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Biological manganese oxidation in a lake II: A thermodynamic consideration of the habitat utilization of *Metallogenium* sp.

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With 6 figures and 4 tables in the text

Abstract

Factors governing the occurrence and the vertical distribution of the manganese-oxidizing bacterium *Metallogenium* sp. in a lake were examined by the thermodynamic treatments. The oxidation of Mn^{2+} was found to be favorable thermodynamically in the habitat of *Metallogenium*, and the distribution of this organism seemed to be determined solely by the thermodynamic balances between oxidants (O_2, Mn-oxide) and reductants (Mn^{2+}, Fe^{2+}) in the redox transition layer of this lake. This suggested that the ecological niche of *Metallogenium* was due to kinetic effectiveness of this organism in the oxidation of Mn^{2+}. The possibility of the chemolithotrophy of *Metallogenium* was also discussed from both the ecological and energetic points of view.

Introduction

Metallogenium is the most widespread manganese-oxidizing bacterium ever known in freshwater environments and has been recognized to exert a significant impact on the geochemical behavior of manganese (Zavarzin, 1981). As concluded in the preceding paper (Miyajima, 1992), the oxidative precipitation of manganese at the redox gradient in the dredged area of Lake Biwa was largely controlled by the activity of this organism. Since *Metallogenium*-like organisms also occur in the vicinity of the sediment/water interface of the north (main) basin of Lake Biwa, ecological information of this organism seems indispensable for considering the overall geochemical behavior of manganese in this lake.

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Furthermore, it is also known that microfossils strikingly resemblant to *Metallogenium* abound in Proterozoic iron formations (CLOUD, 1965; WALTER et al., 1976). Thus of paleoecological interest are such informations as the conditions which allow the occurrence of *Metallogenium* in present-day basins.

However, *Metallogenium* has not been cultivated successfully in the laboratory as yet except as binary cultures with heterotrophic fungi or otherwise by the use of a medium supplimented with serum. For this reason a difficulty accompanies with the direct experimental investigation of the nutrition and the growth condition of this organism. It seems unrealistic that sufficient supply of either fungal biomass or such complex organics as serum would be ensured in natural habitats of *Metallogenium* in lake waters.

In this study, the conditions for *Metallogenium* to occur and grow in a lake are examined on the basis of the results of in situ investigation. Both ecological and energetic aspects of the manganese oxidation mediated by *Metallogenium* will be discussed with the aid of thermodynamic consideration.

Methods

The study site and the data source for particular parameters used in this study are common to those of the foregoing paper (MIYAJIMA, 1992), to which the reader is referred for the protocols of sampling, chemical analyses, and counting of microbes.

In general, the redox half-cell equilibrium between Mn^{2+} and manganese oxide (MnO_x) is expressed as follows:

$$n\,MnO_x + (2n + 4)\,H^+ + 4\,e^- = n\,Mn^{2+} + (n + 2)\,H_2O. \quad (1)$$

The oxidation number of the oxide MnO_x is $2 + 4/n$, or $x = 1 + 2/n$. The equilibrium constant (K) of the above reaction is given by using the change in the standard free energy of formation (ΔG_f°), namely,

$$K = \exp(-\Delta G_f^\circ/RT), \quad (2)$$

where R is the gas constant and T is the absolute temperature (which is assumed here to be 298.15 K). If the concentration of Mn^{2+} and the pH value are known, then the pE value of the equilibrium (1) can be calculated as follows:

$$pE = (\log K - n \log (Mn^{2+}) - (2n + 4)\,pH)/4, \quad (3)$$

where log means the common logarithm and (Mn^{2+}) indicates the activity of Mn^{2+}, so that,

$$(Mn^{2+}) = \gamma_{Mn}\,[Mn^{2+}]. \quad (4)$$

The bracket [ ] indicates the analytical concentration and γ_{Mn} is the activity coefficient of Mn^{2+} which is a function of the ionic strength of the solution.

Similarly, the equilibrium of the O_2/H_2O half-cell and its pE value are expressed as

$$O_2 + 4H^+ + 4e^- = 2H_2O \tag{5}$$

and

$$pE = (\log K_{ox} - 4\,pH + \log pO_2)/4, \tag{6}$$

respectively. In the equation (6), K_{ox} is the equilibrium constant of (5) and pO_2 means the partial pressure of O_2.

When Mn^{2+} is oxidized with O_2 as the sole electron acceptor, the overall oxidation reaction is given by subtracting the equation (1) from (5), that is,

$$n\,Mn^{2+} + O_2 + n\,H_2O = n\,MnO_x + 2n\,H^+. \tag{7}$$

If pH and the partial pressure (or concentration) of O_2 are known, the equilibrium concentration (that is to say, the solubility) of Mn^{2+} with regard to the oxygenation (7) can be calculated as follows:

$$\log(Mn^{2+}) = (\log K - \log K_{ox} - 2n\,pH - \log pO_2)/n. \tag{8}$$

Furthermore, the free energy change in the system of the equation (7) in the course of the oxygenation of Mn^{2+}, say ΔG_{sys}, can also be calculated as follows:

$$\Delta G_{sys} = \Delta G_f^\circ + RT \ln((H^+)^{2n}/(Mn^{2+})^n\,pO_2), \tag{9}$$

where ΔG_f° means the change in the standard free energy of formation for the reaction (7) and ln is the natural logarithm. If chemolithotrophic bacteria are to catalyze the manganese oxidation reaction (7) in an energy-conservative way, they would be provided through this reaction with, at maximum, the free energy of $-\Delta G_{sys}$ which is given by the equation (9).

