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Experimental modeling of $CaCO_3$ precipitation in the presence of oxygenic and anoxygenic phototrophic bacteria

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Carbonate biomineralization is considered as one of the main natural processes controlling CO_2 levels in the atmosphere both in the past and at present time. In contrast to a large amount of studies devoted to morphological characterization of biomineralisation processes and products, quantitative assessment of macroscopic links between bacterial activity (growth rate, cell number), physico-chemical parameters of the medium (pH, temperature, calcium and carbonate ions concentrations) and rate of $CaCO_3$ precipitation is impossible at the present time. Among various aquatic microorganisms, phototrophic bacteria (cyanobacteria and anoxygenic phototrophs) are the most interesting from the view point of carbonate mineral formation. These microorganisms are known to form calcium carbonate as a result of their metabolism, in particularly, rising the pH of the external solution due to oxygenic photosynthesis (uptake of HCO_3/CO_3^{2-} ions and release of OH^-) or anoxygenic photosynthesis (uptake of organic ligands such as acetate$^-$ or malate^{2-} and release of OH^-). Due to the extremely large variety of bacterial species, their different metabolisms and the different physical, structural and chemical properties of their surfaces, no unique methodological and conceptual approach in the experimental study of biomineralization can be recommended at the present time. As a result, it is very important to “scan” a large

variety of bacteria species to assess their passive or active role in mineral precipitation.

The present study is aimed at establishing the first-order relationship between the growth rate of phototrophic bacteria and associated calcium carbonate precipitation. Cyanobacteria *Gloeocapsa* sp. f-6gl from the culture collection of the Institute of Microbiology RAS (Moscow) isolated from hot springs (30-40^{o}C) in Kamchatka were cultured at pH = 8.0-8.2. Two strains of anoxygenic phototrophic bacteria: haloalcaliphilic *Rhodovulum* sp. A-20s isolated from soda lake in central Siberia, and halophilic neutrophilic *Rhodovulum* sp. S-17-65 from hypersaline water body in Crimea steppe, represent another large group of phototrophic bacteria likely to be involved in $CaCO_3$ formation in soda and saline lakes. These bacteria are known to use acetate as substrate for non-oxygenic photosynthesis and thus may mediate $CaCO_3$ precipitation without CO_2 consumption in highly-saline, highly-alkaline, $NaHCO_3$-rich solutions.

Experiments consisted of culturing cyanobacteria in phosphate-free media similar to the natural environment in order to facilitate $CaCO_3$ precipitation at low supersaturation index. Initial concentrations of both Ca and HCO_3^- ranged from 0.01 to 0.001 M. Two types of experiments were performed with cyanobacteria: with atmospheric air bubbling, to keep the pH constant (8-8.5) and without air bubbling, to let the pH rise to 9-10 as a result of photosynthesis. In the course of experiments, pH, optical density (cell biomass), [Ca], and [Alkalinity] were measured as a function of time. Experiments with haloalcaliphilic (*Rhodovulum* sp. A 20s) and halophilic neutrophilic (*Rhodovulum* sp.S-17-65) anoxyphotobacteria were conducted in closed vials in normal and $NaHCO_3$-enriched media (0, 10, 20 and 30 g/L) at pH from 8 to 9 with Ca concentration ranging from 1 to 10 mM. Control experiments with cell-free media or autoclaved cells were always performed. At the end of experiments, reaction products were examined via scanning electron microscopy, X-ray diffraction and EDS analysis.

First preliminary results demonstrated a high capacity for both oxygenic and anoxygenic phototrophs to decrease Ca concentration in the medium corresponding to $CaCO_3$ precipitation. The rates were determined from [Ca] vs. time dependencies and normalized to biomass. Among studied bacteria, *Gloeocapsa* sp. has the highest ability to sequester calcium carbonate, whereas alkaliphilic A-20s grown on $NaHCO_3$-rich media can act as an important substrate for $CaCO_3$ nucleation. Experiments on Ca adsorption on the surface of active and dead bacteria allowed quantifying the number of surface sites able to participate in $CaCO_3$ precipitation via Ca^{2+} binding at the cell surface. Results of this study demonstrate the possibility to quantify $CaCO_3$ precipitation rates in well-constrained experimental systems in the presence of metabolizing bacteria.