

Use of Poisons in Determination of Microbial Manganese Binding Rates in Seawater

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A method was developed to determine whether microorganisms mediate the precipitation of manganese(II) in the marine environment. Radioactive ⁵⁴Mn(II) was used as a tracer to measure the precipitation (binding and oxidation) of Mn(II) [i.e., the ⁵⁴Mn(II) trapped on 0.2- μ m membrane filters] in the presence and absence of biological poisons. A variety of antibiotics, fixatives, and metabolic inhibitors were tested in laboratory control experiments to select poisons that did not interfere in the chemistry of manganese. The poisons were deemed suitable if (i) they did not complex Mn(II) more strongly than the ion-exchange resin Chelex 100, (ii) they did not interfere in the adsorption of ⁵⁴Mn(II) onto synthetic δ MnO₂ (manganate), (iii) they did not cause desorption of ⁵⁴Mn(II) which had been preadsorbed onto synthetic manganate, and (iv) they did not solubilize synthetic ⁵⁴manganate. In addition, several known chelators, reducing agents, and buffers normally added to microbiological growth media or used in biochemical assays were tested. Most additions interfered to some extent with manganese chemistry. However, at least one inhibitor, sodium azide, or a mixture of sodium azide, penicillin, and tetracycline was shown to be appropriate for use in field studies of ⁵⁴Mn(II) binding. Formaldehyde could also be used in short incubations (1 to 3 h) but was not suitable for longer time course studies. The method was applied to studies of Mn(II) precipitation in Saanich Inlet, British Columbia, Canada. Bacteria were shown to significantly enhance the rate of Mn(II) removal from solution in the manganese-rich particulate layer which occurs just above the oxygen-hydrogen sulfide interface in the water column.

Bacteria catalyze redox transformations in various environments. Since many of these reactions can occur spontaneously under environmental conditions, bacterial catalysis is not always necessary to explain the final chemical states observed. When the rates of reactions are too rapid to be explained by chemical reaction kinetics or diffusion processes alone, bacterial activities are often inferred. Similar inferences are drawn from physiological characterization of pure cultures of bacteria known to be abundant in certain environments. However, since bacteria can regulate their metabolism in response to environmental conditions, the presence of a given bacterial type, even in great abundance, although suggestive, cannot always be taken as proof of its activity *in situ*.

The preferred method to establish bacterial catalysis is the direct measurement of their activities. Such activity measurements have been performed for a variety of microbial activities, including transformations of sulfur and nitrogen (9-13, 20). For Mn(II) oxidation, however, direct measurements of activities *in situ* or in freshly collected samples have not usually been presented. Rather, microbial activities have been inferred from studies of the abundance and potential activity of bacterial populations in soils and aquatic environments (4, 5, 14). Kinetic studies of the chemistry of several environments have also concluded that Mn(II) oxidation is too rapid to be accounted for by purely inorganic chemical mechanisms (6, 7, 23). Reliable, direct measurements would be very helpful in resolving the actual contribution of bacteria to Mn(II) oxidation in natural environments.

One method to estimate *in situ* microbial activities involves the use of poisons to block metabolic activities. Such an approach would be of great value for the study of microbial Mn(II) oxidation in the environment, comparing

unpoisoned and poisoned bacterial populations in radioactive binding experiments to distinguish biological from autocatalytic oxidation, respectively. However, great care must be taken when introducing poisons into natural or artificial environments (2) because, if the poisons interfere with autocatalytic processes, artifactual microbial activities might be inferred. We present here the results of experiments designed to define suitable poisons that inhibit microbial ⁵⁴Mn(II) binding and oxidation without significantly interfering with either manganese(II) adsorption to preformed manganese(IV) oxides (manganates) or the autocatalytic oxidation of manganese(II).

MATERIALS AND METHODS

Solutions of inhibitors and other compounds. Stock solutions of all compounds to be tested were made in distilled water. The only exceptions were rifampin, which was first solubilized in 0.3 ml of 95% ethanol and then diluted with distilled water, and dichloromethylurea (DCMU; dissolved in 95% ethanol and added directly). The pH of these solutions was adjusted to 7.5, except where noted. Usually, 0.5 ml of a stock solution was added to a 5-ml assay.