In practice, the equilibrium constants and the energetics of the manganese oxidation reactions are dependent on the mineralogy of the manganese oxides produced by these reactions. Any naturally occurring oxide minerals of manganese are insoluble solids. It is known that, in neutral or mildly alkaline solutions, chemical (abiotic) oxidation of Mn^{2+} usually yields manganese oxides of which the oxidation number does not exceed 3 (such as Mn_3O_4, β-MnOOH, and γ-MnOOH; see Stumm & Giovanoli (1976), Hem & Lind (1983), Murray et al. (1985)). On the other hand, biological oxidation of Mn^{2+} in freshwaters is known to yield manganese oxides of higher oxidation state (δ-MnO_2 or its related minerals; see Chukhrov et al. (1980), Tipping et al. (1984), Zavarzin (1989)), although this is not always the case in marine environments where γ-MnOOH (Grill, 1982) or Mn_3O_4 (Hastings & Emerson, 1986; Mann et al., 1988) are often precipitated by manganese-oxidizing bacteria. Therefore, the present author examined in this study the thermodynamic consistency of the oxidative precipitations of δ-MnO_2, γ-MnOOH, and Mn_3O_4 in lake water.

The ionic strength of lake water of the dredged area of Lake Biwa was assumed to be ca. 10^{-3}, according to the data of major ion concentrations (Table 1) previously published by Kawashima et al. (1983). In this case, the activity coefficients of Mn^{2+} and Fe^{2+} are to be 0.87 after the extended Debye-Hückel law (Stumm & Morgan, 1981).

The redox systems which are considered in this paper are O_2/H_2O, O_2/H_2O_2, δ-MnO_2/Mn^{2+}, γ-MnOOH/Mn^{2+}, Mn_3O_4/Mn^{2+}, and amorphous FeOOH/Fe^{2+}. The equilibrium constants were calculated from the standard free energies of formation of rel-

Table 1. Major ion concentrations in lake water of the dredged area of Lake Biwa (summarized from the data published by KAWASHIMA et al. (1983)).

Ca^{2+}	0.2 –0.3 mmoles/l
Mg^{2+}	0.05–0.1 mmoles/l
K^+	0.02–0.05 mmoles/l
Na^+	0.25–0.35 mmoles/l
Cl^-	0.2 –0.3 mmoles/l
SO_4^{2-}	0 –0.1 mmoles/l
NO_3^-	0 –0.01 mmoles/l
HCO_3^- [1]	0.5 –0.7 mmoles/l

[1] Determined in this study using a dissolved carbon analyzer (Shimadzu TOC-500). In the stagnation period, HCO_3^- in anoxic hypolimnion reached 2 mM at maximum.

Table 2. Values of pE for unit activities of oxidants and reductants in neutral water (pH = 7.0, 25 °C), and equilibrium constants of six redox reactions used in the text.

Reaction	pE (at pH 7)	log K
(i) $\frac{1}{4}\ O_2(g) + H^+ + e^- = \frac{1}{2}\ H_2O$	+13.75	20.75
(ii) $O_2(g) + 2\ H^+ + 2\ e^- = H_2O_2$	+ 4.55	23.1
(iii) δ-$MnO_2(s) + 4\ H^+ + 2\ e^- = Mn^{2+} + 2\ H_2O$	+ 7.84	43.68
(iv) γ-$MnOOH(s) + 3\ H^+ + e^- = Mn^{2+} + 2\ H_2O$	+ 4.35	25.28
(v) $Mn_3O_4(s) + 8\ H^+ + 2\ e^- = 3\ Mn^{2+} + 4\ H_2O$	+ 2.81	61.63
(vi) *am*-$FeOOH(s) + 3\ H^+ + e^- = Fe^{2+} + 2\ H_2O$	– 5.01	15.99

evant species which were cited from the tables published in GARRELS & CHRIST (1965) or STUMM & MORGAN (1981). The above six redox half-cells and their equilibrium constants were listed in Table 2.

Results

The vertical distribution of the manganese-oxidizing bacterium *Metallogenium* was always confined within the vicinity of the upper boundary of the redox transition layer of the water column. Fig. 1 shows the relationships of the vertical distribution of *Metallogenium* with the ambient pE and pH values. The pE values were calculated here as the electrode-measured redox potential (E_h) divided by (RT ln 10)/F (= 59 mV). When the hypolimnion had become virtually dysoxic (June 15, July 6, Aug. 4, Sep. 4), the diagram of the vertical variation of pE and pH was lambdoidal. Lowered pE values in the hypolimnetic water in these cases were presumably due to the presence of electroactive, reductive substances such as Fe^{2+} and HS^- which were originally supplied from sediment. On the other hand, elevation of pH in the epilimnetic water was caused by the consumption of CO_2 through photosynthesis of phytoplankton. The abundance peak of *Metallogenium* was found to be always

