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Microbial colonization and weathering of sulphide minerals at deep-sea hydrothermal vents: in situ exposure experiments

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Introduction

The abundant metal sulphide minerals present at deep-sea hydrothermal vents represent an important potential source of energy for chemolithotrophic microorganisms. While less energetically efficient than the oxidation of dissolved hydrogen sulphide, the oxidation of metal sulphides could potentially support chemosynthesis long after hydrothermal activity had ceased, even in well-buffered seawater (e.g. Eberhard et al., 1995; McCollom & Shock, 1997).

Newly formed sulphide deposits at deep-sea hydrothermal vents are invariably colonized by a variety of microorganisms, often forming extensive films and mats directly on the sulphide substrata (e.g. Karl, 1995; Taylor et al., 1999). In addition, sulphide minerals are subjected to oxidation upon contact with ambient seawater. They typically show micro-scale weathering features e.g. etch pits on mineral surfaces (Verati et al., 1999), large-scale orange-brownish coatings (especially on inactive chimneys) consisting mainly of secondary Fe-oxyhydroxide minerals, and evidence of talus and mass wasting (Scott, 1997). Despite these observations, the mechanisms involved in the weathering of seafloor sulphides and the role of microorganisms are still poorly understood.

To date, most geomicrobiological studies at deep-sea hydrothermal vents have focused on S-oxidizing or metal accumulating bacteria (Ehrlich, 1990; Juniper & Tebo, 1995). In their study of bacterial mats on sulphide deposits at the TAG and Snake Pit sites on the Mid-Atlantic Ridge, Eberhard et al. (1995) isolated obligate chemoautotrophic, non-acidophilic S-oxidizing bacteria that could oxidize sterilized sulphide minerals at near-neutral pH. They also assayed S-oxidation of sulphides by aerobic, mesophilic S-oxidizing bacteria via in situ shipboard and laboratory experiments. In all cases, substantial fixation of $^{14}CO_2$ occurred at near-neutral pH by natural populations of bacteria covering sulphide minerals. Avery et al. (2000) proposed that sulphide minerals at locations away from active venting sites along the Juan de Fuca Ridge were being used as a local energy source by S-oxidizing bacteria. They found a higher than normal density of prokaryotes on sulphide minerals with associated pits and irregular grain boundaries on the sulphide minerals.

While there is some evidence to suggest that hydrothermal sulphide deposits could potentially serve as localized, long-lived energy sources for chemolithoautotrophic microorganisms, the influence of this microbial activity on the weathering of these sulphides remains to be determined. To investigate the mechanisms of sulphide mineral weathering, and the possible role of microorganisms, we have taken a multifaceted approach that combines in situ and laboratory-based weathering experiments, along with examination of naturally-altered sulphide deposits. In this contribution, we present the first results from long-term, in situ sulphide exposure experiments on the Juan de Fuca Ridge.

Materials and methods

Natural hydrothermal chimney sulphides (mixed mineralogy) cut into disks of approximately 25 mm in diameter or blocks of ~ 20 mm x 30 mm, and pyrite cubes of approximately ~ 1 cm^3 (Ward's Scientific) were fixed onto aluminium SEM stubs with epoxy and screwed or tied down to plastic dishes. These dishes were in turn fixed to experimental arrays (galvanized steel), which were designed primarily for larval recruitment/colonization studies. Several arrays were deployed at various distances from active vent sites at Axial Volcano, Juan de Fuca Ridge; arrays were placed at Cloud Vent, a low-temperature vent (~10 °C) on the eastern part of the caldera and at ROPOS Vent, a slightly warmer vent (~30 °C) in the ASHES vent field, on the western part of the caldera. One array was recovered in the summer of 2001 after a short-term deployment, and 3 more were recovered in the summer of 2002 after 12 months of exposure. Details of these experiments are summarized in Table 1. Remaining experimental arrays will be recovered in the summer of 2003 after about 2 years of continuous exposure. Experimental arrays were placed in specially designed Lexan recovery boxes and transported to the surface by the ROV *Ropos*. Observations of the experimental arrays in place were done using a 3-chip CCD video camera and a 5 megapixel digital still camera on *Ropos*.

