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# Experimental modelling of carbonate mineral precipitation in the presence of cyanobacteria

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## Abstract

Carbonate mineral precipitation in the presence of cyanobacteria is at the forefront of scientific research due to its importance for understanding paleo-environments of mineral formation and for optimizing conditions of mineralogical CO<sub>2</sub> sequestration *via* biological pathway. Stromatolites are amongst the oldest known biological formations, and they provide insight into early Earth environments and climates. It is therefore essential to understand the processes governing their formation. Numerous field studies were carried out to characterize these bio-formed rocks and their way of formation showing that various parameters could be involved in the processes of formation of carbonate rocks. Thus, reproducing natural environments under laboratory-controlled conditions is an efficient approach to better understand the role of each parameter. The present chapter aims to present some results of these laboratory studies on the biomineralization of Ca, Ca-Mg and Mg carbonates, *via* analyzing and discussing mechanisms of mineral formation, providing examples of several case studies, assessing, based on available information, the stoichiometry of inorganic carbon removal in the form of carbonate minerals and organic carbon sequestered in the form of bacterial biomass, and finally recommending future research directions in this actively developing field of science.

## Keywords

Cyanobacteria, calcium carbonate, magnesium carbonate, precipitation, kinetics, stoichiometry, mechanisms, exopolysaccharides

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## Introduction

Geochemical modeling and laboratory experiments demonstrated the importance of physico-chemical mineral nucleation/precipitation processes rather than specific metabolic reactions in overall microbially-induced  $\text{CaCO}_3$  biomineralization processes occurring for most of Earth's history (Bosak and Newman, 2003). From the early Proterozoic and certainly since Precambrian-Cambrian boundary until the end of the Cretaceous, calcifying cyanobacteria frequently occurred in normal marine environments (Riding, 2006; Ries, 2010). At the present time, calcification by cyanobacteria occurs almost exclusively in freshwater, alkaline and hypersaline or brackish water (Merz, 1992).

While there were calcium carbonate organominerals and probably calcium carbonate biominerals in the Proterozoic, it was not until the Phanerozoic that abundant

fossil evidence of calcium carbonate biominerals was found in marine deposits. It is generally thought that the surface oceans through the Phanerozoic were supersaturated with respect to calcium carbonate minerals, with possible exception of episodes with high atmospheric and surface ocean carbon dioxide over time scales too short to allow the ocean circulation to return higher-alkalinity water to the surface. As a result, it is difficult to model accurately the calcium carbonate saturation states in past oceans (Raven and Giordano, 2009). Through early lithification, cyanobacterial mats produced vast amounts of  $\text{CaCO}_3$  on Precambrian carbonate platforms (before 540 Myr ago). The superposition of lithified cyanobacterial mats forms internally laminated, macroscopic structures known as stromatolites. Similar structures can be important constituents of Phanerozoic carbonate platforms (540 Myr to present). Early lithification in modern marine cyanobacterial mats is thought to be driven by a metabolically-induced increase of the  $\text{CaCO}_3$  saturation state ( $\Omega_{\text{CaCO}_3}$ ) in the microbial mat. Calcium ion has a crucial role as a metabolic and morphogenetic messenger for early eukaryotes (Kazmierczak and Degens, 1986). However, it is uncertain which microbial processes produce the  $\Omega_{\text{CaCO}_3}$  increase and to which extent similar  $\Omega_{\text{CaCO}_3}$  shifts were possible in Precambrian oceans whose chemistry differed from that of the modern ocean. Numerical models could help answering these questions (Aloisi, 2008).

Microbial carbonate precipitation is a way to couple the transfer of carbon between the atmosphere and terrestrial carbonate mineral reservoirs. Deep-surface weathering and secondary carbonate precipitation are apt to be similarly influenced by microflora underground, as the metabolic activity of many microorganisms is known to influence alkalinity and pH, thus inducing the carbonate precipitation (Ferris et al., 1994). Cyanobacteria have evolved an extremely effective single-cell  $\text{CO}_2$  concentrating mechanism (CCM), which plays crucial role in carbonate mineralization (Kamennaya et al., 2012). The CCM components include at least four modes of active inorganic carbon uptake, which serve to accumulate  $\text{HCO}_3^-$  in the cytosol of the cell (Badger and Price, 2003).

