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Biogeosciences Discussions

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

J. Einen et al.

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The authors are thankful for the review by the anonymous referee #3. A revised manuscript is being prepared based on her/ his comments. Below are our comments to the different issues raised by the reviewer.

General comments:

The reviewer claims that the inoculum does not represent the true diversity of microorganisms on the seafloor, as the microbial community undoubtedly changes during sampling and storage, we agree. However, we did not have the facilities to start an experiment of this scale on board the ship, so we had to start the experiment onshore. Two options were left open, either to inoculate the samples immediately on return from the cruise, or the store the inoculum. When the basalts used for inoculum were stored on flasks filled with sterile seawater, there was probably an abrupt change in the community, as organisms with a high growth rate probably proliferated. In order to reduce this

"bottle effect", we decided to let the inoculum age for one year, so that the high growth rate specialist would not be so numerous when we inoculated the microcosm. Whether this was a flaw in the experimental set-up, is a matter of opinion. We don't claim that this experiment explains all what happens on the seafloor, but we believe that the results from this experiment will help understanding the processes of microorganisms / basalt interactions better.

The reviewers concerns about the TEM results are understandable, as the manuscript treat these issues too lightly. The manuscript will be rewritten with this in mind.

Specific comments:

Title: 1: The title will be changed to "Microbial colonization of seafloor lavas and its influence on basaltic glass degradation - an experimental approach"

Abstract: 2a: The abstract will be changed, which removes line 1-5. 2b: see above (2a) 2c: This will be corrected in the revised manuscript 2d: Line 22-29: Throughout the manuscript the assignment of 16S rRNA gene fragments to the different taxa is done conservatively. This leads to that some sequences which have low identity to 16S rRNA sequences from the nearest cultured organisms only is assigned to a specific family and not genus.

Introduction: 3a: Some changes will be made in the revised manuscript 3b: Glass is mostly found in the 1-2 cm outer rim of the pillow lava, this will be made clearer in the revised manuscript. 3c: Changed as suggested 3d: In order to clarify, line 14-17 will be changed to: "Electron acceptors such as O2, NO3-, SO42- are supplied with the seawater to microorganisms deep within the basaltic layer (D'Hondt et al. 2004). The basalts are reduced in comparison to the oxidized seawater and microorganisms may catalyze redox reaction between the two in order to gain energy." 3e: The introduction has been rearranged omitting any references to the two species. 3f: Its not entirely clear for us what the reviewer means. 3g: The introduction has been somewhat restructured in order to increase readability.

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Materials and methods: 4a: It is correct that phototrophs were prevalent in the microcosms, especially in the oxic microcosms. With exception for one sequence from an algae plastid, all were from the families Rhodospirillaceae and Rhodobacter. These sequences are probably derived from organisms capable of anoxic photosynthesis or heterotrophic growth under oxic environments, and not from organisms with oxygenic photosynthesis in the seawater column. An organism with a 16S rRNA gene clustering in the midst of the Rhodospirillaceae bacterium PH30 cluster, has later been isolated from other sources of seafloor basalt, and are able to grow heterotrophic under oxic conditions. 4b: Changed to “crushed in a mortar” 4c: Changed sentence to:” The crushed glass was transferred to 100 mL serum bottles filled with either oxic or anoxic seawater, previously aged for six months and then autoclaved.” The level of organic carbon in this seawater was unknown; it is collected at a depth of 50 meters, and aged in darkness in order to reduce the level of easily available nutrients. 4d: Changed sentence to: The five different microcosms (1B to 5B) were each set up in four replicas, and four parallel sterile controls.” 4e: In order to clarify the inoculation process the following sentences will be added to the manuscript: “The flasks with the inoculum were shaken to re-suspend small glass fragments. Then inoculum was withdrawn from the flask and introduced into microcosms using a syringe with a 0.5mm thick needle.” 4f: Microcosm 1B and 3B had 10 vol% CH4 and H2 respectively. This information will be added in the manuscript. 4g: Sentence changed to : In order to observe microbial colonization and cell morphology on glass surfaces, glass grains were dehydratedĚ” 4h: The reviewer is corrected, however the kit was called BIO101 when we used it for our analyses.

Results 5a: To enumerate cells on glass fragments is not easily done, by fluorescence microscopy due to a patchy distribution of cells. The relatively large irregular surfaces of glass grains also make such analysis difficult. The use of confocal microscopy would help to resolve some of the difficulties, but it would still be a problem on how to interpret the results, as many cells would be undetected attached to the “underside” of the glass grains. We have developed a quantitative PCR assay for determining bacteria

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and Archaea in basalts, and this would be a better method to use, however this was not in use when we started the experiments, and the small amount of glass 0.8g in each microcosm, was not enough for such an additional analysis. Regarding the sentences 10-13, page 281, we agree that they don't confer any clear information these sentences will be removed. 5b: We will implement the reviewers comment throughout the manuscript. 5c: TEM results will be described more thoroughly in the revised manuscript 5d: The precipitates were identified as calcium carbonated by use of energy dispersive spectroscopy. This will be explained in the revised manuscript. 5e: We agree, this will be done in the revised manuscript

Discussion 6a: We will restructure the discussion part, by rearranging sections and moving parts of the conclusions into the discussion, but we will not move any parts from discussion to results. 6b: Some new points are discussed in the discussion part of the manuscript. 6c: The biomass production in the microcosms could be explained without considering energy yielding reactions from basalt weathering, as stated. 6d: The glass alteration may have been described to vaguely in the original manuscript, but the alteration feature observed in our experiments is probably dissolved glass and precipitates of secondary minerals. It is thus impossible to compare the alteration feature observed in our experiments with dissolution experiments. This will however be discussed in the revised manuscript. 6e: The decrease in the DGGE pattern complexity is a response to the conditions in the different microcosms. The exact factors governing the community complexity is unknown and not discussed in this manuscript. 6f: It is difficult to say why calcite is precipitating out, one possibility is that Ca concentration is increasing due to glass dissolution; this will be discussed in the revised manuscript. 6g: We can not dismiss the theory that the decrease in CH4 concentration is due to abiotic reasons such as a leaky container. We have however checked the integrity of the containers prior to the experiments. The suggestion that any methanotrophs in the inoculum would have died during the one year storage, is something that we cannot argue against, as we have not been able to cultured methanotrophs from samples stored in this way or on basalts fresh from the sea. We have however cultured methanogens

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from samples stored in the same manner as the inoculums after three years of storage. 6h: The significance about the trends of the developing microbial community in the microcosms during the experiment lies in that as the community stabilizes the remaining/dominating organisms are those most likely to play an role in bacterial alteration of basalts, as they appear when most of the easily degradable carbon is used by fast growing organisms, the one left are those who are adapted to an oligotrophic environment. 6i: We have not said that the majority of the cells in the microcosms are free-living.

Conclusions: The conclusions will be completely rewritten in the revised manuscript.

References: Several new references are added in the revised manuscript.

Interactive comment on Biogeosciences Discuss., 3, 273, 2006.