Sulphides from the weathering experiments were kept at 4 °C after collection. They were then rinsed with filtered distilled water, air-dried, and gold sputter coated (except for the pyrite cubes) for analysis with a Hitachi S-2300 scanning electron microscope (SEM), equipped with a Kevex energy dispersive X-ray analyser. The SEM was operated at 7 kV for optimal imaging or 20 kV for X-ray analysis. Identical control samples (unexposed) were also analysed by SEM for comparison with the exposed samples.

Results and Discussion

To our knowledge, this is the first report of results from long-term (12 months) exposure experiments at deep-sea hydrothermal vents using natural, massive sulphide mineral substrata. Guezennec et al. (1998) performed similar experiments in diffuse vent fluids (5-20 °C), but used artificial metal and plastic substrata, and experiments lasted 1-12 days. They found that, after as little as 4 days, rapid microbial colonization of all substrata had taken place. However, microbial biomass was found to be most elevated on stainless steal and titanium (i.e. inert) substrata. Lipid analyses revealed signatures of sulphate-reducing bacteria and filamentous sulphur-oxidizing bacteria. No filamentous forms were observed to colonize a control experiment placed outside the influence of the hydrothermal fluids. Similarly, Taylor et al. (1999) observed that an artificial titanium substrate was rapidly colonized by filamentous, H_2S oxidizing, mat-forming bacteria after only 2-11 days in 20-40 °C diffuse vent fluids. In similar exposure experiments using various sulphide minerals, Edwards & McCollom (2001) found that the abundance of iron-deposits that apparently form as the result of bacterial activity is correlated with mineral reactivity and porosity. They also found an abundance of iron-bacteria associated with certain minerals and they postulated that growth of these organisms is supported by mineral dissolution.

Unlike in previous studies, we did not observe any significant microbial colonization of sulphides in the short-term (Array K5) deployment. This may be more related to location and distance from vent, or vent fluid composition, than to time of exposure. In comparison, long-term deployments at the vent sites (e.g. Array L6) typically showed abundant colonization of all substrata by large, white, filamentous bacteria (Fig. 1). These filamentous bacteria, resemble the giant sulphide-oxidizers, such as *Beggiatoa spp.*, commonly found at hydrothermal vents (e.g. Nelson et al., 1989; Karl, 1995), and they appeared to unselectively colonize all substrata of the arrays. An array next to array E7 at Cloud Vent was partially covered by similar filamentous bacteria, however E7 did not show this abundant bacterial colonization, presumably because it was located further (~1 m) from the vent. The sulphides and plastic substrate holders on Array E7 did show a thin orange-red oxidized surface coating. At the periphery site (e.g. Array G9), little obvious colonization by bacteria was observed, but the pyrite cube and massive sulphides showed an orange-red oxidized surface coating (Table 1).

Scanning electron microscope observations confirmed the presence of large, filamentous bacteria on the substrata from Array L6. These were recognized as aggregates of spherical sulphur granules aligned in forms resembling filaments, both in size and shape (Fig. 2A). The presence of

Table 1. Details of seafloor sulphide exposure experiments and summary of observations.

Array	Location	Type of Substrate	Duration	Observations
K5 (short-term)	Near Cloud Vent	Single Pyrite Cube Massive Zn-Fe-sulphides	7 Days	No changes evident
E7	~ 1 m from Cloud Vent	Massive Zn-Fe-sulphides	12 months	Sulphides and sample holder show orange-red coating on surfaces; SEM shows small, Fe-Si encrusted bacteria
G9 (Periphery)	~8 m from Cloud Vent Massive sulphides	Single Pyrite Cube	12 months	Pyrite cube heavily oxidized; no obvious bacteria; massive sulphides show some oxidation on surface
L6	ROPOS Vent	Massive Zn-Fe-sulphides	12 months	All substrates heavily covered by white, filamentous bacteria; no oxidation evident; SEM shows abundant sulphur granules aligned as filamentous forms

Figure 1. Photograph of experimental arrays near Ropos vent, Axial Volcano, after deployment periods of several days (left) and 12 months (right). All surfaces of the array on the right are completely covered by giant, filamentous bacteria. The arrays are ~ 65 cm in width.

these sulphur granules, along with their location in the vent fluid, indicates that these filaments are the remains of *Beggiatoa*-like sulphur-oxidizing bacteria. Other cellular components were not preserved following the air-drying of samples. Disorganized aggregates of smaller, partially mineralized, filamentous forms were also present, though less common. In general, the sulphide surfaces were very heterogeneous, though no obvious alteration features could be detected when compared to the controls.

