Geochemistry of the Earth’s Surface meeting, GES-10

Microbial colonization of bare rocks: laboratory biofilm enhances mineral weathering

F. Seiffert\textsuperscript{a}, N. Bandow\textsuperscript{a}, J. Bouchez\textsuperscript{b}, F. von Blanckenburg\textsuperscript{c}, A. A. Gorbushina\textsuperscript{a}

\textsuperscript{a}Department of Materials and Environment, Federal Institute for Materials Research and Testing, Unter den Eichen 87, 12205 Berlin, Germany
\textsuperscript{b}Geochemistry and Cosmochemistry, Institut de Physique du Globe de Paris, 1 rue Jussieu, 75232 Paris 05, France
\textsuperscript{c}Earth Surface Geochemistry, Helmholtz Centre Potsdam GFZ German Research Centre for Geosciences, Telegrafenberg, 14473 Potsdam, Germany

Abstract

A laboratory biofilm consisting of the phototrophic cyanobacterium \textit{Nostoc punctiforme} ATCC 29133 and the rock-inhabiting ascomycete \textit{Knufia petricola} CBS 726.95 was tested for its mineral weathering potential. Minerals with different grain sizes and mineralogy were incubated with and without biofilm in batch and in flow-through column experiments. After incubation, the mineral dissolution was quantified analysing (i) leachate chemistry via ICP-OES/MS (inductively coupled plasma optical emission spectrometry/mass spectrometry) and (ii) the residual grains as thin polished sections via SEM/TEM-EDX (scanning electron microscopy/transmission electron microscopy-energy dispersive X-ray spectrometry). Mineral dissolution was enhanced in biotic experiments as compared to abiotic ones, for both batch culture and flow-through approaches. Analyses of thin polished sections confirmed the leaching of these elements near the surface of the mineral grains. These results clearly indicate a biotic effect on the weathering of minerals produced by the laboratory biofilm.

© 2014 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/3.0/).

Keywords: Biotic weathering; flow-through cell; albite; forsterite; olivine

1. Introduction

Mineral weathering is not only the first step of soil formation\textsuperscript{1} but can also promote the decay of cultural heritage\textsuperscript{2-5}.

*F. Seiffert, Tel: +0049-30-8104-4477, fax: +0049-30-8104-1407.
E-mail address: franz.seiffert@bam.de

*A.A. Gorbushina, Tel: +0049-30-8104-1400, fax: +0049-30-8104-1407.
E-mail address: anna.gorbushina@bam.de

1878-5220 © 2014 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/3.0/).
Peer-review under responsibility of the Scientific Committee of GES-10
doi:10.1016/j.proeps.2014.08.042
Soil formation is intrinsically connected with microbial colonization at the atmosphere-lithosphere interface. Life is ubiquitous on rock surfaces all around the world but the quantification of its influence on weathering is possible only in well-controlled and simplified laboratory models. While most of the studies dealing with microbial mineral dissolution so far focused on soil organisms\textsuperscript{6-8}, the weathering potential of primary colonizers on bare rocks is poorly understood. Due to the extreme conditions on such bare rocks only certain stress tolerant microorganisms can prevail initially in this ecological niche: phototrophic cyanobacteria and oligotrophic microcolonial fungi\textsuperscript{9-14}. In a previous study\textsuperscript{15} a laboratory rock biofilm consisting of the heterotrophic microcolonial fungus \textit{Knufia petricola} and the nitrogen-fixing cyanobacterium \textit{Nostoc punctiforme} was established. In the present work this biofilm was used as a model to study the biological impact on mineral weathering.

2. Methods

2.1 Minerals
Calcite (Klaus Lenz GmbH, Berlin, Germany), olivine (Mineraliengroßhandel Hausen GmbH, Telfs, Austria) and forsterite (produced in a microcrystalline form by mixing liquified MgO and SiO\textsubscript{2} in a melting furnace) were used for batch experiments. Albite (Rheinisches Mineralien-Kontor GmbH, Bonn, Germany) was used for weathering experiments in flow-through columns.

2.2 Microorganisms
\textit{Nostoc punctiforme} ATCC 29133 was obtained from Jack Meeks (University of California, USA), \textit{Knufia petricola} CBS 726.95 was isolated from a weathered marble monument in Athens (Greece).

