Laboratory Investigation of the Role of Bacteria in the Weathering of Basalt Near Deep Sea Hydrothermal Vents

Christopher J. Daughney, Jean-Philippe Rioux, Danielle Fortin, and Thomas Pichler

The principal goal of this study was to assess the potential role of bacteria on the weathering of basalts near deep-sea hydrothermal vents (DSHVs). Natural basalt samples were collected from the vicinity of Axial Seamount on the Juan de Fuca plate during the New Millennium Observatory (NeMO) 2000 cruise and characterized by scanning electron microscopy (SEM) and X-ray diffractometry. Bacteria were isolated from the naturally weathered basalt samples and used in a laboratory batch experiment. The bacteria (identified as marine gamma proteobacteria SWAT4) and unweathered basalt fragments were placed in artificial seawater in the presence and absence of an organic carbon supplement (analogous abiotic control systems were also included). These systems were incubated in the dark (4°C, ambient pressure) for four months, and optical density, pH, Eh and concentrations of dissolved organic carbon, cations and anions were measured over time. Despite the limited data available (i.e., one system per set of chemical conditions) and the fact that contamination occurred in one abiotic system, we were still able to draw conclusions with respect to the role of bacteria in basalt weathering. The presence of the bacteria induced the release of Fe and Mn from the basalt relative to the abiotic controls, especially with the addition of the organic carbon supplement. At the conclusion of the experiment, the basalt fragments from the batch experiment were examined by SEM. The surfaces of the basalt fragments from the biotic systems, which were colonized by bacteria and showed evidence of secondary Fe-mineral precipitation, were very similar to the naturally weathered basalts. The results of this study therefore suggest that chemoorganotrophic bacteria are involved in the cycling of Fe and Mn and the weathering of basalt near DSHVs.

Keywords: bacteria, basalt, dissolution, hydrothermal vent, marine, mineral

INTRODUCTION

Deep sea hydrothermal vents (DSHVs) are chemically and ecologically unique ecosystems that play an important role in several global chemical budgets (Seyfried and Mottl 1995; Van Dover 2000, and references therein). DSHVs form near oceanic plate margins, where shallow magma chambers heat seawater that is convecting in hydrothermal cells in the ocean crust. The interaction between the seawater and the ocean crust creates hydrothermal brines that can reach temperatures as high as 350°C, and that are acidic, oxygen-poor, and rich in dissolved metals, such as Fe, Mn, and S (Van Dover 2000). DSHVs are the sites where these hydrothermal solutions emerge from the seafloor. Mixing of the hydrothermal solutions with the surrounding seawater creates strong chemical and temperature gradients. The resulting fluids become enriched in reduced chemical species which can be used by chemolithotrophic bacteria as a source of energy (Van Dover 2000). These chemolithotrophs are the base of the food chain in an ecosystem that also supports chemoorganotrophic microorganisms and diverse macrofauna.

The activity of bacteria can potentially influence the weathering of minerals in several ways (Erlich 1996). First, chemolithotrophic microbes may directly cause the dissolution of primary minerals and/or the precipitation of secondary minerals through enzyme-catalyzed oxidation or reduction (Lovley and Phillips 1988; Francis and Dodge 1990, 1991; Roden and Zachara 1996; Zachara et al. 1998). Second, chemolithotrophic or chemoorganotrophic microbes may indirectly solubilize minerals by producing ligands (e.g., organic acids, metabolites, siderophores, polysaccharides) that form complexes with...
mineral-forming ions, thereby causing ligand-promoted mineral dissolution (Francis and Dodge 1990, 1991; Barker and Banfield 1996; Ullman et al. 1996; Ransom et al. 1999; Kalinowski et al. 2000; Lierrmann et al. 2000; Welch and Banfield 2002). Third, bacterial surfaces may act as templates for the precipitation of secondary minerals, and potentially cause precipitation from undersaturated solutions (Ferris et al. 1986; Konhauser 1993; Fortin et al. 1997, 1998). Such bacterially catalyzed mineral dissolution and precipitation reactions have been observed in both the laboratory and the field.

Relatively few studies have focused on the role of bacteria in the weathering of basalts, particularly in marine environments at or near DSHVs. Thorseth and colleagues have investigated microbial alteration of basaltic glass, both as observed in the marine environment and in the laboratory (Torseth et al. 1991, 1992, 1995a, 1995b; Fisk et al. 1998). These studies strongly suggest that bacteria are able to colonize basaltic glass, resulting in the generation of pitted surface features and the mobilization of some elements relative to the parent material (changes in the chemical composition of the aqueous phase were not followed in these investigations). Fortin et al. (1998) showed that bacteria near DSHVs were coated with secondary Fe and Mn oxides and Fe silicates, although it was not clear if the bacteria played a direct ( enzymatic) or passive role in the formation of the precipitates (see also Juniper and Tebo 1995).

