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.

Received and published: 1 August 2008

Manuscript Number bgd-2008-0022

Reply to Referee #2

We thank the referee #2 for the positive general comments and reply to the specific comments point by point.

1- Referee general comment: The subject fits well into the scope of Biogeoosciences and the methodology represents the state of the art. However, I find it rather surprising that no XRD or SAED was collected to identify the oxide. Could that possibly be handed in when revising the manuscript?
Author reply: XRD analyses and SAED have been performed on the minerals of the crust. However, the results do not show any diffraction pattern suggesting the amorphous or poorly-ordered state of iron oxides. The nanoparticulate organisation of Ferrihydrite concretions was confirmed by Mössbauer spectroscopy and TEM observations.

2- Referee comment: Furthermore, I propose to use the EDX results in a qualitative way only (see below) and to considerably revise the discussion of these data. I also differ about the importance of the fact that ferrihydrite was found here: In my opinion, neither the presence nor a certain Fe/O ratio of ferrihydrite point to its biogenic origin. Finally, I consider this paper to be an interesting contribution to the ongoing discussion about bio-induced Fe oxidation, but the main message could be told much more directly and precisely. This is why I recommend a clear reduction in text, possibly by combining the sections results and discussion.

Author reply: We roughly agree with the referee except for the use of the EDS quantitative data that are better explained in the results. Moreover, we prefer keep results and discussion apart. But the discussion has been considerably rewritten and we have tried to be clearer about the biologically-mediated origin of the iron oxides. All the results have not to be considered as proofs indicating the biogenic nature of the minerals but constitute several clues suggesting that ectosymbiotic bacteria influence the mineral precipitation within the branchial chamber of Rimicaris exoculata.

3- Referee comment: Specific comments Throughout the text the authors use "thin slices", I only know the term "thin section".

Author reply: We prefer to use thin slices (20-50 µm thick) for polished sections of mineral to avoid confusion with histological thin sections (5-20 µm thick), semi-thin sections (1-2 µm thick) and ultra-thin sections (80 nm thick). For thin slices, pieces of branchiostegite were dehydrated in an ethanol and propylene oxide series, and then embedded in epoxy resin (EpoFix, Struers) for geological specimens with a low Cl content. Polished-thin slices on glass slides were realised by abrasion on diamond
disks and finally mirror polished with a non-aqueous 1 µm diamond suspension (ESCIL, PS-1MIC). The polished-thin slices were surrounded by conductive silver paint and then carbon-coated in a Balzers BAF-400 rotative evaporator.

4- Referee comment: Figure 1, I would appreciate an additional picture of the whole animal. It is not clear to me how the dashed line in Figure 1a relates to 2b or the rest of paper. Besides, for 2b, I propose to draw in the three different layers, which are distinguished in this and the previous study.

Author reply: We don’t consider that a general view of the shrimp is required in this paper because it was extensively described as also the bacterial colonisation in previous papers (see references section: Casanova et al. (1993); Segonzac et al. (1993); Zbinden et al. (2004); Corbari et al. (2008)).

5-Referee comment: The term "mineral density" is used in a misleading way. What in fact is observed are differences in the aggregate density or the mutual dilution of mineral- and bacterial matter.

Author reply: The referee is right, we have change the term "mineral density" by aggregate density, throughout the text. Moreover, the term particle was used in a misleading way. That’s why we have used also the term of concretion to define the large aggregates of mineral particles.

6-Referee comment: P. 1827: Line 5 (also P. 1830, line 8 and P. 1831, line 6), "structure" is misleading, since it implies crystal structure (architecture, morphology, spatial relation to bacteria...).

Author reply: Ok, we have modified this sentence as follows: "The structure and elemental composition of the mineral concretions associated with these bacteria have been investigated by using LM, ESEM, TEM STEM and EDX microanalyses".

7-Referee comment: In Line 7 I would prefer "EDX" instead of "X-ray microanalyses". Beside I wonder if all microscopes have to be listed in the abstract, or if mentioning
SEM, TEM and EDX would suffice.

Author reply: OK, we have change the terminology of the different microscopes (Abstract) and modified X-ray microanalysis by EDX microanalysis.

8-Referee comment: Line 9, mentioning of the location is at a strange place in the abstract. Put it in the beginning (in that case, also note TAG) or leave it out.

Author reply: OK, we have removed the rainbow location from the abstract. This information is only present in the Materials & Methods section.

9-Referee comment: Line 11, at a first reading I couldn’t understand ;step-levels of mineral crust;. I would prefer terms like &amp;#8220;layers&amp;#8221; or zones

Author reply: We would like to keep this terminology because it refers to the previous article (Corbari et al., 2008), in which we suggest that the three layers of the mineral crust could be considered as representative of the different steps of its formation. It is important for us to illustrate the dynamic process of the crust formation.

