

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

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This study deals with an interesting topic, providing an experimental test of the hypothesis that lysis of cyanobacterial cells by virus may trigger Ca-carbonate precipitation by helping overcoming the energy barrier of nucleation. If true, this means that such biological events may change the apparent solubility of carbonates in eg seawater. While the study overall provides some results which are convincing to me, there are several flaws which need to be corrected first before acceptance for publication.

Reply: Thank you very much for your appreciation on the overall performance of the research work. Improvements have been done following the suggestion from the reviewer.

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The most important ones are: 1) there is a strong incoherence between XRD results showing crystalline aragonite and TEM data suggesting ACC. TEM data should be revised.

Reply: We understand the concern pointed out by the reviewer regarding to mineral composition of the precipitation. For ACC, which is metastable hydrated phase, it is thought to be poorly ordered and lack of distinct peaks within XRD pattern (Rodriguez-Blanco et al., 2008). Thus, bulk samples for XRD analysis are composed by aragonite. ACC in TEM images are confirmed by the diffuse rings in the selected area electron diffraction patterns (Fig. 6b). ACC and aragonite also can be seen from the SEM images. We can add the SEM images as a supplementary figure in revised version.

2) It is not convincingly explained what happens around day 8 and how DIC and total alkalinity follow such different paths.

Reply: Growth of cyanobacteria directly results in the DIC uptake. With regards to the total alkalinity, its changes depend on the other sources of base added to the medium. On day 6-8, two treatments grow at similar rates and reach similar cell density. Moreover, there are no cations removed from the medium. Thus, only DIC decreased and TA values remained relatively constant. As DIC transport by cyanobacterial replication, the pH of the medium increase, leading to the immobilization of calcium and magnesium and total alkalinity decrease delaying.

3) The Mg story is really not convincing. How can you explain that the solution goes from supersaturated to undersaturated with brucite. I detail thereafter these comments and add several other ones which should be addressed

Reply: The geochemical condition of the medium is largely influenced by the growth of cyanobacteria. Photosynthetic bicarbonate uptake and its conversion within the cell to CO<sub>2</sub> by carbonic anhydrase lead to increase of pH in the immediate vicinity of the cell (Reviewed by Riddings 2011). In present study, when lytic rates run over the bacterial replication, photosynthesis ceases and atmospheric CO<sub>2</sub> dissolve in the water and this

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will change the acid-base balance of the system.

Introduction: L29: sentence is awkwardly written. It should read "Dissolved inorganic carbon in the typical..." (dissolved CO<sub>2</sub> is not HCO<sub>3</sub>-)

Reply: Thanks for your correction. It has been rephrased.

Page 2: formation and dissolution of carbonate is one of the most instead of the most.  
I guess photosynthesis is at least as important

Reply: Thanks for your correction. It has been rephrased.

L4: Needs rewriting "seawater is considered supersaturated with several calcium carbonate phases such as xx (calcite?), with saturation index..." a solution is supersaturated with. A carbonate is not

Reply: It has been rephrased. "Modern sea water is considered supersaturated with several calcium carbonate phases such as calcite, with  $\Omega$  values ranging from 2 to 4."

L13 : you do not need to get into that debate about whether cyanobacteria formed the first stromatolites or not. Many studies now argue against this idea and I agree this is beyond the scope of your paper to debate about that. You should rephrase

Reply: It has been deleted.

L18: Instead of furthermore, I would write "In contrast", since it has been suggested that intracellular precipitation might be controlled in opposition to non-control as mentioned on line 16. And I would remind that this is true for some species of cyanobacteria, not all. Last, it was recently showed that it can occur in undersaturated solutions (Cam et al., 2018 Geobiology). I am wondering if the same could be imagined in some environments with viruses lysing cyanobacteria. Maybe as a perspective?

Reply: We agree with the suggestion. We highly appreciate the latest reference shared by the reviewer. Cam et al., (2018) is included as a reference for the introduction of intracellular calcification. We can design some viral calcification experiments in un-

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dersaturated medium in the near future to discuss the viral influence in intracellular precipitation.

L30: you should specify that this increase would be very local

Reply: We agree with the suggestion. It has been rephrased. The theoretically saturation index change is at the cellular level.

Page 3, L19: Synechococcus spp. This is such a broad name encompassing so different bacteria. Could you specify at this point the name of the strain? Or specify that the strain was isolated in the present study?

Reply: Cyanobacteria are identified to the group of Synechococcus spp. based on the morphology of cell. DNA work will be carried out to precise the statement in the revised manuscript version.

P4: Is the strain axenic?

Reply: During all the treatment, operations are asepsis strictly.

L7: I guess the other not-mentioned treatment is a control where no virus has been added? Please specify this

Reply: Yes. The other treatment is not inoculated with virus. We re-write completely the "Experimental setup" paragraph to make our statements more accurate.

