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Biogeosciences Discussions

Interactive comment on "Kinetics of calcite precipitation by ureolytic bacteria under aerobic and anaerobic conditions" *by* Andrew C. Mitchell et al.

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We thank the reviewers for their comments on our manuscript. We were very pleased that both reviews were positive suggesting publication with revisions. We have therefore revised the manuscript according to their suggestions. The reviewer comments and responses are shown below:

Reviewer #1 This is a useful study exploring the kinetics of urea hydrolysis by a group of bacteria and its link to microbial-induced calcite precipitation. Three strains of bacteria are tested (S. pasteurii, B. sphaericus 21776 and B. sphaericus 2178), grown at 30C while also varying redox conditions to compare rates of urea hydrolysis be-

tween aerobic and anaerobic conditions. Further comparison involves using different terminal electron acceptors in the case of anaerobic experiments, presumably because metabolic pathways based on these TEAs are also known to drive up pH.

The main finding is that only two (S. pasteurii and B. sphaericus 21776) of the three bacterial strains tested hydrolyse urea and lead to calcite precipitation, and that rates were higher for B. sphaericus 21776 than S. pasteurii, although the differences are not tested statistically. In addition, urea hydrolysis rates are higher when calcium is absent, and biomass (and protein) data is given to show that calcite precipitation arrests the growth of the bacteria through cell entombment, which reduces diffusion of urea to cells. Paradoxically, cell growth is also compromised under anaerobic conditions but this does not appear to affect urea hydrolysis rates.

The study is of wide interest to readers of Biogeosciences and deserves to be published following some minor modifications.

Comment 1: There is apparent confusion about the meaning of rate being zero order with respect to biomass being interpreted as X=0, which invalidates both equations 9 and 10. I believe what they mean is that X is constant so the non-normalised rate = kobs[Urea], and kurea = kobs/[X]. In this context, the calculation of kobs is simply a step towards the calculation of the real kurea and so is not in itself a separate method. Consider this in the same way that observed mineral dissolution rates from solution data are normalised to surface area. The consequence of this is that the comparison with other studies should only be on the biomass-normalised rate constants.

Response: We apologize for the oversight and thank both reviewers for pointing out this issue. This should have stated X = X0 and not X = 0. We modified the text as follows (Page 7, line 11-20):

"Biomass-normalized ureolysis rates were calculated. Firstly, it was assumed that biomass (X) is constant in Eqs. 10 and 11, as performed in other studies of ureolysis kinetics (Cuthbert et al., 2012;Ferris et al., 2004;Mitchell and Ferris, 2005, 2006;Schultz

et al., 2011;Tobler et al., 2011). Secondly, the obtained first order rate coefficients with respect to urea concentration (kurea) were normalized to the biomass concentration by dividing the ureolysis rate coefficient by the biomass at the onset of precipitation (i.e. urea hydrolysis rates were normalized to the absorbance reading of initial biomass, OD600, and CFU mL-1; SI section 2.2), which was equivalent to the initial biomass in each system (X = X0). This is an appropriate choice of model, since the biomass analysis indicated that the cell density was constant for the duration of CaCO3 precipitation and was equivalent to the initial biomass in the systems, as presented in the results section. The biomass-normalized ureolysis rates were compared to other parameters previously published (Ferris et al., 2004;Fujita et al., 2000;Stocks-Fischer et al., 1999;Tobler et al., 2011). "

Comment 2: In the context of Equation 10, a simpler analysis would have been simply to linearize the function by plotting ln [Urea] against time. I am concerned that other orders were not tested but perhaps it would be useful to provide fitting constraints information (residual sum of squares, r-squared values) for the non-linear fitting presented. Significantly, urea hydrolysis is an enzymatic reaction, but this analysis is purely abiotic, while the real rate is likely cell-surface controlled, requiring enzymatic analysis approaches (e.g. Michaelis-Menten).

