Todorokite Formation in Seawater by Microbial Mediation

Noburu Takematsu†, Hiroy Kusakabe†, Yoshio Sato† and Shiro Okabe†

Abstract: Microbial manganese oxidation in seawater was carried out in enrichment cultures which were obtained from the seawater supply system at the Marine Science Museum, Tokai University (Shimizu-shi, Japan). The manganese oxide formed was well-crystallized todorokite. The major element composition was within the range of marine manganese concretions and the O/Mn molar ratio was 1.8. The conditions for formation of manganese oxide minerals in marine environments are discussed on the basis of these results.

1. Introduction
Manganese oxidizing bacteria are ubiquitous and well-known, although very few are well characterized (Ehrlich, 1981; Ghiorse, 1984). The importance of bacterial oxidation of manganese has been demonstrated in various environments (Chapnick et al., 1982; Emerson et al., 1982; Tebo et al., 1984). Further, many manganese oxides have been formed by bacteria in the laboratory (Tyler and Marshall, 1967; Bromfield and David, 1978; Rosson and Nealson, 1982; Hastings and Emerson, 1986). However, only a few papers referred to their mineralogy. According to Hastings and Emerson (1986), the initial oxidation product catalyzed by spores of a marine Bacillus was hausmanite (Mn₃O₄) and then amorphous oxides of MnO₁₋ₓ were formed by the disproportionation of MnO₄⁻. The manganese oxides formed by bacterial mediation from oxygenated lake water rich in Mn (II) was amorphous MnOₓ (x > 1.8) (Tipping et al., 1984). On the other hand, the most highly oxidized manganese compound by air, which was precipitated inorganically from an oversaturated manganese solution of nearly neutral pH was manganite (γ-MnOO)(Bricker, 1965; McKenzie, 1971; Murray et al., 1985). However, almost pure manganese oxides found in marine environments are todorokite and birnessite (Corliss et al., 1978).

In the seawater supply system at the Marine Science Museum, Tokai University, todorokite and birnessite are precipitated from aerated well seawater (Takematsu et al., 1984). The oxidation rate of Mn (II) is high and the microbial contribution to manganese oxidation was inferred (Sato et al., 1984). In this paper, manganese oxides formed by enrichment cultures of seawater from the seawater supply system are discussed.

2. Materials and methods
Seawater used for experiments was sampled from the precipitation tank of the seawater supply system at the Marine Science Museum, Tokai University (Fig. 1). The seawater pumped from the well is free of dissolved oxygen and contains ca. 1 ppm of manganese, ca. 25 μM of ammonia, ca. 100 μM of silicate and ca. 2 μM of phosphate. In the waterway covered with translucent plastic plates, bog moss and some diatoms grow and supply organic matter and siliceous skeletons to the precipitation tank. The amount of seawater used for aquaria is about 500 m³ per day.

The experimental procedures used to examine microbially mediated manganese oxidation are shown in Fig. 2. A sterile manganese sulfate solution was added to seawater in 20-liter polyethylene bottles immediately after aseptic sampling. The final concentration of manganese in the seawater was 50 ppm. A part was incubated in a room light (procedure I) and the other part in the dark (procedure II) in order to suppress

* Received 29 March 1988; in revised form 23 August 1988; accepted 19 September 1988.
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Fig. 1. Schematic diagram of the seawater supply system at the Marine Science Museum, Tokai University.

Fig. 2. Experimental procedures used to examine manganese oxidation by microbial mediation. [Oxide should be oxidation.]


Another seawater sample was allowed to stand in a room light in order to grow phytoplankton. After suitable growth, a sterile manganese sulfate solution was added (50 ppm) and the seawater was incubated under room conditions (procedure III). A mixture of poisons to manganese oxidizing bacteria (Rosson et al., 1984) and the sterile manganese sulfate solution were added to the other sample of seawater with phytoplankton growth and the seawater was incubated under room conditions (procedure IV). Procedures I and III are almost the same but the high concentration of manganese (50 ppm) may inhibit the growth of phytoplankton in procedure I. Phytoplankton and their decomposition products serve as a substrate for bacteria.