Competition with Chelex 100 for ⁵⁴Mn(II) binding. Chelex 100 (Bio-Rad Laboratories, Richmond, Calif.) columns (5 ml) were preequilibrated with natural seawater. Each compound to be tested was added to seawater containing 1.0 μ M MnCl₂ plus tracer carrier-free ⁵⁴MnCl₂ (0.2 μ Ci ml⁻¹; New England Nuclear Corp., Boston, Mass.). After a 10-min incubation at room temperature, a portion (0.1 to 0.5 ml) was added to a Chelex column and eluted with 2.5 ml of seawater. Radioactivity in the eluate was counted on a Biogamma II (Beckman Instruments, Inc., Irvine, Calif.). Elution of antibiotics was confirmed by bioassay with sensitive manganese-oxidizing bacteria. A suspension of sensitive bacteria was spread on K media (19) agar plates, and

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TABLE 1. Binding of $^{54}\text{Mn(II)}$ by test compounds in competition with Chelex 100^a

Compound	Concn	% ^b in eluate	
		Compound	Radioactivity
Antibiotic			
Chloramphenicol	200 µg/ml	2	3
Gentamicin sulfate	100 µg/ml	9	0
Kanamycin monosulfate	200 µg/ml	12	0.1
Nalidixic acid	100 µg/ml	25	7
Penicillin G	2,000 µg/ml	100	0
Puromycin dihydrochloride	200 µg/ml	0.8	0
Rifampin/ethanol	100 µg/ml	50	0
Streptomycin sulfate	200 µg/ml	44	87
Sulfanilamide	200 µg/ml	—	0
Tetracycline-hydrochloride	200 µg/ml	57	0
Chelator (EDTA [pH 7.5])	2 mM	—	100
Fixative (formaldehyde [pH 7.5])	3.7%	—	0
Inhibitor			
Potassium cyanide	50 mM	—	74
Sodium azide	15 mM	86	0

^a A compound was incubated with 1.0 µM MnCl₂ plus tracer ^{54}Mn (ca. 10⁵ cpm). A portion of the mixture was added to a Chelex 100 column, and the column was eluted with natural seawater. The eluate was counted and analyzed for the presence of the compound as described in the text.

^b Percentage is based on initial compound concentration or radioactivity added. —, not determined.

concentration disks saturated with the eluate were placed on the plates. A positive test for antibiotics in the eluate was demonstrated by a zone of growth inhibition around the disk. Sodium azide in the eluate was determined spectrophotometrically at a wavelength of 240 nm.

Interference with $^{54}\text{Mn(II)}$ adsorption onto synthetic manganate(IV). δMnO_2 was prepared by oxidizing Mn(II) with permanganate by the method of Murray (17), producing a manganese oxide with an approximate elemental composition of MnO_{1.95} (J. Murray, personal communication). The flocculent manganate(IV) was washed with deionized water (two to three times) and seawater (two times) before being suspended in filtered (0.2-µm filters) natural seawater (pH 7.5). All experiments were carried out in disposable sterile polystyrene tubes (17 by 30 mm; BD Labware, Oxnard, Calif.) except in experiments testing chloroform, for which glass test tubes were used. Tracer, carrier-free $^{54}\text{MnCl}_2$ (0.1 to 0.2 µCi ml⁻¹) was added to a seawater suspension of δMnO_2 (10 µg ml⁻¹) plus the compound being tested. Duplicate samples (0.1 to 0.2 ml) were filtered at various times through 0.2-µm triacetate membrane filters (GA8; Gelman Sciences, Inc., Ann Arbor, Mich.), and the filters were immediately washed with 5 ml of filtered seawater (pH 7.5). Radioactivity on the filters and in the filtrates was determined as described above. In addition, the total radioactivity in unfiltered samples was monitored throughout each experiment to detect any soluble and insoluble radioactivity that might adsorb to the solids of the reaction tubes.

Desorption of preadsorbed $^{54}\text{Mn(II)}$ from synthetic manganate(IV). Flocculent δMnO_2 (10 µg ml⁻¹) was preincubated with $^{54}\text{MnCl}_2$ (0.2 to 1.5 µCi ml⁻¹) for 30 to 60 min, until all added soluble radioactivity was adsorbed. A given compound (0.5 ml of stock solution) was then added to this suspension (total, 5 ml), and the samples were filtered and

washed. The radioactivity on the filters and in the filtrates was determined as described above.