The principal objective of this study was to evaluate the potential role of bacteria in the weathering of basalts near DSHVs. The rate of reaction was not determined in the present study. Natural fresh (<2 years) and aged (2–10 years) basalt samples were collected near Axial Volcano, on the Juan de Fuca Plate, during the New Millennium Observatory (NeMO) 2000 cruise. The naturally weathered surfaces of the basalts were physically and mineralogically characterized, then fresh basalt fragments were weathered in artificial seawater (ambient pressure, 4°C) in a laboratory batch experiment, both in the presence and absence of bacteria isolated from the DSHV environment. Changes in solution chemistry were tracked during the laboratory experiment, and the mineralogy and texture of the laboratory-weathered basalts were investigated and compared to the naturally weathered samples.

MATERIALS AND METHODS

Location, Sample Collection, and Isolation of Bacteria

Two basalt samples were collected from the seafloor during the New Millennium Observatory (NeMO) 2000 cruise over the Juan de Fuca Plate, in the vicinity of Axial Seamount, approximately 600 km west of the Oregon coast (Figure 1). One basalt sample was collected at 42° 29’ 76’’ N, 50° 85’ 28” W, south of Coquille Vent, from a depth of 1533 m. This sample was formed during the most recent eruption of Axial Seamount (January, 1998; Embley et al. 1999), and is therefore referred to as “young basalt” for the remainder of this paper. The second basalt sample was collected at 42° 37’ 78’’ N, 50° 88’ 44” W, northeast of Castle Vent, from a depth of 1,523 m. This second sample formed in the second-most recent eruption of Axial Seamount, and is between 2 and 10 years old (Embley et al. 1999). For the remainder of this paper, this sample is referred to as “old basalt.”

Immediately after its collection, a small portion of the oxidized coating from the old basalt sample was removed and used to inoculate a basic marine growth medium. The medium was composed of 10.0 g/l tryptone peptone, 1.0 g/l yeast extract, 2.0 g/l MgSO₄·7H₂O, and 20 g/l NaCl in distilled deionized (DDI) water. The medium was adjusted to pH 7.0 and then autoclaved (121°C, 20 min). Once inoculated with the oxidized coating, the medium was incubated at ambient pressure at 4°C in the dark until visible growth was observed. The suspension was then used to inoculate a fresh, sterile volume of the marine medium, which, after 2 weeks of growth, was used in the laboratory batch experiment (see below). The inoculum used for the batch experiments was further characterized by DNA extraction, PCR amplification and sequencing of 16S rRNA (A.L. Reysenbach, Department of Biology, Portland State University).

Characterization of the Naturally Weathered Basalt Samples

The mineralogy and texture of the naturally weathered (young and old) basalt samples were examined using Scanning Electron Microscopy (SEM), Energy Dispersive Spectroscopy (EDS) and X-ray Diffraction (XRD). To facilitate these analyses, the young basalt sample was cut into small cubes. The old basalt was friable, and so was broken into pieces by hand.

For SEM analyses, small fragments of the samples were glued onto glass slides using epoxy. The mounted samples were
sputter-coated sequentially with carbon then Au/Pd using a Sputter Hummer 07-UII. Fresh fractures and polished surfaces of both the young and old basalt samples were examined using a JEOL JSM-6400 scanning electron microscope equipped with a Link eXLLZ4 X-ray analyzer.

The mineralogy of the basalt samples was characterized by XRD. Several small portions of the weathered surface were removed using a scalpel, then powdered using an agate mortar and pestle. The samples were placed into a Phillips X’pert diffractometer with a Cu source (operating at 45 kV and 40 mA) and a Kevex Si(Li) solid-state detector. All samples were run in step-scan mode, with steps of 0.05°, a step time of 2.0 s, and limiting angles of 4° and 90°.

**Laboratory Investigation of Basalt Weathering**

To assess the potential role played by bacteria in the weathering of basalt, a batch experiment was performed using 2 l flasks stoppered with cotton wool and closed with aluminum foil. Each flask (no replicate systems were set up) contained 250 ml sterile artificial seawater (see Table 1 for molar concentrations) and a small, sterilized piece of fresh (young) unweathered basalt (Table 2). Certain elements (Ca, Mn, Fe, Al) were omitted from the artificial seawater to facilitate their detection should they be released in small concentrations during the weathering of the basalt. All four systems were adjusted to pH 7.7 using 1 M HCl.

