Production of Biogenic Mn Oxides by *Leptothrix discophora* SS-1 in a Chemically Defined Growth Medium and Evaluation of Their Pb Adsorption Characteristics

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Biogenic Mn oxides were produced by the bacterium *Leptothrix discophora* SS-1 (= ATCC 3182) in a chemically defined mineral salts medium, and the Pb binding and specific surface area of these oxides were characterized. Growth of SS-1 in the defined medium with pyruvate as a carbon and energy source required the addition of vitamin B_{12}. Complete oxidation of Mn(II) within 60 h required the addition of ≥0.1 μM $FeSO_4$. Pb adsorption isotherms were determined for the biogenic Mn oxides (and associated cells with their extracellular polymer) and compared to the Pb adsorption isotherms of cells and exopolymer alone, as well as to abiotic Mn oxides. The Pb adsorption to cells and exopolymer with biogenic Mn oxides (0.8 mmol of Mn per g) at pH 6.0 and 25°C was 2 orders of magnitude greater than the Pb adsorption to cells and exopolymer alone (on a dry weight basis). The Pb adsorption to the biogenic Mn oxide was two to five times greater than the Pb adsorption to a chemically precipitated abiotic Mn oxide and several orders of magnitude greater than the Pb adsorption to two commercially available crystalline MnO_2 minerals. The N_2 Brunauer-Emmet-Teller specific surface areas of the biogenic Mn oxide and fresh Mn oxide precipitate (224 and 58 m^2/g, respectively) were significantly greater than those of the commercial Mn oxide minerals (0.048 and 4.7 m^2/g). The Pb adsorption capacity of the biogenic Mn oxide also exceeded that of a chemically precipitated colloidal hydrous Fe oxide under similar solution conditions. These results show that amorphous biogenic Mn oxides similar to those produced by SS-1 may play a significant role in the control of trace metal phase distribution in aquatic systems.

Fe and Mn oxides have long been recognized as important adsorbing phases governing the cycling of trace metals in aquatic environments (33, 35, 46, 62, 64). Fe oxides are often considered more important than other adsorbing phases because the high specific surface area of amorphous or colloidal Fe oxides is expected to result in high adsorption capacity, and thus trace metal adsorption to Fe oxides has been studied extensively (e.g., see reference 20). Less attention has been given to trace metal adsorption by Mn oxides, even though it is likely that fresh biologically oxidized Mn is poorly crystallized or amorphous (41, 58, 65) and could exhibit much greater trace metal adsorption than crystalline Mn oxides.

The Mn oxidation states and mineral forms of biogenic Mn oxides have been characterized in several prior investigations (3, 23, 24, 41, 57); however, specific surface areas and trace metal binding characteristics of biogenic Mn oxides have not been reported previously. Moffett and Ho (44) reported that Co, Zn, Ce, and trivalent lanthanides could be incorporated into biogenic Mn oxides by being "coprocessed" via the same putative enzymatic pathways as those used for Mn oxidation, but adsorption of these elements to already formed Mn oxides was not described. He and Tebo (32) measured the surface area of Mn-oxidizing *Bacillus* sp. strain SG-1 spores and Cu adsorption to these spores, but Cu adsorption to Mn oxides formed by the spores was not reported. Tessier et al. (59) observed trace metal adsorption to freshwater sediment extracts containing Mn oxides, Fe oxides, and organic materials, but the specific role of Mn oxides was not elucidated because the extracted Mn oxides were contaminated with Fe oxides and residual organic material. Additional information on the metal adsorption capacity and specific surface area of biogenic Mn oxides under controlled laboratory conditions is needed to assess the relative importance of biogenic Mn oxides in controlling trace metal adsorption in natural aquatic environments.

