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David J. Burdige
Old Dominion University, dburdige@odu.edu

Kenneth H. Nealson

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Microbial Manganese Reduction by Enrichment Cultures from Coastal Marine Sediments

DAVID J. BURDIGE†* AND KENNETH H. NEALSON

Marine Biology Research Division, Scripps Institution of Oceanography, University of California, San Diego, La Jolla, California 92093

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Manganese reduction was catalyzed by enrichment cultures of anaerobic bacteria obtained from coastal marine sediments. In the absence of oxygen, these enrichment cultures reduced manganates when grown on either lactate, succinate, or acetate in both sulfate-free and sulfate-containing artificial seawaters. Sodium azide as well as oxygen completely inhibited microbial manganese reduction by these enrichment cultures, whereas molybdate had no effect on them. The addition of nitrate to the medium slightly decreased the rate of Mn^{2+} production by these enrichment cultures. These findings are consistent with the hypothesis that the manganese-reducing organisms in these enrichment cultures use manganates as terminal electron acceptors and couple manganese reduction in some way to the oxidation of organic matter.

Microbes often catalyze manganese reduction, that is, the production of reduced Mn^{2+} from solid Mn^{3+}, Mn^{4+} oxides known as manganates, manganese oxides, or MnO_x (where x is generally less than 2). Manganese reduction can occur when reduced metabolic end products are excreted and react abiotically with these oxides. For instance, sulfate-reducing bacteria can be considered (indirect) manganese-reducing organisms because of the rapid reaction between sulfide and manganates (D. J. Burdige, Ph.D. thesis, University of California, San Diego, 1983; D. J. Burdige and K. H. Nealson, manuscript in preparation). Some organic compounds also reduce manganese oxides (28), suggesting another type of indirect microbial manganese reduction because many reduced organics may be produced as metabolic end products during bacterial fermentation.

Bacteria can also directly reduce manganates under a wide range of experimental conditions (3, 10, 11, 17, 21, 22, 29–33). Many of these studies, as well as studies of the geochemistry of marine sediments (7, 9, 15) and thermodynamic considerations, suggest that bacteria link manganese reduction to their oxidation of organic substrates, with manganates serving as an alternative electron acceptor when oxygen and nitrate are depleted. However, some laboratory studies with pure bacterial cultures have observed that the presence of oxygen does not inhibit microbial manganese reduction (29–31). Trimble and Ehrlich (29) showed that oxygen does not interfere with manganese reduction by the two organisms they examined and that O_2 and manganates do not compete with one another as electron acceptors. Further studies with one of these organisms (10, 11, 30) showed that it possesses a manganese-reductase system whose activity is inducible by Mn^{2+} and is coupled to an electron transport chain (with glucose as an electron donor) but was unaffected by the presence or absence of O_2.

Bromfield and David (3) observed that a soil *Arthrobacter* sp. capable of oxidizing Mn^{2+} could also catalyze the reduction of manganese oxides, depending on the experimental conditions. Zehnder and Brock (33) noted that manganese oxides stimulated anaerobic methane oxidation by both freshwater sediments from Lake Mendota, Wis., and digested sewage sludge. However, it was not clear whether the manganese oxides were reduced or what role they played in methane oxidation. Wollast et al. (32) showed that an enrichment culture from the upper Scheldt estuary (Belgium) reduced manganates under anaerobic conditions in a synthetic medium containing inorganic salts, manganates, and acetate as the sole carbon source. Sterile controls in this experiment showed no reduction activity.

The biochemical study of Hochster and Quastel (13) demonstrated that under anaerobic conditions manganese oxides could substitute for oxygen and serve as an electron acceptor in a number of enzyme-catalyzed biological redox reactions. In many cases a reversible electron carrier such as methylene blue or ferricyanide was required at low concentrations to couple the anaerobic oxidation of substrates such as ethanol, lactate, or succinate to manganese reduction. These experiments were performed with cell extracts of a variety of eucaryotic organisms and tissues, which contain enzymes and respiratory chain compounds similar to those found in bacteria and are suggestive of ways manganese reduction could be coupled to bacterial metabolic processes.

Given these observations, the experiments described here were undertaken to further study manganese reduction by microorganisms. Anaerobic organisms were enriched for in a medium in which (i) manganates were the sole possible inorganic electron acceptors (O_2, nitrate, sulfate, and ferric iron were excluded from the medium), and (ii) there were low concentrations of organics (yeast extract and peptone) and carbon sources that are difficult to ferment (such as succinate or acetate rather than glucose). These criteria were aimed primarily at excluding the growth of sulfate-reducing and fermentative bacteria in the enrichments, because the metabolic end products of these types of organisms can lead to the indirect microbial reduction of manganates. With this medium, enrichment cultures containing manganese-reducing organisms were obtained from two coastal marine sediments. Experiments were then performed with these

* Corresponding author.
† Present address: Marine Sciences Program, University of North Carolina at Chapel Hill, Chapel Hill, NC 27514.

enrichment cultures to examine some of the constraints on microbial manganese reduction.

MATERIALS AND METHODS

All of the experiments described here were performed under anaerobic conditions in a Coy anaerobic chamber (Coy Laboratory Products, Ann Arbor, Mich.) containing 10% H_2–90% N_2. The manganate used was prepared by the oxidation of Mn^{2+} by permanganate under basic conditions by the reaction $3Mn^{2+} + 2MnO_4^- + 2H_2O \rightarrow 5\ \delta MnO_2 + 4H^+$. It was inferred to be the mineral-phase vernadite or δMnO_2 on the basis of the average oxidation state of this solid (O:Mn ratio, 1.94 ± 0.02) and its X-ray diffraction pattern (2, 5, 18). The δMnO_2 was stored in a desiccator as a fine, freeze-dried powder, and before use in any of the experiments described here it was reequilibrated in sulfate-containing artificial seawater (ASW; see below). This preparation was a modification of previously published methods (2, 18) and is described in detail elsewhere (Burdige, Ph.D. thesis). In this paper, δMnO_2 prepared in this manner will be referred to as preconditioned δMnO_2.

