

Experimental Assessment of Microbial Effects on Chemical Interactions Between Seafloor Massive Sulfides and Seawater at 4 °C

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Abstract

To assess the effects of microbes on the exchange of Cu, Zn, and P between seafloor massive sulfide (SMS) deposits and seawater, we monitored the variation of the concentrations of Cu, Zn, and P in the artificial seawater of reaction systems that did or did not also include slabs and microbes originating from an SMS sample at 4 °C for 71 days. Dissolution of Cu and Zn from the slabs was observed when microbes were present or absent. Zinc from the slabs dissolved 1.4–2.3 fold more rapidly when microbes were present. In the presence of slabs and microbes, the rate of removal of P from the artificial seawater was the sum of the individual removal rates associated with the slabs and microbes. Six bacterial phylotypes including *Halomonas* and *Marinobacter* were present at the end of the experiment as shown by PCR-based analysis targeting 16S rRNA genes. These bacteria probably contribute to the release of Zn from the SMS slab and removal of P from the artificial seawater. Our results provide further insights into the role(s) of microbes on the geochemical interactions between SMS deposits and seawater.

Keywords

Bacteria • Batch experiment • Elemental cycle • Microbial dissolution • Seafloor massive sulfides

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9.1 Introduction

Seafloor massive sulfide (SMS) deposits occur at and around the plate boundaries, e.g., mid-ocean ridges, back-arc basins and volcanic arcs (Hannington et al. 2011). These SMS deposits consist of relatively insoluble sulfide minerals, e.g., pyrite (FeS_2), chalcopyrite (CuFeS_2), sphalerite (ZnS), and galena (PbS), and also contain trace elements such as Au, Ag, Co and in some cases, Ni (Herzig and Hannington 1995). The hydrothermally active and inactive vents of chimney- and mound-shaped SMS deposits are microbe-rich environments, with microbial densities of up to 10^{10} cells (g sulfide) $^{-1}$ (Kato et al. 2010; Schrenk et al. 2003; Suzuki et al. 2004; Takai and Horikoshi 1999), which are densities comparable with those in soils and animal bowels (Whitman et al. 1998). Dissolved H_2 , H_2S , CH_4 and Fe^{2+} supplied from hydrothermal fluids in the active sulfide vents serve as energy sources for the microbial communities. Conversely, microbial communities in inactive sulfide vents apparently use metal sulfides as energy sources (Edwards et al. 2003a). An in situ examination of SMS ores with time suggested that sulfide minerals on the seafloor are highly weathered by microbes (Edwards et al. 2003b). However, a quantitative assessment of the elements released from or adsorbed by SMS deposits mediated by microbes had not been done prior to this report.

Considering the high levels of heavy metals in the SMS deposits and the ubiquity of these deposits on the seafloor (Hannington et al. 2011; Herzig and Hannington 1995), an experimental study of the change with time of the SMS components via microbial activity is important to understand how such events impact the oceanic biogeochemical cycles. The concentrations of certain heavy metals, e.g., Fe, Zn, Mn, Cu and Ni, which are essential for oceanic microbial viability, are extremely small ($<0.1 \mu\text{M}$) in the ocean (Morel and Price 2003; Sohrin and Bruland 2011). The exchange of these heavy metals between seawater and SMS deposits is potentially needed for the maintenance of important oceanic microbial ecosystems.

Here, we report the effects of microbes on the exchanges of elements between SMS samples and seawater by simulating the environmental conditions of deep seafloor (i.e., low temperature, weakly alkaline pH and a small amount of organic carbon). The goals of this study were (i) to measure the kinetics of the dissolution of certain metals and P from a recovered SMS sample and (ii) to assess if and how microbial communities affect the kinetics.