Solubilization of synthetic $^{54}\text{manganate(IV)}$. $\delta^{54}\text{MnO}_2$ was prepared by the method of Murray (17), with the modification that $^{54}\text{MnCl}_2$ was added during the preparation, so that the resulting δMnO_2 was radioactive (10,000 cpm µg⁻¹). The solubilization of manganate(IV) in response to treatment with a variety of compounds was followed by detecting soluble radioactivity as described above.

Field measurements with Saanich Inlet water. Water from various depths was collected in 5-liter Niskin bottles. Triplicate samples (100 ml each) were removed into 8-ounce (ca. 240-ml) polypropylene bottles. One bottle was untreated, one received a poison mixture (sodium azide, 1 mg ml⁻¹; penicillin G, 50 µg ml⁻¹; tetracycline-hydrochloride 50 µg ml⁻¹), and the third received a borate-buffered formaldehyde (pH 8.1). The experiment was initiated by the addition of $^{54}\text{MnCl}_2$ (0.02 µCi ml⁻¹). Duplicate samples (10 ml) from each bottle were filtered onto 0.2-µm membrane filters as a function of time, and the radioactivity on the filters and in

TABLE 2. Effect of test compounds on adsorption of $^{54}\text{Mn(II)}$ onto δMnO_2 ^a

Compound	Concn	% ^b Interference at (h):		
		0.5	2.0	24.0
Antibiotic				
Chloramphenicol	50 µg/ml	1	0	0
Gentamicin sulfate	50 µg/ml	15	—	—
Kanamycin monosulfate	50 µg/ml	11	—	—
Nalidixic acid	50 µg/ml	—	0	0
Penicillin G	50 µg/ml	12	—	—
Puromycin dihydrochloride	50 µg/ml	5	—	—
Rifamin-ethanol	50 µg/ml	98	—	99
Rifampin (powder)	50 µg/ml	98	—	99
Streptomycin sulfate	50 µg/ml	—	0	0
Tetracycline-hydrochloride	50 µg/ml	12	—	—
Fixative				
Formaldehyde (pH 7.5)	3.7%	32	—	68
Formaldehyde (pH 8.1)	3.7%	0	7	32
Inhibitor				
Dichloromethylurea	20 µM	0	0	0
2,4-Dinitrophenol	20 mM	71	65	71
Mercuric chloride	10 mM	2	—	1
Potassium cyanide	50 mM	32	43	69
Sodium azide	15 mM	0	0	—
Poison mix (pH 7.4)				
Penicillin G	50 µg/ml			
Sodium azide	15 mM	9	—	20
Tetracycline-hydrochloride	50 µg/ml			
Miscellaneous				
Chloroform	Saturated solution	3	1	10
Dithiothreitol (pH 7.4)	10 mM	100	100	100
Ethanol	3.0%	0	0	0
HEPES buffer (pH 7.4)	10 mM	0	0	0
Sodium sulfide (pH 7.4)	10 µM	—	97	98
Tris buffer (pH 7.5)	10 mM	0	0	0

^a A compound was added to natural seawater containing flocculent δMnO_2 (10 µg ml⁻¹). After the addition of $^{54}\text{MnCl}_2$ (10⁵ cpm ml⁻¹), samples were taken as a function of time and filtered through 0.2-µm membrane filters, and the radioactivity on the filters and in the filtrates was determined.

^b Percentage is based on initial radioactivity added.

the filtrates was determined as described above. Mn(II) concentrations were measured spectrophotometrically by the method of Brewer and Spencer (1).

RESULTS

Competition with Chelex 100 for $^{54}\text{Mn(II)}$ adsorption. Seventeen different antibiotics, fixatives, or metabolic inhibitors, dissolved in natural seawater, were tested for complexation of manganese [Mn(II)] in competition with Chelex 100 (Table 1). Chelex 100 adsorbs Mn(II) even in the presence of the mono- and divalent cations found in seawater (bulletin 2020; Bio-Rad). Since the inhibitors might also adsorb onto the Chelex columns, it was necessary to analyze the eluate for both radioactivity (^{54}Mn) and, by bioassay, the presence of the compound being tested. A number of the antibiotics tested, including penicillin, rifampin, and tetracycline, and the respiratory inhibitor sodium azide did not complex Mn(II) more strongly than Chelex 100. As expected, EDTA and potassium cyanide formed strong complexes with Mn(II). It was not possible to designate some compounds as either complexing or noncomplexing agents. For example, with formaldehyde, no radioactivity was detected in the eluate. But, since a bioassay was not performed on the eluate, formaldehyde may not have passed through the column, and hence the absence of radioactivity in the eluate may only relate to the retention of both radioactivity and the compound on the column.