Two types of artificial seawater were used in this study. Sulfate-free artificial seawater (SF ASW contained (per liter of distilled water) 17.55 g of NaCl (300 mM); 0.76 g of KCl (10 mM), 1.5 g of $CaCl_2 \cdot H_2O$ (10 mM), and 10.2 g of $MgCl_2 \cdot 6H_2O$ (45 mM). Total chloride in this solution was 420 mM, and the ionic strength was 0.475 (approximately two-thirds that of natural seawater). ASW contained the major cations of seawater in their natural concentrations and was prepared with (per liter of distilled water) 23.8 g of NaCl (407 mM), 11 g of $MgCl_2 \cdot 6H_2O$ (54 mM), 1.5 g of $CaCl_2 \cdot 2H_2O$ (10 mM), 4 g of Na_2SO_4 (28 mM), and 0.76 g of KCl (10 mM).

Media for both initial enrichment cultures and experiments were prepared by first dissolving 0.2 g of yeast extract and 0.1 g of Bacto-Peptone (Difco Laboratories, Detroit, Mich.) in either 1 liter of SF ASW or 667 ml of ASW and 333 ml of distilled water and adjusting the pH to 7.6. These two solutions had approximately the same ionic strength (0.475 and 0.462, respectively). Hereafter, the latter solution will be referred to as 2/3I ASW. After either of these solutions was autoclaved, the following were aseptically added from sterile, stock solutions: 10 mM HEPES buffer (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; pH 7.8), 2 mM HCO_3^-, and either 15 mM succinate, 20 mM lactate, 30 mM acetate, or a carbon mixture containing 7 mM pyruvate, 5 mM succinate, and 10 mM acetate (all concentrations are final concentrations). Stock solutions were sterilized by autoclaving and were prepared with distilled water and sodium salts, except for lactate which was prepared by diluting 60% lactate syrup with distilled water. The initial pH of these media was 7.5 ± 0.1.

Enrichment procedures. The medium used for enrichment cultures was prepared with SF ASW and the three-carbon mixture (pyruvate, acetate, and succinate). Aliquots (10 ml) were then aseptically placed in presterilized screw-cap test tubes containing preconditioned δMnO_2 (initial concentration, 0.05 to 0.1 mg/ml = 575 to 1,150 mM). These were then stored in the anaerobic chamber until used (minimum equilibration time before use was overnight).

Enrichment cultures used in these experiments were obtained by inoculating these tubes with surface sediments (1 to 10 cm) collected from Skan Bay, Alaska (24), and the San Clemente basis off southern California (8). All enrichments were maintained at room temperature (25°C) in the anaerobic chamber and were transferred (0.1 ml) to tubes containing fresh medium upon depletion (on the basis of visual inspection) of the solid δMnO_2. After three to five transfers in this medium, enrichments were transferred to a medium containing SF ASW and succinate. For consistency among experiments, these were used to inoculate all experiments.

Studies of manganese reduction by enrichment cultures. Media for these experiments were prepared with either ASW or SF ASW (as described above) with either lactate, acetate, or succinate as the single carbon source. Portions (20 ml) of these media were aseptically dispensed into presterilized screw-cap test tubes, and the tubes were equilibrated overnight in the anaerobic chamber before use.

Before an experiment was begun, preconditioned δMnO_2 was added to the experimental tubes from a sterile, anaerobic stock suspension. The initial concentration of δMnO_2 was either 0.05 or 0.1 mg/ml. After inoculation with 0.2 ml of an enrichment culture (see above), samples (generally 0.5 ml) were removed as a function of time to measure the amount of Mn^{2+} produced in the experiment. All experimental tubes were gently agitated for the duration of the experiment on an orbit shaker set inside the anaerobic chamber. A sterile glass bead was placed in each tube so that this agitation would keep the solid manganate in suspension. Uninoculated parallel blank experiments were run for all experiments.

The extent of manganese reduction was determined by measuring the total amount of Mn^{2+} produced (i.e., the end product of the reduction reaction). Because manganate suspensions above pH 5 to 7 have large capacities for binding or adsorbing Mn^{2+} (18, 19), a method was developed to measure both free, soluble Mn^{2+} and Mn^{2+} which is bound or adsorbed to the surfaces of the remaining δMnO_2 particles. Samples were removed from the tubes and filtered (inside the anaerobic chamber) through a 0.2-μm-pore-size membrane filter (Gelman Sciences, Inc., Ann Arbor, Mich.) by using a 13-mm (diameter) Swinnex filter holder (Millipore Corp., Bedford, Mass.) and a 5-ml disposable plastic syringe. The filters were then removed from the filter holder and soaked for 2 h in 2 ml of an anaerobic 10 mM $CuSO_4$ solution (pH 4.7), allowing the bound Mn^{2+} ions to be replaced by the excess cuprous ions. The $CuSO_4$ solution was then filtered. Dissolved manganese was measured in both filtrates by flame atomic absorption spectrophotometry with an air-acetylene flame. Values were corrected for sample dilution and summed to obtain the amount of Mn^{2+} in a given sample. This method was found to desorb >90% of the Mn^{2+} bound to a δMnO_2 surface (Burdige, Ph.D. thesis).