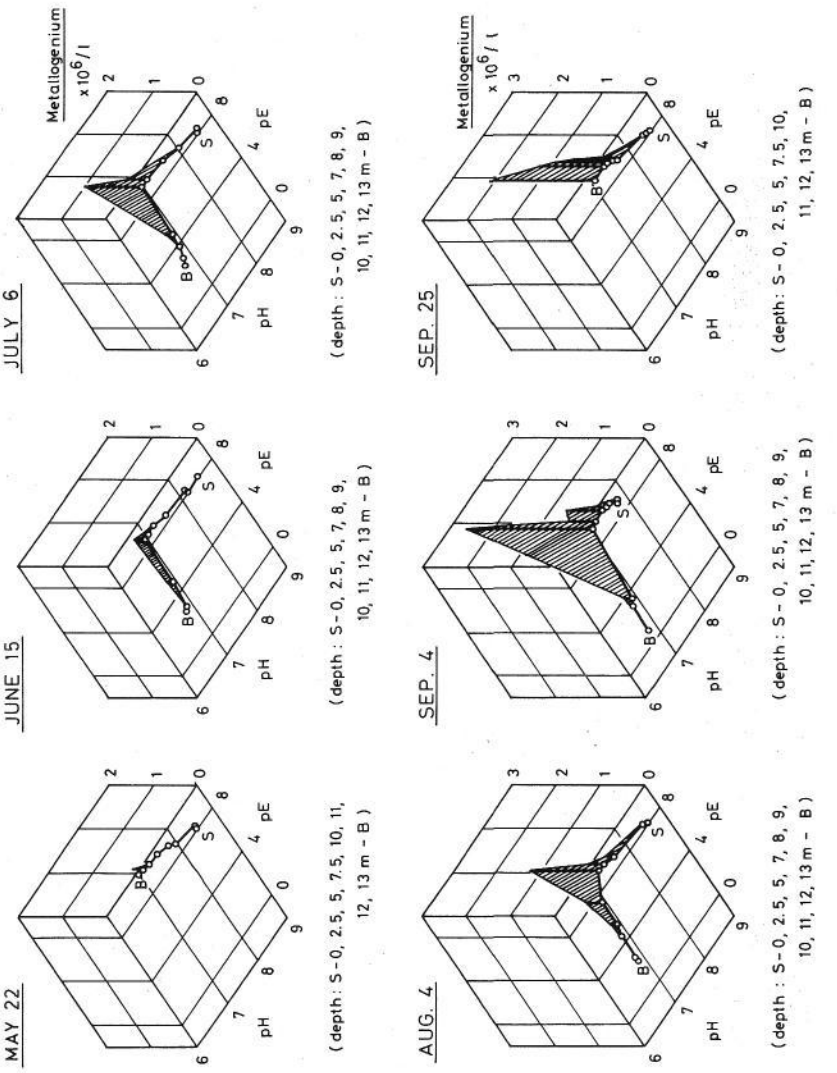

Fig. 1. Relationships among the ambient pH and pE values and the distribution of *Metallogenium* in lake water on six sampling dates. The vertical changes in pH and pE are plotted on the pH-pE planes and the distribution of *Metallogenium* at each sampling depth is shown along the ordinates. The sampling depths from 0 m ("S") to the bottom layer ("B") are denoted below the figure of each sampling date.

located just at the corner of the lambdoid of the pE-pH diagrams. Below this depth, *Metallogenium* apparently grew no longer despite the presence of Mn^{2+} in abundance. Thus it was expected that *Metallogenium* was incapable of oxidizing Mn^{2+} at $pE < 8$.

Table 3. pE values which were computed from the electrode-measured E_h values (pE measured) and those which were calculated by assuming the equilibria of (i) Mn_3O_4/Mn^{2+}, (ii) γ-$MnOOH/Mn^{2+}$, (iii) δ-MnO_2/Mn^{2+} (pE calculated) within the habitat of *Metallogenium*. The density of *Metallogenium* is also shown in the rightmost column.

Date	Depth	pE measured	pE calculated			Density of *Metallogenium*[1]
			(i)	(ii)	(iii)	
May 22	12 m	9.82	11.14	9.86	10.54	0.11
	13 m	9.86	11.01	9.80	10.56	N.D.[2]
July 6	7 m	9.18	10.88	9.53	10.13	0.04
	8 m	9.41	12.55	10.80	11.01	0.32
	9 m	9.37	13.15	11.25	11.31	1.21
	10 m	4.64	11.14	9.93	10.68	0.05
Sep. 4	5 m	9.27	10.48	9.25	9.98	0.07
	7 m	9.27	10.90	9.57	10.20	0.15
	8 m	9.32	11.31	9.86	10.38	0.76
	9 m	9.44	11.52	10.76	10.95	0.57
	10 m	9.25	12.05	10.48	10.86	2.86
	11 m	3.39	10.47	9.50	10.48	0.13
	12 m	2.92	10.54	9.55	10.52	0.05

[1] $\times 10^6$ coenobia per liter.
[2] Not determined.

Table 3 provides comparisons of the pE values in lake water which were calculated from the electrode-measured E_h values versus those which were expected from the equilibria of the MnO_x/Mn^{2+} half-cells. The former was regarded as an indicator for the average redox status of the environment. The latter was calculated as to three manganese oxides ((i) Mn_3O_4, (ii) γ-MnOOH, and (iii) δ-MnO_2) by substituting the ambient pH values and the concentration of Mn^{2+} into the equation (3). Table 3 revealed that the measured pE values (that is, those calculated from the electrode-measured E_h values) were nearly equilibrated with those expected from the manganese equilibria (particularly the γ-$MnOOH/Mn^{2+}$ equilibrium) in the layers at and above the abundance peak of *Metallogenium*. The former was found to be much lower, by contrast, than the latter below the abundance peak of *Metallogenium*. Similar results are shown graphically in Fig. 2, where the measured pE was plotted against the expected one as to the γ-$MnOOH/Mn^{2+}$ equilibrium. The points in box A which correspond to the layers at or above the abundance peak of *Metallogenium* are scattered in the vicinity of the $y = x$ line, from which deviated downward are the points in box B which correspond to the layers below the abundance peak.