Are culture bottles closed to air exchange or are they open? This is important to understand the evolution of DIC in your system I guess

Reply: They are air exchangeable. So the DIC increase after the viral lysis of cyanobacteria and inhibition on photosynthesis.

L8-9: please rephrase. I guess you do not measure OD on a fixed and filtered suspension.

Reply: We did not measure OD but enumeration the abundance of cyanobacteria by

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autofluorescence.

And Why do you fix the cells before measuring the chemical composition of the solution. Could fixation modify the chemical composition of the solution?

Reply: Although we collect the DIC and total alkalinity subsample in bottles without headspace, carbonate chemistry parameters of solution may change during the storage. So, the subsamples were poisoned with HgCl<sub>2</sub>. The same methods had been used for seawater sampling and analyses elsewhere (Cao and Dai 2011)

L11: what is a TA sample?

Reply: It is total alkalinity.

2.3: Electron microscopy instead of electronic microscope

Reply: It has been correct.

L19: what is the stable phase? Do you mean stationary phase (after exponential)? But what do you mean by the end of it?

Reply: Yes. Stationary phase is used more common than stable phase in literature. The particulate of

L26 what is abs?

Reply: It refers to "selected-area electron diffraction"

L27: Technically you do not sputter coat with carbon. This is achieved by evaporation. You can rewrite as: before being carbon coated

Reply: The change is done accordingly "The samples were carbon coated and before being sputter coated with carbon for 2–3 min. Samples were examined with an Apreo scanning electron microscope (Thermofisher Scientific)."

Page 5: L 3-4: the lag phase lasted 4-5 days

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Reply: The sentence has been rephrased.

L5: you say slightly lower but I see on Fig 4a a DO of 3 vs 14. This sounds like a very big difference to me

Reply: The sentence has been rephrased. "The cell abundance of the viral group is slightly lower than the non-treated group on the fifth day to eighth day."

Fig. 4a: you said you ran duplicates. Where are the error bars then in your curves?. Could you show the pH curve?

Reply: Error bars were added in panel Fig 4 (a, b). DIC samples were measured in one set in other lab, so we cannot show the error bars of DIC but the accuracy of measurements of measuring are given. pH values are calculated from the geochemical program and can be showed in revised manuscript.

Why TA does not match with alkalinity? Which other species contribute to alkalinity here? Do you think that they also might vary differently? I think of N species? Or P species? TA sharply decreases at day 8 while alkalinity starts decreasing at day 6. How do you explain that?

Reply: I think the reviewer want to know the reason why TA does not match with DIC during the day 6–8. During growth of cyanobacteria, the DIC drawdown by photosynthetic carbon uptake exceeds the slow reequilibration with the atmosphere. Thus, chemical speciations of the DIC pool shift toward higher CO<sub>3</sub><sup>2-</sup> concentration and pH. TA will not decrease until the precipitation of Ca and Mg, which is responsible for the base removal from the medium. That explains the DIC decrease first (day 6) and TA decrease later (day 8) by the precipitation of CaCO<sub>3</sub> and Mg(OH)<sub>2</sub>.

Why is there such a sharp decrease of Ca in all conditions at day 8?

Reply: Despite the inoculation of cyanophage, culture with and without cyanophage grow at similar rates and reach the similar cell density during the first 8 days. The DIC drawdown by photosynthetic carbon uptake exceeds the slow reequilibration with

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the atmosphere, causing chemical speciation of the DIC pool to shift toward higher CO<sub>3</sub><sup>2-</sup> concentration and pH. At day 8-10, the geochemical condition influenced by photosynthesis promote the Ca and Mg precipitation.

Why does dissolved Mg increase again in viral treatment after 14 days? L21: you do not mention that Mg also redissolve in the viral treatment.

Reply: There is a strong positive correlation between Mg<sup>2+</sup> and DIC recovered after the 12th day, which is the time point when the lytic rate begins to dominate cell replication. In present open system, atmospheric CO<sub>2</sub> is dissolved in water and changes acid-base balance of the system. Hence, unstable mineral phases (like bructie) can dissolve with acidification during the culture of cyanobacteria. The dissolution of brucite in seawater is also discussed elsewhere, which is responsible for the mineral not well preserved over long time (Nothdurft et al., 2005).

Fig 6, caption: by definition ACC is not crystalline so you should not write about crystallization of ACC but precipitation or formation

Reply: The change is done accordingly

Fig 7: please show a spectrum. Maps are not enough. Why is these are phosphates? Only spectra could show that there is no P peak

Reply: EDS spectra of the nanoparticle shows that a small peak of element P. But P signal from the STEM mapping is not consistence with the nanoparticles. So it is not included in the early version of the manuscript.

