Dear editor:

We highly appreciate the opportunity for submitting a revised version of our manuscript. We are thankful for all the valuable comments and suggestions. Here, we submit a thoroughly revised version and marked-up version of our manuscript, which has been modified according to the reviewers' suggestions.

- *The methods have been re-organized following the reviewer's suggestion. Each of the method is given a sub-section in revised version of manuscript.*
- *The discussion of Mg and carbonate chemistry is improved. Carbonate chemistry changes influenced by growth of cyanobacteria and viral lysis of cyanobacteria are discussed in two separate chapters.*
- *The paragraph about whiting and Mg/Ca ratios are deleted following the advice from reviewers.*

Below we have pasted in the entire review, and we have inserted our responses to the suggestions (blue font).

Sincerely,

Xiaotong Peng, on behalf of the co-authors.

Response to the comments by referee#1

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.

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.

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.

We understand the concern pointed out by the reviewer regarding to mineral composition of the precipitation. There are two carbonate mineral phases (ACC and aragonite) precipitating by viral lysis of cyanobacteria in the medium. The particulate fraction of the medium (~1 L) was harvested via centrifugation (13000 g, 5 min) for electronic microscopy and X-ray diffraction. 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).

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

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. thus, DIC and total alkalinity changed in different paths.

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

Brucite, which is an unstable minera, may not be preserved over long times and can be dissolved in undersaturated state (Nothdurft et al., 2005). 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_2 by carbonic anhydrase lead to increase of pH in the immediate vicinity of the cell (Reviewed by Ridding 2011). In present study, when lytic rates run over the bacterial replication, photosynthesis ceases and atmospheric CO_2 dissolve in the water and this will change the acid-base balance of the system. There is a strong positive correlation between Mg^{2+} and DIC recovered after the 12th day in Group A, which is the time point when the lytic rate begins to dominate cell replication. It support the deduction that atmospheric CO_2 is dissolved in water and changes the acid-base balance of the system. Hence, brucite can dissolve with acidification during the culture of cyanobacteria:

Introduction: L29: sentence is awkwardly written. It should read "Dissolved inorganic carbon in the typical..." (dissolved CO2 is not HCO3-)

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

Thanks for your correction. It has been rephrased.

L4: Needs rewritting "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

It has been rephrased.
"Modern sea water is considered supersaturated with several calcium carbonate phases, such as calcite and aragonite."

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

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 occurs 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?

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

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

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 as 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?

Cyanobacteria are firstly 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. 16S rRNA genes of the strain were amplified by PCR and shows that the strain have the close relationship with Synechococcus sp. PCC 7177.

P4: Is the strain axenic?

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

Yes. The other treatment is no 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

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.

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

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 $HgCl_2$. The same methods had been used for seawater sampling and analyses elsewhere (Cao and Dai 2011)

L11: what is a TA sample?

It is total alkalinity.

2.3: Electron microscopy instead of electronic microscope

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?

Yes. Stationary phase is used more common than stable phase in literature. The precipitation was harvested at the 16th day, which means at the end of the stable phase.

L26 what is abs?

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

The change is done accordingly
"For SEM analysis, dried precipitates were fixed onto aluminum stubs with two-way adherent abs and allowed to dry overnight. The samples were carbon-coated and examined with an Apreo scanning electron microscope (Thermofisher Scientific)."

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

The sentence has been rephrased.
"After the inoculation, cells exhibited a lag phase and started to grow exponentially for 9 to 13 d before reaching maximum cell numbers."

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

The sentence has been rephrased.
"The cell abundance of Group A was slightly lower than Group B 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?

Error bars were added in panel Fig 4 (a, b). DIC samples were measured in one set in other

Why TA does not match with alkalinity? Which other species contribute to alkalinity here? Do you think that they als 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?

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_3^{2-} concentration and pH. TA will not decrease until the precipitation of Ca and Mg, which is responsible for the base remove from the medium. That explain the DIC decrease first (day 6) and TA decrease later (day 8) by the precipitation of $CaCO_3$ and $Mg(OH)_2$.

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

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 the atmosphere, causing chemical speciation of the DIC pool to shift toward higher CO_3^{2-} 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.

There is a strong positive correlation between Mg^{2+} 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_2 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

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

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?

Based on XRD results, the peak at 10 is loeweite, which is a sodium-magnesium sulphate hydrate [Na12Mg7(SO4)13 15H2O], found only in salt deposits of oceanic origin (Fang and Robinson 1970). Aragonite and ACC can be shown in a figure panel 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.

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^{2+} activity and low pCO_2 (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.

The story of Mg and brucite have been further discussed in revised manuscript according the advice from the reviewer.
First, not all brucite in cyanobacteria culture are dissolved after 8 days, which can be seen from the concentration of Mg. Thus, Brucite is detectable after 8 days.
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_2 is dissolved in water and changes the PCO_2 level and acid-base balance of the system. Hence, brucite can dissolve with acidification during the culture of cyanobacteria. It is suggested that the 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

We appreciate the suggestion of this part of discussion. In revised manuscript version, they were reduced to a single sentence.

Response to the comments by referee#2

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

The methodological have been re-organized following the reviewer's suggestion.

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.

Thanks for the comments. The specific calcium polymorph names are used at the right place in revised manuscript.

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.

We highly appreciate the term names clarification and understand that is preferable avoid any confusing terminology in the carbonate nucleation and precipitation. Nucleation of calcium carbonate mineral in the manuscript are deleted from the text.

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.

There are three alkalinities (total alkalinity, cytoplasmic alkalinity and carbonate alkalinity) used in the manuscript. We agree with the reviewer that we should make them clear to the reader.
Total alkalinity used in the manuscript is the measurement of water's ability to neutralize acids.
Intracellular alkalinity used in the early version of manuscript refer to the alkalinity released from the cell by lysis of bacteria. We revised it to the "Cytoplasmic bicarbonate" following Lisle and Robbins (2016).
Carbonate alkalinity calculates the amount of negatively charged carbonate and bicarbonate atoms in the solution. We talk about the carbonate alkalinity once in the text when we discuss the dissolution of ACC (P7 L28).
P6, L7 has been rephrased.

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.

The geochemical data are provided in a supplemental table.

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 CO2SYS/CO2calc are not always equivalent.

Carbonate chemistry was calculated by the program PhreeqC [version 3.3; Wateq4f database; United States Geological Survey (USGS), Reston, VA, USA], which has been used in previous cyanobacterial calcification research (e.g. Obst et al., 2009). Compared with CO2calc and CO2SYS, PhreeqC is more convenient to calculate magnesium-related mineral. We agree with reviewer that the saturation states are per polymorph and the calculated SI values from different geochemical modeling software are not always equivalent. Under this circumstances, we give the geochemical modeling analyses with CO2calc and Phreeqc separately as supplemental materials.

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.

We understand the concern pointed out by the reviewer regarding to the discussion section. We take the utmost care to refine the part of discussion. In revised manuscript version, some

part of discussion are reduced to a single sentence to expand the importance of viral induced calcification.

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.

The precipitate investigated by XRD showing particles of aragonite in viral lysate and brucite in bacterial culture. XRD results combined with chemical parameters change of the non-infected culture revealing that it is unable to calcify to the extent that a stable $CaCO_3$ precipitate was formed. As atmospheric CO_2 dissolved in water, acid-base balance of the system will be changed. Unstable mineral phases can dissolve with acidification. However, with the aid of the viral cycle and the lysis of the host, the dissolution of carbonate seemed not to happen in viral treatment, and a more stable mineral formed. Thus, we conclude that viral lysis of cyanobacteria 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.

We decided to tone down our statement and reformulate the discussion regarding intracellular calcification.

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?

It has been rephrased.
"Formation and dissolution of calcium carbonate is one of the most important process that can change the carbonate chemistry in sea water"

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.

Thanks for your advice. They are removed.

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

$CaCO_3$ biomineralization by cyanobacteria are considered as exclusively extracellular. The sheath structure of some species of cyanobacteria may play a role in the calcification process, but environmental influence are also crucial: the saturation state of the adjacent

water, which affects precipitation of calcium carbonate minerals, and the availability of dissolved CO_2, which affects photosynthesis (reviewed by Riding 2012/Science, VOL 336). Although it is the truth that calcification by coccoliths or bivalves are controlled mineralization and are significant in marine chemistry, cyanobacterial calcification processes introduced in our manuscript are non-controlled.

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.

Present manuscript focus on the important role of viruses in precipitation of carbonate minerals. This passage reviews the direct effects of viral lysis of microbe in marine system and illustrates why we turn to virus to discuss potential viral effects on CaCO3 biomineralization. Thus, it is important to the experimental design. We value the comments by the reviewer. We reorganize the passage and make it more reasonable and readable.

Pg 2: Ln 33. Which of the calcium carbonate polymorphs was capable of homogenous nucleation in the cited study?

The thermodynamics calculation proposed by Lisle and Robbins (2016) reveal that the activation energy for nuclei formation thresholds for all three polymorphs is significantly reduced but only vaterite nucleation is energetically favored. It has been described precisely in revised version.

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.

We appreciate the suggestion from the reviewer. Mg story is included in introduction in the revised version.
"Displacements of acid-base carbonic equilibrium in seawater can not only form calcium carbonate minerals, but also can lead to the precipitation of Mg(OH)2 (brucite) (Möller, 2007). It has been proposed that the dissolution of brucite in seawater is favourable for CaCO3 precipitation (Nguyen Dang et al., 2017)."

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

This passage reviews the research on viral particle acting as nucleation sites for different mineral precipitation. We refine the passage to make it more concise.

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.

Thanks for your reminding. It has been reparsed.
"Virus-related carbonate minerals are also reported in recent studies of biofilms from hypersaline lakes, where hypersaline carbonate minerals can precipitate at the surface of viral particles (Pacton et al., 2014; Lisle and Robbins, 2016; Perri et al., 2017). However, the pathway of precipitation of calcium carbonate onto the surface of viruses remains poorly understood."

(2) Here is an example of the confusing use of nucleation and precipitation in the same sentence.

The change is done accordingly.
"When combined with the release of cytoplasm-associated bicarbonate, which results in the formation of carbonate mineral energetically favored, and available viral capsids for surface-induced precipitation, the comprehension of viral influence on the precipitation of carbonate is extremely limited. "

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.

"Cyanophages, which infect this ecologically important group of cyanobacteria were isolate from the surface seawater from Sanya Bay." TEM image of cyanophage by negative staining is included in the revised version of manuscript.

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.

Thanks for your suggestion.
Although isolation and identification of cyanobacteria and cyanophage are out of scope of the main goal of the manuscript, they are used for simulation experiment. Detailed methods for isolation and identification are provided as supplemental file in revised version.

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

We agree with the reviewer that each of the method should be given a sub-section in revised version of manuscript. Changes are done accordingly.
"2.1 Cyanobacteria and Viruses
2.2 Culture conditions and calcification experiments
2.3 Measuring methods
2.3.1 Total alkalinity and dissolved inorganic carbon
2.3.2 Calcium and magnesium cations
2.3.3 Enumeration of cells and viruses
2.3.4 Electronic microscopy
2.3.5 X-ray Diffraction
2.4 Saturation indices calculation"
"Supplement
    Synechococcus sp. PCC 7177
    Viruses that infect Synechococcus sp. PCC 7177
    Saturation indices calculation"

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.

Data used for geochemical calculating are provided in revised version. The PHREEQC code used in the early version of the manuscript, as referred, is Wateq4f database. In order to make it more directly, we list it in the supplemental data files together with data calculated by CO2calc.

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.

The change is done accordingly. They growth curve is determined by the counting of autofluorescence during the culture of cyanobacteria.

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.

Cyanophage were enumerated from the culture by epifluorescence microscopy with SYBR Green I staining (Patel et al., 2007).

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 nonfluorescent 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.

Cyanobacteria have been isolated and purified using standard microbiological techniques. During all the treatment, operations are asepsis strictly.
Even if there is non-host and non-fluorescent bacterium, the culture media inoculation of the cyanophage will be turbid, which is caused by the growth of mixed bacteria. But Fig.3 b and d show the lysate are clear (Fig 3b and d). Even though it is not stained, non-fluorescent bacterium, if there is, may be seen from the optical microscope images. But this is conflicts with the optical microscope result. Under these conditions, we believe they are pure culture.

(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

In the earlier version of manuscript, we made the conclusion that cyanophage were responsible for the host mortality after comparing the growth curve of culture with and without inoculation of cyanophage. But even if it is, we agree with the reviewer that the abundance of virus particles are needed.

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.

Most of this section has been rewritten in revised version of manuscript.

(2) Throughout this section there are repeated references to different subsamples 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 unrealistic 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.

We understand the concern pointed out by the reviewer regarding to the data acquisition and data view. We have included in the supplemental material a table of raw data and a comparison between different geochemical model outputs.

(3) Was pCO2 measured during these experiments? If so, this method needs to be included and described.

We do not measure the pCO_2 during the experiments. Alternatively, we calculate pCO_2 with total alkalinity and DIC with the known temperature, salinity and pressure. This is included in the supplemental material in revised version.

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?

It refers to the photosynthetic bicarbonate uptake uptake and its conversion within the cell to carbohydrate.

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.

Data used for geochemical calculating are provided in a supplemental data files in revised

version.

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.

The change has been done accordingly.

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

We agree with the reviewer that more information and comments about the geochemical factors can be helpful. Data used for geochemical calculating are provided in a supplemental data files in revised version. The precipitation and dissolution of calcium and magnesium is mainly derived from the ion concentration in the medium. When we know the ion concentration change, we calculate the saturated state of the medium and show the calculated results supporting the precipitation or dissolution.

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.

We decided to tone down our statement and reformulate the discussion regarding intracellular calcification.
The part 4.3 in the early version of manuscript is reduced following the suggestion from the reviewer.

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.

Thanks for reminding. The discussion about whiting is deleted following the reviewer's suggestion.

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.

These two data sets are included in the revised version of manuscript.

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.

Change was done accordingly

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.

F/2 mediums reported in literature are prepared by stocks adding to filtered seawater. Only $NaNO_3$, NaH_2PO_4, Na_2SiO_3, trace mental stock and vitamin stock are defined. Here, the artificial seawater base modified from Harrison et al (2010).
We apologize for the mistaking of repeating Na_2MoO_4 $2H_2O$ in the table and missing $Na_2EDTA \cdot 2H_2O$.
The final pH of the media was adjust to 8.