9.2 Materials and Methods

9.2.1 Sample Collection

A portion of massive sulfide ore (sample ID, D903-R1) was collected from a hydrothermal vent field, called the Archaean site ($12^\circ 56.35' \text{N}$, $143^\circ 38.0' \text{E}$; depth, 3,076 m), in the Southern Mariana Trough during the YK05-09 cruise (July to August 2005) by the manned submersible *Shinkai 6500* (JAMSTEC, Japan) of the R/V *Yokosuka* (JAMSTEC, Japan). A bathymetric map of the vent field is shown in a previous report (Kato et al. 2010) and also in Seama et al. (Chap. 17). The SMS sample (Suppl. 9.1a) was washed with filter-sterilized seawater and crushed into fist-sized, sub-surface, non-oxidized samples, using an autoclave-sterilized hammer and chisel in a clean box on board. Some of the samples were stored at -80°C in DNA/RNA-free plastic tubes for DNA extraction and the others were stored at 4°C for inoculation and mineralogical studies.

9.2.2 Experimental Medium

The artificial seawater (ASW) was a modification of that described in the previous report (Jannasch et al. 1996) and contained 20.0 g NaCl; 3.0 g $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$; 6.0 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$; 1.0 g $(\text{NH}_4)_2\text{SO}_4$; 0.2 g NaHCO_3 ; 0.3 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$; 0.5 g KCl; 0.015 g KH_2PO_4 , 1 mL of vitamin solution (DSMZ medium 141; <http://www.dsmz.de>) and 1 mL of marine trace element solution (DSMZ medium 511), in 1 L of distilled water. The pH value of the ASW was 7.3 before autoclaving and 8.1 afterwards. To simulate the low concentrations of organic compounds found in deep seawater, Bacto yeast extract (BD Difco, NJ, USA) was added from an autoclaved stock solution to render the concentration to be 10 mg (corresponding to 3.3 mg of carbon) L^{-1} in the final medium as described previously (Jannasch et al. 1996).

9.2.3 Batch Experiments

A sample of the interior of the SMS was cut into small slabs ($10 \times 10 \times 1$ mm; Suppl. 9.1b) using a low speed diamond saw (Struers Minitor, Westlake, OH, USA). Each slab weighed 0.35 ± 0.05 g. The relative surface area of each slab was $5.72 \pm 0.01 \text{ m}^2 \text{ g}^{-1}$, as determined by the BET- N_2 method (Seishin Enterprise Co., Ltd., Tokyo, Japan). The slabs were cleaned and sterilized by soaking them in ethanol

and acetone for 2 h each according to Edwards et al. (2000) and then dried by flushing with N₂ gas at room temperature.

Sterile 250-mL glass culture flasks, each containing 100 mL of ASW and capped with sponge plugs, were used to contain the incubations. Each flask contained one of the following systems: ASW with slabs and an inoculum; ASW with only slabs; ASW with only an inoculum; only ASW. The contents of all systems were prepared in duplicate, except for the system of only ASW. The inoculum was prepared from a slurry of the mostly oxidized surface of the SMS sample. The cell density of the slurry was $3.62 \pm 0.65 \times 10^7$ cells mL⁻¹ (corresponding to $2.22 \pm 0.38 \times 10^6$ cells (g sulfide)⁻¹) determined by fluorescence microscopy (Kato et al. 2009a). The systems containing slabs and an inoculum are denoted as MC1 and MC2; those with only slabs as C1 and C2; those with only an inoculum as M1 and M2; and the negative control, without slabs and an inoculum as N (i.e., blank test), respectively. MC1, MC2, C1 and C2 each contained 10 slabs. One milliliter of the slurry was added into each of MC1, MC2, M1 and M2. These reaction systems were incubated in a cold room at 4 °C.

An aliquot of 3 mL (1 mL for direct cell counting and 2 mL for chemical analyses) was removed from each culture with a sterile disposable syringe at 4, 42, 140, 332, 782, and 1698 h after the start of the experiment. Before sampling, each medium was gently withdrawn and returned by action of a sterile disposable syringe three times. To determine the number of cells, each sample was filtered through a black polycarbonate filter (pore size, 0.2 µm; diameter, 13 mm; Advantec, Tokyo, Japan). Each filter was washed twice with ultra-pure water, and then was dried in a sterilized Petri dish and stored at -20 °C. For the chemical analyses, each sample was filtered through a polycarbonate filter (pore size, 0.2 µm; diameter, 25 mm; Advantec, Tokyo, Japan), and the filtrates were individually stored in an acid-cleaned 2-mL polypropylene tube. Approximately 300 µL of each filtrate was used for the pH measurement.