Two of the four flasks were inoculated with the bacterial consortium isolated from the old basalt sample (see above). To prepare the inoculum, a growing culture (9 ml) was transferred to 200 ml sterile marine broth. The culture was allowed to grow in the dark for five days at ambient pressure and 4°C. After this period of growth, the cells were removed from the growth medium by centrifugation (6,000 rpm, 15 min), then resuspended in 200 ml sterile 35 g/l NaCl. The centrifugation and resuspension were repeated two more times, to ensure that most of the growth medium was removed from the bacterial suspension. Finally, 20 ml of the washed bacterial suspension were used to inoculate two of the four flasks in the batch experiment. In addition, an organic carbon supplement (0.1 g/l tryptone peptone and 0.02 g/l yeast extract) was added to one of the two flasks that had been inoculated with the bacteria, and to one of the two flasks without bacteria. For the remainder of this paper, the four flasks in the batch experiment are referred to as systems A (abiotic system without organic carbon supplement), A + O (abiotic system with organic carbon supplement), B (biotic system without organic supplement), and B + O (biotic system with organic carbon supplement) (Table 2).

The four flasks were incubated in the dark at ambient pressure at 4°C for 4 months, with selected chemical and biological parameters monitored over time. Subsampling was performed every two weeks in order to assess the role played by bacteria on mineral weathering in each system. The experiment was not designed to determine weathering rates, but to measure and compare the quantity of metals released in each system. Each measurement (2 weeks apart) therefore represents a replicate measurement over the course of 4 months, because steady-state conditions were reached within the first weeks of the experiment (see next). The length of the experiment (4 months) was chosen to ensure microbial growth and colonization of the basalt surfaces. A 17-ml subsample was aseptically extracted from each flask at the start of the experiment, and again after every second week. Filtered (0.2 µm) portions (10 ml) of the subsamples were acidified (100 µl 10% OmniTrace nitric acid) and analyzed for dissolved metals (K, Ca, Mg, Sr, Mn, Fe, Al, Si) by ICP-OES (Actlabs, Ancaster, Ontario, Canada). Separate portions (3 ml) of the subsamples were filtered (0.2 µm) and acidified (30 µl 10% OmniTrace nitric acid) for analysis of dissolved organic carbon (DOC) with an O-I Analytical 1010 Total Organic Carbon Analyzer (using the persulfate oxidation method). A filtered (0.2 µm) but unacidified portion of each subsample was analyzed for dissolved SO$_4$ using a Hach DR-2010 spectrophotometer. Optical density (OD) was measured on unfiltered portions of the subsamples using quartz cells and a Beckman Du-65 spectrophotometer operating at 600 nm. The remaining portion of the subsample was analyzed for pH and E$_{H}$, pH measurements were made with a VWR Scientific 8005 meter and combination electrode calibrated using VWR pH standards (4.01 and 7.00)

<table>
<thead>
<tr>
<th>Substance</th>
<th>Concentration (mM)</th>
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<tbody>
<tr>
<td>Cl</td>
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<tr>
<td>Na</td>
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<tr>
<td>K</td>
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<tr>
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<tr>
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<tr>
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<tr>
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<tr>
<td>NO$_3$</td>
<td>0.183</td>
</tr>
<tr>
<td>Sr</td>
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<tr>
<td>Ba</td>
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<table>
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<td>1.7571</td>
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</tr>
<tr>
<td>A + O</td>
<td>3.8682</td>
<td>246:1</td>
</tr>
<tr>
<td>B</td>
<td>5.4053</td>
<td>176:1</td>
</tr>
<tr>
<td>B + O</td>
<td>3.3166</td>
<td>286:1</td>
</tr>
</tbody>
</table>
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at 4°C. Eh measurements were made with the VWR Scientific 8005 meter and a Corning W/RJ combination electrode tested using Zobell’s solution (Nordstrom 1977).

After the batch experiment had run for four months, the basalt fragments were removed and examined by SEM and XRD as described above, with the aim of comparing any weathering features produced in the lab to those observed on the natural (young and old) basalt samples. The computer speciation code PHREEQC (Parkhurst 1995) and the MINTEQA2 database (Allison et al. 1991) were used to assess the degree of mineral supersaturation for each experimental solution over the course of the experiment.

