Arch. Hydrobiol.	124	3	317–335	Stuttgart, Mai 1992

Biological manganese oxidation in a lake I: Occurrence and distribution of *Metallogenium* sp. and its kinetic properties

By Toshihiro Miyajima

Otsu Hydrobiological Station, Kyoto University[1, 2]

With 8 figures in the text

Abstract

Biologically mediated oxidation of manganese occurring at the redox gradient of a lake was studied from the kinetic viewpoint. With regard to the microbial oxidative precipitation, the residence time of Mn^{2+} at the redox gradient was estimated to be 2–5 days, which was much shorter than those expected for any abiotic oxygenations of Mn^{2+} at pH ~7. The manganese precipitation at this site was found to be principally owed to the activity of the planktonic manganese-oxidizing bacterium *Metallogenium personatum* inhabiting at the redox gradient in the stagnation period, forming there a MnO_x/Mn^{2+} boundary. The population growth of *Metallogenium* seemed to be limited simply by the diffusional supply of Mn^{2+} from the underlying anoxic layer to its habitat. The kinetics for the precipitation of manganese by *Metallogenium* was consistent with the Michaelis-Menten's formula, with K_m = 2.40 μM and V_{max} = 34.2 fmoles Mn^{2+} hr^{-1} per individual coenobium of *Metallogenium*.

Introduction

Physical and chemical forms of manganese existing in lake water are severely affected by the ambient redox conditions. Under such an oxidative condition as in surface lake water which is constantly exposed to atmosphere, manganese usually exists as insoluble oxides assuming the oxidation numbers of 3 or 4, whereas the aqueous divalent cation Mn^{2+} is the most stable manganese species under more reductive conditions such as those encountered in the anoxic hypolimnion of well stratified lakes (Stumm & Morgan, 1981). Consequently, there is often formed an MnO_x/Mn^{2+} boundary at the redox gradient which is established between the oxygenated epilimnion and the an-

[1] Address of the author: Otsu Hydrobiological Station, Kyoto University, Shimosakamoto, Otsu 520-01, Japan.

[2] Present address: Center for Ecological Research, Kyoto University, Shimosakamoto, Otsu 520-01, Japan.

0003-9136/92/0124-0317 $ 4.75

oxic hypolimnion in a stratified lake, and intense redox reactions of manganese can take place at this boundary. Mn^{2+} which diffuses up from underlying anoxic hypolimnion or sediment can be readily oxidized at this boundary, presumably at the expense of dissolved O_2, into insoluble oxide minerals (MnO_x), which then settles down to the anoxic hypolimnion and is reduced and solubilized again into Mn^{2+}. As a result, the MnO_x/Mn^{2+} boundary is observed in natural lakes as a peak of the vertical concentration profile of particulate (that is, the oxidized forms of) manganese. On the other hand, dissolved manganese (Mn^{2+}(aq)) is accumulated exclusively below this boundary (DAVISON, 1985). It is known in many lakes ever studied that the oxidative precipitation of Mn^{2+} at the MnO_x/Mn^{2+} boundary is mediated by manganese-oxidizing bacteria (NEALSON et al., 1988).

Lake Biwa is the largest, mesotrophic lake in Japan. In the north (main) basin of this lake, the whole water column is oxygenated throughout the year and then the redox cline as well as the MnO_x/Mn^{2+} boundary always stays within the sediment or at the water/sediment interface. It has been reported that particulate manganese but not Mn^{2+} is accumulated in the deepmost layer of this basin during the summer stagnation (KAWASHIMA et al., 1988). By contrast to the other part of Lake Biwa, a seasonal bottom anoxia occurs in summer at a dredged area of the south basin of this lake (TERASHIMA & UEDA, 1982), and a typical MnO_x/Mn^{2+} boundary appears in the middle of the water column. In the latter case, the importance of biological processes relative to chemical ones in the precipitation of manganese has already been evidenced by the biological inhibition techniques (KAWASHIMA et al., 1988).

In this and the following papers, the present author reports some ecological features and geochemical activity of the manganese-oxidizing bacteria which occur in the dredged area of Lake Biwa. The kinetic aspect of the biological manganese oxidation and the seasonal population dynamics of the manganese-oxidizing bacteria are discussed mainly in this paper, whereas some factors which control the occurrence and distribution of the manganese-oxidizing bacteria are examined in some detail in the second paper.

Material and Methods

1. Study site

This study was carried out mainly during the stagnation period of 1989. The study site was a dredged area located in the south basin of Lake Biwa (Fig. 1). It is ca. 500 m × 500 m in area and ca. 13 m in depth. Though the other part of the south basin of Lake Biwa is so shallow (ca. 3.5 m in depth) that thermal stratification cannot be formed throughout the year, the dredged area is sufficiently deep and lake water is thermally stratified in summer (April to September). For the detailed hydrography of this site, the reader is referred to TERASHIMA & UEDA (1982).