At the end of the incubations, a 60 mL aliquots of each solution was filtered through a polycarbonate filter (pore size, 0.2 µm; diameter, 13 mm; Advantec, Tokyo, Japan). The filters were stored at -80 °C prior to DNA analysis. To remove the microbes that were loosely attached to the slabs, the slabs were gently swished in sterilized Petri dishes containing 30 mL of ultra-pure water with tweezers. The water was replaced three times. Five slabs from each experiment were stored at -80 °C for DNA analysis, and the other five slabs were fixed with 3.7 % (w/v) formalin at 4 °C over night and then stored in a 1:1 ethanol/phosphate-buffered saline mixture at -20 °C until microscopy was performed.

9.2.4 Chemical Analysis

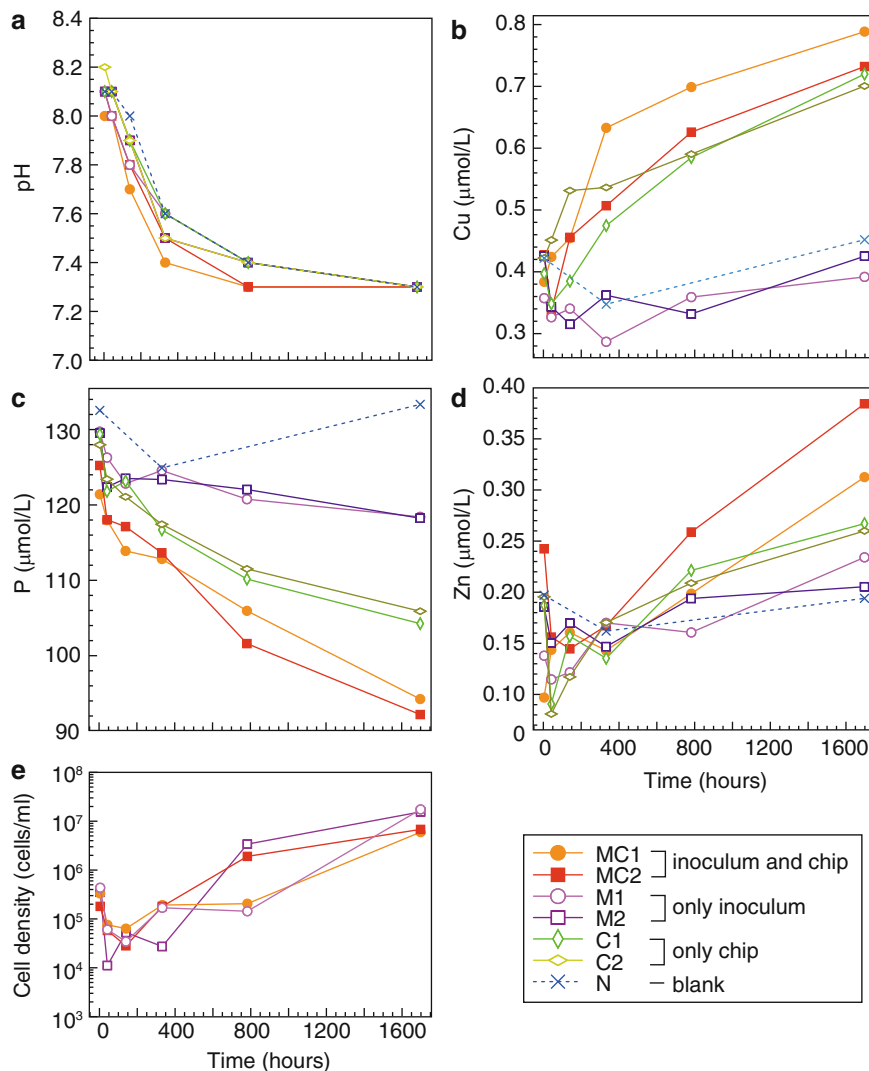
To determine the chemical composition of the retrieved SMS sample, 0.1 g of the slabs was pulverized and decomposed in HNO₃-HF-HClO₄ solution at 90 °C. The solution was evaporated at 90 °C. The residue was dried at 140 °C and then dissolved in 1.25 mL HCl-HNO₃ solution at 90 °C. The concentration of each element was determined by multi-channel inductively coupled plasma-optical emission spectrometry (ICP-OES) (SPS5500; SII NanoTechnology, Chiba, Japan). The chemical composition of the sample was 42.9 wt% Fe (weight per total weight); 694 ppm Zn; 594 ppm Al; 114 ppm Co; 106 ppm Cu; 97.4 ppm Mg; 72.9 ppm Ba; 46.0 ppm Ca; 32.4 ppm Cd; 30.0 ppm Ni; 23 ppm Na; 9.3 ppm V; 6.90 ppm Rb; 6.80 ppm Sr; 4.0 ppm Mn. We assumed that the remaining solid, which could not be quantified, was sulfur. The uncertainties for the ICP-OES analysis were within ±5 % for Al, Ba, Ca, Cd, Cu, Fe, Mg, Rb, Sr and Zn, and approximately ±10 % for Co, Mn, Na, Ni and V. The detection limit for the aforementioned elements was <10 ppb. Ikehata et al. (Chap. 22) characterized the mineral content of a bulk sample of this SMS of D903-R1, and showed that it was primarily composed of cryptocrystalline pyrite and marcasite with lesser amounts of sphalerite, chalcopyrite, and barite.

