Enzymatic microbial Mn(II) oxidation and Mn biooxide production in the Guaymas Basin deep-sea hydrothermal plume

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Abstract

Microorganisms play important roles in mediating biogeochemical reactions in deep-sea hydrothermal plumes, but little is known regarding the mechanisms that underpin these transformations. At Guaymas Basin (GB) in the Gulf of California, hydrothermal vents inject fluids laden with dissolved Mn(II) (dMn) into the deep waters of the basin where it is oxidized and precipitated as particulate Mn(III/IV) oxides, forming turbid hydrothermal “clouds”. Previous studies have predicted extremely short residence times for dMn at GB and suggested they are the result of microbially-mediated Mn(II) oxidation and precipitation. Here we present biogeochemical results that support a central role for microorganisms in driving Mn(II) oxidation in the GB hydrothermal plume, with enzymes being the primary catalytic agent. dMn removal rates at GB are remarkably fast for a deep-sea hydrothermal plume (up to 2 nM/h). These rapid rates were only observed within the plume, not in background deep-sea water above the GB plume or at GB plume depths (1750–2000 m) in the neighboring Carmen Basin, where there is no known venting. dMn removal is dramatically inhibited under anoxic conditions and by the presence of the biological poison, sodium azide. A conspicuous temperature optimum of dMn removal rates (~40 °C) and a saturation-like (i.e. Michaelis–Menten) response to O2 concentration were observed, indicating an enzymatic mechanism. dMn removal was resistant to heat treatment used to select for spore-forming organisms, but very sensitive to low concentrations of added Cu, a cofactor required by the putative Mn(II)-oxidizing enzyme. Extended X-ray absorption fine structure spectroscopy (EXAFS) and synchrotron radiation-based X-ray diffraction (SR-XRD) revealed the Mn oxides to have a hexagonal birnessite or δ-MnO2-like mineral structure, indicating that these freshly formed deep-sea Mn oxides are strikingly similar to primary biogenic Mn oxides produced by laboratory cultures of bacteria. Overall, these results reveal a vigorous Mn biogeochemical cycle in the GB hydrothermal plume, where a distinct microbial community enzymatically catalyzes rapid Mn(II) oxidation and the production of Mn biooxides.

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1. INTRODUCTION

Manganese plays important roles in the biogeochemistry of the oceans. The dissolved form, Mn(II), serves as a crucial micronutrient for marine organisms, and the particulate form, Mn(III/IV) oxide, is a highly reactive mineral phase that participates in a wide range of redox and adsorptive reactions (Tebo et al., 2004). Due to their high sorptive capacity, Mn oxides sequester a variety of metals and have earned the nickname “scavengers of the sea” (Goldberg, 1954). Deep-sea hydrothermal vents are a significant source of Mn for the world’s oceans (Edmond et al., 1982). Reducing hydrothermal fluids, often enriched in Mn greater than a million times over ambient seawater, are injected into the deep sea at vents along mid-ocean ridges, forming hydrothermal plumes that can rise hundreds of meters above the seafloor and be transported thousands of kilometers from their source. In hydrothermal plumes, hydrothermal fluids mix with oxygenated deep-seawater, and dissolved Mn(II) (dMn) is scavenged onto particles and/or oxidized and precipitated to form Mn oxide minerals. These particles sink towards the seafloor, scavenging trace elements and contributing to metalliferous sediments (Edmond et al., 1982). Because of its high concentration in hydrothermal plumes relative to background seawater, Mn is a valuable and commonly used tracer of hydrothermal activity (Baker et al., 1995; Lilley et al., 1995).

The conversion of dMn to the particulate phase may occur by several processes, including adsorption onto particles or microbial cells, uptake into cells, or oxidative precipitation as Mn oxides. We use the term “dMn removal” to include all of these processes, however, Mn(II) oxidation is thought to be the dominant process in environments where Mn cycling is prevalent (Tebo and Emerson, 1985; Sunda and Huntsman, 1987; Tebo et al., 1991). The chemical oxidation of Mn(II) by O₂ is thermodynamically favorable but kinetically slow, and in many environments microorganisms catalyze Mn(II) oxidation to rates that are orders of magnitude faster than those due to abiotic processes (Tebo et al., 2004). At deep-sea hydrothermal vents, microorganisms have been implicated in dMn removal by several lines of evidence: (1) inhibition of dMn removal by poisons such as sodium azide (Cowen et al., 1986, 1990; Mandernack and Tebo, 1993; Cowen et al., 1998), (2) observation of Mn-encrusted microbial cells by transmission electron microscopy (TEM) (Cowen et al., 1986, 1998; Campbell et al., 1988), and (3) isolation of Mn(II)-oxidizing bacteria from vent sites (Ehrlich, 1983, 1985; Dick et al., 2006). Despite the importance of microorganisms in mediating dMn removal at deep-sea hydrothermal vents, little is known about the mechanistic or ecological factors that underpin this process. In Mn(II)-oxidizing bacteria that have been studied in culture, Mn(II) oxidation is enzymatic (Rosson and Nealson, 1982; Francis et al., 2001; Francis and Tebo, 2002), but evidence that this is the operatic mechanism in deep-sea hydrothermal plumes is lacking.

Mandernack and Tebo (1993) showed that dMn removal rates and the relative contribution of microbial activity versus chemical processes (e.g. adsorption or coprecipitation with iron oxyhydroxides) vary dramatically among different hydrothermal vent sites. At the Galapagos spreading center microbial activity was very important, but at the Endeavor Ridge (Juan de Fuca spreading center) Mn scavenging occurred primarily via an abiotic mechanism (Mandernack and Tebo, 1993). Guaymas Basin (GB), located in the Gulf of California (Fig. 1), is an oft-cited example of a deep-sea hydrothermal system where microbially-mediated dMn removal dominates. This reputation is based on a geochemical modeling study that calculated (based on the flux and standing stock of dMn) extremely short residence times of dMn, and found that Mn oxide particles at GB are dominated by Mn-encrusted microorganisms (Campbell et al., 1988). dMn removal experiments have been reported for only one GB sample, but they showed a high turnover rate and short residence time for dMn, and removal was dominated by oxidation rather than adsorption (Mandernack and Tebo, 1993). However, no studies have directly measured dMn removal rates at GB or analyzed the importance of microorganisms in catalyzing them. In this study, we confirm that GB Mn removal rates are extremely rapid and test the null hypothesis that biological activity is not involved. Based on a series of experiments we refute this null hypothesis and present evidence that microbial enzymes are driving the oxidation of Mn(II) and production of biogenic Mn oxides.