RESULTS

Characterization of the Naturally Weathered Basalt Samples

Both the young and old basalt samples displayed visible alteration rims. A cross section through the young basalt sample revealed a red-orange weathered surface (RWS-y) extending from the edge of the sample inward approximately 4 mm. The interior of the young basalt sample was also pervasively altered, albeit less intensely than the surface RWS-y. A cross section through the old basalt sample also displayed a red-orange weathered surface (RWS-o), approximately 10-mm thick. On some areas of the old basalt, a yellowish weathered surface (YWS-o) extended from the base of RWS-o a further 5–10 mm towards the center of the sample. Some areas of the old basalt also displayed a brown weathered surface (BWS-o). The center of the old basalt sample appeared largely unaltered.

SEM was used to examine the weathered and unweathered surfaces of the two naturally weathered basalt samples (Figure 2). The outermost weathered surface of the young basalt (RWS-y) displayed secondary precipitates with both linear and cubic morphologies overlying the matrix (Figure 2a). The outermost weathered surface on the old basalt (RWS-o) was characterized by elongated mineral precipitates and features interpreted to be rod-shaped bacteria (Figure 2b). The boundary between the brown weathered surface (BWS-o) and the unaltered portion of the old basalt was sharply demarcated, with elongated precipitates and bacterial cells in much greater concentration in BWS-o (Figure 2c). Freshly exposed surfaces of the young

![Figure 2. SEM images of the naturally weathered basalt samples. A) The outermost weathered surface on the young basalt (RWS-y). Precipitates with linear and cubic morphologies (P) are visible above the matrix (M). B) The outermost weathered surface on the old basalt (RWS-o). Rod-shaped bacteria (B) and elongated mineral precipitates (P) are visible above the matrix (M). C) The boundary between the unweathered matrix (M) and the brown weathered surface (BWS-o) on the old basalt sample. Elongated precipitates (P) and rod-shaped bacteria (B) are present in significant abundance on BWS-o, but are absent on the unweathered portion of the sample. D) Freshly exposed, unweathered surface of the young basalt shown by SEM and with corresponding EDS spectrum (Cl peak is an artifact introduced by drying the sample; the unidentified peaks correspond to Au and Pd from the sputter coating).](image)
basalt sample showed numerous pits and fissures, and a corresponding EDS spectrum indicated the presence of Na, Mg, Al, Si, Cl, Ca, and Fe (Figure 2d).

XRD analyses were conducted to identify the major mineral components of the two naturally weathered basalt samples (Figure 3). The unaltered, interior sections of both the young and old basalt samples were composed of diopside (CaMgSi$_2$O$_5$) and anorthite (CaAl$_2$Si$_2$O$_8$) with trace amounts of quartz (SiO$_2$). The outermost reddish weathered surface on the young basalt (RWS-y) was composed primarily of quartz, halite (NaCl, an artifact produced by drying the sample prior to analysis), lepidocrocite ($\delta$-FeOOH) and celadorite (K(Mg, Fe$^{2+}$, Fe$^{3+}$)(Fe$^{3+}$, Al)Si$_4$O$_{10}$(OH)$_2$). The outer reddish weathered surface on the old basalt (RWS-o) was composed of diopside, anorthite, halite, lepidocrocite, celadorite and gypsum (CaSO$_4$·2H$_2$O). The brown weathered surface on the old basalt (BWS-o) contained anorthite, lepidocrocite and nontronite (Na$_0$·Fe$^{3+}$·(Al, Si)$_4$O$_{10}$(OH)$_2$·nH$_2$O), and the yellowish weathered surface on the old basalt (YWS-o) was composed of quartz, lepidocrocite, halite, gypsum, and nontronite.

Analysis of 16S rRNA was used to identify the species present in the inoculum. All isolates (Four in total) were identified as Marine gamma proteobacteria SWAT4 (genbank # AF366024, minimum homology 99%), suggesting that the inoculum was in fact a pure culture. SWAT4 was originally isolated off La Jolla, California (Long and Azam 2001). It is important to mention here that the isolates used in the batch experiments might not be representative of the in situ populations present on the sea floor and that they might represent a small fraction of the consortia present on the basalt surface.

**Figure 3.** XRD results of the unaltered sections of the young basalt, the unaltered section of the old basalt, the red weathered surface on the young basalt (RWS-y), the red weathered surface on the old basalt (RWS-o), the yellowish weathered surface on the old basalt (YWS-o), and the brown weathered surface on the old basalt (BWS-o). (A: anorthite, D: diopside, C: celadorite, H: halite, L: lepidocrocite, Q: quartz, G: gypsum, N: nontronite).