The pH of each system was measured with a TWIN pH meter (HORIBA, Kyoto, Japan). Concentrations of Cu, Zn and P in the ASW samples with time were measured using the ICP-OES system as described above. The detection limits were 2.25 nmol L⁻¹ for Cu, 239 nmol L⁻¹ for P, and 5.54 nmol L⁻¹ for Zn. The uncertainty for the P measurement was ±5 % and that for the Cu and Zn measurement was approximately ±10 %. We also assessed the concentrations of the other elements that had been detected in the SMS sample; however, no clear temporal trends were observed because of their low concentrations (data not shown). The concentration of each element was corrected for the decrease in the sample volume and the loss of element mass during the sampling using the following equation (Eq. 4 in Wu et al. 2007):

$$C'_{j,i} = \frac{C_{j,i}[V_0 - (j-1)V_s] + \sum_{h=1}^{j-1} C_{h,i}V_s}{V_0} \quad (9.1)$$

where $C'_{j,i}$ is the corrected concentration of element i in the j th sample ($j = 1, 2, \dots, 7$), and $C_{j,i}$ is the measured concentration. V_0 is the initial volume (0.1 L), V_s is the sample volume (0.003 L), and the term $\sum_{h=1}^{j-1} C_{h,i}V_s$ accounts for the total mass of element i extracted during the samplings.

Fig. 9.1 Changes in cell density, pH, the concentrations of Cu, P and Zn during the course of the experiments. (a) pH values. Concentrations of (b) Cu, (c) P, and (d) Zn. (e) Cell density. Symbol for the systems are shown in the *box* at the lower right of the figure (see text for details)



The rate constant (dC'/dt) of dissolution for each element was calculated for the 332–1,698 h period (Suppl. 9.2), a time when the pH values of the ASW were between 7.3 and 7.5 (Fig. 9.1a and Suppl. 9.3). The release or removal rate (R) of each element to or from the ASW, respectively, was calculated using the corresponding rate constant (dC'/dt), the initial fluid volume (V_0 , 0.1 L), the relative surface area (A , $5.72 \text{ m}^2 \text{ g}^{-1}$), the total mass of the slabs in each system (m , 3.11–3.33 g), and Eq. 5 in Wu et al. (2007):

$$R = \frac{dC' \cdot i}{dt} \frac{V_0}{Am}, \quad (9.2)$$

The values for R are listed in Table 9.1.

9.2.5 16S rRNA Gene Clone Library Construction and Phylogenetic Analysis

The 16S rRNA gene analysis was performed as described (Kato et al. 2009a, b). Partial 16S rRNA genes in extracted genomic DNA were amplified by PCR with the prokaryote-universal primer set, Uni515F and Uni1406R (Kato et al. 2009a). The PCR products were cloned and the nucleotide sequences of randomly selected clones were determined. Nucleotide sequences were aligned using ClustalW 2.0.12 (Larkin et al. 2007). Sequences with at least 97 % similarity according to DOTUR (Schloss and Handelsman 2005) were treated as the same phylotype. Maximum-likelihood (ML) trees were constructed using PHYML (Guindon and Gascuel 2003). Bootstrap values were calculated using 100 replicates.