10-Referee comment: Lines 12, delete &amp;#8220;heavy&amp;#8221;

Author reply: OK, "heavy" has been changed by "large"

11-Referee comment: Lines 12-13, the occurrence of SiO2, sulfates and phosphates was not evidenced and is even found less probable than adsorption in P.1843, lines 9ff. Separate mineral phases cannot be assumed to stabilize ferrihydrite.

Author reply: We agree with the referee, we have largely rewritten the results and discussion sections. We have reconsidered the occurrence of SiO2, sulfates and phosphates (see new table 3) and thus we have modified the abstract (line 12-13) as follows: "This multidisciplinary approach has revealed that the three layers of mineral crust in the Rimicaris exoculata shrimps consist of large concretions formed by aggregated nanoparticles of two-line ferrihydrite and include other minor elements as Si, Ca, Mg, S and P, probably present as silicates cations, sulphates or phosphates respectively that
may contribute to stabilise the ferrihydrite form of iron oxides." The precipitation or adsorption of such mineral ligands in iron oxides was already suggested by Chatellier et al. (2001, 2004); Fortin and Langley (2005). Regarding the elemental proportion of Si, Ca, Mg, S and P, we have considered that there are very probable mineral constituents of the iron oxides concretions.

12-Referee comment: Line 14, "Morphological observations"; sentence should be phrased more precisely.

Author reply: OK, we have modified this sentence: "TEM-observations on the bacteria have revealed their close interactions with these minerals."

13-Referee comment: Lines 16ff: this conclusion could also be explained more clearly.

Author reply: OK, we have rephrased this sentence as follows: "The difference of the bacterial density in the three-mineral crust layers could be correlated to the importance of the iron oxide concretions and suggest that the first mineral particles precipitates on the lower layer which could be considered as the most likely location of iron-oxidizing bacteria."

14-Referee comment: Page 1834 Line 4, results should not start with the results of a previous study.

Author reply: OK, we have modified this sentence.

15-Referee comment: Line 14ff, description of the three layers is not very clear. For example, the individual ferrihydrite crystals or particles can have a diameter of only several nm (as usually observed and as shown by Mössbauer), everything larger must thus be described as aggregates or concretions of ferrihydrite. The different types of contact between bacteria and minerals are addressed separately (line 19f, line 22f), which blurs the observation that both types do occur. Or is precipitation/deposition on exopolysaccharides much more abundant?

Author reply: The referee is right; we have changed particles by concretions throughout.
the MS and used aggregates when it was appropriate. For the bacteria, we have chosen to separate the different bacteria-mineral interactions in function of the location where they are dominant. So, the precipitation on exopolysaccharides has been more frequently observed within the lower layer of the mineral crust.

16-Referee comment: What is meant by "the minerals are diffuse" (line 22-23)?

Author reply: We agree with the referee. It is true that this terminology was not very adequate to characterise mineral structure. We proposed to modify this sentence by "the minerals appear as diffuse precipitates on secretions of the bacteria".

17-Referee comment: 15-P. 1835 Line 16, (and later e.g., p. 1838); Rosette-like; is used for gypsum roses and the like, the observed habit is better described by like grape-like rounded forms, botryoidal, concentrically banded

Author reply: We agree with the referee. We have changed "rosette-like" by "botryoidal structure" or "grape-like forms" throughout the MS.

18-Referee comment: 16-Line 8, could the ghosts also be artefacts, e.g. produced during the polishing, or can that be excluded?

Author reply: The bacteria ghosts can’t be artefacts because they are only observed as membranes remains on ultra-thin sections (TEM) of glutaraldehyde-fixed epoxy resin embedded samples what is the classical procedure to observe intact tissue samples in TEM.

19-Referee comment: P. 1836 Lines 1ff, I do not really understand the Mössbauer approach. Why were these two types of sample used? Why was the temperature dependence only measured on one of the samples? What can be learned from figure 5a? Do the Mössbauer results unequivocally identify ferrihydrite?

Author reply: Mössbauer spectroscopy is a powerful tool in analysing Fe-bearing compounds. The method identifies Fe-bearing minerals, determines Fe oxidation states
with high accuracy, quantifies the distribution of Fe between mineralogical phases, and provides clues about crystallinity and particle sizes. It is particularly useful in systems where the iron oxide may be too low in concentration or in crystallinity to be detected by XRD, for example in soils, sediments, rust and in organisms. Mössbauer spectroscopy is based on the recoil-free fluorescent resonance of gamma-rays discovered in 1958 by R. L. Mössbauer. Because of the natural line width of the gamma-ray, the hyperfine interactions between nucleus and electrons can be investigated by the Mössbauer effect from the static and dynamic point of view. The Mössbauer effect can be observed on solid samples in the energy range from a few keV to 100 keV, for a few tens of rather heavy nuclei in the periodic table. The most useful is Fe-57. A Mössbauer spectrometer is equipped with a gamma-ray source, which usually moves with a constant acceleration between two extreme velocities. The gamma-ray energy is thus Doppler shifted. The gamma-ray is absorbed by a stationary absorber and detected by a nuclear type counting system as a function of the source velocity. The spectrum is recorded as the relative transmission as a function of the source velocity, which is a measurement of the gamma-ray energy. The analyses in this paper have been performed with a Fe-57 spectrometer in which the temperature of the sample may be varied between 4 and 300 K.


