II) and UO_2 [3]. The intermediate oxidation state, Mn(III), is unstable as an ion under normal environmental conditions unless complexed with organic or inorganic ligands (see below) [1]. Mn(III) also occurs as insoluble Mn(III) oxy(hydrox)oxide phases or in mixed Mn(III,IV) oxides. Mn(III,IV) oxides also sorb metals and other compounds from the environment; as a result, Mn(II) oxidation can control the distribution of many other elements (e.g. copper, cobalt, nickel, lead, iron, radium, uranium and rare earth elements) [4,5].

A one-electron transfer for the oxidation of the soluble Mn(II) species $Mn(H_2O)_6^{2+}$ by O_2 is thermodynamically unfavourable at pH < 9, but a two-electron oxidation is favourable at pH > 3 [6]. Complexed forms of Mn(II) can oxidize at neutral pH, but abiotic Mn(II) oxidation at circumneutral pH is quite slow under normal environmental conditions; bacteria and fungi can greatly increase the rate of this reaction by up to 5 orders of magnitude [1]. Thus it is thought that the bulk of environmental Mn(II) oxidation is carried out by micro-organisms. In the present mini-review, we focus specifically on Mn(II)-oxidizing bacteria.

Mn(II)-oxidizing bacteria are widespread and found in diverse environments such as soil, natural waters and sediments. These organisms are phylogenetically diverse, with representatives in the Firmicutes, Actinobacteria and the Alpha-, Beta- and Gamma-proteobacteria [7]. The physiological role Mn(II) oxidation plays in these species is unknown. Because Mn(IV) formation is thermodynamically favourable, the bacteria could derive energy from the reaction; however, this has not been shown conclusively for any organism. The oxidation of Mn(II) may also help to protect the cells from ROS (reactive oxygen species) or other free radicals [8]. Alternatively, the Mn(IV) oxides formed in this reaction are themselves highly reactive and may be used to oxidize refractory organic material that can then be utilized by the micro-organism as a carbon source. Other possible functions for the oxides are as terminal electron acceptors, manganese storage or, since the solids coat the cell, protection from environmental hazards such as UV radiation, predation or phage infection [7,9].

Mn(III,IV) oxide deposits can be found in many environments [1,10]. These include ferromanganese nodules in the deep sea, lakes and soils and terrestrial ferromanganese crusts, also called rock varnish. Mn(IV) oxides are also found in ore deposits and metalliferous sediments associated with spreading centres and as ferromanganese crusts on seamounts on the ocean floor. Given that the oxides reflect the redox conditions of the local environment, they are unsurprisingly

Key words: manganese(II), manganese(III), manganese oxidation, manganese oxide, multicopper oxidase.

Abbreviations used: MCO, multicopper oxidase.

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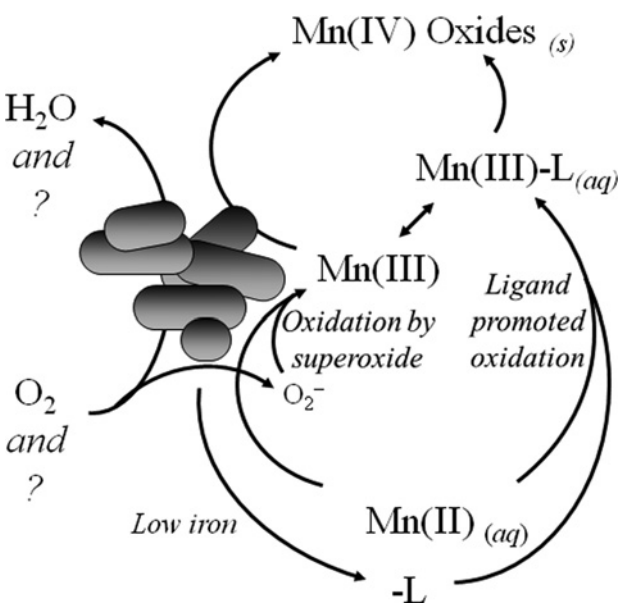
Figure 1 | **The updated manganese cycle**



often found associated with oxic–anoxic interfaces in sediments and water. The extent to which microbial activity is responsible for these formations is unclear. However, the ubiquity of Mn(II)-oxidizing organisms and their presence in environments where Mn(IV) oxides are found suggest that their role is substantial.