In the present work, Pb adsorption and specific surface area were measured for biogenic Mn oxides produced by the bacterium *Leptothrix discophora* SS-1 in a chemically defined medium. *L. discophora* is a well-characterized, model Mn-oxidizing bacterium whose structure, physiology, and phylogeny have been studied extensively (8, 26–28). In pure cultures of *L. discophora*, Mn oxidation has been shown to occur extracellularly (1–3, 8, 16, 17, 21, 22, 54, 58). The Mn oxides formed by *L. discophora* were previously shown to be mixed Mn(III, IV) oxides or oxyhydroxides with an average oxidation state of 3.6 (3). Pb adsorption by the biogenic Mn oxide (with cells and exopolymer) was compared to Pb adsorption by *L. discophora* cells and exopolymer alone (without Mn oxide). The adsorption properties of the biogenic Mn oxides were also compared to those of Mn oxides of abiotic origin.

To unambiguously assess Pb adsorption to Mn oxides produced by *L. discophora*, it was necessary to grow the bacterium in a defined medium free of competing trace metals and undefined organic ligands or mineral precipitates that could interfere with Pb adsorption. Previous work with another strain, *L. discophora* SP-6, had shown that this organism could be grown in a defined mineral salts medium that contained 10 μM $FeSO_4$ and a suite of vitamins (3). Therefore, to facilitate our experiments, it was first necessary to determine the minimal

TABLE 1. Composition of the MMS liquid medium used for growth of and Mn oxidation by *L. discophora* SS-1[a]

Component	Concn in ddH_2O	
	mg/liter	μM
Pyruvate	240	2,900
$CaCl_2 \cdot 2H_2O$	30	200
$MgSO_4 \cdot 7H_2O$	35	140
$(NH_4)_2SO_4$	120	910
KNO_3	15	150
$NaHCO_3$	0.84	10
KH_2PO_4	0.70	5
Vitamin B_{12}	0.002	0.0015
$FeSO_4$	0.015	0.1

[a] The initial pH of the medium was 6.0, and the ionic strength was adjusted to 0.05 M with $NaNO_3$. The concentrations of the different lead species in MMS medium (in the absence of vitamin B_{12} and $FeSO_4$) for a total dissolved lead concentration of 1.0 μM were as follows: Pb^{2+}, 0.89 μM; $PbSO_4$, 0.09 μM; and $PbOH^+$, 0.012 μM.

vitamin and Fe requirements of strain SS-1. These experiments revealed that vitamin B_{12} was required for SS-1 growth and also revealed that supplemental Fe enhanced Mn oxidation in the defined medium. Development of a chemically defined medium based on these determinations resulted in more accurate and meaningful measurement of Pb adsorption to the resulting biogenic Mn oxides.

MATERIALS AND METHODS

Culture and medium. *L. discophora* SS-1 (= ATCC 43182) cultures (1, 29) were maintained at 4°C on plates of peptone-Trypticase-yeast extract-pyruvate medium that contained 0.25 g of peptone (Difco Laboratories, Detroit, Mich.) per liter, 0.25 g of Trypticase (BBL Becton Dickinson Microbiology Systems, Cockeysville, Md.) per liter, 0.50 g of yeast extract (Difco Laboratories) per liter, 0.6 g of $MgSO_4 \cdot 7H_2O$ (Fisher Scientific, Pittsburgh, Pa.) per liter, 0.07 g of $CaCl_2 \cdot 2H_2O$ (Fisher Scientific) per liter, 10 μM $FeSO_4$ (Fisher Scientific), 2.38 g of HEPES buffer (U.S. Biochemical Corp., Cleveland, Ohio) per liter, 0.3 g of pyruvate (Aldrich Chemical Co., Milwaukee, Wis.) per liter, and 1.6% agar (Difco Laboratories). Inocula from these plates were first grown in a minimal mineral salts (MMS) liquid medium (Table 1) lacking $FeSO_4$ and vitamin B_{12} but containing 0.25 mg of peptone per liter and 0.50 mg of yeast extract per liter. Mn oxidation experiments were conducted in MMS liquid medium containing filter-sterilized $MnSO_4$ at a concentration of 50 μM. For the Mn oxidation experiments, *L. discophora* cultures were inoculated with 1% (vol/vol) inocula containing residual peptone and yeast extract. Thus, the maximum peptone and yeast extract concentrations in final media used for Mn oxidation experiments were 2.5 and 5.0 μg/liter, respectively. Cell dry weights were determined by analyzing the total suspended solids in the broth (standard method 2540 D [5]).