Guillard R.R.L. and Ryther J.H. (1962) Studies of marine planktonic diatoms. I. Cyclotella nana Hustedt and Detonula confervacea Cleve. Can. J. Microbiol. 8: 229-239.
Guillard R.R.L. (1975) Culture of phytoplankton for feeding marine invertebrates. in "Culture of Marine Invertebrate Animals." (eds: Smith W.L. and Chanley M.H.) Plenum Press, New York, USA. pp 26-60.
Harrison P J, Waters R E, Taylor F J R. A BROAD SPECTRUM ARTIFICIAL SEA WATER MEDIUM FOR COASTAL AND OPEN OCEAN PHYTOPLANKTON. Journal of Phycology, 2010, 16(1):28-35.

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.

The pH values were calculated from the PhreeqC. Photosynthetic bicarbonate uptake and its conversion within the cell to CO2 by carbonic anhydrase lead to increased pH in the immediate vicinity of the cell (Reviewed by Ridding 2011).
In present study, when lytic rates run over the bacterial replication, photosynthesis ceases making the atmospheric CO2 dissolved in the water and changing the acid-base balance of the system. In present study, SI value care and Ω were determined following:
$SI = log\ \Omega = log[\ IAP/K_{sp\ Mineral}]$ wherein, $IAP = [Ca^{2+}][CO_3^{2-}]$
Thus, SI values in table 2 are higher than typical marine water.
We calculate the Ω of aragonite and calcite in CO2Calc in revised Supplemental table and the results also support our conclusion.

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

We agree with the reviewer and give the descriptions of cyanobacteria and cyanophage in a supplemental material in revised version of manuscript.

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

The citation is done accordingly.

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.

We agree with the reviewer that morphological evidence is not enough to discriminate the microbial and mineral. We attempted to take EDS or EDS-mapping of the encrusted structure. Unfortunately, SEM under EDS model could not acquire the morphology of particle with hundreds nanometers. Since we have decided to tone down our statement regarding intracellular calcification, electron microscopic photographs are revised following the reviewers suggertion.

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.

Fig. 10 are revised following the suggestion of reviewer on the mechanism of viral induced calcification. We try our best to explained it well in revised version of 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.

We understand the concern pointed out by the reviewer regarding to the supplementary Figure. Since the method of isolation and characterization of cyanopahge are needed in the revised manuscript, we interpret this figure together with the characterization of cyanophage in supplementary material.

Precipitation of Calcium Carbonate Mineral Induced by Viral Lysis of Cyanobacteria

Hengchao Xu[1, 2], Xiaotong Peng[2*], Shijie Bai[2, 3], Kaiwen Ta[2], Shouye Yang[1], Shuangquan Liu[2], Ho Bin Jang[3], Zixiao Guo[2]

[1]School of Ocean and Earth Science, Tongji University, Shanghai, China
[2]Deep-sea Science division, Institute of Deep-sea Science and Engineering, Chinese Academy of Science, Sanya, Hainan, China
[3]Department of Microbiology, The Ohio State University, Columbus, OH, USA

Correspondence to: X. Peng (xtpeng@idsse.ac.cn)

Abstract. Viruses have been acknowledged ~~as~~ to be important components of the marine system for the past two decades, but ~~the understanding of~~ their role in the functioning of the geochemical cycle ~~remains poor~~has not been thoroughly elucidated to date. Vir~~us~~al induced rupturing of cyanobacteria ~~is~~are theoretically capable of releasing intracellular bicarbonate and inducing the homogeneous nucleation of calcium carbonate~~,~~; ~~but~~ however, experiment~~al~~-based support for vir~~us~~al induced calcification is lacking. In this laboratory study, both water carbonate chemistry and precipitates were monitored during the ~~cyanophage~~viral infection and lysis of host cells. Our results show that viral lysis of cyanobacteria can influence the carbonate equilibrium system remarkably and promotes the ~~nucleation~~ formation and ~~precipitation~~stabilization of carbonate minerals. Amorphous calcium carbonate (ACC) and aragonite were evident in the lysate, compared to the brucite precipitate in non~~-~~infect~~ion~~ed cultures, implying that a different precipitation process had occurred. Based on the carbonate chemistry change and microstructure of the precipitation, ~~the viral induced calcification may initiate by rapid intracellular calcification because of the unfettered intracellular access of Ca²⁺ and react with cytoplasmic alkalinity after the virus attacks and breaks down the cell wall.~~we propose that viral lysis of cyanobacteria can construct a calcification environment where carbonate is the dominant inorganic carbon species. Numerous virus particles available in lysate may coprecipitate with the calcium carbonate. The experimental results ~~raised here~~presented in this study, first demonstrate the pathway and result regarding how viruses influence the mineralization of carbonate minerals. Furthermore, our results also imply that viruses play a crucial role in seawater carbonate chemistry and may balance the geochemical element budget within the earth system.

1 Introduction

Over the past several~~few~~ years, several studies have highlighted the urgent need to better understand the changes in sea water carbonate chemistry, which is one of the fundamental processes in the carbon cycle on both the global and regional scales~~.,~~ This is mainly because it is the primary buffer for the acidity of water in the earth-surface environment (Ridgwell and Zeebe, 2005; Martin, 2017; Zeebe, 2012). The ocean is recognized as a large carbon reservoir that contains approximately~~about~~

sixty times more carbon in the form of dissolved inorganic carbon than that in the pre-anthropogenic atmosphere (Zeebe and Wolf-Gladrow, 2009). Dissolved ~~inorganic~~ carbon ~~dioxide~~ in ~~the~~ typical surface seawater, with a pH of 8.2, is ~~occurs~~ mainly in the form of [HCO$_3^-$], compared to the speciation of [CO$_3^{2-}$] and [CO$_2$], with a ratio ~~at~~of 89 : 10.5 : 0.5 (Zeebe and Wolf-Gladrow, 2009). Formation and dissolution of calcium carbonate is one of the most important processes that can change ~~the~~

carbonate chemistry in sea water (Equation 1).

$$Ca^{2+} (aq) + 2HCO_3^- (aq) \rightleftarrows CaCO_3 (s) + CO_2 (aq) + H_2O \tag{1}$$
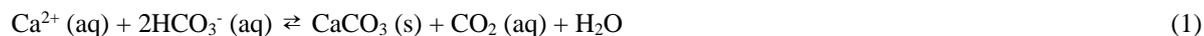
~~Carbonate in m~~Modern sea water is considered supersaturated with several calcium carbonate phases, such as calcite ~~saturation index ranging from 2 to 4~~ and aragonite. ~~But~~ However, there is no persistent precipitation of calcium carbonate in sea water because of the inability to overcome the energetic threshold for homogeneous precipitation. Marine calcium carbonate mineral is traditionally considered to be calcification ~~by~~production of coral, coccolithophores, foraminifera or pteropods. ~~When~~ The recent identification of an unaccounted fraction of marine calcium carbonate particles in seawater ~~was identified recently,~~suggests that ~~–~~ additional environmental factors affecting the formation of calcium carbonate should be further investigated (Heldal et al., 2012).

Cyanobacteria, which are ~~the~~ubiquitously abundant organisms and play important roles in most aquatic environments, are usually known to influence ~~the~~ CaCO$_3$ precipitation by taking up inorganic carbon via photosynthesis (Obst et al., 2009b; Planavsky et al., 2009; Yang et al., 2016; Kamennaya et al., 2012; Kranz et al., 2010; Semesi et al., 2009; Riding, 2011; 2012; Merz-Preiß, 2000) ~~and are recognized as major players in the formation of carbonate sedimentary deposits such as stromatolites (Riding, 2012;Altermann et al., 2006), the oldest one of which was formed by cyanobacteria and is possibly as old as 3.5 billion years (Schopf, 1993)~~. Although there is great importance in ~~the~~ calcium carbonate formation mediated by cyanobacteria in ~~the~~ sedimentary deposits, the ~~involved~~ mechanisms involved are still controversial~~poorly understood~~. In many cases, the precipitation of CaCO$_3$ by cyanobacteria ~~have~~has been invariably considered a non-controlled process, promoted either by photosynthetic uptake of inorganic carbon, raising the pH adjacent to cyanobacterial cells (Riding, 2006) or induced by cell-surface properties for the nucleation of CaCO$_3$ minerals (Obst et al., 2009b). In contrast~~Furthermore~~, diverse cyanobacterial taxa have been shown recently to form amorphous calcium carbonate minerals intracellularly, with a~~the~~ diameter of several hundreds of nanometers (Couradeau et al., 2012; Benzerara et al., 2014). The intracellular carbonate makes the mechanisms involved in calcification more confusing (Cam et al., 2015; 2016; 2018; Li et al., 2016;~~Cam et al., 2016~~). Thus, new pathways, in which ~~that~~ some biological processes alter the carbonate system, are ~~thus~~ important to evaluate.

Viruses, which are vital parasites of unicellular cyanobacteria, can modulate microbial production and, in some cases, can terminate plankton blooms (McDaniel et al., 2002; Bratbak et al., 1993; Bratbak et al., 1996; suttle, 2005; 2007). It has been established that 3-31% of free-living bacteria are infected by viruses, which can occur in excess of 10^5 infectious units ml^{-1} (Suttle and Chan, 1994). Hence, viral lysis of microbes is certainly thought to have direct effects on both ecosystem function involving the release of nutrients back into the environment and the host-involved geochemical reaction (Brussaard et al., 2008; Rohwer and Thurber, 2009; Weitz and Wilhelm, 2012; Jover et al., 2014).

There are an estimated 10³⁰ viruses in the ocean, the majority of which infect microbes, including bacteria, archaea, and microeukaryotes, and all of which are vital players in global geochemical cycle of key nutrient elements (Suttle, 2005, 2007;Brussaard et al., 2008). It has been established that viruses infecting specific hosts can be extremely abundant. For example, 3-31% of free-living bacteria are infected by viruses and can occur in excess of 10⁵ infectious units ml⁻¹ (Suttle and Chan, 1994). Hence, virus lysis of microbe is certainly thought to have direct effects on both ecosystem function involving the release of nutrients back into the environment and some host involved geochemical reaction (Brussaard et al., 2008;Rohwer and Thurber, 2009;Weitz and Wilhelm, 2012;Jover et al., 2014).

The thermodynamic calculation proposed by Lisle and Robbins (2016) infers that virus induced cyanobacteria lysate theoretically can elevate the saturation index of carbonate minerals at the cellular level, by releasing of cytoplasmic-associated bicarbonate (Lisle and Robbins, 2016). This thermodynamics calculation proposed by Lisle and Robbins (2016) also highlights that the released cytoplasmic-associated bicarbonate can be as much as ~23-fold greater than in the surrounding seawater, which can shift the carbonate chemistry toward the homogenous nucleation of calcium carbonate (such as vaterite). However, theoretical calculationsit does not take into account the condition in theoretically calculation that magnesium in seawater may influence the properties and behaviours of carbonatecalcite in seawater (Morse et al., 2007). Displacements of acid-base carbonic equilibrium in seawater can not only form calcium carbonate minerals, but also can lead to the precipitation of Mg(OH)₂ (brucite) (Möller, 2007). It has been proposed that the dissolution of brucite in seawater is favourable for CaCO₃ precipitation (Nguyen Dang et al., 2017).

Furthermore, viral particles could act as nucleation sites for precipitation of different minerals precipitation. In the past few years, researchers have investigated the theory that the capsid of viruses can interact directly with elements in solution,s and thus, potentially mediate the formation and precipitation of different minerals (Daughney et al., 2004; Kyle et al., 2008; Peng et al., 2013; Pacton et al., 2014; De Wit et al., 2015; Laidler and Stedman, 2010; Orange et al., 2010). It has been widely studied that viruses can interact with iron minerals under both marine system (including experimental conditions) (Daughney et al., 2004;Bonnain et al., 2016) and in the natural low pH acid mine drainage environment (Kyle et al., 2008;Kyle and Ferris, 2013). Thus, the iron-based flocculation of viruses has already been accepted as a simple and effective method to concentrate marine viruses for ecological study (John et al., 2011). Long-term and short-term experimental silicification was also studied for the simulation of hot spring mineralization and checking the preservation of viruses in the mineralized structure (Laidler and Stedman, 2010;Orange et al., 2011). The study of the hot spring biofilm by Peng et al. (2013) proved that viruses can be preserved in the natural environment. Recent studies have shown that hypersaline carbonate minerals can precipitate at the surface of viral particles and have implication to the nano-sized calcium carbonate structures in various geological settings (Pacton et al., 2014;Lisle and Robbins, 2016;Perri et al., 2017). All of the above viral mineralization studies are focused on heterogeneous nucleation, which occurs at nucleation sites on surfaces and is assumed to be much more common than homogeneous nucleation (Sear, 2014). When combined with the release of cytoplasmic-associated bicarbonate, which induces the homogeneous nucleation of calcium carbonate and available nucleation site for heterogenous nucleation, the comprehending of viral influence on the precipitation of carbonate is extremely poorVirus-related carbonate minerals are also

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reported in recent studies of biofilms from hypersaline lakes, where hypersaline carbonate minerals can precipitate at the surface of viral particles (Pacton et al., 2014; Lisle and Robbins, 2016; Perri et al., 2017). However, the pathway of precipitation of calcium carbonate onto the surface of viruses remains poorly understood. When combined with the release of cytoplasm-associated bicarbonate, which results in the formation of carbonate mineral energetically favored, and available viral capsids for surface-induced precipitation, the comprehension of viral influence on the precipitation of carbonate is extremely limited.

Laboratory ~~study~~ studies of viral calcification ~~was~~ were adopted here by culturing viruses and their host *Synechococcus* spp. PCC 7177 ~~and cyanophage~~. Such ~~modeling~~modeling experiments do not intend to mimic the processes occurring within the cells, which remain unknown, and generally do not provide an ultimate and direct answer as to which geobiological processes are involved in biomineralization. However, these experiments constrain, to some extent, the chemical conditions necessary to predict the geochemical processes similar to those in the aquatic environment. Carbonate parameters and cultural status were monitored to calculate the carbonate equilibrium system and saturation index. Precipitates of the culture were also characterized, to identify the microstructure of the minerals. Our results provide ~~gross~~ large-scale support of the importance of carbonate ~~nucleation~~ formation and ~~stabilization~~ precipitation during ~~viral~~ virus induced cyanobacteria mortality in the marine system. The extension of the viral role in mediating sea water carbonate systems will also provide an important, but previously ignored, carbon cycle in the earth system.

