

Bacterial Manganese Reduction and Growth with Manganese Oxide as the Sole Electron Acceptor



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of 75 to 300 km. Both the number of events and the cumulative moment per unit depth decrease by about two orders of magnitude in this depth interval. In contrast, the total along-strike length of all subducted slabs decreases by less than one order of magnitude as depth increases from 75 km to 300 km. Therefore, the numerator of Eq. 4 decreases more rapidly with increasing depth than does the denominator, and as a result the (seismic) down-dip

strain rates in this depth interval tend to decrease with increasing depth.

23. B. Hager and R. J. O'Connell, *Tectonophysics* 50, 111 (1978).
24. Giardini and Woodhouse (5) argue that the Tonga slab has deformed by shear at a rate comparable to the one that I calculate.

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Bacterial Manganese Reduction and Growth with Manganese Oxide as the Sole Electron Acceptor

CHARLES R. MYERS AND KENNETH H. NEALSON

Microbes that couple growth to the reduction of manganese could play an important role in the biogeochemistry of certain anaerobic environments. Such a bacterium, *Alteromonas putrefaciens* MR-1, couples its growth to the reduction of manganese oxides only under anaerobic conditions. The characteristics of this reduction are consistent with a biological, and not an indirect chemical, reduction of manganese, which suggest that this bacterium uses manganic oxide as a terminal electron acceptor. It can also utilize a large number of other compounds as terminal electron acceptors; this versatility could provide a distinct advantage in environments where electron-acceptor concentrations may vary.

MICROBIAL REDOX REACTIONS are important mechanisms for mobilizing metals and organic compounds in natural, anaerobic, aquatic environments. Important microbial reactions include those involving the oxidation of organic matter coupled to the reduction of nitrate (NO_3^-), ferric iron [Fe(III)], manganese oxides [containing Mn(IV) and Mn(III)], or sulfate (SO_4^{2-}), and the conversion of organic matter to carbon dioxide (CO_2) and methane (CH_4) (1).

Iron (2) and manganese may represent the primary electron acceptors for organic matter oxidation in sedimentary environments where they are enriched. In marine sediments, Fe and Mn reduction would likely be important in the zone between the region of oxygen removal and the region of sulfate reduction; in freshwater sediments, which are characteristically low in both NO_3^- and SO_4^{2-} , metal reduction would occur between the regions of oxygen depletion and CO_2 reduction (methanogenesis).

Bacterially mediated Mn reduction can occur indirectly when reduced, metabolically excreted end products, such as sulfide (3) and certain organic compounds (4, 5), react abiotically with manganese oxides. Results from several studies (6–9), as well as thermodynamic considerations (10), suggest that some bacteria may link Mn reduction to the oxidation of organic substrates. Some bacteria have been shown to reduce Mn(IV)

under both aerobic and anaerobic conditions (6, 9). In contrast, Burdige and Nealson (7) suggested that certain bacteria could do this only under anaerobic conditions, but they did not work with pure cultures and were thus unable to identify the Mn-reducing microbes. We have isolated and characterized a bacterium that reduces Mn(IV) only under anaerobic conditions and couples its growth to this reduction.

The Mn-reducing bacterium *Alteromonas putrefaciens* strain MR-1 was isolated from the anaerobic sediments of Oneida Lake, New York. These sediments, although aero-

bic at the water-sediment interface, become anaerobic a few millimeters beneath the interface. During the summer months extensive Mn(IV) reduction occurs, which results in pore water Mn(II) concentrations of greater than $100 \mu\text{M}$ and a rapid upward flux of Mn(II) into the lake water (11, 12).

We established enrichment cultures of the anaerobic sediments by the use of LO medium (13) with 0.75% agar, succinate or acetate as the carbon sources, and MnO_2 (approximately 1 mM) as the electron acceptor. These cultures were incubated at room temperature, and after a period of 2 to 7 weeks the MnO_2 was reduced. Secondary enrichments were established from these cultures by use of the same LO medium. After the MnO_2 in the secondary enrichments had been reduced, the cultures were transferred under anaerobic conditions (13) to agar plates of the LO medium containing an overlay of MnO_2 in 0.75% agar. In less than 1 week, zones of visible reduction (that is, clearing) in the MnO_2 top agar were evident; no visible colonies were noted in these zones, although MR-1 was repeatedly isolated in pure culture from such zones. MR-1 apparently stopped growing in an area after the local supply of MnO_2 was depleted. This implies that MR-1, a motile bacterium that could move through the MnO_2 -rich overlay, requires physical contact with insoluble MnO_2 to grow. In contrast, if MR-1 mediated Mn reduction through the release of a diffusible reductant, then visible colonies within the zones of Mn reduction would be expected as is observed with sulfide-generating bacteria (3). We identified MR-1 as a strain of *A. putrefaciens* by conventional biochemical identification tests (14).

