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Comment

Interactive comment on “Iron oxide deposits associated with the ectosymbiotic bacteria in the hydrothermal vent shrimp Rimicaris exoculata” by L. Corbari et al.

L. Corbari et al.

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We wish to thank the referee#1 for his objective and clear review as well as the detailed technical suggestions.

Reply to Referee #1

We thank the referee #1 for the positive general comments and reply to the specific comments point by point.

1-Referee comment: The overarching question for this work is the potential role of iron oxidising bacteria as an energy-source for the shrimp. A more comprehensive

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statement of the information that underlies this hypothesis is needed. Particularly, it is unclear why previous assumptions concerning sulfide-oxidizing bacteria should be reconsidered. This would allow the reader to better appreciate the originality of this model.

Author reply: Several studies (Wirsén et al. 1993, Polz & Cavanaugh 1995) suggested that the bacterial symbionts acquire energy from sulphide oxidation but it has never been confirmed experimentally. The assumptions about the presence of sulfo-oxidizing bacteria have been reconsidered because of the presence and abundance of iron oxides deposits and their close associations with bacteria. Finally, these minerals have been hypothesised to result from bacterial metabolism, suggesting, therefore, the presence of chemoautotrophic iron-oxidisers among the bacterial community (Zbinden et al. 2004). But it has never been dismissed that sulfo-oxidizing bacteria could be present within the bacterial community and we have rather hypothesized that iron oxidation could be the dominant bacterial metabolism. A recent paper from Zbinden et al. (2008) reveal from molecular analysis that three metabolic types (iron, sulphide and methane oxidation) may co-occur within the ectosymbiotic community associated with *Rimicaris exoculata*. This new insight on the diversity of the ectosymbiotic bacterial community will be included in our paper. We have added a new comment in the introduction.

2-Referee comment: Quantitative estimates of the contribution of inorganic compounds stabilizing the oxide structure are important as these ligands are expected to influence the surface properties of the oxide. As indicated in Table 3, they have been calculated under the assumption of most probable occurrence. More details should be provided on this assumption and on its relevance to hydrothermal conditions. Conditions of mineral formation in hydrothermal systems differ significantly from those of natural aquatic systems in equilibrium with the atmosphere. As an example, iron sulphides (FeS , FeS_2) are abundant in hydrothermal environments and may therefore be deposited in the branchial chamber. The authors did not consider these environmental contaminants as potential components of the crust. Rejecting this hypothesis should

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be more robustly constrained in the discussion. If this is not possible, the uncertainty arising from the possible occurrence of these minerals on the average composition of the oxide should be considered.

Author reply: The presentation of the ligands and of their most probable occurrence has been changed in the text and in table 3. The relevance of their most probable form is detailed in the results section and justified in the discussion according to the work of Châtellier et al. (2001, 2004). Iron sulphides are rare and clearly identified on BSE images as large crystals (2-10 μm) as previously shown by Zbinden et al. (2004). The Mössbauer analysis show that Fe³⁺ represents more than 98% of the Fe present in the mineral crust and suggest that iron sulphides (Fe²⁺) are negligible. Moreover they are not possible contaminants homogeneously distributed within the iron oxide concretions of the crust.

3-Referee comment: A main conclusion of this paper is that bacteria must participate to the oxide formation. As presented above, there are clear arguments in the manuscript to support this assumption. There is however some confusion in the discussion regarding this idea. The assertion that results indicate the biogenic origin of the iron formed (abstract) is in contradiction with the indication that both biotic and abiotic mineral particles coexist in the *R. exoculata* ectosymbiosis (p1839).

Author reply: Concerning the abstract, we agree with the referee. We have modified the sentence (P1827, ln15-16) as follows: "TEM-observations on the bacteria have revealed their close interactions with these minerals. Abiotic and biotic precipitation could occur within the gill chamber of *Rimicaris exoculata*, suggesting the biologically-mediated formation of the iron oxide deposits."; The discussion was modified to explain this idea (see also below).

4-Referee comment: In fact, the results are consistent with a bacterial influence but do not prove that all oxides in the branchial chamber are biogenic. As indicated by Fortin and Langley (2005), iron oxides formed in association with microorganisms dis-

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play physicochemical characteristics that are similar to their abiotic counterpart. 2I-Ferrihydrite is among the minerals formed by these microbes but cannot constitute a proof of biologically-mediated formation as it can also be produced in abiotic conditions (Majzlan et al. 2004 *Geochimica et Cosmochimica Acta*, Vol. 68, No. 5, pp. 1049-1059). The authors should acknowledge this more clearly in the discussion.

Author reply: As mentioned by the editor, it is very difficult to differentiate biogenic oxides from those formed as a result of abiotic reaction. Considering the formation of iron oxides, it is evident that they are biologically-mediated, at least initiated, because the first mineral particles occur in close contact with the bacterial cell walls. If iron oxidising bacteria are present as supposed by Zbinden et al (2008), Fe³⁺ oxides could be likely a by-product of the bacterial metabolism and thus be truly considered as biogenic. However the referee is right and we have to moderate some statements on ferrihydrite as a proof of the bacteriogenic origin of the mineral crust. We have considerably modified some statements on the biogenic origin of iron oxides, especially in the discussion. We suggest rather that this is an additional clue for understanding the bacterial influence on the iron oxide precipitation.

5-Referee comment: Furthermore, iron oxidizing microbes are known to compete with their own by-products as iron oxidation is autocatalytic. Rentz et al. 2007 *Environ. Sci. Technol.*, 41, 6084- 6089 have shown that iron oxides in bacterial mats are composed of both biogenic and abiotic products. In their review Fortin and Langley (2005) conclude: it remains very difficult to differentiate biogenic oxides from those formed as a result of abiotic reaction in natural samples containing neutrophilic iron-oxidisers. What is particularly lacking in this article is a more explicit mention of the potential heterogeneity of oxides in the crust and a discussion of the limits of the averaging approach used. This is particularly important to decipher whether the difference in Fe/O ratios results of an artefact due to preservation or simply reflect microscale variation in the oxide composition resulting of different formation processes. In conclusion, a more cautious discussion of the results with regard to the complexity of processes leading to

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the formation of bacteria-associated oxides should be proposed. Particularly, it would deserve a clearer definition of what is meant here by: biogenic origin.

Author reply: The definition of "biogenic iron oxides" has been now clearly exposed in the introduction part and referred to the definition of Fortin & Châtellier (2003): "the term biogenic iron oxides refer to iron oxides formed in the presence of bacteria. This general term includes iron oxides formed as a direct result of microbial activities (i.e. from enzymatic reactions) or of passive mechanisms whereby bacterial exudates trigger the formation and precipitation of iron oxides minerals". The introduction and discussion parts have been extensively modified.

Because of the similar comments of both referees, we propose to pool all our answers about the arguments on the biogenic origin of the iron oxides (Ferrihydrite, Fe/O ratio, influence of the ligands) in a general comment.

Referee comment: Specific comments p1827_line9: Longitude for the Rainbow site is missing. p1838_line7: U nor Ur p1838_line8: Remove: with p1838_line16: occur p1839_line14: missing verb? p1842_line 26: replace: element by compounds or molecule p1843_lines 13-16: Unclear. P1855_line 5 and table: Format for SO42-PO43-

Author reply: OK, all these specific comments have been corrected in the MS.

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