Measurement of Mn oxidation. *L. discophora* SS-1 cultures were grown at 25°C in 500 ml of MMS liquid medium (Table 1) in 1-liter flasks on a rotary shaker (Innova 2000; New Brunswick Scientific, Edison, N.J.) operated at 150 rpm. The medium was not buffered, and the pH increased from 6.0 to 7.8 during growth. Mn(II) oxidation was monitored by aseptically removing 20-ml aliquots of broth, centrifuging them at 13,400 × *g* for 30 min with a Centra MP4R centrifuge (IEC, Needham Heights, Mass.), and measuring the supernatant Mn concentrations by atomic absorption spectroscopy using an AAnalyst 100 instrument (Perkin-Elmer, Norwalk, Conn.) with an acetylene flame. To distinguish between Mn oxidation and Mn adsorption to cells, the procedure of Bromfield and David (10) was used, as follows. $CuSO_4$ (5 mM) was added to biogenic Mn oxide suspensions at pH 4.2. These mixtures were centrifuged as described above, and each supernatant was analyzed for desorbed Mn^{2+} by atomic absorption spectroscopy. Since Cu^{2+} was not observed to displace Mn^{2+}, we concluded that Mn was oxidized and not merely adsorbed.

Preparation of abiotic Mn oxide precipitates. Mn oxide precipitates were prepared by reacting $MnCl_2$ with $KMnO_4$ as described previously (6, 12, 45). $KMnO_4$ (8 g) was dissolved in 200 ml of distilled deionized water (ddH_2O) and heated to 90°C, and then 10 ml of 5 N NaOH was added. Fifteen grams of $MnCl_2 \cdot 4H_2O$ was dissolved in 75 ml of ddH_2O, and the resulting solution was added slowly to the basic $KMnO_4$ solution. The resulting suspension was heated at 90°C for 1 h. This procedure has been reported to produce an Mn solid phase with a low degree of crystallinity and an X-ray diffraction pattern attributed to δMnO_2 (45). After the precipitate was cooled, it was washed several times by centrifugation and resuspension in ddH_2O. A portion of the washed precipitate was resuspended in MMS medium and used immediately for Pb adsorption experiments. The remaining precipitate was lyophilized and stored in a desiccator. Pb adsorption was also measured by using suspensions prepared from lyophilized Mn oxide.