The formation of natural carbonate minerals *via* microorganisms occurs in multiple environments (Chagas et al., 2016). External microbial precipitation of low-Mg calcite appeared to be the main mechanism involved in the early stage of ooid formation in freshwater Lake Geneva. Although in this lake photosynthetic activity was found to be the main factor of precipitation (Plee et al., 2010), the variety of bacterial communities could result in different precipitation processes. The presence of eukaryotic picoplankton could lead to the formation of small calcite crystals whereas cyanobacterial picoplankton induced the formation of micritic carbonate (Dittrich et al., 2004). In eutrophic basins, calcite could be produced during daylight *via* the photosynthesis and partly dissolved at night when dissolved  $\text{CO}_2$  concentration decreases due to respiration (Cicerone et al., 1999). The photosynthetic increase of pH could not always reflect the occurrence and significance of photosynthesis-induced  $\text{CaCO}_3$  precipitation (Shiraishi, 2012). In a UK-travertine deposit stream, the relative importance of degassing, photosynthesis within algal colonies and photosynthesis by the total aquatic flora depended upon the position of the colonies

with respect to the spring-fed sources, the discharge of water, and environmental factors affecting photosynthesis rates such as irradiance and water temperature (Pentecost and Spiro, 1990). In such travertine streams, already supersaturated with respect to  $\text{CaCO}_3$ , the annual growth rate of the cyanobacterium *Homoeothrix crustacea* colonies was  $1.25 \text{ mm y}^{-1}$  (Pentecost, 1988) whereas the calcification rate of another cyanobacterium, *Rivularia haematites*, ranged from  $12\text{-}14 \mu\text{m d}^{-1}$  during the summer to  $< 2 \mu\text{m d}^{-1}$  during winter (Pentecost, 1987). Carbonate precipitation peaks during early spring and late summer, and low-Mg calcite crystals mostly occurred in close association with filamentous and coccoid cyanobacteria (Plee et al., 2008). Recently, important role of viruses in calcium carbonate precipitation in the presence of bacteria has been demonstrated (de Wit et al., 2015; Lisle and Robbins, 2016; Xu et al., 2019; Slowakiewicz et al., 2021).

Biofilms play a substantial role in low-Mg calcite ooid cortex formation. The formation of algal biofilms in hardwater streams, rivers and lakes is strongly associated with calcite deposition (Hartley et al., 1996). Biofilms were also found to control the formation of stromatolites in karst-water creeks (Bisset et al., 2008). In these studies, the application of microelectrodes to the study of algal biofilms permits the direct measurement of chemical activity close to biofilm surfaces. Calcium carbonate precipitation in the photic zone of a hypersaline microbial mat is primarily controlled by photosynthesis-induced pH and carbonate concentration increase (Ludwig et al., 2005). Biomineralization could also take place under hydrothermal conditions. In this case, high  $\text{pCO}_2$  boosts the generation of acidic extracellular polysaccharides (a part of exopolymeric substances or EPS produced by microorganisms) that adsorb Ca and Mg cations, support mineralization and aggregate cells to form ballasted carbonate particles (Kamennaya et al., 2018). The importance of EPS has been recently demonstrated for dolomite precursor formation in cyanobacteria dominated microbial mat from a sabkha (Paulo et al., 2020).

The bio-induced formation of Ca carbonate can be useful to artificial applications, such as the restoration of concrete (Zhu et al., 2015). Several sorts of limestones are used for building and sculpture. Among these, two categories may be distinguished according to the size of their constituents and their porosity. The first category includes fine-grained limestones, which may have high porosity but be formed of small (less than  $10 \mu\text{m}$ ) pores. This is the case of the famous Tuffeau limestone which is a kind of siliceous chalk used widely in historic monuments in Western France. The second category is composed of generally bio-detrital limestones of variable porosity formed of both larger grains and larger pores (more than  $10 \mu\text{m}$  wide), such as the Saint-Maximin limestone and the Saint-Vaast limestone. The biomineralization process consists of first spraying the entire surface to be protected with a suitable bacterial suspension culture. Afterward, the deposited culture is fed daily or every two days with suitable medium to create a surficial calcareous coating scale, the "biocalcin". The nutritional medium is designed to stimulate the active and passive bacterial production of carbonate through the nitrogen cycle metabolic pathways which are the only pathways to be activated in operational condi-

tions, i.e. in aerobiosis and microaerophily (Castanier et al., 2000). Other applications of the biomineralization of Mg carbonate minerals could be the rehabilitation of asbestos mine sites and *in situ* carbon sequestration (Power et al., 2011; McCutcheon et al., 2016).

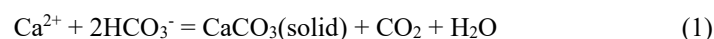
The above considered examples underline the importance of understanding the mechanisms of carbonate mineral precipitation under the influence of microbes. To achieve this understanding, however, well-constrained laboratory approaches are needed. Experimental modeling of naturally-occurring processes allows straightforward identification of governing parameters *via* decreasing the number of freedoms in the system and systematically testing various controlling factors. Below we consider laboratory experiments on Ca and Mg carbonate formation in the presence of cyanobacteria. This is by far the most important process, controlling carbonate cycle on the planet since early Proterozoic.

