

Interactive comment on “Carbonic anhydrase is involved in benthic foraminiferal calcification” by Siham de Goeyse et al.

Anonymous Referee #2

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General comments

The author present a set of experiments, performed on a high number of foraminifera specimen of a same common symbiotic species. In this experimental work, the effect of an extracellular inhibitor of the carbonic anhydrase (CA) enzyme is compared to the effect of a photosynthesis inhibitor, as well as the sole light deprivation. Biomineralisation change is evaluated through measurements of DIC concentration and alkalinity change, and using solely that approach to evaluate “biomineralisation yield” is also a main aspect of the article. These results evidence, in my opinion, the role of carbonic anhydrase, but I do believe that additional simple information should be given in order to confirm that no other phenomenon can explain, or interfere with, those results. If those information can be given (see below) and the role of CA is confirmed, then

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the scientific significance of these results is excellent. The scientific quality is good, the method and experimental aspects are good despite the few information lacking, as great effort were provided to replicate the experiments and perform them on a significant amount of specimens. The discussion however and the manuscript text in general is not as good as I believe necessary for publication in an international journal. There are not enough references backing information, several aspects of the results are not discussed, a part of the discussion is just a description of results, there are words missing in some sentences, one name on a figure and a table do not match, one figure permitting the comparison of all results is missing, and there are several typography mistakes. I am not able to properly judge the english, but I found the manuscript perfectly understandable. If the text of the manuscript can be improved by the authors, I would recommend publication of the article as the results constitute a major advance in the understanding of biomineralisation by foraminifera (and in my opinion, it gives insight on biomineralisation mechanism in general considering how widespread is CA). For that reason, I hope the authors will improve the text, scientific content and discussion of the article in order to provide these interesting results the context they deserve to become a well referred to article.

Specific comments

#1 I understand that solely using chemical solution parameters to describe the evolution of biomineralisation is one of the suggestion of the article, I however believe it is not enough as some other parameters can affect DIC concentration and alkalinity: ex: microbial proliferation or open system phenomenon (improved gas /liquid phase exchanges in one experiment because of slightly different pH, or temperature differences due to the use of aluminium foil etc. .). In my opinion the interest of a laboratory experiment on living organism cultured in vials is to be able to observe directly these organisms, which is not possible in other type of experiments. Are, in the end of the experiment, the vials clean enough with no particular microbial proliferation in one treatment? What proportion of foraminifera survived the experiment in all setups?

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How are the new chambers? The author used calcein, they should thus be able to image the new chambers formed in each media. I believe any experiment of that type should present some kind of imaging, or at least a description of the visual aspects of the experiment, validating that new chambers formed, and evaluating that no microbial proliferation could have explained death of several microorganisms, that could explain less biomineralisation. For example, in the acetazolamide experiment, if a microbial proliferation occurred and a third of the foraminifera died, while the other survived and biomineralised regularly, wouldn't it give the impression, just by measuring DIC and alkalinity variation that only biomineralisation was affected by acetazolamide? These are simple information that would strengthen the results and the methods, that should be provided in the manuscript before publication.

#2 In my opinion, authors should find a way to represent the results of all the different experiments together in one figure to ease comparison. As an example they could use the “corresponding g/L precipitated calcite” calculated for each experiment.

#3 When discussing the effect of photosynthesis on calcification (line 192) the author do not mention the effect of lowering ATP production and rather suggest that photosynthesis promotes the production of molecules that are used in organic templates of calcification. The role of ATP in chamber formation is, in my opinion, impossible to ignore, the author must discuss it in the manuscript. On the other hand, organics produced by the symbionts may help biomineralisation (this indeed need further investigation), but it should be mentioned that there are (many?) benthic foraminifera with a hyaline test that do not bear symbionts. This should be discussed by the authors as well.

Technical corrections

Missing words or information:

L29: “saturation state” the author should specify that it is towards calcium carbonate

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L35-36: The authors could specify the foraminifera species (benthic ? planktonic ? Amphistegina ?)

L44: “Since this uptake. . .” The sentence sounds odd, a word is probably missing, it should be rephrased.

L48: There is a dot after the bracket

L48: The sentence states “It was recently suggested that CO₂ uptake by foraminifera is achieved through proton pumping” is that correct or did the authors used a shortcut to say that proton pumping (and thus ATP consumption) is used to modify pH and thus favor CO₂ uptake/or that a proton pump is used to actively cotransport CO₂? This imprecision should be corrected.

L50: In my understanding Bentov paper rather says that CO₂ gets concentrated in low pH vesicles, and that from there it diffuses to the high pH vesicles where it converts into charged DIC species and is thus trapped inside the vesicle. The authors should clarify that point if I am not mistaking.

Line 112: I am not familiar with the “T” symbol signification next to each alkalinity species, could it be clarified?

L115: Is that equation calculating “the alkalinity” or “the change in alkalinity”, this should be clarified. Figure 4 caption: there is a dot after “represents”

L193: the sentence is missing a word

L198 : “it has been shown that symbiotic dinoflagellates and zooxanthellae can trigger the activity of carbonic anhydrase (CA) in their host organisms [. . .] thereby explaining how photosynthesis enhances calcification”. The authors need to specify whether they mention the symbiont CA or if they refer to the host CA. Additionnally the link between CA and photosynthesis must be explained.

Missing references:

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L27: “1/3rd of the carbon. . .” a reference should be provided

L40: Who suggested it? a reference is missing

L52: “many procaryotes and virtually all eukaryotes” a reference should be provided.

L60: “ a membrane impermeable inhibitor of this enzyme” a reference must be added. (A reference attesting that DCMU inhibits photosynthesis should be added as well if not provided in the manuscript).

L207 : “Ca promotes [...] into the calcicoblastic space” this information should be supported by a reference

L214: this reference and thus probably the whole sentence (except if another reference can be given) must be suppressed (as mentioned on Biogeosciences website “Works cited in a manuscript should be accepted for publication or published already”).

Other comments :

#1 Given that TA is measured with a $3\mu\text{mol/kg}$ precision and given the errors given in the tables, the decimals should be suppressed. #2 L78: What is the final concentration of dimethyl sulfoxide in the final flasks? The effect of dimethyl sulfoxide at that concentration on foraminifera should have been checked in a control experiment, if not, it should at least be discussed. #3 Figure 3 and 4, error bars should be represented or mentioned in the caption if smaller than symbols, or, even better, each 3 point replicates could be represented. #4 Can the author explain why on figure 4 and 3 the control point is not at the same position (is there an explanation for these two different control results?). Additonnally, in table 1 and figure 3 there is two different names for one treatment, “No AZ” and “ $0\mu\text{M}$ ”, please choose one wording. #5 From line 159 to 170 it is a summary of the results that should not be, in my opinion, in the discussion. #6 Line 175: The “extracellular” specificity of CA is mentioned here but not discussed in part 4.3 and then comes back later in the manuscript. This should be restructured to clarify the message of the auhtors. #7 Line 183: “the discrepancy between results

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may be caused by differences in the process involved in calcification between these species” Can the author mention one or more process they are referring to?

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