Biogeosciences Discuss.,
https://doi.org/10.5194/bg-2018-194-RC2, 2018

Biogeosciences Discussions
Open Access
EGU

Interactive comment on "Precipitation of Calcium Carbonate Mineral Induced by Viral Lysis of Cyanobacteria" *by* Hengchao Xu et al.

Anonymous Referee #2

Received and published: 16 May 2018

The manuscript by Xu, et al. describes the isolation of a cyanobacterium and cyanophage and the use of this host/bacteriophage system to induce mineral precipitation in artificial medium under laboratory conditions. Using observational data, mineral characterization technologies, microscopic cyanobacterial counts and chemical analyses the authors conclude the presence of cyanophage in a culture of the host cyanobacterium lyses the host and releases cellular constituents into the culture medium. The author propose this release of dissolved and particulate cellular constituents promotes the precipitation of specific polymorphs of calcium carbonate and magnesium hydroxide.

General Comments: 1. The manuscript is not well organized, with some sections lacking adequate methodological information. Collectively, these issues makes the

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manuscript a bit difficult to read and interpret. Examples will be specifically described in the Specific Comments section.

2. The use of “calcium carbonate” is used throughout the manuscript but in most instances, this is too general a descriptor within the context of this study. The use of the specific calcium carbonate polymorph names [e.g., amorphous calcium carbonate (ACC), vaterite, aragonite, calcite] will be more appropriate when applicable.

3. The mineral precipitation mechanisms of homogenous and heterogeneous nucleation are not clearly delineated throughout the manuscript and appear to be used interchangeably in some instances. Additionally, mineral nucleation and precipitation are also used interchangeably. This conflation of terms, phrases and concepts makes it more difficult for the reader to read and interpret the manuscript. The experimental design for this study does not allow the detection or characterization of nucleation events, only gross precipitation that can only be indirectly assumed the result of one or both mechanisms of nucleation.

4. Throughout the manuscript total alkalinity (TA) and alkalinity are used interchangeably. When working with marine carbonate chemistry there is a significant difference between the ways these two alkalinities are calculated. Make it clear to the reader which one is being used or referred to.

5. There is repeated mention of carbonate chemistry in the text and tables. However, there is no listing of the geochemical data used for or the output from the geochemical modeling analyses. These data sets need to be included.

6. Saturation indices (SI) are a central component of this manuscript but there is no description of how these were calculated. When working with carbonate chemistry in marine waters most calculations on saturation states are per polymorph, like aragonite and calcite. These calculations are not normally performed within commonly used geochemical modeling programs but rely on CO2SYS or a recently developed application, CO2calc. The calculated SI values from the geochemical modeling software and

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CO2SYS/CO2calc are not always equivalent.

7. The Discussion section is too long and not focused on placing the data and interpretations from this study in context of previously published papers. There is a considerable amount of text dedicated to introducing and developing concepts that are on the periphery of the stated objectives and generated data of this study. This section needs to be edited to remove these passages and re-written to focus the discussion in a focused and concise style.

Specific Comments:

Abstract

Pg 1: Ln 10-22. (1) The data do not support the statement that the presence of viruses stabilizes the carbonate minerals detected in this study. (2) There's no evidence in this study of rapid intracellular calcification due to intracellular calcium concentrations.

Introduction

Pg 2: Ln 1-2. The precipitation and dissolution of calcium carbonate does later sea water chemistry but do these processes have a more significant influence on the carbonate chemistry than carbon dioxide flux from the atmosphere?

Pg 2: Ln 11-15. This passage describes sedimentary processes and stromatolites. Consideration should be given to removing this passage from the manuscript. This concept is not relevant to the stated theme of this manuscript.

Pg 2: Ln 16. The precipitation of calcium carbonate, regardless of the mechanism, is always a controlled geochemical or biogeochemical process.

Pg 2: Ln 23-29. All of the information in this passage is true. However, it's not clear how this information is relevant to the stated objectives and experimental design of this study. Considering should be given to removing this passage from the manuscript.

Pg 2: Ln 33. Which of the calcium carbonate polymorphs was capable of homogenous

nucleation in the cited study?

Pg 3: Ln 1-2. The authors state the cited study does not consider the role of magnesium in their calculations. However, they don't tell the reader why it's important in this study. Since the role of magnesium in mineral precipitation is one of the objectives, this would be the place to provide the reader with some background information that will put the data and interpretations in the proper context.

Pg 3: Ln 5-14. The information in this passage is not relevant to this study based on the stated objectives and experimental design. Its inclusion is a distraction from the Consideration should be given to removing this passage from the manuscript.