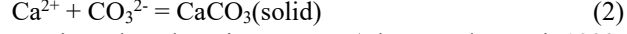
## ***1 Calcium carbonates***

Cyanobacteria are known to promote the precipitation of Ca-carbonate minerals by the photosynthetic uptake of inorganic carbon. This process has resulted in the formation of carbonate deposits which is a fossil record of high importance for deciphering the evolution of cyanobacteria and their impact on the global carbon cycle. Calcite precipitation induced by cyanobacteria is not a mere side effect of photosynthesis in carbonate-rich water. Precipitation might serve as an effective buffer against a pH rise in alkaline environments and could be the protection of the bicarbonate pump, which is possibly inhibited by carbonate ions. The major physicochemical factor controlling the precipitation of calcite is the saturation state of the water with respect to calcium carbonate. Calcite formation by cyanobacteria can be extracellular, in this case the ambient water has to be supersaturated with respect to calcium carbonate. However, the precipitation is not always observed in supersaturated hard water lakes. Besides, different dissolved substances can inhibit the precipitation. On the other hand, calcite nucleation may occur in the supersaturated microenvironment that we cannot access in the field. Factors leading to calcite precipitation have to be determined under controlled conditions in order to monitor and separate their importance.

### **1.1 Mechanisms of precipitation**

According to Jansson et al. (2010), the precipitation of CaCO<sub>3</sub> can occur by either or both the following reactions:





with reaction (2) being the main path, at least in seawater (Zhong and Mucci, 1993; Zuddas and Mucci, 1998). The ion  $\text{HCO}_3^-$  is ubiquitous in water and is formed *via* dissolution of gaseous  $\text{CO}_2$ :



In carbonate mineral-precipitating environments, the quantity of carbonic acid  $\text{H}_2\text{CO}_3$  is usually small, so the dissolved  $\text{CO}_2$  from reactions (3) and (4) occurs predominantly as  $\text{HCO}_3^-$ , a fraction of which dissociates to form carbonate  $\text{CO}_3^{2-}$ :



The supersaturation of solution with respect to calcium carbonate ( $\Omega_{\text{CaCO}_3}$ ) is defined as:

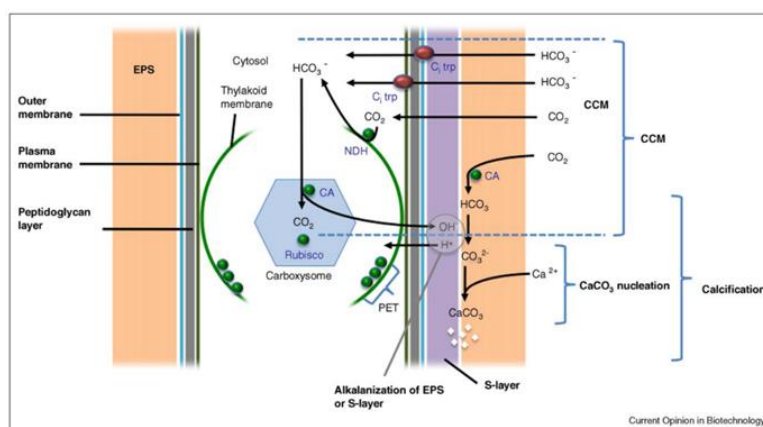
$$\Omega_{\text{CaCO}_3} = \frac{a_{\text{Ca}^{2+}} \cdot a_{\text{CO}_3^{2-}}}{K_{\text{S}_{\text{CaCO}_3}}} \quad (6)$$

where  $a_{\text{Ca}^{2+}}$  and  $a_{\text{CO}_3^{2-}}$  are activity of free ions  $\text{Ca}^{2+}$  and  $\text{CO}_3^{2-}$  in solution, respectively, and  $K_{\text{S}_{\text{CaCO}_3}}$  is the solubility product of calcium carbonate.

Although oceans are supersaturated with  $\text{Ca}^{2+}$  and  $\text{CO}_3^{2-}$ , spontaneous precipitation of  $\text{CaCO}_3$  in the absence of calcifying (micro)organisms is rare due to various kinetic barriers (Berry et al., 2002), such as  $\text{Mg}^{2+}$ , phosphate ions, and dissolved organic matter (Pokrovsky and Savenko, 1994a, b; 1995; Pokrovsky, 1998). Cyanobacteria and eukaryotic microalgae exhibit a carbon-concentrating mechanism (CCM), a metabolic system that allows the cells to enrich the amount of  $\text{CO}_2$  at the site of Rubisco (the first enzyme in the Calvin cycle that assimilates  $\text{CO}_2$  into organic carbon compounds) up to 1000-fold over that in the surrounding medium. The salient features of the CCM in cyanobacteria are shown in Figure 1. Details difference between cyanobacteria, and the mechanisms are not fully understood but the general arrangement consists of transport of  $\text{HCO}_3^-$ , the major uptake form of inorganic carbon in cyanobacteria, across the outer membrane and the plasma membrane, through  $\text{HCO}_3^-/\text{Na}^+$  symports or ATP-driven uniports, as well as diffusion of  $\text{CO}_2$ , into the cytosol. Conversion of cytosolic  $\text{CO}_2$  is carried out by NADP dehydrogenase (NDH) complexes on the thylakoid and plasma membranes. The bicarbonate ion then enters the carboxysome, the protein-enclosed compartment that houses most of the Rubisco population, where it is converted to  $\text{CO}_2$  in a reaction catalyzed by carbonic anhydrase (CA):