The observed discrepancy between the measured pE values (low) and the ones expected from the manganese equilibria (high) in the layers deeper than the abundance peak of *Metallogenium* can be reasonably explained only when

the coexistence in lake water of those layers of electroactive, reductive substances such as Fe^{2+} which have lower oxidation potentials than Mn^{2+} is taken into account. In fact, Fe^{2+} was abundantly accumulated in the hypolimnetic layers (Fig. 3), and where the concentration of Fe^{2+} exceeds 10^{-6} M, the pE value is expected to be around or lower than zero from the am-FeOOH/Fe^{2+} equilibrium (Table 2, (vi)). Thus the electrode potential in these deeper layers seems to have been determined principally by the iron equilibrium rather than

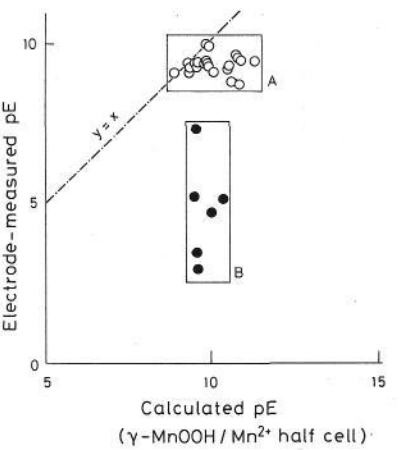

Fig. 2. The pE values which were computed from electrode-measured E_h's ("measured pE") are plotted against those which were calculated from the γ-MnOOH/Mn^{2+} equilibria ("calculated pE"). Among 56 sampling cases already presented in Fig. 1, 28 samples in which *Metallogenium* was detected were plotted in this figure. The points in box A and B correspond to the layers above and below the abundance peak of *Metallogenium*, respectively.

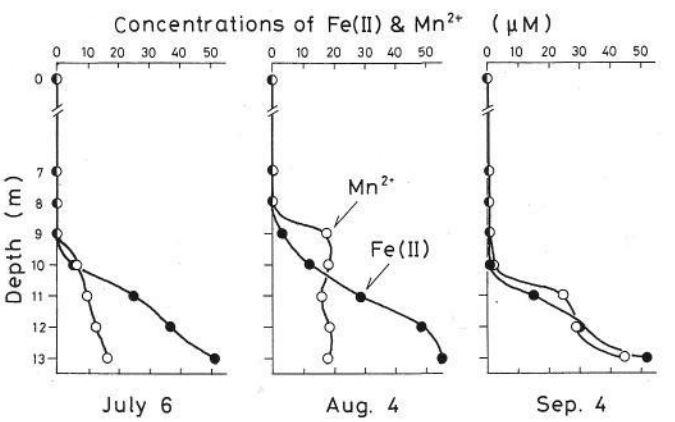

Fig. 3. The vertical distributions of Fe(II) and Mn^{2+} in lake water of the dredged area.

the manganese one. Due to such reductive species as Fe^{2+}, the environmental condition in these layers would have favoured the reduction of MnO_x rather than the oxidation of Mn^{2+}.

It should be noted here that the actual redox conditions in lake waters of the layers above the abundance peak of *Metallogenium* should have been more oxidative than expected from the electrode potential. Although the MnO_x/Mn^{2+} half-cell was apparently nearly equilibrated with the ambient redox status (Fig. 2, box A), this is perhaps the result of that the lake water of these layers would have lacked any electroactive species other than Mn^{2+}. In these layers dissolved oxygen was always distributed at measurable levels ($> 10^{-5}$ M), but the electrode potential can hardly reflect the potential of O_2 because of low exchange current of the O_2/H_2O half-cell (STUMM & MORGAN, 1981). Where a measurable concentration of O_2 is distributed, the pE value is predicted to range 12–13 from the O_2/H_2O half-cell (Table 2 (i)), and this value is considerably high compared with the pE values of the natural habitat of *Metallogenium* expected from the manganese equilibria (Table 3). This means that the concentration of Mn^{2+} therein should have been supersaturated as regards the oxygenation.

Table 4 shows the measured concentration of Mn^{2+} in lake water in which *Metallogenium* was detected, comparing them with the solubilities of Mn^{2+} with respect to the oxidative precipitations of (i) Mn_3O_4, (ii) γ-MnOOH, and (iii) δ-MnO_2. The latter was calculated by the equation (8) with the pH value and the concentration of O_2 in lake water. It is convinced by these results that Mn^{2+} was always supersaturated with regard to any oxygenation reactions in

Table 4. The actual concentrations and the equilibrium concentrations (solubilities) of Mn^{2+} within the habitat of *Metallogenium*. The solubilities are calculated as regards the oxidative precipitations of (i) Mn_3O_4, (ii) γ-MnOOH, (iii) δ-MnO_2. The density of *Metallogenium* is also presented in the rightmost column.

Date	Depth	Actual conc.[1]	Solubilities[1]			Density of *Metallogenium*[2]
			(i)	(ii)	(iii)	
June 15	9 m	6.22	7.14	9.18	11.39	0.04
	10 m	5.92	6.76	8.78	10.95	0.14
	11 m	5.34	6.72	8.75	10.93	0.02
July 6	7 m	6.89	8.27	10.31	12.52	0.04
	8 m	6.72	7.22	9.22	11.29	0.32
	9 m	6.72	6.94	8.95	11.06	1.21
	10 m	5.22	6.80	8.80	10.87	0.05
July 24	8 m	6.17	6.95	9.01	11.02	12.4
	10 m	4.89	7.02	9.06	11.00	0.49

[1] $-\log_{10}[Mn^{2+}]$. $[Mn^{2+}]$ indicates the molar concentration of Mn^{2+}.
[2] $\times 10^6$ coenobia per liter.

the habitat of *Metallogenium*, that is, the manganese oxidation reaction catalyzed by *Metallogenium* was itself a thermodynamically spontaneous reaction. The degree of supersaturation was the most prominent for the precipitation of δ-MnO_2, and relatively moderate for the precipitation of Mn_3O_4.