Fig. 1. Manganese reduction in liquid medium by MR-1. (A) Manganese reduction versus time for different numbers of cells: no cells (\square); 9.33×10^5 cells ml^{-1} (\blacktriangle); 1.87×10^6 cells ml^{-1} (\circ); 3.73×10^6 cells ml^{-1} (\blacksquare); 7.46×10^6 cells ml^{-1} (\triangle); 1.49×10^7 cells ml^{-1} (\bullet). Cell number was determined by colony counts on LB medium (21) with 1.5% agar. The concentration of Mn^{2+} was determined by the measurement of free Mn^{2+} and did not include Mn^{2+} that may have been bound to insoluble MnO_2 (22). (B) Manganese reduction as a function of relative cell number; these data were obtained from (A). In three independent experiments, the y-axis intercept was equal to $0.5 \pm 0.3 \mu\text{M}$. (C) Temperature optimum of Mn reduction by MR-1 at pH 7.4; media at all temperatures contained 6.36×10^6 cells ml^{-1} . (D) Optimum pH of manganese reduction by MR-1 at 24°C ; media at all pH values contained 1.83×10^6 cells ml^{-1} . The net Mn reduction values in (C) and (D) were obtained by subtracting the values for Mn reduction in the absence of cells from reduction in the presence of cells. Experiments were conducted in LO medium (13) as described (23). The data shown in (C) and (D) were reproducible in duplicate experiments.

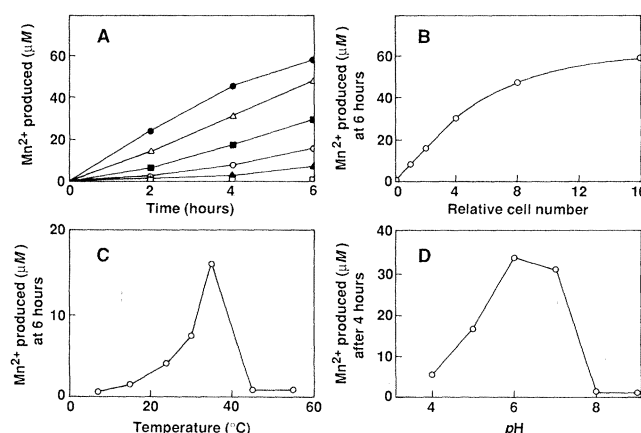


Fig. 2. Effects of oxygen and metabolic poisons on the reduction of Mn by MR-1. Experiments were conducted in LO medium (13) as described (23); all flasks were inoculated under anaerobic conditions (13) with anaerobically grown cells.

(A) Manganese reduction in the presence (○) and absence (●) of O_2 . (B) Manganese reduction in the presence of various metabolic inhibitors: (●) no inhibitor; (△) 0.2% formaldehyde; (■) 100 μM antimycin A; (□) 100 μM CCCP (carbonyl cyanide *m*-chlorophenyl hydrazone); (▲) no cells. Manganese reduction was inhibited 62% by formaldehyde, 67% by antimycin A, and 75% by CCCP. Other metabolic poisons also inhibited Mn reduction by MR-1 (percentage of inhibition observed): 1 mM sodium azide (36%), 10 μM 2-heptyl-hydroxyquinoline-*N*-oxide (HQNO) (39%), 100 μM dicumarol (53%), 100 μM dinitrophenol (43%), and 50 μM zinc sulfate (71%). Sodium cyanide (200 μM) did not affect Mn reduction by MR-1. Data were reproducible to within approximately 5% in duplicate experiments.

