

Interactive comment on “Archive of bacterial community in anhydrite crystals from a deep-sea basin provides evidence of past oil-spilling in a benthic environment in the Red Sea” by Yong Wang et al.

Yong Wang et al.

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Reviewer 2 The manuscript submitted by Wang et al. describes the diversity of organisms in anhydrite crystals and further states a high abundance of *Alcanivorax* microbes. The authors see the specific community composition of the 750 years old crystals as indicator of an oxic oil-spilling benthic environment. Overall, the approach and results are highly interesting and definitely worth reporting in Biogeosciences. I, however, suggest revising and clarifying several parts of the manuscript before final publication. General remarks: While I do not doubt the results of the metagenomic investigation per se, I am

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honestly wondering what the meaning of the quantification of microbes based on traces of DNA is and how you could find intact cells being preserved for 750 years in crystals, which are still traceable using FISH. Response: Since 2009, we have been working on this sediment layers for several years to answer the question regarding the elevated organic carbons in the ABS183 layer. The hypothesis is the preservation of cells in the crystals as we also revealed eukaryotic and archaeal sequences from metagenome. It was very surprising for us to find intact microbes in the crystals. I am missing a detailed explanation and discussion on that topic as also for me the microscopic pictures are not entirely satisfying. Response: we have to apologize for the quality of the pics. We selected these pictures to illustrate the presence of the microbes from hundreds of pictures. Although the quality is still not satisfying for you, the result is almost our best for readers. As we can understand, these microbes had been preserved for hundreds of years. It's difficult to find out well-preserved and well-labeled microbes. Additionally I would like to see some proof of the assembled genome and a metabolic map. Further, the conclusion on past oil-spilling is a bit too loosely connected to the other parts of the story, both in the abstract and the results and discussion parts. This could be done by introducing geochemical data earlier and by rephrasing some of the respective text parts. The references are not in Copernicus style. Response: thanks for these comments. We have finished a new schematic map for the metabolism based on the gene profile. About the context of oil-spilling, we have rephrased the Abstract and inserted several lines in the Introduction (lines 139-157) to introduce the geochemical background in the oil-producing hydrothermal sediment. Specific comments: Abstract: First sentence should be rephrased as a hypothesis. Response: yes I.33: which binning tool? Replace separated by assembled Response: we assembled the reads and then conducted binning of the contigs for a draft genome. I. 35. Remove 'the' I.33-I.37: you are jumping back and forward between the metagenome and the cell identification Response: yes, these sentences are reorganized. I. 40 sentence is misplaced, here. Should probably be moved upwards. Response: yes I. 41-45: As written this seems to be highly speculative Response: yes, we have changed the tone. Introduction L. 54:

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Repetition of previous sentence. Response: yes , deleted. I. 57: I don't understand this sentence. Response: rephrased! I. 59: replace 'prediction' by 'interpretation' Response: yes I. 60: replace 'have been' by 'are' Response: yes I. 63: The previous sentence already started with 'although'- replace. Response: yes, used 'but' now. I. 64: I would like to see a reference for this. Response: yes, two refs inserted. I. 69: replace 'become' by 'may get' and add a reference for this statement. Response: yes. I. 79: the bodies are the biomarkers? Actually lipids, DNA or also pigments may be used as biomarkers. Not sure whether the word 'body' is really the right expression dead microbial cells. Response: yes, it was replaced with 'evidence'. Line 118 I. 81: similar to what? Response: changed. Line 120 I. 95: 'Hence' is contradictory to 'probably' Response: yes. I. 97: Which other markers? Why probably? This would actually be the place to explain the geochemical background. Response: yes, we inserted several lines for the introduction. I. 100-104. This belongs to the methods part Response: this part was simplified. Material and methods I. 110: A map of the sampling location would be beneficial. Response: we used a suppl figure S1. I. 138: Commonly, all abbreviations should be introduced when used for the first time. Response: yes. I. 146: this is a very low amount of DNA, how representative is this for community analysis? In how far is the quantification of OTUs and the genome identification trustworthy based on such low amounts of DNA? What about different degradation patterns of different organisms? Response: We did not use up all the extraction. In total, there was not 1ng of DNA, so we have to use the amplification. The DNA might be used for many amplifications actually. The kit may allow for unbiased amplification of all sorts of DNA. We have a publication to support the linear amplification of microbial genomic DNA. I. 150: Is this a random amplification? Response: the theoretical basis and our test support the random amplification for the trace DNA. I. 156: Which chemistry and protocol? Some statistics on the sequencing (how many reads per run, how many of the identifiable, how many reads related to 16S rDNA, etc.) would be desirable. Response: we should have put all the information. The sequencing was done in a service centre. We don't know how many sequences were produced in one run. Perhaps there were

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many samples in one runs. I. 169: How? Response: we inserted the command for this work (line2 239-240). I. 169: Based on the low amount of DNA and amplification steps in between, I doubt the quantitative aspects of the analysis. Response: we admit that this step is a brief statistics of the community. The abundance of the species could not be quantified in an accurate manner. I.175: remove gap after). Rstudio will not make any visualization without a prober script, so please provide details on your script. Also, provide the correct reference instead of the link to the homepage. Response: yes. We inserted a linkage for the R scripts. See lines 247-248. I. 181: It is unclear how the draft genome was assembled. What is the completeness? A genome plot would increase the credibility. Response: the assembly work was introduced at line 236. The assembly made the reads into long contigs. Then the binning process grouped the contigs according to their distinctive coverage levels and tetranucleotide frequencies. The completeness was assessed firstly by the number of single-copy genes. We have used Fig. S2 to illustrate the genomic alignment between Sk2 and ABS183. From the alignment, there are no notable gaps between the two genomes, indicating the high genomic completeness of the ABS183 strain. I. 207: replace 'examine' by 'validate' Response: yes I. 223 MQ- please spell Response: yes I doubt that FISH produces a reliable signal if you only get 20pg of DNA out of the crystals. Response: actually we obtained far more than 20pg of DNA. We concentrated the cells into a membrane, so that the microbes from many crystals were released and condensed on one membrane. Results I.232, 236, 238, 246, 249 : Check numbering of figures. Also, where do you refer to Fig. 5? Response: yes, there are mistakes in the figures. The figure 5 was described in line 364. I. 262: archaea, fungi without capital letters. Response: yes. I. 265: Why is that surprising? Response: deleted. I. 269 onwards: this needs a genome plot and a Kegg metabolic map. Response: we described the metabolic map of Figure 6. Lines 392-403. I. 278: I don't buy the quantitative aspect, here. Response: we deleted it. I. 346: postulate seems to be a bit strong for the line of evidence provided, here. Response: we used 'proposed' now. I. 346 ff: This information is what you need to put into the abstract to make it convincing Response: this is a nice comment. See

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the second sentence in the Abstract.

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