Response: Our previously published work demonstrates that a first-order ureolysis rate model is appropriate in this study, rather than a Michaelis-Menten model, specifically because maximum urea concentrations are 330mM (also see further comments in relation to reviewer 2). We have therefore included a paragraph to justify the use of the first-order ureolysis rate model over the Michaelis-Menten model in our study (Page 7, lines 2-5):

"Indeed, while the Michaelis-Menten model has been used when evaluating ureolysis, studies of ureolysis-induced calcium carbonate precipitation have demonstrated that the first-order ureolysis rate model fits well for urea concentrations of approximately 330 mM or below (Lauchnor et al., 2015; Connolly et al., 2015), which is the concentration

range used in this study."

Comment 3: Figure 1 is slightly misleading as it implies urea concentration was measured when it was derived from NH4+ measurements. I wonder if NH4+ data should also be given. Importantly, there is need for an explanation of the drop in Ca concentration in Figure 1C given the limited urea hydrolysis for this bacteria.

Response: We included a sentence stating that urea concentrations were calculated based on ammonium measurements on page 6, line 14. Because we clarify this, we don't believe it's necessary to present raw NH4+ data which would just be extraneous.

The decrease in Ca concentrations in the B. sphaericus 21787 treatments has now been discussed in much more detail at a number of points in the manuscript.

Specifically on page 8, line 27, we have added: "B. sphaericus 21776 and S. pasteurii exhibit urease activities approximately twice that of B. sphaericus 21787 when Ca is present (Hammes et al., 2003a) supporting our observations of limited ureolysis and delayed CaCO3 precipitation by B. sphaericus 21787. Nevertheless, the decrease in Ca concentrations in the absence of significant urea hydrolysis for B. sphaericus 21787 suggests there was a sufficient carbonate ion concentration in the artificial medium and from ureolysis to initiate some early CaCO3 precipitation. Hammes et al. (2003a) observed B. sphaericus 21787 was also able to precipitate CaCO3 despite lower urease activity in the presence of Ca, suggesting this strain may enhance precipitation via other mechanisms such as enhanced nucleation on cell surfaces or via organic exudates (Mitchell et al, 2006b)."

Then in the urea kinetics section, page 10, line 30, we have added: "B. sphaericus 21787 has been shown to have urease activity about half that of S. pasteurii and B. sphaericus 21776 in the presence of Ca (Hammes et al. 2003a) consistent with the observed low kurea values. However, in the absence of Ca, urease activity for B. sphaericus 21787 is about twice that of B. sphaericus 21776 (Hammes et al., 2003a) which does not support our experimental results. This suggests that while B. sphaer-

icus 21787 has high potential to generate comparable rates of urea hydrolysis to the other strains, under the experimental conditions used in this study, B. sphaericus 21787 exhibits limited ureolytic capabilities."

And also, in relation to the precipitation kinetics on page 11, line 36: "The kprecip for B. sphaericus 21787 was lower than the other strains (0.21 h-1), although as noted, rate constants for this strain are not statistically significant. The lag time for CaCO3 precipitation was 3.3 h for B. sphaericus 21776 and S. pasteurii, which reflects the similar kurea values, and thus the similar time it took to reach CaCO3 saturation and induce precipitation, whereas the longer lag time for B. sphaericus 21776 reflects the significantly lower kurea value."

And in the conclusion, on page 14: "Although B. sphaericus 21787 showed poor ureolysis, some CaCO3 precipitation was observed, suggesting this strain may enhance precipitation via other mechanisms such as enhanced nucleation on cell surfaces or via organic exudates."

Comment 4: In line 15, page 8, I am always concerned when control data is "not shown" or presented. It is the only data that gives us confidence that the experimental observations relate to our manipulations rather than chance.

Response: As per the reviewer's suggestion, we have now included the values of urea concentrations for the control (abiotic experiments). New Figure 1 and caption file attached.

Comment 5: In Figure 5, neither of the radial sections show the bright spots which I assume represent the calcium in the cells (arrowed). It would be helpful to clarify why their appearance is orientation-dependent.