The number of bacteria, the concentration of manganese and the pH were measured before and after incubation. The number of manganese oxidizing bacteria was counted by the MPN method on K medium (Nealson, 1978): 2 g of peptone, 0.5 g of yeast extract, 0.2 g of MnSO₄·4H₂O, 0.1 mg of FeSO₄·7H₂O, 15 g of agar and 1 liter of 75% seawater (pH 7.6). Colonies showing an intense blue color with benzidine-acetic acid reagent (Bromfield, 1956) were regarded as manganese oxidizing bacteria.

Manganese oxides formed on the wall of the 20-liter polyethylene bottles in procedures I, II and III were scoured off with a nylon brush, and seawater was removed by filtration. The manga-
nese oxides were air-dried without washing with distilled water, because the major element composition was changed by washing (Sato et al., in preparation). The content of sea salt in the oxides was estimated from that of chloride. The samples were decomposed with nitric acid and hydrogen peroxide in order to determine the major elements and chloride. Sodium, K, Mg and Ca were determined by ICP spectrometry. In order to determine chloride, the solution after decomposition was passed through a column of H-form cation exchange resin, and the effluent was neutralized with sodium bicarbonate and evaporated to an adequate volume. Chloride was determined by titration with mercuric nitrate.

The oxidation state of manganese in the samples was determined by the method of Gattow (1961). The samples were dissolved in 4 N H$_2$SO$_4$ solution containing KI. The liberated I$_2$ was titrated with standardized 0.1 N Na$_2$S$_2$O$_3$ solution, and the content of manganese was measured by atomic absorption spectrometry. Minerals in air-dried samples were examined by X-ray diffraction (Cu-Kα; graphite monochromator).

Manganese oxidizing bacteria were isolated from the original and incubated seawater by the method described by Neelson (1978). The pure cultures were incubated in a continuous culture system for the oxidation of manganese (Dubininia, 1980) and in a recirculatory culture system (Tyler and Marshall, 1967). The broth used for the oxidation of manganese was K medium without agar.

3. Results and discussion

The total number of bacteria in the original seawater which grew on K medium varied between $5 \times 10^6$ and $2 \times 10^8$ ml$^{-1}$ with the time of sampling and most of them had the ability of oxidizing manganese. The number of bacteria which grew on K medium was almost the same as that on ZoBell 2216E which does not contain manganese. This suggests that bacteria in the original seawater are tolerant of 50 ppm of manganese in K medium. The low number of bacteria is attributed to the fact that the seawater pumped from under the ground is free of dissolved oxygen, in which aerobes will not survive. The high concentration of Mn(II) in the seawater is favorable to the growth of manganese oxidizing bacteria and their substrate is supplied from the waterway where some diatoms grow (Fig. 1). Therefore, the precipitation tank is considered to be a kind of a continuous culture system for manganese oxidizing bacteria.

The total number of bacteria in the seawater for procedures I and III increased to about $5 \times 10^8$ ml$^{-1}$ but the ratio of manganese oxidizing bacteria to the total was about one third. The number of bacteria in the seawater for procedure II was about $5 \times 10^4$ ml$^{-1}$ and half of them were manganese oxidizing bacteria (Table 1). In the case of procedure IV, as was expected, bacteria were not found in the seawater.

The pH of the seawater increased from 7.6 to about 8.5 with the growth of phytoplankton and decreased with the oxidation of manganese. The pH of the seawater was ca. 6.4 when the concen-