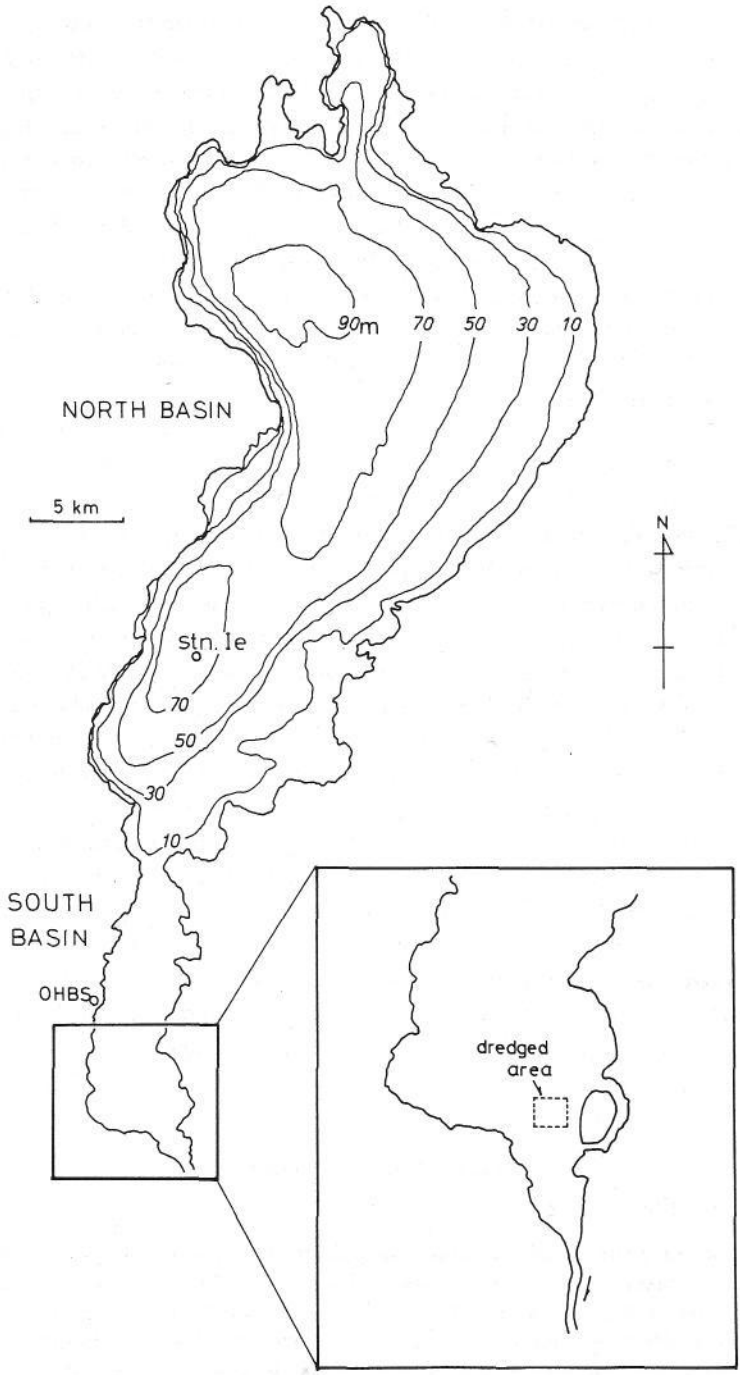

Fig. 1. Location of a dredged area in Lake Biwa. OHBS indicates the Otsu Hydrobiological Station.

2. Sampling and sample preparation

Lake water and sediment were collected with a Kitahara-type water sampler (500 ml in volume) and a gravity corer (∅ 50 mm), respectively. Water temperature was determined in situ with a thermistor thermometer. Samples assigned for microscopic observations were fixed immediately with glutaraldehyde solution (final 1 %) and stored at 4 °C. The color development for the photometric determination of Fe(II) was also performed immediately after sampling to preclude the oxidation during storage. After transported on ice to the laboratory, an aliquot of each lake water sample was filtered through a glass fiber filter (Whatman, GF/F) which had been prewashed with 1 N HCl (overnight) and then three times with deionized water. The sediment pore water was extracted from each sediment sample by centrifugation (6000 g, 20 min, at 5 °C). These sediment pore waters and the filtered and unfiltered aliquots of water samples were acidified with a few drops of 6 N HCl after the determination of pH and E_h (see below) and stored at −20 °C.

3. Chemical analyses

The pH and E_h values of lake water were determined with a pH meter (HORIBA, model F-8L), using unfiltered samples, within 3 hours after sampling. The pE values were calculated as E_h divided by (RT ln 10)/F (Stumm & Morgan, 1981). Dissolved oxygen was determined by the Winkler titration with the azide modification.

The concentration of total Fe(II) was measured colorimetrically with 1, 10-phenanthroline solution (Moss & Mellon, 1942). For the analysis of total Fe (Fe(II) + Fe(III)), unfiltered water samples were digested with HCl (final 0.5 N, 110 °C, 10 min), reduced with hydroxylammonium solution, and determined as Fe^{2+} by the phenanthroline method. PO_4^{3-} was determined by molybdenum blue method using the filtered samples (Murphy & Riley, 1962). The concentration of Mn^{2+} was determined with formaldoxime solution after the method of Brewer & Spencer (1971) using the filtered lake water samples or the pore water samples. Manganese contained in these samples were all regarded as Mn^{2+}(aq), assuming that all other manganese species such as oxide and carbonate existed as the particulate form.

4. Estimation of manganese oxidation rates

The rate constants of manganese oxidation in situ were estimated by the precipitation experiment. The lake water sample used for this experiment was collected originally from the vicinity of the MnO_x/Mn^{2+} boundary (10 m layer on September 4), and then it was diluted prior to the experiment with deionized, sterilized water (vol : vol = 1 : 4) because it contained originally a significant amount of Mn^{2+}. By this dilution, sufficient oxygen was also brought into the sample so that the partial pressure of oxygen in the experimental system was supposed to be approximately constant (0.2 atm) throughout the experimental period. 500 ml portions of the diluted lake water were poured into sterilized glass bottles, enriched aseptically with various concentrations of Mn^{2+} (added as $MnSO_4$), and then incubated in the dark at 15 °C with occasionally shaken. Appropriate amounts of subsamples were withdrawn from these bottles at intervals. The precipitates contained in these subsamples were then trapped on Millipore HA filters and analyzed for Mn content by the formaldoxime method – manganese oxide trapped on the filters were readily solubilized by formaldoxime solution itself. The amount of precipitated manganese was considered approximately identical to

that of oxidized manganese, although the former might have included some Mn^{2+} adsorbed on the surface of the precipitates.

The average precipitation rate during the first 100 hours in each bottle was plotted against the initial concentration of Mn^{2+} which was measured using the filtrates of subsamples at the starting point. The kinetic constants were determined according to the Michaelis-Menten's formula.

5. Differential filtration experiments

In some instances, the differential filtration technique was applied in order to separate the particulate material contained in lake water into size fractions. Aliquots of water samples were passed through Nuclepore membrane filters of various pore sizes (0.2, 1.0, 3.0, and 10.0 μm). Particulate material trapped on each filter was then digested with 0.5 N HCl (20 min at 110 °C), whereby oxide and sulfide of iron as well as oxide of manganese could be solubilized completely. $Fe^{2+} + Fe^{3+}$, Mn^{2+}, and PO_4^{3-} liberated into the aqueous phase were then determined as described above. The amounts of these ingredients belonging to each size fraction were calculated by subtraction.

6. Microscopy

The numbers of total bacterial cells were counted by the standard AODC method. Countings of metal-depositing microorganisms were carried out as follows (Jones, 1981). A 10–20 ml portion of the lake water sample (prefixed with glutaraldehyde) was passed through a Millipore HA membrane (∅ 25 mm). This filter was dried at ca. 40 °C and cleared with a few drops of immersion oil for microscope, and the material trapped on it was observed with an interference microscope (Nikon, OPTIPHOT). The coenobium of manganese-oxidizing bacterium *Metallogenium personatum* is readily distinguished by its characteristic trichospherical morphology (usually 5–10 μm in diameter) assuming brownish color of manganese oxide (Zavarzin, 1989). Individual cells of *Metallogenium* involved presumably within the coenobium could not be visualized by this method or by the AODC method, even when deposited manganese oxide was removed from the coenobium by oxalic acid solution. For this reason, the numbers of coenobia were counted as to *Metallogenium*. Other morphologically distinct microorganisms such as iron bacteria and anoxygenic phototrophic bacteria could also be detected on the same filters. Among iron bacteria, the encapsulated types belonging to the family Siderocapsaceae were rather dominant and the filamentous types of the *Leptothrix*-group were generally rare in the dredged area of Lake Biwa. In this study, *Ochrobium* sp., one of the most dominant species of the Siderocapsaceae, was counted in order to compare its habitat zonation pattern with that of the manganese-oxidizing bacteria. *Ochrobium* looks like a torus under the microscope because of iron oxide deposited on its extracellular structure (Jones, 1981) and a botryoidal coenobium is occasionally formed which consists of some tens of cells. The numbers of individual cells were counted as to *Ochrobium* sp. The counting procedure for the metal-depositing bacteria is similar to that of the standard AODC method. Countings of the metal-depositing microorganisms was performed as early as possible after the fixation with glutaraldehyde, though any visible morphological alterations of these microorganisms were not detected under the microscope during storage of fixed samples for more than one month.