Molecular biochemistry of Mn(II) oxidation

Despite the diversity of organisms capable of the reaction, there are common themes in bacterial Mn(II) oxidation [9]. The product is primarily $Mn(IV)O_2$, with oxidation occurring through two sequential one-electron steps [Mn(II)→Mn(III)→Mn(IV)] that require O_2 [11,12] (Figure 1). Organisms commonly initiate Mn(II) oxidation at the onset of stationary phase and deposit the oxides on their outer surface [1,9]. However, some variations on these common themes occur. Both fungal Mn(II)-oxidizers and the alphaproteobacterium *Erythrobacter* sp. SD-21 produce Mn(III), which is stabilized by biogenic ligands [13–16]. Another less direct mechanism of Mn(II) oxidation is through the action of enzymatically produced superoxide, as has been observed with the alphaproteobacterium *Roseobacter* sp. AzwK-3b [17–19]. The iron-chelating siderophores produced by bacteria under iron-limiting conditions are also able to bind with high affinity to manganese; this interaction itself can promote Mn(II) oxidation [20–23].

The enzymes responsible for bacterial Mn(II) oxidation have been identified in several species and fall into two general categories. The alphaproteobacteria *Aurantimonas manganoxydans* SI85-9A1 and *Erythrobacter* sp. SD-21 both employ calcium-binding haem peroxidases named MopA to oxidize Mn(II) [16]. Many other species possess MCO (multicopper oxidase) Mn(II) oxidase enzymes (Figure 2). These include MofA in *Leptothrix* sp. and the twin Mn(II) oxidase MCOs of *Pseudomonas putida* GB-1, MnxG and McoA ([24], and Geszvain, K., McCarthy, J.K. and Tebo, B.M., unpublished work). MCO enzymes catalyse the one-electron transfer from substrates as diverse as Fe(II) and lignin to ultimately reduce O_2 to H_2O [26]. MCOs incorporate multiple copper ligands, including a type I copper-binding site and a trinuclear centre made up of two type II and one type III site. One electron is removed from the substrate at the type I copper then shuttled to the trinuclear centre where it is ultimately added to O_2 to form H_2O. As the diversity of substrates would suggest, this family of enzymes is very diverse; identification of family members is through conservation of characteristic copper-binding sites, identified both at the sequence level and by their spectroscopic characteristics [26,27].