Table 9.1 Rates of dissolution of Cu and Zn from the SMS slabs and of removal of P from the ASW of the samples containing both microbes and slabs (MC1, 2) microbes, and only slabs (C1, 2)

Sample ID	Dissolution rate (mol m ⁻² s ⁻¹)		
	Cu	P	Zn
MC1	2.4.E-16	-2.0.E-14	1.9.E-16
MC2	1.7.E-16	-2.2.E-14	2.3.E-16
C1	2.5.E-16	-1.3.E-14	1.3.E-16
C2	1.9.E-16	-1.3.E-14	1.0.E-16

9.2.6 Fluorescence Microscopy

Microbes on the filters and slabs were stained with SYBR Green I. Microscope images were recorded using a fluorescence microscope BX60 (Olympus, Tokyo, Japan) and a cooled CCD camera (Penguin 600CL, Pixera, San Jose, CA, USA). At least 30 fields of the images of each filter and slab were used for cell counting.

9.2.7 Accession Numbers

Sequence data were submitted to the DDBJ database under the accession numbers AB600466 to AB600509 for the 16S rRNA genes recovered from the inoculum, and AB600510 to AB600530 for the 16S rRNA genes recovered at the end of the experiment.

9.3 Results and Discussion

9.3.1 Concentrations and Release/Removal Rates of Elements to/from the ASW Samples

The initial pH values of the incubations were between 8.2 and 8.0. They decreased to 7.6–7.4 by 332 h and finally to 7.3 (Fig. 9.1a) after 1,698 h. The pH drop may have resulted from dissolution of CO₂ from the air because the drop was observed in all samples including N. The change in the concentrations of each element differed during the 4–332-h and 332–1,698-h periods; Fig. 9.1b–d, see also Suppl. 9.3). A dC'/dt value for each element was calculated for the 332–1,698-h period when the pH of the systems remained between 7.3 and 7.5 (Fig. 9.1a). Results of the calculation are shown in Suppl. 9.2.

Leaching of Cu and Zn, which were relatively abundant in the slabs (see above), was observed in the presence and absence of the microbes (Fig. 9.1b, d). In particular, R for Zn dissolution was greater (1.4–2.3 folds) when microbes were present (Table 9.1). The dC'/dt values for Zn were similar for each pair of duplicated samples, i.e., MC1 and MC2, and C1

and C2 (Fig. 9.1d and Suppl. 9.2). In contrast, no differences for the R values of Cu associated with the MC1 and MC2, and C1 and C2 samples were observed.

When SMS slabs were present in a reaction system, P was removed even if microbes were not (Fig. 9.1c). P was also removed in the presence of microbes when slabs were absent (Fig. 9.1c). The dC'/dt absolute values for P were greater when only slabs were present (C1 and C2) than when only microbes were present (M1 and M2; Fig. 9.1c, Suppl. 9.2), indicating that the rate of precipitation or adsorption of P onto the sulfide slabs was faster than the rate of uptake by the microbes. dC'/dt for P in the systems that included slabs and microbes (MC1 and MC2) were corresponding to the sums of the values for M1 or M2 and C1 or C2 (Suppl. 9.2).

9.3.2 Microbial Communities

The temporal changes in cell densities are shown in Fig. 9.1e, with decreases seen in the MC1, MC2, M1 and M2 systems to 10⁴–10⁵ cells mL⁻¹ at 140 h and increases up to 10⁶–10⁷ cells mL⁻¹ by 1,698 h. The cell densities in the C1, C2 and N systems could not be reliably counted by microscopy (<10³ cells mL⁻¹). Cell colonies were observed on the MC1 and MC2 slabs at the end of the experiment (6.92 ± 4.55 and 5.76 ± 3.28 × 10⁵ cells cm⁻², respectively; Suppl. 9.4).