<table>
<thead>
<tr>
<th>Procedure I</th>
<th>Procedure II</th>
<th>Procedure III</th>
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<tbody>
<tr>
<td>Initial</td>
<td></td>
<td></td>
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<tr>
<td>Total number of bacteria</td>
<td>$5 \times 10^9$~$2 \times 10^9$ ml$^{-1}$</td>
<td>$5 \times 10^3$ ml$^{-1}$</td>
</tr>
<tr>
<td>Ratio of manganese oxidizing bacteria</td>
<td>$&gt;90%$</td>
<td>$&gt;90%$</td>
</tr>
<tr>
<td>Concentration of manganese</td>
<td>50 ppm</td>
<td>50 ppm</td>
</tr>
<tr>
<td>pH</td>
<td>7.6</td>
<td>7.6</td>
</tr>
<tr>
<td>Final</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total number of bacteria</td>
<td>$\sim 5 \times 10^6$ ml$^{-1}$</td>
<td>$\sim 5 \times 10^4$ ml$^{-1}$</td>
</tr>
<tr>
<td>Ratio of manganese oxidizing bacteria</td>
<td>$\sim 1/3$</td>
<td>$\sim 1/2$</td>
</tr>
<tr>
<td>Concentration of manganese</td>
<td>21.9±0.7 ppm</td>
<td>18.9±2.0 ppm</td>
</tr>
<tr>
<td>pH</td>
<td>6.32±0.02</td>
<td>6.32±0.02</td>
</tr>
<tr>
<td>Period of incubation</td>
<td>3 weeks</td>
<td>3 months</td>
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tration of Mn (II) decreased from 50 ppm to ca. 20 ppm. In this case, the content of dissolved oxygen decreased to 70% of the saturation. In procedures I and III, about 30 ppm of Mn (II) was oxidized within three weeks, while it took about three months for this oxidation to occur in procedure II. The long period required for manganese oxidation in procedure II is attributed to the low concentration of manganese oxidizing bacteria, which in turn is probably due to the low concentration of phytoplankton, relative to procedures I and III.

No discernible difference was found between procedures I and III. This suggests that phytoplankton living in the waterway covered with translucent plastic plates (Fig. 1) can grow in seawater with a high manganese concentration (50 ppm). In prolonged incubations using procedure I (about 6 months), the pH and manganese concentration were ca. 6.4 and ca. 20 ppm, respectively. This suggests that the reaction involved in manganese oxidation ceased within three weeks and that pH was the limiting factor (Hastings and Emerson, 1986).

Manganese oxides formed in procedures I, II and III were the same and well-crystallized 10 Å manganate (Fig. 3). No manganese oxide was formed in procedure IV, because of the absence of manganese oxidizing bacteria. The oxides formed were stable in air for at least a year, but transformed to poorly-crystallized 7 Å manganate in a desiccator of silica gel. The O/Mn molar ratio was 1.8, although lowering of the apparent oxidation state of the manganese oxides formed should be due to the adsorbed Mn (II), as reported by Murray et al. (1984). These characteristics are similar to those of synthetic buserite (Giovanoli et al., 1970a, b; Tejedor-Tejedor and Paterson, 1979). However, buserite is synthesized by the oxidation of manganous hydroxide in an extremely alkaline solution, while 10 Å manganate formed in this study was precipitated directly from almost neutral seawater. Manganese nodules with characteristics similar to those of 10 Å manganate formed in this study have been found in MANOP Site H where the environment is suboxic (Dymond et al., 1984). Therefore, 10 Å manganate formed is todorokite rather than buserite, although the relationship between todorokite and buserite is obscure (see Burns et al., 1983).

The chemical composition of major elements (Table 2) was within the range of marine manganese concretions (Moorby et al., 1984). The contents of minor transition metals were not measured but the maximum content of the sum of Co, Ni, Cu and Zn was estimated to be 0.006% from their concentrations in the original sea-

Fig. 3. Typical X-ray diffraction pattern of manganese oxides formed in seawater by microbial mediation in procedures I, II and III.
water (Takematsu et al., 1983).

Ten strains with high activity for manganese oxidation were isolated from both the original and incubated seawater. All of them were motile, Gram-negative rod-shaped bacteria. The broth of pure cultures turned brown in a few days, but less than 5% of Mn (II) in the broth was oxidized. The broth used was not suitable for the oxidation of manganese but good for bacterial growth. After two weeks of incubation in the recirculatory culture system, dark brown particles consisting mostly of bacterial cells were separated by a centrifuge. The particles changed to dark blue with benzidine-acetic acid reagent, which produces a dark blue color upon reaction with manganese (III) and (IV) oxides. However, no X-ray diffraction lines for any manganese oxides were observed from the particles, probably because of the small amount of manganese oxides present. A suitable broth for manganese oxidation should be developed for experiments of this nature.