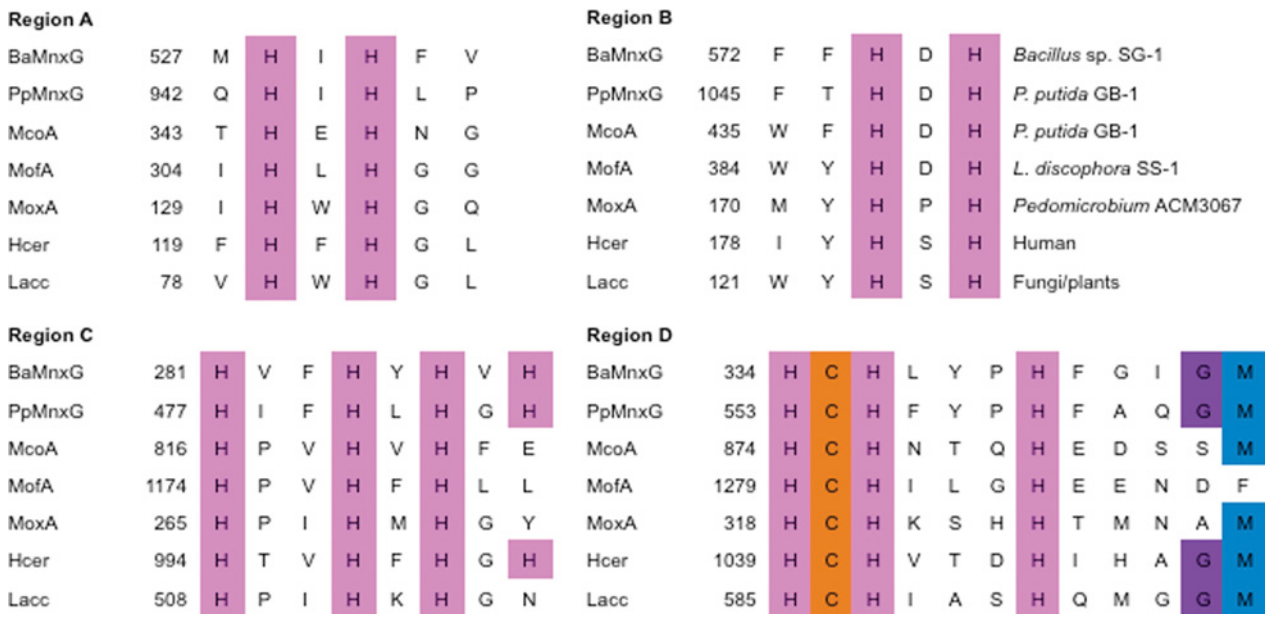
The Mn(II) oxidase from *Bacillus* sp., MnxG, is the best characterized oxidase enzyme to date. MnxG was first implicated as the *Bacillus* Mn(II) oxidase via transposon mutagenesis. Several transposon insertions were identified within an operon of eight genes (*mnxA–mnxG*) that resulted in mutant strains that failed to oxidize Mn(II) [28,29]. The last gene in the operon, *mnxG*, encodes an MCO, based on the presence of five putative copper-binding domains. The previous observation that the addition of copper stimulated Mn(II) oxidation supported the idea that an MCO was the Mn(II) oxidase in this organism [29]. Subsequent partial purification of the Mn(II) oxidase activity from *Bacillus* sp. PL-12 exosporium followed by mass spectrophotometry identified MnxG in the active fraction, supporting the identification of this MCO as the Mn(II) oxidase [30,31].

MCO enzymes catalyse the one-electron oxidation of their substrates, whereas Mn(II) oxidation appears to require two electron transfers, making the mechanism of Mn(II) oxidation a subject of intense study. This mechanism has been investigated using exosporium prepared from *Bacillus* sp. SG-1 [30–32]. Exosporium preparations are capable of oxidizing Mn(II) to Mn(IV); in the presence of pyrophosphate, a stable Mn(III)–pyrophosphate complex forms that can be monitored by measuring the absorbance at 258 nm [32]. From these experiments, it was concluded that Mn(II) oxidation in fact proceeds through an Mn(III) intermediate [32] and that oxygen was required for each step [12]. Both the Mn(II) to Mn(III) and Mn(III) to Mn(IV) reactions were absent from exosporium prepared from a strain in which *mnxG* was disrupted by a transposon insertion [30]. Therefore MnxG carries out each electron transfer, differentiating Mn(II) oxidase MCOs from the typical MCO which performs just one electron transfer.

The large size and domain organization of MnxG makes it somewhat similar to human caeruloplasmin, a ferroxidase [29,30]. Previous crystallographic and biochemical studies of caeruloplasmin make it possible to propose a model of the mechanism of Mn(II) oxidation by MnxG [8] (Figure 3). In caeruloplasmin, Fe(II) binds to a site adjacent to the type 1 copper; after electron transfer, the product Fe(III) moves several angstroms away to a 'holding site'. MnxG

Figure 2 | **Copper-binding motifs from Mn(II) oxidases and other MCOs**

Residues implicated in copper binding are highlighted. Hcer, human caeruloplasmin; Lacc, laccase.



may similarly oxidize Mn(II) to Mn(III). The presence of a solvent-accessible Mn(III) holding site on MnxG could account for the complexation of Mn(III) by pyrophosphate. Since mononuclear Mn(IV) is a powerful oxidant, MnxG probably forms a polynuclear Mn(IV) oxide. Thus it has been proposed that MnxG has a binuclear Mn(III) site adjacent to the holding site, with a third Mn(III) ion serving as an electron shuttle between the binuclear site and the type I copper [12]. Future work with purified MnxG will make it possible to investigate in detail the molecular mechanism of Mn(II) oxidation.