Results

1. Vertical and seasonal changes in the physicochemical environments

The thermal stratification of lake water in the dredged area of Lake Biwa persisted from April to September in 1989, though a partial circulation was caused by a hit of a typhoon at the beginning of September. Fig. 2 shows the vertical and seasonal trends in some representative environmental factors during the stagnation period in 1989. The distributions of water temperature and oxygen (Fig. 2 (a), (b)) are the most representative of the vertically stratified structure. In summer, lake water seemed to be circulating constantly only above 5 m, and isolated bottom water has become virtually anoxic in July and August. The pH values in the epilimnion was elevated to near 9 owing to the CO_2 depletion by photosynthesis of planktonic algae (Fig. 2 (c)). The pE value,

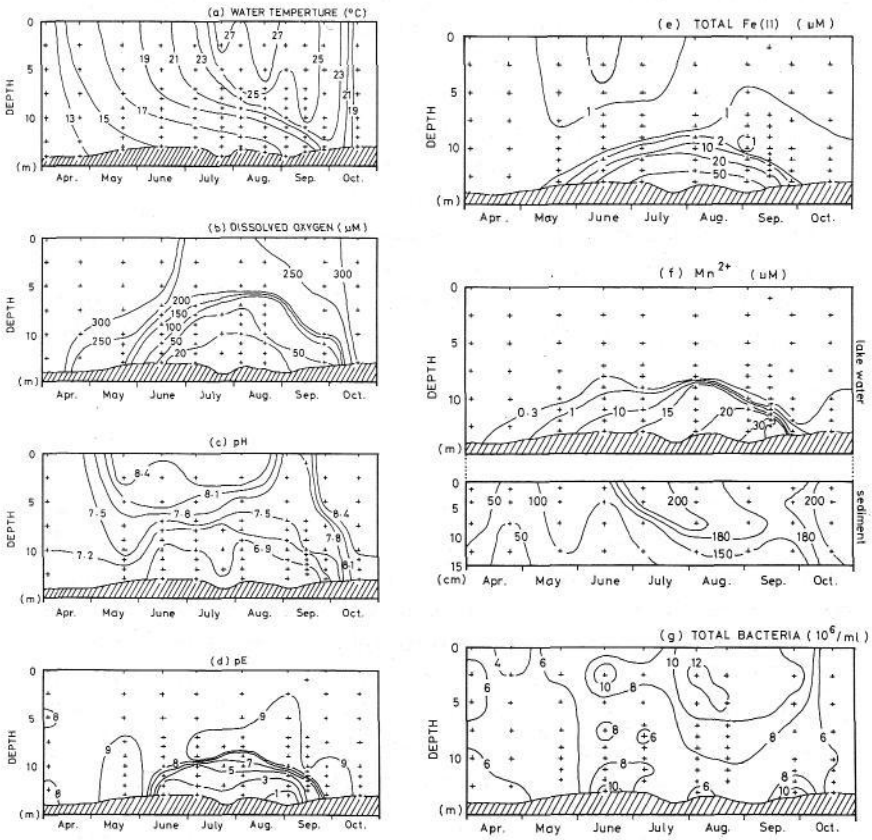

Fig. 2. The vertical and seasonal variations in water temperature (a), dissolved oxygen (b), pH (c), pE (d), concentrations of total Fe(II) (e) and Mn^{2+} (f), and density of the total bacterial cells (g) in the stagnation period in 1989. The pE value was calculated as the electrode-measured E_h divided by (RT ln 10)/F. The shaded area is the sediment.

which is the electrode-measured E_h [mV] divided by 59 mV (= (RT ln 10)/F), fell down to around zero in the bottom anoxia, while it reached higher than 8 in the surface oxygenated layer (Fig. 2 (d)), although the latter value is not very reliable (STUMM & MORGAN, 1981) because the surface oxic water seems to have lacked any electroactive species in detectable levels. As comparing Figs. 2 (b) and (d), it is found that the steepest gradient in the vertical profile of oxygen was formed in shallower layer than that in the vertical profile of pE throughout the stagnation period. Both Fe(II) and Mn^{2+} (Figs. 2 (e), (f)) were accumulated grossly in anoxic hypolimnion, but Mn^{2+} rather than Fe(II) showed a tendency to be the more densely accumulated as time went on. A steep vertical gradient in the concentration of Mn^{2+} was observed in summer and autumn, which is representative of the MnO_x/Mn^{2+} boundary formed at the redox gradient. As shown later by comparing with the distribution of manganese-oxidizing microorganisms, the layer in which the biological manganese oxidation occurred most intensely seems to have located most likely in the vicinity of the 1 μM-isopleth of Mn^{2+} in Fig. 2 (f). The vertical distribution of Mn^{2+} in the pore water showed the reverse trend to that in the water column in the stagnation period, because the concentration of Mn^{2+} in surficial sediment usually exceeded that in deeper subsurface sediment. As the Mn^{2+} concentration in pore waters was always higher than that in the hypolimnetic water, the diffusion of Mn^{2+} from the sediment to the overlying lake water is thought to have persisted throughout the stagnation period.

The density of total bacterial cells was usually more than 10^7 cells/ml for the epilimnion and 10^6-10^7 cells/ml for the meta- and hypolimnion (Fig. 2 (g)).