Except where noted, all experiments were performed with 2/3I ASW. In one set of experiments, 20 mM sodium molybdate or 20 mM sodium molybdate and 10 mM sodium azide were added to experimental tubes 6 h after they were inoculated. Molybdate was added an an inhibitor of sulfate-reducing bacteria (20, 27), whereas azide was added to poison organisms that possesses an electron transport system and generate at least a portion of their ATP by oxidative phosphorylation (12). The combination of azide and molybdate should, under anaerobic conditions, only allow the growth only of fermentative bacteria.

The effect of nitrate on manganese reduction was assessed by adding 200 μM $NaNO_3$ to the medium before inoculation. To study the effect of oxygen on manganese reduction, we covered another set of tubes with Belco cap closures (rather than screw caps), removed them from the anaerobic chamber just after inoculation, and then mixed them vigorously for 2 to 3 min to ensure that the medium was fully equili-

brated with atmospheric oxygen at the start of the experiment. These were then also shaken (under laboratory air) for the duration of the experiment.

RESULTS

The two enrichment cultures used in these studies were isolated from coastal marine sediments. Enrichment culture SK-13 came from surface sediments collected in Skan Bay, Alaska, and after being returned to the laboratory was transferred three times before use in these experiments. The predominant organism in this culture was an oblate-shaped rod approximately 4 to 6 μm long and 1 to 2 μm wide (estimated by light microscopy at ×1,000 magnification). Most of the cells were nonmotile and occurred both individually and as linked chains of 3 to 10 bacteria. Enrichment culture SC-44 was obtained from surface sediments taken from the San Clemente basin off southern California. This culture was transferred four times before being used in these studies. The dominant organisms in this enrichment culture were long thin rods (7 to 11 by 0.6 to 1.2 μm). They were almost exclusively nonmotile, and many seemed to have, or were forming, terminal endospores.

The results from these experiments are shown in Fig. 1 to 7 and summarized in Tables 1 to 3. The rates listed in the tables were obtained by linear least-squares fitting of the data. Data points were omitted from the calculation when (i) the Mn^{2+} values in the early part of the experiment had not increased above background levels, suggesting that the culture had not yet come out of lag phase (see Fig. 4, 6, and 7), or (ii) the manganese profile appeared to level off near the end of an experiment owing to depletion in either the total δMnO_2 added to the experiment (see Fig. 1) or, possibly, other nutrients, leading to growth limitation (see Figs. 3 and 6). With this fitting procedure, we did not consider the possibility that in mixed enrichment cultures such as these the observed rate of Mn^{2+} production does not necessarily have to be constant. Because the activities of the different manganese-reducing organisms in the enrichment cultures may vary over the course of an experiment, it is possible that the resulting Mn^{2+} production could be nonlinear over time. However, as we will show below and in comparison of rates among different experiments, we believe that to at least a first approximation this fitting procedure was valid in analyzing our data.

Relative to uninoculated parallel blank experiments, these enrichment cultures were able to reduce δMnO_2 when grown

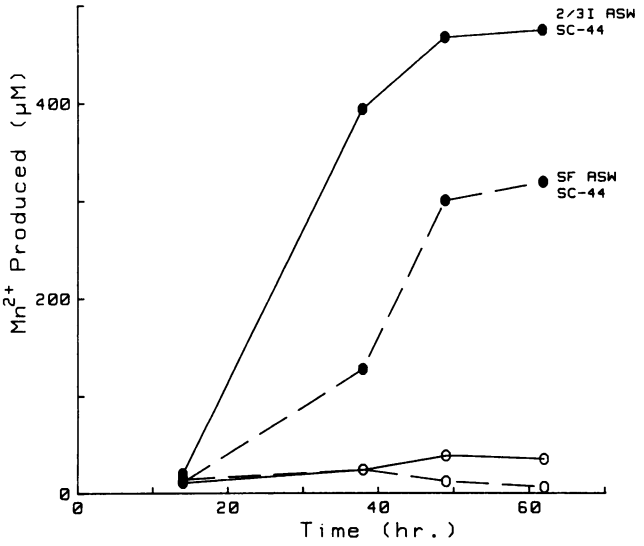

FIG. 1. Mn^{2+} production by enrichment culture SC-44 when grown with lactate as the carbon source in SF ASW (closed symbols, broken line) or 2/3I ASW (solid line). Parallel uninoculated experiments are indicated by open symbols and the same line type.

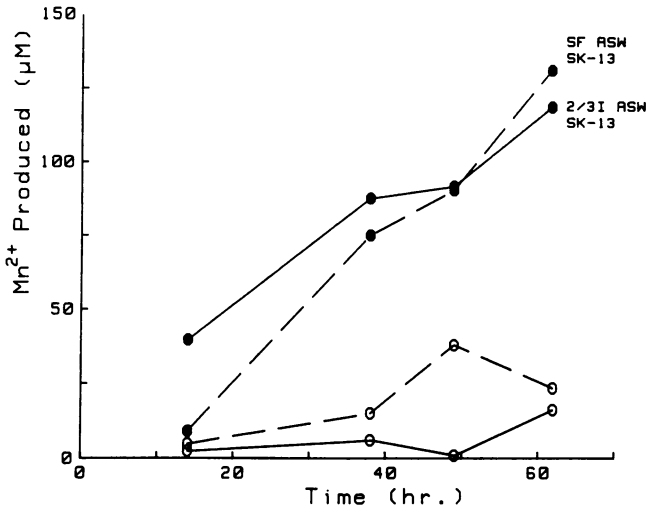

FIG. 2. Mn^{2+} production by enrichment culture SK-13 when grown with succinate as the carbon source in SF ASW (closed symbols, broken line) or 2/3I ASW (solid line). Parallel uninoculated experiments are indicated by open symbols and the same line type.