Environmental geochemistry of soluble Mn(III)

Much work on the environmental roles of manganese species has been carried out in the Black Sea, a useful environmental laboratory due to its permanent halocline, a broad suboxic layer characterized by O_2 levels below 5 μM and undetectable H_2S and an anoxic H_2S-rich layer below ~150 m. Therefore the Black Sea can be used as a model oxic–suboxic–anoxic transition zone. The suboxic zone is thought to be maintained by microbially driven, O_2-dependent Mn(II) oxidation depleting the last traces of O_2, whereas the Mn(IV) oxides serve to cap the vertical flux of H_2S. However, the O_2 consumption to form manganese oxides is not sufficient to account for the H_2S depletion. Originally, it was proposed that Mn(II) oxidation was coupled to nitrate reduction, but no system has yet been identified in which nitrate serves as the electron acceptor in Mn(II) oxidation; in all cases studied so far, oxidation has required O_2.

Mn(III) historically has not been considered to be important in the environment because it was thought to be too reactive to exist in natural environments. Furthermore, manganese chemistry has been operationally defined, with Mn(II) being the species that passes through 0.4 μm filters (i.e. soluble), whereas Mn(III,IV) oxides would be trapped on the filters. However, Mn(III) stabilized by complexation to organic or inorganic ligands can be soluble, pass through filters and be measured as Mn(II). Work in the laboratory suggested that Mn(III) complexed with siderophores is stable and soluble, raising the possibility that such soluble Mn(III) is an even more important redox agent in the environment than O_2.

To determine soluble Mn(III) levels in the Black Sea, it was necessary to devise methods to routinely detect it in environmental samples. A sensitive technique to measure Mn(III) in water samples by employing the addition of the siderophore desferrioxamine B to complex Mn(III) and subsequent quantification using cyclic voltammetry was developed [33]. With this method, it was shown that Mn(III) levels in the suboxic zone were in fact quite high (4–5 μM) and constituted up to 100% of the dissolved manganese (passing through a 0.2 μm filter). The ligand-complexed Mn(III), which could be both a reductant and an oxidant, could therefore be responsible for capping the H_2S flux in the suboxic zone. Subsequent modelling results support the conclusion that the H_2S is consumed by oxidized forms of manganese with no need to propose the presence of alternative electron acceptors such as nitrate [34,35].

Similar work has been carried out with sediments collected along the Lower Saint Lawrence Estuary, using a manganese speciation method based on a metal substitution reaction with soluble porphyrin [11,36]. This work also detected up to 65 μM soluble Mn(III), therefore the effect of this species on environmental redox cycling may be widespread and

Figure 3 | **Proposed mechanism of bacterial Mn(II) oxidation**

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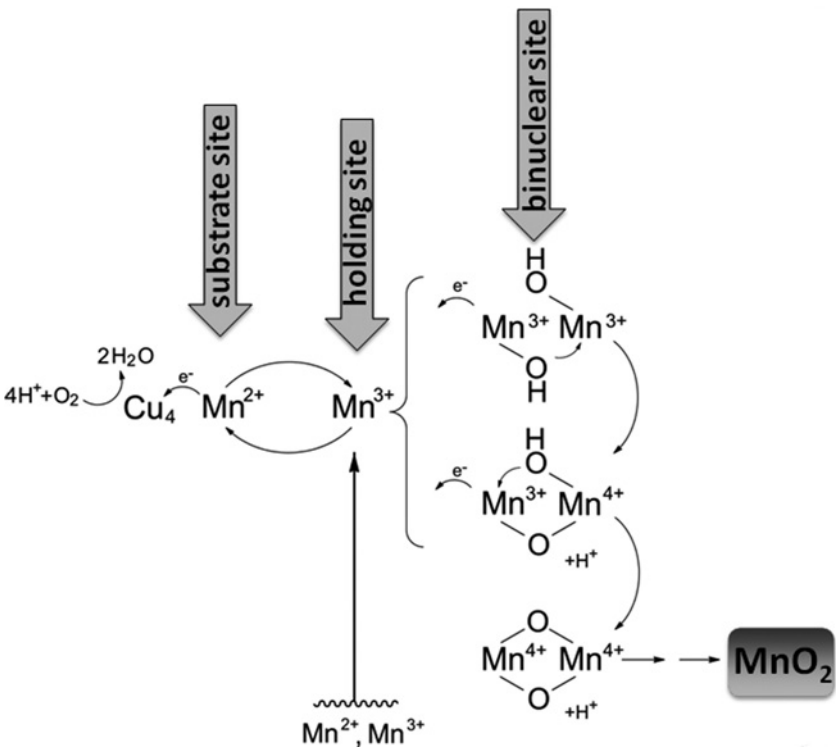