Measurement of Pb adsorption to Mn oxides. Pb adsorption to biogenic and abiotic Mn oxide suspensions was measured with a series of jacketed, 500-ml beakers. The inside surfaces of the beakers were treated with $(CH_3)_2SiCl_2$ (Eastman Kodak Co., Rochester, N.Y.) to produce a hydrophobic surface and minimize Pb adsorption to the glass. Water was circulated through the beaker jackets with a recirculation bath in order to maintain a constant temperature at 25 ± 0.5°C. Glass autoclavable pH probes (Ingold Electrodes, Wilmington, Md.) were installed in each beaker and connected to individual pH controllers (Chemcadet; Cole Parmer, Vernon Hills, Vt.) in order to maintain the pH at 6.00 ± 0.05 by automatic addition of 0.01 N HNO_3 (glass distilled; GFS Chemicals, Columbus, Ohio). To each beaker we added 300 ml of an Mn oxide suspension in MMS medium (Table 1) prepared without vitamin B_{12}, $FeSO_4$, or pyruvate. Pb^{2+} was added to the suspensions from a reference solution containing 1,000 mg of Pb per liter in 2% HNO_3 (Fisher Scientific) in order to produce an initial concentration range of 0.1 to 4.0 μM. The speciation of Pb in these solutions was calculated with MINEQL (52, 66), and approximately 89% of the metal was present as the aquo Pb^{2+} ion (Table 1). Biogenic Mn oxide suspensions (consisting of cells and associated exopolymer and Mn oxide) were diluted 20-fold with MMS medium (which contained no vitamins, $FeSO_4$, or pyruvate) in order to obtain measurable final equilibrium concentrations of dissolved Pb. Both the abiotic Mn oxide and the biogenic Mn oxide mixtures were equilibrated for 24 h with slow magnetic stirring (kinetic experiments indicated that adsorption was complete within 18 h). After equilibration, 20-ml aliquots were centrifuged for 30 min at 13,400 × *g* in Teflon centrifuge tubes, and 7.5 ml of supernatant was pipetted from each centrifuge tube and acidified by adding 100 μl of 15% HNO_3. The Pb concentrations in centrifuged and uncentrifuged samples were measured by graphite furnace atomic absorption spectroscopy with a Perkin-Elmer AAnalyst 100 instrument equipped with a model HGA 800 graphite furnace and a model AS-72 autosampler. The final Pb concentrations in control solutions (no adsorbent) were within 5% of the initial concentrations after 24 h of equilibration. At pH 6.0, a portion (typically about 30%) of the biogenic Mn oxide dissolved during equilibration, and the amount of Mn dissolved was subtracted from the total amount of Mn in order to calculate Pb adsorption on a per mole of Mn basis. Pb adsorption was also measured with two commercially available Mn oxides: a chemically derived granular βMnO_2 (Fisher Scientific) and a powdered MnO_2 (ICN Pharmaceuticals, K&K Laboratories, Plainview, N.Y.), by the same methods. The observed adsorption data were fitted to Langmuir adsorption isotherms having the form $\Gamma = \Gamma_{max} \cdot K \cdot [Pb^{2+}]/(1 + K \cdot [Pb^{2+}])$, where Γ is the Pb adsorption (millimoles of Pb per mole of Mn), Γ_{max} is the maximum Pb adsorption, and K is the Langmuir equilibrium constant.

Surface area measurements. The surface areas of the biogenic and abiotic Mn oxides were measured by adsorption of N_2 gas from a mixture containing 30% N_2 and 70% He with a surface area analyzer (Quantasorb, Syosset, N.Y.) and were calculated by a single-point Brunauer-Emmet-Teller (BET) method (11). Biogenic and fresh abiotic Mn oxides were lyophilized (Flexi-Dry μP; FTS Systems, Stone Ridge, N.Y.) before their surface areas were measured. All samples were outgassed under N_2 at 110°C for 4 h prior to surface area measurement. The surface area of the biogenic Mn oxide was calculated by subtracting the experimentally determined surface area of *L. discophora* cells without Mn (approximately 7 m^2/g), a value that was very small compared to the surface area of the biogenic Mn oxide.

RESULTS

Vitamin B_{12} requirement for *L. discophora* SS-1 growth and Fe requirement for Mn oxidation. Growth of *L. discophora* SS-1 was not observed in MMS liquid medium (Table 1) without vitamins. However, growth was observed after 2 μg of vitamin B_{12} per liter was added to the growth medium. Microscopic examination of the white, turbid suspensions confirmed the presence of long filamentous cells containing inclusions of polyhydroxyalkanoate that are characteristic of sheathless *L. discophora* SS-1 cells (1).

Although *L. discophora* SS-1 grew in MMS medium containing vitamin B_{12}, Mn oxidation did not occur during growth in this medium as it did in peptone-Trypticase-yeast extract-pyruvate medium containing Mn. However, addition of Fe as $FeSO_4$ stimulated Mn oxidation. To determine the minimum amount of Fe required for Mn oxidation, *L. discophora* SS-1 cultures were amended with $FeSO_4$ at concentrations ranging from 0.01 to 10 μM. Cell growth was observed in all cultures, as indicated by increases in optical density at 600 nm. When 50 μM $MnSO_4$ was added at the time of inoculation along with the added $FeSO_4$, complete Mn oxidation occurred simulta-

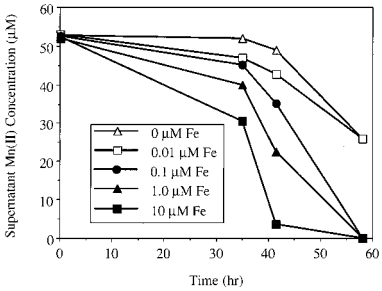

FIG. 1. Fe requirement for Mn oxidation by *L. discophora* SS-1 in MMS medium (Fe and Mn were added at the time of inoculation).