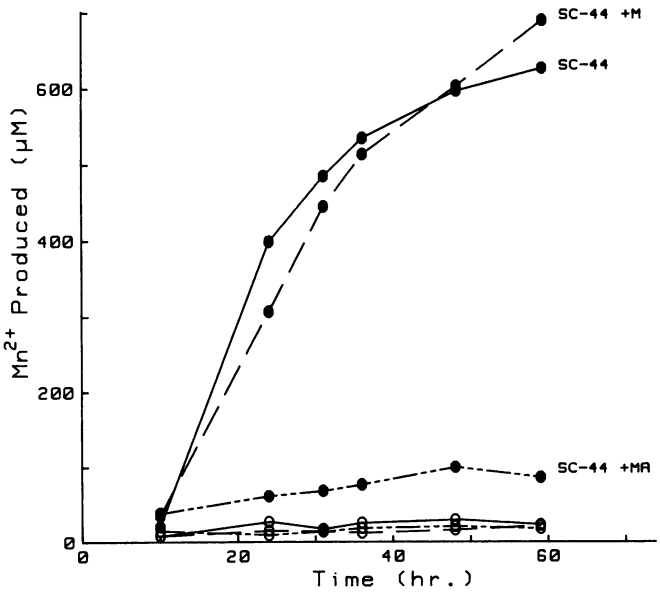

FIG. 3. Mn^{2+} production by enrichment culture SC-44 grown with lactate as the carbon source and no added inhibitors (closed symbols, solid line), 20 mM molybdate (broken line, +M), or 20 mM molybdate and 10 mM azide (dot-dash line [+MA]). As discussed in the text, these inhibitors were added 6 h after inoculation. Parallel uninoculated experiments are indicated by open symbols and the same line type.

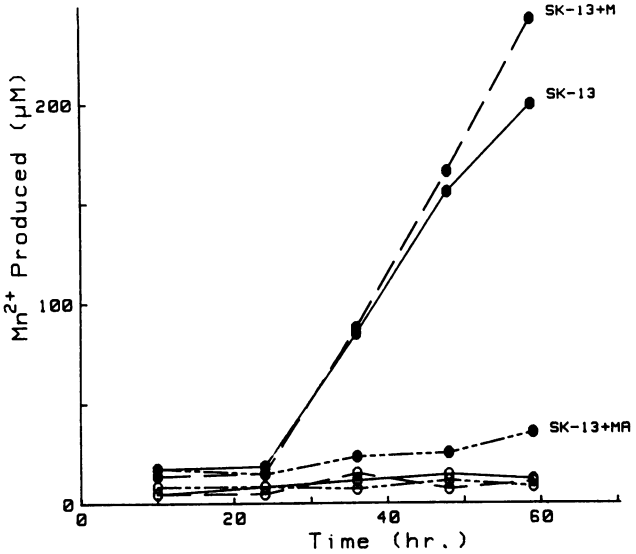

FIG. 4. Mn^{2+} production by enrichment culture SK-13 grown with succinate as the carbon source and no added inhibitors (closed symbols, solid line), 20 mM molybdate (broken line [+M]), or 20 mM molybdate and 10 mM azide (dot-dash line [+MA]). As discussed in the text, these inhibitors were added 6 h after inoculation. Parallel uninoculated experiments are indicated by open symbols and the same line type.

with either lactate, succinate, or acetate as a carbon source in both SF ASW and ASW. Two typical experiments are shown in Fig. 1 and 2, and the results of all experiments performed are summarized in Tables 1 to 3.

When SC-44 was grown on lactate (Fig. 3) and SK-13 was grown on succinate (Fig. 4), the addition of 20 mM molybdate had little effect on manganese reduction. Although molybdate caused a 13% decrease in the rate of Mn^{2+} production by SC-44 when it was grown on lactate (Table 1),

TABLE 1. Summary of rate data from Mn^{2+} production by enrichment culture SC-44

Exptl conditions[a]	Initial δMnO_2 (mg/ml)	Rate of Mn^{2+} production (μM/h)	Linear correlation coefficient (r)	Time range of calculation (h)	Figure
SF, L	005	693	0960	14–62	1
L	005	1324	0980	14–49	1
SF, S	005	176	0988	14–62	—
S	005	278	0979	14–62	—
SF, A	005	067	0983	14–62	—
A	005	080	0949	14–62	—
L	01	1514	0936	10–48	3
L + M	01	1313	0968	10–59	3
L + MA	01	112	0911	10–59	3
S	01	170	089	10–59	5
S + M	01	1042	0987	10–59	5
S + MA	01	053	065	10–59	5
L	01	1408	0921	22–58	6
L + N	01	1188	0988	22–69	6
L + 0	01	081	0737	22–69	6

[a] Abbreviations: SF ASW (unless noted by SF, all experiments were performed in 2/3I ASW which contains sulfate [see the text]); L, lactate added as the carbon source; S, succinate added as the carbon source; A, acetate added as the carbon source; M, 20 mM molybdate added; MA, 20 mM molybdate and 10 mM azide added; N, 200 μM nitrate added; O, experiment was removed from the anaerobic chamber and oxygenated (equilibrated with atmospheric O_2) at the start of the experiment.

[b] —, not shown here but shown in Burdige, Ph.D. thesis.

