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Discussions

Interactive comment on “Carbonate precipitation by the thermophilic archaeon *Archaeoglobus fulgidus*: a model of carbon flow for an ancient microorganism” *by* L. L. Robbins et al.

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Reviewers Report

Title: Carbonate precipitation by the thermophilic archaeon Archaeoglobus fulgidus: A model of carbon flow for an ancient microorganism Authors: L. L. Robbins, K.A. Van Cleave, and P. Ostrom

General Comments This paper reports on experiments designed, using carbon isotopes, to determine the relative abundance of organically derived carbon incorporated into carbonate minerals where precipitation was induced by Archaeoglobus fulgidus, a strictly anaerobic, hyperthermophillic, sulphate-reducing archaeon, during the oxidation of lactate to CO2. The experiments also aimed to provide an insight into links between the carbon cycle, microbial metabolism and mineral precipitation processes, while adding to our understanding of how cellular physiology affects calcification processes and thereby developing a reliable geochemical tool for identification of microbial biomarkers in carbonates.

The paper recognizes the potential importance of microbial mediation in carbonate precipitation, and provides experimental proof of its occurrence; it therefore represents an important contribution to our understanding of the links between the biosphere and lithosphere. However, the paper is poor on referencing, leaves some issues incompletely addressed, and also fails to ask some necessary questions. For example, the authors should support their statement that “Archaeons are thought to be one of the most ancient forms of life to exist on Earth, appearing during the Archaean Era around 3.9 billion years ago” (3411, lines 8-9) with references.

We need a clear explanation from the outset on why Archaeoglobus fulgidus was chosen for the experiment, what habitats it typically occupies, whether or not it precipitates carbonate in the natural environment and a fuller discussion of the processes involved. We should also be told the nature of the carbonate involved – is it calcite, aragonite, dolomite or some other phase?

The reader would also benefit from a fuller explanation of the techniques employed, particularly in the 14C experiments.

Abstract Line 2: it is important that the reader understands at the outset that Archaeoglobus fulgidus is an anaerobic, sulphate-reducing hyperthermophile. This should therefore be included in the first sentence.

Introduction The first sentence makes an unsupported claim and requires references. The authors may consider that a helpful source is Wright and Oren (2005), and references therein, which discusses the quantitative contribution of microbes to calcium carbonate precipitation in the environment, and traces studies from the 19th Century showing that many types of microbes were involved in some way in calcification.

3410 lines 18-19. The ‘significant magnitude of microbial calcification’ has in fact been recognized for some time by some workers, but their contributions have largely been passed over in favour of physico-chemical models. Thus several papers (including some co-authored by the present lead author!) have concluded that lacustrine and marine whitings are induced by blooms of picoplanktonic cyanobacteria (e.g. Ellis and Milliman, 1986; Shinn et al., 1989; Thompson and Ferris 1990; Robbins and Blackwelder, 1992; Robbins et al., 1996; Thompson, 2000), while experimental work by Yates and Robbins (1998) estimated sediment production rates of 1.6 x 106 kg of CaCO3 per 12 hour day in a single planktonic bloom, contributing significantly to the carbonate sediment budget.

This is also true in the case of dolomite where theories or models of dolomitization from pre-existing limestone, first postulated by Von Buch (1822), has long dominated geological thought, leading to an emphasis on inorganic models of low-temperature sedimentary dolomite formation which lacked experimental or empirical support. Research by e.g. Wright (1997) led over 10 years ago to propositions that microbial mediation had made a significant contribution to dolomite occurrences from the Precambrian onwards.

So when the authors state (3410; lines 19-21) that “The difficulty in recognizing biogenic versus abiogenic minerals has been, in part, often due to similarities in morphologies and lack of unambiguous biomarkers”, they are correct but understate the problem. The problem has been a long-term emphasis on a physico-chemical origin for common carbonate deposits such as whitings and dolomite, while the potential of large-scale microbially-mediated carbonate has been recognised but not accepted in the mainstream of geological thought – perhaps until now, after nearly 2 centuries of accumulating evidence.

Microbial influences on carbonate precipitation extend well beyond the examples listed

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and attributed to Sumner (1997), e.g. Bosnak and Newman (2003) reported experiments showing that dead microbial cells stimulate in situ carbonate precipitation. Calcium carbonate precipitation in anaerobic zones characterized by sulphate reduction activity has been documented in the hypersaline Solar Lake, Sinai, Egypt (Lyons et al., 1984), although other mechanisms may be active there in the calcification of cyanobacterial mats below the upper zone of photosynthesis (Jørgensen and Cohen, 1977; Krumbein and Cohen, 1977). Hendry (1993) showed that calcite formed during sequential manganese, iron and sulphate reduction in Jurassic shallow marine carbonates. Moreover, Wright and Oren (2005) have argued persuasively that a number of kinetic inhibitors to carbonate precipitation in saline aqueous media have to be overcome before carbonate precipitation can occur, regardless of saturation levels.