16S rRNA gene clone libraries were constructed from the genomic DNA extracted from the ASW samples and SMS slabs at the end of the experiment. The Inoc library is that from microbes in the original inoculum. The MC1lq, MC2lq, M1lq and M2lq libraries are from microbes in the MC1, MC2, M1 and M2 ASW samples, respectively. The MC1cp and MC2cp libraries are from microbes on the MC1 and MC2 slabs, respectively. The total numbers of the analyzed clones are 91, 45, 46, 47, 48, 20 and 20 for Inoc, MC1lq, MC1cp, MC2cp, MC2lq, M1lq and M2lq, respectively. 16S rRNA genes were not detected by PCR from the ASW samples and slabs from the C1 and C2 systems or from the ASW of the N system.

The clones in the Inoc library were affiliated with the following taxonomic groups (Fig. 9.2 and Suppl. 9.5): *Actinobacteria* (5.4 % of the total number of clones), *Bacteroidetes* (2.2 %), *Chloroflexi* (1.1 %), *Planctomycetes* (6.5 %), *Alphaproteobacteria* (16.3 %), *Deltaproteobacteria* (2.2 %), *Gammaproteobacteria* (63.0 %), *Zetaproteobacteria* (1.1 %), *Verrucomicrobia* (1.1 %) and *Euryarchaeota* (1.1 %). Many of the phylotypes in the Inoc library are similar to uncultured environmental clones recovered from the sulfide chimneys of the inactive vents (Kato et al. 2010), from the oceanic basaltic rocks (Santelli et al. 2008) and marine sediments but are distantly related to known species with <95 % of 16S rRNA gene similarity. Phylotypes (the