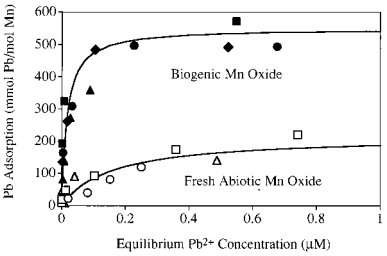

FIG. 3. Pb adsorption to biogenic Mn oxide and fresh abiotic δMnO_2 precipitates. Symbols: ●, biogenic replicate 1; ■, biogenic replicate 2; ▲, biogenic replicate 3; ◆, biogenic replicate 4; ○, abiotic replicate 1; □, abiotic replicate 2; △, abiotic replicate 3.

neously with growth within 60 h only in cultures containing 0.1 μM or more $FeSO_4$ (Fig. 1). The average Mn oxidation rate in the presence of 0.1 μM $FeSO_4$ and 50 mg of cells (dry weight) per liter was 1.9 μM/h. This rate is about five times higher than the rates reported for freshwater lakes (61). In contrast, the Mn oxidation rate was much lower and oxidation was not complete in 60 h when 0.01 μM $FeSO_4$ or no $FeSO_4$ was added to the medium.

The biological role in the Mn oxidation was confirmed by inhibiting the cell culture with sodium azide (51). No decrease in the soluble Mn(II) level was observed with controls containing *L. discophora* SS-1 cells plus 10 μM $FeSO_4$ in the presence of 0.15 mM sodium azide. Since Fe oxidation was likely to occur prior to the addition of Mn and the azide inhibitor, the possibility that Fe oxide catalyzed Mn oxidation in these experiments was also excluded by these controls.

Because of the possibility that the added Fe stimulated growth rather than Mn oxidation, the experiment was repeated with cultures of *L. discophora* SS-1 that were first grown to the stationary phase (75 h at 25°C) with and without 0.1 μM $FeSO_4$ (optical density at 600 nm, 0.060 ± 0.005; Spectronic 20 colorimeter; Bausch and Lomb, Rochester, N.Y.). The final turbidity values with and without Fe added were similar (within 10%). Mn oxidation was rapid and complete within 20 h when 0.1 μM $FeSO_4$ was added, while Mn oxidation was slow and incomplete when Fe was not added. Therefore, the Fe requirement appears to have been primarily for oxidation of Mn rather than for growth.

Based on the results of these experiments, the growth medium used for subsequent experiments contained 2 μg of vitamin B_{12} per liter and 0.1 μM $FeSO_4$ as shown in Table 1.

Pb adsorption to biogenic and abiotic Mn oxides and surface areas. Pb adsorption to *L. discophora* cells without Mn followed a linear isotherm (Fig. 2A). Pb adsorption to *L. discophora* cells with biogenic Mn oxide deposits (0.8 mmol of Mn/g) was much greater and followed a Langmuir isotherm (Fig. 2B). Figure 2 shows that Mn oxide on the cell surface increased Pb adsorption at least 2 orders of magnitude compared to cells without Mn (note the difference in scales in Fig. 2A and B).

