Biogeosciences Discussions

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Comment on bg-2022-89

Anonymous Referee #2

Referee comment on "Do bacterial viruses affect framboid-like mineral formation?" by Paweł Działak et al., Biogeosciences Discuss., https://doi.org/10.5194/bg-2022-89-RC2, 2022

General comments:

The experiments seem to have been well done, the issue is interesting, but a deep understanding of what's happening in the experiments seem to be lacking.

The English language is reasonably good, but key concepts and interpretation seem misunderstood, or are expressed in awkward ways. I do not know if it is a problem of understanding basic concepts, or if it is a language problem. For instance, viruses cannot attract ions; they have chemical motifs able to bind ions. Using the words used in the text: attraction is very short-range and fast, then there is binding, which lasts and change the material's properties, including the z-potential. Adsorption also fits in this context. The idea that the viruses attract ions is repeated throughout the text, as pointed below. I do not know how much of this is a matter or language, or if the authors simply misunderstood the meaning of their results on z-potential.

The main finding (formation of framboids in experiments containing the phage P1, Fe and Na2S) is not convincing. Spheres containing high amounts of Fe and S are shown by SEM, but there is no images showing that the spheres are composed by several crystallites, all of them similar in size, shape and composition, which is the main characteristic of framboids.

Knowledge on how minerals are nucleated and grow seem to be lacking. I suggest to read classic books and reviews on biomineralization, to understand the basics of interaction between organic molecules and structures with ions and minerals. For example, see the volume 54 of Reviews in Mineralogy and Geochemistry, published in 2003, particularly the chapter by De Yoreo and Vekilov, Principles of Crystal Nucleation and Growth, Reviews in Mineralogy and Geochemistry (2003) 54 (1): 57–93 (https://doi.org/10.2113/0540057).

A major revision is required, where most of the text must be rewritten before this manuscript can be accepted for publication.

Minor comments:

Lines 14-16: Please re-write or delete this phrase. In the context of biomineralization, ions are not attracted by biological surfaces – or are capsids electrically charged, or magnetic? Please explain how could a virus change the electrochemical properties of precipitated minerals.

Line 18: They are different viruses, and thus it was expected different outcomes in the experiments. The presence of a lipid envelope is certainly a difference between the two viruses, but note that cells have membranes and are frequently involved in biomineralization.

Lines 21-22: (i) see comments for lines 14-16; (iii) what colloid? The structure of framboids indicate they form as a sphere of crystallites, not by agglomeration.

Lines 28-29: Microbes can oxidize both sulfide and Fe(II) from pyrite and other sulfide minerals, producing sulfate, Fe(III) minerals and protons, leading to decrease in pH.

Lines 60-65: Please re-write these sentences. The hypothesis/objectives are not clear. It is unclear what kind of experiments were done. In addition, the importance of your findings is over-emphasized – data presented are not sufficient to access the importance of the formation of framboids associated to viruses in nature.

Lines 111-117: Were the solutions treated to remove dissolved oxygen? How? Was O2 in the solutions quantified?

Lines 127-128: ions are not attracted by viruses: they adsorb, bind, etc.

Line 130: Were the viral capsids isolated? How? How binding of Fe, Cu and sulfide to viruses were measured?

Lines 139-143: Please explain how samples were prepared for XRD. Of crucial importance is how samples were stored, and how long they were stored.

Line 141: there is no "CoKα lamp" in X-ray diffractometers.

Line 148: Are you sure it was a glass slide? Glass coverslips are widely used to mount samples for SEM.

Line 148: Are you sure it was 20 nm? Or was this an estimate from the device?

Lines 170-171: …are differences in the samples with and without viruses (or bacteriophages). Were the differences noted in the z-potential or the conductivity?

Lines 171-172: were the significant differences found between the FeS and CuS groups, or among the three treatments for the same metal?

Lines 177-178: Was the z-potential in the last column measured with virus + metal + Na2S, or with virus + Na2S?

Lines 188-194: Any differences in the mineral composition were probably masked by sample oxidation. This is stated in lines 193-194. But the sentence in line 188 states that "Phase composition of samples did not differ significantly". You could re-arrange the text to make clear in the beginning of the paragraph that the X-ray data should be analyzed knowing that the sample has been oxidized.

Are mohrite, blutlerite and jarosite products of oxidation of the sample? You should make a list of probable original products and oxidation products for the FeS and CuS experiment, to help the reader unfamiliar with these minerals.

Lines 197-198: How did you recognize the mineral phases in the SEM to record the EDS spectra? Maybe the use of the term "mineral phase" is a mistake. Do you mean "minerals", "mineral particles", or something like that?

Line 200: the use of "mineral phases" is probably a mistake here too.

Lines 200-201: The spectra “1” and “2” seem very similar, not distinct.

Line 204: O and Na are not negligible in spectrum 4, and Na is not negligible in spectrum 5.

Lines 209-216: The text is very confusing. From the images in Figure 6, it seems that the shapes and sizes of the particles are not really different in the three first groups of images (a-l). In figures m-p they seem smaller, and sometimes are arranged in spheres.

Line 210: “Experiments with bacteriophages gave similar structures”. Similar to what? To each other?

Lines 222-223: From the images, images in Figure 7 not seem to be really different from images in Figure 6 without bacteriophages.

Lines 234-235: The differences observed with the naked eye must be diverse from that observed at the SEM – the magnification are orders of magnitude different.

Line 242: Please provide a reference for the DLVO theory.