2.3 Experimental setting
Batch experiments were done in cell culture flasks (Orange Scientific, Braine-l’Alleud, Belgium) containing a nutrient solution (0.1 \% glucose, 0.3 mM Na\textsubscript{2}SO\textsubscript{4}, 0.3 mM Na\textsubscript{2}HPO\textsubscript{4} and 10 \textmu M thiamine-hydrochloride in Milli-Q water) for 60 d, as triplicates. Mineral (calcite, forsterite, olivine) grain size ranged from 5 to 200 \textmu m and the flasks were inoculated with either (a) single cultures of \textit{K. petricola} (b) single cultures of \textit{N. punctiforme} (c) mixed cultures of both microorganisms and (d) nothing (abiotic controls). Additionally, some experiment were performed with Milli-Q water only instead of the nutrient solution, and some without minerals. The starting cell number for biotic experiments was $2.5 \times 10^{5}$ cells/ g mineral for each of both organisms.

Flow-through experiments were performed for 180 d in slightly modified percolation columns used in German standard DIN 19528\textsuperscript{16}. Briefly, columns were filled with 750 g albite grains (1-6 mm size) and a nutrient solution (0.1 \% glucose and 10 \textmu M thiamine-hydrochloride in MilliQ-water) was provided from the top using a peristaltic pump. For each column, one single container served both as reservoir for nutrient solution and as eluate collector, such that the experiments operated as closed systems (Fig. 1). Here we report results for a column not inoculated ("abiotic experiment") and for a column initially inoculated with $10^{5}$ cells per g mineral for each of both organisms.

All experiments were done in Persival climate chambers (Geneva Scientific, Fontana, USA) at 25°C and 90 \textmu moles photons of photosynthetically active light per m\textsuperscript{2} per s for 24 h/d.
2.4 Analysis
Analyses of the solutions were done by ICP-OES (Varian, Palo Alto, USA) or ICP-MS (ICAP-Q, ThermoScientific, Waltham, USA), analyses of polished mineral sections were done by SEM-EDX (XL30, Fei, Hillsboro, USA) or TEM-EDX at Helmholtz Zentrum Berlin. Cell numbers of the microorganisms were quantified via qPCR (quantitative polymerase chain reaction) with specific primer pairs.17,18

3. Results
3.1 Batch experiments with monominerals
For calcite experiments, no difference in Ca release was observed between the different types of biotic experiments, and between biotic and abiotic experiments. However, Ca release was higher in the presence of the nutrient solution than with Milli-Q water alone (as also observed in the olivine experiment). The relative dissolution of Mg from forsterite and olivine was increased in two types of biotic experiments (with mixed cultures and single cultures of *K. marina*) compared to the third type of biotic experiment (with single cultures of *Nostoc*) and to abiotic experiments (Fig. 2). For forsterite this increase was higher in the presence of mixed cultures. The pH values decreased in a similar way during all experiments (Table 1) with a starting pH of the nutrient solution at 7.8.
Cell numbers at the end of the experiments were always higher when mixed cultures had been inoculated. *Nostoc* cell numbers were very similar between experiments with different minerals and in control experiments without mineral. *Knufia* cell numbers were similar for samples without mineral or with forsterite and on a significant lower level for those with calcite or olivine (not shown).

![Fig. 2. Relative dissolution of Ca from calcite (left Y axis) and Mg from forsterite and olivine (right Y axis) as mass %.
Increased dissolution of Mg in forsterite and olivine is observed for biotic samples, Ca dissolution is similar for biotic and abiotic samples. The boxplots show the triplicate data per sample as cross lines and the mean values as squares. For the water controls of calcite and olivine only one data point was above the quantification limit. Measurements were done via ICP-OES.](image)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Calcite experiments</th>
<th>Forsterite experiments</th>
<th>Olivine experiments</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH before the experiment</td>
<td>8.8</td>
<td>8.4</td>
<td>8</td>
</tr>
<tr>
<td>pH after incubation with mixed culture</td>
<td>7</td>
<td>7</td>
<td>7.2</td>
</tr>
<tr>
<td>pH after incubation with single <em>Knufia</em> culture</td>
<td>7</td>
<td>7</td>
<td>7.1</td>
</tr>
<tr>
<td>pH after incubation with single <em>Nostoc</em> culture</td>
<td>7</td>
<td>7.1</td>
<td>7</td>
</tr>
<tr>
<td>pH after incubation with nutrient solution</td>
<td>7.2</td>
<td>7.2</td>
<td>6.9</td>
</tr>
<tr>
<td>pH after incubation with Millipore-water</td>
<td>7.3</td>
<td>7.1</td>
<td>7</td>
</tr>
</tbody>
</table>