2 Methods and Materials

2.1 Cyanobacteria and ~~Cyanophage~~Viruses

Cyanobacteria, which have a long evolution history and are widespread in the marine environment, are key primary producers in the surface of the world ocean system. *Synechococcus* sp. PCC 7177 and the viruses that infected it were isolated from surface seawater from Sanya Bay (Supplement material). *Synechococcus* is a unicellular cyanobacterium that is very widespread in the marine environment and thus, is well-adapted for the present experiment (Fig. S1).~~(Fig. 1) and virus infected cyanobacteria (known as cyanophage) (Fig. 2) were isolated from the surface seawater from Sanya Bay. The isolation, purification and identification of cyanobacteria *Synechococcus* spp. is followed methods from Waterbury (2006). Cells of this strain are coccoid-shaped, and ~1.3 μm in diameter. Cyanophages were isolated using the double-layer agar technique with the method modified from Millard (2009).~~ Isolated virus particles, which are ~53 nm in diameter (Fig. S2), are classified as podovirus, based~~d~~ on ~~the~~ morphology and metagenomic analysis ~~(Supplement 1)~~(Fig. S3).

2.2 ~~Experimental Setup~~Culture conditions and calcification experiments

Cyanobacteria and viruses were grown at 25°C, under a photon irradiance of 6000 lux, with a 12 h light/dark cycle. Before the precipitation experiments, cyanobacteria were cultivated to harvest fresh cells for calcification experiments.

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Precipitation experiments were performed in sterile 4-L borosilicate bottles~~Cultures of cyanobacteria were grown at 25°C~~, in 0.2-µm-filtered artificial medium (based on F/2 media, Table 1). To minimise gas exchange with air, filtration was performed by means of a peristaltic pump. ~~Light intensity was provided in a 12:12 h light: dark cycle. Experiments were carried out in sterile 4-L borosilicate bottles.~~ To avoid artifacts caused by residues from previous precipitation experiments, the bottles were treated overnight in 0.1 M hydrochloric acid, rinsed with water several times and finally, stored overnight filled with water. 2000 ml of medium was added into each bottle. The headspaces of the bottle were continuously exchanged with ambient air via a plastic membrane. The inoculation and subsampling of each experiment was performed aseptically.

Two milliliters of fresh ~~For each treatment,~~ cells from the precultures were~~as~~ inoculated into each of 2 L of culture media. After the lag phase (day 5), ~~In one treatment, v~~5 ml of vir~~uses~~al stock ~~were~~ ($\sim 10^9$ virus particles per milliliter) was inoculated ~~at the fifth day~~in one treatment (Group A) to detect the potential viral influence on carbonate chemistry. The other treatment without inoculation of viruses was left as a control (Group B). Both treatments were run in duplicate, and subsamples from these incubations were taken simultaneously over the course of the incubations.

During the course, subsamples were taken from both treatments for analysis of the cell and virus concentration, total alkalinity (TA), dissolved inorganic carbon (DIC), calcium and magnesium concentration, and morphology of minerals. First, salinity was determined by measuring the apparent electrical conductivity. Cells and viruses were enumerated with a 1.5 ml solution. Filtrates (through 0.22 µm filters) were collected for TA, DIC, calcium and magnesium analysis. TA samples (30 ml) were stored in borosilicate bottles at room temperature. DIC samples (5 ml) were stored in borosilicate flasks without headspace at 4 °C. Subsamples for DIC and TA were poisoned with $HgCl_2$ solution to inhibit growth (Cao and Dai, 2011). At the end of the stationary phase (day 16), the particulate fraction of the medium (~1 L) was harvested via centrifugation (13000 g, 5 min) for electronic microscopy and X-ray diffraction study, using the methods adopted from Peng et al. (2013).

2.3 Measuring methods

2.3.1 Total alkalinity and dissolved inorganic carbon

Total alkalinity (TA) was determined by titration of 25.00 ml of medium samples with HCl solution from the volume of HCl required. The instrument and program ran automatically by *Metrohm* 916 Ti-Touch. The approximately 0.1 N HCl solution was ascertained by titration of solutions made from dried high-purity sodium carbonate and borax. The fluctuations of our total alkalinity determinations were approximately limited to 10 µM/kg.

DIC was measured by acidification of 0.5-1.0 ml of water samples at the Stable Isotope Laboratory, Third Institute of Oceanography, State Oceanic Administration, China. Measurements were performed with continuous flow isotope ratio mass spectrometry (Delta V Advantage, Thermo-Fisher Scientific Inc., USA), coupled with a GasBench II device.

2.3.2 Calcium and magnesium cations

Concentration of ~~M~~magnesium and calcium ~~cations~~ cations were determined by ion chromatography (*Dionex* ICS-900), after ~~0.02 μm filtering and~~ acidification by 1 ~~M~~N HCl. The precision of the IC method used ~~i~~was 2 ppm for Ca^{2+} and 5 ppm for Mg^{2+}.

2.3.3 Enumeration of cells and viruses

For determination of cell numbers, samples of 0.5ml were filtered on black nuclepore filters (25 mm, 0.2μm pore size, Whatman) under low vacuum (200 mbar). Cyanobacteria were counted within 72h under a *Leica* fluorescence microscope (Leica DM6B) with autofluorescence. The growth curves of the cultures were drawn through constant survival cell counts.

Enumeration of viruses from the culture was following Patel et al., (2007). Subsamples were first filtered (25 mm, 0.2μm pore size, Whatman) to remove bacteria and large mineral particles. Aliquots of filtered supernatant were filtered through 0.02μm- poresize Anodisc 25 membrane filter (Whatman, Inc.). The Anodisc filters were then stained with a final concentration of 25X SYBR Green for 15 min, mounted on glass microscope slides, and treated with an antifade solution. The slides were examined using an epifluorescence microscope (Leica DM6B) within 72 h. A minimum of 10 fields of view was examined per slide.

2.3.4 Electronic microscopy

Subsamples for the TEM study were fixed by the addition of glutaraldehyde (to 4% final concentration); they were then rinsed in distilled water to remove salts, mounted on copper grids and air-dried. The TEM analysis was conducted on a JEM-2100F field emission electron microscope operated at an accelerating voltage of 200 kV. Elemental analysis was conducted at 200 kV using an Oxford INCA Energy TEM X-ray energy dispersive spectrometer. Elemental maps were acquired in a STEM DF mode operating at 200 kV, with a focused electron beam (1 nm). The mineralogy of the structures in the areas of interest were determined using selected-area electron diffraction (SAED).

~~Subsamples from these incubations were taken to measure the microbial density and chemical composition of the medium after filtering and fixing. Cyanobacteria were counted under a *Leica* fluorescence microscope with autofluorescence. Magnesium and calcium cations were determined by ion chromatography (*Dionex* ICS-900) after 0.02 μm filtering and acid by 1 M HCl. The precision of the IC method used is 2 ppm for Ca²⁺ and 5 ppm for Mg²⁺. The TA samples (30 ml) were sterile filtered through 0.2 μm filters and stored in borosilicate bottles at room temperature and analyzed by potentiometric titration (*Metrohm* 916 Ti-Touch) within one week. DIC samples (5 ml) were sterile filtered by 0.2 μm filters and stored in borosilicate flasks without headspace at 4 °C. Subsamples for DIC and TA were poisoned with HgCl₂ solution. Salinity was determined by measuring the apparent electrical conductivity. To determine the activity of carbonate species and the degree of saturation in the solutions sampled, the geochemical computer program PHREEQC Interactive [version 3.3; Wateq4f database; United States Geological Survey (USGS), Reston, VA, USA] was used.~~

~~2.3 Electronic microscope and XRD~~

~~At the end of the stable phase, the particulate fraction of the residual medium (~1 L Fig. 1) was harvested via centrifugation (13000 g, 5 min) for electronic microscopy and mineral study with the methods adopted from Peng et al. (2013). In brief, subsamples for TEM study were fixed by the addition of glutaraldehyde (to 4% final concentration), rinsed in distilled water~~

~~to remove salt, mounted on copper grids and air dried. The TEM analysis was conducted on a JEM 2100F field emission electron microscope operated at an accelerating voltage of 200 kV. Elemental analysis was conducted at 200 kV using an Oxford INCA Energy TEM X-ray energy dispersive spectrometer. Elemental maps were acquired in a STEM DF mode operating at 200 kV with a focused electron beam (1 nm). The mineralogy of the structures in the areas of interest were determined using SAED.~~ For SEM analysis, dried ~~precipitation~~ precipitates ~~was~~ were fixed onto aluminum stubs with two-way adherent abs and allowed to dry overnight. 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).

2.3.5 X-ray Diffraction

XRD was employed to characterize the bulk mineralogy of the precipitates. The subsamples were thoroughly ground, followed by analyses using a LabX XRD-6100 X-ray Diffractometer with Cu Kα radiation (λ = 1.54056 nm) and a 2θ angle in the range of 10° to 80° at a speed of 1° min^{-1}.

2.4 Saturation indices calculation

To determine the activity of the carbonate species and the degree of saturation in the solutions sampled, the geochemical computer program PHREEQC Interactive [version 3.3; Wateq4f database; United States Geological Survey (USGS), Reston, VA, USA] was used. The saturation index (SI), defined as SI = log Ω = log[IAP/K$_{sp}$ Mineral], where IAP is the ion activity product (For CaCO$_3$, IAP= [Ca^{2+}][CO$_3$$^{2-}$]) and K$_{sp}$ represents the solubility product for a given temperature, was also calculated with PHREEQC. Although PHREEQC is more convenient for calculating magnesium-related minerals, we also used CO2Cal [version 4.0.9; United States Geological Survey (USGS), Reston, VA, USA] to calculate the carbonate system **for comparison.**

3 Results

3.1 ~~The g~~Growth of cyanobacteria culture

~~Cell growth was monitored over the course of 20 days. After the inoculation, cells did not exhibit an exponential phase for 4~5 days (Fig. 4a). For one treatment, cyanophage was inoculated to allow the adsorption and infection of cyanobacteria. The cell abundance of the viral group is slightly lower than the non-treated group (Fig. 4a). Although the cell abundance increases at the first few days, the growth rates of viral treatment are slightly lower and the cell number is reduced to 1.7×10^7 cell/ml compared to the 1.3×10^8 cell/ml of the non-virus treatment.~~ The color of the culture medium varied daily between the two treatments after ~~viral~~ virus induced lysis. ~~On the 13th day, viral treatment began to coagulate.~~ The supernatant of ~~the~~ Group A, which was inoculated to allow the adsorption and infection of cyanobacteria, ~~viral treatment~~ became clarified on the 14th day and seemed completely clear on the 17th day (Fig. 1b, d). ~~The non-virus treatment~~Group B, in contrast, ~~has~~ had some turbidity and higher cell density at the corresponding times (Fig. 1a, c). By the time cells were lysed~~deposited~~, the white

precipitation phase emerged in ~~the virus treatment~~Group A (Fig. ~~3~~1e~~b~~, d). Cell growth was monitored over the course of 20 days by counting the autofluorescence of cyanobacteria. After the inoculation, cells exhibited a lag phase and started to grow exponentially for 9 to 13 day before reaching maximum cell numbers (Fig. 2a). The cell abundance of Group A was slightly lower than Group B on the fifth day to eighth day (Fig. 2a). Although the cell abundance increased at the first few days, the growth rates of Group A were slightly lower and the cell number was reduced to 1.7×10^7 cell/ml, compared to the 1.3×10^8 cell/ml of Group B (days 19, Fig. 2a). The maximum number of virus particles occurred on the 9th day (Fig. 2a). Unlike the traditional viral one-step growth curve, virus particle in the present calcification experiment decreased with the ongoing process of calcification.

3.2 Change of carbonate parameters

The carbonate chemistry of the two treatments showed similar patterns during the ~~early and mid-exponential growth phases (<~~first 10 days~~.)~~ but started to deviate strongly in term~~s~~ of the total alkalinity (TA) and dissolved inorganic carbon (DIC) after~~wards, when~~ the cell lysis rates were greater than cell replication rates in Group A (Fig. ~~4b~~2b, c). As DIC transportation by the growth of cyanobacteria, there was a negative correlation between DIC and cell growth in Group B (Fig. 2a, c). When cultures were at the end of the exponential phase (Group B, days 14), the DIC declined to the lowest values. In group A, by contrast, the lowest DIC values was found on the 12th day, when the lytic rate began to dominate cell replication. DIC then rose to the initial level, because of re-equilibration with the atmosphere in the present open system~~The change of DIC was well-coordinated with the cell growth. When cultures were at the end of exponential phase (days 14), the DIC declined to the lowest. However, when cells lysed by the phage, the DIC rose again to the initial level because of reequilibration with atmosphere and increase of PCO₂~~. The TA of the two treatments also dropped ~~from the initial concentration of 3866 µmol/kg to the 1252 µmol/kg at~~during the exponential phase, reflecting a removal of cations from the solution (Fig. 2b). Compared to Group A, TA in Group B dropped to the lower level. During the lytic phase in Group A, TA increased again to values of 2936 µmol/kg in three days and ~~kept~~maintained balance during the lytic cycle. ~~Compared to the TA and cell lysis, there is an early TA increase.~~ Both calcium and magnesium cations were removed from the solution at the early-exponential growth phases (Fig. ~~5~~3). It is interesting to find that precipitated ~~cations~~calcium re-dissolve~~d~~ into the solution in ~~non-infection treatment~~Group B. In striking contrast, there ~~is~~was a persistent calcium removal within the viral lysate, indicating that robust vir~~us~~al-induced calcification had ~~happened~~occurred (Fig. ~~5~~3 a, b).

3.3 Microstructure of vir~~al~~us-induced carbonate precipitation

SEM and TEM images of the white precipitates from the viral lysate show~~ed~~ numerous calcium nanoparticles scattered or aggregated. These particles ~~are~~were in a spherical morphology, having diameters ranging from dozens of nanometers to hundreds of nanometers (Fig. ~~6, 7, 8~~4, 5). STEM mappings and XEDS analysis showed ~~that there is~~ evidence of calcium accumulation all around the particle surface (Fig. ~~7~~5), as well as ~~and~~ selected-area electron diffraction (SAED) patterns with ~~their~~ diffuse halos (Fig. ~~6b~~5b), confirming that they ~~are~~were amorphous calcium carbonate (ACC) (Rodriguez-Blanco et al.,

2008). Although Mg ~~is~~ was not dominant in the particles, there were signs of enrichment of Mg around the particles (Fig. ~~7c~~5c). SEM images of the nano-particle are attached to the surface of the infected cells and usually have an encrusted structure (Fig. ~~8~~4d)~~. However, occasionally, the inner core of the nanoparticles may lose (Fig. 8 d)~~. The bulk mineralogy of the cultural deposits, based on XRD analyses, were dominated by brucite in the non-infection treatment, compared to aragonite in the lysate (Fig. ~~9~~6).