**Laboratory Investigation of Basalt Weathering: Characterization of Basalt Samples**

SEM was used to examine the basalt fragments at the completion of the laboratory batch experiment (Figure 4). After four months of simulated weathering in the batch experiment, the surface of the basalt fragment from system A displayed numerous precipitates with irregular morphology, typically clustered around fissures and steps on the basalt surface (Figure 4a). An EDS spectrum of one cluster of precipitates indicated reduced concentrations of Ca, Fe and Si and increased Mg relative to the matrix of the unaltered basalt (see EDS spectra in Figures 4a and 2d). The surface of the basalt fragment from system A + O was not investigated using SEM or EDS, because OD measurements indicated that the system had become contaminated with an unknown microorganism (see below). After the conclusion of the batch experiment, the surface of the basalt fragment from system B also displayed irregular mineral precipitates, but in addition, it was colonized by abundant rod-shaped bacteria (Figure 4b). The distribution of both the particulates and the bacteria was nonuniform, with both being more concentrated near fissures and steps on the basalt surface. An EDS spectrum taken on a cluster of precipitates and bacteria indicated an increase Fe and a reduction in Ca and Al relative to the matrix of the unweathered basalt (see EDS spectra on
Figure 4. SEM images of the surface of the basalt fragments after the batch experiment. A) System A. Mineral precipitates with irregular forms (P) were evident. The EDS spectrum corresponds to an area of irregularly shaped mineral precipitates (nonidentified peaks correspond to Au and Pd (sputter coating)). B) System B. Mineral precipitates with irregular shapes (P) were evident, and the surface was colonized by rod-shaped bacteria (B). The EDS spectrum corresponds to one of the clusters of P and B (nonidentified peaks correspond to Au and Pd (sputter coating)). C) System B + O. Mineral precipitates with irregular shapes (P) and rod-shaped bacteria (B) were evident above the basalt matrix. The EDS spectrum corresponds to one of the clusters of P and B (nonidentified peaks correspond to Au and Pd (sputter coating) and Cl peak is an artefact introduced by drying the sample).

Figures 4b and 2d). Following the batch experiment, the surface of the basalt fragment from system B + O appeared very similar to that of system B, with abundant irregular particulates and bacteria present (Figure 4c). An EDS spectrum of a cluster of the precipitates and the bacteria indicated an increase in Fe and a reduction in Ca and Al relative to the matrix of the unweathered basalt (see EDS spectra on Figures 4c and 2d).

XRD could not be used to characterize the mineralogy of the basalt surfaces after the batch experiment, simply because
secondary minerals and weathering products were not present in sufficient quantities.

**Laboratory Investigation of Basalt Weathering: Chemistry of the Aqueous Phase**

Analyses of subsamples extracted during the batch experiment indicated that several parameters changed significantly over time, and behaved differently in the four synthetic systems, while other parameters remained essentially constant over time (Figure 5). OD in systems B and B + O increased from zero to 0.018 and 0.076, respectively, within the first two weeks, then declined gradually for the following 3 months. OD in system A was not monitored, but the solution remained visibly clear for the duration of the experiment. OD in system A + O remained low for the first half of the experiment, but then

![Figure 5](attachment:image.png)

**Figure 5.** Chemical and biological parameters monitored over time during the batch experiment.
began to increase. Because the increase in OD in system A + O was indicative of microbial growth (and therefore contamination), all measurements on this system were stopped after two months. Initial DOC concentrations for systems B and B + O were roughly equal (5.21 and 5.04 mg/l, respectively), and were slightly higher than for systems A and A + O (1.78 and 2.02 mg/l, respectively). In general, DOC concentrations changed very little for the first month, decreased significantly during the next two weeks, then increased gradually for the following two months. The pH of all four systems decreased during the first week of the experiment. Following the initial decrease, pH in systems B and B + O increased gradually for the remainder of the batch experiment, while the pH of systems A and A + O continued to decrease with time. The $E_{H}$ of all four systems increased from approximately 0.2 V to 0.5 V during the first month and remained essentially constant thereafter. The concentrations of several dissolved ions remained constant in all four systems for the duration of the experiment (K, Sr, Mg, Sr, Al, Si, SO$_4^{2-}$), while the concentrations of other ions changed significantly (Ca, Mn, Fe).

