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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 grateful for the thorough review by Professor Dr. J. Peckmann. A revised manuscript is being prepared based on his comments. Below are our comments to the different issues raised by the reviewer.

General comments:

The role of Archaea and fungi:

We have not analysed the microcosms for the presence of Archaea and Eukaryotes due to time restrictions. This being said, if any signs of fungi had been seen during the SEM analysis, primers for fungal 18S rRNA genes whould have been included in the study. As for Archaea, in an article submitted to Applied and Environmental Microbiology, we have estimated cell numbers and relative abundance of Bacteria and Archaea in Arctic seafloor basaltic glass, the same type of samples as used for inoculating our microcosms, and found that Bacteria accounts for 99.9% of the total prokaryotic com-

munity. Based on these facts we feel that failing to screen the microcosms for Archaea and Eukarya were not a serious flaw in our experiments. We agree that a reason for not including Archaea, as well references to experiments finding fungi and Archaea in basalts should be included. This is done in the revised manuscript.

The discussion part of the manuscript has been rearranged. The two first sections of the conclusions have been moved to the front of the discussion, and the discussion has been rewritten to make reading easier. Some additional points are also discussed.

We are thankful for the useful references given by Dr. Peckmann, and they are now used in the manuscript.

We changed the language throughout the manuscript, though without adding or removing any content, in order to make the manuscript easier to read.

Specific comments:

1: Changed title to “Microbial colonization of basaltic glass- an experimental approach”

2: Changed as suggested

3: In line 2-3, I did not mean to imply that lithotrophic metabolism is responsible for alteration of the basaltic glass, although I believe it to be important. I meant that the release of Fe and other biological important elements during the process of alteration, whether biotic or abiotic, is important for the microbial community in basaltic glass. This section of the abstract is however removed in the revised manuscript.

4: Changed as suggested

5: Changed to:” bladed aggregates”

6: Changed to:” dendric”

7: We will be more precise in description of bacterial metabolisms, throughout the manuscript. The term oligotrophic confers the information that the organisms can grow

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with very little organic carbon present in the environment, which is an important element in the understanding of the results from our experiments. In line 22-28 we will restructure the sentences and remove specific references to metabolisms in order to keep the abstract easily understandable. The section now reads: “The family Rhodospirillacae, the family Hyphomicrobiaceae; the genus Rhizobium; and the genus Sphingomonas. Although no bioalteration of glass could be confirmed from our experiments, oligotrophic surface adhering bacteria such as the Sphingomonas sp. and Hyphomicrobium sp. may nevertheless be important for bioalteration in nature”

8: The two references are cited as suggested, also a sentence about the relative abundance of Bacteria and Archaea in artic seafloor basalts are added to clarify why we focused on Bacteria. See general comments. The new section will be changed to: “Culture independent molecular phylogenetic techniques have also proved that basaltic glass is colonized by a diverse and unique microflora, consisting mainly of Bacteria (2, 4). Quantitative PCR experiments suggest that more than 99.9% of the prokaryotic community in artic seafloor basalts is from the domain Bacteria (Einen et al. in preparation). When deep (1300-1400m) Hawaiian terrestrial basalts were analyzed, Archaea was found closely associated to alteration features (1). Even fossilized fungi have been found in seafloor within carbonate filled glass vesicles in subseafloor basalt (3).”

9: The glass was crushed into pieces ranging from dust to pieces small enough to be put into the 100mL serum bottles (Ø 1.4 cm). The microcosms were inoculated with samples through a syringe needle with diameter of 0.5mm. To clarify the process of inoculation, the following sentence will be added in line 26:” The inoculum was introduced into all microcosms using a syringe with a 0.5mm thick needle”

10: We agree, but this equipment was only recently available for our use.

11: We did not consider the presence of Archaea, see general comments.

12: Changed as suggested

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13: Changed as suggested

14: Sentence in Line 26 will be changed to: “No band with this position was observed in the inoculum, but appeared after tree months of incubation and became increasingly dominant during the experiment.”

15: Changed to:” The most diverse DGGE profile in 3B was observed.”