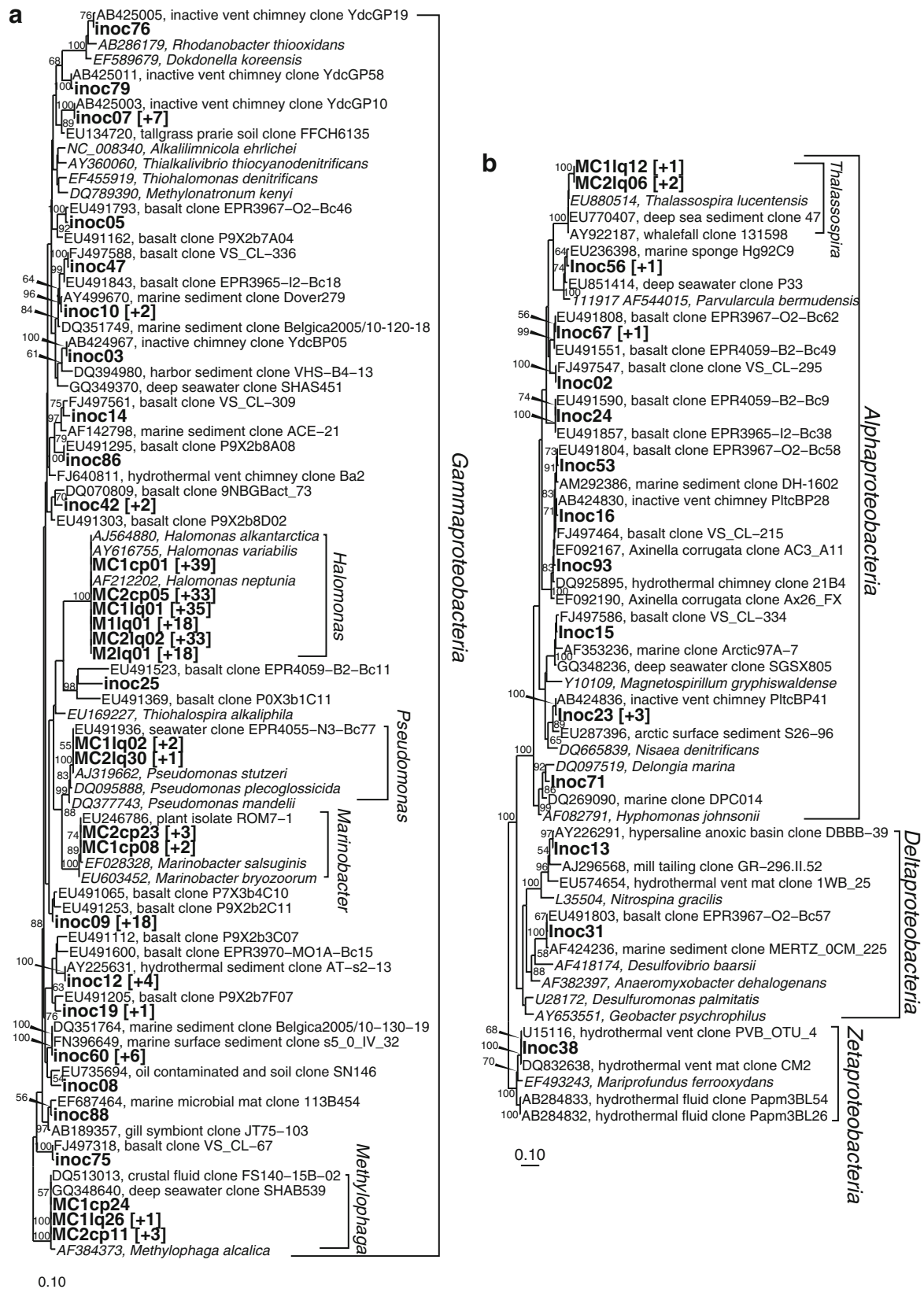


Fig. 9.2 Phylogenetic trees for *Proteobacteria*. The ML trees for (a) *Gammaproteobacteria* and (b) *Deltaproteobacteria*, *Alphaproteobacteria* and *Zetaproteobacteria* were inferred using 753 and 752 homologous positions in the alignment dataset, respectively. *Mariprofundus ferrooxydans* (EF493243) or *Halomonas aquamarina*

(AJ306888) were used as the out groups for each tree (data not shown). Bootstrap values (>50 %) based on 100 replicates are shown at the branch points. The scale bar represents 0.1 nucleotide substitutions per sequence position. The clone numbers are shown in bold type. The numbers that follow in brackets indicate the number of detected clones

Table 9.2 Number of PCR clones detected in the ASW samples and slabs at the end of the experiments

	Sample ID					
	MC1cp	MC1lq	MC2cp	MC2lq	M1lq	M2lq
<i>Bacteroidetes</i>						
<i>Sphingobacteria</i>						
<i>Algoriphagus</i>	1 (2.2)	3 (6.5)	5 (10.6)	9 (18.8)	1 (5.0)	1 (5.0)
<i>Proteobacteria</i>						
<i>Alphaproteobacteria</i>						
<i>Thalassospira</i>		2 (4.3)		3 (6.3)		
<i>Gammaproteobacteria</i>						
<i>Halomonas</i>	40 (88.9)	36 (78.3)	34 (72.3)	34 (70.8)	18 (90.0)	17 (85.0)
<i>Marinobacter</i>	2 (4.4)	2 (4.3)	3 (6.4)	1 (2.1)		
<i>Methylophaga</i>	1 (2.2)	2 (4.3)	4 (8.5)			2 (10.0)
<i>Pseudomonas</i>	1 (2.2)	1 (2.2)	1 (2.1)	1 (2.1)	1 (5.0)	
Total	45 (100)	46 (100)	47 (100)	48 (100)	20 (100)	20 (100)

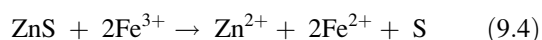
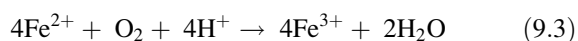
Numbers in parentheses are the percentage of the clones in each taxon

representative clones: inoc38 and 51) related to *Mariprofundus ferrooxydans* (95 % similarity) of *Zetaproteobacteria* and *Ferrimicrobium acidiphilum* (89 % similarity) of *Actinobacteria*, which are iron-oxidizing bacteria, were also detected in the Inoc library. In contrast to the great diversity of the microbial community in the original inoculum, only six phylotypes were detected at the end of the experiment (Table 9.2). These phylotypes were completely different from those in the Inoc library (Fig. 9.2 and Suppl. 9.5), probably because the original phylotypes had been out-competed during the incubation periods.