4 Discussion

4.1 Carbonate chemistry influenced by the growth of cyanobacteria~~Carbonate chemistry influenced by viral lysis of cyanobacteria~~

Various studies in recent years have demonstrated the direct effects of carbonate chemistry shifts over the course of cyanobacteria growth (Dittrich et al., 2003; Kranz et al., 2010; Millo et al., 2012; Obst et al., 2009b; Yang et al., 2016). In cases where photosynthesis occurred, ~~it~~this results in the stimulation of cell division and DIC uptake, but no total alkalinity changes because no other sources of base are added during photosynthetic carbon uptake~~as follows~~:

$$HCO_3^- + H_2O \rightarrow CH_2O + OH^- + O_2 \tag{2}$$

Studies of cyanobacteria calcification always attribute the increase of pH to the growth of cyanobacteria which construct a favorable calcification environment where carbonate is the dominant inorganic carbon species and induces calcification by the incorporation of carbonate ions into a growing $CaCO_3$ crystal (Lee et al., 2004; Obst et al., 2009a; Kranz et al., 2010). It ~~is~~has been interpreted by the majority of ~~research~~ studies that cyanobacteria calcification is restricted to certain species (Merz-Preiß, 2000; Lee et al., 2004). The calcification~~mineralization~~ induced by photosynthetic acid-base equilibrium by *Synechococcus* sp~~p.~~. PCC 7177 in the present study seems to be transitional and unable to calcify to the extent that ~~a~~ stable $CaCO_3$ precipitate~~s~~ ~~was~~are formed. This~~It~~ is inferred based on the observation that cations are released again in~~to~~ the solution in ~~non-infection treatment~~Group B. Fixed Ca^{2+} redissolves to the concentration equivalent to the former concentration ~~and Mg^{2+} partial release~~ (Fig. ~~5a~~3a, b). On the 8th day~~8~~, however, there was an evident~~ly~~ decrease ~~of~~in calcium concentration, accompanied by a decrease of TA, which ~~imply~~implies~~ing~~ that calcium carbonate~~s~~ ~~were~~was formed and separated from the filtrate (Fig. ~~5a~~3a). Photosynthetic carbon uptake (Equation 2) raised the pH values of the medium, leading to the formation of $CaCO_3$. This $CaCO_3$ phase can be recognized as amorphous calcium carbonate, based on electron microscope images (Fig. 4, 5) and the fact that it is unstable. ACC~~It is the result of the fixation of calcium by high concentration of carbonate at high pH values.~~, which received relatively little attention as one of metastable $CaCO_3$ phases, has been increasingly recognized as a precursor for the formation of crystalline calcium carbonate (Cartwright et al., 2012; Weiner and Addadi, 2011). ACC may precipitate virtually instantaneously, when conditions promote high local supersaturation for short periods of time (Blue et al., 2017; Cartwright et al., 2012). Although the saturation index (SI) of the

ACC <0 (Table 2), implying nonspontaneous ACC formation within the solution, the growth of cyanobacteria in the present experiment created an ACC favorable microenvironment on days 8-10, reflected by the removal of Ca^{2+}.

Magnesium, which is actually precipitated as brucite from the solution, is also responsible for removing TA from the cyanobacteria culture. It has been demonstrated that supersaturated $Mg(OH)_2$ can precipitate at local alkaline conditions with pH > 9.30 (Möller, 2007). Saturation indices (SI), which are determined using the software PHREEQC, yielded values > 0 for brucite ($Mg(OH)_2$) during the first 8 days (0.34 ~ 1.15) and values < 0 after the 10th day (-1.47 ~ -0.15) in Group B (Table 2). As DIC transportation by cyanobacteria proceeded, the pH of the growth medium increased (Table 2, S1), thus, leading to the formation of $Mg(OH)_2$ in the supersaturated state (Equation 2 and 3). The emergence of brucite crystal formation have also been reported in coral microbial biofilms (Nothdurft et al., 2005) and cultures of diatoms (Tesson et al., 2008), where high pH and low pCO_2 microenvironments are created by biological activities such as the cyanobacteria in the present study (PCO_2 data can be found from the Table S1, calculated by CO2Cal).~~Consequently, with the growth of cyanobacteria, carbonate alkalinity limitation leads to the redissolve of ACC.~~

~~Magnesium, which is actually precipitated from the solution, is responsible eventually for removing TA from the cyanobacteria culture:~~

$$Mg^{2+} + 2OH^- \rightarrow Mg(OH)_2 \hspace{4cm} (3)$$

Upon the mass consumption of DIC by photosynthesis and fixation of calcium, cell growth seems to slow down (days 8-9, Fig. 2a). In the present open system, atmospheric CO_2 is dissolved in water and changes the acid-base balance of the system. Dissolved carbon is present in the form of bicarbonate at the present pH level:

$$2CO_2 + CO_3^{2-} + OH^- + H_2O \rightarrow 3HCO_3^- \hspace{3cm} (4)$$

The reaction represented by equation 4 in Group B, based on the continuous decrease of DIC (days 10-14). On days 9 ~10, there is an insufficient amount of carbon for photosynthetic carbon to be concentrated in the form of CO_2 or HCO_3^- (Miller et al., 1990). Nevertheless, cyanobacteria grew vigorously during days 10 -14 (Fig. 2a). The consumption of CO_3^{2-} and OH^- led the unstable minerals, such as ACC and brucite, to dissolve in the solution. Especially for the ACC, robust carbon concentration inhibits the transformation of ACC to a more stable carbonate phase such as aragonite. pH values calculated by PHREEQC after the 10th day were reduced to 8.66, at which $Mg(OH)_2$ formation cannot occur spontaneously. Consequently, with the growth of cyanobacteria, carbonate alkalinity limitation leads to the redissolution~~dissolve~~ of ~~ACC~~calcium and magnesium minerals.~~-~~

4.2 Carbonate chemistry influenced by viral lysis of cyanobacteria

~~As DIC transport by cyanobacteria proceeded, the pH of the growth medium increased, leading to the formation of Mg(OH)₂ under the supersaturate state (Equation 2 and 3). 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).~~

~~There is no surprise that biologically mediated brucite crystal formation emerges under high pH and low pCO_2 microenvironments created by cyanobacteria, which are similar to coral microbial biofilms (Nothdurft et al., 2005) and cultures of the diatom (Tesson et al., 2008).~~

~~In regard to calcium, despite the SI~~ ~~aragonite~~ ~~>0, the formation of stable carbonate (aragonite or calcite) was documented as improbable because of saturation states that could not develop or persist to overcoming the activation energy barriers for nucleation (Morse and He, 1993).~~ It seems that the two treatments of the tested culture grew at similar rates and reached similar cell densities during the first ~~10~~8 days, despite ~~the~~ inoculation ~~of~~with the ~~cyanophage~~viruses (Fig. ~~4a~~2a). However, when lytic rates r~~u~~an over the bacterial replication (day 8), Mg^{2+} ~~has been~~began to recover~~ed~~ to the initial level, but ~~the further removed~~ Ca^{2+} was ~~further removed~~ simultaneously present~~ing~~ ~~with~~ distinct variations between the two treatments~~compared to the non-infection treatment~~.

In regard to magnesium, which is immobilized from the solution in the form of brucite, the resulting mineral grains are unstable. It has been suggested that brucite may not be preserved over longer time frames, possibly being dissolved in the undersaturated state (Nothdurft et al., 2005). There is a strong positive correlation between Mg^{2+} and DIC recovered after the 12th day in Group A, which is the time point when the lytic rate begins to dominate cell replication. In the present open system, atmospheric CO_2 is dissolved in water and changes the ~~PCO_2 level and~~ acid-base balance of the system. Hence, ~~some of unstable mineral phases~~brucite can dissolve with acidification during the culture of cyanobacteria:

$$Mg(OH)_2 + \text{~~H_2CO_3~~}CO_2 \rightarrow Mg^{2+} + HCO_3^- + \text{~~2~~}H_2O \tag{~~4~~5}$$

~~The release of CaCO_3 + H_2CO_3 → Ca^{2+} + 2HCO_3^-~~ $\tag{5}$

extra bicarbonate releasing during the dissolution of brucite also contributes to the TA recovery after the 12th day.

On the other hand~~However~~, it should be noted that, with the aid of the virus, photosynthetic uptake of inorganic carbon decreases. Calcium, which is in the form of ACC during the growth of cyanobacteria, is formed in a stable carbonate environment, where Equation 4 cannot take place (days 8-12). The atmospheric CO_2 exchange is neutralized by $Mg(OH)_2$, as discussed above (days 12-16). ~~calcification is consistent and more stable aragonite minerals are formed.~~ Brucite dissolution in seawater controls the pH and Mg/Ca ratios and is recognized as a key factor inhibiting calcite and vaterite growth and precipitation (Nguyen Dang et al., 2017). Thus, a microenvironment favorable for calcification is available after the viral lysis of cyanobacteria and deposition of ~~stable~~ aragonite carbonate minerals~~are deposited~~.

~~4.2 Precipitation of carbonate mineral induced by virus lysis of cyanobacteria~~

~~The precipitate was investigated by means of XRD showing particles that can be described best as aggregates of aragonite in viral lysate and brucite in bacterial culture (Fig. 9). XRD results combined with chemical parameters change of the non-infected culture revealing that, despite the chemical environment, it may be favorable for calcium nucleation during the growth of cyanobacteria, which is unable to calcify to the extent that a stable CaCO_3 precipitate was formed. Only partial~~

Mg²⁺ may precipitate under the supersaturated state with the fact that both bivalent cations (Mg^{2+}, Ca^{2+}) cycled to the dissolved phase when the growth curves are in stable phase (Fig. 4a, b).

It has been extensively investigated that various physicochemical factors control the formation of the $CaCO_3$ polymorph and aragonite tends to precipitate under a high molar ratio of Mg/Ca (Folk, 1974;Berner, 1975). The microenvironment maintained by the growth of *Synechococcus* spp. could not overcome the activation energy barriers for the nucleation of aragonite. TEM images revealed no order in the majority of the detected particles, which was confirmed by the diffuse rings in the selected area electron diffraction patterns but the appearance of nanodomains within the ACC particles (Rodriguez-Blanco et al., 2008). However, with the aid of the viral cycle and the lysis of the host, the dissolution of carbonate seemed not to happen, and a more stable mineral formed.

4.3 Virus induced carbonate precipitation

The aggregates of aragonite in viral lysate were confirmed by the means of XRD (Fig. 6). It has been extensively investigated that various physicochemical factors control the formation of the $CaCO_3$ polymorph and aragonite tends to precipitate under a high molar ratio of Mg/Ca (Folk, 1974; Berner, 1975). Nevertheless, the microenvironment maintained by the growth of *Synechococcus* sp. PCC 7177 could not overcome the activation energy barriers for the formation of aragonite, according to the evidence presented by XRD and the carbonate chemistry changes in Group B. TEM images revealed no order in the majority of the detected particles (Fig. 4, 5), which was confirmed by the diffuse rings in the selected area electron diffraction patterns and the appearance of nanodomains within the ACC particles (Rodriguez-Blanco et al., 2008). Unlike Group B, with the aid of the viral cycle and the lysis of the host, the dissolution of carbonate seemed not to occur, and a more stable mineral formed in Group A. Although the theoretical calculation proposed by Lisle and Robbins (2016) indicates viral induced rupturing of cells released by cytoplasmic-associated bicarbonate, thereby dramatically reducing the activation energy for nucleation of carbonate polymorphs, experimental-based processes for the nonclassical multistep calcification is lacking.

Here, a possible model for calcium carbonate precipitation induced by viral lysis of cyanobacteria is proposed by regarding the carbonate chemical changes and microstructures (Fig. 7). As bicarbonate transport by cyanobacteria and intracellular conversion to CO_2 for photosynthesis proceeded, the pH of the ambient waters increased, leading to the formation of brucite and ACC. If no viruses were available, cell growth and replication would consume the bicarbonate. Atmospheric CO_2 dissolves in water but cannot do so in the form of CO_2 or HCO_3^- for photosynthesis, because of the high pH. Alternatively, dissolved CO_2 reacts with OH^- and $Mg(OH)_2$ resulting in dissolved carbon in the form of HCO_3^- for cyanobacteria. However, when cells are infected by viruses, there are a few percentages of uninfected cells that require bicarbonate. Only $Mg(OH)_2$ dissolved to neutralize the dissolution of CO_2 and precipitation of calcium carbonate will continue.

Preliminary investigations have also demonstrated that viruses from hypersaline lakes are incorporated in biogenic carbonate, suggesting that viruses may be mistaken for nanobacteria and may play a role in initiating calcification (De Wit et

al., 2015; Pacton et al., 2014; Perri et al., 2017). The viral drive during the biogenic carbonate precipitation in hypersaline lakes is attributed to either an indirect route, involving silicified viruses as an intermediate phase during diagenesis (Pacton et al., 2014) or a direct incorporation of amino acids polymerized with viral proteins into growing high-Mg calcite crystals (De Wit et al., 2015). The encrusted structure indicated by SEM images may support the hypothesis of carbonate formation on and near the virus particles (Fig. 4d). Coprecipitation of viruses and calcium carbonate is also supported by the number of viruses floating in the solution. Subsamples for enumeration of virus particles were filtered to remove precipitated minerals. The yield of the filtrate may preclude viruses incorporated in minerals. This is a reasonable explanation as to why virus numbers did not increase exponentially, despite the bursting of host cells (Fig. 2a). Viral infection~~The lysogenic cycle of Synechococcus spp.~~ does not result in the immediate lysi~~ng~~s of the host cell~~,~~; ~~but~~ dormancy occurs, while~~by~~ integrating their genome with host DNA and the replicati~~ng~~on along with it is relatively harmless. When the reproductive cycle initiates, the virus attacks and breaks down the cell wall peptidoglycan, which is an essential structure that protects the cell protoplast from mechanical damage and from osmotic rupture (Middelboe and Jørgensen, 2006). Thus, we propose that Ca^{2+} has unfettered access to the intracellular space and reacts with cytoplasmic alkalinity (Fig. ~~10~~7).