2. GEOLOGICAL SETTING AND SITE DESCRIPTION

Guaymas Basin (GB) is one of a series of deep basins that occur along the central and southern Gulf of California (Fig. 1). The formation of the Gulf and its deep basins has been driven by sea floor spreading, with rifting giving rise to hydrothermal activity. Based on ³He and silica data, only GB is thought to be currently hydrothermally active (Lupton, 1979; Campbell and Gieskes, 1984). GB contains two spreading centers that occur in troughs; hydrothermal activity is best documented in the southern trough (Lonsdale and Becker, 1985) and subsequent submersible dives, exploration, and experimental work over the past two decades have been focused there. GB is an unusual setting for a deep-sea hydrothermal vent system due its location in a basin and proximity to the coast. Because hydrothermal fluids are injected into the deep waters of a semi-enclosed basin, inputs accumulate to higher concentrations than at mid-ocean ridges where venting occurs at topographic highs into the open ocean. GB sits under productive surface waters (van Andel, 1964) and high sedimentation rates have blanketed the spreading axis with a ~400 m layer of sediment (Lonsdale and Becker, 1985). Reactions between this sediment layer and ascending hydrothermal fluids influence the chemistry of hydrothermal fluids that emerge from the seafloor. In particular, the pH is raised, resulting in precipitation and removal of metal sulfide-forming metals such as Fe. dMn is more stable at higher pH and remains in solution to a greater extent than Fe; thus, GB hydrothermal fluids have an unusually high Mn:Fe ratio ranging from 1.25 to 10, with dMn concentrations of 128–236 μmol/kg (Von Damm et al., 1985a). Hydrothermal inputs are the major source of...
Mn for the basin (Campbell et al., 1988). In the plume, dMn is rapidly converted to a particulate phase consisting of 2–5 μm particles that resemble Mn-encrusted microbes (Campbell et al., 1988). These particles form a turbid “hydrothermal cloud” that sits in the basin (Campbell et al., 1988) and is easily detectable by a light transmission anomaly.

3. METHODS

3.1. Sample collection

Samples from GB were collected aboard the R/V New Horizon on three cruises: GoCAL1 (July 2004), GoCAL2 (January–February 2005), and GoCAL3 (July–August 2005). GB was sampled on all three cruises by “tow-yo” casts (Baker et al., 1995), whereby the CTD is lowered and raised from ~1700 to 2000 m depth while being dragged by the ship at a speed of ~1 knot. Eleven tow-yos were done in the Southern Trough (station 1) and 10 tow-yos in the Northern Trough (station 2). Two vertical casts were done at Carmen Basin (CB) (station 3) on GoCAL3. Station locations are shown in Fig. 1. Hydrothermal plumes were detected by turbidity anomalies as measured by an air-calibrated transmissometer (WetLabs) on a CTD rosette (Sea-Bird). All samples were collected in 10 L niskin bottles.

3.2. Determination of dissolved and total Mn concentrations

Samples were transferred from niskin bottles to acid-washed 50 ml polypropylene tubes. Samples for dMn were filtered through 0.2 μm acid-washed nucleopore polycarbonate filters within 1 h of collection. Filtrate (dMn) and unfiltered total Mn samples (tMn) were stabilized by acidification with Optima grade nitric acid to a pH of <2 and stored at 4 °C until analysis. All shipboard manipulations were performed in a laminar flow hood with clean techniques. Mn concentrations were determined on a Thermoquest Finnigan Element 2 double focusing, single collector, magnetic sector inductively coupled plasma mass spectrometer (ICP-MS) at the SIO unified laboratory analytical facility. ICP-MS was done at low resolution following instrument and induction parameters described previously (Field et al., 1999). Samples were diluted 1:50 in 2% nitric acid in quartz-distilled (QD) water prior to analysis. A calibration curve was prepared as described previously (Rodushkin and Ruth, 1998) using matrix-matched external standards made with 2% natural seawater stripped of metals by precipitation with Optima ammonium hydroxide. Indium (1 ppb) was used as an internal standard in all standards and samples. Standard additions (Willard et al., 1965) were used to rule out a matrix effect. To confirm analytical accuracy, reference waters CASS-4 and NASS-5 (Verplank et al., 2001) were included in the analysis as samples. Our experimentally determined average Mn concentration for CASS was 56 ± 10 nM (reported to be 51 nM) and for NASS-5 it was 16 ± 8 nM (reported to be 17 nM). Six samples from station 1 were collected, processed and analyzed in duplicate. The average standard deviation was 8 nM, which includes variation due to both sampling and analytical error.