16: We will substitute “three” for “six” in line 19. The new sentence will read: “After six months of incubation, rods and prosthecate organisms”

17-18: Changed the section to: “Calcium carbonate precipitations showed bladed aggregates and columnar and dendritic habits in the microcosms as well as in the sterile controls. As a consequence, the crystal habit was not diagnostic to test whether or not microbial metabolism contributed to secondary mineral formation. Bladed aggregates and columnar crystal shapes were observed during the whole experimental run, whereas the dendritic calcium carbonate precipitations appeared after three months and disappeared after six months, showing that these dendritic precipitations under the experimental conditions will go in solution or re-crystallize to more stable forms by the time.”

19: There is a possibility that anaerobic environments developed in the CH4 amended microcosms. We however don’t think that this happened as the corks of the microcosms were open to the 5L glass vessel filled with air and CH4. We especially doubt that reduced conditions and thus reverse methanogenesis could occur. We didn’t not find any know sulphur, nitrate or iron reducing bacterial counterpart for anaerobe methane oxidation in this microcosms, but this don’t disprove the possibility for a archaea counterpart, or that the whole process occurred in the methanogen (going in reverse).

20: Changed as suggested

21: Not changed, these structures are abiotic as they were found in the sterile controls.

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22: Changed as suggested

Technical corrections:

1: As a consequence of other corrections (Specific comments comment 3), this correction is no longer applicable.

2: Changed as suggested

3: Changed as suggested

4: Changed as suggested

5: seawater changed to sea water and seafloor to sea-floor

6: Sentence changed to “Among the samples there were both freshly erupted, glassy black glass and older, oxidized, rust coloured glass.” for better readability.

7: Changed was to were

8: Changed was to were

9: Changed was to were

10: Changed were to was

11: Changed as suggested

12: Changed as suggested

13: Changed as suggested

14: Changed as suggested

15: Rephrased the section to “Bands with the same vertical migration (Band 44, 50, 53, and 55) were observed in the DGGE profile of microcosm 4B after three, six, nine and twelve months of incubation. Sequence analysis of these bands showed that they contained DNA sequences similar to the Sphingomonas sp. sequence found in 3B. At

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the end of the experiment the Sphingomonas sp band dominated 4B DGGE profile."

16: Changed as suggested

17: Changed as suggested

18: Removed reference to the asterisk in the text, also in line 14

19: Changed as suggested

20: Changed as suggested

21: Changed as suggested

22: Changed as suggested

23: Changed as suggested

24: Rephrased section to:" Clustering analysis based on DGGE banding pattern, grouped samples from 3B together with 4B and 5B, this indicates that H2 amendment had little impact on the microcosm. The DGGE analysis showed a band emerging after three months which prevailed throughout the experiment. Sequencing results revealed that this band belonged to an organism with phylogenetic affiliation to Sphingomonas sp., an obligate aerobic heterotrophic rod-shaped bacterium (Yabuuchi 1990) often with extensive biofilm production. Organism with this morphology, although with few signs of extra-cellular material, dominated the glass surface in the end of the experiment. The DNA and SEM based results indicates that microcosm 3B, 12 months after incubation, was dominated by an organisms belonging to the genus Sphingomonas. The fact that all known species in the genus Sphingomonas are an obligate aerobic organisms, suggest that the microcosms were in fact microaerophilic rather then anoxic." for better readability.

25: Changed in correction 24

26: Changed section to: Many of the DNA sequence obtained from this microcosm,

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affiliated with the order Rhizobiales, an order containing many N2 fixating organisms."

27: Changed section to:" Several of the bands, including the dominating in the 12 month DGGE profile showed phylogenetic affiliation to the genera Hyphomicrobium."

28: Changed as suggested

29: Changed as suggested

30: Changed as suggested

31: Changed as suggested

32: Changed as suggested

33: Changed as suggested

34: Changed as suggested

35: Changed to:" bacterially formed pit marks"

36: Corrected

37: Corrected

38: Changed as suggested

39: Changed as suggested

40: Changed as suggested, Xe also substituted with X

41: Note for editor

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