

## ***Interactive comment on “Microbial colonization and alteration of basaltic glass” by J. Einen et al.***

### **Anonymous Referee #3**

Received and published: 15 June 2006

**GENERAL COMMENTS:** The goal of this manuscript is to observe natural microbial communities that colonize basalt glass at the seafloor and determine if they contribute to glass alteration. Previous studies suggest that microorganisms may play an important role in alteration of the ocean crust, but little is known about which communities are responsible. Unfortunately, the experimental design is flawed in that the microbial communities were allowed to incubate for an entire year prior to inoculation of the experiment. This is effectively creating an enrichment culture in which organisms capable of growing well under laboratory conditions were selected for. This preparation does not represent the true diversity of microorganisms at the seafloor and may account for the lack of initial phylogenetic diversity, in comparison to what would be expected based on molecular biological analyses of endolithic communities in natural basalt glasses (refer to Lysnes et al., 2003). The organisms that may thrive in this one year pre-incubation period may not at all represent organisms that are active and abundant at the seafloor.

Additionally, one of the major conclusions is that the microbes are not contributing to basalt alteration. This is based entirely on an observation of thickness (based on TEM analyses) of the alteration rind that forms on the glass surface. However, this alteration rind is never presented to the readers, and no mineralogical analyses were performed to determine if it truly is altered basalt glass or just something coating the surface such as a biofilm or minerals precipitating from seawater. For a result so pertinent to the conclusions of this study, I am perplexed as to why the results are not shown. Was there truly a systematic evaluation performed? Additionally, no geochemical analyses of elements relevant to basalt weathering were performed (especially Fe, Mn, Ca, Si, K, etc.) to determine if microbes are controlling the chemistry which could be relevant to alteration. The results of these experiments are not discussed as far as relevance to the experiment at hand or to the broader implications of these findings.

**SPECIFIC COMMENTS:** 1) Title: The title is somewhat misleading. Emphasis should be placed on the fact that this is a microcosm experiment. Also, the results suggest that microorganisms are not contributing to the alteration of basalt.

2) Abstract: (a) p. 274, lines 1-2. The association of microorganisms and weathered glass is an observation in these studies, there is no direct evidence for microorganisms contributing to the alteration process. (b) p. 274, lines 3-4. This sentence needs to be re-worded because it is combining two independent points: Why basalt weathering is important for microorganisms, and why basalt weathering is important geochemically. While iron is a biologically essential element, it is likely not the most vital reason why microbes colonize the substrate. Redox gradients that form from the reduced Fe, S, and Mn species in basalt and oxygenated seawater are more important. Also, this would be a good opportunity to state other element cycles that may be affected by basalt weathering (i.e. S, C, etc.). (c) TEM was also used to visualize glass alteration. (d) p. 274, lines 15-18. Avoid mixing family and genus names. Try to be consistent if at all possible.

3) Introduction: (a) The opening paragraph should grab the reader's attention as well as

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introduce the topic of the paper. The first several sentences do not do either, and there are some incorrect statements. Why are the experiments being performed? Maybe the first half of the second paragraph (p. 275, lines 18-24) should be included in the opening paragraph. (b) Glass is not only on the surface of pillow lavas, it is also a major component in the interior of most basaltic lava flows (pillows or sheet flows). The lack of crystallinity is probably far more important than surface area for dissolution kinetics (for example, clays have a considerable surface area but are not easily dissolved). (c) p. 275, line 11. The elemental composition of basalts is not identical, but similar, to basalt glass. (d) p. 275, lines 14-17. This sentence is confusing and poorly worded. What are the oxidized compounds and carbon sources? Clarify. Why are they important? Also, does glass need to be dissolved in order for microorganisms to utilize? I believe this is not understood currently. Stress the importance of redox conditions and reactions. This is more likely what is driving microbial communities on basalt surfaces. This is hinted at later on in p. 276, lines 1-2. (e). need references for Gallionella and Leptothrix. Also, Leptothrix has not been demonstrated to grow autotrophically. (f) p. 276, lines 91-20. Alteration of basalt glass was also followed with changes in colonization and experimental conditions. (g) The overall structure of the introduction is disjointed. It jumps around subject to subject. For example, in paragraph two, the authors discuss molecular techniques used to identify microbial communities on natural basalts in one sentence and microbial dissolution mechanisms in the very next sentence. This is a common problem throughout the introduction.