~~Preliminary investigations have also demonstrated that viruses from the hypersaline lake occur and are incorporated in biogenic carbonate, suggesting that virus may be mistaken for nanobacteria and play a role in initiating mineralization (De Wit et al., 2015;Pacton et al., 2014;Perri et al., 2017). The viral drive during the biogenic carbonate precipitation in hypersaline lakes is attributed to either an indirect route, involving silicified viruses as an intermediate phase during diagenesis (Pacton et al., 2014) or a direct incorporation of amino acids polymerized with viral proteins into growing high-Mg calcite crystals (De Wit et al., 2015). The encrusted structure indicated by SEM images may support the heterogeneous nucleation of carbonate formation (Fig. 8).~~ When Ca^{2+} access to intracellular space, the existence of the capsid synthetized by viral DNA provides a ~~nucleation site for~~surface for the initial calcification (Fig. ~~10~~7). ~~This heterogeneous c~~Calcification involves the addition of material to the preexisting viral surface~~s~~, which may be similar to the mineralized virus in microbial mat from hot springs (Peng et al., 2013) or~~-~~ hypersaline lakes (Pacton et al., 2014; De Wit et al., 2015; Perri et al., 2017).

Viruses are vital parasites of unicellular marine cyanobacteria modulating microbial production and, in some cases, terminating plankton blooms (McDaniel et al., 2002; Bratbak et al., 1993; Bratbak et al., 1996). The pathway ~~that~~ of vira~~lus~~-induced calcification during the lysis of the host cells was determin~~retriev~~ed by experimental study and expands the roles of viruses in marine ~~carbonate precipitation~~geochemical cycles.

~~4.3 Significance of viral induced carbonate precipitation~~

Owing to the fact that biologically mediated $CaCO_3$ precipitation is one of the fundamental processes in the carbon cycle (Ridgwell and Zeebe, 2005; Planavsky et al., 2009; Riding, 2011; Kamennaya et al., 2012), the study of viral impact is important ~~toward~~ in an understanding of the carbon cycling on both the global and regional scales. For example, ~~The~~ so-called "Whiting events", which refers to ~~an~~ events of high levels of suspended, fine-grained $CaCO_3$ precipitation, have long been ~~a~~ spectacular and extensively investigated $CaCO_3$ precipitation event~~s~~ due to their controversial~~. Although whiting events were recognized to be of abiotic~~ origin~~, mechanisms of formation and maintenance of "Whiting" are still debated~~ (Wright and Oren,

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2005; Morse et al., 2003). ~~Regarding the fact that cyanobacteria are the most abundant primary product and play important roles in the global carbon cycle, biologically-induced CaCO₃ formation is much more frequent than the abiotic processes under different conditions of seawater chemistry in the geological past (Obst et al., 2009b;Riding, 2011). The influence of photosynthesis on CaCO₃ precipitation in general is based either on the uptake mechanism of inorganic carbon (Riding, 2006;Riding, 2011) or surface induced mechanisms involving the cell surface or extracellular polymers as preferential sites for mineral nucleation (Obst et al., 2009b). With regard to viruses numbering, usually ten times more than their hosts (Suttle, 2005, 2007), viruses have been recognized as new agents of the nucleation site for the carbonate mineral (De Wit et al., 2015;Perri et al., 2017) and can influence carbonate chemistry notably (Lisle and Robbins, 2016). However, the viral influence on the "whiting event" is hardly know.~~ Clear evidence of net carbonate precipitation from the waters culturing cyanobacteria and ~~cyanophage~~viruses suggest that release of the virus during plankton bloom may stimulate vir~~alus~~-induced CaCO₃ precipitation, representing one potential whiting mechanism for CO_2 sequestration.

~~Plankton blooms are frequently known in few recent years as a result of extensive accumulation of algal and cyanobacteria population impulses by nutrient enrichment. Cyanophages are vital parasites of unicellular marine cyanobacteria modulating microbial production and, in some cases, terminating plankton blooms (McDaniel et al., 2002;Bratbak et al., 1996;Bratbak et al., 1993). Experimental studies proposed in the present work give a glimpse of carbonate precipitation during plankton blooms influenced by viruses and reinforced the great importance of viruses in global geochemical cycles.~~Furthermore, ~~I~~in view of the marine vir~~al us~~-induced carbonate deposit~~ion~~ and the increased Mg/Ca ratio of the medium (Fig. ~~5~~3c), the possibility that viral processes alter ~~the~~ seawater Mg/Ca rations, which are an important proxy for reconstructing the paleoenvironment (Lear et al., 2000), is thus important to evaluate.

~~Mg/Ca in benthic foraminiferal calcite have often been used as proxies to reconstruct the paleoenvironment. The basis for the Mg/Ca paleothermometry method lies in the determination that past seawater Mg/Ca (Lear et al., 2000). The reconstruction of seawater chemistry reveals that the Mg/Ca ratios were lower from the mid-Jurassic to the Oligocene than at present (Coggon et al., 2010). The higher Mg/Ca is also reflected by the greater abundance of aragonitic non-skeletal and biogenic marine carbonate since the early Cenozoic (Stanley and Hardie, 1998). The processes responsible for the increase in the seawater Mg/Ca ratio during the Oligocene were concluded to river discharge, sediment burial and hydrothermal exchange (Coggon et al., 2010). Cyanobacteria, which were recognized as the most ancient group, were responsible for the original oxidization of the Earth's atmosphere and dominated the elemental cycles over geological time scales (Des Marais, 2000;Hohmann-Marriott and Blankenship, 2011). Virologists have placed the viruses in the earliest phases of life's evolution and associated them with the primitive precursors of the cellular systems (Holmes, 2011). In view of the marine viral-induced carbonate deposit and increased Mg/Ca ratio of the medium (Fig. 5c), the possibility that viral processes alter the paleoenvironment is thus important to evaluate.~~

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5 Conclusion

First, we provide ~~here~~ a detailed view of <u>changes in</u> carbonate chemistry ~~change~~ and, mineral composition during viral infection and lysis of cyanobacteria. Amorphous calcium carbonate and aragonite were evident in the lysate, which differed substantially from <u>the lack of</u>~~no~~ calcification in the non-infect~~ion~~<u>ed</u> culture. We ~~also~~ inferred that ~~not only~~ <u>viral lysis of cyanobacteria can construct an environment of calcification, where carbonate is the dominant inorganic carbon species. Moreover, potential mechanisms involving viruses acting as nucleation sites are also discussed.</u> ~~photosynthetic acid-base equilibrium influence the carbonate chemistry; but that viruses may also play important roles in either releasing cytoplasmic alkalinity or acting as nucleation sites to balance the carbonate system.~~ Altogether, our results expand the <u>role of</u> vir~~al role~~<u>uses</u> in mediating sea water carbonate systems and provide new insights in certain ~~aspects of the~~ global geochemical process<u>es</u>.

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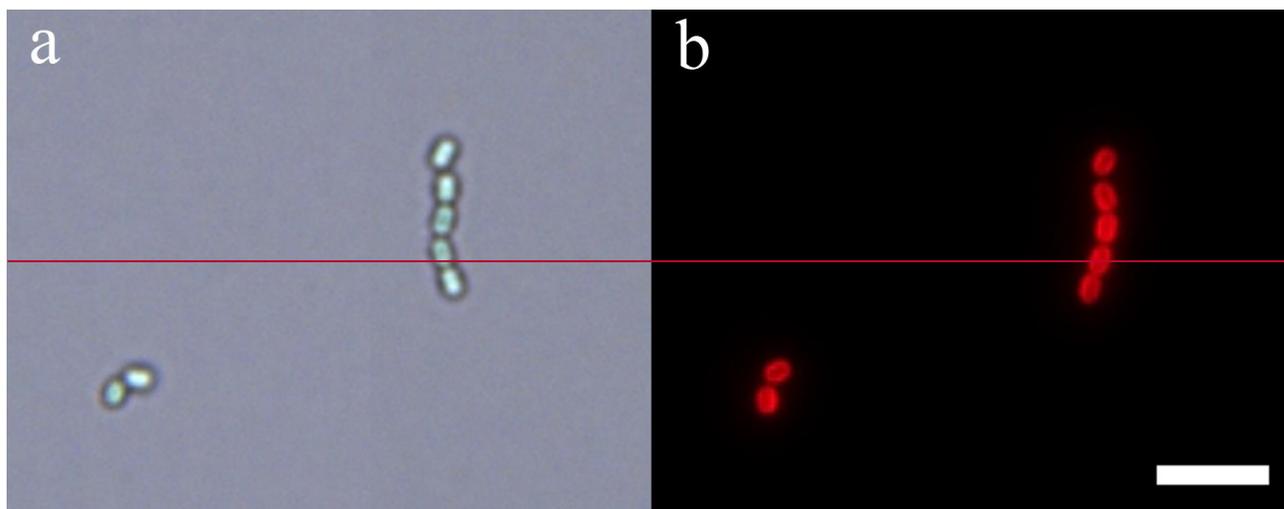
Figure 1: Strain of *Synechococcus* spp. isolated from Sanya Bay. Scale bar = 10 μm.
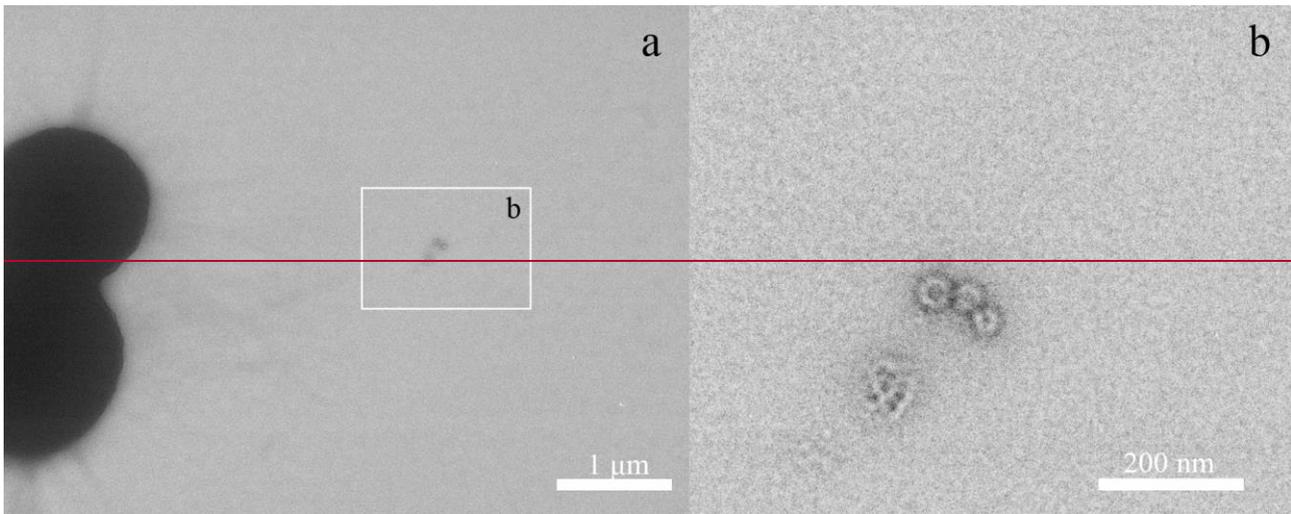
Figure 2: ~~Cyanophage isolated from Sanya Bay. (a) Cyanophage will infect the host cell of Synechococcus spp. (b) enlarged view of the cyanophage particles.~~
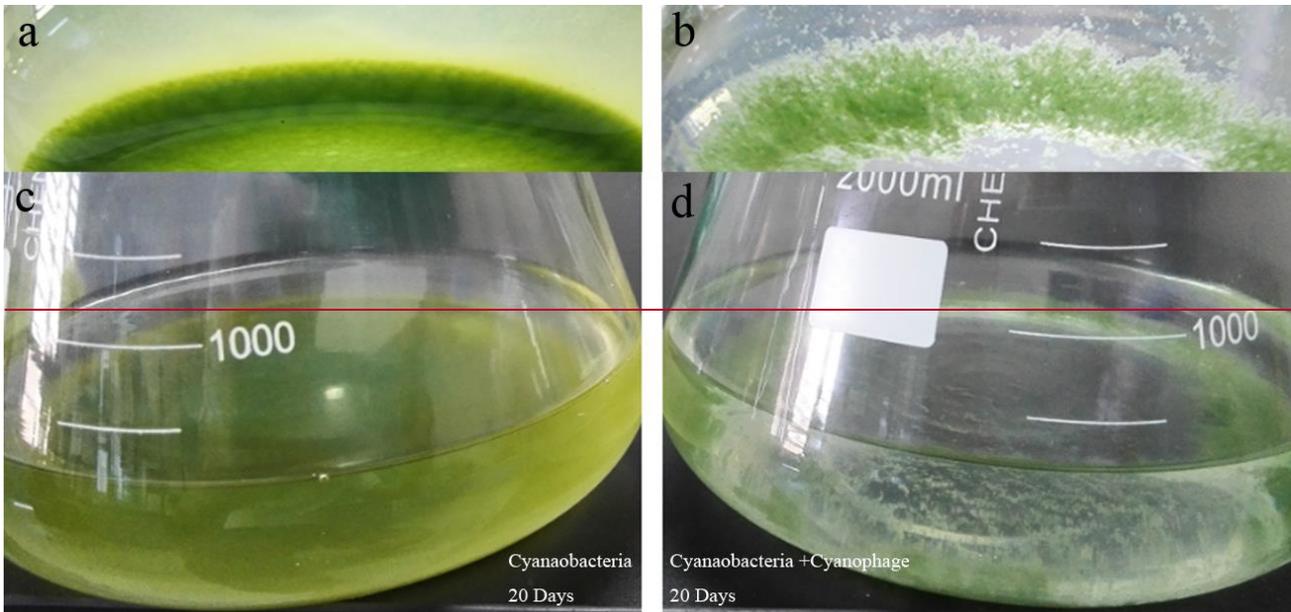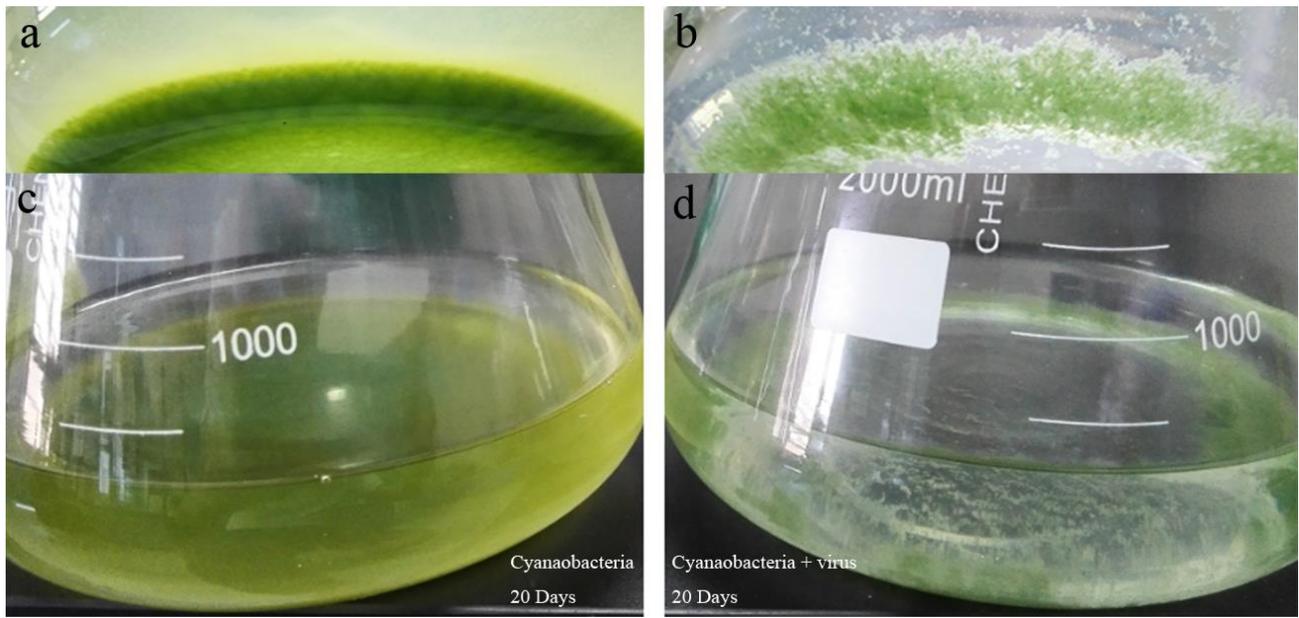
Figure 3~~1~~: Photos of the culture medium at the end of the experiments. (a and c) without inoculation of the ~~cyanophage~~virus. (b and d) white precipitates are evident in viral lysis of cyanobacteria.