profound. The nature of the ligands responsible for stabilizing soluble Mn(III) is unknown. However, in the Black Sea, Mn(II)-oxidizing bacteria have been isolated that also produce siderophores that are capable of binding Mn(III) [37].

Siderophores have been traditionally studied for their role in iron sequestration and uptake. The realization that they also bind manganese, resulting in high concentrations of Mn(III) in the environment, makes it necessary to rethink the role that they play in metal cycling. In many regions of the ocean, levels of available Fe(III) are low and levels of iron-complexing ligands resembling siderophores are high. Clearly, the cycling of manganese, as is evident from recent studies of manganese oxidation, is mechanistically much more complex than depicted by the paradigm of manganese that passes through a filter is dissolved Mn(II) and that retained by the filter is Mn(III,IV) oxide (Figure 1).

Conclusions

Mn(II)-oxidizing bacteria are widespread and diverse, and the enzymes directly responsible appear to fall in to two general categories: haem peroxidase and MCO. Indirect oxidation of Mn(II) may also occur through the enzymatic production of superoxide. Soluble Mn(III) has been found in a variety of environments and is expected to be present wherever there is a redox gradient. As both an oxidant and a reductant, soluble Mn(III) can profoundly affect redox cycling in complex ways. Furthermore, since iron-chelating siderophores also bind Mn(III), there is competition between Fe(III) and Mn(III) for the same ligands, complicating the effect of soluble Mn(III) on iron biogeochemistry. Mn(III) may also be linked to other biogeochemical cycles, such as nitrogen, sulfur and phosphorus, but the importance of the coupling of manganese cycling with these other elemental cycles is completely unknown. Clearly, study of Mn(II) oxidation at the molecular, biochemical and environmental levels has only just begun.

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References

1 Tebo, B.M., Bargar, J.R., Clement, B.G., Dick, G.J., Murray, K.J., Parker, D., Verity, R. and Webb, S.M. (2004) Biogenic manganese oxides: properties and mechanisms of formation. Annu. Rev. Earth Planet. Sci. **32**, 287–328

2 Brouwers, G.J., Vijgenboom, E., Corstjens, P.L.A.M., de Vrind, J.P.M. and de Vrind-de Jong, E.W. (2000) Bacterial Mn^{2+} oxidizing systems and multicopper oxidases: an overview of mechanisms and functions. Geomicrobiol. J. **17**, 1–24

3 Chinni, S., Anderson, C.R., Ulrich, K., Giammar, D.E. and Tebo, B.M. (2008) Indirect UO_2 oxidation by Mn(II)-oxidizing spores of *Bacillus* sp. strain SG-1 and the effect of U and Mn concentrations. Environ. Sci. Technol. **42**, 8709–8714

4 Miyata, N., Tani, Y., Sakata, M. and Iwahori, K. (2007) Microbial manganese oxide formation and interaction with toxic metal ions. J. Biosci. Bioeng. **104**, 1–8

5 Spiro, T.G., Bargar, J.R., Sposito, G. and Tebo, B.M. (2010) Bacteriogenic manganese oxides. Acc. Chem. Res. **43**, 2–9

