

Biogeosciences Discuss., author comment AC2
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Reply on RC2

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.,
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RC2: 'Comment on bg-2021-146', Anonymous Referee #2, 05 Sep 2021 reply

This paper investigates the capability of cyanobacterium *D. flosaquae* to induce MICP. The results of the study rely on the measurement of the growth parameters, and a few solution chemistry parameters (Ca, pH, TA, NH₃). Based on these datasets, the authors claimed that the *D. flosaquae* induces precipitation of CaCO₃ (calcite). I find that assertion poorly supported by the presented data and paucity of other (necessary) datasets (saturation state, microscopy/spectroscopy observations, abiotic controls) needed to support the findings claimed in the paper. In light of these issues, I find that study is not up to the standards of the journal and will be more appropriate for another journal. Below are my more elaborate comments:

1) The introduction lacked a clear motivation for the study. Several statements in the introduction were not properly referenced, which is surprising given that MICP is a very well-studied field.

Done

2) Lack of any abiotic control in the experiment design makes it difficult to infer whether the formation of CaCO₃ was due to *D. flosaquae* or merely due to changes in the solution chemistry.

BG11 medium is daily used in our lab and doesn't contain carbonate to precipitate calcium.

3) No information on the saturation state of the solution with respect to carbonate and other minerals is included in the results and discussion.

The process of MICP, which under study at this work is (micro)biological; all other physical and chemical components are equal at all experimental samples i.e. their impact is equal, if any.

4) The time evolution data on the growth parameters, and dissolved Ca concentrations are not included, which again makes it challenging to fully understand the evolution of the

system under different treatments

Kinetics of the process was not targeted in this work; only the net product or net result was the aim. Once again, a microbiological system is different from the physico-chemical system.

5) In the current design of the incubations, the effect of Ca concentrations and anions cannot be decoupled. This results in confusing interpretations of the Figures. It has been actually decoupled. Three different anions are included to show the effect of each anion at a certain calcium concentration and different calcium concentrations to show the effect of calcium.

6) Lastly, the authors refer to the possible CaCO_3 phase as calcite, but yet provide no data that confirms whether the carbonate is calcite.

We have never referred to our possible CaCO_3 phase as calcite since we did not plan to identify it; the word calcite has been mentioned only in the cited literature and reference list.

Specific comments:

Line 41: references missing.

Added.

Line 52-55: The phrase is difficult to understand at first. Recommended to revise it

Reformulated

Line 56: why is "ate" in carbonate in parentheses?

Carbon(ate) refers to carbon or carbonate.

Now, it is changed to carbon/carbonate in the submitted version.

Line 56: The use of co-precipitation to define CaCO_3 is incorrect. Co-precipitation implies that 2 or more phases precipitating simultaneously. In CaCO_3 , it's just one phase.

OK, changed to "precipitation".

This is although the two phases, in the course, are calcium and carbonate, which coprecipitate forming calcium carbonate.

Line 58: "Calcium and phosphate also coprecipitate..." phrasing unclear. Do Ca-phosphate co-precipitates with CaCO_3 forming a solid solution? Please revise

Yes, but the terminology used in this case is "binding" as follows:

Furthermore, any calcium carbonate precipitate is a good binder of phosphate (Yanamadala 2005), mentioned in Line 247-248.

Line 62: it would be helpful to add some references or some examples of key industrial

applications.

Reference and some applications have been added.

Line 64: misuse of word co-precipitation (see comment on Line 56)

OK, changed

Line 76-77: Why is the cyanobacterium *D. flosaquae* chosen for this study? What is its environmental importance? A brief description of the cyanobacterium itself and reference to any previous studies would be helpful to better understand the context of this manuscript.

Done

The hypothesis of this work is to explore whether *Dolichospermum flosaquae* is able to perform MICP in freshwater lakes.

Line 87: where was the strain procured from? Is it axenic?

D. flosaquae is a major temperate cyanobacterium; the strain used in this work has been isolated from Stechlinsee lake (IGB Berlin). It was not axenic.

Line 90-93: There is no sterile control for the experiment (i.e. BG11 with no cells). This is a crucial problem as the authors are proposing the *D. flosaquae* is able to induce CaCO₃ precipitation.

Done

BG11 medium is daily used in our lab and doesn't contain carbonate to precipitate calcium.

Line 151-153: The way the incubations are set up, it's not possible to discriminate between the effects of Ca concentrations and the effect of citrate, acetate, or chloride anions, on the growth of *D. flosaquae*. Using perhaps, sodium salts to assess the effect of anions would be a more logical choice.

Sodium salts do not precipitate.

Line 160: how was pH set?

The pH of BG11 medium was adjusted to pH 7.0 using acid(HCl)/alkali(NaOH).

Line 260-261: I don't agree with the authors that solely based on the alkalinity levels, pH, and Ca concentrations; it could be asserted that *D. flosaquae* induces MICP. These datasets don't eliminate the possibility of the formation of other Ca phases (such as phosphates). A simple saturation index calculation (for e.g using Visual Minteq or PHREEQC) would be easiest to confirm what mineral phases would be over-saturated under the experimental condition. Alternatively, microscopic observation could also confirm with confidence what mineral is actually precipitated.

The process of MICP, studied in this work, is (micro)biological; all other physical and chemical components are equal at all experimental samples i.i. their impact is equal.

Line 311: "The high ability of *D. flosaquae* to shift the pH to alkalinity ..." high compared to what? There's no comparison of *D. flosaquae* versus other cyanobacteria inducing extracellular carbonates. It would be useful to clarify how authors calculate or assign the "high ability" to *D. flosaquae*.

It is high, not higher; no comparison was there. It is high because the pH elevated from 7.0 at the beginning of the experiment to pH 12 at the end of the experiment.