The conversion of  $\text{CO}_2$  to  $\text{HCO}_3^-$  via the NDH complexes relies on CA-like activities in associated proteins. The active transport of  $\text{HCO}_3^-$  is dependent on extra ATP generated by cyclic electron transport around Photosystem I (PSI) in the photosynthetic electron transport chain (PET). This model of carbon-concentrating mechanism (CCM) and calcification in a cyanobacterial cell is represented on Figure 1. The different steps of nucleation, phase transition, crystallization and aggregation in the biomineralization processes still need to be understood, as well as the structural and functional characteristics of the EPS and S-layers during calcification. Note that some experimental studies demonstrated that the precipitation of  $\text{CaCO}_3$  is significantly hindered when the function of extracellular carbonic anhydrase in *Synechocystis* sp. was inhibited (i.e., Yang et al., 2016).



**Fig. 1.** Model of the carbon-concentrating mechanism (CCM) and calcification in a cyanobacterial cell.  $\text{CO}_2$  enters the cells mainly via active transport of  $\text{HCO}_3^-$  and also through diffusion of  $\text{CO}_2$ , which is converted to  $\text{HCO}_3^-$  during the uptake. Cytosolic  $\text{HCO}_3^-$  is subsequently imported to the carboxysome. CA, carbonic anhydrase; Ci, inorganic carbon; EPS, exopolysaccharide sheath; NDH, NADPH dehydrogenase; and PET, photosynthetic electron transport. From Jansson et al. (2010).

According to Liang et al. (2013), while there is evidence that picocyanobacteria play a crucial role in  $\text{CaCO}_3$  formation, the underlying mechanisms are a matter of controversial debates (Obst et al., 2009; Robbins et al., 1992). One hypothesis suggests that photosynthetic activity of bacterial cell leads to  $\text{CaCO}_3$  formation by  $\text{CO}_2$  uptake that influence calcium–carbonate equilibrium in the surrounding microenvironment, and thus shifts the chemical equilibrium in favor to  $\text{CaCO}_3$  precipitation merely via increasing ( $\Omega_{\text{CaCO}_3}$ ) (Kranz et al., 2010). An alternative mechanism of  $\text{CaCO}_3$  formation is that cell surfaces serve as a template for  $\text{CaCO}_3$  nucleation, and crystal growth is possible due to oversaturation in respect of  $\text{CaCO}_3$  in the vicinity



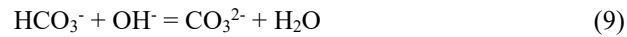
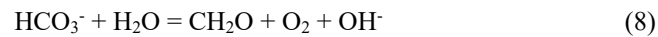
of cells. Picocyanobacterial cells have a highly reactive surface due to their large surface area-to-volume ratio (Dittrich et al., 2004; Lee et al., 2004) that can be an ideal template for crystal nucleation. The negatively charged functional groups at the cell surfaces can bind cations (i.e.,  $\text{Ca}^{2+}$ ) to promote nucleation process and subsequently lead to mineral formation (Schultzelam et al., 1994). The proposed mechanism of  $\text{CaCO}_3$  biomineralization suggests a formation of amorphous calcium carbonate (ACC) through aggregation of stable, amorphous, pre-critical clusters or particles are absorbed onto a surface, and crystallization of ACC to generate the final stable crystal product (Gebauer et al., 2008; Stephens et al., 2011). In their study, Liang et al. (2013) showed that each of the three tested *Synechococcus* strains (ARC21, LS0519 and PCC8806) had a different impact on the physicochemical conditions in bulk solution. These variations were linked to the ion exchange capacity at the cell surface-solution interface. These results suggested that *Synechococcus* cells facilitate aggregation of ions on organic templates *via* surface charge and crystallization of stable  $\text{CaCO}_3$  under low oversaturation. The same type of mechanism is proposed by Bundeleva et al. (2014), where cyanobacteria *Gloeocapsa* sp. cells induced the precipitation of  $\text{CaCO}_3$  *via* binding of  $\text{Ca}^{2+}$  with the negatively-charged groups of the cell surfaces and cell exopolymeric substances (EPS).