TABLE 2. Summary of rate data for Mn^{2+} production by enrichment culture SK-13

Exptl conditions[a]	Initial δMnO_2 (mg/ml)	Rate of Mn^{2+} production (μM/h)	Linear correlation coefficient (r)	Time range of calculation (h)	Figure
SF, L	0.05	2.02	0.988	14–62	—[b]
L	0.05	4.29	0.992	14–62	—
SF, S	0.05	2.49	0.995	14–62	2
S	0.05	1.59	0.985	14–62	2
SF, A	0.05	1.06	0.924	14–62	—
A	0.05	1.89	0.980	14–62	—
S	0.1	5.24	0.997	24–59	4
S + M	0.1	6.48	0.999	24–59	4
S + MA	0.1	0.54	0.968	24–59	4
S	0.1	5.42	0.991	22–69	7
S + N	0.1	4.16	0.950	22–69	7
S + O	0.1	0.07	0.32	22–69	7

[a] For a definition of the abbreviations, see footnote *a* to Table 1.

[b] —, not shown here but shown in Burdige, Ph.D. thesis.

the rate of manganese reduction by SK-13 grown on succinate increased 24% in the presence of 20 mM MoO_4^{2-} (Table 2). However, when SC-44 was grown on succinate (Fig. 5), the addition of molybdate actually caused a five- to sixfold enhancement in both the rate of manganese reduction and the amount of Mn^{2+} produced after 59 h. This rate was only slightly lower than the rate of Mn^{2+} production when SC-44 was grown on lactate (Table 1).

In these same experiments, the simultaneous addition of azide and molybdate caused a large decrease in microbial manganese reduction. When SC-44 was grown on lactate, the rate in the presence of both compounds was only 7.4% of the rate in their absence (Table 1). For SK-13 grown on succinate, these inhibitors decreased the rate of manganese reduction to 9.7% of the unpoisoned rate (Table 2). For comparison, rates in uninoculated experiments were 0.2 to 1.9% of the rates in unpoisoned, inoculated experiments (Table 3). When SC-44 was grown with succinate, the rate of

TABLE 3. Summary of rate data for Mn^{2+} production in uninoculated experiments

Exptl conditions[a]	Initial δMnO_2 (mg/ml)	Rate of Mn^{2+} production (μM/h)	Linear correlation coefficient (r)	Time range of calculation (h)	Figure
SF, L	0.05	–0.18	–0.49	14–62	1
L	0.05	0.56	0.932	14–62	1
L	0.1	0.3	0.65	10–59	3
L + M	0.1	0.24	0.89	10–59	3
L + MA	0.1	0.16	0.65	10–59	3
L	0.1	1.78	0.974	10–69	6
L + N	0.1	1.04	0.997	10–69	6
L + O	0.1	0.17	0.36	10–69	6
SF, S	0.05	0.51	0.75	14–62	2
S	0.05	0.22	0.64	14–62	2
S	0.1	0.17	0.89	10–59	4,5
S + M	0.1	0.12	0.53	10–59	4,5
S + MA	0.1	0.03	0.31	10–59	4,5
S	0.1	0.33	0.963	10–69	7
S + N	0.1	0.12	0.937	10–69	7
S + O	0.1	–0.09	–0.42	10–69	7
SF, A	0.05	0.02	0.07	14–62	—[b]
A	0.05	0.53	0.58	14–62	—

[a] For a definition of the abbreviations, see footnote *a* to Table 1.

[b] —, Not shown here but shown in Burdige, Ph.D. thesis.

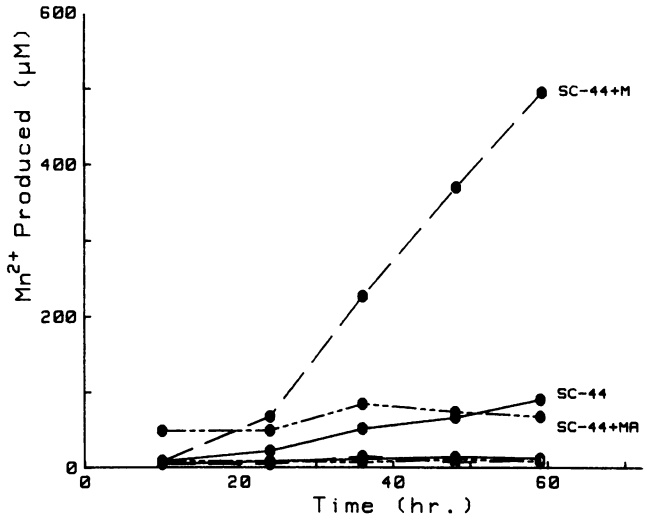

FIG. 5. Mn^{2+} production by enrichment culture SC-44 grown with succinate as the carbon source and no added inhibitors (closed symbols, solid line), 20 mM molybdate (closed symbols, broken line [+M]), or 20 mM molybdate and 10 mM azide (dot-dash line [+MA]). As discussed in the text, these inhibitors were added 6 h after inoculation. Parallel uninoculated experiments are indicated by open symbols and the same line type.