Discussion

The manganese-oxidizing bacteria referred to the genus *Metallogenium* have been included to the Mycoplasmatales (HIRSCH, 1974; ZAVARZIN, 1989), although there are still some debates about the origin and the viability of naturally occurring "*Metallogenium*-like structures" (MARGULIS et al., 1983; MAKI et al., 1987; EMERSON et al., 1989). The stable cultivation of *Metallogenium* under the laboratory condition has not been reported except the binary cultures with some imperfect fungi. However, DUBININA (1984) has found that some non-manganese-oxidizing bacteria such as *Arthrobacter pascens* could be infected with *Metallogenium symbioticum* with had originally parasitized on a fungus. Also, the present author has isolated a number of heterotrophic bacteria which had a manganese-oxidizing ability from lake water and sediment of Lake Biwa, and found that virtually all of these isolates showed the ability to produce "*Metallogenium*-like structures" encrusted with manganese oxide when incubated in proper liquid media (Figs. 4, 5). These findings suggest that the manganese-oxidizing ability of many, if not all, of so-called "manganese-oxidizing bacteria" would be owed to parasitic *Metallogenium* and that the "*Metallogenium*-

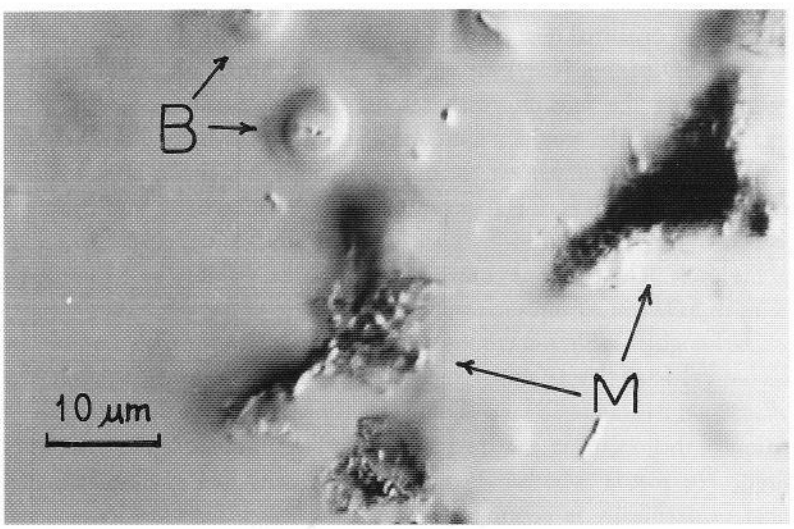

Fig. 4. *Metallogenium*-like structure (M) which was developed in association with the growth of capsulated *Bacillus* sp. (B) in a liquid medium. Bar, 10 µm.

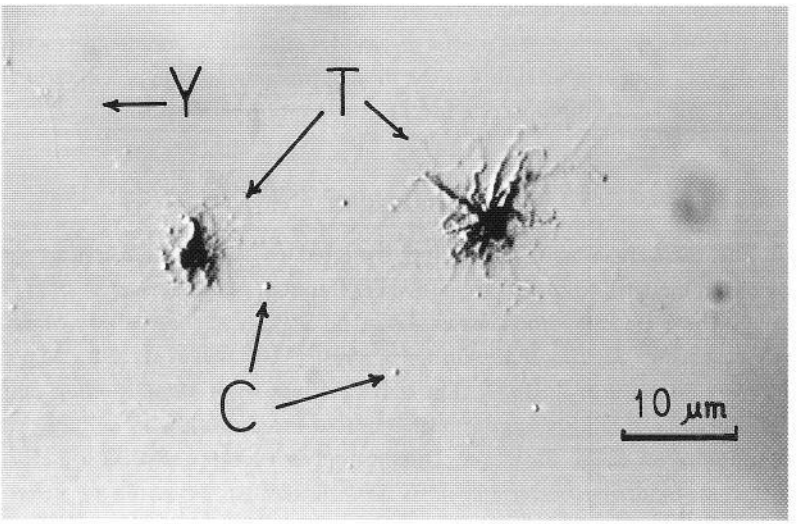

Fig. 5. Typical trichospheric structures (T) of *Metallogenium* which appeared in association with the growth of unidentified Gram-positive bacteria (not photographed). Putative coccoid stage (C) and young trichospheric stage without manganese encrustation (Y) are also seen. Bar, 10 μm.

like structures" which occur in so many natural lakes would originate in such parasitism. Thus it seems also feasible that the apparent diversity in genera which has often been reported for aquatic manganese-oxidizing bacteria (e.g. GREGORY & STALEY, 1982) would have been resulted in part from the broad spectrum of potential host bacteria for the parasitism by *Metallogenium*. BALASHOVA & DUBININA (1989) succeeded in axenic cultivation of *Metallogenium* by amending the medium with serum. But the fastidious nature of this organism about the trophic requirement and the growth condition renders it less likely for *Metallogenium* to follow a wholly free-living life cycle in its natural habitat in lakes. It is thus probable that *Metallogenium* would grow as a parasite of native planktonic bacteria in the natural habitat, at least in a part of its life cycle. The previous controversy about the viability of *Metallogenium* seems to have come from such a peculiar life cycle of this organism.

Although possible autotrophy of *Metallogenium* on the oxidation of Mn^{2+} has been claimed (KELLY, 1981), the difficulties in the axenic cultivation of *Metallogenium* has hindered severely the experimental investigations into the nutrition of this organism and the physiological significance of the oxidation of Mn^{2+}. No matter whether *Metallogenium* is cultivated symbiotically with fungi or axenically in serum-containing media, *Metallogenium* cannot grow without supply of appropriate organic substrates. By contrast, it is also known that the supply of Mn^{2+} is not a *sine-qua-non* condition for sustaining the

viability of *Metallogenium* (Zavarzin & Epikhina, 1963). For these reasons, the Soviet microbiologists contended that *Metallogenium* is among the chemoorganotrophic microorganisms (Zavarzin, 1989).