For the samples, we have used two types because it was the first time we used "hydrothermal" iron oxide analyses with Mössbauer spectroscopy and thus have pooled branchiostegites of three shrimps, in order to get a sufficient signal. Finally, the signal was very good and we had prefer economise the samples, that's why for the second
sample, one shrimp has been analysed. As a reminder, Mössbauer spectroscopy is not a quantitative but rather a powerful qualitative technique.

20-Referee comment: Line 13, the chemical compositions of ferrihydrite is still under discussion, it should be mentioned that this is only one of the proposed formulas

Author reply: It is true, we have added this precision.

21-Referee comment: P. 1837, Table 3 Lines 3ff, I'm in doubt about the quantitative EDX analyses: 1) because of the small particle size the EDX signal will originate from the oxide and the resin. This will affect the ZAF correction and the final composition. To correct for the latter, i.e. the additional C, O and Cl signals from the embedding resin, the authors subtracted the spectrum of the resin from that of the sample. How was that done? In addition to the carbon content of the resin the C-coating and the organic matter from the sample will add to the signals of O and C, while the Cl-signal is only very small; I assume that this will lead to a higher error of the analysis. I do not concur with the interpretation of the EDX analysis, i.e. with the calculated mineral phases. Too many assumptions must have been made: the correction mentioned above, the not measurable H, the possibility of other oxidation states of Fe, the possibility of other anions like sulfides (or can they be excluded?). The calculation can only be used to check if the presence of the proposed minerals is plausible. But even then, I cannot understand why (Ca, Mg)SO4, (Ca,Mg)PO4 and SiO2 should be the most probable minerals in a shrimp or why these elements must be accommodated in separate mineral phases. Their presence can also be explained by substitution in ferrihydrite (Si, Mg), sorption to the large surface of ferrihydrite (SO4, PO4) or maybe even shrimp-matter; The mineral calculation is particularly strange because even the authors seem not to be convinced as they discuss surface complexation in great detail on p. 1842. I propose to go without Table 3 and simply discuss the possibility of additional mineral phases, adsorption and substitution. (This means that the respective parts of the abstract and the conclusions have to be changed as well).
Author reply: We agree with the referee. We have deeply changed the discussion and modified the table 3. As the comments of both referees are similar on these points, we propose some explanations in a general answer.

22-Referee comment: Lines 27ff, the discussion concerning the glutaraldehyde preparation is very important for future work and the interpretation of Gloter et al., 2004. However, Mössbauer spectroscopy would have been much better suited to quantify the Fe(II)/Fe(III) than the Fe/O measured by EDX (O is generally hard to quantify, additional error is to be expected because of the resin correction AND the correction of the presumed phosphates and sulfates). Furthermore the Fe/O will be dependent on the drying procedure. Is there a reason why glutaraldehyde treated ferrihydrite was not investigated by Mössbauer? Could that still be done? P. 1838 Line 4f, the parallel distribution of Fe and Si is a further argument not to believe in a separate SiO2 phase

Author reply: See general answer

23-Referee comment: P. 1840 Line 21, Michel et al. 2007 is an odd reference, this paper doesn’t deal with beam damage or high vacuum problems

Author reply: The referee is right, this reference has not been placed in the correct section

24-Referee comment: Line 22ff, I disagree: ferrihydrite can easily be formed abiotically and can show a wide range of crystallinity and composition, i.e. a wide range of Fe/O. (I’m afraid this question can only be addressed experimentally.)

Author reply: We agree with this comment, we have to moderate our conclusions about the biologically mediated origin of the Ferrihydrite

25-Referee comment: P1842 Lines 5ff, why should a higher Fe/O indicate bacterial influence??

Author reply: See general answer
26-Referee comment: P. 1844 Lines 17ff, some organic matter will probably suffice to stabilize ferrihydrite. The exopolysaccharides could do the trick or something else inside the shrimp (compare Schwertmann, Inhibitory effect of soil organic matter on crystallization of amorphous ferric hydroxide. Nature 1966, 212, 645-646).

Author reply: This is true, organic matter could stabilize Ferrihydrite but in the case of the hydrothermal vent shrimp, it remains difficult to evaluate the influence of the organic matter versus the inorganic element. Moreover, most of the publications on iron-oxidising bacteria and their FeO minerals associated have been performed on very well-known bacteria such as Leptotrix or Gallionella. In our case, the iron-oxidising bacteria have not yet been identified and so consequence it is difficult to evaluate the impact of the expolysaccharides on the stabilisation of ferrihydrite. Concerning the ectosymbiosis, we can just compare it to a bacterial biofilm which could promote and influence the FeO precipitation and stabilisation.

Interactive comment on Biogeosciences Discuss., 5, 1825, 2008.