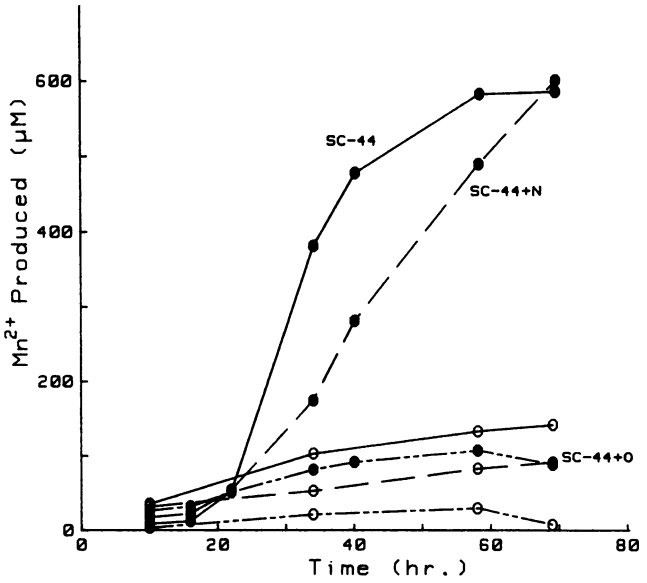

FIG. 6. Mn^{2+} by enrichment culture SC-44 grown with lactate as the carbon source and no additional electron acceptors (closed symbols, solid line), 200 μM NO_3 (broken line [+N]), or oxygenated after inoculation (dot-dash line [+O]). Parallel uninoculated experiments are indicated by open symbols and the same line type.

Mn^{2+} production in the presence of azide and molybdate was 31.2% of the rate in their absence; however, this was only 5.1% of the enhanced rate observed when molybdate alone was present (Table 1).

The results in Fig. 6 and 7 show the effects of NO_3^- and oxygen on manganese reduction by these enrichments. The addition of 200 μM NO_3^- to the medium led to a slight decrease in the rate of Mn^{2+} production by both enrichment cultures (approximately 20%; Tables 1 and 2), whereas oxygen caused an almost complete cessation of microbial manganese reduction. Under aerobic conditions, the rate of manganese reduction by SK-13 grown with succinate was only 1.3% of the rate under anaerobic conditions; for SC-44 grown with lactate, the rate of Mn^{2+} production in the presence of O_2 was 5.7% of the rate observed in its absence.

DISCUSSION

The results presented here serve to illustrate a few points and to generate several questions. In agreement with the results of other workers, the presence of viable bacteria led to a great enhancement in the rate of manganese reduction. However, in contrast to other studies (11, 29–31), our enrichment cultures reduced manganese under anaerobic but not aerobic conditions (Fig. 6 and 7). Whether the individual organisms responsible for manganese reduction are facultative or obligate anaerobes is not yet known, but here the process of microbial manganese reduction is clearly dependent on the exclusion of oxygen. Troshanov (31) noted that the manganese-reducing organisms he isolated from lake sediments were microaerophiles that were unaffected by the presence of oxygen. Trimble and Ehrlich (29) observed that not only did oxygen not interfere with manganese reduction by a marine *Bacillus* species and an unidentified marine coccus but that oxygen was required to adapt the cultures to utilize manganates. In addition, further studies with the *Bacillus* sp. (11) showed that 10 mM azide stimulated manganese reduction by this organism, whereas in our experiments this concentration of azide inhibited essentially all microbial manganese reduction (Fig. 3 to 5). These differences suggest that the manganese-reducing organisms in our enrichment cultures are metabolically distinct from the others that have been studied previously. Pure culture studies should resolve the detailed nature of these differences.

In our initial enrichment cultures, SF ASW was used in conjunction with carbon sources which favor respiratory metabolism in an effort to eliminate the possibility of isolat-

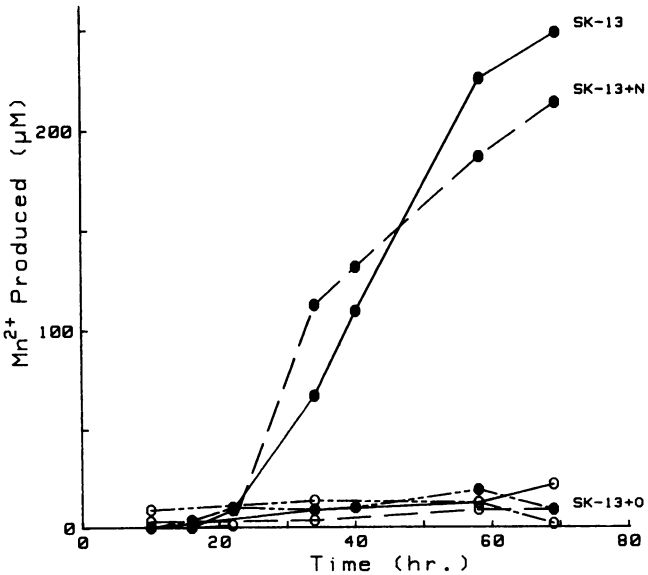

FIG. 7. Mn^{2+} by enrichment culture SK-13 grown with succinate as the carbon source and no additional electron acceptors (closed symbols, solid line), 200 μM NO_3^- (broken line [+N]), or oxygenated after inoculation (dot-dash line [+O]). Parallel uninoculated experiments are indicated by open symbols and the same line type.