Interference with Mn(II) adsorption onto synthetic manganese(IV). The adsorption of Mn(II) onto flocculent δMnO_2 is well documented (15, 18). In natural environments, a similar

TABLE 3. Desorption of preadsorbed $^{54}\text{Mn(II)}$ from δMnO_2 by test compounds^a

Compound	Concn	% ^b Desorption at (h):		
		0.5	1.0	24.0
Antibiotic				
Gentamicin sulfate	50 $\mu\text{g/ml}$	0	—	0
Kanamycin monosulfate	50 $\mu\text{g/ml}$	0	—	0
Penicillin G	50 $\mu\text{g/ml}$	0	—	0
Rifampin-ethanol	50 $\mu\text{g/ml}$	22	—	100
Tetracycline-hydrochloride	50 $\mu\text{g/ml}$	1	—	1
Chelator (EDTA [pH 7.4])				
	10 mM	99	—	99
Fixative				
Formaldehyde (pH 7.4)	2.0%	—	44	72
Glutaraldehyde (pH 7.4)	2.5%	—	19	63
Inhibitor				
Mercuric chloride (pH 4.0)	10 mM	15	—	22
Mercuric chloride (pH 6.0)	10 mM	—	1	11
Sodium azide (pH 7.4)	15 mM	0	—	0
Miscellaneous				
Chloroform	Saturated solution	—	0	0
Cupric sulfate (pH 4)	10 mM	—	15	22
Hydroxylamine (pH 7.3)	3 mM	100	100	—
Sodium sulfide (pH 7.4)	10 μM	—	98	98

^a A compound was added to natural seawater containing flocculent δMnO_2 ($10 \mu\text{g ml}^{-1}$) onto which $^{54}\text{Mn(II)}$ (10^5cpm ml^{-1}) had been preadsorbed. Samples were removed as a function of time and filtered through 0.2- μm membrane filters. The radioactivity on the filters and in the filtrates was determined.

^b Percentage is based on initial radioactivity added. —, Not determined.

TABLE 4. Solubilization of $\delta^{54}\text{MnO}_2$ by a variety of test compounds^a

Compound	Concn	% MnO_2 solubilized at (h):		
		0.5	24.0	96.0
Antibiotic				
Penicillin G	50 $\mu\text{g/ml}$	0	0	0
Rifampin-ethanol	50 $\mu\text{g/ml}$	0	15	15
Tetracycline-hydrochloride	50 $\mu\text{g/ml}$	0	0	0
Chelator (EDTA [pH 7.4])				
	10 mM	96	99	98
Inhibitor				
Dichloromethylurea	20 μM	0	0	0
Mercuric chloride (pH 6)	10 mM	0	21	27
Sodium azide (pH 7.4)	15 mM	0	0	0
Poison mix (pH 7.4)				
Penicillin G	50 $\mu\text{g/ml}$			
Sodium azide	15 mM	0	1	9
Tetracycline-hydrochloride	50 $\mu\text{g/ml}$			
Fixative (formaldehyde [pH 7.5])				
	3.7%	7	32	39
Miscellaneous				
Dithiothreitol (pH 7.4)	10 mM	97	100	100
HEPES buffer (pH 7.4)	10 mM	0	0	0
Hydroxylamine (pH 7.4)	10 mM	97	100	100
Resazurin	0.4 mM	35	41	42
Sodium sulfide (pH 7.4)	10 μM	16	25	23

^a A compound was added to natural seawater containing flocculent $\delta_{54}\text{MnO}_2$ ($10 \mu\text{g ml}^{-1}$; 10^5cpm ml^{-1}). Samples were removed as a function of time and filtered through 0.2- μm membrane filters. The radioactivity on the filters and in the filtrates was determined.

binding reaction occurs and is followed by autocatalytic oxidation of Mn(II) (16). A variety of inhibitors, singly and in combination, as well as other compounds of interest, was tested for interference in such binding (Table 2). Antibiotics such as gentamicin sulfate, penicillin G, and tetracycline-hydrochloride showed limited interference. One antibiotic, rifampin, inhibited $^{54}\text{Mn(II)}$ binding to δMnO_2 . Borate-buffered formaldehyde caused some inhibition at pH 8.1 and was unsuitable for use at pH 7.4 or lower. Sodium azide and mercuric chloride showed no measurable interference.