2. Manganese precipitation rate

The estimation of the precipitation rate of Mn^{2+} in the vicinity of the MnO_x/Mn^{2+} boundary (September 4, 10 m layer) is presented in Fig. 3. The results of the time-course precipitation experiment for various initial concentrations of Mn^{2+} are shown in Fig. 3 (a), and the average precipitation rate between 0 and 100 hrs was plotted against the initial concentration of Mn^{2+} in Fig. 3 (b). The kinetic curve in Fig. 3 (b) appeared to be consistent with the Michaelis-Menten's type of kinetics and the Michaelis-Menten constants of K_m = 2.40 μM Mn^{2+} and V_{max} = 19.35 nmoles Mn^{2+} l^{-1} hr^{-1} were obtained based on these data. Because the crude lake water sample collected from the vicinity of the MnO_x/Mn^{2+} boundary was five-fold diluted prior to the experiment (see, Material and Methods, section 4), the actual V_{max} value in situ should have been 96.75 nmoles Mn^{2+} l^{-1} hr^{-1}. As the ambient concentration of Mn^{2+} in the vicinity of the MnO_x/Mn^{2+} boundary seemed to range 1–10 μM, the residence time of Mn^{2+} therein was expected to range 2–5 days, although the res-

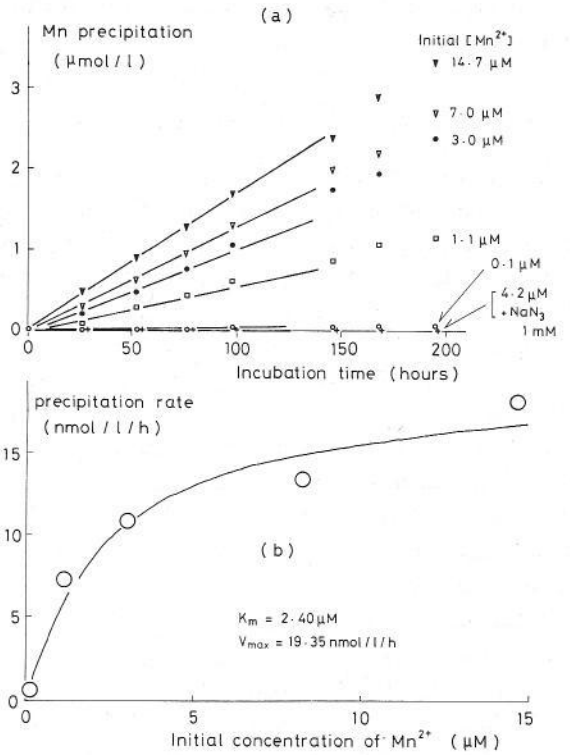

Fig. 3. (a) The time-course experiment for the precipitation of Mn^{2+} by *Metallogenium*. The symbols correspond to the initial concentrations of Mn^{2+} listed on the right side. The lake water used in this experiment was originally collected from 10 m layer on September 4 and was five-fold diluted with sterilized water prior to the experiment. (b) The average precipitation rates plotted against the initial concentrations of Mn^{2+}.

idence time was thought to be more or less dependent on the density of manganese-oxidizing bacteria which increased toward late summer (see below).

It must be borne in mind that the manganese precipitation rates shown in Fig. 3 are not strictly identical to the manganese oxidation rate because Mn^{2+} sorbed onto the surface of manganese oxide was also determined as precipitated manganese in the course of analysis. The precipitation rates presented here, however, rather simulated the actual geochemical flux of manganese, for manganese oxide particles should also have deposited in nature with sorbed Mn^{2+}.

In addition, Fig. 3 (a) also shows that the oxidation of Mn^{2+} in lake water was stopped completely by the addition of a respiratory inhibitor NaN_3 (1 mM), which supported that the precipitation process was associated with the metabolic activity of the manganese-oxidizing microorganisms. For the more strict evaluation of both microbiological and pure-chemical contributions to

the precipitation process of Mn^{2+} at this site, the reader should be referred to KAWASHIMA et al. (1988).

3. Size fractionation analyses of particulate manganese

Fig. 4 shows the vertical profiles of manganese, iron, and phosphate separated into three size fractions for the case of September 14. A peak of the profile of particulate manganese (fraction 1 of Mn in Fig. 4) appeared at 11 m layer associated with the MnO_x/Mn^{2+} boundary, just above the peak of the profile of dissolved manganese (Mn^{2+}, fraction 3). It is also found that almost all of the manganese particles accumulated at the MnO_x/Mn^{2+} boundary belonged to the fraction 1 (larger than 3 μm in diameter) in contrast to particulate iron and phosphate which were accumulated in deeper layers.

Fig. 4 also shows the vertical distributions of pH and the concentration of total carbonate. Using these data with the equilibrium constants cited from literature (e.g. STUMM & MORGAN, 1981), the solubility of Mn^{2+} in lake water with respect to the precipitation of $MnCO_3(s)$ can be calculated. Then, it was proved that lake water was undersaturated for the precipitation of $MnCO_3$ in the vicinity of the MnO_x/Mn^{2+} boundary ($IAP/K_{sO} < 0.15$ for 10.5–11.5 m). Thus, the accumulation of particulate manganese at this boundary can be explained exclusively by the oxidative precipitation of Mn^{2+}.

A more detailed size fractionation analysis is presented in Fig. 5. In this case (July 24), the MnO_x/Mn^{2+} boundary occurred near 8 m (see also Fig. 2 (f)). Fig. 5 (a) revealed again that almost all (83 %) of particulate manganese ac-

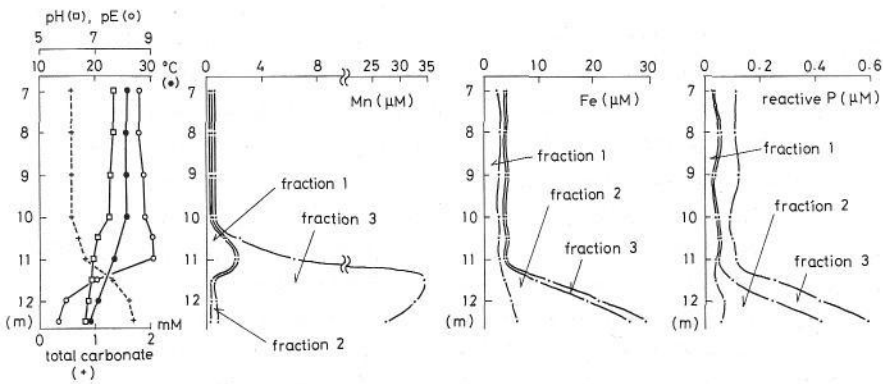

Fig. 4. Vertical distributions of Mn, Fe, and phosphate separated into three size fractions in the vicinity of the MnO_x/Mn^{2+} boundary on September 14. Fractions 1, 2, and 3 indicates the particle size classes of > 3.0 μm, 0.2–3.0 μm, and < 0.2 μm in diameter, respectively. Fraction 3 was considered here as the dissolved fraction such as $Mn^{2+}(aq)$, $Fe^{2+}(aq)$, and $PO_4^{3-}(aq)$. Distributions of pH, pE, and total carbonate concentration are also shown in the leftmost panel.