3.3. dMn removal experiments

The general experimental design for determining dMn removal rates was as described previously (Sunda and Huntsman, 1987; Tebo et al., 1991; Mandernack and Tebo, 1993). Experiments were performed shipboard using $^{54}$Mn(II) as a radioactive tracer to measure the conversion
of dMn to the particulate phase, as defined by retention on a 0.2 μm pore-size filter. Water samples were transferred from niskin bottles to the radiation van in polycarbonate bottles (0.25–4 L), and care was taken to keep them cold at <4 °C at all times. All incubations were performed in the dark, typically being started within 4 h of sample collection and always within 12 h. For time course experiments, 100 ml of sample were transferred to 250 ml polycarbonate bottles, and amended with 0.14–0.26 μCi carrier-free $^{54}$MnCl$_2$. Time courses were done in replicate bottles, with duplicate 5–10 ml samples from each bottle filtered and processed at each time point as described below. Rates were determined based on the linear part of the curve, usually the first 4–6 h. For end-point experiments (depth profiles, anoxic and desorption experiments), 40 ml of sample were transferred to 60 ml polycarbonate bottles, and amended with 0.010–0.026 μCi $^{54}$MnCl$_2$. End point experiments were done in triplicate bottles, and incubation times ranged from 4 to 10 h. All incubations were done at 4 °C (except temperature optimum experiments described below). The quantity of $^{54}$Mn converted to the particulate phase was determined by vacuum filtration through 0.2 μm multilayered ester membrane filters. After filtration of sample, filters were rinsed with 3–5 ml 0.2 m-NaCl solution. $^{54}$Mn was counted from three fractions – total (sampled before filtration), soluble (filtrate), and particulate (filter) – so that mass balance could be calculated. To dissolve and evenly disperse particulate Mn prior to counting, 1 ml 0.1% hydroxylamine hydrochloride (H-HCl) was added to 2 ml total samples, and 3 ml H-HCl was added to filters. Samples (3 ml) were counted on a LKB Wallac 1282 Compgamama CS Universal (Perkin-Elmer) gamma counter. The portion of $^{54}$Mn retained on the filter over time as a fraction of the total gives the fraction of $^{54}$Mn removed, which multiplied by the dMn concentration equals the dMn removal rate.

The important processes and mechanisms contributing to dMn removal were assessed by manipulating $^{54}$Mn incubation experiments under a variety of conditions. Biological processes were assessed by including the metalloenzyme inhibitor sodium azide at a concentration of 0.1% (w/v). To determine the fraction of $^{54}$Mn present as (or scavenged by) extracellular Mn oxide, ascorbic acid (pH adjusted to 8 at with NaOH), an efficient reductant of Mn oxide, was added to replicate bottles to a final concentration of 40 μM at the end of incubations (30 min prior to filtration). Anoxic experiments were done in 60 ml glass serum bottles with rubber stoppers sealed with aluminum caps. Prior to addition of $^{54}$Mn, serum bottles were bubbled with N$_2$ and CO$_2$ using a gas proportioner to maintain pH at 7.6–7.8 as described previously (Clement et al., 2009). Desorption experiments were conducted by the addition of cold (non-radioactive) MnCl$_2$ to exchange with bound $^{54}$Mn (Buridge and Nealson, 1986; Sunda and Huntsman, 1987; Myers and Nealson, 1988; Mandernack and Tebo, 1993). After initial filtration of sample, the vacuum was broken and the filter was overlaid with 3 ml MnCl$_2$ in filtered seawater, which was then filtered through after 15 min. We tried several different concentrations of MnCl$_2$ including 100 μM (sample 3-1-12), 1 mM (3-1-3), and 10 mM (2-1-6). In one experiment (2-1-6), desorption was also investigated by adding MnCl$_2$ directly to the incubation bottles (to a final concentration of 10 mM) 15 min prior to filtration. For copper addition experiments, CuCl$_2$ was added at the beginning of incubations, prior to addition of $^{54}$Mn. Temperature optimum experiments were done by pre-equilibrating samples to the appropriate temperature (4, 20, 40, 55, and 70 °C) in water baths prior to addition of $^{54}$MnCl$_2$. After addition of tracer, the bottles were quickly returned to the water baths. For heat treatment experiments, samples were incubated in a water bath at 80 °C for 20 min, transferred to a 4 °C water bath for equilibration, then spiked with $^{54}$MnCl$_2$ and incubated at 4 °C for the remainder of the experiment.

3.4. Mineralogy of biogenic Mn oxides

Mn oxides were collected by gas pressure (N$_2$, <5 psi) filtration of water directly from niskin bottles onto 142 mm Supor membranes filters (Paul Corp.). Samples were frozen shipboard at ~20 °C, then transferred to ~80 °C after the cruise and kept frozen until analyzed. Samples were prepared for Mn K-edge X-ray absorption spectroscopy (XAS) by loading sections of the membrane filters into polychlorotrifluoroethylene (PCTFE) sample holders. Spectra were collected in fluorescence geometry at room temperature at the Stanford Synchrotron Radiation Laboratory (SSRL) SPEAR3 at beam line 11-2 using a collimating mirror for harmonic rejection. X-ray energy was tuned using a variable-exit Si(220) double-crystal monochromator. Data were collected in step-scan mode, which required 35 min per scan (one to two scans per sample). Energy calibration was monitored by use of the pre-edge peak of K$_2$MnO$_4$ (6543.34 eV). Multi-hour exposure tests of the near-edge spectra (XANES) provided no evidence that the incident X-ray beam was reducing Mn(IV) to Mn(II).

Spectra were background-subtracted and normalized according to standard methods, and then fit using SIXPACK (Webb, 2005), which uses IFEFFIT for its primary fitting algorithm (Newville, 2001). Mn EXAFS were fit using a model based on a layered phyllomanganate structure as described previously (Ressler et al., 1999; Webb et al., 2005a). This model explicitly accounts for: (a) splitting of the Mn–O and Mn–Mn distances in the phyllomanganate MnO$_6$ octahedral layer structure due to Jahn-Teller distortions, (b) out-of-plane bending of the octahedral layer (particularly important with Mn–Mn multiple scattering), (c) aqueous and surface bound Mn(II), and (d) vacancies present in the manganese octahedral layer. The model has been extensively validated on many Mn oxide minerals and found to provide a good description of the local structure up to $R \sim 6.0 \AA$. Specific details on the model and its parameters are discussed in detail in Webb et al. (2005a).

GB particles were also examined using synchrotron radiation powder diffraction (SR-PD). The membrane collection filters were placed in an aluminum transmission sample cell having polycarbonate windows to prevent desiccation of the sample. Transmission XRD intensity data were collected at SSRL SPEAR3 beam line 11-3 using a MAR 345 image plate. X-ray energy was set to ~0.975 Å using a Si(311) asymmetric cut, bent crystal monochromator.
Wavelength and detector distance calibrations were maintained by measuring intensity data from a LaB₆ powder. The bent monochromator crystal provides sagittal focusing to a beam size of approximately $0.15 \times 0.15$ mm. The typical scan required about 2–4 min of X-ray exposure for data acquisition. Transmission data were corrected for geometric effects and analyzed using FIT2D. Separate XRD patterns were collected for the empty cell (lexan windows), a water-filled cell, and a blank filter membrane. These scattering patterns were then directly subtracted out of the sample spectra, allowing the scattering arising from GB particles to be accurately examined.