The presence of abundant, filamentous microbial biomass may have decreased available substratum surface, thus inhibiting colonization by other organisms, such as mineral sulphide oxidizers. These filamentous microbes may also have locally diminished dissolved oxygen, to a point where other organisms requiring oxygen for mineral sulphur and/or iron oxidation were unable to compete. The sulphides from this location showed no evidence of iron oxidation, either as a primary precipitate or as a product of sulphide mineral oxidation.

Array E7 showed a pervasive, though discontinuous Fe-rich surface coating of irregular thickness along with an abundance of much smaller filamentous forms than on array L6 (Fig. 2B). In some cases, the filaments do not appear to be mineralized or encrusted, though in other cases they appear to be partially, or completely covered by the Fe-rich coating. This coating also shows abundant desiccation cracks, suggesting that it was originally composed of hydrated oxyhydroxide minerals. These forms are morphologically similar to iron-oxidizing bacteria, such as *Leptothrix spp*., that are present in low-temperature iron-oxide deposits at hydrothermal vents (e.g. Emerson & Moyer, 2002; Kennedy et al., in press). In addition, small (~1 µm diameter) hollow, sheath-like structures are encrusted by a Fe-Si-rich precipitate (Fig. 2C). In general, pores in the sample contain more filaments than on the surface, suggesting that the Fe-rich surface coating may mask the presence of similar features.

The E7 array was likely too far (~1 m) from the influence of the hydrothermal vent opening to support an abundance of large, filamentous S-oxidizers. Instead, smaller, filamentous, putative Fe-oxidizing bacteria were most abundant. Similarly, the Fe-Si-mineral-encrusted tubes may represent mineralized sheaths of Fe-oxidizing bacteria. These organisms likely influenced the formation of the Fe-rich surface coating by oxidizing dissolved ferrous iron and nucleating Fe-oxide precipitation (e.g. Kennedy et al., in press). Since the plastic substratum holder was also covered by a similar coating, formation of the Fe-rich coating was not necessarily related to the sulphide substrate. Although iron-oxidizers are likely abundant at vents, and they likely oxidize reduced iron in vent fluids (Emerson & Moyer, 2002; Kennedy et al., in press), it is not clear if they can use mineral substrata for growth.

Array G9 (periphery) showed little evidence of colonization by S- or Fe-accumulating bacteria or any biomineralization. The pyrite cube showed much more extensive surface corrosion compared to the unexposed control pyrite (Fig. 2d). This corrosion consisted of ubiquitous, irregular depressions of approximately 1 µm in size. Although the corrosion (etch) pits on the pyrite surface are similar in size to bacteria (i.e. ~1 µm), such features can be produced on pyrite without bacterial influence (Edwards et al., 2001). In addition, a loosely-attached, Fe-rich coating of regular thickness (~5 µm) was found on all exposed surfaces of the cube (Fig. 2d). Unlike in array E7, this Fe-oxide coating was limited to the pyrite cube, and to a lesser extent the massive sulphide. This coating showed many desiccation cracks, reflecting its original hydrated nature (likely Fe-oxyhydroxides?). Spherical, Si-rich features (~5-20 µm in diameter) were also common throughout the sample. In some cases, larger, hollow tubes were surrounded by these spheres. Since Fe-oxidizers typically require microaerophilic conditions for optimal growth (Ehrlich, 1990; Emerson & Moyer, 2002), the peripheral experiment may have been placed in water that was too oxidizing for Fe-oxidizers to grow in. Such organisms are more likely to be found closer to vents where seawater and vent fluids mix (e.g. Array E7), or possibly within the pores of inactive sulphide deposits where geochemical gradients may be maintained.