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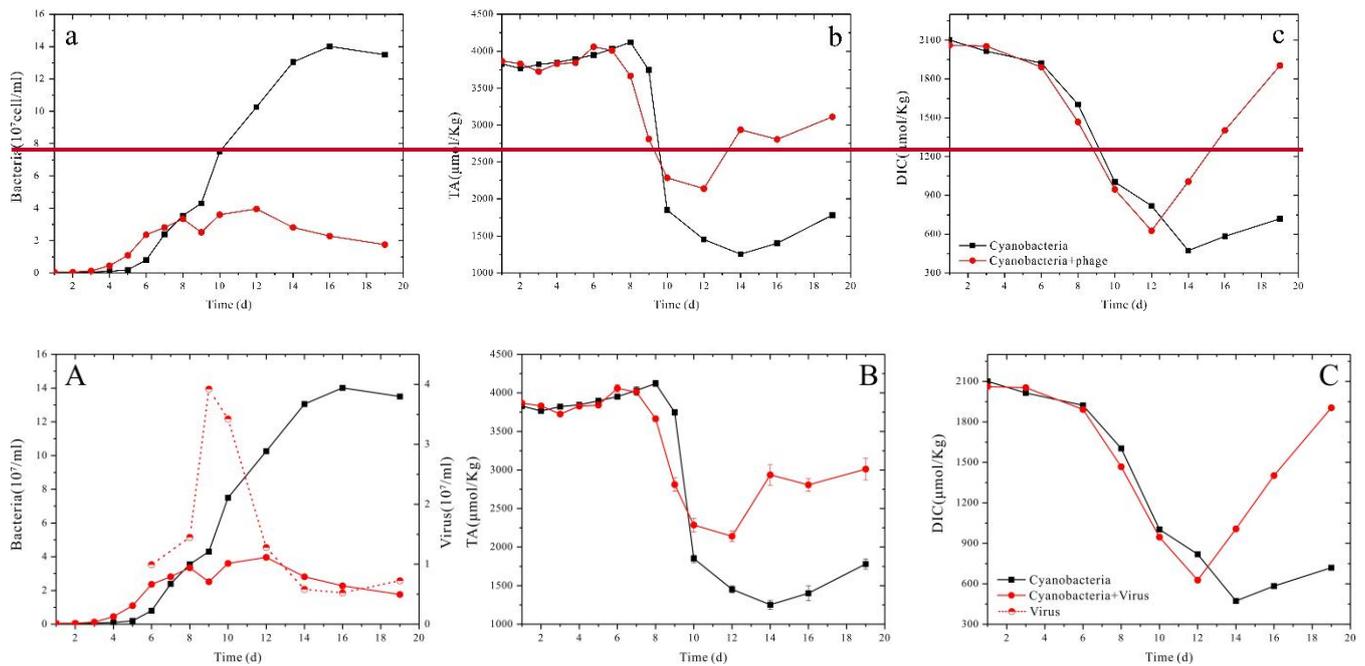
Figure 4~~2~~: Changes in the solution bacteria <u>and virus</u> concentration (a), total alkalinity (b) and dissolved inorganic carbon (c)
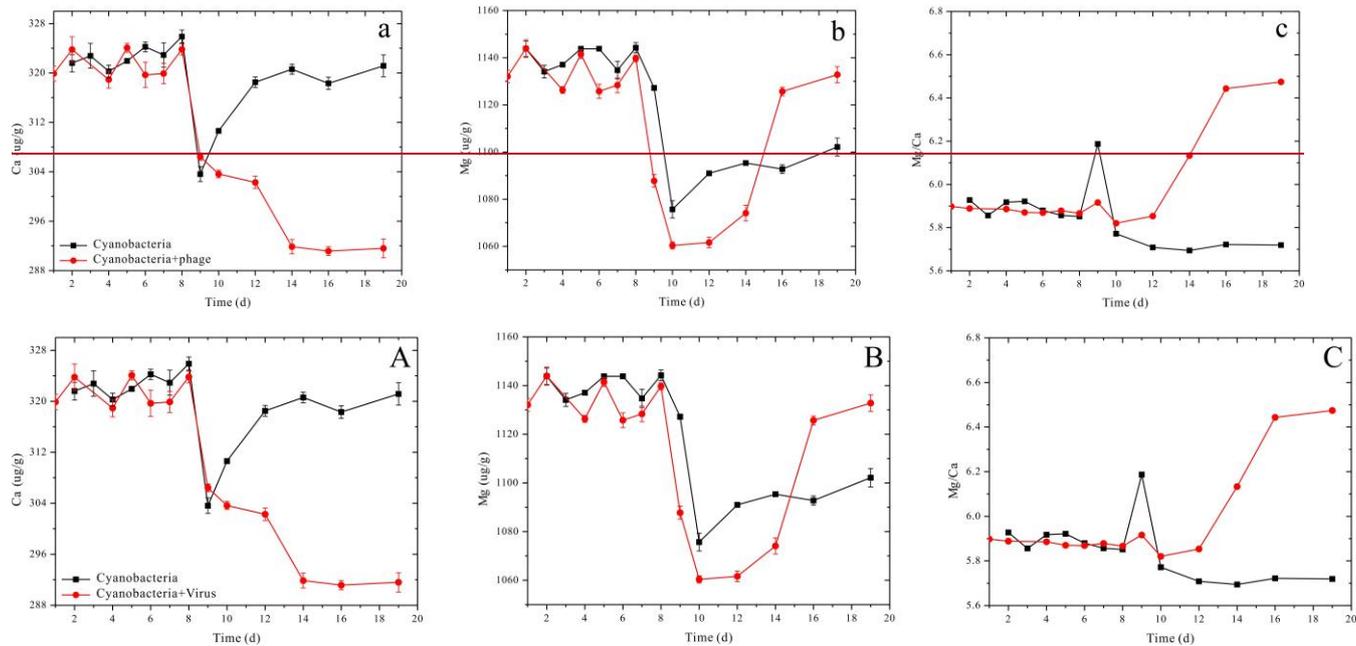
Figure 5̶3: Changes in the solution calcium concentration (a), magnesium concentration (b) and Ma/Ca atomic ratio (c).
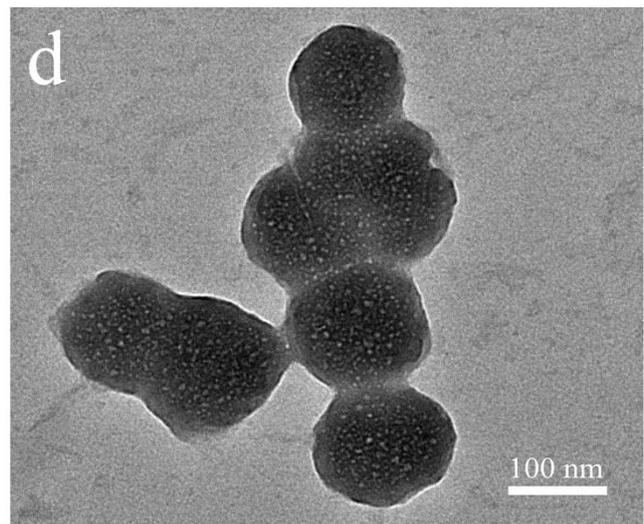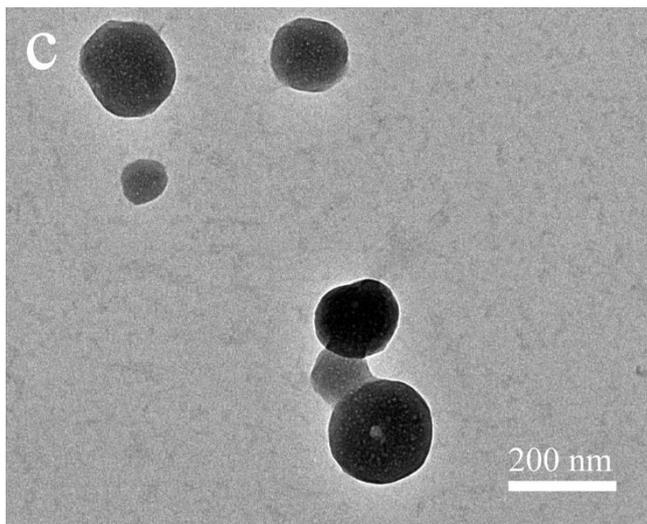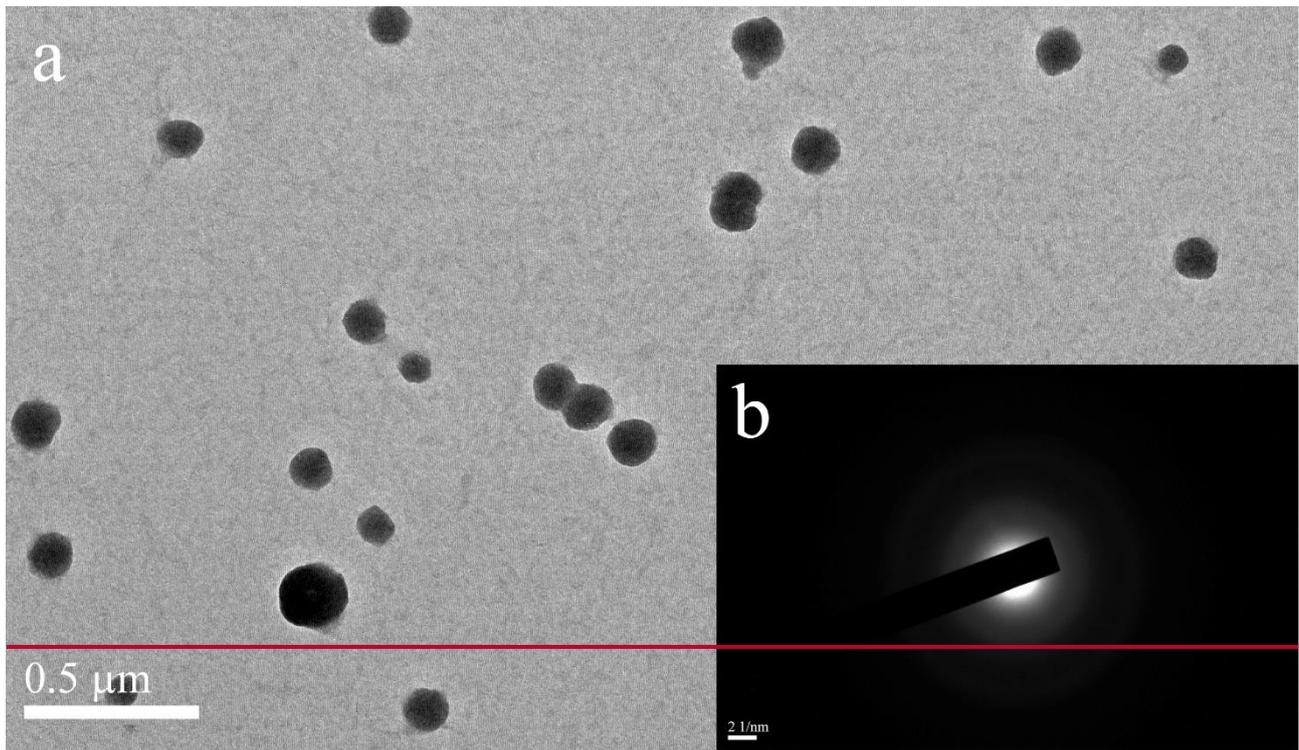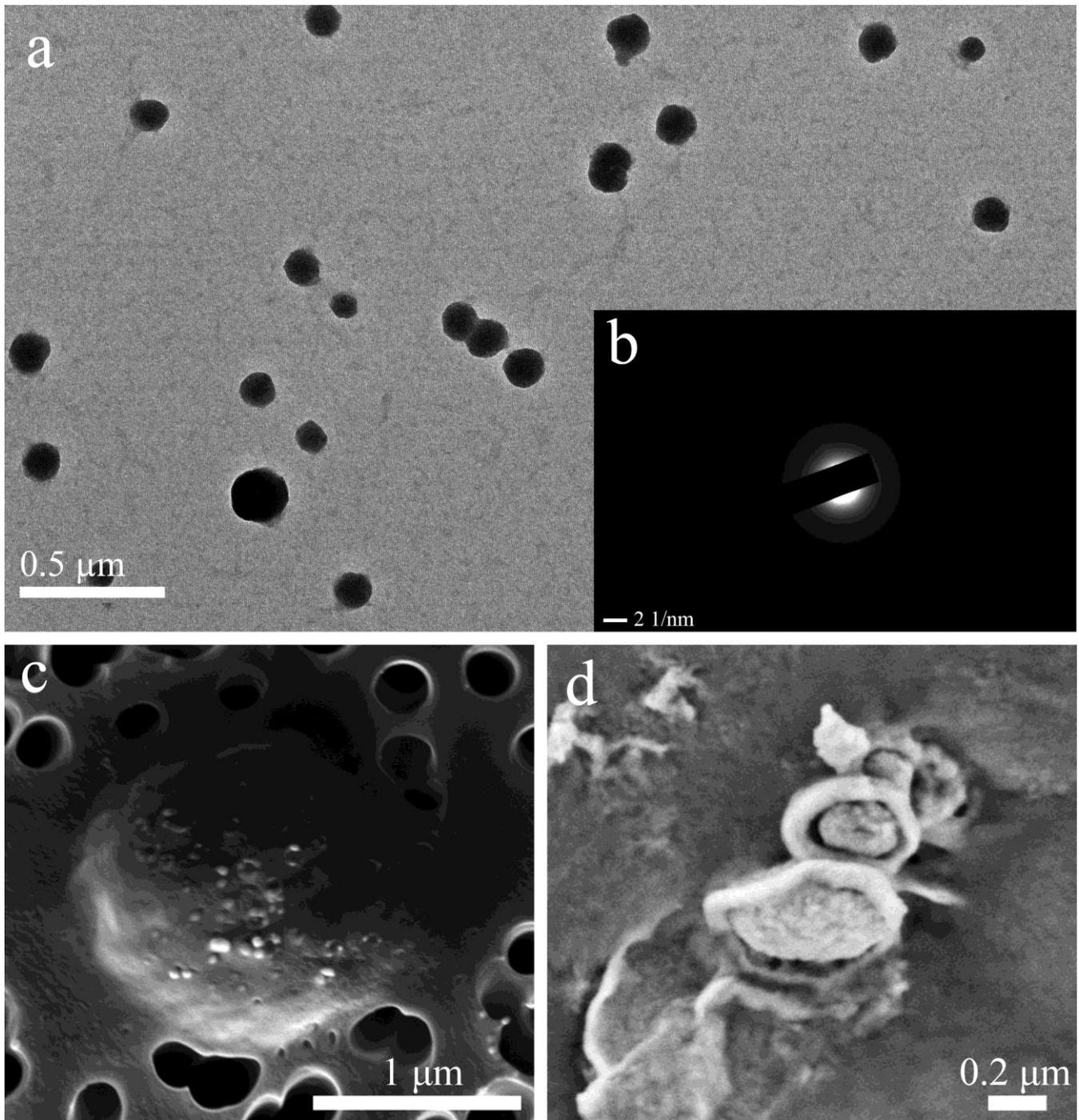
Figure ~~6~~4: ~~TEM~~ **Electron microscope** images show the ~~crystallization~~**formation** of ACC nanoparticles in the viral lysate. (a) Nanoparticles with a diameter **of** approximately 50-200 nm are scattered in the viral lysate. The insert ~~in~~ **at** the bottom right image (b) shows a selected area **of the** electron diffraction pattern of ACC, revealing only diffuse rings**,** related to poorly ordered materials **(c)** ~~and (d) Enlarged view of ACC nanoparticles showing that some of them aggregate together.~~ **Back scattered-electron imaging photomicrographs of host cells infected by virus and mineral particles. (d) Nano-particles with an encrusted structure.**