**DISCUSSION**

**Characterization of the Naturally Weathered Basalt Samples**

Both the young and old basalt samples displayed surface characteristics that were comparable to other similar samples previously described in the literature. Studies conducted by Torseth et al. (1995a, 1995b) indicated that bacteria had a high affinity for the surfaces of basaltic glass, and were attached to it via extracellular polymers. The glass was shown to be depleted in all cations except Al and Si, which had accumulated as secondary minerals in/on the bacteria and biofilms attached to the glass. An investigation of the Columbia River basalts (McKinley and Stevens 2000) showed that the weathered rock surfaces were covered with secondary iron oxyhydroxides and ferrous smectites intermingled with organic structures interpreted to be bacteria. A study of a naturally weathered amphibole syenite (Barker and Banfield 1996) revealed the development of an extensive surface biofilm composed of intact cells and extracellular polymers, and selective mobilization of elements from the primary minerals accompanied by the formation of secondary iron and aluminum phyllosilicates. In general, then, naturally weathered igneous rock surfaces are coated with microorganisms and their biopolymers, and these organic components are intimately associated with and secondary mineral phases. Such bacterial adhesion to mineral surfaces is driven, at least in part, by the search for nutrients, such as phosphate, as shown by Rogers et al. (1998). However, the co-occurrence of bacteria and secondary minerals does not provide unequivocal evidence that bacteria are directly responsible for either the dissolution and mobilization of elements from primary minerals, or the formation of secondary phases.

**Laboratory Investigation of Basalt Weathering: Characterization of Basalt Samples**

On the basis of SEM images, the basalt fragments taken from the biotic systems at the end of the experiment appeared to be very similar to the naturally weathered (young and old) basalts. Both the natural and the laboratory weathered basalts were colonized by bacteria, with the cells being concentrated around pits, fissures, and irregularities on the rock surfaces. In addition, both the natural and the laboratory-weathered basalts displayed secondary Fe-bearing mineral precipitates in intimate association with the bacteria. These observations are in agreement with previous investigations (Torseth et al. 1995a, 1995b; Barker and Banfield 1996; McKinley and Stevens 2000), which have shown that naturally weathered igneous rock surfaces are coated with intermixed microorganisms, their biopolymers, and secondary mineral phases.

The basalt fragments taken from the abiotic systems differed from the naturally weathered basalt samples in that bacteria were not present, and secondary minerals were composed primarily of Mg and not Fe. Although this is indicative of differences between the biotic and abiotic systems, these differences cannot be unequivocally and directly related to the presence of the bacteria. To assess the role of the bacteria in the weathering of the basalts, consideration of the chemistry of the aqueous phase is required.

**Laboratory Investigation of Basalt Weathering: Chemistry of the Aqueous Phase**

Some of the physical and chemical changes observed during the laboratory investigation of basalt weathering were due to abiotic processes, whereas other changes were directly related to the presence of the bacteria. This discussion considers the changes in OD, DOC, $E_{H}$, pH and dissolved ion concentrations that occurred during the batch experiment, and relates these changes to features developed on the basalt surfaces as evidenced by SEM and EDS.

The quantity of bacteria present in each system was reflected by the OD values over time. Both systems B and B + O had initial OD values greater than zero, as a result of the bacteria introduced with the inoculum. A slight increase in OD was observed after two weeks in system B, indicating that the bacterial population was increasing, even in the absence of a source of organic carbon. The subsequent gradual decline in OD in this system indicated that the cells were responding to the lack of dissolved organic carbon by dying off. The organic carbon initially present in system B + O permitted the bacterial population to increase more dramatically during the first 2 weeks of the experiment (relative to system B). As the organic carbon was consumed, the cells died off and the OD decreased. Bacteria did not grow in system A, as indicated by the visually clarity of the solution at the end of the experiment. However, an increase in OD in system A + O indicated that microorganisms had been introduced to the system some time in the first two months of the experiment.
As stated above, changes in the DOC concentration were likely caused by growth and decline of the bacterial population, and thus were related to changes in OD. It is however clear that OD measurements do not represent direct bacterial counts and should therefore be interpreted with caution. Systems B and B + O displayed nearly identical initial concentrations of DOC, even though an organic carbon supplement was not added to system B. Similarly, nearly identical initial DOC concentrations were observed in systems A and A + O. This suggested that the organic carbon supplements introduced to systems A + O and B + O did not represent a significant fraction of the total DOC. We surmise that the majority of the DOC initially present in systems B and B + O was introduced along with the bacterial inoculum, and although the composition of this DOC was not characterized in this study, it may have been some form bacterial exudate and/or metabolite.

Unlike the changes in DOC, changes in E_H were entirely abiotically controlled, as evidenced by the identical trends observed in all four experimental systems and their poor correlation to biomass. The E_H values were initially low due to the expulsion of dissolved oxygen from the solutions during autoclaving. The increase in E_H values during the first month of the experiment was due to the slow diffusion of atmospheric oxygen into the flasks, which were only sealed with cotton wool and aluminum foil, and were therefore permeable to oxygen. The E_H values therefore suggested that equilibrium with respect to atmospheric gases was obtained within about one month.