Pg 3: Ln 17-18. (1) The authors state the understanding of viral influences on the precipitation of carbonate is poorly understood but they in the previous 16 lines of text they list several published studies that do characterize this process. (2) Here is an example of the confusing use of nucleation and precipitation in the same sentence.

Pg 3: Ln 30. (1) A virus infected cyanobacterium is not a cyanophage. (2) Interpreting Figure 2 as showing a cyanophage and its host bacterium is a bit of a reach. These images could be almost anything.

Pg 3: Ln 31-32. There needs to be, at a minimum, an abbreviated description of the cited method used to isolate, purify and identify the cyanobacterial specie. It should not be incumbent on the reader to run the most basic description of a method.

Pg 4: Ln 1-2. (1) As noted in the previous comment, at a minimum, an abbreviated description of the cited method for the cyanophage isolation needs to be provided. (2) There is a reference to metagenomics analysis, including a supplemental data file. The method of sample collection, processing, sequencing and sequence analysis (including the bioinformatics) is not mentioned or described anywhere in this manuscript. This is a significant deficiency in this version of the manuscript.

Pg 4: Ln 3-17. (1) This section is very poorly organized and developed with respect to

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the different methods mentioned. There is no reasonable way a person could replicate this research effort or interpret the data from these methods using this section for guidance. (2) In this reviewer's opinion there are seven distinct methods: culture growth conditions; cyanobacteria counts; ion chromatography; salinity, total alkalinity and DIC measurements; geochemical modeling of carbonate chemistry; metagenomics analysis. Consider giving each of these their own sub-section within Experimental Setup and develop each section so the reader will understand how the methods were performed. (3) List the incubation or experimental times for each experiment type. (4) What is meant by a "pre-culture"? (5) A "one treatment" is mentioned. Are there other treatments? If so, describe those treatments and their respective differences. (6) List the volumes for each experimental container, the volumes of the sub-samples and the times at which the sub-samples were collected. (7) For the cyanobacterial growth cultures provide the light wavelength and dose.

Pg 4: Ln 15-17. There is reference to geochemical modeling using a specific program. However, there is no mention or listing of the data on which the geochemical analyses were performed or the outputs from those analyses (e.g., activities of carbonate species). Both of these data sets need to be included in this manuscript. Additionally, the PHREEQC code used for these analyses needs to be included, most likely as part of the Supplemental Data files.

Pg 4: Ln 19. Here and in several later passages, there are references to "phases" of the cyanobacterial cultures. Based on the references it's assumed these phases are similar to those commonly measured during the growth of bacteria in the laboratory (i.e., lag, exponential, stationary). However, the methods used to determine these growth phases and the data from those experiments are not provided. This is another method that should be included in the Experimental Setup section.

Results

Pg 5: Ln 4-5. How many cyanophage were inoculated into the cyanobacterium culture?

Were the cyanophage titered? If so, the titer data need to be included. Without these data you cannot know the ratio of host cells-to-cyanophage (i.e., MOI), which has a significant influence on infection rates.

Pg 5: Ln 5-7. (1) Based on the brief description of the counting method and Figure 1b, it appears the cyanobacterial host abundances are based solely on the autofluorescence of the photosynthesizing microorganism. How can you be sure that non-fluorescent bacteria are not present in these cultures? Without knowing this, the possibility that some or all of the observed responses are due to a non-host bacterium or bacteria cannot be ruled out. (2) From this passage, it appears the cyanobacterial host abundances in the two culture types were measured using fluorescent microscopy but the cyanophage abundances were not determined in the co-culture. How can you conclude the cyanophage were responsible for any of the observations if there is no indication of how many were added to the host culture at time zero and their abundances at the different time points are not known? These cyanophage abundance counts from the different sub-samples should show increases as the host abundances decrease. Without the cyanophage abundance data you can't support the reductions in host abundances as being solely due to cyanophage induced lysis.

Pg 5: Ln 13-23. (1) This section needs to be revised into a more organized and concise presentation of the geochemical data. It's very difficult to interpret in its current format. (2) Throughout this section there are repeated references to different sub-samples and geochemical data associated with those sub-samples. This suggests sub-samples were collected at specific time points during the incubations and those sub-samples were then analyzed for the presence and concentrations of analytes required for the geochemical modeling of changes in the carbonate chemistry. The times of the sub-samples are not provided, the analytical data from those sub-samples is not presented and the outputs from the geochemical modeling analyses are not listed. Collectively, this information and data have to be included for the proper interpretation of the documented observations. For example, reference is made to seemingly unre-

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alistic changes in total alkalinity, DIC and calcium and magnesium removal over the different growth phases of the host culture. The geochemical modeling data need to be presented to support these observations. (3) Was pCO2 measured during these experiments? If so, this method needs to be included and described.