The Pb adsorption by Mn oxidized by *L. discophora* was significantly greater than the Pb adsorption observed with fresh abiotic Mn oxide precipitates (referred to here as δMnO_2) that were prepared by reaction of $MnCl_2$ with $KMnO_4$ (Fig. 3). At the concentrations used, the Pb adsorption by the biogenic Mn oxide was two to five times greater than the Pb adsorption by the fresh precipitates. The levels of Pb adsorption to both biogenic and fresh abiotic δMnO_2 precipitates were similar for several different replicate preparations of material and for freeze-dried and fresh (not dried) precipitates (biogenic replicate 3 and abiotic replicate 3 [Fig. 3] were not freeze-dried prior to measurement of Pb adsorption). The *Leptothrix*-oxidized Mn and the fresh abiotic δMnO_2 precipitate both adsorbed several orders of magnitude more Pb than commercially obtained Mn(IV) oxides (Fig. 4 and Table 2). In all cases, adsorption followed Langmuir isotherms, with r^2 values ranging from 0.83 to 0.87 (Table 2). The Pb adsorption of the biologically oxidized Mn was also 2 orders of magnitude greater than the Pb adsorption previously determined by Nelson et al. for colloidal Fe oxide deposits under the same solution conditions (49) (Fig. 4).

The N_2 BET specific surface area of *Leptothrix*-oxidized Mn was about four times the BET specific surface area of fresh

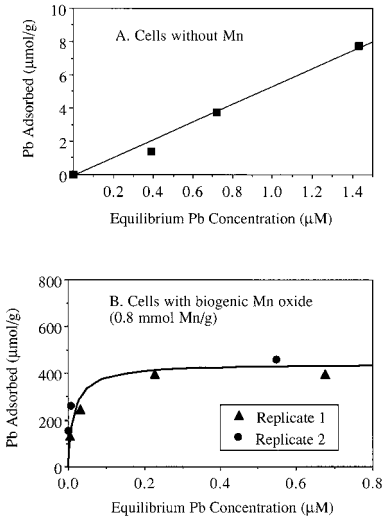

FIG. 2. Pb adsorption isotherms for *L. discophora* SS-1 cells. (A) Without Mn. (B) With biogenic Mn oxide (0.8 mmol/g). The temperature was 25°C, the pH was 6.0, and the concentration of total suspended solids was 63 mg/liter.

TABLE 2. Langmuir adsorption isotherm parameters for Pb adsorption to biologically oxidized Mn and abiotic Mn oxides

Mn oxide	Langmuir parameters		
	Γ_{max} (mmol of Pb/mol of Mn)	K (liters/μmol of Pb)	r^2
Leptothrix-oxidized Mn[a]	550	62	0.83
Abiotic Mn oxide precipitate[b]	220	6.4	0.85
Crystalline powdered MnO_2 (ICN)	1.2	4.8	0.83
Crystalline β-phase MnO_2 (Fisher)	0.031	3.3	0.87

[a] Freeze-dried and aged for 29 days.
[b] Freeze-dried and aged for 13 days.

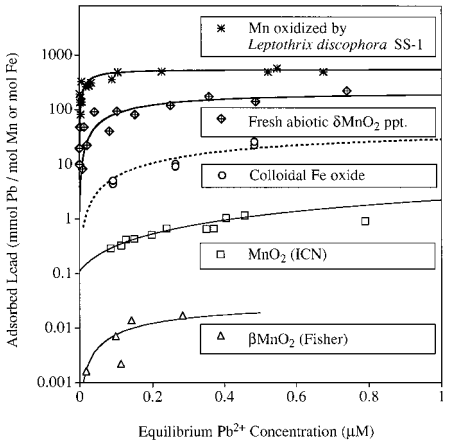

FIG. 4. Comparison of Pb adsorption by biogenic Mn oxide, by abiotic δMnO_2 precipitates, by commercial Mn oxide minerals, and by colloidal Fe oxide (log scale).

abiotic δMnO_2 precipitates, and the surface area of the fresh δMnO_2 was 1 to 3 orders of magnitude greater than the surface area of the more crystalline Mn oxide minerals (Table 3). Pb adsorption increased with increasing specific surface area (Fig. 4 and Table 3).