6 Luther, G.W. (2009) The role of one- and two-electron transfer reactions in forming thermodynamically unstable intermediates as barriers in multi-electron redox reactions. Aquat. Geochem. **16**, 395–420

7 Tebo, B.M., Johnson, H.A., McCarthy, J.K. and Templeton, A.S. (2005) Geomicrobiology of manganese(II) oxidation. Trends Microbiol. **13**, 421–428

8 Daly, M.J. (2009) A new perspective on radiation resistance based on *Deinococcus radiodurans*. Nat. Rev. Microbiol. **7**, 237–245

9 Tebo, B.M., Geszvain, K. and Lee, S. (2010) The molecular geomicrobiology of bacterial manganese(II) oxidation. In Geomicrobiology: Molecular and Environmental Perspective (Barton, L.L., Mandl, M. and Loy, A., eds), pp. 285–308, Springer, Berlin

10 Nealson, K.H., Tebo, B.M. and Rosson, R.A. (1988) Occurrence and mechanisms of microbial oxidation of manganese. Adv. Appl. Microbiol. **33**, 279–318

11 Madison, A.S. (2012), Biogeochemical cycling of soluble manganese(III) in marine (pore)waters. Ph.D. Thesis, University of Delaware, Newark, DE, U.S.A.

12 Soldatova, A.V., Butterfield, C., Oyerinde, O.F., Tebo, B.M. and Spiro, T.G. (2012) Multicopper oxidase involvement in both Mn(II) and Mn(III) oxidation during bacterial formation of MnO_2. J. Biol. Inorg. Chem., doi:10.1007/s00775-012-0928-6

13 Hofer, C. and Schlosser, D. (1999) Novel enzymatic oxidation of Mn^{2+} to Mn^{3+} catalyzed by a fungal laccase. FEBS Lett. **451**, 186–190

14 Schlosser, D. and Hofer, C. (2002) Laccase-catalyzed oxidation of Mn^{2+} in the presence of natural Mn^{3+} chelators as a novel source of extracellular H_2O_2 production and its impact on manganese peroxidase. Appl. Environ. Microbiol. **68**, 3514–3521

15 Glenn, J.K., Akileswaran, L. and Gold, M.H. (1986) Mn(II) oxidation is the principal function of the extracellular Mn-peroxidase from *Phanerochaete chrysosporium*. Arch. Biochem. Biophys. **251**, 688–696

16 Anderson, C.R., Johnson, H.A., Caputo, N., Davis, R.E., Torpey, J.W. and Tebo, B.M. (2009) Mn(II) oxidation is catalyzed by heme peroxidases in "*Aurantimonas manganoxydans*" strain SI85–9A1 and *Erythrobacter* sp. strain SD-21. Appl. Environ. Microbiol. **75**, 4130–4138

17 Hansel, C.M., Zeiner, C.A., Santelli, C.M. and Webb, S.M. (2012) Mn(II) oxidation by an ascomycete fungus is linked to superoxide production during asexual reproduction. Proc. Natl. Acad. Sci. U.S.A. **109**, 12621–12625

18 Hansel, C.M. and Francis, C.A. (2006) Coupled photochemical and enzymatic Mn(II) oxidation pathways of a planktonic *Roseobacter*-like bacterium. Appl. Environ. Microbiol. **72**, 3543–3549

19 Learman, D.R., Wankel, S.D., Webb, S.M., Martinez, N., Madden, A.S. and Hansel, C.M. (2011) Coupled biotic–abiotic Mn(II) oxidation pathway mediates the formation and structural evolution of biogenic Mn oxides. Geochim. Cosmochim. Acta **75**, 6048–6063

20 Duckworth, O.W., Bargar, J.R. and Sposito, G. (2009) Coupled biogeochemical cycling of iron and manganese as mediated by microbial siderophores. Biometals **22**, 605–613

21 Duckworth, O.W. and Sposito, G. (2005) Siderophore–manganese(III) interactions. I. Air-oxidation of manganese(II) promoted by desferrioxamine B. Environ. Sci. Technol. **39**, 6037–6044