Furthermore, it cannot be taken for granted that the electron acceptor used in the biological oxidation of Mn^{2+} in general would be molecular oxygen. Dubinina (1978) showed that the oxidation of Mn^{2+} mediated by *Leptothrix pseudoochracea* occurred with H_2O_2 as an oxidant, and suggested that this strain could take an advantage by removing at the expense of Mn^{2+} toxic H_2O_2 that resulted from its own respiration. She also noted without showing data that the peroxide mechanism of the oxidation of Mn^{2+} could be applied to other manganese-oxidizing bacteria such as *Metallogenium*. On the other hand, Boogerd & De Vrind (1987) evidenced that an extracellular protein excreted by *Leptothrix discophora* could oxidize Mn^{2+} at the expense of molecular oxygen. Similarly, the oxidation of Mn^{2+} by crude cell-free extracts which has been reported for various manganese-oxidizing bacteria (Nealson et al., 1989) cannot necessarily be interpreted by the peroxide mechanism alone. Thus the majority of manganese-oxidizing bacteria seem to have the ability to oxidize Mn^{2+} with O_2. It seems possible, however, that some strains can utilize H_2O_2 as an alternative electron acceptor to oxidize Mn^{2+} when available H_2O_2 is supplied in abundance. H_2O_2 is also known to cause the oxidation of Mn^{2+} abiotically in alkaline condition, though this reaction yields γ-MnOOH rather than MnO_2 (Bricker, 1965).

In this study, the present author examined from the thermodynamic point of view the factors which would be critical in the occurrence and the distribution of *Metallogenium* in a lacustrine environment. One of the conclusions of this study is that the oxidation of Mn^{2+} with O_2 was thermodynamically spontaneous in the natural habitat of *Metallogenium*. That is to say, there was a disequilibrium in the redox transition layer of the water column between the half-cell potentials of O_2/H_2O (high) and of MnO_x/Mn^{2+} (low). This can be explained by the inertness of Mn^{2+} against the oxygenation reaction (Stumm & Morgan, 1981). Despite the thermodynamic tendency the abiotic oxidation of Mn^{2+} with O_2 should not advance so rapidly as it could match up to the diffusional supply of Mn^{2+} from contiguous reductive layer owing to high activation energy for the oxygenation of Mn^{2+}. Such a disequilibrium which favors the oxidation of Mn^{2+} seems to be a necessary condition for *Metallogenium* to occur and precipitate manganese in lake water. Where a micromolar level of Mn^{2+} is furnished constantly, *Metallogenium* can remove it by the oxidative precipitation much more effectively than any non-biological removal processes expected in lake water (Miyajima, 1992). Thus, in summary, the kinetic superiority of *Metallogenium* to abiotic processes in the oxidation of Mn^{2+} seems to provide this organism with an ecological niche in the redox transition layer of lakes where Mn^{2+} is supplied sufficiently.

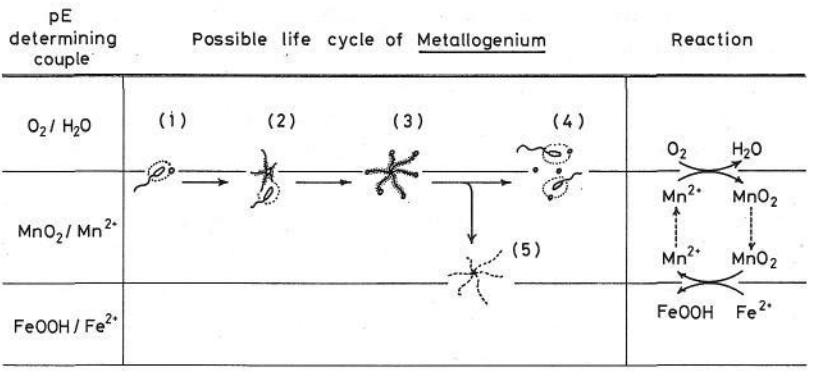

Fig. 6. Simplified vertical structure of water column in relation to the pE determining redox couples and habitat zonation pattern of *Metallogenium*. Developmental stages of *Metallogenium* are: (1) coccoid stage, associated with other ordinary bacteria, (2) germination of coccoid cell, (3) trichospheric stage, encrusted with manganese oxide, forming new coccoids at the tips of branches, (4) re-infection of coccoid cells on other bacteria, (5) deposition of the aged trichospheric body, followed by reductive dissolution of manganese oxide. Physiological implication of the parasitic association of *Metallogenium* with other bacteria (see text) is unknown. Information of the life cycle of *Metallogenium* is partially owed to ZAVARZIN (1989).

The second finding in this study is that, below the depth at which the vertical abundance peak of *Metallogenium* was located, the overall redox status of environment seemed to be controlled principally by certain reductive species such as Fe^{2+} which had lower oxidation potentials than Mn^{2+}. Existence of such reductive species should have caused reductive dissolution of manganese oxide produced by *Metallogenium*. As for Fe^{2+}, the reductive dissolution followed by the pseudomorphic replacement of manganese oxides by iron has been reported (GOLDEN et al., 1988). So the lower boundary of the habitat of *Metallogenium* should have been also determined by the appearance of such reductive species in deeper layers.

Thus, the occurrence and the vertical distribution of *Metallogenium* can be explained solely by the thermodynamic terms: namely, the thermodynamic disequilibrium between O_2 and Mn^{2+} in the habitat of *Metallogenium* and that between manganese oxide and Fe^{2+} (or other reductants) below the habitat. Such a situation is illustrated in Fig. 6. *Metallogenium* should, as a result, grow and dwell in the environmental gradients of O_2, Mn^{2+}, and reductive species such as Fe^{2+}, sorting condition the most comfortable for its growth in essentially uneven environment, which should result in the steeply peaked vertical distribution of *Metallogenium* as illustrated in Fig. 1.