### 3.2 Flow-through experiments with albite

Although SEM-EDX analyses showed only minor anorthite fractions in the albite grains, significant release of Ca (higher than that of Na, Fig. 3) was observed during the experiment. The final Ca concentration in solution was higher for the biotic experiment compared to the abiotic experiment. Ca leaching from the outer layers of feldspar
grains was confirmed via SEM-EDX measurements down to a depth of 5 µm (not shown). Na leaching was demonstrated to occur over depths of less than 2 µm by TEM-EDX (Fig. 4).

4. Discussion

4.1 Batch experiments with monominerals
As mineral dissolution was enhanced in the presence of Knufia cells or for mixed cultures but not with Nostoc only (Fig. 2), we infer that the presence of the fungus alone is sufficient for biotic enhancement of weathering. However, growth for both biofilm partners was increased in mixed cultures, which suggests a helping function of Nostoc cells through a symbiosis. The growth on minerals compared to that in nutrient solution only was not enhanced in the presence of olivine and calcite, suggesting that there was no benefit from mineral dissolution. Altogether, our observations suggest that weathering is enhanced through an indirect process resulting from the metabolism of the fungi. For example, production of organic acids or CO2 would lower the pH which is a possible factor in biodeterioration of minerals19,22. The fact that pH decreased in the same way in all experiments (Table 1) despite varying extent of dissolution (Fig. 2) is not contradictory with this explanation as pH values in the direct micro-environment of cells between a biofilm and its substrate can differ significantly from those in the macro-environment23. The lack of enhancement of calcite dissolution in the presence of micro-organisms might reflect that the solution in the vicinity of the minerals is close to saturation with respect to calcite for all experimental conditions.

4.2 Flow-through cell experiments with albite
Higher concentrations of Ca compared to Na in solution after 180 d flow-through column conditions for the albite with minor anorthite fractions imply a faster dissolution of Ca (Fig. 3, 4). Increasing dissolution rates with increasing anorthite fractions within plagioclases is known24,25. This is additionally confirmed through Ca leaching up to 5 µm into the substrate and Na leaching up to only less than 2 µm from the interface.

![Fig.3. Release of Ca (white) and Na (grey) from albite after 180 d incubation in the flow-through experiment with (right) and without (left) addition of a mixed culture of Knufia and Nostoc. Dissolved Ca concentrations are higher in the biotic experiments and compared to dissolved Na concentrations, despite the relative low anorthite-content in the used albite. Measurements were done via ICP-MS.](image-url)
5. Concluding remarks

The present work demonstrates that silicate dissolution is enhanced in the presence of biofilms under batch and flow-through conditions. This effect is most likely indirectly caused by the metabolism of Knufia alone, but the growth of Knufia in mixed cultures with Nostoc is enhanced through symbiotic mechanisms. Interestingly, the major nutrient Ca is preferentially released from Na-rich feldspar grains. The mechanism of the biodeteriorating process still has to be elucidated. Acidification in the micro-environment between biofilm and mineral surface is a hypothesis that can be tested via high-spatial resolution pH measurements.

Acknowledgements

This work was funded by the Federal Institute for Materials Research and Testing. We thank Ines Feldmann for her help with SEM-EDX measurements, Peter Schubert-Bischoff for TEM-EDX measurements and polished sections preparation, Katja Nordhauß for ICP-MS measurements as well as Dr. Jan Schüssler (GFZ Potsdam) for ICP-OES measurements. Furthermore we thank Dr. Ute Kalbe for providing the columns and know-how for the flow-through columns and Dr. Ralf Milke (FU Berlin) for forsterite production.

References