The initial oxidation product catalyzed by spores of a marine Bacillus was hausmanite (Mn₃O₄) and then amorphous oxides of MnO₁·₉ were formed by the disproportionation of Mn₃O₄ (Hastings and Emerson, 1986). In procedures I, II and III of this study, the initial solid phases were not examined, but numerous black spots were visible on the wall of the 20-liter polyethylene bottles within a few days in the case of procedure I. No manganese oxides were formed in procedure IV in which the activity of bacteria was inhibited. Therefore, it is considered that todorokite was initially formed by bacterial mediation according to:

\[
\text{Mn}^{2+} + \frac{1}{2} \text{O}_2 + \text{H}_2\text{O} \xrightarrow{\text{bacteria}} \text{MnO}_2 + 2\text{H}^+ 
\]

As described above, the measured low oxidation state of manganese (MnO₁·₉) is due to the adsorption of Mn (II) on Mn (V) oxides, because the final concentration of Mn (II) in the seawater after incubation was high (about 20 ppm). The oxidation of Mn (II) to MnO₂ in marine environments is believed to be a two step process in which Mn (II) is oxidized to metastable Mn (III) oxides or oxyhydroxides and then the metastable solids are transformed to more stable Mn (IV) oxides (Hem, 1980; Hem and Lind, 1983; Hastings and Emerson, 1986). However, the direct oxidation of Mn (II) to Mn (IV) oxides is reasonable when adequate catalysts such as manganese oxidizing bacteria are present, because Mn (IV) oxides are thermodynamically the most stable phase inoxic environments (Bricker, 1965) and Mn (III) oxides or oxyhydroxides have not been found in marine environments. This is the first report in which well-crystallized todorokite is formed by microbial mediation in seawater with nearly neutral pH. However, this does not mean that bacteria control the mineralogy of manganese oxides. Bacteria catalyze only the oxidation of Mn (II) to Mn (IV), as suggested by McKenzie (1971). The mineralogy is controlled by the chemical environments.

Todorokite is abundant in marine manganese nodules which are almost buried in various oozes, where the concentration of manganese is high and the environment is less oxidizing as the result of diagenesis (Froelich et al., 1979; Usui, 1983/1984; Heggie et al., 1986). In hydrothermal regions, mineralogically pure todorokite is precipitated, which contains very little iron and the other transition elements (Corliss et al., 1978; Lalou et al., 1983). In hydrothermal regions, the concentration of manganese in seawater is high and hydrothermal solutions are reducing (Klinkhammer et al., 1977; Edmond et al., 1979). In the precipitation tank of the seawater supply system at the Marine Science

<table>
<thead>
<tr>
<th>Na (%)</th>
<th>K (%)</th>
<th>Mg (%)</th>
<th>Ca (%)</th>
<th>Mn (%)</th>
<th>Fe (%)</th>
<th>O/Mn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manganese oxides formed by procedures I, II and III (this study)</td>
<td>1.63</td>
<td>0.263</td>
<td>1.58</td>
<td>1.69</td>
<td>52.3</td>
<td>0.05</td>
</tr>
<tr>
<td>±0.06</td>
<td>±0.045</td>
<td>±0.30</td>
<td>±0.13</td>
<td>±0.3</td>
<td>±0.01</td>
<td>±0.008</td>
</tr>
<tr>
<td>Hydrothermal manganese-oxide deposits (Moorby et al., 1984)</td>
<td>2.4</td>
<td>0.76</td>
<td>1.7</td>
<td>1.8</td>
<td>41</td>
<td>0.9</td>
</tr>
<tr>
<td>±0.5</td>
<td>±0.23</td>
<td>±0.5</td>
<td>±0.6</td>
<td>±9</td>
<td>±1.3</td>
<td>—</td>
</tr>
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</table>

Table 2. The major element composition and the oxidation state of manganese in manganese oxides formed by bacteria and hydrothermal manganese-oxide deposits.
Museum, todorokite is precipitated from aerated well seawater which contains 1 ppm of manganese. The O/Mn molar ratio of todorokite is 1.8 (Takematsu et al., 1984). In this study, todorokite is precipitated from seawater containing 20 ppm of manganese and about 70% of the saturated content of dissolved oxygen. Considering the common points of these four cases, it is concluded that todorokite is precipitated in less oxidizing environments from seawater or interstitial water enriched in Mn (II).