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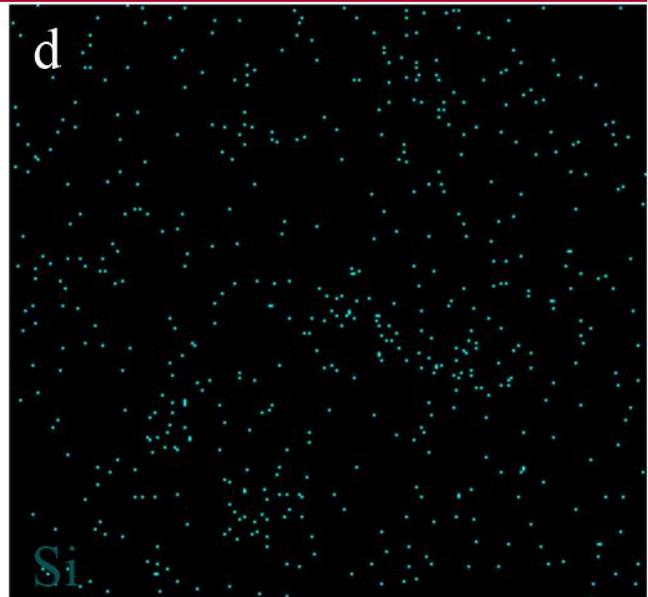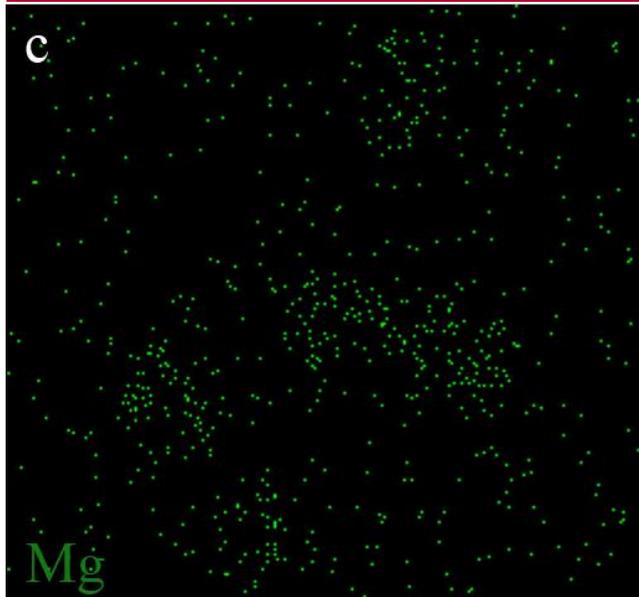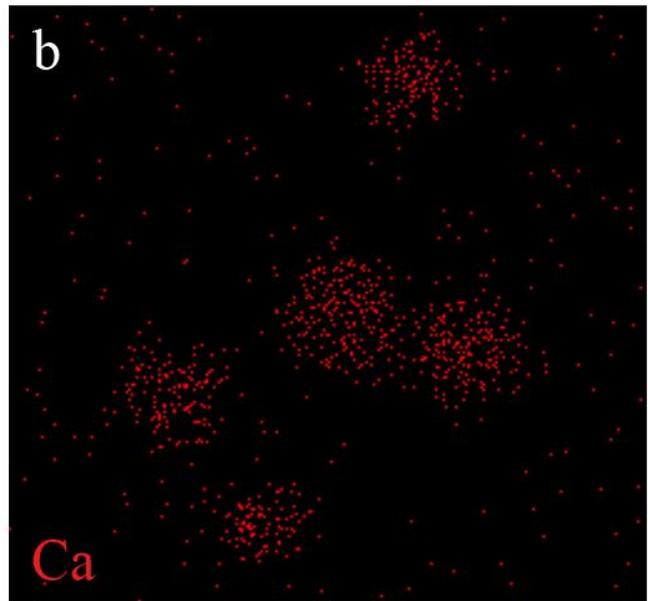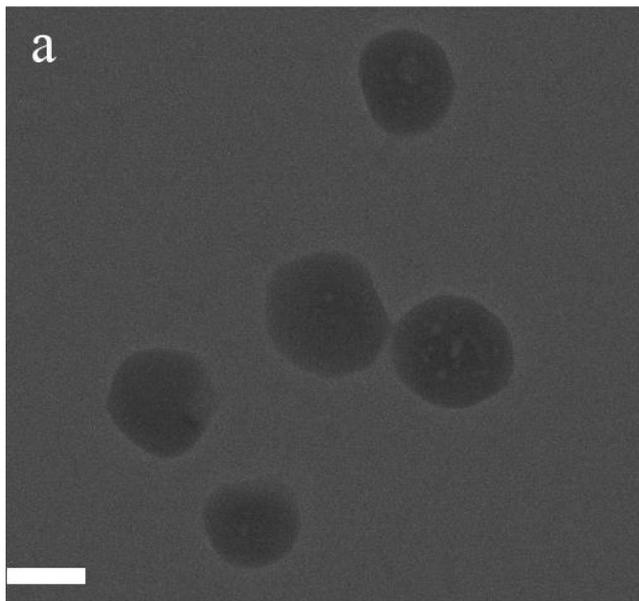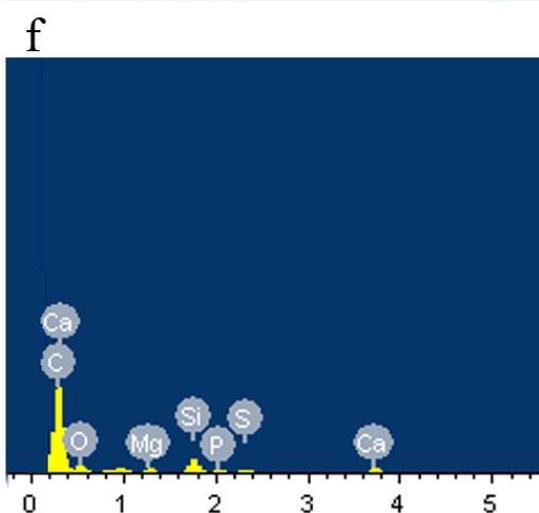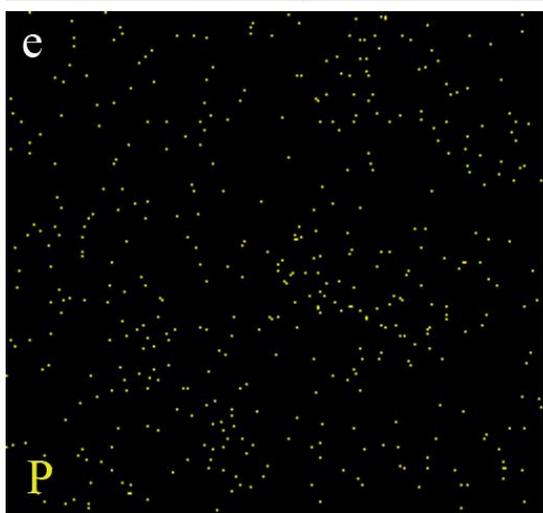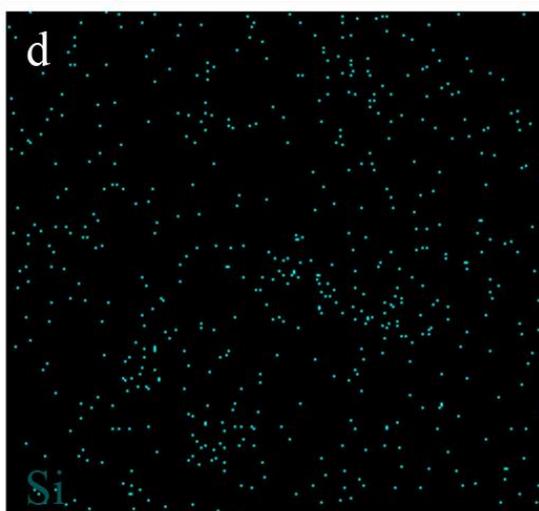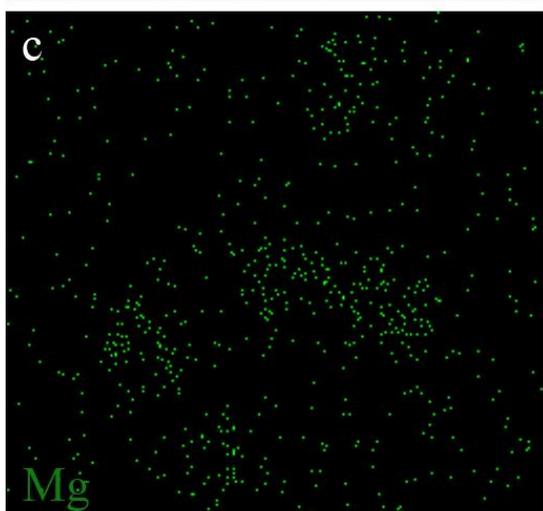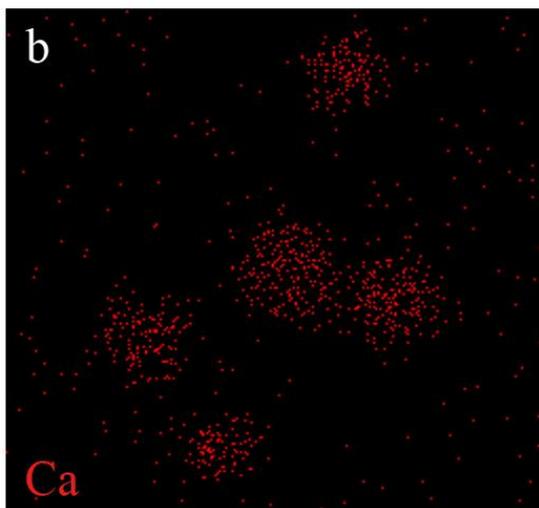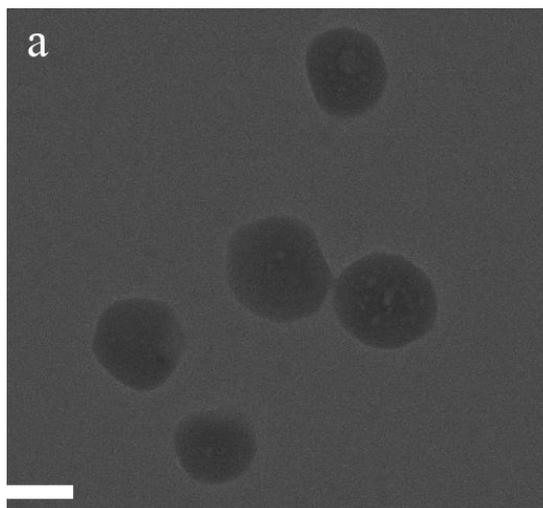
Figure ~~7~~5: **Chemical composition of ACC nanoparticles. (a) STEM images showing ACC nanoparticles. (b, c, d) XEDS maps of Ca, Mg and Si~~,~~ respectively showing that ACC is composed mainly of Ca. EDS spectra of the nanoparticle shows a small peak of element P (f). However, the P signal from the STEM mapping is not consistent with the nanoparticles (e).**
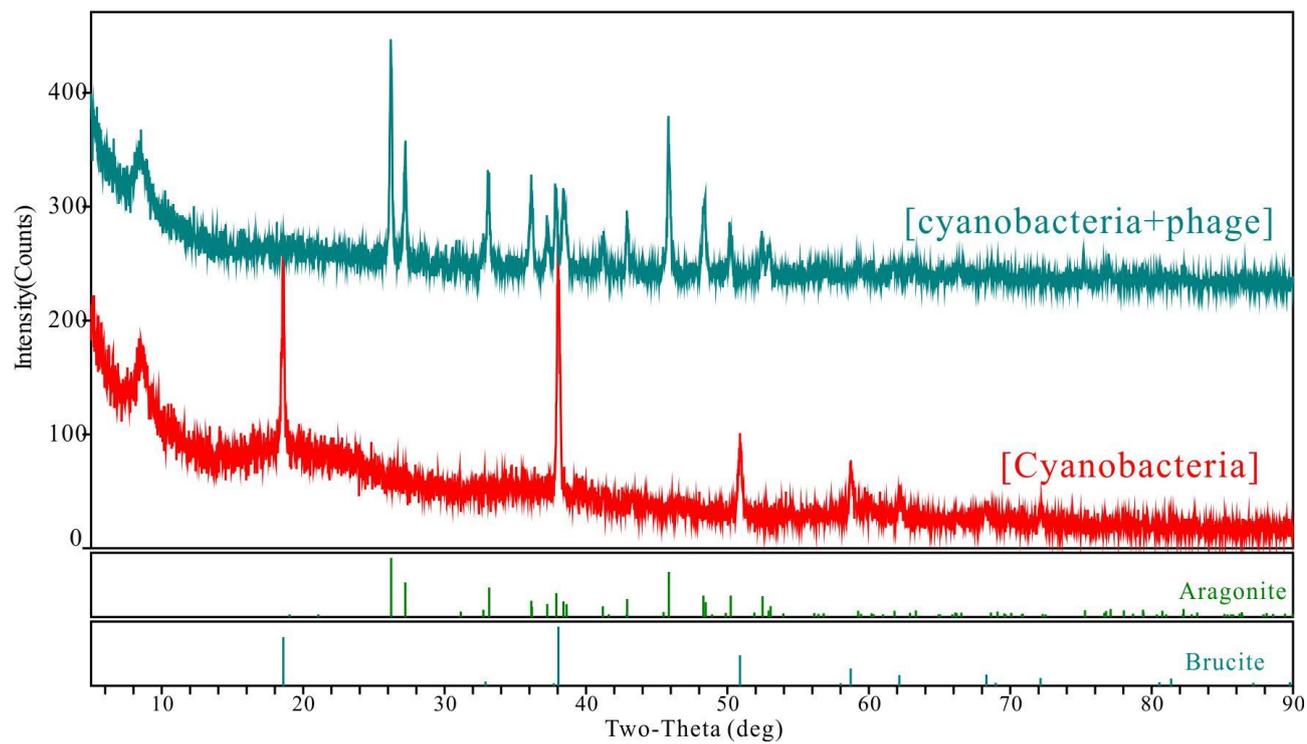
Figure 96: Typical X-ray diffraction patterns collected for each polymorph that formed in this study.
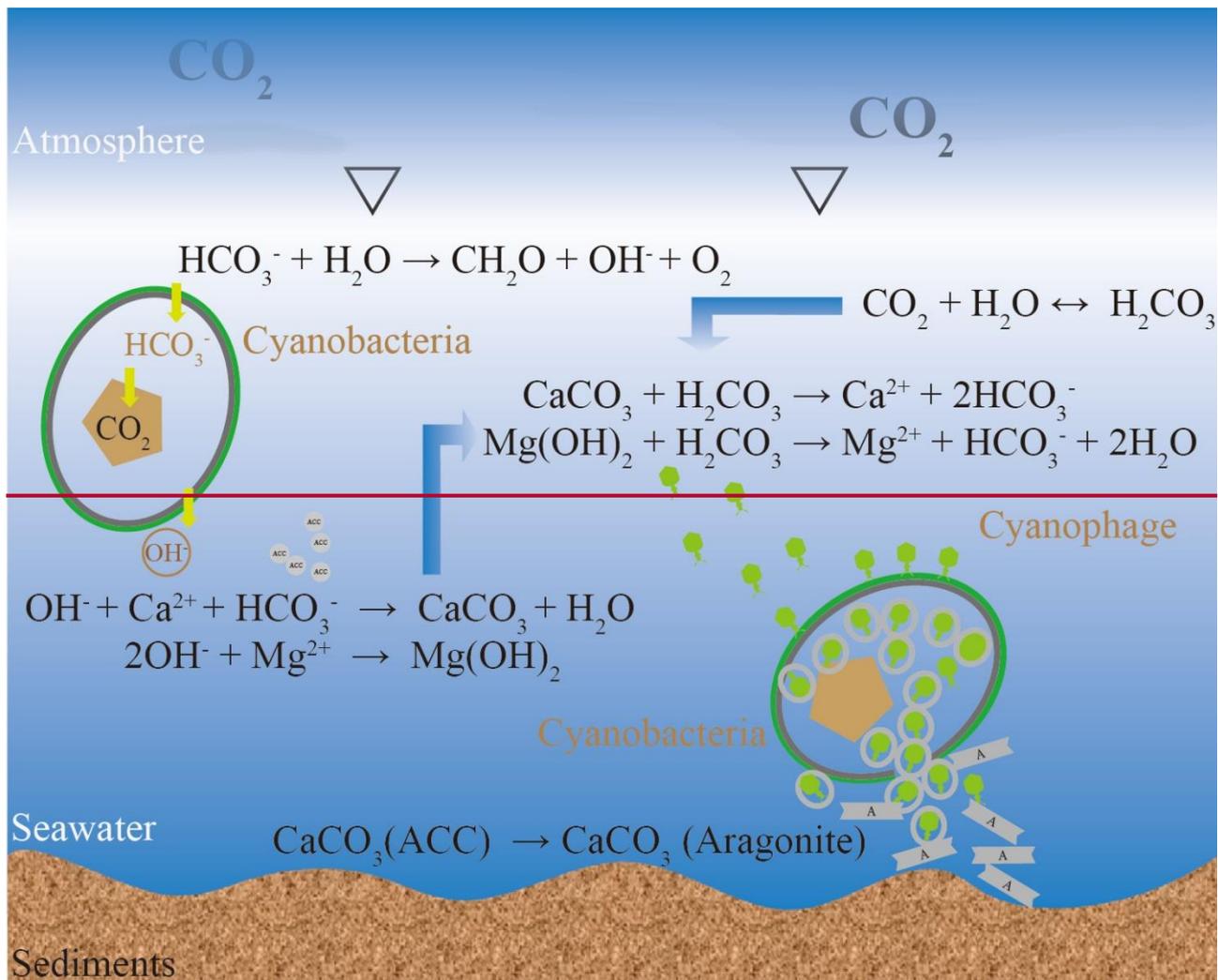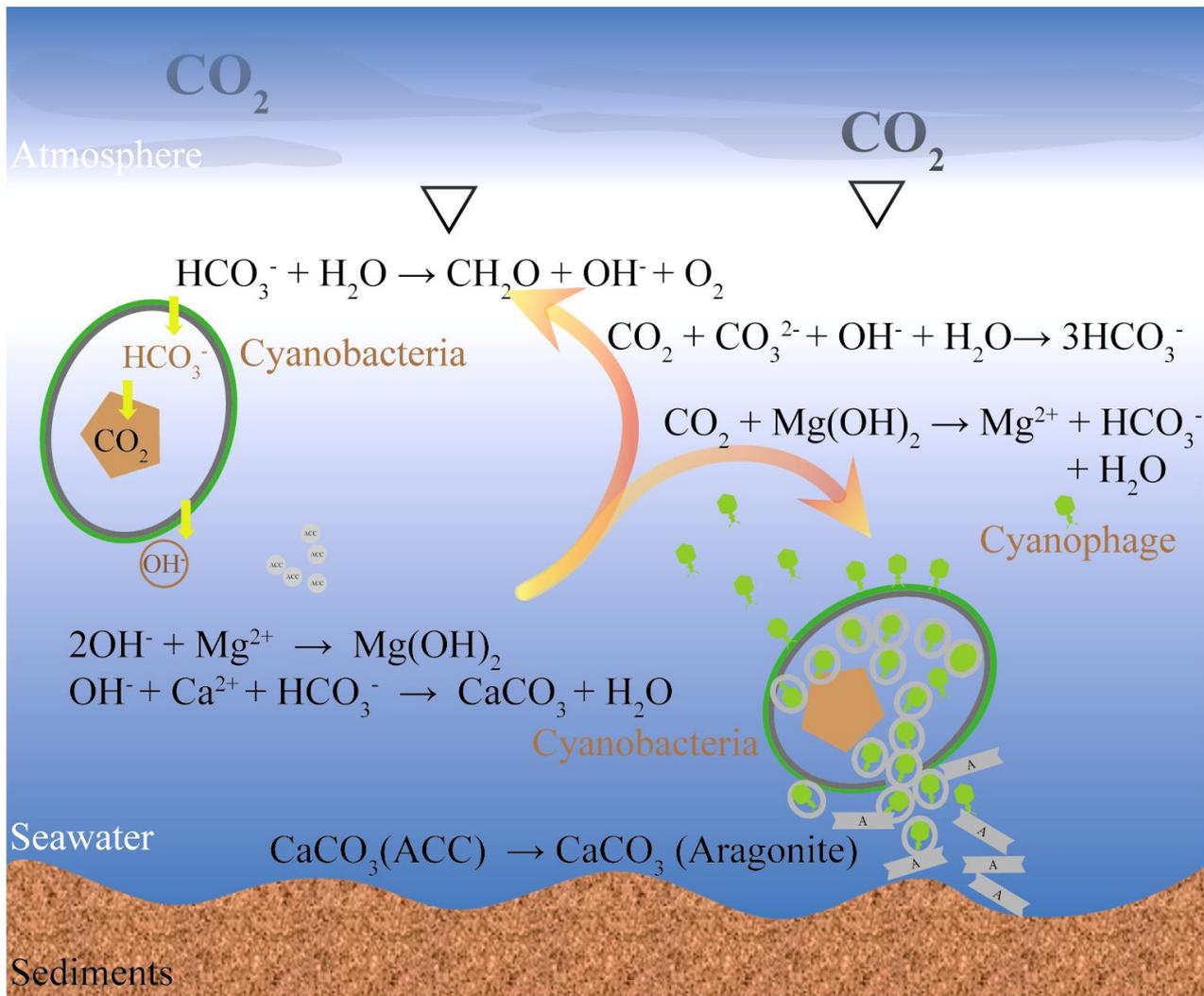
Figure ~~10~~7: Formation model of the carbonate mineral induced by viral lysis of the cyanobacteria. Although the chemical environment may be favorable for mineral ~~nucleation~~ precipitation during photosynthesis, it is unable to facilitate mineralizatione to the extent that stable $CaCO_3$ precipitates are formed. During the viral lysis of cyanobacteria, there are a few percentages of uninfected cells that require bicarbonate, $Mg(OH)_2$ dissolved to neutralize the dissolution of CO_2. Viral lysis of cyanobacteria thus construct a calcification environment where carbonate is the dominant inorganic carbon species. Numerous virus particles available in lysate may coprecipitate with the calcium carbonate.~~calcification was initiated by rapid intracellular calcification because of the unfettered intracellular access of Ca²⁺ reacts with cytoplasmic alkalinity after virus attacks and breaks down the cell wall. Meanwhile, more available nucleation sites (e.g., viral capsid) also stimulate the calcification.~~