TABLE 4. Rates of manganese reduction and first-order reduction rate constants from a number of environments and for enrichment cultures of manganese-reducing bacteria

Source[a]	k_{red} (yr^{-1})	Mn^{2+} production rate (upper limit; μM/day)
East equatorial Atlantic sediments (4)	.0015–.0021	0.38
Chesapeake Bay sediments (14)	.0173	0.57
Lake Michigan sediments (25)		0.47
Long Island Sound sediments (1)	8.2–25	576
Anoxic water column Saanich Inlet (6)		220
Enrichment culture SC-44 (this work)		363
Enrichment culture SK-13 (this work)		156

[a] Numbers in parentheses refer to references in which the rate data were reported.

ing sulfate-reducing or fermentative organisms. However, because some strains of sulfate-reducing bacteria can grow on pyruvate in the absence of sulfate (23), it is possible that the enrichment procedure described here could have selected for such a sulfate-reducing organism. The lack of molybdate inhibition of manganese reduction (Fig. 3 to 5) indicates that this did not occur. The concentration of molybdate we used has been shown to be an effective inhibitor of sulfate reduction in both laboratory (pure culture) and field studies of this process (20, 27; Burdige, Ph.D. thesis). As a result, it appears that the reduction of δMnO_2 by these two enrichment cultures is not due to the presence of sulfate reducers (and the sulfide they produce) in either of the cultures.

The fact that the simultaneous addition of azide and molybdate stopped virtually all manganese reduction (Fig. 3 to 5) indicates that the organisms responsible for manganese reduction in these enrichment cultures coupled manganese reduction to electron transport and respiration. Although it is possible that this azide inhibition was the result of a non specific inhibition of other enzymes by azide, we are not familiar with any studies which illustrate such a phenomenon and we have assumed that, as has been shown previously (11, 12), azide was acting here as an inhibitor of electron transport.

If manganates were used here as terminal electron acceptors, then the slightly lower rates of Mn^{2+} production in the presence of nitrate (Fig. 6 and 7, Tables 1 and 2) may be due to the fact that some of the manganese-reducing bacteria in these enrichment cultures preferentially utilized nitrate as an electron acceptor. The reduction of nitrate can be more efficient (in terms of free energy gained per mole of carbon substrate utilized), depending on how far nitrate is reduced (NO_2^- or N_2) and the manganate phase available for reduction. Given that there is strong evidence indicating that marine and freshwater bacteria capable of reducing nitrate or ferric iron can reduce the other compound as well (16, 21, 26), it is not unlikely that such a relationship might also exist for manganese reducers.

If solid manganates are indeed used as electron acceptors, then one must also address the question of how electron transport is effected from the bacteria to an insoluble substrate. For example, do they attach themselves directly to the manganate particles (as the photomicrographs in Ghiorse and Ehrlich [11] indicate for the manganese-reducing organism they studied), or do the organisms use a diffusible reversible electron carrier which shuttles between their electron transport system and the manganate particles. The works of Hochster and Quastel (13) and Stone and Morgan (28) suggest that compounds such as ferricyanide, methylene blue, or certain quinones could serve in such a role. As with the above questions, these can be answered only with pure cultures of manganese-reducing organisms, which are now being isolated in our laboratory.

Finally, how do these results compare with other reported rates of manganese reduction? Table 4 lists rates and rate constants for a number of environments (rates of manganese reduction are reported here as Mn^{2+} production rates). First-order rate constants were obtained from geochemical models, assuming that the rate of manganese reduction is first order with respect to the concentration of solid manganate present (1, 4, 14). These environmental rates are upper limits, because solid-phase manganese generally goes to zero in the zone of manganese reduction. Also listed in Table 4 are the maximum rates of manganese reduction observed in the experiments presented here. The fact that these experimental microbial rates are generally higher than environmental rates could be the result of a number of factors, including the following. (i) The density of manganese-reducing organisms in our laboratory experiments was higher than in the environments examined. (ii) The concentration of nutrients (including manganates) in our experiments was greater than is commonly observed in nature. (iii) The manganate phase used in these experiments was kinetically more reactive than natural manganates. (iv) Variability in other physical parameters (e.g., pH, E_h, or temperature) may have existed between these experiments and the environments studied. It should also be kept in mind that these are modeled, not measured, rates and they do not take into account the possibility of simultaneous oxidation and reduction of manganese in, perhaps, microenvironments of these regions. Such an occurrence would lead to an underestimation of true activity. It is also possible that in many environments, including some of those listed in Table 4, mechanisms of manganese reduction other than those studied here (such as indirect microbial manganese reduction mediated by sulfide or reduced organics) may be responsible for the observed activity. Careful microbiological field studies will be required to address these questions.

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LITERATURE CITED

1. **Aller, R. C.** 1980. Diagenetic processes near the sediment-water interface. II. Fe and Mn. Adv. Geophys. **22**:351–415.
2. **Balistrieri, L. S., and J. W. Murray.** 1982. The surface chemistry of δMnO_2 in major ion seawater. Geochim. Cosmochim. Acta **46**:1041–1052.
3. **Bromfield, S. M., and D. J. David.** 1976. Sorption and oxidation of manganous ions and the reduction of manganese oxide by cell suspensions of a manganese oxidizing bacterium. Soil Biol. Biochem. **8**:37–43.
4. **Burdige, D. J., and J. M. Gieskes.** 1983. A pore water/solid phase diagenetic model for manganese in marine sediments.