The changes in pH observed during the first month of the experiment were also due, in part, to infiltration of the atmosphere into the flasks. The initial pH values were relatively high due to the exclusion of dissolved carbon dioxide from the solutions during autoclaving. The pH decreased in all four systems as the carbon dioxide redissolved in the solutions. Indeed, a model simulation performed with the chemical speciation code PHREEQC and the MINTEQA2 database indicated that the artificial seawater, when isolated from the atmosphere, would have a pH of 9.4, whereas if in equilibrium with the atmosphere (at 4°C), it would have a pH of 8.3. These calculated values were in general agreement with the pH values observed in system A at time zero and after one month, respectively. After one month, the pH in system A + O was 8.1, suggesting that the organic carbon supplement had a slight effect on pH. The pH values in systems B and B + O were initially lower than those in the abiotic systems, suggesting that the bacteria provided a significant degree of acidity to these systems. The data indicated that pH-altering reactions continued to occur after equilibration with the atmosphere, and that different reactions were occurring in the biotic and abiotic systems.

The changes in pH observed after the first month were likely related to mineral dissolution or precipitation. The experimental solutions reached equilibration with respect to the atmosphere after the first month, as indicated by the plateaus in the E_H values, and thus any changes in pH that occurred thereafter were caused by different processes. Such pH changes were observed in all four systems. In systems A and A + O, the pH continued to decrease slowly over time for the duration of the experiment, whereas in systems B and B + O, the pH increased gradually with time. Many dissolution and precipitation reactions result in a gradual change in the pH of the solution phase, although the extent of the pH change depends on which minerals are dissolving, which (if any) secondary minerals are forming, and the kinetics of the reaction(s) (Langmuir 1997). Thus, although the pH changes observed in the latter portion of the batch experiment were generally consistent with mineral dissolution and/or precipitation, additional corroborating evidence was required to determine if and which minerals were actually forming or dissolving.

The observed pH changes, coupled with other experimental data, and supported by chemical speciation calculations, suggested that magnesite was precipitating in the abiotic systems. The chemical speciation code PHREEQC, coupled with the MINTEQA2 database, was used to assess the degree of mineral supersaturation in the four systems. At the beginning of the experiment, both systems A and A + O were oversaturated with respect to magnesite (MgCO_3). The precipitation of magnesite would have resulted in a gradual decrease in pH, as was observed in the abiotic systems. Indeed, a PHREEQC simulation indicated that the artificial seawater, if equilibrated with atmospheric CO_2 and magnesite at 4°C, would have a final pH of 7.9. This predicted pH value was in reasonable agreement with the observed value in system A at the end of the experiment. The simulation also indicated that only about 2% of the Mg in solution would precipitate during equilibration with magnesite, in agreement with the observed constancy of Mg concentrations in all systems (Figure 5). The increase in Mg on the surface of the basalt fragment from system A, relative to a fresh unweathered surface (compare Figures 2d and 4a), suggested that a Mg-bearing mineral was indeed forming in this system. The PHREEQC simulation also suggested that barite (BaSO_4), strontianite (SrCO_3), chalcedony or quartz (SiO_2), and talc (Mg_3Si_2O_5(OH)_2) may have precipitated in the abiotic systems, but it was not possible to confirm this with the chemical and EDS analyses available.

In contrast to the abiotic systems, the experimental data and chemical speciation calculations suggested that secondary magnesite was not precipitating in the biotic systems. Systems B and B + O had lower initial pH values than the abiotic systems, and based on PHREEQC models, they were not oversaturated with respect to magnesite. In addition, an increase in solid-phase Mg was not observed on the basalt fragments from systems B or B + O, supporting the hypothesis that Mg-bearing minerals were not forming in these systems. As in the abiotic systems, barite, strontianite, chalcedony, quartz and/or talc may have precipitated in systems B and B + O, but the data do not provide sufficient evidence to confirm this.

The precipitation of magnesite in the abiotic systems can be ascribed to the absence of bacteria. The abiotic systems had higher initial pH values than the biotic systems, which made the precipitation of magnesite possible. In the biotic systems, the bacteria produced organic acids, or acted as acids themselves,
lowering the initial pH values and preventing the formation of secondary Mg-hydroxides and -carbonates. This, however, should be considered an indirect effect, in that if a base were added to a biotic system to raise the pH to 9, precipitation of Mg-minerals would also likely occur.