Conclusion

These long-term, in situ exposure experiments provide us with fundamental temporal and spatial data on bacterial colonization of natural sulphide mineral substrata. These results can be used as baseline information for future experiments. They show that proximity to vent fluid and exposure time are likely key variables in submarine sulphide mineral weathering. An exposure time of one week does not lead to significant sulphide weathering, whereas exposure times of one year leads to significant oxidation of sulphide minerals, and can also lead to formation of Fe-oxide coatings on pyrite crystals. Proximity to hydrothermal vent fluids may also favour colonization of substrata by

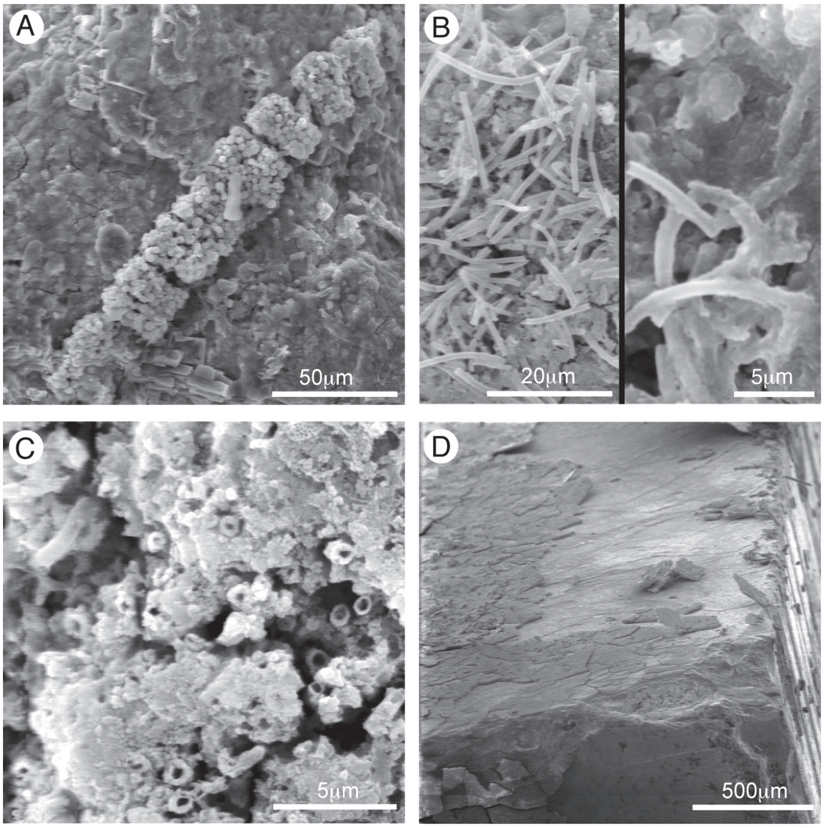

Figure 2. SEM photomicrographs of sulphide substrata from long-term exposure experiments. A. The remains of a giant, filamentous sulphur oxidizing bacteria (from Array L6). Intracellular sulphur granules are all that remain of this organism. X-ray analysis shows that the granules consist of nearly 100% S. B. Left Panel - Filamentous, tubular forms morphologically similar to iron-oxidizing bacteria like *Leptothrix* spp. from Array E7. Right Panel – Close-up of similar forms partially encrusted in a Fe-Si-rich surface coating (Both panels from Array E7). C. Fe-Si-mineral encrusted tubular forms similar to those in B, likely from a biofilm-like surface coating. The tubes may represent the mineralized sheaths of Fe-oxidizing bacteria (from Array E7). D. View of the surface of an oxidized pyrite cube. A thick (~5 µ) iron-oxide coating has developed on the pyrite surface as a result of dissolution and oxidation of the pyrite, as indicated by the abundant corrosion features (pitting) on the pyrite surface (from Array G9).

sulphide (H_2S) and iron oxidizers rather than microbes directly attacking the sulphide minerals. Therefore, caution should be used when preparing and performing such experiments since microbial processes such as iron oxidation and precipitation can be prevalent near hydrothermal vents.

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