Desorption of preadsorbed $^{54}\text{Mn(II)}$ from synthetic manganese(IV). δMnO_2 is a scavenger of divalent cations (16, 18), and the addition of an inhibitor or other compound might cause desorption of preadsorbed Mn(II). If this desorption occurs, the specific activity of the tracer would be diluted, thus leading to an underestimation of binding. Of the compounds tested (Table 3), rifampin, EDTA, hydroxylamine hydrochloride, mercuric chloride (to a limited extent), and formaldehyde caused desorption.

Solubilization of synthetic manganese(IV). The interference of a compound with Mn(II) adsorption onto δMnO_2 or the desorption of preadsorbed Mn(II) from δMnO_2 could be the result of solubilization of the δMnO_2 . Such solubilization is apparently caused by rifampin, mercuric chloride, EDTA, hydroxylamine hydrochloride, and formaldehyde, as well as by a few other compounds tested (Table 4).

Microbial manganese binding in water samples as a function of depth. A profile of the initial rate of manganese binding by particulates (0.2 μm), for a 1-h incubation, in the presence and absence of formaldehyde as a function of depth is presented in Fig. 1 (essentially identical results were obtained with the poison mixture; 7). There was a broad peak

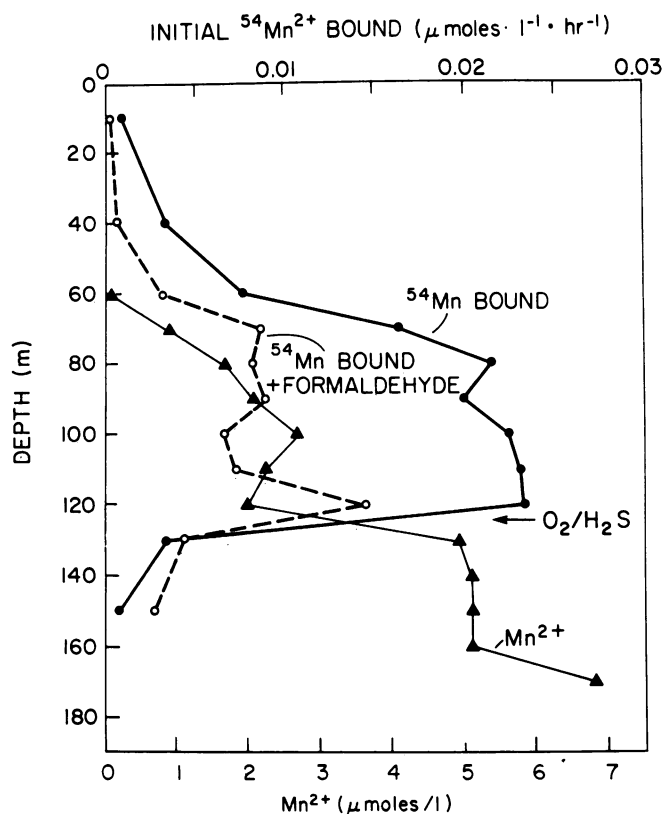


FIG. 1. Inhibited and uninhibited $^{54}\text{Mn(II)}$ binding to particulates as a function of depth. $^{54}\text{Mn(II)}$ binding assays were performed as described in the text. The radioactivity bound at 1 h was plotted as a function of depth. The difference between $^{54}\text{Mn(II)}$ bound (●) and $^{54}\text{Mn(II)}$ bound plus formaldehyde (○) represents the microbial Mn(II) binding rate. Dissolved manganese(II) was defined as that which passes through a $0.4\text{-}\mu\text{m}$ membrane filter and was determined by the colorimetric formaldoxime method of Brewer and Spencer (1).

of Mn(II) binding, corresponding to a microaerophilic zone above the $\text{O}_2\text{-H}_2\text{S}$ interface (profiles of oxygen and sulfide have been previously published [7]). A significantly lower rate of binding was measured in samples treated with formaldehyde (or poison mix [data not shown]; 7). Therefore, significant biologically catalyzed manganese binding (resulting in oxidation) occurs just above the $\text{O}_2\text{-H}_2\text{S}$ interface in the water column.