4) Materials and Methods: (a) Although the samples were handled aseptically once on board the vessel, they were not collected in this manner (because they are dredge samples). This contamination matter should be addressed somewhere in the paper, especially since some phototrophic bacteria are prevalent in the study. (b) p. 277, line 1, the samples were either crushed in a mortar or crushed with a mortar and pestle, rather than in a pestle. (c) p. 277, line 4, what do you mean by aged sterile seawater. How long was it aged, how was it sterilized, is there dissolved organic matter in the seawater? (d) p. 277, line 22, Was there a sterile control for each condition? For

example, was there an oxic methane amended control, an acetate amended control, an anoxic control, etc? This is unclear. (e). p. 277, line22-23, was there basalt chips included in the inoculum, or was it just fluid from the initial enrichment culture. If it was just unattached cells, then the experiment is likely further selecting for organisms that may not contribute to alteration. (f) p. 277, lines 24 and 26. What was the concentration of methane and argon in microcosms 1B and 3B respectively? (g). p 278, line 23, cell concentrations/densities were not actually recorded using SEM. (h) p. 279, line 15. I'm not positive, but I think the extraction kits are no longer called Bio101, rather fastDNA.

5) Results: (a) Why were cell densities on surfaces not measured, especially considering one goal of the experiment is to determine if microorganisms contribute to glass alteration? This could easily be done by fluorescent DNA staining and imaging with a confocal microscope. Descriptive terms of “highest” and “lowest” on p. 281, lines 10-13 do not give the reader a clear understanding. (b) the authors give the impression earlier that the 30 nm alteration rind was observed with TEM, but refer to SEM images. SEM should not be used for this purpose as it is not possible to determine if the “rim” is a result of basalt glass alteration or if it is a layer of mineral precipitates or biofilm. (c) The TEM results are never discussed here, nor are images of the TEM analyses ever shown. These analyses MUST be shown if the major conclusions that are drawn are based on these observations. (d) p. 281, line 25, How were the mineral precipitates identified as calcium carbonate? This is never addressed. (e) p. 282, line 27, The Rhodospirillaceae bacterium should be referenced and better described. For example, what is known about where it is isolated from, what are its metabolic capabilities, etc? This statement applies to other identified species throughout the manuscript.

6) Discussion: (a) Much of the discussion section should be moved to the results section. (b) The discussion section as written does not enhance the paper. There is very little interpretation of the results and what they suggest and how it contributes to the overall scientific understanding of basalt alteration and how microorganisms play a role in weathering. (c) Is the observed biomass production expected based on basalt

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weathering/bioenergetic calculations? Refer to Bach and Edwards, GCA (2003) paper. Calculations in the first paragraph on p. 286 suggest that cells are growing regardless of the basalt substrate. (d) Is 30 nm/year alteration consistent with what would be expected about known basalt weathering rates (S.R. Gislason and E.H. Oelkers have written many papers on this subject) (e) Why did diversity decrease? What is this the result of? Is it experimental design? (f) Why is there calcite precipitating out of solution? This is not discussed. It possibly suggests a problem with the experimental setup or conditions. (g) p. 287, line 13. Could there not be an abiotic explanation for the disappearance of methane? After one year of initial enrichment of microorganisms prior to inoculation for the experiments, it's more likely that any methanotrophs, if they initially existed, are no longer present. (h) What is significant about the trends of microorganisms observed with time? How does it relate to basalt weathering? (i) If the majority of microbes are free living, why should they contribute to alteration? This may be a better time to discuss microbial alteration mechanisms, rather than the introduction.

7) Conclusions: (a) No conclusions are made or even suggested about the patterns of microbes observed. (b) p. 290, lines 1-15. This should go into the discussion, rather than the conclusions. (c) There is no mention of the alteration thickness, which is one of the major findings. (d) p 289, line 1. What is meant by "same levels found in natural environments? Need clarification and references. (e) The overall conclusions do not

8) References: The majority of papers cited are from the authors' group. There is significantly more literature regarding microorganisms, ocean crust, and basalt alteration mechanisms. Adding more outside references could also contribute to the discussion section of the manuscript. Look for papers by: N. Banerjee, J. Huber, H. Staudigel, J. Cowen, C. Daughney, W. Bach, M. Fisk, S. Giovannoni and others previously mentioned in review.

## TECHNICAL CORRECTIONS

The use of the English language is OK, but there are numerous grammatical errors

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such as misspelled words, incorrect sentence structure, and incorrect word choice. Also, many words are overused and border on monotony. For example, on p. 288 lines 11-20, the word “showed is used in four consecutive sentences. It is suggested that the authors seek a native English speaking reviewer prior to re-submission.

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