Table 1 Composition of the artificial seawater (modified F/2 media, final pH was adjust to 8). Regents are purchased from Sinopharm and Sigma-Aldrich

Chemical	Amount (g/L)	Chemical	Amount (10^{-5} g/L)
NaCl	21.19	$CuSO_4 \cdot 5H_2O$	0.98
Na_2SO_4	3.55	$ZnSO_4 \cdot 7H_2O$	2.20
KCl	0.60	$CoCl \cdot 6H_2O$	1.00
$NaHCO_3$	0.29	$MnCl_2 \cdot 4H_2O$	18.0
KBr	0.09	$Na_2MoO_4 2H_2O$	0.63
H_3BO_3	0.02	$Na_2EDTA Na_2MoO_4 \cdot 2H_2O$	436
NaF	0.003	$FeCl_3 \cdot 6H_2O$	315
$MgCl_2\ 6H_2O$	9.59	vitamin B1	0.01
$CaCl_2$	1.01	Vitamin Biotin	0.00005
$SrCl_2\ 6H_2O$	0.02	Vitamin B12	0.00005
$NaNO_3$	0.075		
$NaH_2PO_4\ H_2O$	0.005		
$Na_2SiO_3\ 9H_2O$	0.03		

5

Table 2 Saturation indices calculated from culture system by PHREEQC.

	Time (d) saturation index	6	8	10	12	14	16
	pH	9.56	9.96	8.66	8.95	9.32	9.29
	Aragonite	1.34	1.35	0.56	0.69	0.65	0.73
Cyanbacteria	Brucite	0.34	1.15	-1.47	-0.89	-0.15	-0.2
	ACC	-0.72	-0.71	-1.50	-1.37	-1.41	-1.33
	vaterite	0.92	0.93	0.14	0.27	0.23	0.31
Cyanbacteria + Phage	pH	9.65	9.85	8.87	9.89	9.93	9.74
	Aragonite	1.36	1.3	0.68	0.92	1.12	1.1

Brucite	0.52	0.92	-1.06	0.93	1.06	-0.02
ACC	-0.70	-0.76	-1.38	-1.14	-0.94	-0.96
vaterite	0.94	0.88	0.26	0.50	0.70	0.68