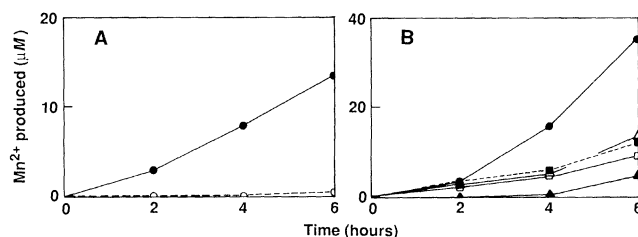
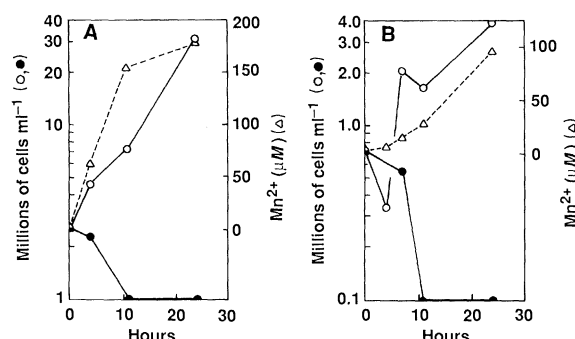


Fig. 3. Anaerobic growth of MR-1 with MnO_2 as the sole terminal electron acceptor. Growth in the presence of MnO_2 (2 mM) was assessed by increases in cell number (○); the particulate MnO_2 in each sample was reduced with a small amount of sodium dithionite ($Na_2S_2O_4$) just prior to plating the cell counts. Increases in microbial growth paralleled reduction of manganese (△), whereas no growth was noted in the absence of a terminal electron acceptor (●); the cell counts shown at baseline on the x-axis represent $<10^3$ cells ml^{-1} .

The experiment in (A) was conducted in defined medium (15) and that in (B) was conducted in LO medium (13), with succinate as the carbon source in both. Growth of MR-1 coupled to the reduction of Mn was demonstrated in duplicate experiments with succinate as the carbon source and in a single experiment with lactate as the carbon source. After 24 hours, cell counts in the presence of MnO_2 leveled off and declined by approximately 50%; Mn reduction rates continued unchanged in LO medium but declined in the defined medium. Concentrations of Mn^{2+} as low as 200 μM retard aerobic growth of MR-1, which suggests that Mn^{2+} toxicity possibly caused the cell number to decline after 24 hours. Concentrations of Mn^{2+} in the pore water of Oneida Lake sediment range from 10 to 150 μM (11), below the concentration that is potentially toxic for MR-1.



Rates of reduction of MnO_2 by MR-1 in LO medium were proportional to cell number (Fig. 1, A and B) except at high cell concentrations (Fig. 1B). For the data in Fig. 1A, absolute rates of Mn reduction were 0.76×10^{-9} to 1.2×10^{-9} μmol $hour^{-1}$ per cell; in a total of 16 experiments in this same medium, reduction rates ranged from 0.2×10^{-9} to 8.7×10^{-9} μmol $hour^{-1}$ per cell. For analogous experiments in defined medium (15), rates ranged from 10.4×10^{-9} to 42.4×10^{-9} μmol $hour^{-1}$ per cell. The differences in the rates for these two media may be the result of pH differences: the initial pH of the LO medium was 7.4, but it increased to approximately 7.8 by the end of the experiments, whereas the pH of the defined medium remained at pH 7.4, close to the optimal pH of between 6 and 7 for reduction by MR-1 (Fig. 1D). The rates of Mn reduction by MR-1 are comparable to those rates that have been measured for sulfate-reducing bacteria, which reduce manganese oxides indirectly by the generation of sulfide (3).

The optimum temperature for Mn reduc-

tion by MR-1 was 35°C (Fig. 1C). Reduction of Mn by MR-1 was also detectable at 7° and 15°C. The temperature of the sediment-water interface in Oneida Lake is typically 4°C during the winter and approximately 20°C during the summer (11). Rates of Mn reduction declined markedly at temperatures higher than 35°C, as expected for a biologically mediated (but not a strictly chemical) process. In controls without MR-1, there was essentially no reduction of Mn at any of the tested temperatures.

Conditions of low pH and redox potential favor abiotic reduction of Mn (4), but rates of Mn reduction by MR-1 were optimal at pH 6 to 7. In controls without MR-1, detectable reduction of Mn occurred at pH 4 and 5; essentially no reduction of Mn occurred in controls with a pH above 6. The pH of the Oneida Lake sediments ranges from 7.5 to 8.2 (11), which is in the range of Mn reduction by MR-1.