Almost pure birnessite is found in hydrothermal manganese crusts and nodules which contain a little iron (Corliss et al., 1978; Lonsdale et al., 1980; Moorby et al., 1984). The birnessite-rich crusts at Tonga-Kermadec Ridge are considered to be precipitated after hydrothermal fluids have been well mixed with bottom seawater and oxygenated (Moorby et al., 1984). In the precipitation tank of the Museum, todorokite is transformed into birnessite with time, and the transformation is due to the oxidation of Mn (II) in todorokite (Takematsu et al., 1984). In the storage tank next to the precipitation tank of the seawater supply system, manganese crusts are precipitated, which are pure birnessite (Takematsu et al., 1981). Birnessite is readily synthesized by various methods (Buser et al., 1954; McKenzie, 1971), and is one of the most common minerals of manganese in soils (Taylor et al., 1964). Therefore, birnessite is the most stable form of manganese oxides in highly oxidizing marine environments, when the concentration of iron in seawater is low relative to that of manganese.

Vernadite-rich nodules and crusts contain almost equivalent amounts of manganese and iron, and are exposed on the pelagic clay or in topographic highs such as seamounts and mid-ocean ridges (Calvert and Price, 1977; Cronan, 1980). Vernadite is considered to be the epigenetic growth of proto-birnessite on FeOOH\(\cdot\)xH\(_2\)O\(_2\), and this intergrowth inhibits the formation of the ordered layer structure of birnessite (Burns and Burns, 1975). According to Calvert and Price (1977), vernadite is precipitated from seawater. Seawater contains almost equivalent amounts of manganese and iron (Quinby-Hunt and Turekian, 1983).

In hydrothermal fluids of a 350°C end-member, the concentrations of manganese and iron are in almost the same range but are several orders of magnitude higher than those in seawater (Edmond et al., 1982). While hydrothermal fluids ascend through fissures and fractures, iron is preferentially removed as sulﬁde and Fe-rich silicate in reducing zones. On contact with seawater, hydrothermal fluids are immediately oversaturated with respect to ferric iron, owing to the high oxidation rate of ferrous iron, and iron is removed preferentially from the oxygenated hydrothermal fluids as Fe oxides or Fe-Mn oxides (Corliss et al., 1978; Edmond et al., 1979). After the segregation of iron, manganese is precipitated first as todorokite from seawater with a high manganese concentration and then as birnessite in highly oxidizing environments during the diffusion of oxygenated hydrothermal fluids.

Manganese oxidizing bacteria have been isolated from various marine manganese nodules (Ehrlich, 1963, 1968; Ehrlich et al., 1972; Schütt and Ottow, 1978; Ghirose and Hirsch, 1982). A large number of viable manganese oxidizing bacteria (10\(^3\)-10\(^4\) g\(^{-1}\)) were found, not only on the surface but also in the central core of manganese nodules (Ehrlich et al., 1972; Schütt and Ottow, 1978). Even from Mn-Fe oxides on glass slides which were placed for 10 months in the vicinity of hydrothermal vents (Janasch and Wirsch, 1981), manganese oxidizing bacteria were isolated (Ehrlich, 1983). Manganese oxidizing bacteria are abundant in marine sediments (Schütt and Ottow, 1978; Edenborn et al., 1985). The microbial contribution to manganese oxidation is important in marine environments and the mineralogy of manganese oxides is controlled by chemical conditions.

Acknowledgements

The authors would like to thank Dr. K. Suzuki, The Institute of Physical and Chemical Research, for an introduction to the identification of bacteria. They are also grateful to Prof. K. Suzuki and Mr. K. Hiroki, Tokai University, for their help in making the surveys. Thanks are also extended to Mses. M. Tsuchiya, N. Imahashi and M. Suzuki, The Institute of Physical and Chemical Research, for their technical assistance.
References


微生物の媒介によって海水中で生成した蹂石

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要旨：微生物の媒介するマンガンの酸化を、東海大学海洋科学博物館の海水給水系から採取した海水の増菌培養

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によって調べ、生成したマンガン酸化物は結晶度の良い蹂石であった。酸化物の主要元素組成は海産マンガン蹂石のそれに近いため、O/Mn モル比は 1.8 であった。これらの結果に基づいて、海洋環境におけるマンガノ酸化物蹂石の生成条件を議論した。