Lines 242-243: Again: viruses are not nuclei which attract ions from solution. If they attracted ions from solution, they would be soon encased in minerals and would not be effective in infecting cells (which are the sine-qua-non condition for virus replication).

Line 244: the work cited states that capsids can bind iron and nucleate iron minerals.

Line 249 Viruses do not attract ions; they can bind, or adsorb, ions.

Lines 251-252: bind, not attract. If they bind, you can measure differences relatively to the sample without Na2S.

Lines 254-255: It is probably much more complex than just charge interactions. For example, sulfide ions may interact with -SH groups of cysteine residues.

Line 261: Are the two groups statistically different from each other? Or each experiment is different from the others in the same group?

Lines 268-268: The measurements are not erroneous if the effects of aggregation inherent to the technique are considered in interpretation. Even if the numerical results do not represent real sizes, they can still be used to compare samples.

271-273: Was the P1 virus chosen because of the icosahedral shape, or the icosahedral shape was a coincidence? Several minerals can show icosahedral morphologies, including macroscopic specimens of natural pyrites. Crystal shapes depend mainly on the arrangement of atoms, and this is the case of icosahedral pyrites. Conversely, there are several icosahedral viruses. The fact that pyrite in framboids and in your experiments is icosahedral does not mean that they were nucleated by icosahedral viruses, since they can be produced by purely chemical processes.

Lines 300-302: The idea or virus capsids attracting ions again – please consider that binding is the important thing to consider for mineral precipitation. To precipitate a mineral, it is needed several layers of each type of ion.

Lines 318-319: Again the idea of viruses attracting ions from solution. Here, attract and bind seem to have been used as synonyms. Are you sure that the lipid envelope is the cause of the differences observed between the viruses? They surely have other differences too.

Lines 322-323: Fe(II) and S(II-) are not oxidized in anoxic environments, and FeS precipitation is not limited in these environments. Most of the Earth's crust is anoxic, including deep soils and sediments.

Figure 2: Please provide scale bars for the light micrographs.

Figure 2 (caption): Magnification in printed micrographs is almost useless – it depends on how much the image is enlarged. Please write the bacterial genus *Pseudomonas* in italics. Please state in the caption the technique used (fluorescence light microscopy or transmission electron microscopy) in each image.

Figure 3b: Please make a legend without colors for Figure 3b, to make it clear the differences between conductivity and z-potential in the graph.

Figure 3 (caption): Please explain what where the solutions used for measurements (phosphate buffer, saline, etc) in each plot. In (b), there is a plot of conductivity which is not mentioned in the caption. In (c), it is shown the z-potential of complex materials in suspension, not the "attraction of ions by bacteriophages" – there are other ingredients in the mixture. In (d), it is shown the size of FeS and CuS particles. Does ** means statistical differences? It is not clear what data were compared in statistical tests.

Figure 4: Please highlight the peaks for the minerals which were not oxidized during sample preparation.

Figure 4 (caption): Please separate Fe and Cu minerals in the list at the end of the caption, and provide a chemical formula for each mineral. You can see mindat.org for mineral formulae. In the figure, is "Ja" for jarosite?

Figure 5: The lettering in the scale bars are too small. Please increase them.

Figure 5 (caption): How was this material prepared? With viruses, or not?

Figures 6 and 8 (captions): It is mentioned in the captions that the experiments were made with the P1 bacteriophage, but a-d and i-l were prepared without bacteriophages.

Figure 10: The idea of icosahedral viruses serving as nuclei for the synthesis of icosahedral FeS mineral particles, expressed in the drawings and also in the text, is not based on your data, nor on data from the literature. It seems pure imagination. A second issue is that there are two possibilities for formation of spherical structures: agglomeration as suggested in Figure 10, or they are formed already as a sphere. For framboidal pyrite, it seems more likely that it forms as a sphere: otherwise, how could they be so well organized by aggregation of pre-existing particles? The "framboids" shown in other figures of this manuscript are too smooth to have been formed by aggregation of pre-existing particles.

Technical comments:

Lines 13 and 19: do you mean *Enterobacter*, or enterobacteria? If it is *Enterobacter* (genus), it should be written in italics; if it is enterobacteria (common word), it should be written without capital letter.

Lines 14 and 19: *Pseudomonas* should be written in italics, since it is the name of a bacterial genus.

Line 29: sulphide.

Line 33: Please add a space before the parenthesis. Is it greigite?

Line 34: euxinic?

Line 40: I suggest to begin a new paragraph to explain the basics of viruses and bacteriophages.

Lines 42-43: the same idea is expressed better in lines 51-53.

Line 49: I suggest to begin a new paragraph to present the phages used in this work.

Lines 51-53: Consider using “can” only once.

Lines 60-61: I think “in this work” would be better.

Line 75: laminar flow cabinet?

Line 89: You may use “used” instead of “added”.

Line 137: the device.

Line 164: “both” is not suitable here.

Line 192: There is a misspelling here, please write “chalcanthite”.

Figure 4, caption: There is some misspelling: synthesized, in the mineral names, please write “chalcanthite” and “troilite” (you can see mindat.org for correct mineral names).

Lines 197-198: EDS spectra are not measured, they are obtained.

Lines 209-210: visibly small

Line 244: viral

Lines 287-288: the hydration of the $FeSO_4$ is excessive detail.

Lines 295-297: Try writing a single phrase comparing your results with others’.

Lines 298-299: Try rewriting this phrase using “stir” only once.