Manganese reduction by MR-1 was inhibited by molecular oxygen (O_2) (Fig. 2A), and by various metabolic poisons, including inhibitors of electron transport-linked respi-

Table 1. Terminal electron acceptors for MR-1. Compounds were considered to be electron acceptors if they supported growth of MR-1 on LO medium (13) and defined medium (15) with succinate or lactate as the carbon source. Aerobic growth of MR-1 on these media (in the absence of other electron acceptors) was used as the criterion for use of oxygen as an electron acceptor; MR-1 was unable to grow on these media in the absence of oxygen or other alternative electron acceptors. The ability of all other electron acceptors to support growth of MR-1 was tested under anaerobic conditions (13) at concentrations of 2 mM. The formation of visible colonies within 48 to 72 hours was considered a positive result.

Terminal electron acceptor
Oxygen (O_2)
Nitrate (NO_3^-)
Nitrite (NO_2^-)
Ferric iron (Fe^{3+})*
Thiosulfate ($S_2O_3^{2-}$)*
Sulfite (SO_3^{2-})*
Tetrathionate ($S_4O_6^{2-}$)
Glycine
Fumarate
Manganese dioxide (MnO_2)†
Trimethylamine N-oxide (TMAO)
Not used as terminal electron acceptor
Sulfate (SO_4^{2-})
Molybdate (MoO_4^{2-})
Carbon dioxide (CO_2)

*Recent studies (24) also demonstrate that other strains of *A. putrefaciens* grow using these compounds as electron acceptors. †Colonies were not visible within the zones of Mn reduction. See Fig. 3 for evidence of use of MnO_2 as a terminal electron acceptor.

ration (Fig. 2B). These relations imply that MR-1 may use MnO_2 as a terminal electron acceptor.

MR-1 was unable to ferment any of the carbon sources tested (16); like other non-fermentative bacteria under anaerobic conditions, it must obtain its energy from respiratory reactions in which terminal electron acceptors other than oxygen are used (Table 1). The variety of compounds that MR-1 can use to support growth (Table 1) demonstrates its remarkable respiratory versatility. Such versatility could provide a distinct advantage in environments where the concentration of certain electron acceptors may change with time and sediment depth.

In the absence of other electron acceptors, the growth of MR-1 is obligately coupled to the reduction of Mn (Fig. 3). MR-1 did not grow in the same medium if MnO_2 , or an alternate electron acceptor, was lacking (Fig. 3). If the value of 2.8×10^{-13} g per cell for dry cell weight (17) is used, the observed cell number increases (Fig. 3) for MR-1 give molar growth yields of 9 to 45 g of cells per mol of MnO_2 reduced. These values are similar to those reported by Stouthamer (18) for the obligately respiratory bacterium *Paracoccus denitrificans* (16 to 39 g of cells per mol of nitrate or oxygen reduced). Even

though more quantitative growth yield experiments (18) are needed to define more precisely growth yields for MR-1, these values clearly indicate that MnO_2 functions as an electron acceptor for MR-1. A dissimilatory Fe(III)- and Mn(IV)-reducing bacterium, GS-15, was recently isolated by other investigators from the sediments of the Potomac River, Maryland (19); GS-15 can couple its growth to the reduction of Fe(III) or Mn(IV). GS-15 has not yet been classified taxonomically, but it is clearly different from MR-1 in that it is an obligate anaerobe.

Microbial Mn reduction has been reported in marine, freshwater, and terrestrial environments (6–9, 20), which implies that Mn could play an important role in carbon mineralization. The ability of MR-1 to grow by the use of manganese oxide as a terminal electron acceptor suggests that MR-1, and other bacteria with similar properties, may play a significant role in the cycling of Mn and in organic matter mineralization in anaerobic sediments that contain abundant Mn, such as in Oneida Lake, Green Bay, and Lake Michigan.