DISCUSSION

L. discophora SS-1 requires vitamin B_{12} for growth and 0.1 μM Fe for complete Mn oxidation under the conditions used in this study. When vitamin B_{12} was added, SS-1 grew in a defined medium containing no additional trace metals. Any cellular requirements for trace metals were probably satisfied by trace impurities in the reagents used for medium preparation. Although the previous work of Emerson and Ghiorse (21) showed that a suite of vitamins and 10 μM Fe were required for growth of *L. discophora* SP-6 (a sheathed strain) in a mineral salts medium, nutritional requirements have not been reported previously for growth of SS-1 or for biological Mn oxidation by either SS-1 (1, 2) or SP-6 (21). The requirement for 0.1 μM Fe for Mn oxidation suggests that Fe may be a cofactor in the extracellular Mn-oxidizing system of SS-1, but further research is needed to elucidate the exact role of Fe in Mn oxidation. Copper (a contaminant in the $FeSO_4$ reagent) may have been responsible for the observed stimulation of Mn oxidation, but we believe that this is unlikely because the calculated concentration of copper in 0.1 μM $FeSO_4$ reagent-grade salt would have been less than 0.5 nM. The contribution of 0.1 μM Fe to the Pb adsorption observed should be negligible compared to the contribution of the 50 μM Mn used in the investigation of Pb binding to *Leptothrix*-oxidized Mn. Indeed, cells grown with 0.1 μM Fe but without added Mn exhibited minimal Pb adsorption (Fig. 2A).

TABLE 3. BET surface areas of biologically oxidized Mn and abiotic Mn oxides

Source of Mn oxide	BET surface area (m^2/g of Mn oxide)	
	Avg	SD (n = 3)
Leptothrix-oxidized Mn[a]	224	7
Abiotic Mn oxide precipitate[b]	57.6	0.4
Crystalline powdered MnO_2 (ICN)	4.7	0.1
Crystalline β-phase MnO_2 (Fisher)	0.048	0.003

[a] Freeze-dried and aged for 29 days.
[b] Freeze-dried and aged for 13 days.

TABLE 4. Reported N_2 BET surface areas of synthetic δMnO_2

Reference author(s)	Reference	BET surface area (m^2/g of Mn oxide)
Catts and Langmuir	14	290
Murray	45	263
Loganathan and Burau	37	160
Godtfredsen and Stone	31	137
Balistrieri and Murray	6	74
Nelson et al.	This study	58
Burdige et al.	13	45
Kanungo and Mahapatra	34	15

Pb adsorption to Mn oxide deposits on *Leptothrix* cells overshadowed direct Pb adsorption to cells alone by 2 orders of magnitude. These results underscored previous reports which revealed that hydrous metal oxides played a greater role than cellular surfaces in governing Pb phase distribution. These reports were based on both laboratory adsorption experiments (33, 35, 62) and selective extraction of natural sediments (4, 7, 9, 36, 38). However, the biological catalysis of Mn oxidation has an indirect but very significant influence on Pb adsorption. This role should be large in circumneutral aquatic environments in which Mn oxidation is biologically mediated. Abiotic oxidation of Mn at circumneutral pH values is thermodynamically favorable but kinetically unfavorable (55), and thus it is thought that Mn oxidation in natural aquatic environments requires active enzymatic catalysis by microorganisms (8, 19, 30, 48, 58). In some cases, phototrophic organisms have been shown to facilitate the oxidation of Mn by increasing the local pH and the dissolved oxygen concentration (50). However, studies of the biogeochemical mechanisms and molecular genetics of Mn-oxidizing microorganisms (15, 47, 54, 58, 63) have indicated that enzymatically linked microbial processes are extremely influential in controlling global Mn cycles. Field studies have provided evidence that biological oxidation of Mn occurs in marine environments (43, 48, 60), estuarine environments (42), and freshwater environments (40, 48). Tessier et al. (59) found that Mn oxides in freshwater sediments were poorly crystallized and had diagenetic origins. Cycling of Mn between oxidizing aquatic environments and sediments or bottom waters under reducing conditions occurs over short time scales, making it likely that suspended Mn oxides in the water column are freshly oxidized (48, 56, 65) and therefore poorly crystalline or amorphous.