4. RESULTS AND DISCUSSION

4.1. General water column features and location of hydrothermal plumes

During a series of three cruises we investigated the rate and mechanism of dMn removal from both the northern and southern spreading troughs of GB (Fig. 1). Hydrothermal plumes were sampled with CTD and had profiles of dissolved and particulate Mn, oxygen, and turbidity similar to those observed previously (Fig. 2) (Campbell et al., 1988). In general, higher concentrations of dMn and greater turbidity structure were observed in the southern trough, above areas of previously described active hydrothermal venting. The strongest plume signal was encountered at station 1 at a depth of 1996 m (dMn = 258 nM, total Mn (tMn) = 356 nM). Northern trough profiles were less dynamic and typically featured increasing Mn and turbidity with depth (Fig. 2).

Due to the semi-enclosed nature of GB, hydrothermal inputs are retained and the entire deep basin is influenced by hydrothermal activity. To sample a suitable non-plume control two deep casts were conducted in Carmen Basin (CB), the next deep basin south of Guaymas, which is not thought to host hydrothermal activity (Lupton, 1979; Campbell and Gieskes, 1984). Water column data for CB (Fig. 2c) show a slight light transmission anomaly present at just deeper than 1500 m, which likely represents spillover of GB plume water at the basin sill depth (1560 m). Between ~1750 and 2000 m (the depth interval of the GB plume), light transmission is higher and Mn concentrations are much lower than that of GB, consistent with the other deep basins of the Gulf of California that are not influenced by hydrothermal activity (Campbell et al., 1988). Below 2000 m, Mn concentrations begin to increase, and there are turbidity, dMn, and dMn removal maxima at ~2200 m. There is no oxygen minimum at this depth, so reduction and mobilization of Mn from sediments followed by lateral transport is not a plausible source of this dMn. Further, the total Mn concentration in the deepest CB sample we analyzed (2715 m) was more than 100 nM higher than background levels found in other non-hydrothermally active deep basins (~50 nM) (Campbell et al., 1988). These data suggest a possible hydrothermal input to the deep waters of the CB. Such inputs are not entirely unexpected since there are several hydrothermal hotspots throughout the Gulf of California region (Prol-Ledesma et al., 2004).

However, further work (e.g. $^3$He, silica, Mn/Al ratios) is required to confirm the hydrothermal origin of this Mn.

4.2. Dissolved Mn removal rates and processes

Time course measurements of dMn removal in the GB hydrothermal plume using $^{54}$Mn(II) as a radioactive tracer demonstrated a generally linear dMn removal rate for the first 4–6 h (Fig. 3) that ranged from 0.10 to 1.96 nM/h (Table 1). The fastest rate was measured in areas directly above known sites of hydrothermal venting in the southern trough (sample 3-1-3 #1; sample designations are presented throughout as ‘cruise-station-cast bottle #’) (Table 1). However, rapid rates were also recorded in the northern trough, away from known vent fields (1-2-27a #1, 3-2-10 #11). It should be noted that there are several potential sources of error in the determined dMn removal rates relative to the actual in situ rate deriving from the experimental methods we employed. Experiments were conducted at saturated $O_2$, whereas concentrations in the GB plume are typically 25–30 $\mu$M, thus our measurements reflect potential removal rates that are greater than the in situ rate. The impact of this difference can be estimated from the $O_2$ response curve of dMn removal (see Section 4.4 and Fig. 6 below); while significant, it is less than 25%. On the other hand, two aspects of our method may contribute to measured dMn removal rates actually being underestimates of the in situ Mn(II) oxidation rate. First, experiments were done shipboard at atmospheric pressure, whereas dMn removal is enhanced at in situ pressure conditions (Cowen, 1989; Mandernack and Tebo, 1993). Second, our filtration-based method (see Section 3) is insensitive to dissolved Mn(III), a potential product of Mn(II) oxidation which was recently reported to occur at marine suboxic zones where Mn cycling is prevalent (Trouwborst et al., 2006) and is also a known intermediate of Mn(II) oxidation (Webb et al., 2005b). Even if the potential effects of these experimental considerations are taken into account, the dMn removal rates we report here are remarkably rapid for a deep-sea hydrothermal plume. Previously reported dMn removal rates from other vent environments include (in nM/h): 0.0004–0.1004 at the Galapagos spreading center (Mandernack and Tebo, 1993), 0.0002–0.1033 at the Endeavor Ridge (Mandernack and Tebo, 1993), and 0.0002–0.0023 at the Cleft Segment (Cowen et al., 1990) (both of the Juan de Fuca spreading center). Residence time for dissolved Mn was calculated from:

$$\tau = \frac{A_{Mn}}{\frac{d(dMn)}{dt}}$$

where $A_{Mn}$ is the dMn concentration and $d(dMn)/dt$ is the measured dMn removal rate. Residence time ranges from 24 to 86 h (Table 1), fitting within an upper limit for residence time (1–2 weeks) predicted by a geochemical model (Campbell et al., 1988), and shorter than the 26 days calculated from one GB sample previously (Mandernack and Tebo, 1993). The residence times of dMn we calculate for GB are much shorter than those of GB surface waters (Delgado-Hinojosa et al., 2006), the open ocean (Landing and Bruland, 1987), or some hydrothermal vent environments (Mandernack and Tebo, 1993), which are on the time scale...
of years. But comparable residence times have been recorded in other areas of intense Mn cycling, such as the Galapagos spreading center (as low as 28 days) (Mander-}
{nack and Tebo, 1993), and the oxic/anoxic interfaces of
Saanich inlet (2–5 days) (Emerson et al., 1982) and the Black Sea (as low as 0.6 days) (Tebo et al., 1991).
Extremely rapid dMn removal rates suggest catalysis by microorganisms. To test the null hypothesis that microor-

