Biogeosciences Discussions

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Reply on RC1

Refat Abdel-Basset et al.

Author comment on "Manifestations and environmental implications of microbially-induced calcium carbonate precipitation (MICP) by the cyanobacterium *Dolichospermum flosaquae*" by Refat Abdel-Basset et al., Biogeosciences Discuss., https://doi.org/10.5194/bg-2021-146-AC1, 2021

RC1: 'Comment on bg-2021-146', O.S. Pokrovsky, 02 Sep 2021 reply

This paper is devoted to experimental study of cyanobacterium-induced precipitation of CaCO3. The topic is generally well studied, and fits the scope of the journal. However, the overall quality of this research, its design and interpretation are below the standards of academic journal and rather suit for some applied journal audience.

The major problems are the following:

1) There is no information on $CaCO_3$ saturation state during the experimental run and even at the beginning of experiments. One cannot study CaCO3 precipitation without having an idea of solution saturation state

The process of CaCO3 formation, dealt with in this work, is microbially induced, which depends on (micro)biological metabolism, rather than on chemical or physical conditions including solution saturation. Microbial walls (or sheaths) act as nucleation sites, which concentrate calcium ions regardless of solution concentration and microbial metabolism (particularly urease activity) catalyze precipitation.

2) There is no kinetic assessment of pH and Ca concentration evolution in the course of experiment. It is thus impossible to assess the rate of the process and the dynamics of bacterially induced precipitation. The 4-weeks duration of experiment is not justified; the growth curve Is not presented.

Neither the rate nor the dynamics were targeted in this work; only the final magnitude of pH, growth and product were concerned. Rate and dynamics may need a sperate work.

3) The effects of anions and Ca are not distinguished. In addition to Ca salts, Na salts should be used if the authors aim to characterize the effect of citrate, for instance.

Why should Na citrate be used, for instance? Sodium salts do not precipitate. To distinguish the effect of anions, three different anions citrate, acetate, and chloride are studied. Meanwhile, only cation, calcium, is at concern.

4) 100% BG-11 used in the experiments contain unreasonably high PO4 concentration, totally irrelevant to natural settings, especially for P-limited lakes. Not only PO4 is a strong

inhibitor of calcite precipitation, it also provide unrealistical conditions for cyanobacterial growth. The application of obtained results to lakes is unwarranted.

BG11 is a standard enrichment medium widely used for growth and enrichment of cyanobacteria and algae since a long time (since Rippka and Herdman 1993). Yes, PO4 in BG11 is high as it is used as phosphate buffer of the medium. The cyanobacterium used has been isolated from a freshwater lake (Stechlinsee, Germany) but the unialgal culture is not applicable for natural lakes but shows the ability of the organism at defined conditions of not only phosphorus but rather at constant temperature, pH, nutrients, etc.., which are not the case in nature.

5) Alkalinity titration of unfiltered solution is not suitable. Part of H+ will be used for i) cell surface adsorption, ii) HCO3- neutralization, and iii) CaCO3 dissolution. The authors cannot distinguish between these 3 processes

This is a standard method used in the literature for titrating carbonate; all fractions are supposed to be constant e.g. cell surface adsorption.

Several specific comments below.

L 27-29 unclear. What is the driving force, photosynthesis or precipitation?

Photosynthesis was the driving force for precipitation

L32, unclear, why Chl a is not dependent on cell number in a monocultural experiment

The confusing part has been removed from the text.

L64 Ca does not coprecipitate. It precipitates as CaCO3

OK, changed in the text.

The link between 2nd and 3rd § of the Introduction is unclear

The text and information in the two paragraphs have been modified to clear confusion and be more synchronized.

L179 It is unclear where these ratios are shown

They were existing in a former version but removed from this version, as one of the reviewers recommended that; these are now indicated in the manuscript as "data not shown"

L203-205 How do we know that this release is not dependent on the identity and concentration of anion? Otherwise it is inconsistent with what is stated in L 154-157

This is an assumption that the cells of the same organism release similar amounts of calcium since the release itself has been found. No link between these two results; one for calcium release and one for growth.

L 246-248 This is not shown in the resent work ; no phosphate analysis !

The sentence is "Calcium precipitates carbon in the form of calcium carbonate either chemically or microbially and precipitates phosphorus in the form of calcium phosphate."

It does not refer to experimental analysis; it is a fundamental scientific information

supported by references on several occasions in the manuscript.

L252-253 contradicts to what is state din L203-204

L252-253:

The studied concentrations (0, 1, 1.5, 2 and 4 mg Ca2+ L-1) were chosen from previous records in the literature (Weyhenmeyer et al 2019), which considered these concentrations critical.

L203-204:

Therefore, a virtual concentration of total calcium is given to account for the externally supplemented concentration of calcium (0, 1, 1.5, 2 or 4 mg Ca2+ L-1) and the amount of calcium found at calcium-deprivation i.e., 2.26 mg Ca2+, which is assumed to be equally released by each culture.

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No contradictions or even a link

The first is cited from the literature while the second are validated values based on experimental estimates.

L257-258 This is self-contradictory. Why 1.5 ppm if cells released 2.26 ppm?

The "1.5 ppm" refers to the externally supplemented concentrations before cells grow and secrete.

L263265 unclear, and unsupported. What about bicarbonate level and buffering?

Removed

L277 Not really? S.I. of CaCO3 is more important

A lot of references support the notion that alkalinity is a prerequisite for microbial CaCO3 formation. e.g.:

"Most calcite precipitation occurs under alkaline conditions of pH values from 8.7 to 9.5 (Ferris et al 2003; Dupraz et al 2009)."

The superiority of carbonate over alkalinity may be found in physico-chemical systems as microbially induced CaCO3 precipitation may rely on collecting CO2 with water to form bicarbonate then carbonate.

L287-288 Irrelevant without pH/pCO2 parameters

L287-290:

"In addition, it has been stated that calcium carbonate can be formed at very low solubility levels in pure water before precipitation; its solubility in pure water is as low as 13 mg L-1 at 25°C (Aylward et al 2008); it increases relatively with decreasing temperature and increases in rainwater saturated with carbon dioxide, due to the formation of more soluble calcium bicarbonate."

This is a statement from the cited references, not mine.

L290 What does it mean, solid phase

The sentence states: “soluble”

L290-293 This is not correct. There are many quantitative laboratory studies of $CaCO_3$ precipitation kinetics and mechanisms in the presence of cyanobacteria

I could not find any publications despite several google searches. Contribution of cyanobacteria are rich but as a population in ancient structures.

L294-297 This is irrelevant to the discussion of results of this study

It is actually in the discussion of this study.

L308 This is not correct. The OH^-/HCO_3^- exchange during photosynthesis is by far the most important process

- However, this line does not deal with anything about photosynthesis.

L345-346 This is not assessed in this study

The conclusion is a “citation” supported by the reference.

L364-366 This has been shown well fifty years before Berry

Yes. The paragraph also contains a sentence referenced at the year 1975.

L354-382: The purpose of this § and its relevance to the present work are unclear. This is not a discussion of obtained results

Removed

L393-394 Unclear

Reformulated

L400-401 The cost will be quite elevated and thus commercially not interesting

OK

Figures: legend is unclear

Revised and reformulated

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Oleg S Pokrovsky