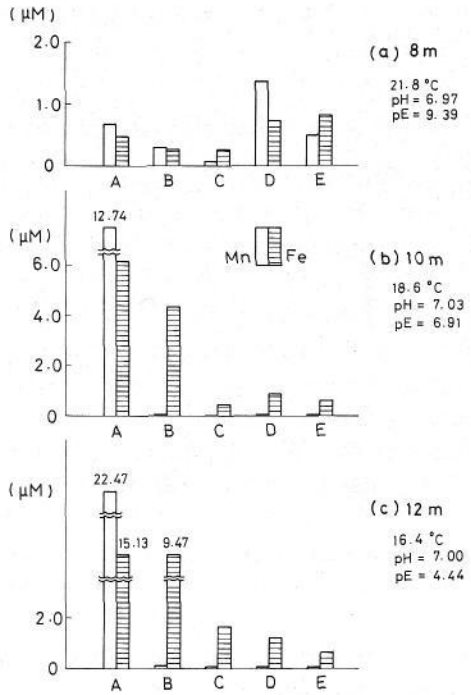

Fig. 5. Particle size distributions of manganese and iron in lake waters collected from 8 m, 10 m, and 12 m layers on July 24. Symbols indicate the following size fractions: A – < 0.2 μm, B – 0.2–1.0 μm, C – 1.0–3.0 μm, D – 3.0–10.0 μm, E – > 10.0 μm. Fraction A was regarded here as the dissolved fraction. Water temperature, pH, and pE are also listed.

cumulated in the vicinity of the MnO_x/Mn^{2+} boundary belonged to the size fractions D and E (3.0–10.0 and > 10.0 μm in diameter, respectively), and below this boundary (Fig. 5 (b), (c)) manganese existed almost exclusively as a dissolved form (Mn^{2+}, fraction A).

The manganese oxide particles larger than 3 μm in diameter can be readily distinguished under the microscope by brownish color which is characteristic of manganese oxide minerals. It was found by microscopy that the particles of manganese oxide larger than 3 μm collected from the vicinity of the MnO_x/Mn^{2+} boundary were of trichospheric form, ranging in diameter from 5 to 15 μm, which is the characteristic morphology of the coenobium of a manganese-oxidizing bacterium *Metallogenium personatum* Perfil'ev & Gabe. Fig. 6 is a photomicrograph of the particulate material contained in the lake water sample of 8 m layer collected on a Millipore HA filter on July 24. The darkly stained trichospherical particles found in this picture are the typical coenobia of *Metallogenium* inhabiting near the MnO_x/Mn^{2+} boundary in the dredged

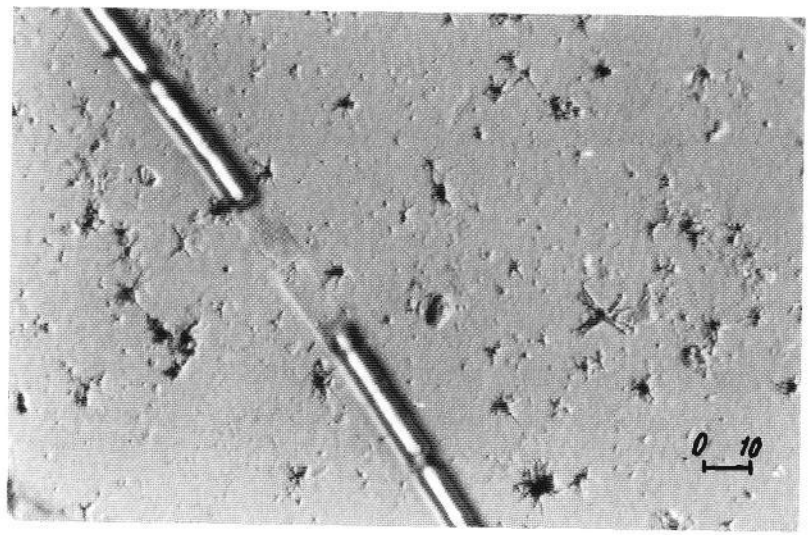

Fig. 6. Particulate material in lake water collected from 8 m layer on July 24. Particles of arborescent form seen in this picture are the coenobia of *Metallogenium*. Bar, 10 μm.

area of Lake Biwa. All these microscopic observations combined with the results of the size fractionation analyses led to the conclusion that the principal agent in the oxidative precipitation of Mn^{2+} at the MnO_x/Mn^{2+} boundary in this site was the manganese-oxidizing bacterium *Metallogenium*.

In contrast to manganese, particulate iron found in the hypolimnion (Figs. 5 (b), (c)) had a size distribution pattern characteristically biassed toward finer fractions. As far as judged by its black color, particulate iron accumulated in these layers seemed to consist of colloidal ferrous sulfide rather than ferric hydroxide. Fig. 5 (a) shows, on the other hand, that particulate iron in 8 m layer was concentrated in the fractions D and E, similarly to particulate manganese. Although coenbia of *Metallogenium* might have contained some amount of iron oxide, particulate iron of such a large size could also have been attributed at least partially to allochthonous particles and phytoplankton biomass; thus, whether *Metallogenium* contributed to the iron precipitation or not could not be determined unambiguously in this study.

4. Seasonal and vertical distributions of *Metallogenium* and *Ochrobium*

The vertical and temporal distributions of a manganese-oxidizing bacterium *Metallogenium* and an iron bacterium *Ochrobium* are shown semiquantitatively in Fig. 7. *Metallogenium* was first detected near the sediment surface in May and then its habitat gradually moved upward. The abundance peak of *Metallogenium* occurred at 7 to 8 m layer during July and August, and moved

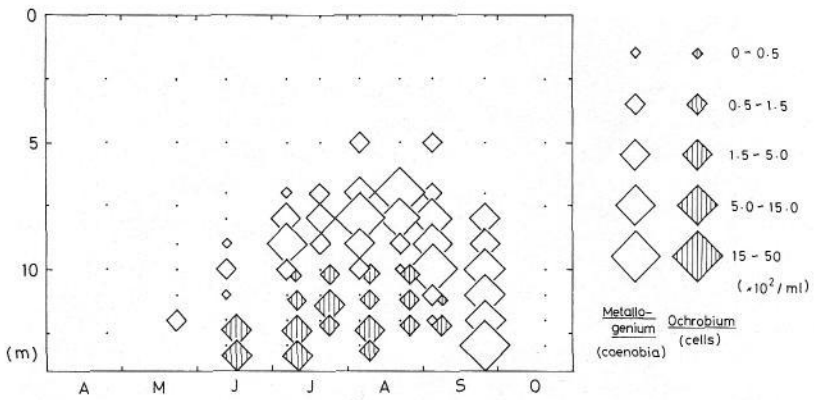

Fig. 7. Vertical and seasonal distributions of a manganese-oxidizing microorganism *Metallogenium* (coenobia ml^{-1}) and an iron-depositing bacterium *Ochrobium* (cells ml^{-1}) in the stagnation period in 1989.