Fig. 2. Representative depth profiles of turbidity, concentrations of dissolved Mn (dMn) and total Mn (tMn), and dMn removal rates. Plumes are evident by the decrease in light transmission and increase in Mn concentrations. (a) Guaymas Basin southern trough [cruise 1, station 1, cast 1]; (b) Guaymas Basin northern trough [cruise 1, station 2, cast 8]; (c) Carmen Basin [cruise 3, station 4, cast 2]. Error bars represent standard deviation of $^{54}$Mn removal from triplicate incubation bottles.
ganisms are not involved, we assayed the dMn removal rate in the presence of sodium azide, a metalloenzyme inhibitor. Azide treatment is thought to be suitable for estimation of microbially-mediated dMn removal because it interferes little with Mn solution chemistry or adsorption onto particles in seawater (Emerson et al., 1982; Rosson et al., 1984; Clement et al., 2009). Azide dramatically inhibited dMn removal compared to untreated samples (Figs. 3 and 4), providing a strong basis for refutation of the null hypothesis, and suggesting that biology is central to the rapid dMn removal rates in the GB plume.

To determine if the biologically-mediated dMn removal was due to precipitation on cellular surfaces or cellular uptake, we treated incubations with ascorbic acid, a strong reducing agent that readily dissolves Mn oxides (Stone, 1983; Sunda and Huntsman, 1987) but does not lyse cells (Anderson and Morel, 1982). Addition of ascorbic acid after termination of the experiment consistently and completely reduced all particulate $^{54}\text{Mn}$, indicating that dMn removal was due to extracellular oxidation and/or adsorption onto preexisting Mn oxides rather than intracellular uptake (data not shown). To further assess the importance of Mn(II) oxidation versus adsorption onto preexisting Mn oxides, dMn removal experiments were performed under anoxic conditions or with rinses/additions of “cold” (non-radioactive) MnCl$_2$ to exchange $^{54}\text{Mn(II)}$. dMn removal was greatly inhibited under anoxic conditions, where dMn removal rates accounted for 27–41% of oxic controls (Fig. 4). About 0–15% of $^{54}\text{Mn(II)}$ was displaced by cold MnCl$_2$ rinses or additions, consistent with sorption being a potentially significant but not major factor in dMn removal (Fig. 4). Taken together, our dMn removal experiments indicate that biologically-mediated, extracellular oxidation and precipitation of Mn oxides is the primary process responsible for driving the rapid dMn removal that occurs in the GB hydrothermal plume.

4.3. Depth profiles of dMn removal rates in the Guaymas and Carmen Basins

To determine whether fast dMn removal rates are a common feature throughout the GB water column, we measured dMn removal across a range of depths. Rapid dMn removal rates were observed only at the depths of the hydrothermal plume (Fig. 2a and b). Similar rates were observed in the southern trough, where there is active venting, and in the northern trough, where hydrothermal deposits have been recovered (Lonsdale et al., 1980) but plumes

Table 1

Summary of dMn Removal Rate Measurements

<table>
<thead>
<tr>
<th>Samplea</th>
<th>Date</th>
<th>Depth (m)</th>
<th>dMn (nM)</th>
<th>tMn (nM)</th>
<th>dMn Removal Rate (nM/h)</th>
<th>dMn Residence time (h)</th>
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<tbody>
<tr>
<td>1-1-2 #3</td>
<td>7/18/04</td>
<td>1851</td>
<td>25</td>
<td>235</td>
<td>0.66 (0.04)$^b$</td>
<td>38</td>
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<tr>
<td>1-2-27a #1</td>
<td>7/15/04</td>
<td>1950</td>
<td>40</td>
<td>288</td>
<td>1.70 (0.02)$^b$</td>
<td>24</td>
</tr>
<tr>
<td>2-1-1 #9</td>
<td>1/30/05</td>
<td>1800</td>
<td>37</td>
<td>247</td>
<td>0.43 (0.06)$^b$</td>
<td>86</td>
</tr>
<tr>
<td>3-1-3 #1</td>
<td>8/5/05</td>
<td>1905</td>
<td>83</td>
<td>178</td>
<td>1.96 (0.19)$^c$</td>
<td>42</td>
</tr>
<tr>
<td>3-2-10 #11</td>
<td>8/1/05</td>
<td>1890</td>
<td>35</td>
<td>159</td>
<td>1.44 (0.09)$^c$</td>
<td>24</td>
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<tr>
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<td>7/12/04</td>
<td>1762</td>
<td>5</td>
<td>138</td>
<td>0.10 (0.02)$^d$</td>
<td>55</td>
</tr>
<tr>
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<td>1953</td>
<td>19</td>
<td>219</td>
<td>0.43 (0.08)$^d$</td>
<td>44</td>
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<tr>
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<td>1883</td>
<td>15</td>
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<td>0.61 (0.08)$^d$</td>
<td>25</td>
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<tr>
<td>2-1-6 #24</td>
<td>2/5/05</td>
<td>1800</td>
<td>11</td>
<td>140</td>
<td>0.26 (0.01)$^d$</td>
<td>42</td>
</tr>
<tr>
<td>2-1-6 #21</td>
<td>2/5/05</td>
<td>1900</td>
<td>15</td>
<td>313</td>
<td>0.90 (0.05)$^d$</td>
<td>17</td>
</tr>
<tr>
<td>2-1-6 #7</td>
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<td>1963</td>
<td>62</td>
<td>315</td>
<td>2.35 (0.15)$^d$</td>
<td>26</td>
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<tr>
<td>2-2-4 #5</td>
<td>1/30/05</td>
<td>1895</td>
<td>22</td>
<td>210</td>
<td>0.28 (0.14)$^d$</td>
<td>79</td>
</tr>
<tr>
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<td>7/28/05</td>
<td>1900</td>
<td>53</td>
<td>130</td>
<td>1.16 (0.22)$^d$</td>
<td>46</td>
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<tr>
<td>3-1-12 #1</td>
<td>8/6/05</td>
<td>1975</td>
<td>15</td>
<td>61</td>
<td>0.20 (0.04)$^d$</td>
<td>72</td>
</tr>
<tr>
<td>3-1-17 #1</td>
<td>8/7/05</td>
<td>1959</td>
<td>52</td>
<td>207</td>
<td>0.69 (0.09)$^d$</td>
<td>75</td>
</tr>
</tbody>
</table>

$^a$ Samples designated as ‘cruise-station-cast bottle #’.