DISCUSSION

One way to directly measure rates of microbial manganese oxidation is to administer $^{54}\text{Mn(II)}$ and then follow changes in manganese solubility as a function of time. Laboratory experiments with pure cultures of bacteria have shown that binding or removal of Mn(II) from solution is a prerequisite for bacterially catalyzed oxidation (19, 21). Since particulate manganese also adsorbs Mn(II) (15, 18), one must distinguish inorganic removal and autocatalytic oxidation from microbially catalyzed manganese binding and oxidation. This can be accomplished by inhibiting microbial metabolism and measuring the residual rate of removal of Mn(II) from solution, i.e., the inorganic autocatalytic oxidation rate. However, if the poisons interfere with the inorganic chemistry of manganese, one may either overestimate or underestimate the microbial activity.

Most of the inhibitors tested interfered to some extent with inorganic manganese chemistry by complexing Mn(II) or solubilizing δMnO_2 . Some of the poisons that did not interfere were sodium azide, chloramphenicol, and streptomycin sulfate. Other compounds that interfered only to a limited extent were penicillin G, tetracycline-hydrochloride, formaldehyde, mercuric chloride, and a poison mixture (penicillin G, sodium azide, tetracycline-hydrochloride). Organic solvents have been used to poison microbial metabolism, and ethanol did not interfere in any of our experiments, although chloroform partially inhibited Mn(II) binding to δMnO_2 . Chloroform may have damaged the cellulose triacetate membranes used in this study, allowing particulate manganese, which normally would have been trapped, to pass through the filters.

The effects of a variety of other compounds were tested for potential interference with $^{54}\text{Mn(II)}$ adsorption onto δMnO_2 . Two buffers, HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) and Tris, did not interfere in any way and, therefore, can be used to buffer water samples, if desired. As expected, a number of the compounds tested solubilized MnO_2 and hence strongly interfered with manganese chemistry. These included EDTA, dithiothreitol, hydroxylamine hydrochloride, resazurin, and sodium sulfide. Therefore, one must use caution in the use of complexing and reducing agents or if reducing agents are found in natural samples which are being tested. In addition, cupric sulfate has been used to desorb bound but not oxidized Mn(II) from bacteria, thus providing a method to estimate the percentage of bound manganese that had been oxidized (3, 21). Our results (Table 3) demonstrate that in a 24-h incubation with 10 mM cupric sulfate only 22% of the $^{54}\text{Mn(II)}$ prebound to δMnO_2 was desorbed. The possibility still exists that the Mn(II) was actually oxidized during the course of the incubation; however, this is unlikely since the rate of autocatalytic Mn(II) oxidation is very slow (16, 22).

Although there was some variability in Mn(II) adsorption or desorption both in the presence of a given compound from one δMnO_2 preparation to the next and with the age of a given manganese preparation, the activities measured for any single batch were always reproducible. The results of the four methods used in this study are in good agreement, with the following two exceptions: (i) rifampin did not bind Mn(II) when in competition with Chelex 100 but strongly inhibited Mn(II) adsorption onto, and enhanced desorption from, δMnO_2 , and (ii) streptomycin sulfate complexed Mn(II) more strongly than Chelex 100 but showed no interference in any of the Mn(II)- δMnO_2 binding experiments. The manganese(II)-manganate(IV) system used in these studies approximates the primary inorganic manganese reactions, Mn(II) autooxidation, and adsorption of Mn(II) by δMnO_2 . We feel, therefore, that the results of the Chelex method can be disregarded in favor of the data from the manganese(II)-manganate(IV) method for selection of suitable poisons. In addition, although streptomycin activity was measured in the eluate, a significant portion was probably retained on the Chelex 100 column, and the test cultures may have been sensitive to low concentrations.