down thereafter in accordance with the breakdown of the thermal stratification of lake water. Toward late summer, the vertical ranging of the distribution of *Metallogenium* spread significantly, and also its population size in the whole water column seemed to have increased as much. As soon as the stratification of lake water was entirely broken down in October, *Metallogenium* disappeared completely from the water column, and *Metallogenium* was not detected during the holomictic period (from October to March). Comparing Fig. 2 (f) and Fig. 7, it was found that the concentration of Mn^{2+} ranged $1-10\,\mu M$ in the stagnation period at the depth where the abundance peak of *Metallogenium* existed. Below this depth, *Metallogenium* seemed no longer capable of growing in spite of more abundant Mn^{2+} coexisting. In the layers where *Metallogenium* seemed to be growing actively, the environmental factors varied during the stagnation period within the following ranges:

$$pE > 8,\ pH = \text{ca. } 7,$$
$$\text{dissolved oxygen: } 50-200\,\mu M \text{ (usually } < 80\,\mu M),$$
$$Mn^{2+}: 1-10\,\mu M,\ Fe(II): < 2\,\mu M.$$

Ochrobium always inhabited deeper layers than *Metallogenium* and the habitats of these two species were well separated vertically. The seasonal variance in the density of *Ochrobium* were moderate compared with *Metallogenium*. Such an abundance peak as was seen in the vertical distribution of *Metallogenium* was far less prominent in the case of *Ochrobium*. The environmental factors in the habitat of *Ochrobium* ranged as follows:

$$pE < 6,\ pH = \text{ca. } 7,$$
$$\text{dissolved oxygen: } < 50\,\mu M,\ Fe(II): > 10\,\mu M.$$

Discussion

In many lakes ever studied, the oxidative precipitation of manganese in the redox transition layer is known to be kinetically too effective to be explained solely by non-biological reactions (DIEM & STUMM, 1984), and in some cases the bacterial mediation to the environmental manganese oxidation has been demonstrated directly by using the radioactive tracer and the biological inhibition techniques (NEALSON et al., 1988). This is not always the case, however, for the circulated surface waters (epilimnia), where scavenging of Mn^{2+} seems to be promoted principally by abiotic or indirectly biological processes (KAWASHIMA et al., 1988; RICHARDSON et al., 1988). Where the bacterial precipitation of Mn^{2+} occurs in the redox gradient, the trichospheric coenobia of *Metallogenium* have been often detected by the microscopic observation of manganese-containing particles collected therefrom (DUBININA, 1973; KLAVENESS, 1977; GREGORY et al., 1980; GIOVANOLI et al., 1980; JAQUET et al., 1982; SCHMIDT & OVERBECK, 1984; TIPPING et al., 1985). *Metallogenium* is known to occur also in the freshwater sediments (PERFIL'EV & GABE, 1965). But occurrence of *Metallogenium* has not been reported as yet in marine environments even when the bacterial mediation of manganese oxidation was demonstrated, except for one case of the sediment of Western Indian Ocean (NOVOZHILOVA & BEREZINA, 1984). The important role of this organism in the lacustrine manganese geochemistry has been contended by the authors mentioned above, on the basis of the microscopic examination of the suspended matter in lake water or the settling matter collected with sediment traps. MAKI et al. (1987), on the other hand, determined directly the vertical flux of manganese in Lake Washington with the aid of radiotracer, and questioned the importance of *Metallogenium* relative to other bacterially mediated precipitation processes in the overall manganese flux in that lake.

The present author showed that the manganese precipitation at the redox transition layer of the dredged area of Lake Biwa was mediated principally by the activity of *Metallogenium,* as demonstrated by the combination of the microscopic observation and the size fractionation analysis of manganese-containing suspended material. At particular depth in the water column appeared a density peak of the vertical distribution of particulate manganese larger than 3 μm in diameter, and manganese-containing particles of this size class consisted predominantly of coenobia of *Metallogenium* encrusted with manganese oxide. Mn^{2+} which diffused up from the underlying reductive layer appeared to be oxidized and precipitated entirely within the habitat of *Metallogenium,* because the vertical distribution of Mn^{2+} was almost completely enclosed below the habitat of *Metallogenium* throughout the stagnation period. The abundance peak of *Metallogenium* was located at a particular depth within the redox gradient, which implies that below that depth *Metallogenium* could no

longer oxidize Mn^{2+} presumably because of oxygen deficiency and/or presence of excess reductive substance such as Fe^{2+}. It can be supposed consequently that the population growth of *Metallogenium* was limited principally by the diffusional supply of Mn^{2+} from the underlying layer to its habitat. The diffusion rate of Mn^{2+} can be expected to be proportional to both the vertical eddy diffusivity of lake water and the concentration gradient of Mn^{2+} in the vicinity of the MnO_x/Mn^{2+} boundary. The latter became steeper as time went on toward late summer as shown in Fig. 2 (f). Also, the gradual breakdown of the vertical temperature gradient in late summer (Fig. 2 (a)) is thought to have increased the vertical eddy diffusivity. These effects were presumably responsible for vertical spreading of the habitat of *Metallogenium* and the increase of its population size in areal basis observed in late summer (Fig. 7). Similar proliferation phenomena of *Metallogenium* accompanied with vernal and autumnal turnover of lake water have also been reported in other lakes (KLAVENESS, 1977; GREGORY et al., 1980).

The appearance of the density peak of particulate manganese at the redox gradient revealed by itself the kinetic effectiveness of *Metallogenium* in the precipitation of Mn^{2+}. For such a peaked profile of particulate manganese to be realized, the potential oxidation rate of Mn^{2+} therein must be rapid compared with both the vertical diffusion rate of Mn^{2+} and the gravitational sinking rate of produced particulate manganese. This kinetic condition is not satisfied a priori because of the high activation energy for the oxygenation of Mn^{2+}. In homogeneous solution with no adequate catalyst, Mn^{2+} remains unoxidized over a few years despite the presence of O_2 (DIEM & STUMM, 1984). The residence time of Mn^{2+} in the redox gradient of natural lakes is usually much shorter, and in the case of the dredged area of Lake Biwa, it was estimated to be only 2–5 days (September 4, 10 m layer). Such a rapid oxidation of Mn^{2+} has not ever been attained experimentally, except in the presence of adequate surface catalysts in excess or alternatively in the presence of the manganese-oxidizing bacteria.

As regards the surface catalysts, it has been reported that the manganese oxidation is strongly enhanced by the presence of $MnCO_3$, $Mn(OH)_2$, Mn oxides (DIEM & STUMM, 1984), γ-FeOOH (SUNG & MORGAN, 1981), or some other oxide minerals of Fe, Mn, Al, and Si (DAVIES & MORGAN, 1989). The authigenic formation of $MnCO_3$ and $Mn(OH)_2$ in lake water of Lake Biwa seems unrealistic because the concentrations of HCO_3^- and OH^- are insufficient. The oxide minerals of Fe, Mn, Al, and Si, but not $MnCO_3$ and $Mn(OH)_2$, may be supplied significantly to lake water as allochthonous particles. However, the densities of these minerals suspended in lake water of this lake seem generally low (e.g. the concentration of total Fe(III) in lake water of the dredged area had seldom exceeded 10^{-5} M). And besides, for these minerals to affect the manganese oxidation rate to a significant degree, the ambient pH value must be

higher than 8 (SUNG & MORGAN, 1981; DAVIES & MORGAN, 1989). Although the pH value often exceeds 8 in the epilimnion of Lake Biwa, it is not the case around the redox transition layer. Thus it can be concluded that any non-biological catalysts could affect the oxidation rate of Mn^{2+} sparingly in this environment, and that the manganese precipitation at the redox gradient should not occur so effectively without the bacterial mediation.