$^b$ Standard deviation based on rate determined for duplicate time courses is shown in parentheses.

$^c$ Standard deviation based on rate determined for triplicate time courses is shown in parentheses.

$^d$ Standard deviation based on end-point measurements of triplicate incubations.
had not been detected previously (Lonsdale and Becker, 1985) (Table 1; Fig. 2a and b).

The deep waters of CB and GB derive from the same source water (Pacific Deep Water) (Lavin and Marinone, 2003) and have similar overall physicochemical conditions (temperature, salinity, O₂). At depths in CB corresponding to the GB plume (1750–2000 m), Mn concentrations and light transmission are consistent with background deep Gulf of California waters; thus, this depth interval represents a suitable non-plume control. Here, dMn removal rates are low – comparable to those observed in background seawater throughout the water column – indicating that the rapid rates observed at 1750 to 2000 m depth in GB are specific to the hydrothermal plume rather than to those depths. Regardless of the origin of the elevated Mn concentrations in the deep waters of CB, the rapid Mn(II) oxidation rates observed in the GB hydrothermal plume are specific to the plume since rates either directly above the plume or at GB plume depths (~1750–2000 m) in CB are >20 times slower. The known physical and chemical determinants of the Mn(II) oxidation rate – temperature and oxygen concentration – are not elevated in the GB plume. Given the biological nature of the dMn removal, we infer that the rapid dMn removal rates observed in the GB plume are driven by microbial activities that are unique to the GB hydrothermal plume.

4.4. Mechanism of Mn(II) oxidation

The mechanism of microbially-mediated Mn(II) oxidation in deep-sea hydrothermal plumes has been suggested to be either enzymatic (Cowen et al., 1986; Dick et al., 2006) or via binding of Mn to organic polymers of microbial capsules (Cowen et al., 1986, 1990; Cowen, 1989). Mn(II) oxidation proceeds by an enzymatic mechanism in pure bacterial cultures (Rosson and Nealson, 1982; Francis et al., 2001; Francis and Tebo, 2002) and biogeochemical evidence from several studies indicates this is also the case for at least certain sites in the upper water column of the ocean (Tebo and Emerson, 1985; Moffett and Ho, 1996). Several features of dMn removal in the GB plume are indicative of an enzymatic mechanism of Mn(II) oxidation. There is a conspicuous temperature optimum for dMn removal which is not apparent in azide-treated or anoxic incubations (Fig. 5), indicating that the temperature response is biological in nature and dependent on the presence of oxygen. Temperature optimum is one of the most effective indicators of biological mediation of a geochemical process (Brock, 1978), and it has been used to demonstrate that enzymes catalyze Mn(II) oxidation in natural samples (Tipping, 1984; Tebo and Emerson, 1985); enzymatic activity increases with temperature to a point where activity decreases due to denaturation of a protein’s complex structure. In contrast, under the null hypothesis of dMn removal by an abiotic chemical mechanism or by binding to simple organic polymers (e.g. cell surface polysaccharides) dMn removal would be expected to increase monotonically with temperature. Although a reaction rate temperature optimum could theoretically be explained by a change in mechanism of some unknown chemical process, we are unaware of scenarios that would plausibly explain the oxygen-

Fig. 4. dMn oxidation/desorption experiments. dMn removal rates were determined from the percentage of ⁵⁴Mn retained on the filters after no treatment (control), addition of sodium azide (azide), sparging to remove O₂ (anoxic), and treatment with non-radioactive Mn to exchange the adsorbed ⁵⁴Mn fraction (desorption). For desorption-A, filters were rinsed with filtered seawater containing MnCl₂ at either 100 μM (3-1-12 #1) or 10 mM (2-1-6 #7). For desorption-B, MnCl₂ was added directly to the incubation bottles to a final concentration of 10 mM 15 min prior to filtration. Note: the desorption columns indicate dMn removal during the incubation, calculated after desorption treatment (rather than dMn displaced by desorption); the fact that this is near-identical to the control indicates that little dMn was displaced by the desorption treatment. See Section 3 for further details. N.D., not determined.

Fig. 5. Temperature optimum of dMn removal (3-1-3 #1; see Table 1 for depth and Mn concentration data). Error bars represent standard deviation of ⁵⁴Mn removal from triplicate incubation bottles.
dependent temperature optimum we observe for conversion of dissolved Mn to the particulate phase.

An enzymatic mechanism of Mn(II) oxidation is also evident in the response of dMn removal rates to O₂ concentration. dMn removal rates measured at controlled O₂ levels resulted in a hyperbolic curve as O₂ increases from 0 to 7.5 μM (Fig. 6). This response is shifted towards higher O₂ concentrations relative to previous results from the oxic/anoxic interface of Saanich Inlet (Tebo and Emerson, 1985) and the suboxic zone of the Black Sea (Clement et al., 2009). Unfortunately no data points were obtained between 7.5 μM and saturated O₂ (285 μM); while this lack of data precludes accurate determination of the half saturation constant (Kₘ, a measure of an enzyme’s affinity for substrate), the response of dMn removal rate to O₂ is consistent with Michaelis–Menten enzyme kinetics with an estimated Kₘ of ~10 μM.