The study of Obst et al. (2009) is also in favor of a heterogenous nucleation process. Indeed, they performed long-term precipitation experiments in presence of cyanobacteria *Synechococcus leopoliensis* PCC 7942 with stabilized  $\text{CO}_2$  concentration and carbonate equilibrium in order to differentiate between *i*) a direct influence of the photosynthesis uptake of inorganic carbon on  $\text{CaCO}_3$  nucleation by local oversaturation next to the cell and *ii*) an indirect effect of photosynthesis uptake of bicarbonate raising the saturation of the bulk solution. In contrast to previous work, this study differentiated the impact of the cyanobacteria on the individual steps of bacterial biomineralization by continuously equilibrating the system with an atmosphere of constant  $\text{pCO}_2$  at low nutrient conditions. Although cyanobacteria can influence their chemical environment and, therefore, the thermodynamics and kinetics of  $\text{CaCO}_3$  precipitation, under nutrient-poor conditions and sufficient  $\text{CO}_2$  supply, neither photosynthetic uptake of  $\text{CO}_2$  nor active ion exchange processes across the cell membrane directly influenced kinetics of the mineral nucleation step on the cell surface. These authors conclude that the nucleation step of calcite biominerals on the surface of *S. leopoliensis* is a passive process, wherein the cell surface and/or extracellular polymers provide preferential sites for mineral nucleation.

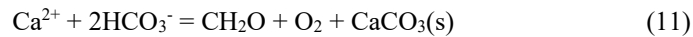
The study of Dittrich et al. (2003) on the precipitation of calcite in the presence of *Synechococcus* sp. cells with two different  $\text{HCO}_3^-/\text{Ca}$  ratios also suggests the importance of cells as nucleation centers in the precipitation mechanisms. This study investigates the role of the solution composition on calcite precipitation using solutions with different compositions, but at similar saturation state. Calcite precipitations were mediated by the addition of cyanobacteria *Synechococcus* strain PCC 7942 in a solution of calcium bicarbonate at different concentrations of inorganic

carbon and dissolved calcium. The composition of solutions strongly influenced the calcite precipitation, as the precipitation was higher when  $\text{HCO}_3^-/\text{Ca} > 1$ . However, these laboratory results suggested that the precipitation was mostly controlled by the conditions near to the cell walls rather than the supersaturation degree in the bulk solution.

Martinez et al. (2016) used a mixed flow bio-reactor set-up consisting of 2 stock solutions, one inorganic Ca stock solution and one cyanobacteria *Synechococcus* sp. PCC 7942 biomass stock solution, which were pumped through a sealed reactor vessel to maintain steady-state conditions. The inorganic carbon was provided *via* air bubbling. They observed the precipitation of  $\text{CaCO}_3$  phase and a gradual pH decrease which can be explained by the formation of  $\text{CaCO}_3^{\circ}(\text{aq})$  or  $\text{CaHCO}_3^+(\text{aq})$  complexes in alkaline solution, with the rapid deprotonation of dissolved  $\text{CO}_2$  ( $\text{H}_2\text{CO}_3$ ) to  $\text{CO}_3^{2-}$  and  $\text{HCO}_3^-$  and the slow exchange of  $\text{H}_2\text{CO}_3$  with the atmosphere. This supports the following mechanism:



with an overall reaction that can be written as:



The precipitation was confirmed by Raman spectroscopy and SEM observations, showing calcite crystals generated in the mixed-flow reactor. Overall, the results of this study propose that the photosynthetic activity of the planktonic cyanobacteria species *Synechococcus* sp. could aid in carbonate biomineralization at the large scale. Martinez et al. (2016) showed the generation of a supersaturated solution with respect to calcite with dissolving  $\text{CO}_2$  as the only source of inorganic carbon where active cyanobacteria maintained highly alkaline pH conditions. Although, in principle, this system has the potential to continuously biomineralize  $\text{CO}_2$  to store it in the form of calcite, further studies would be needed before a large-scale application of this system can be implemented.

Indeed, the role of photosynthesis in the precipitation processes still remains controversial. For example, Kosamu and Obst (2009) studied the precipitation of

CaCO<sub>3</sub> with *Synechococcus* sp. PCC 7942, with and without a photosynthesis inhibitor (Diuron). With sufficient supply of atmospheric CO<sub>2</sub>, precipitation was accelerated when photosystem II was blocked. This calls into question the role of photosynthesis in precipitation mechanisms. These results are in opposition with other studies (eg Stipp 1999; Thompson et al., 1997) which suggested that photosynthesis was essential in biomineralization processes.

## 1.2 Extracellular precipitation of Ca carbonates

Various cyanobacteria strains have proved to be able to calcify and produce extracellular precipitation of calcium carbonates. For example, Lee et al. (2004) showed that different strains of *Synechococcus* and *Synechocystis* have the ability to calcify when grown in microcosms enriched with HCO<sub>3</sub><sup>-</sup> and Ca<sup>2+</sup>. Among them, only two were able to calcify to the extent that a calcite precipitation was formed. Factors that had the greatest effect on calcification were CO<sub>2</sub> removal and subsequent generation of alkaline pH. Precipitation of CaCO<sub>3</sub> was also observed in the presence of cyanobacteria *Synechococcus* PCC 8806 and PPC 8807 (Lee et al., 2006). The adsorption of Ca<sup>2+</sup> cations on the outer membrane of cyanobacterial cells could form nuclei for the following precipitation of CaCO<sub>3</sub> (Obst et al., 2006).