Such ecological properties of *Metallogenium* as cited in the above paragraphs seem to have some parallelism with those of other bacteria that mediate electron transfer reactions between inorganics such as nitrification, sulfur ox-

idation, Fe^{2+} oxidation (KRUMBEIN, 1983), as well as with those of the methylotrophic bacteria (HANSON, 1980). Many of those bacteria which catalyze the oxidation of inorganics are chemolithotrophs in such a sense that they can utilize those reactions as an energy source for their metabolisms. The manganese oxidation catalyzed by *Metallogenium* in lake water is also a thermodynamically spontaneous (hence exergonic) reaction. For example, if *Metallogenium* oxidizes Mn^{2+} with O_2 into δ-MnO_2, the free energy released by this reaction into the surroundings ($-\Delta G_{sys}$ [kJ mole^{-1} O_2]) is given based on the equation (9) as:

$$-\Delta G_{sys} = -7.84 + 5.71\,(4\,pH + 2\log(Mn^{2+}) + \log(O_2)), \quad (10)$$

where the bracket ( ) means the activity. In the case where $(Mn^{2+}) = 10^{-6}$, $(O_2) = 10^{-5}$, and pH = 7.0, this reaction yields 54.3 kJ per 1 mole of O_2 consumed, which is enough, for example, to synthesize 1 mole of ATP from ADP + P_i (which requires 30.6 kJ for unit activity of reactants). The kinetics of the manganese oxidation of an individual coenobium of *Metallogenium* (v_{ind} [fmoles Mn^{2+} hr^{-1}]) is expressed as follows (MIYAJIMA, 1992):

$$v_{ind} = 34.2\,C_{Mn}/(2.40 + C_{Mn}), \quad (11)$$

where C_{Mn} [μM] is the concentration of Mn^{2+}. Then, for $(Mn^{2+}) = 10^{-6}$, one coenobium of *Metallogenium* would oxidize ca. 10 fmoles of Mn^{2+} per hour at the expense of 5 fmoles of O_2, and henceforth 5 fmoles of ATP could be synthesized per hour. This amount is not small as an energy budget. Suppose that one mole of carbon dioxide be converted into cellular biomass via autotrophic pathway with about 7.9 moles of ATP consumed (SCHLEGEL, 1975), the supply of 5 fmoles of ATP would yield some 0.63 fg-at.C of cellular standing block. For reference, NAGATA (1986) reported that the carbon content per unit volume of natural bacterial cells collected from Lake Biwa ranged 3–15 fg-at. μm^{-3}. Thus, in theory, the manganese oxidation reaction seems to be able to support the chemolithotrophic growth of bacteria.

The energetics of the oxidation of Mn^{2+} with H_2O_2 as an oxidant can be also treated qualitatively by relating it with the equilibrium between O_2 and H_2O_2 (Table 2 (ii)). The oxidation reaction of Mn^{2+} with H_2O_2 is written as follows:

$$Mn^{2+} + H_2O_2 = \delta\text{-}MnO_2 + 2H^+. \quad (12)$$

If the half-cell potential of O_2/H_2O_2 is in equilibrium with that of O_2/H_2O, the amount of free energy liberated by the oxidation of Mn^{2+} with H_2O_2 (12) is equivalent, by definition, to that liberated by the oxidation of Mn^{2+} with O_2. But in real lake waters, the concentration of H_2O_2 may be still much higher than predicted by the direct equilibrium between O_2 and H_2O_2, due to respiration of bacteria and certain photochemical processes which both should produce significant H_2O_2 against the thermodynamic tendency. In such a case

the oxidation of Mn^{2+} with H_2O_2 yields more energy than the oxidation with O_2 as the sole oxidant, and thus the use of the peroxide mechanism of the manganese oxidation might set up the manganese-oxidizing bacteria with an energetic advantage if constant supply of peroxide is ensured.

Thus, the chemolithotrophy of *Metallogenium* on the oxidation of Mn^{2+} seems rather possible from both the ecological and energetic points of view, no matter whether Mn^{2+} was oxidized with oxygen or with hydrogen peroxide as the oxidant. Of course this does not reject other possibilities, but if the physiological significance of the manganese oxidation for *Metallogenium* were to be elsewhere as suggested by e.g. DUBININA (1978), then another question should arise. Namely, why would not the energy-yielding metabolism on the manganese oxidation occur in nature, for all the thermodynamic consistency? At present, more detailed knowledge of the biochemistry and physiology of manganese-oxidizing bacteria is needed for interpreting without ambiguity the ecological meaning of the manganese oxidation for them and predicting their occurrence and behavior in natural lakes. Now that the close association of *Metallogenium* with heterotrophic bacteria and fungi has been recognized, it seems of particular importance to elucidate the role of these host microorganisms in the development and manganese oxidation of *Metallogenium*.

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References

BALASHOVA, V. V. & DUBININA, G. A. (1989): The ultrastructure of *Metallogenium* in axenic culture. – Mikrobiologiya **58:** 841–846.

BOOGERD, F. C. & DE VRIND, J. P. M. (1987): Manganese oxidation by *Leptothrix discophora*. – J. Bacteriol. **169:** 489–494.

BRICKER, O. (1965): Some stability relations in the system $Mn-O_2-H_2O$ at 25° and one atmosphere total pressure. – Am. Miner. **50:** 1296–1354.

CHUKHROV, F. V., GORSHKOV, A. I., RUDNITSKAYA, E. S., BERESOVSKAYA, V. V. & SIVTSOV, A. V. (1980): Manganese minerals in clays: A review. – Clays Clay Miner. **28:** 346–354.

CLOUD, P. E. Jr. (1965): Significance of the Gunflint (Precambrian) microflora. – Science **148:** 27–35.

DUBININA, G. A. (1978): Functional role of bivalent iron and manganese in *Leptothrix pseudoochracea*. – Mikrobiologiya **47:** 783–789.

– (1984): Infection of prokaryotic and eukaryotic microorganisms with *Metallogenium*. – Curr. Microbiol. **11:** 349–356.