4.5. Characteristics of GB Mn(II)-oxidizing microorganisms and enzymes

An important unresolved question concerns the origin and nature of Mn(II)-oxidizing microorganisms in the plume: are they indigenous to the deep sea or are they entrained from near-vent environments such as chimneys or sediments? GB hydrothermal plumes entrain fine sediment particles as they rise from the seafloor (Campbell and Gieskes, 1984) and TEM studies have suggested that sediments are generally enriched in encapsulated or Mn-encrusted bacteria (Cowan and Bruland, 1985; Campbell et al., 1988). Further, Mn(II)-oxidizing Bacillus have been isolated from GB hydrothermal sediments and plumes (Dick et al., 2006). These Bacillus species oxidize Mn(II) as otherwise metabolically dormant spores with an enzyme that resides in the outer layer of the spore coat (Francis et al., 2002). Two features of the dMn removal at GB are characteristic of Mn(II) oxidation by Bacillus spores. First, the temperature response of Mn(II) oxidation by Bacillus spores isolated from GB plumes and sediments (Dick et al., 2006) and coastal sediments (Rosson and Nealson, 1982) is strikingly similar to the profile we observed in GB plume waters (Fig. 5). Second, in most cases dMn removal in the GB plume was heat resistant (Fig. 7), a characteristic used to select for Bacillus spores in environmental samples (Lee, 1994; Dick et al., 2006); spores survive this treatment, whereas other organisms are killed. Heat tolerance extends to the Mn(II)-oxidizing enzyme, and has been used to assess the contribution of spores to Mn(II) oxidation rates in the environment (Lee, 1994).

Despite these similarities in characteristics of Mn(II) oxidation between the GB plume and Bacillus spores, other evidence suggest that the observed environmental Mn(II) oxidation cannot be attributable solely to Bacillus spores. Cell-specific Mn(II) oxidation rates have been estimated at ~3 x 10⁻⁸ nM Mn(II) h⁻¹ spore⁻¹ (de Vrind et al., 1986), which would require ~7 x 10⁴ spores ml⁻¹ to account for dMn removal rates reported here for the GB plume (~2 nM/h). Although there are certainly potential sources of error in extrapolating laboratory rate measurements to the environment, this estimate suggests that Bacillus species would represent an appreciable portion of total microbial cells within the GB plume (2 x 10⁵ cells ml⁻¹) (Lam, 2004). In a molecular survey, Bacillus species were not detected in the plume microbial community (Dick, 2006), thus it seems unlikely that they are the dominant catalysts of Mn(II) oxidation in the GB plume. As Bacillus spores are among the most robust Mn(II)-oxidizing...
organisms to be studied in pure culture, these inferences extend to other known Mn(II) oxidizers as well. Overall, these considerations suggest that (i) organisms responsible for oxidizing Mn(II) in the GB plume must be abundant community members and (ii) the key microorganisms catalyzing Mn(II) oxidation in the GB hydrothermal plume have yet to be identified.

Multicopper oxidase (MCO) enzymes have been implicated in Mn(II) oxidation in diverse Mn(II)-oxidizing bacteria (van Waasbergen et al., 1996; Corstjens et al., 1997; Brouwers et al., 1999; Francis et al., 2001; Ridge et al., 2007; Dick et al., 2008a,b), and in laboratory cultures Cu stimulates Mn(II)-oxidizing activity (van Waasbergen et al., 1996; Brouwers et al., 1999, 2000; Larsen et al., 1999). We examined the effect of CuCl₂ additions on dMn removal rates in GB plume waters (Fig. 7). In one case, one nanomolar added CuCl₂ stimulated dMn by a small yet significant amount (sample 2-8 #3), but the more dramatic effect was inhibition of dMn removal at higher CuCl₂ concentrations. In two different samples, 10 nM added CuCl₂ (approximately equal to the dMn concentration) inhibited dMn removal by ~50%. In one sample where higher concentrations of CuCl₂ were added, inhibition of dMn removal approached 100% (Fig. 7). The nature of this effect of Cu on dMn removal is unclear and does not really address the null hypothesis since, in addition to toxic or stimulatory effects of Cu²⁺ on cells, Cu²⁺ could potentially compete with Mn²⁺ binding to cells or preformed Mn oxides and thereby inhibit both biotic or autocatalytic Mn(II) oxidation. The observed inhibition does not necessarily exclude MCOs as the major catalysts of Mn(II) oxidation in the GB plume; Cu addition experiments to Mn(II)-oxidizing bacterial cultures have shown a Cu optimum, with inhibition at higher Cu concentrations (van Waasbergen et al., 1996; Brouwers et al., 2000). Therefore it is possible that the Cu additions presented here were above the optimum Cu concentration. Whatever the mechanism of inhibition, Cu is enriched in hydrothermal fluids relative to seawater and therefore could potentially compete with Mn²⁺ binding to cells or preformed Mn oxides and thereby inhibit both biotic or autocatalytic Mn(II) oxidation.

4.6. Mineralogy of GB Mn oxides

We analyzed the mineralogy of the GB plume Mn oxides with synchrotron radiation-based X-ray diffraction (SR-XRD), X-ray absorption near-edge structure (XANES), and extended X-ray absorption fine structure spectroscopy (EXAFS). XRD of the particles showed very little diffraction, even though the filters are strongly colored with Mn oxides, suggesting that the particles are extremely small and/or amorphous. The energy position of the X-ray absorption spectroscopy absorbance maximum lies at 6562 eV, indicative of the Mn(IV) peak of Mn(IV) oxides such as δ-MnO₂ or hexagonal acid birnessite (Fig. 8) (Baragar et al., 2000). This suggests that the GB Mn oxides have an average oxidation state between 3.7 and 4.0 (Villalobos et al., 2003) and that solid-phase pure Mn(III) and Mn(II/III) intermediates or minerals are not present in abundance. Analysis of EXAFS spectra of the GB Mn oxides indicated that they are structurally most similar to hexagonal birnessite or δ-MnO₂-like layered (phyllomanganate) minerals (Fig. 9). Quantitative fitting of spectra showed the agreement between data from GB samples and hexagonal birnessite is very good except for some minor amplitude differences specifically at the positive antibodies between k = 9 and 10 Å⁻¹ (Fig. 9). The EXAFS amplitude at these locations arises from the presence of hexagonal unit cell symmetry and in-layer Mn(III) content (sharper peaks for lower Mn(III) content). We can therefore conclude that slight differences between the models and data indicate qualitatively greater hexagonal character and/or lower