Fig 9: what is the peak at 10\_? If XRD sees aragonite (ie crystalline phase) how does it come that you see amorphous Ca-carbonates? Is aragonite amorphizing under the TEM beam?

Reply: Based on XRD results, the peak at 10 is loeweite, which is a sodium-magnesium sulphate hydrate [Na<sub>12</sub>Mg<sub>7</sub>(SO<sub>4</sub>)<sub>13</sub> 15H<sub>2</sub>O], found only in salt deposits of oceanic origin (Fang and Robinson 1970). Aragonite and ACC can be shown in a figure panel

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by SEM. Combine with the SEM and TEM, the formation of ACC during the cultivation can be clarified.

Discussion: P25: the authors claim that this is no surprise that brucite forms but to my knowledge this has never been really shown by previous studies on cyanobacteria cultures; How do they explain that they produced brucite and not the other groups.

Reply: It has been rephrased. We agree with the reviewer that few studies show the precipitation of brucite from cyanobacteria culture. Literature review indicates that brucite precipitation may result from increased pH coupled with increased Mg<sup>2+</sup> activity and low pCO<sub>2</sub> (Nothdurft et al., 2006). When the cyanobacteria grow during the log phase, DIC are removed from the solution and the pH of the growth medium increased (Table 2). The chemical change leads to the supersaturate state of brucite (Table 2). Thus, Brucite can formation in our culture.

Moreover, they detect brucite by XRD but they mention that the solution became undersaturated with brucite after 8 days. How is it possible. I would expect in the worst case a SI of 0. Not below. I see that Mg is released after 8 days even in the viral treatment. This could be consistent with the undersaturation of the solution with brucite. But how do we switch from supersaturated to undersaturated? Precipitation of brucite should take it to saturated. And since there is no Mg going to aragonite and there is no carbonate in brucite, this cannot be explained by aragonite precipitation and changes in DIC.

Reply: The story of Mg and brucite have been further discussed in revised manuscript. Saturation indices (SI), which are determined using the software PHREEQC, yielded values > 0 for brucite during the first 8 days (0.34 ~ 1.15) and values < 0 after the 10th day (-1.46). The culture bottle are open to the air. So when DIC uptake by photosynthesis is slow compared to the log phrase, atmospheric CO<sub>2</sub> is dissolved in water and changes the PCO<sub>2</sub> level and acid-base balance of the system. Hence, brucite can dissolve with acidification during the culture of cyanobacteria. It is suggested that the

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brucite microbialite of Holocene corals may not be preserved over longer time, which is easy to dissolved in undersaturated solution.

End of discussion is too long. The paragraph on page 9 from L 6 to 17 could be skipped or at least significantly reduced since this is quite far away from the main scope of the paper

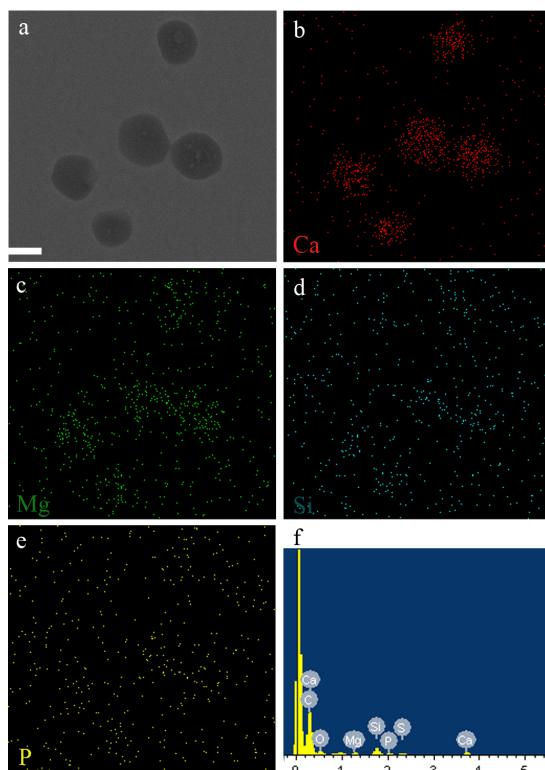
Reply: We appreciate the suggestion of this part of discussion. In revised manuscript version, they were reduced to a single sentence to expand the importance of viral induced calcification.

Please also note the supplement to this comment:

<https://www.biogeosciences-discuss.net/bg-2018-194/bg-2018-194-AC1-supplement.zip>

Interactive comment on Biogeosciences Discuss., <https://doi.org/10.5194/bg-2018-194>, 2018.

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**Fig. 1.** EDS spectra of the nanoparticle shows that a small peak of element P. But P signal from the STEM mapping is not consistence with the nanoparticles.

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