This paper deals with the selection of suitable poisons for use in studies of microbially catalyzed manganese(II) binding and oxidation in seawater. Many of these poisons may also be useful for freshwater studies. Poisons that showed the least interference in the seawater manganese(II) adsorption studies were tested with distilled water substituted for seawater (data not shown). Sodium azide, penicillin, and formaldehyde showed limited interference in distilled water,

comparable to a seawater control. Tetracycline, mercuric chloride, and the poison mixture exhibited moderate-to-strong inhibition of manganese(II) binding to δMnO_2 . It would appear, therefore, that the results from our seawater system cannot be extrapolated to a freshwater system without rigorous determination of the extent of interference a poison may introduce.

Using the acceptable poisons, we estimated the microbial contribution to manganese precipitation in water samples from a fjord, Saanich Inlet, British Columbia, Canada. Emerson et al. (6) had previously suggested that manganese-oxidizing bacteria are primarily responsible for the rapid accumulation of oxidized manganese in a particulate zone enriched with manganese, iron, carbon, and nitrogen. The $^{54}\text{Mn(II)}$ binding assay, with and without added poisons, showed that microbially catalyzed manganese oxidation accounts for greater than 50% of the total Mn(II) removal rate (Fig. 1; 7). This value probably underestimates the actual microbial binding and oxidation potential for at least two reasons. First, these experiments were run with saturating oxygen levels, in contrast with the low levels of ambient oxygen in situ (5 to 15 μM) between 70 and 130 m, which would significantly reduce the background levels of inorganic oxidation (22). The effect on microbial rates of these low oxygen conditions is not known, but the organisms might be less affected. Second, an additional control, in which Saanich particulates were sonicated to kill bacteria, showed that both formaldehyde and the poison mixture enhanced $^{54}\text{Mn(II)}$ binding (data not shown; 7). This is in contrast to our control experiments with flocculent δMnO_2 (Tables 2, 3, and 4) and those with nonsonicated Saanich particulates (7). In any case, if the time course examined is short then the potential interference by an added compound is minimal.

The poison mixture and the formaldehyde used in $^{54}\text{Mn(II)}$ binding studies in Saanich Inlet (Fig. 1; [7]) have very different mechanisms of inhibition. Formaldehyde is a fixative, whereas the poison mixture is composed of metabolic and respiratory inhibitors. Therefore, these poisons may affect different manganese-oxidizing bacteria to different extents. Manganese oxidation resulting from direct (enzymatic) or indirect (a product of microbial metabolism, such as a change in local pH) microbial metabolic activity will be inhibited by both types of poisons. However, manganese binding and oxidation that may be independent of metabolism, such as an interaction with organic polymers or an organic matrix on the cell surface, may or may not be inhibited (8). It is therefore advantageous to use both types of inhibitors when comparing poison-treated samples with untreated samples (Fig. 1; 7). Furthermore, additions of divalent cations such as Hg^{2+} or Cu^{2+} might be expected to interfere to some extent with manganese chemistry as they could directly compete with the adsorption of divalent Mn(II) onto manganese oxides. In seawater, which contains an assortment of ions, this effect may be less pronounced. However, this may explain why the mercuric chloride strongly interfered with the distilled water system.

The poisons tested in this study will be useful for in situ manganese binding and oxidation experiments. However, since natural manganates may differ in their ion-exchange capacity, especially as a function of age (17), the added poisons may potentially interfere in unknown ways with Mn(II) adsorption. Incubation experiments with sonicated and nonsonicated natural samples, as well as in situ experiments, will permit accurate estimation of microbial manganese binding and oxidizing potentials in situ. Independent of the technique used to measure microbial manganese binding

and oxidizing potentials, one must carefully select the compounds added to ensure that they do not interfere with inorganic autocatalytic activities.