9.3.3 Microbial Effects on Chemical Interaction on Sulfide Deposits

The bacterial species, e.g., *Halomonas* and *Marinobacter*, found at the end of the experiment should be minor constituents of the microbial community of the in situ SMS deposits because they were not detected in the original inoculum. The species present at the end of the experiment were probably selected by the experimental conditions. These species may have influenced the release of Zn and removal of P. Remarkably, these species have been detected in SMS samples in various deep-sea fields (Edwards et al. 2003b; Kaye et al. 2010; Rogers et al. 2003), suggesting that they may play a role in the release of Zn and removal of P in situ.

Our results suggest that microbial action accelerated Zn dissolution from the SMS slabs. Iron-oxidizing bacteria convert Fe^{2+} to Fe^{3+} , such that Fe^{3+} can then react with sphalerite (ZnS) as oxidants to release Zn^{2+} (Fowler and Crundwell 1999) as follows:



In fact, the SMS samples contain sphalerite (Ikehata et al. Chap. 22). The genus *Marinobacter* includes an iron-oxidizing bacterium that has been isolated from an SMS sample (Edwards et al. 2003c). In addition, local acidification on the sulfide slabs by microbes might also have accelerated the dissolution of Zn in our systems, similar to that of silicate dissolution by *Arthrobacter* sp. (Liermann et al. 2000). The genus *Halomonas* includes acid-producing bacteria (Sanchez-Porro et al. 2010). Our 16S rRNA gene analysis indicates that the *Marinobacter* sp. and *Halomonas* sp. were attached to the slabs. Thus, the activity of these species likely contributed to the acceleration of the Zn dissolution from the sulfide slabs. In contrast to the dissolution of Zn, we did not observe a significantly accelerated Cu dissolution from the slabs.

P was removed from the ASW when microbes and/or slabs were present. Microbes use P as a nutrient. An increase in cell numbers with time was observed in the systems to which the inoculum had been added (Fig. 9.1e). Other reports have suggested that P is removed from seawater by adsorption onto the solid surfaces of sediments and rocks (Berner 1973; Wheat et al. 1996), which is consistent with our observation that P is removed from the systems containing only slabs (Fig. 9.1c). Notably, P was removed from the ASW more rapidly when only slabs were present, than when only microbes were present (Fig. 9.1c); however, rate of P removal from seawater on actual environments by SMS deposits and by microbes in situ should fluctuate according to the in situ physicochemical conditions and the degree of microbial activity.

9.3.4 Conclusion and Perspective

In the present study, we conducted batch experiments with and without the SMS samples and microbes at 4 °C and monitored certain chemical and microbial changes in the

reaction systems for 71 days. The dissolution rate of Zn from the SMS slabs was faster when microbes were present than in the absence of microbes, suggesting that the microbes accelerated Zn dissolution. Removal of P from the ASW was observed when microbes were present or absent and slabs were present. Our results should lead to a better understanding of the roles played by SMS deposits with microbes in the oceanic biogeochemical cycles.

Between 1 and 90 million-tons of the SMS deposits are estimated to be present in each hydrothermal field (Herzig and Hannington 1995). Given that the total amount of SMS deposits on the seafloor and their relative surface areas are approximately 100 million-tons and $6 \text{ m}^2 \text{ g}^{-1}$, respectively, the flux of P adsorbed onto SMS deposits can be calculated as $4 \times 10^8 \text{ mol year}^{-1}$ with the use of the averaged R value from Table 9.1 for the MC systems. This value is approximately one-hundredth of the input of P yearly into the oceans from rivers ($3 \times 10^{10} \text{ mol year}^{-1}$) (Elderfield and Schultz 1996), which suggests that adsorption of P by SMS deposits with microbes helps control the levels of oceanic P. Similar calculations suggest that the yearly release of Zn from the SMS deposits (approx. $\sim 2 \times 10^6 \text{ mol year}^{-1}$) should be much smaller than that from rivers (approx. $\sim 10^{10} \text{ mol year}^{-1}$) (Elderfield and Schultz 1996). Our estimates are rough approximations because the total amount of SMS deposits are probably underestimated and the necessary in situ microbial and physicochemical information has not been adequately delineated. Additional investigations are needed to more accurately calculate the fluxes of elements associated with SMS deposits.

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