BG-2018-477

Kinetic of Calcite Precipitation by Ureolytic Bacteria under Aerobic and Anaerobic Conditions

Andrew C. Mitchell, Erika J. Espinosa-Ortiz, Stacy L. Parks, Adrienne Phillips, Alfred B. Cunningham, and Robin Gerlach

The manuscript describes laboratory determinations of the urea hydrolysis (both in the presents and absence of calcite precipitation) and calcite precipitation rates for 3 microorganisms (*S. pasteurii*, *B. sphaericus* 21776, and *B. sphaericus* 2178) at a temperature of 30 °C. The motivation for the study was the quantification of microbial induced calcite precipitation technology. The reported experiments were conducted under both aerobic and anaerobic conditions (screening for terminal electron acceptors). The manuscript concluded that rates (as measured by first order rate constants) for both urea hydrolysis and calcite precipitation under aerobic condition were slightly higher for *B. sphaericus* 21776 than for *S. pasteurii*. Given the scatter among the replicate measure of rate constants (Table SI2.1 CMM+) a more defensible statement would be that there is no apparent difference in rate constants between the two microorganisms. This statement is further supported by the observation that for the calcium absent experiments the mean urea hydrolysis rate constant (Table SI2.1 CMM-) for *S. pasteurii* is greater than for *B. sphaericus* 21776; the opposite of what was observed for the calcium present experiments.

The manuscript also reported that calcite precipitation inhibits the ability of the microorganisms to hydrolysis urea (presented as the ratios of the hydrolysis rate constants constants) and proposed (based on TEM observations and solid state diffusion calculations) that precipitating calcite entombs some fraction of the microorganisms and that the entombed organisms, while still alive, do not contribute to further hydrolysis. The manuscript also describes experiments assessing the potential for urea hydrolysis by *S. pasteurii* under anaerobic conditions. The manuscript concludes that although population growth (as measure by OD_{600}) did not occur in the absence of oxygen, urea hydrolysis does occur at rate similar to those for aerobic conditions. Additionally, growth resume following anaerobic exposure by the re-introduction of oxygen.

The manuscript could be strengthen by additional analysis of the results. Equations 9 (and 10) represents a first order rate approximation of enzymatically catalyzed urea hydrolysis. This approach is justified because a) rates were not measured directly (rather urea [or NH_4] concertation as a function of time was measured) and b) the integrated rate expression fits the observations (not surprising given the scatter in the observation both within and between experiments). However enzymatic (urease) kintetics are better describe by the Michaelis-Menten expressions that reduces to 1^{st} order kinetics at low urea concentrations and 0^{th} order at high urea concentrations. This results in the apparent rate varying between 1^{st} and 0^{th} order as a function of urea concentration potentially over the course of a single experiment. With this in mind the normalization process needs to be consider carefully especially in the comparison to results of other studies using different concentration and temperatures. An alternative approach would be to consider a simple linear regression model of apparent k_{urea} values in term of optical density (OD_{600}) and temperature (Kelvin). Using the results for *S. pasteurii* reported in Table 3

supplemented with the 10 and 15 °C results of Ferris et al. (2004; this is incorrectly reference in both Table 3 and the reference list in the SI as 2003) yields:

$\log k_{urea} = 34.3\ (9.5) + 1.22\ (0.47)*\log OD_{600} - 10{,}070\ (2{,}660)/T_{Kelvin}$

where the values in parentheses represent the standard errors for the parameters. The fit has an R value of 0.77 (n=13) with the fit parameters having p-values of 0.027 (OD_{600}) or less. If Equation 9 in the manuscript is the proper representation of the kinetics and OD_{600} is the measure of cell concentration (proportional to enzyme concentration), the expect coefficient for log OD_{600} would be 1. The regression value of 1.22 is less than half the standard error different from 1 suggesting that the assumption of equation 9 are adequate given (or because of?) the scatter in the experimental results, although one cannot exclude the possibility that k_{urea} may also show a dependences on urea concentration (e.g., Michaelis-Menten kinetics). In addition, the coefficient for the temperature can be used to derive an apparent activation energy thus providing a temperature depended (10 – 30 °C) expression for estimating urea hydrolysis rates.

A couple of text issues:

Page 7 line 5 – assuming the reaction is zero order with respect to biomass is not the same as stating that X=0 (if X=0 then d[Urea]/dt in equation 9 is also 0). Perhaps this section could be revised to consider this a pseudo-first order reaction where the apparent rate constant $k^*_{urea} = k_{urea}$ [X] and d[X]/dt = 0.

Page 8 line 16 (and SI page 4 line 6) – the statement that approximately 25.5 mM of urea need to hydrolyze in order to achieve super-saturation is incorrect. Figure SI1.1 shows that a DIC concertation of 25.45 mM is required to reach saturation with respect to calcite. Given that the medium starts with DIC concentration of 25 mM (as NaHCO3; Table SI1.1) only 0.45 (~0.5) mM of urea hydrolysis is required to achieve calcite saturation.