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- Preliminary estimates of Mn(II) flux obtained with bottom flux chambers indicate a flux of 1 to 3 mmol $\text{m}^{-2} \text{day}^{-1}$ (K. H. Nealson and M. Enzien, unpublished data). Given the observed experimental rates of Mn reduction by MR-1 (see text discussion of Fig. 1), approximately 1×10^5 to 5×10^5 MR-1 cells ml^{-1} could account for the reduction rates observed in the summer of 1987.
- LO medium consisted of Oneida Lake water with 10 mM Hepes [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid] buffer (pH = 7.4), 0.01% Bacto-peptone, 0.02% Bacto-yeast extract (Difco Laboratories, Detroit, MI); after autoclaving, sodium acetate or succinate and sodium bicarbonate were added from sterile stock solutions to yield final concentrations of 15 mM and 2 mM, respectively. Manganese oxide (δMnO_2) was prepared (3, 7), ground to a fine powder with mortar and pestle, and sterilized by dry heat (200°C) for several hours. Before addition to the cultures, the δMnO_2 was suspended in pH 11 sterile water and sonicated to reduce particle size. Experiments were conducted under anaerobic conditions (10% hydrogen plus 90% nitrogen) with a Coy anaerobic chamber (Coy Laboratory Products, Ann Arbor, MI) (3, 7).
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- Defined medium (pH = 7.4) consisted of 15 mM sodium succinate ($\text{Na}_2\text{C}_4\text{H}_4\text{O}_4$), 9.0 mM $(\text{NH}_4)_2\text{SO}_4$, 5.7 mM K_2HPO_4 , 3.3 mM KH_2PO_4 , 2.0 mM NaHCO_3 , 1.01 mM MgSO_4 , 0.485 mM CaCl_2 , 67.2 μM Na_2EDTA , 56.6 μM H_3BO_3 , 10.0 μM NaCl , 5.4 μM FeSO_4 , 5.0 μM CoSO_4 , 5.0 μM $\text{Ni}(\text{NH}_4)_2(\text{SO}_4)_2$, 3.87 μM Na_2MoO_4 , 1.5 μM Na_2SeO_4 , 1.26 μM MnSO_4 , 1.04 μM ZnSO_4 , 0.2 μM CuSO_4 , L-arginine HCl (20 $\mu\text{g ml}^{-1}$), L-glutamine (20 $\mu\text{g ml}^{-1}$), and DL-serine (40 $\mu\text{g ml}^{-1}$). This defined medium, when supplemented with alternative appropriate terminal electron acceptors (Table 1), was able to support the growth of MR-1.
- The carbon sources tested were: arabinose, cellobiose, fructose, galactose, glucose, inositol, maltose, malonic acid, mannose, mannitol, melibiose, raffinose, rhamnose, sorbitol, sorbose, sucrose, xylitol, and xylose.
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- For the pH experiments (Fig. 1D), the pH of the medium was adjusted to the appropriate values with 1 M hydrochloric acid or sodium hydroxide, and the medium was filter sterilized. Cells grown anaerobically for 2 to 3 days on LB agar were suspended in sterile medium and added to the flasks. Sterile sonicated MnO_2 (10 mg ml^{-1}) was added to the medium in a final concentration of 0.2 mM; the time of the MnO_2 addition represented time zero. The experiments in Fig. 1, A, B, and D, and Fig. 2 were conducted in 10-ml quantities of LO medium in 50-ml Erlenmeyer flasks, agitated on a rotary platform shaker (75 rpm) at 24°C throughout the course of the experiments. The experiment in Fig. 1C was set up in 5-ml amounts of media in sterile Hungate tubes. The tubes were tightly stoppered and removed from the anaerobic chamber to water baths of the appropriate temperature; the tubes were returned to the anaerobic chamber for sampling. Samples (1.0 ml) were taken at intervals and filtered through Gelman GA-8 polysulfone 0.2- μm filters (Gelman Sciences, Ann Arbor, MI). The filtrates were made acidic by addition of 3.0 μl of 12M hydrochloric acid to prevent the Mn^{2+} from adhering to vessel walls before analysis. The concentration of Mn^{2+} in the filtrates was determined by flame atomic absorption spectrophotometry. The initial MnO_2 concentration for all experiments in Figs. 1 and 2 was 0.20 mM.
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Potassium Salt Microinjection into *Xenopus* Oocytes Mimics Gonadotropin Treatment

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Gonadotropin stimulates protein synthesis and growth in ovarian oocytes. The hormone is also known to modify transfollicular K^+ fluxes and is now shown to cause increased intraoocytic K^+ activity (a_K). The hormone's effect on a_K was duplicated by microinjecting K^+ salts into oocytes which were incubated in paraffin oil. This treatment mimicked the influence of gonadotropin on both the rate of protein synthesis and the synthesis of specific polypeptides. These findings suggest that gonadotropin-stimulated oocyte growth is attributable largely to the hormone's influence on transfollicular K^+ fluxes. They support the hypothesis that the K^+ flux and a_K changes observed during cell activation are critical in causing subsequent increases in protein synthesis and growth.

IN MANY VERTEBRATE SPECIES, OVARIAN oocytes undergo cycles of quiescence and hormone-activated protein synthesis and growth (1, 2). Quiescent *Xenopus*

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