In order to convey this while maintaining brevity, it may be better to state that bacteria may play an important role in the precipitation of carbonates as a consequence of their metabolic activity, which alters the physicochemical environment toward increased alkalinity, removes kinetic barriers and provides nucleation points (citing references).

3411 lines 8-12: the authors should state where Archaeoglobus fulgidus is found in the natural environment, and how closely the experimental conditions simulated the typical microbiochemical milieu, if they wish to extrapolate the results of their experiment to the natural world. They should also explain what an S-layer is, and why “an S-layer composed of glycoprotein subunits in hexagonal array” can act as a mineralization template. Also (3411, lines 19-20) the cells of Archaeoglobus fulgidus consist of more than an S-layer!

E.g. ‘S-layers are self-assembled proteinaceous subunits commonly formed on the surfaces of prokaryotes as planar, monomolecular-thick crystalline lattices. These regular crystalline surface layers may represent the earliest (and simplest) cell wall structures. The S-layer is anchored to the underlying cell wall components and in addition to their presumptive original role as protective coats, they may have adapted new functions, e.g., as molecular sieves, or attachment sites for extracellular molecules.

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Recently, S-layer technologies have provided new approaches for controlled biomineralization. Etc.

Final paragraph: how did the authors know at the outset that the experiments would result in microbially-produced carbonate?

Materials and Methods – 2.2 3412 line 16. The first sentence should begin with either the definite or indefinite article.

3412 lines 16-20. How was the 13C labelling of the medium ingredients achieved? Cysteine HCL has a formula C3H7NO2S•HCl – how might this affect the 13C value of the precipitation medium? Presumably the flushing CO2 was inorganic?

I would prefer that the authors do not assume that every reader is familiar with technical terms. E.g., they could perhaps explain, in brackets, that a supernatant is “the soluble liquid fraction of a sample after centrifugation or precipitation of insoluble solids”. Similarly – what are ‘the pellets’? These have not been described hitherto. Why not explain that these comprise (e.g.) ‘the remaining microbial cells/solids’?

3413 Line 27. The sentence is poorly constructed, and the definite article should be introduced before the words ‘microbial precipitation medium’.

I am unsure what is meant by “The percentage of carbonate C and the percentage of organic C in sample OC for each time interval were determined…...” – what is sample OC?

Figure 1 refers to drying ovens at 70 degrees – but this is not referred to anywhere in the text. This should be explained.

Materials and Methods – 2.3 The general reader might ask what is, and what is the purpose of a ‘scintillation vial’? What is ‘LCD cock-

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tail’? Please explain. It would also be useful to have a flow chart for the 14C experiment.

Results In general, the reporting of the results lacks clarity, and I found it difficult to connect between the 2 experiments. The two experiments should be explicitly referred to the appropriate figures, and the described time phases, since they occur at different times both within the same sample, and between different samples, should be more clearly illustrated in Fig. 4.

The described correlations in Fig. 3 look tenuous for the pH and the percentage of carbonate C in OC between 12 and 24 hours. Why does not pH fall as carbonate is precipitated? More explanation is needed in both text and figure caption.

3416 line 16. The term ‘phases’ should be qualified as time phases, so as to avoid confusion with e.g. carbonate phases.

3416 lines 24-25. “Phase II was characterized by cell, carbonate, supernatant, and total (cells, carbonate, and supernatant) and supernatant trends…”. The terminology here is very awkward – ‘supernatant’ occurs 3 times in a single phrase, and the meaning is lost.

3417 Lines 10-14. This paragraph is confusing: “The three different phases (I, II, and III) observed in samples A, B, C, and D for the precipitation of 14C carbonates occurred in a specific sequential order which is present in all four samples. At some point during the microbial-precipitation process, phase I, phase II, phase III, and phase I occurred in consecutive order for samples A, B, C, D”. Please explain logically.

Discussion 3417 line 16. “These experiments demonstrate that Archaeoglobus fulgidus plays a role in the precipitation of carbonate minerals….”. Only in the context of the experiments: the authors have not demonstrated that carbonate precipitation by Archaeoglobus fulgidus takes place, or is likely to take place, in the natural environment (although I am sure that they could so).

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3418 line 16. “Our experiments using [3−14C] lactic acid determined that CO2 evolved from the oxidation of lactate by A. fulgidus was concomitantly incorporated into carbonate minerals”. How do the authors consider that this happened? Was the 14C incorporated into the carbonate as a gas, HCO3- or CO32-?! Where, how and why?

3418 lines 25-26. “The S-layer is hexagonally patterned and forms a meshwork of open pores that promote highly efficient carbonate nucleation”. But the authors have not demonstrated that Archaeoglobus fulgidus does this in the natural environment, only in the experimental culture medium – so such a general statement cannot be made.

3419 lines 17-19. “This may indicate that between 9 and 15 h the majority of carbonate precipitation has become regulated by strictly inorganic processes, which would explain the transition from smooth symmetrical spheroids to almost angular crystals”. What are these “strictly inorganic processes” – or the organic process for that matter? Alternatively, the transition in mineral morphology may indicate that the carbonate precipitation process is no longer influenced by the S-layer template. Carbonate can hardly continue to precipitate on the S-layer once it has become ‘coccooned’, so the subsequent precipitation may be controlled by the same (microbially-mediated) process while the morphology is subject to other influences – such as rate of precipitation, saturation, change in pH (as the authors themselves argue for some stages), nature of mineral surface etc.