Another phenomenon which could impact CaCO<sub>3</sub> biomineralization is viral lysis of cyanobacteria: this could release intracellular bicarbonate and induce the homogeneous nucleation of calcium carbonate. Indeed, Xu et al. (2019) demonstrated that viral lysis of cyanobacteria *Synechococcus* sp. PCC 7177 induced the formation of amorphous calcium carbonate and aragonite, compared with non-infected cultures, where only brucite (Mg(OH)<sub>2</sub>) precipitated. Viral lysis of cyanobacteria can create a calcification environment where carbonate is the dominant inorganic carbon species.

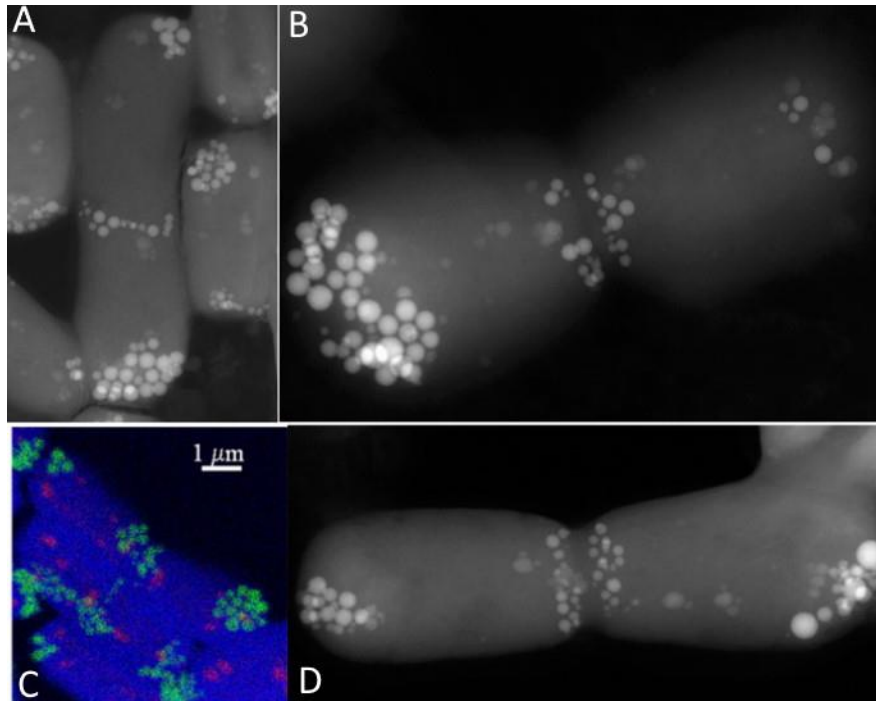
Organic compounds, such as extrapolymeric substances (EPS) secreted by cyanobacteria, could influence CaCO<sub>3</sub> polymorphism. In the study of Kawaguchi and Decho (2002), EPS from the lithified layer of stromatolites induced the formation of aragonite crystals whereas calcite crystals were formed in presence of EPS from the unlithified layer. For marine calcifiers, it has been shown that the chitin-rich skeletal organic matrix of coralline algae plays a key role in the biocalcification process, by enabling the formation of Mg-calcite (Rahman et al., 2019). The quantity of phosphorous in the medium is another factor that can impact carbonate precipitation rates and mineral morphology (Paulo et al., 2018).

Experiments with anoxygenic phototrophic bacteria demonstrated that they were able to form CaCO<sub>3</sub>, whose amount was directly correlated with the amount of organic C in the bacterial biomass produced (Bundeleva et al., 2012). There was no solid phase at the surface or in the vicinity of the cells, suggesting the existence of certain cell protection mechanism against carbonate precipitation at the cell surface. The same protective mechanism was observed with cyanobacteria *Synechococcus*

sp. and *Planktothrix* sp. (Martinez et al., 2010). These bacteria were capable of regulating their surface electric potential *via* decreasing the magnitude of surface charge or even generating positive charges in highly alkaline solutions (Martinez et al., 2008). From the one hand, this helped to electrostatically attract bicarbonate anions at alkaline pH, required for photosynthesis. From the other hand, maintenance of a positive surface charge at alkaline pH could protect active cyanobacteria against  $\text{Ca}^{2+}$  adsorption and subsequent carbonate precipitation on their surfaces. On the contrary, cyanobacteria *Gloeocapsa* sp. did not show any protection mechanism: experiments showed cell encrustation by  $\text{CaCO}_3$  precipitated in the form of nano-spheres adjacent to the cell surface (Bundeleva et al., 2014). This is most likely linked to the specific cellular organization of this species, which involves several cells in one single capsule. As such, planktonic cultures of *Gloeocapsa* sp. exhibit significant calcifying potential, making them important  $\text{CO}_2$  fixing microorganisms for both paleo-environmental reconstructions and technological applications.