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LITERATURE CITED

1. Brewer, P. G., and D. W. Spencer. 1971. Colorimetric determination of manganese in anoxic waters. *Limnol. Oceanogr.* **16**:107-112.
2. Brock, T. D. 1978. The poisoned control in biogeochemical investigations, p. 717-725. *In* W. E. Krumbein (ed.), *Environmental biogeochemistry and geomicrobiology*, vol. 3. Ann Arbor Science Publishers, Ann Arbor, Mich.
3. Bromfield, S. M., and D. J. David. 1976. Sorption and oxidation of manganous ions and reduction of manganese oxide by cell suspensions of a manganese oxidizing bacterium. *Soil Biol. Biochem.* **8**:37-43.
4. Ehrlich, H. L. 1963. Bacteriology of manganese nodules. I. Bacterial action on manganese in nodule enrichments. *Appl. Microbiol.* **11**:15-19.
5. Ehrlich, H. L. 1978. Conditions for bacterial participation in the initiation of manganese deposition around marine sediment particles, p. 839-845. *In* W. E. Krumbein (ed.), *Environmental biogeochemistry and geomicrobiology*, vol. 3. Ann Arbor Science Publishers, Ann Arbor, Mich.
6. Emerson, S., R. E. Cranston, and P. S. Liss. 1979. Redox species in a reducing fjord: equilibrium and kinetic considerations. *Deep-Sea Res.* **26**:859-878.
7. Emerson, S., S. Kalhorn, L. Jacobs, B. M. Tebo, K. H. Nealson, and R. A. Rosson. 1982. Environmental oxidation rate of manganese(II): bacterial catalysis. *Geochim. Cosmochim. Acta* **46**:1073-1079.
8. Ghiorse, W. C., and P. Hirsch. 1979. An ultrastructural study of iron and manganese deposition associated with extracellular polymers of *Pedomicrobium*-like bacteria. *Arch. Microbiol.* **123**:213-226.
9. Indrebø, G., B. Pengerud, and I. Dundas. 1979. Microbial activities in a permanently stratified estuary. I. Primary production and sulfate reduction. *Mar. Biol.* **51**:295-304.
10. Indrebø, G., B. Pengerud, and I. Dundas. 1979. Microbial activities in a permanently stratified estuary. II. Microbial activities at the oxic-anoxic interface. *Mar. Biol.* **51**:305-309.
11. Jørgensen, B. B. 1978. A comparison of methods for the quantification of bacterial sulfate reduction in coastal marine sediments. I. Measurement with radiotracer techniques. *Geomicrobiol. J.* **1**:11-27.
12. Jørgensen, B. B. 1978. A comparison of methods for the quantification of bacterial sulfate reduction in coastal marine sediments. II. Calculation from mathematical models. *Geomicrobiol. J.* **1**:29-47.
13. Jørgensen, B. B. 1978. A comparison of methods for the quantification of bacterial sulfate reduction in coastal marine sediments. III. Estimation from chemical and bacteriological field data. *Geomicrobiol. J.* **1**:49-64.
14. Marshall, K. C. 1979. Biogeochemistry of manganese minerals, p. 253-292. *In* P. A. Trudinger and D. J. Swaine (ed.), *Biogeochemical cycling of mineral-forming elements*. Elsevier/North-Holland Publishing Co., Amsterdam.
15. Morgan, J. J., and W. Stumm. 1964. Colloid-chemical properties of manganese dioxide. *J. Colloid Sci.* **19**:347-359.
16. Morgan, J. J., and W. Stumm. 1965. Analytical chemistry of aqueous manganese. *J. Am. Water Works Assoc.* **57**:107-119.
17. Murray, J. W. 1974. The surface chemistry of hydrous manganese dioxide. *J. Colloid Interface Sci.* **46**:357-371.
18. Murray, J. W. 1975. The interaction of metal ions at the manganese dioxide-solution interface. *Geochim. Cosmochim.*

- Acta **39**:505–519.
19. **Nealson, K. H., and J. Ford.** 1980. Surface enhancement of bacterial manganese oxidation: implications for aquatic environments. *Geomicrobiol. J.* **2**:21–37.
 20. **Postgate, J. R.** 1965. Recent advances in the study of the sulfate-reducing bacteria. *Bacteriol. Rev.* **29**:425–441.
 21. **Rosson, R. A., and K. H. Nealson.** 1982. Manganese binding and oxidation by spores of a marine bacillus. *J. Bacteriol.* **151**:1027–1034.
 22. **Stumm, W., and J. J. Morgan.** 1981. *Aquatic chemistry.* John Wiley & Sons, Inc., New York.
 23. **Wollast, R., G. Billen, and J. C. Duinker.** 1979. Behavior of manganese in the Rhine and Scheldt estuaries. *Estuarine Coastal Mar. Sci.* **9**:161–169.