Response: We assume the reviewer refers to Figure 3 since that is the only figure with images in this manuscript. The bright spots are not calcium in the cells. The arrows highlight the breadth of the Ca inclusive precipitate layer which encapsulates

the cell, from the cell wall outwards. We have adjusted the caption in order to clarify this, specifically labelling cell walls (CW) and calcium containing precipitates (CCP) in the images. New Figure 3 and caption file attached.

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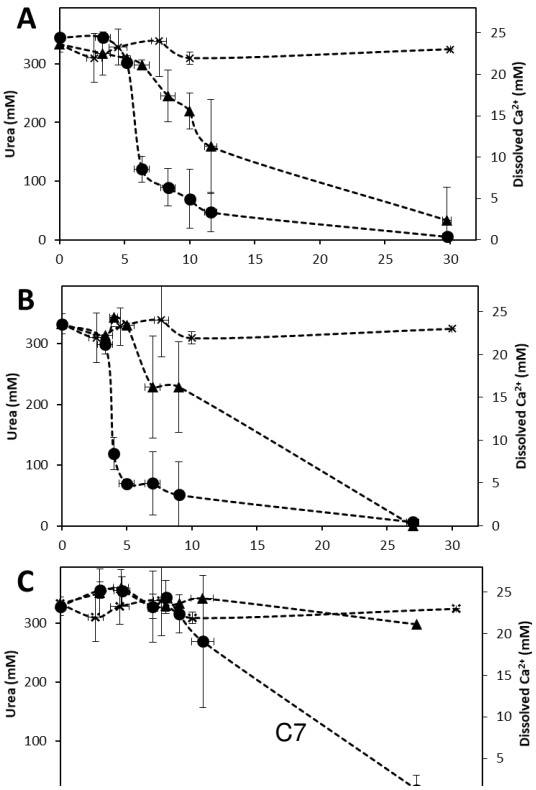
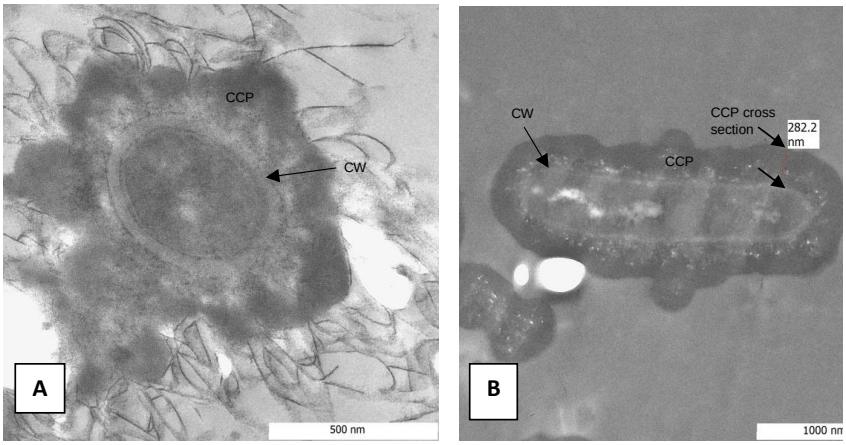

A
B
C
Urea (mM)
Dissolved Ca2+ (mM)
C7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CCP
B
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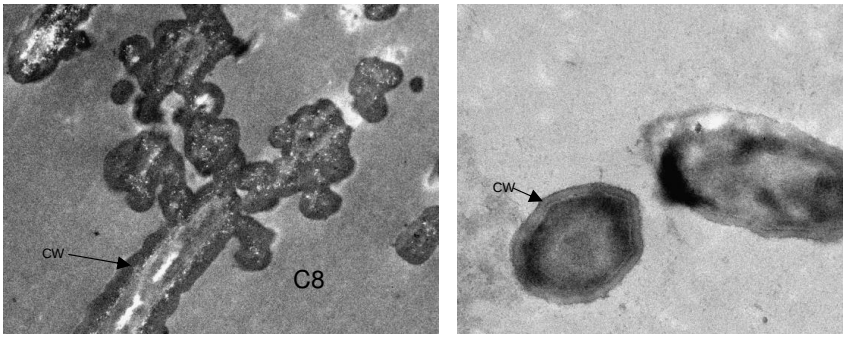
CW
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