The residence times of Mn^{2+} similar to that estimated in this study were also reported previously for the estuarine and oceanic environments (EMERSON et al., 1979; TEBO & EMERSON, 1986; SUNDA & HUNTSMAN, 1987, 1990). The K_m and V_{max} values obtained in these environments were, however, in the order of one-tenth of those values estimated in this study, probably due to the difference in the species composition of relevant manganese-oxidizing microflora or otherwise due to higher pH and ionic strength in sea water.

According to the results obtained in this study, the K_m and V_{max} values of the biological manganese precipitation at the MnO_x/Mn^{2+} boundary were 2.4 μM Mn^{2+} and 96.75 nmoles $Mn^{2+}\,l^{-1}\,hr^{-1}$, respectively, in the case of 10 m layer on September 4. However, the manganese oxidation rate per unit volume of lake water must depend upon the density of *Metallogenium* in it. With the density of *Metallogenium* (2.86×10^6 coenobia l^{-1}) in the same sample taken into account, the average V_{max} value for an individual coenobium of *Metallogenium* can be calculated to be 34.2 fmoles $Mn^{2+}\,hr^{-1}$. Then, the oxidation rate of Mn^{2+} by an individual coenobium (v_{ind} [fmoles $Mn^{2+}\,hr^{-1}$]) is given as

$$v_{ind} = 34.2\,C_{Mn}/(2.40 + C_{Mn}), \qquad (1)$$

where C_{Mn} [μM] indicates the ambient concentration of Mn^{2+}. The oxidation rate of Mn^{2+} per unit volume of lake water is given as the product of v_{ind} with the density of coenobia of *Metallogenium*. If the detailed data on the vertical distributions of Mn^{2+} and *Metallogenium* are available, the oxidation rate of Mn^{2+} in the whole water column will be calculated by integrating this product along depth over the habitat of *Metallogenium* in which it grows and oxidizes Mn^{2+} actively.

Using the v_{ind} value, we can also estimate roughly the turnover time of the biomass of *Metallogenium* in the vicinity of the MnO_x/Mn^{2+} boundary. In the case of 8 m layer on July 24 (Fig. 5), the concentration of particulate manganese larger than 3 μm in diameter (fractions D plus E) was 1.87 μM, which can be regarded approximately identical with the biomass of *Metallogenium*. Taking account of the density of *Metallogenium* in the same layer (12.4×10^6 coenobia l^{-1}), the manganese content of an individual coenobium of *Metallogenium* is calculated to be 151 fmoles Mn. At the same time, the manganese oxidation rate of an individual coenobium (v_{ind}) in this case is 7.46 fmoles $Mn^{2+}\,hr^{-1}$, as computed by substituting the ambient Mn^{2+} concentration of 0.67 μM (fraction A) into the equation (1). Dividing the manganese content of a coenobium

by the oxidation rate of Mn^{2+}, the mean age of coenobia of *Metallogenium* inhabiting this layer is expected to have been 20.2 hours, which also can be assumed to be equivalent to the turnover time of particulate manganese produced in this layer by *Metallogenium*. On the other hand, the manganese oxidation rate per unit volume of lake water is calculated as 92.5 nmoles $l^{-1}hr^{-1}$. Then the residence time of Mn^{2+} in the same layer is expected to be 7.2 hours, as calculated by dividing the concentration of Mn^{2+} by the oxidation rate per unit volume of lake water. As expected from these values, the growth of *Metallogenium* in the vicinity of the MnO_x/Mn^{2+} boundary seemed considerably rapid.

There remain several problems, however, in evaluating the geochemical role of *Metallogenium* in lake water in such a way as described above. First, it must be noted that the manganese oxidation rate might be also dependent upon the ambient concentration of oxygen. Tebo & Emerson (1985) reported that biological oxidation of Mn^{2+} in sea water was about twice as fast under the condition of 67 % air saturation as in 5 % air saturation. Thus the oxidation rate of Mn^{2+} estimated in this study under near 100 % air saturation might have been an overestimation for in situ oxidation rate, because the oxygen saturation in the vicinity of the MnO_x/Mn^{2+} boundary in the study site ranged 10–60 %. Also, the manganese oxidation rate should depend upon the temperature, which varied from 15 °C to 25 °C during the stagnation period at the MnO_x/Mn^{2+} boundary. Therefore, it is desirable to determine experimentally the kinetics of the biological manganese oxidation as a function of the concentrations of both Mn^{2+} and oxygen and of temperature. Second, the accuracy of the evaluation of the manganese precipitation rate in the whole water column must depend upon the quality of informations about the vertical distributions of Mn^{2+}, O_2, and *Metallogenium*. Since a peaked vertical profile of *Metallogenium* as well as steep concentration gradients of Mn^{2+} and O_2 were always observed in the vicinity of the MnO_x/Mn^{2+} boundary (Figs. 4 and 7), more detailed sampling design than that adopted in this study is needed to assess reliably the contribution of *Metallogenium* to the geochemical flux of manganese. Third, the population biological approach used in this study stands upon the assumption that the size and the activity of the coenobium of *Metallogenium* do not differ so much from individual to individual. But the real population of *Metallogenium* is not very homogeneous in size (see Fig. 6). Indeed, the size and the morphology of the coenobium may vary depending on the growth condition. For example, *Metallogenium* also occurs near the lake water/sediment interface in the north basin of Lake Biwa (station Ie, see Fig. 1), where the size of the coenobium usually exceeds 10 μm and the branches of the coenobia are well elongated compared with coenobia collected from the dredged area (see Fig. 8).

Although there are some rooms for improving methodologies as mentioned above, it seems essential to adopt the population biological approach

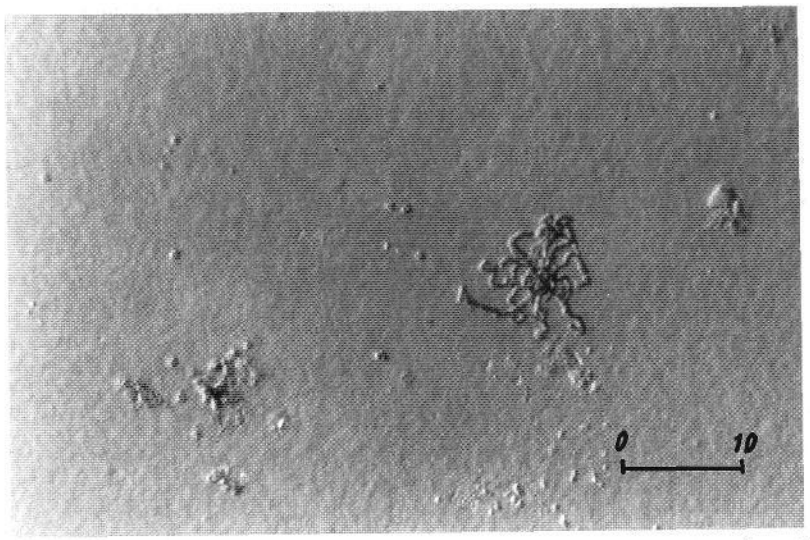

Fig. 8. A coenobium of *Metallogenium* which was found in lake water near the sediment/water interface in the north basin of Lake Biwa (Station Ie, Fig. 1, ca. 75 m in depth). Bar, 10 μm.

such as used in this study for studying such a geochemical process that includes biological factors.