EMERSON, D., GAREN, R. E. & GHIORSE, W. C. (1989): Formation of *Metallogenium*-like structures by a manganese-oxidizing fungus. – Arch. Microbiol. **151:** 223–231.

GARRELS, R. M. & CHRIST, C. L. (1965): Solutions, Minerals, and Equilibria. – Harper & Row, New York.

GOLDEN, D. C., CHEN, C. C., DIXON, J. B. & TOKASHIKI, Y. (1988): Pseudomorphic replacement of manganese oxides by iron oxide minerals. – Geoderma **42:** 199–211.

GREGORY, E. & STALEY, J. T. (1982): Widespread distribution of ability to oxidize manganese among freshwater bacteria. – Appl. Environ. Microbiol. **44:** 509–511.

GRILL, E. V. (1982): Kinetic and thermodynamic factors controlling manganese concentrations in oceanic waters. – Geochim. Cosmochim. Acta **46:** 2435–2446.

HANSON, R. S. (1980): Ecology and diversity of methylotrophic organisms. – Adv. Appl. Microbiol. **26:** 3–39.

HASTINGS, D. & EMERSON, S. (1986): Oxidation of manganese by spores of a marine bacillus: Kinetic and thermodynamic considerations. – Geochim. Cosmochim. Acta **50:** 1819–1824.

HEM, J. D. & LIND, C. J. (1983): Nonequilibrium models for predicting forms of precipitated manganese oxides. – Geochim. Cosmochim. Acta **47:** 2037–2046.

HIRSCH, P. (1974): Budding bacteria. – Annu. Rev. Microbiol. **28:** 391–444.

KAWASHIMA, M., HARA, H., ITASAKA, O., HORI, T., TAKAMATSU, T. & KOYAMA, M. (1983): Chemical changes in Lake Biwa, I. Release from sediment and redox cycle of manganese, iron, and nutrients. – Annu. Rep. Inst. Lake Sci. Fac. Education, Shiga Univ. **22:** 37–72 (in Japanese with English summary).

KELLY, D. P. (1981): Introduction to the chemolithotrophic bacteria. – In: STARR, M. P. et al. (eds.), The Prokaryotes: A Handbook on Habitats, Isolation, and Identification of Bacteria, pp. 997–1004. – Springer-Verlag, Berlin.

KRUMBEIN, W. E. (ed.) (1983): Microbial Geochemistry. – Blackwell, Oxford.

MAKI, J. S., TEBO, B. M., PALMER, F. E., NEALSON, K. H. & STALEY, J. T. (1987): The abundance and biological activity of manganese-oxidizing bacteria and *Metallogenium*-like morphotypes in Lake Washington, USA. – FEMS Microbiol. Ecol. **45:** 21–29.

MANN, S., SPARKS, N. H. C., SCOTT, G. H. E. & DE VRIND-DE JONG, E. W. (1988): Oxidation of manganese and formation of Mn_3O_4 (hausmannite) by spore coats of marine *Bacillus* sp. – Appl. Environ. Microbiol. **54:** 2140–2143.

MARGULIS, L., GROSOVSKY, B. D. D., STOLZ, J. F., GONG-COLLINS, E. J., LENK, S., READ, D. & LÓPEZ-CORTÉS, A. (1983): Distinctive microbial structures and the pre-Phanerozoic fossil record. – Precamb. Res. **20:** 443–477.

MIYAJIMA, T. (1992): Biological manganese oxidation in a lake I: Occurrence and distribution of *Metallogenium* sp. and its kinetic property. – Arch. Hydrobiol. **124:** 317–335.

MURRAY, J. W., DILLARD, J. G., GIOVANOLI, R., MOERS, H. & STUMM, W. (1985): Oxidation of Mn(II): Initial mineralogy, oxidation state and aging. – Geochim. Cosmochim. Acta **49:** 463–470.

NAGATA, T. (1986): Carbon and nitrogen content of natural planktonic bacteria. – Appl. Environ. Microbiol. **52:** 28–32.

NEALSON, K. H., ROSSON, R. A. & MYERS, C. R. (1989): Mechanisms of oxidation and reduction of manganese. – In: BEVERIDGE, T. J. & DOYLE, R. J. (eds.), Metal Ions and Bacteria, pp. 383–411. – Wiley-Interscience, New York.

SCHLEGEL, H. G. (1975): Mechanisms of chemo-autotrophy. – In: KINNE, O. (ed.), Marine Ecology, vol. 2, pp. 9–60. – Wiley-Interscience, New York.

STUMM, W. & GIOVANOLI, R. (1976): On the nature of particulate manganese in simulated lake waters. – Chimia **30:** 423–425.

STUMM, W. & MORGAN, J. J. (1981): Aquatic Chemistry (2nd ed.). – Wiley-Interscience, New York.

TIPPING, E., THOMPSON, G. W. & DAVISON, W. (1984): Oxidation products of Mn(II) in lake waters. – Chem. Geol. **44:** 359–383.

WALTER, M. R., GOODE, A. D. T. & HALL, W. D. M. (1976): Microfossils from a newly discovered Precambrian stromatolitic iron formation in Western Australia. – Nature Lond. **261:** 221–223.

ZAVARZIN, G. A. (1981): The genus *Metallogenium.* – In: STARR, M. P. et al. (eds.), The Prokaryotes: A Handbook on Habitats, Isolation, and Identification of Bacteria, pp. 524–528. – Springer-Verlag, Berlin.

– (1989): Genus "*Metallogenium*" PERFIL'EV & GABE, 1961, 50. – In: STALEY, J. T. et al. (eds.), Bergey's Manual of Systematic Bacteriology, 9th ed., vol. 3, pp. 1986–1989. – Williams & Wilkins, Baltimore.

ZAVARZIN, G. A. & EPIKHINA, V. V. (1963): Symbiotic growth of *Metallogenium.* – Dokl. Biol. Sci. **148:** 151–152.

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