The experimental data also suggested that primary Fe- and Mn-bearing minerals were dissolving in the biotic systems, but not in the abiotic system (system A) (no reference could be made with respect to the A + O system because it became contaminated). Although all four systems were initially undersaturated with respect to Fe- and Mn-bearing minerals, only in the biotic systems were concentrations of dissolved Fe and Mn observed to increase over time. The increase could be an artifact caused the lack of replicates or an experimental error. It is however unlikely, because the release of Fe and Mn in both biotic systems paralleled and peaked with the increase in OD, illustrating the relationship between bacterial population growth and mineral dissolution. The release of Fe and Mn by mineral dissolution is generally accompanied by an increase in pH (Drever 1988), as observed after the first month of the experiment in both biotic systems.

The enhanced rate of Fe and Mn release from the basalt observed in the biotic systems is in agreement with other studies showing that bacteria increase the rate of silicate mineral dissolution (Ullman et al. 1996; Kalinowski et al. 2000; Liermann et al. 2000). It is generally assumed that bacteria increase the rate of silicate mineral dissolution by production of organic compounds that act as metal chelators. However, from the experimental data collected in this study, it is not possible to determine if this was the case, or if the bacteria acted directly (enzymatically) to increase the rate of primary mineral dissolution. Our results were not in agreement with those of Welch and Banfield (2002), who reported a reduced rate of mineral dissolution in the presence of an acidophilic bacterium, which they attributed to surface adsorption of Fe$^{3+}$. It is not possible to calculate meaningful rates for the release of Fe and Mn in the batch experiment, because such rates are dependent on the reactive surface areas of the minerals, which were not determined in this study.

The EDS spectra and saturation calculations indicated that the release of Fe from the primary minerals in the basalt led to precipitation of secondary Fe-bearing minerals in the biotic systems. PHREEQC calculations showed that the solution in system B reached saturation with respect to Fe(OH)$_2$-Cl, goethite (α-FeOOH), lepidocrocite (β-FeOOH) schwertmannite (Fe$_8$O$_8$(OH)$_8$(SO$_4$)$_4$), and K-jarosite (KFey(SO$_4$)$_2$(OH)$_6$) by the second month of the experiment. By the same time, the solution in system B + O had reached saturation with respect to these same phases and, in addition, Na-jarosite (NaFe$_3$(SO$_4$)$_2$(OH)$_6$) and ferrihydrite (Fe(OH)$_3$). Fe concentrations in the abiotic systems remained too low to allow the precipitation of secondary Fe-bearing minerals. None of the experimental solutions reached saturation with respect to Mn- or Ca-bearing minerals. The PHREEQC saturation calculations were in agreement with the EDS spectra, which indicated an accumulation of Fe on the surfaces of the basalt fragments from systems B and B + O, relative to the unweathered material. Fe was not solubilized in the abiotic systems, and the EDS spectra of the basalt fragments from these systems did not show enhanced Fe concentrations.

CONCLUSIONS

Few studies have investigated the role of bacteria in the weathering of basalts by tracking the chemistry of the aqueous phase during the reaction, and by comparing the surface features developed to those of naturally weathered analogues. In this study, evaluation of the relationship between the quantity of bacteria present (as indicated by OD over time) and the values of the other monitored parameters permitted differentiation of chemical and biological reactions. Our experiment was however not designed to calculate weathering rates and our results can only be used to compare biotic and abiotic systems in terms of quantity of metals released from the surface of the basalt. Nonetheless, our results indicate that the weathering of basalts (i.e., the release of metals such as Fe, Mn, and Ca) was increased in the presence of bacteria under laboratory conditions. Our batch experiments also illustrated that basalt fragments weathered in the presence of bacteria developed similar surface features and mineralogies to naturally weathered basalts, whereas the basalt fragments weathered in sterile systems did not. These features were generated during the dissolution of primary minerals, accompanied by the solubilization of Fe and Mn, the colonization of the mineral surfaces by bacteria, and the precipitation of secondary Fe- and Mn-bearing minerals. It was not possible to determine if the development of these features in the biotic systems was the result of a direct metabolic process or an indirect (nonenzymatic) process. It was also not possible to determine if the basalt fragments in the sterile systems would have acquired features similar to the naturally weathered basalts if the experiment were of longer duration.

Caution should be exercised in the extension of the above conclusion to the natural near-DHSV environment. In particular, the laboratory weathering experiments were conducted with what was likely a single species of bacteria, isolated through its ability to grow quickly in the medium and under the culture conditions used here. In nature, the diversity of microorganisms is certainly be greater (Karl 1995), and as a result, a much greater variety of direct and indirect mechanisms may be responsible for basalt weathering. In addition, the effect of increased pressure was not investigated here. Although the effect of pressure on the solubility of the minerals is quantifiable, its effects on microbial processes, and in turn their effects on basalt weathering, are much less well understood.

REFERENCES


BASALT WEATHERING FROM BACTERIA NEAR HYDROTHERMAL VENTS