### 1.3 Intracellular formation of Ca carbonates

Although the mechanisms of cyanobacterial calcification remain poorly understood, this process is invariably thought as extracellular and the indirect by-product of metabolic activity. But contrary to common belief, several cyanobacterial species perform Ca-carbonate biomineralization intracellularly. Indeed, Benzerara et al. (2014) conducted a study of intracellular  $\text{CaCO}_3$  biomineralization on 68 different cyanobacterial strains. For each strain, they performed Scanning Electron Microscopy (SEM) observations, Scanning Transmission X-ray Microscopy (STXM) and phylogenetic analyses. Among studied species, all but eight showed the formation of intracellular carbonate inclusions. Seven of them contained spherical and poorly crystalline Ca carbonate, located specifically at the poles of the cells. Furthermore, they observed at least two phenotypes for intracellular biomineralization, one of which shows an original connection with cell division (Figure 2). These findings open new perspectives on the evolution of cyanobacterial calcification. Other studies, such as Couradeau et al. (2012), Lin et al. (2014 ; 2016), Cam et al. (2018), showed the same type of intracellular precipitation in various cyanobacteria. Cam et al. (2016) realized an abiotic study of the precipitation of Ca- Sr- Ba- Mg- bearing carbonate, to mimic what happens in intracellularly calcifying cyanobacteria. The same size and the poor crystallinity were observed but the partition of these elements between solution and solid was different as in cyanobacterial cells.



**Fig. 2.** STEM-HAADF images of *Synechococcus* sp. PCC 6312. Ca-carbonate inclusions were always observed at the poles of the cells and sometimes at the same location where septation occurs (arrows). Inclusions at the septum are usually smaller than at the poles. Division as observed in A, B, and D seems to partition the Ca-carbonate inclusions between the daughter cells, resulting in the polar distribution observed in all of the cells. STEM-EDX map is shown in C. Calcium, green; phosphorus, red; carbon, blue. From Benzerara et al. (2014).

#### 1.4 Ca carbonate precipitation with heterotrophic bacteria

It is interesting to note that other microorganisms than cyanobacteria could allow the precipitation of calcium carbonate minerals. For example, Silva-Castro et al. (2015) studied the precipitation of calcite and aragonite in presence of cells isolated from seawater and brine in both liquid and solid environments. The cells were identified mostly as heterotrophic bacteria from genera *Bacillus* and *Virgibacillus*. Results showed that precipitation occurred only when organic matter was added in the solution.

Sondi and Matijevic (2001) showed that the nature of Ca carbonate whose formation is catalyzed by urease enzyme was strongly dependent on the agitation of the reacting solution: in an undisturbed system, an amorphous phase was formed,

which then crystallized to vaterite and then turned into calcite, whereas under the influence of magnetic stirring, there was instead smaller particles which aggregated to vaterite and calcite. The amount of the added enzyme had an influence on the kinetics of reaction, a greater quantity of enzyme led to a faster precipitation. But different enzymes can lead to different  $\text{CaCO}_3$  polymorphs. For example, in the study of Sondi and Salopek-Sondi (2005), the presence of *Bacillus* urease induced the formation of spherical and uniform vaterite, whereas only calcite was formed in the presence of *Canavalia* urease. Formation of monohydrocalcite by marine sulfate-reducing bacterium *Desulfovibrio bizertensis* has been recently reported by Bradbury et al. (2020).