However, the Fig. 2 images suggest to me that carbonate precipitation continues at the site of the original nucleation and builds up there, rather than spreading smoothly and coalescing over the S-layer as the authors claim, but do not show.

In the microbially-mediated precipitation of dolomite in the Coorong ephemeral lakes, Wright (1999) showed that initial spherical and subspherical sub-micron dolomite crystals neomorphosed into rhombic crystals with sharp faces. Have the authors observed any evidence for such transitions in their experiments?

3420 lines 8-10. “Cellular activity results in the production of CO2 from the oxidation of lactic acid (Stetter, 1988) leading to the subsequent partial dissolution of carbonate associated with cells”. Where is the SEM evidence for this dissolution? Is this accompanied by an increase in pH/ CO32- activity?

3420 lines 24-26: “the continual increase in carbonate from 0 to 12 h (Fig. 2)” – how is this continual increase demonstrated in Fig. 2?

3420 line 28. “The r-value of 0.997 in Table 2 indicates a strong correlation between pH and the fraction of carbonate in the sample for this time interval.” Where is Table 2?

3421 lines 5-6. “Therefore, the contribution of carbonate and bicarbonate ions to the medium through the oxidation of lactic acid by A. fulgidus has a more significant role in carbonate precipitation during this time interval than it does between 12 and 24 h.” An astute deduction, except that I would argue there is no role for bicarbonate ions in carbonate precipitation (Wright and Oren 2005; Wright and Wacey 2004, 2005; Altermann et al., 2006).

3421 line 8. “Cumulative H2S production (raising pH)….”. How can H2S production raise pH in an aqueous environment? Do the authors mean that sulphate reduction causes a rise in pH? If so, they should say so, because the reactions are important, and it is not H2S production but the breakdown of organic matter and the liberation of ammonia during sulphate reduction which leads to higher pH (e.g. Wright and Oren, 2005). If iron is in the system, it will sequester the H2S as metal sulphide. H2S is extremely sensitive to pH, and is more soluble at lower pH (e.g. http://www.telusplanet.net/public/jcarroll/ION.HTM). On the other hand, the prevailing species H2S, HS-, and S2- that can exist in aqueous media depend upon the pH. Hydrogen sulphide is a weak acid with a dissociation constant that is considerably larger than that of water: H2S(aq) +

H2O(l)  H3O+(aq) + HS-(aq) Ka= 1 x 10-7 H2O(l) + H2O(l)  H3O+(aq) + OH-(aq) Ka= 1 x 10-14 Thus, a 0.01 M H2S solution will have a pH = 4.5. (http://www.juniata.edu/services/ScienceInMotion/chem/labs/gases/microscale/h2s.doc)

3421 line 20. “Because a large portion of the original 14C in the [3−14C] lactate had been evolved as 14CO2 at this point, the 14C values for the cells were lower than they were at the initiation of lactate oxidation. At the end of the lactate oxidation process, a small portion of the 14C was incorporated into the cell while the rest was released as 14CO2.” An important point, which should be backed up by discussion of the fate of the evolved 14CO2 – was it converted into bicarbonate and/or carbonate in the ambient aqueous medium, and where and when? How do you know?

A table or figure showing the variations in 14C of the samples would be most helpful in facilitating comprehension in the Discussion section.

3422 line 12. “Increases and decreases in carbonate trends….”. Is it some value(s) that increases or decreases? I can see that 2 or more trends might be in or out of step – but what is it that is increasing or decreasing?

3422 lines 13-14. “The relatively high 14C values for the carbonate fraction of samples A, B, C, and D…”. Fig. 4 should be referenced here, with fuller explanation in the caption, so that we can check the statement.

Phases present during carbonate precipitation 3426 lines 1-6: “the majority of 14C in the medium is still present as H14CO−3”. Does this support the contention that carbonate ions (not bicarbonate) have been largely incorporated into carbonate, with a consequent fall in pH?

Figures Figures 2-4 and their captions are wholly disappointing. Much greater clarity is needed for Figs 3 & 4, with full explanations in the captions for what is represented. Fig. 2 caption should include the time phases at which carbonate precipitation began and continued.

There are 18 images in Figure 2. However, a single serum bottle was removed from the incubator at 0, 1, 2, 3, 4, 8, 12, 16, 20, and 24 h – making 10 bottles. Many of the images do not appear to show sequential precipitation, as the crystal morphology varies considerably. If dissolution has occurred, the timing and extent should be discussed, and reported in the figure caption. Which of the images relates to which serum bottles? Did precipitation continue in the bottles that were removed from the incubator?

In the caption to Fig 4, what is meant by ‘cell C’ (given that the authors have stated that “Increases and decreases in carbonate trends represent an increase or decrease in the overall amount of 14C present in carbonate minerals.”)?

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