Am. J. Sci. **283**:29–47.
5. **Burns, R. G., and V. M. Burns.** 1979. Manganese oxides, p. 1–46. *In* R. G. Burns (ed.), Marine minerals. Mineralogical Society of America, Washington, D.C.
6. **Emerson, S., R. E. Cranston, and P. S. Liss.** 1979. Redox species in a reducing fjord: equilibrium and kinetic considerations. Deep-Sea Res. **26A**:859–878.
7. **Emerson, S. A., R. Jahnke, M. Bender, P. Froelich, G. Klinkhammer, C. Bowser, and G. Setlock.** 1980. Early diagenesis in sediments from the eastern equatorial Pacific. I. Pore water nutrients and carbonate results. Earth Planet. Sci. Lett. **49**:57–80.
8. **Emery, K. O.** 1960. The sea off southern California. John Wiley & Sons, Inc., New York.
9. **Froelich, P. N., G. P. Klinkhammer, M. L. Bender, N. A. Leudtke, G. R. Heath, D. Cullen, P. Dauphin, D. Hammond, B. Hartman, and V. Maynard.** 1979. Early oxidation of organic matter in pelagic sediments of the eastern equatorial Atlantic: suboxic diagenesis. Geochim. Cosmochim. Acta **43**:1075–1090.
10. **Ghiorse, W. C., and H. L. Ehrlich.** 1974. Effects of seawater cations and temperature on manganese dioxide-reductase activity in a marine *Bacillus*. Appl. Microbiol. **28**:785–792.
11. **Ghiorse, W. C., and H. L. Ehrlich.** 1976. Electron transport components of the MnO_2 reductase system and the location of the terminal reductase in a marine *Bacillus*. Appl. Environ. Microbiol. **31**:977–985.
12. **Heinen, W.** 1971. Inhibitors of electron transport and oxidative phosphorylation, p. 383–393. *In* J. R. Norris and D. W. Ribbons (ed.), Methods in microbiology, vol. 6A. Academic Press, Inc., New York.
13. **Hochster, R. M., and J. H. Quastel.** 1951. Manganese dioxide as a terminal hydrogen acceptor in the study of respiratory systems. Arch. Biochem. Biophys. **36**:132–146.
14. **Holdren, G. R., Jr., O. P. Bricker, III, and G. Matisoff.** 1975. A model for the control of dissolved manganese in the interstitial waters of Chesapeake Bay. Am. Chem. Soc. Symp. Ser. **18**:364–381.
15. **Jahnke, R., D. Heggie, S. Emerson, and V. Grundmanis.** 1982. Pore waters of the central Pacific ocean: nutrient results. Earth Planet. Sci. Lett. **61**:233–256.
16. **Jones, J. G., S. Gardener, and B. M. Simon.** 1983. Bacterial reduction of ferric iron in a stratified eutrophic lake. J. Gen. Microbiol. **129**:131–139.
17. **Mann, P. J. G., and J. H. Quastel.** 1946. Manganese metabolism in soils. Nature (London) **158**:154–156.
18. **Morgan, J. J., and W. Stumm.** 1964. Colloid-chemical properties of manganese dioxide. J. Colloid Sci. **19**:347–359.
19. **Murray, J. W.** 1974. The surface chemistry of hydrous manganese dioxide. J. Colloid Interface Sci. **46**:357–371.
20. **Oremland, R. S., and B. F. Taylor.** 1978. Sulfate reduction and methanogenesis in marine sediments. Geochim. Cosmochim. Acta **42**:209–214.
21. **Ottow, J. C. G., and J. C. Munch.** 1978. Mechanisms of reductive transformation in the anaerobic microenvironments of hydromorphic soils, p. 483–492. *In* W. E. Krumbein (ed.), Environmental biogeochemistry and geomicrobiology, vol. 2. Ann Arbor Science, Ann Arbor, Mich.
22. **Perfil'ev, B. V., D. R. Gabe, A. M. Gal'perina, V. A. Rabiinovich, A. A. Sapotnitskii, E. E. Sherman, and E. P. Troshanov.** 1965. Applied capillary microscopy: the role of microorganisms in the formation of iron-manganese deposits. Consultants Bureau, New York. (Translated from Russian.)
23. **Postgate, J. R.** 1979. The sulfate-reducing bacteria. Cambridge University Press, Cambridge.
24. **Reeburgh, W. S.** 1983. Rates of biogeochemical processes in anoxic sediments. Annu. Rev. Earth Planet. Sci. **11**:269–298.
25. **Robbins, J. A., and E. Callender.** 1975. Diagenesis of manganese in Lake Michigan sediments. Am. J. Sci. **275**:512–533.
26. **Sørensen, J.** 1982. Reduction of ferric iron in anaerobic, marine sediment and interaction with reduction of nitrate and sulfate. Appl. Environ. Microbiol. **43**:319–324.
27. **Sørensen, J., D. Christensen, and B. B. Jørgensen.** 1981. Volatile fatty acids and hydrogen as substrates for sulfate-reducing bacteria in anaerobic marine sediment. Appl. Environ. Microbiol. **42**:5–11.
28. **Stone, A. T., and J. J. Morgan.** 1984. Reduction and dissolution of manganese(III) and manganese(IV) oxides by organics: 2. Survey of the reactivity of organics. Environ. Sci. Technol. **18**:617–624.
29. **Trimble, R. B., and H. L. Ehrlich.** 1968. Bacteriology of manganese nodules. III. Reduction of MnO_2 by two strains of nodule bacteria. Appl. Microbiol. **16**:695–702.
30. **Trimble, R. B., and H. L. Ehrlich.** 1970. Bacteriology of manganese nodules. IV. Induction of an MnO_2-reductase system in a marine Bacillus. Appl. Microbiol. **19**:966–972.
31. **Troshanov, E. P.** 1967. Iron and manganese reducing microorganisms in ore-containing lakes of the Karelian Isthmus. Mikrobiologiya **37**:786–791. (English translation.)
32. **Wollast, R. B., G. Billen, and J. C. Duinker.** 1979. Behaviour of manganese in the Rhine and Scheldt estuaries. Estuarine Coastal Mar. Sci. **9**:161–169.
33. **Zehnder, A. J. B., and T. D. Brock.** 1980. Anaerobic methane oxidation: occurrence and ecology. Appl. Environ. Microbiol. **39**:194–204.