Discussion

Pg 6: Ln 9. Its not clear what this balanced equation is representing? Its not at all clear from the text how formaldehyde (CH2O) is formed from bicarbonate and water or what the significance of CH2O is to the study described by this manuscript. Was the intention to let CH2O be a general reference to a carbohydrate? Or the dissociation of bicarbonate to carbon dioxide?

Pg 6: Ln 14-17, Ln 21-26; Pg 7: Ln 9-10, Ln 16-17, Ln 19-21, Ln 25-29. All of these passages require the reader to have access to the geochemical modeling input data and output results for each sample before an independent interpretation and evaluation of the observations can be made.

Pg 6: Ln 1 and Ln 32. These are the first time brucite is mentioned, other than the abstract. As this mineral seems to a significant product of the processes described in this manuscript, consideration should be given to bring this mineral and its relative importance into the manuscript in the Introduction and the Materials and Methods sections, including the geochemical modeling sub-section.

Pg 6: Ln 22-23. Here and within other passages later in the manuscript, there is reference to the saturation index (SI) as being the metric for determining the saturation, supersaturation and under-saturation state of the culture medium. Though true, the SI alone will not tell you if a mineral will precipitate or dissolve. For example, if this were unconditionally true then average seawater, which has an SI between 2-3, one or more of the calcium carbonate polymorphs would be precipitating out of solution all the time. Instead, average seawater is metastable with no precipitation, indicating there are more geochemical factors other than SI that dictate if a precipitation even will proceed or not.

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Here is another example of where the geochemical modeling data would assist in the discussion of the results from this study.

Pg 8: Ln 1-16 and Ln 24-31; Pg 9: Ln 1-17. These passages do not add anything supportive to the manuscript, based on the stated objectives, experimental design and presented data. Consider deleting these from the manuscript.

Pg 8: Ln 20-23, Ln 28-33. The precipitation of one of the calcium carbonate polymorphs in tropical marine waters, or whitings, has been shown via peer reviewed publications to be driven by biological processes and not the simple physical re-suspension of established carbonate sediments.

Conclusion

Pg 9: Ln 19-20. Without the geochemical data and model outputs a detailed view of carbonate chemistry changes have not been provided. Also, without cyanophage abundance counts the cyanophage infection and lysis of the host cells cannot be definitively stated.

References

Pg 10-13. There are 65 references listed. Based on there being issues with citations being incomplete, having punctuation errors, odd symbols inserted (which may have been a conversion issue) and inconsistency with the DOI information format, there are 36 references that need to be reviewed and corrected if needed. There were too many to list individually.

Table 1. (1) This are several modified f/2 medium formulations, but the recipe listed in this table is not included in those published formulation. If there is a citation for this formulation of f/2 please include it. Also if the mineral and vitamin solutions were purchased then this needs to be noted as well. If these solutions were made in the laboratory, then there are several ingredients that are missing (e.g., EDTA). (2) The final pH (which should be between 7.8-8.2) of the media needs to be included as well.

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Table 2: (1) The pH values for all of the samples are relatively high compared to the initial pH of f/2. Were these measured or the products of the geochemical modeling? (2) The higher pH values are unrealistically high for open marine waters but, interestingly, the SI values are all less than those for the two dominant polymorphs (aragonite and calcite) in typical marine water. At these pH values, higher SI values, relative to typical marine water, would be predicted. This is another example of where having the geochemical modeling data is critical for the proper interpretation and assessment of the research data.

Figures 1 and 2. These images do not contribute additional information to the reader. Consider deleting these from the manuscript.

Figure 5. Panel 5c is not cited in the manuscript.

Figure 8. The simple shape of an object is not enough to proclaim it being a microbial cell, virus or encrusted microbial structure. Unless more definitive proof can be provided that will support the declaration that this images are what's written in the legend, consideration should be given to deleting this figure from the manuscript.

Figure 10. This is a nice graphic but it's not explained well in the manuscript and there is a lot of information which is not even mentioned in the manuscript. This figure would be a nice summary graphic but the information in the image will have to be presented in the manuscript.

Supplementary Figure. The information in this figure stands alone because nothing about or within this figure is mentioned or described in the manuscript. In the manuscript's current format, this figure does not support anything presented in the Results or Discussion sections. For this reason, consideration should be given to removing this figure from the manuscript.

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