Fig. 8. XANES spectra of GB Mn oxides and reference compounds. (a–e) GB Mn oxides: (a) 2-1-6 #24, (b) 2-1-6 #21, (c) 2-1-6 #1, (d) 2-2-4 #5, (e) 1-2-18 #3. (f–h) Reference compounds: (f) Mn(II)Cl₂, (g) Mn(III) pyrophosphate, (h) δ-MnO₂. Mn concentration and dMn removal rate data for the GB samples are provided in Table 1.
Mn oxide deposits (Lalou, 1983; Lalou et al., 1983; Hodework has been done on the mineralogy of hydrothermal elements and their transport to the sediment. Although organic chemistry as well as the availability of inorganic trace elements and their transport to the sediment. Although work has been done on the mineralogy of hydrothermal Mn oxide deposits (Lalou, 1983; Lalou et al., 1983; Hodkinson et al., 1994; Liakopoulos et al., 2001; Rogers et al., 2001) and other naturally occurring Mn oxides (see Tebo et al., 2004 for discussion), to our knowledge these are the first data on the mineralogy of freshly precipitated Mn(III) content in the samples as compared to the hexagonal birnessite model. Overall, however, they are consistent with hexagonal symmetry. We were not able to detect any significant differences in the mineralogical properties of Mn oxides between GB plume samples of varying dMn concentration or dMn removal rate. The structure of the GB Mn oxides is strikingly similar to Mn oxides that are the primary products of enzymatic Mn(II) oxidation in cultures of diverse Mn(II)-oxidizing bacteria (Fig. 9). (Villalobos et al., 2003; Jürgenson et al., 2004; Bargar et al., 2005; Webb et al., 2005a; Saratovsky et al., 2006). In laboratory experiments, these amorphous Mn biooxides have been observed to transition to secondary products such as Ca-pseudo-orthogonal birnessite in seawater over short time scales (several days after formation) (Webb et al., 2005a). The fact that we do not observe any of these phases is consistent with the GB Mn oxides being freshly precipitated minerals. This high surface area mineral is a strong oxidant and scavenger (Tebo et al., 2004) and thus is expected to impact organic chemistry as well as the availability of inorganic trace elements and their transport to the sediment. Although work has been done on the mineralogy of hydrothermal Mn oxide deposits (Lalou, 1983; Lalou et al., 1983; Hodkinson et al., 1994; Liakopoulos et al., 2001; Rogers et al., 2001) and other naturally occurring Mn oxides (see Tebo et al., 2004 for discussion), to our knowledge these are the first data on the mineralogy of freshly precipitated (within days) deep sea hydrothermal Mn oxides. Our data suggest that the layer-type (birnessite) is the primary biogenic mineral, while tunnel-type structures (e.g. todorokite) that have been observed in some deep-sea Mn oxides may be the products of secondary reactions. Natural biogenic Mn oxides have been analyzed with EXAFS and XRD from two other marine environments of rapid Mn(II) oxidation – the suboxic zone of the Black Sea and the oxic/anoxic interface of Saanich Inlet (British Columbia) – and these biogenic Mn oxides are also most similar to δ-MnO2. Despite considerable recent advances, there is still much to be learned regarding the interplay of physical, geochemical and biochemical factors that shape Mn biooxide structure.

5. CONCLUSIONS

The results presented here show that rapid dMn removal rates in the GB hydrothermal plume are predominantly the result of enzymatic microbial Mn(II) oxidation, with the mineral product being Mn bioxide similar to that produced by pure cultures of microorganisms in laboratory. Rapid microbial Mn(II) oxidation is specific to the hydrothermal plume and we propose this reflects unique microbiological dynamics within the plume compared to the background deep sea. Despite early work that showed increased biomass in hydrothermal plumes (Cowen et al., 1986; Winn et al., 1986), little is known about how deep-sea microbial communities respond to the flux of potential nutrients and energy sources (e.g. H2, NH4+, CH4, H2S, Fe3+, Mn2+) from hydrothermal vents. In the Guaymas Basin hydrothermal plume, concentrations of NH4+ and CH4 are particularly high. Biomass, however, is only marginally higher in the plume; total cell numbers within the plume are 2–3 times that of overlying waters (Lam, 2004), and DNA concentrations are not significantly higher than background deep seawater (Dick, 2006). Thus increased biomass in the plume alone cannot account for the ~200-fold increase in dMn removal rates. We conclude that rapid Mn(II) oxidation in the plume is likely due to either a phylogenetically or physiologically distinct microbial population. Based on cell-specific Mn(II) oxidation rates of microorganisms in culture, a large shift in microbial community structure is required to account for the dMn removal rates we report in this study. Such a shift has been reported in the Suiyo Seamount hydrothermal caldera, which is dominated by just two bacterial phylotypes (Sunamiya et al., 2004). Alternatively, the plume-specific rates could be the result of increased metabolic activity, differences in the portion of the microbial community that is active, or differential expression of Mn(II)-oxidizing enzymes. Overall, our work demonstrates that Mn(II) oxidation in the Guaymas Basin hydrothermal plume is actively mediated by microbial enzymes, resulting in the production of highly reactive Mn biooxides. In light of the impact that deep-sea hydrothermal vents have on ocean chemistry and the primary importance of geomicrobial transformations in determining the ultimate fate of these inputs, these results highlight the need for a better understanding of the microbiological dynamics that underpin plume geochemistry.

Fig. 9. Mn K-edge EXAFS of GB and reference Mn oxides. (a–e) GB Mn oxide samples (solid lines) with quantitative fits to hexagonal birnessite (dashed lines): (a) 2-1-6 #24, (b) 2-1-6 #21, (c) 2-1-6 #1, (d) 2-2-4 #5, (e) 1-2-18 #3. (f–i) reference compounds: (f) hexagonal birnessite, (g) triclinic birnessite, (h) todorokite, (i) biogenic Mn oxide from Bacillus sp. strain SG-1 (50 mM NaCl). Mn concentration and dMn removal rate data for the GB samples are provided in Table 1.
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