Acknowledgements

The author is very grateful to Y. Tezuka for comments on the manuscript and also to other researchers of Otsu Hydrobiological Station for discussions. The technical assistance in samplings of A. Kawabata and O. Kawai is also acknowledged.

References

Brewer, P. G. & Spencer, D. W. (1971): Colorimetric determination of manganese in anoxic waters. – Limnol. Oceanogr. **16:** 107–110.

Davies, S. H. R. & Morgan, J. J. (1989): Manganese (II) oxidation kinetics on metal oxide surfaces. – J. Colloid Interface Sci. **129:** 63–77.

Davison, W. (1985): Conceptual models for transport at a redox boundary. – In: Stumm, W. (ed.), Chemical Processes in Lakes, pp. 31–54. – Wiley, New York.

Diem, D. & Stumm, W. (1984): Is dissolved Mn^{2+} being oxidized by O_2 in absence of Mn-bacteria or surface catalysts? – Geochim. Cosmochim. Acta **48:** 1571–1573.

Dubinina, G. A. (1984): Infection of procaryotic and eucaryotic microorganisms with *Metallogenium*. – Curr. Microbiol. **11:** 349–356.

Emerson, S., Cranston, R. E. & Liss, P. S. (1979): Redox species in a reducing fjold: equilibrium and kinetic considerations. – Deep-Sea Res. **26 A:** 859–878.

Giovanoli, R., Brüysch, R., Diem, D., Osman-Sigg, G. & Sigg, L. (1980): The composition of settling particles in Lake Zürich. – Schwei. Z. Hydrol. **42:** 89–100.

GREGORY, E., PERRY, R. S. & STALEY, J. T. (1980): Characterization, distribution, and significance of *Metallogenium* in Lake Washington. – Microb. Ecol. **6:** 125–140.

JAQUET, J.-M., NEMBRINI, G., GARCIA, J. & VERNET, J.-P. (1982): The manganese cycle in Lac Léman, Switzerland: the role of *Metallogenium*. – Hydrobiologia **91:** 323–340.

JONES, J. G. (1981): The population ecology of iron bacteria (genus *Ochrobium*) in a stratified eutrophic lake. – J. Gen. Microbiol. **125:** 85–93.

KAWASHIMA, M., TAKAMATSU, T. & KOYAMA, M. (1988): Mechanisms of precipitation of manganese(II) in Lake Biwa, a fresh water lake. – Water Res. **22:** 613–618.

KLAVENESS, D. (1977): Morphology, distribution, and significance of the manganese-accumulating microorganism *Metallogenium* in lakes. – Hydrobiologia **56:** 25–33.

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.

MOSS, M. L. & MELLON, M. G. (1942): Color reactions of 1, 10-phenanthroline derivatives. – Ind. Eng. Chem. Anal. Ed. **14:** 931–933.

MURPHY, J. & RILEY, J. P. (1962): A modified single solution method for the determination of phosphate in natural waters. – Anal. Chem. Acta **27:** 31–36.

NEALSON, K. H., TEBO, B. M. & ROSSON, R. A. (1988): Occurrence and mechanisms of microbial oxidation of manganese. – Adv. Appl. Microbiol. **33:** 279–318.

NOVOZHILOVA, M. I. & BEREZINA, F. (1984): Iron- and manganese-oxidizing microorganisms in grounds in the north-western part of the Indian Ocean and the Red Sea. – Mikrobiologiya **53:** 129–136.

PERFIL'EV, B. V. & GABE, D. R. (1965): The use of the microbial-landscape method to investigate bacteria which concentrate manganese and iron in bottom deposits. – In: PERFIL'EV, B. V. et al. (eds.), Applied Capillary Microscopy (English translation), pp. 9–54. – Consultants Bureau, New York.

RICHARDSON, L. L., AGUILAR, C. & NEALSON, K. H. (1988): Manganese oxidation in pH and O_2 microenvironments produced by phytoplankton. – Limnol. Oceanogr. **33:** 352–363.

SCHMIDT, W. D. & OVERBECK, J. (1984): Studies on "iron bacteria" from Lake Pluss I. Morphology, finestructure, and distribution of *Metallogenium* sp. and *Siderocapsa geminata*. – Z. Allg. Mikrobiol. **24:** 329–339.

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

SUNDA, W. G. & HUNTSMAN, S. A. (1987): Microbial oxidation of manganese in a North Carolina estuary. – Limnol. Oceanogr. **32:** 552–564.

– – (1990): Diel cycles in microbial manganese oxidation and manganese redox speciation in coastal waters of Bahama Islands. – Limnol. Oceanogr. **35:** 325–338.

SUNG, W. & MORGAN, J. J. (1981): Oxidative removal of Mn(II) from solution catalyzed by the γ-FeOOH (lepidocrocite) surface. – Geochim. Cosmochim. Acta **45:** 2377–2383.

TEBO, B. M. & EMERSON, S. (1985): Effect of oxygen tension, Mn(II) concentration, and temperature on the microbially catalyzed Mn(II) oxidation rate in a marine fjold. – Appl. Environ. Microbiol. **50:** 1268–1273.

– – (1986): Microbial manganese(II) oxidation in the marine environment: a quantitative study. – Biogeochemistry **2:** 149–161.

Terashima, A. & Ueda, T. (1982): Effects of bottom dredging on some environmental factors and benthic animals in the southern basin of Lake Biwa. – Jpn. J. Limnol. **43:** 81–87 (in Japanese with English summary).

Tipping, E., Jones, J. G. & Woof, C. (1985): Lacustrine manganese oxides: Mn oxidation states and relationships to "Mn depositing bacteria". – Arch. Hydrobiol. **105:** 161–175.

Zavarzin, G. A. (1989): Genus "*Metallogenium*" Perfil'ev & Gabe 1961, 50. – In: Staley, J. T. et al. (eds.), Bergey's Manual of Systematic Bacteriology, 9th edition, volume 3, pp. 1986–1989. – Williams & Wilkins, Baltimore.

Submitted: 17 May 1991; accepted: 8 January 1992.