A wide range of N_2 BET surface areas have been reported for δMnO_2 prepared by methods similar to those used in this study (Table 4). Since some researchers have reported surface areas for δMnO_2 that are as large as the surface area observed for the biogenic MnO_2 in this study, it is possible that the trace metal adsorption by synthetic δMnO_2 precipitates could approach the trace metal adsorption by the biogenic MnO_2. However, it is not known if the range of reported surface areas is due to actual differences in properties of the oxides or to difficulties in interpreting N_2 BET surface area measurements (6). In either case, the surface areas of both the biogenic Mn oxide and the fresh δMnO_2 are much greater than the surface area of the more crystalline Mn oxides, supporting the conclusion that biogenic Mn oxides in aquatic environments are much more surface active than crystalline Mn oxides are.

The maximum binding capacity of the biogenic Mn oxide (Γ_{max}) was 550 mmol of Pb/mol of Mn, and this value can be

compared to the metal binding capacities of abiotic MnO_2 reviewed by Luoma and Davis (39). The majority of workers have reported metal binding capacities of MnO_2 that are between 150 and 250 mmol/mol (39), while one group reported a Pb binding capacity as high as 520 mmol/mol (25). Thus, a comparison with previously observed metal binding capacities indicates that the Pb binding capacity of the biogenic Mn oxide described here is at the upper limit of the reported range. The greater adsorption observed for biogenic Mn oxide is likely due to the amorphous nature of the surface compared to the surfaces of the more crystalline abiotic Mn minerals.

The Pb adsorption by *Leptothrix*-oxidized Mn was especially greater than the Pb adsorption by the fresh abiotic Mn oxide precipitate at low Pb concentrations. This high affinity for Pb at low Pb concentrations is reflected by the high *K* value of the Langmuir isotherm for biogenic Mn oxide (Table 2). Since dissolved Pb concentrations are typically very low in aquatic environments, these results suggest that consideration of the metal binding properties of biogenic Mn oxides is particularly important for the development of phase distribution models.

Based on the observations made in this work, the importance of Mn oxides (compared to the importance of Fe oxides) in controlling Pb adsorption may be greater than was previously thought. Fe oxides would be expected to bind more trace metal than crystalline Mn oxides based on the high specific surface area and observed trace metal adsorption of Fe oxides compared to Mn oxide minerals. However, the present work shows that the Pb adsorption of biologically oxidized Mn is much greater than the Pb adsorption of Mn oxide minerals and colloidal hydrous Fe oxide, and thus trace metal scavenging by Mn oxides in aquatic environments could be more significant under some conditions. It is possible that biogenic Fe oxides also exhibit greater trace metal binding capacity than abiotic Fe oxides exhibit. However, Fe oxides in aquatic environments are likely to contain a smaller fraction that has a biogenic origin since Fe is easily oxidized abiotically. We are currently developing experiments to generate biologically oxidized Fe under controlled laboratory conditions in order to measure its trace metal binding capacity.

Further research is needed to fully characterize the trace metal adsorption and other physical and chemical properties of biologically oxidized Mn and to establish the importance of biologically oxidized Mn in natural aquatic systems. The pH was fixed at 6.0 in this research to facilitate comparisons of different Mn minerals and to allow comparisons to prior measurements obtained with Fe oxides. It would be interesting to evaluate Pb adsorption by biogenic Mn oxides at a range of pH values and to similarly examine the adsorption of other trace metals. Experiments of this type could be used to determine parameters for surface complexation modeling of biogenic Mn oxides in order to provide more realistic simulations of trace metal adsorption to natural suspended particulate material (18, 20, 53). It is also interesting to speculate that the high trace metal adsorption capacity of biologically oxidized Mn may have a number of engineering applications. For example, fixed-film bioreactors containing Mn-oxidizing bacteria could be designed to remove trace metals from wastewater. Trace metal adsorption systems based on biogenic Mn oxides could be regenerated by dissolving Mn oxides and the associated trace metals by using a reduction in pH or redox potential.

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