22 Parker, D.L., Sposito, G. and Tebo, B.M. (2004) Manganese(III) binding to a pyoverdine siderophore produced by a manganese(II)-oxidizing bacterium. Geochim. Cosmochim. Acta **68**, 4809–4820

23 Parker, D.L., Morita, T., Mozafarzadeh, M.L., Verity, R., McCarthy, J.K. and Tebo, B.M. (2007) Inter-relationships of MnO_2 precipitation, siderophore–Mn(III) complex formation, siderophore degradation, and iron limitation in Mn(II)-oxidizing bacterial cultures. Geochim. Cosmochim. Acta **71**, 5672–5683

24 Corstjens, P.L.A.M., de Vrind, J.P.M., Goosen, T. and de Vrind-de Jong, E.W. (2009) Identification and molecular analysis of the *Leptothrix discophora* SS-1 *mofA* gene, a gene putatively encoding a manganese-oxidizing protein with copper domains. Geomicrobiol. J. **14**, 91–108

25 Reference deleted

26 Kosman, D.J. (2010) Multicopper oxidases: a workshop on copper coordination chemistry, electron transfer, and metallophysiology. J. Biol. Inorg. Chem. **15**, 15–28

27 Sakurai, T. and Kataoka, K. (2007) Basic and applied features of multicopper oxidases, CueO, bilirubin oxidase, and laccase. Chem. Rec. **7**, 220–229

28 van Waasbergen, L.G., Hoch, J.A. and Tebo, B.M. (1993) Genetic analysis of the marine manganese-oxidizing *Bacillus* sp. strain SG-1: protoplast transformation, Tn917 mutagenesis, and identification of chromosomal loci involved in manganese oxidation. J. Bacteriol. **175**, 7594–7603

29 van Waasbergen, L.G., Hildebrand, M. and Tebo, B.M. (1996) Identification and characterization of a gene cluster involved in manganese oxidation by spores of the marine *Bacillus* sp. strain SG-1. J. Bacteriol. **178**, 3517–3530

30 Dick, G.J., Torpey, J.W., Beveridge, T.J. and Tebo, B.M. (2008) Direct identification of a bacterial manganese(II) oxidase, the multicopper oxidase MnxG, from spores of several different marine *Bacillus* species. Appl. Environ. Microbiol. **74**, 1527–1534

31 Francis, C.A., Casciotti, K.L. and Tebo, B.M. (2002) Localization of Mn(II)-oxidizing activity and the putative multicopper oxidase, MnxG, to the exosporium of the marine *Bacillus* sp. strain SG-1. Arch. Microbiol. **178**, 450–456

32 Webb, S.M., Dick, G.J., Bargar, J.R. and Tebo, B.M. (2005) Evidence for the presence of Mn(III) intermediates in the bacterial oxidation of Mn(II). Proc. Natl. Acad. Sci. U.S.A. **102**, 5558–5563

33 Trouwborst, R.E., Clement, B.G., Tebo, B.M., Glazer, B.T. and Luther, G.W. (2006) Soluble Mn(III) in suboxic zones. Science. **313**, 1955–1957

34 Yakushev, E.V., Pollehne, F., Jost, G., Kuznetsov, I., Schneider, B. and Umlauf, L. (2007) Analysis of the water column oxic/anoxic interface in the Black and Baltic seas with a numerical model. Mar. Chem. **107**, 388–310

35 Yakushev, E., Pakhomova, ,S., Sørenson, K. and Skei, J. (2009) Importance of the different manganese species in the formation of water column redox zones: observations and modeling. Mar. Chem. **117**, 59–70

36 Madison, A.S., Tebo, B.M. and Luther, 3rd, G.W. (2011) Simultaneous determination of soluble manganese(III), manganese(II) and total manganese in natural (pore)waters. Talanta **84**, 374–381

37 Clement, B.G. (2006), Biological Mn(II) oxidation in freshwater and marine systems: new perspectives on reactants, mechanisms and microbial catalysts of Mn cycling in the environment. Ph.D. Thesis, University of California San Diego, San Diego, CA, U.S.A.

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