Microbial cell surfaces and excreted extracellular polymeric substances (EPS) which carry a net negative electric charge and have the capacity to bind  $\text{Ca}^{2+}$  ions are frequently cited as the main sites of carbonate nucleation (Dupraz et al., 2004). However, direct observations of the nucleation processes are lacking and the nucleation sites remain elusive. Aloisi et al. (2006) tested the capacity of sulfate-reducing bacteria *Desulfonatronum lacustre* to serve as nucleation centers in solutions strongly supersaturated with calcite and aragonite. Small globules were only observed attached to the surface of bacterial cells where they were embedded in a thin film of EPS, whereas large globules occurred mostly separated from the cells where they formed aggregates. Chemical analyses of cell surfaces and globules indicated that the surface of *D. lacustre* cells was not the site of carbonate precipitation. Although minor amounts of calcium carbonate were bound to EPS and small globules, both the abundance of large globules and their elevated carbonate content indicated that the bulk of carbonate precipitation occurred in the form of large globules. Indeed, *D. lacustre* promoted calcium carbonate precipitation by producing spherical nucleation centers that calcified extensively when released in solution. The initial steps of globule formation occurred within the EPS film in intimate association with the bacterial cell surface. In the study of Bosak and Newman (2005), the uptake of millimolar sulfate and lactate by the sulfate-reducing bacteria *Desulfovibrio desulfuricans* stimulated the growth of subhedral crystals relative to abiotic controls. Besides, the presence of purified EPS produced by this strain enhanced the formation of crystals with rounded edges at low supersaturation index (SI) of calcite whereas the effect of these organic compounds was reduced at higher SI. These observations indicate that microbes can exert kinetic control by the secretion of exopolymeric substances and by the active uptake or release of primary organic and inorganic metabolites. For example, Ercole et al. (2007) described the formation of calcite crystals by extracellular polymeric substances isolated from *Bacillus firmus* and *Bacillus sphaericus*. Extracellular polysaccharides and capsular polysaccharides extracted from both bacteria were able to mediate  $\text{CaCO}_3$  precipitation. The shapes of the crystals were different depending on the capsular and extracellular polysaccharides fractions isolated from the bacteria.

The morphology of crystals could also depend on the presence of live or dead cells. Both play a passive role in calcium carbonate precipitation by acting as a template for heterogeneous nucleation, whereas live bacteria additionally play an active

role by modifying the physical chemistry of their microenvironment through active alkalization (Chekroun et al., 2004).

Overall, heterotrophic bacterial activity, linked to sulfate reduction, was considered as one of the main mechanisms of carbonate stromatolite buildups (i.e., Vasconcelos et al., 2006; Visscher et al., 1998), although a lot of controversies still remain, given that at relatively low pH of sulfate reduction (6.5 to 7.0), carbonate saturation might not be sufficient to induce large amount of carbonate mineral formation, especially in alkaline Precambrian ocean (Meister et al., 2013).

## 2 Ca-Mg carbonates

Dolomitic rocks, depending on the quantitative proportions of Ca and Mg, include dolomite, protodolomite, high- and low-magnesium calcites. Dolomite has a calcite structure in which the atoms of Ca in every second layer of cations are replaced by atoms of Mg. Protodolomite differs from true dolomite in the increased content of CaCO<sub>3</sub> (up to 10 mol%) and in its structural imperfection. High- and low-magnesium calcites are intermediates between calcite and dolomite. Both high- and low-magnesium calcites are widespread in marine sediments, where they form skeletal remains and cement. There is little doubt that in natural low temperature systems (< 60 °C) dolomite formation occurred via microbially-catalyzed reactions (Petrash et al., 2017). Numerous experiments demonstrated poorly ordered dolomite formation under action of sulfate-reducing bacteria (Baldermann et al., 2015; Vasconcelos et al., 1995; Warthmann et al., 2000), anaerobic microbial consortium (Kenward et al., 2009), methanogens (Kenward et al., 2013) and halophiles (Al Disi et al., 2017; Kenward et al., 2013; Sánchez-Román et al., 2009a). Thus, Qiu et al. (2017) demonstrated dolomite precipitation in a batch culture of *Haloferax volcanii*, a halophilic archaeon and argued that under high salinity conditions, more carboxyl groups were presented on the cell surface to resist the high osmotic pressure, which may result in the subsequent promotion of dolomite formation. Moderately halophilic bacteria *Halobacillus trueperi*, isolated from hypersaline sediments of the Great Salt Lake in Utah, were able to produce Mg and Ca carbonates in both solid and liquid media of different salt concentrations, most of the time during the stationary phase of growth. This mechanism involves the adsorption of Ca<sup>2+</sup> and Mg<sup>2+</sup> and the production of CO<sub>2</sub> and NH<sub>4</sub><sup>+</sup> ions, which increases pH, during the metabolism of the nitrogenated organic materials. This process could be described as a biologically induced biomineralization, a process which takes place in an open medium without the participation of macromolecules or specific cells (Rivadeneira et al., 2004). Nineteen other moderately halophilic bacteria also formed Mg and Ca carbonates whose size and quantity increased with the incubation time (Sanchez-Roman et al., 2007). In the latter study, with increased Ca<sup>2+</sup> ion concentration in the culture media, the Mg<sup>2+</sup> content in calcite decreased, the time required for carbonate

precipitation decreased, and the amount of precipitate increased. Studies of sulfate reducing heterotrophic bacteria mediation in the precipitation of Mg-Ca carbonate minerals showed that the associated extracellular polymeric substance rather than the bacterial respiration played an important role in the nucleation of dolomite (Bontognali et al., 2008; 2014).

A number of studies addressed Ca isotope fractionation during microbial-induced Ca and Mg carbonate formation (Krause et al., 2018; Bradbury et al., 2020). In the presence of pure cultures of the sulfate-reducing bacterium *Desulfobulbus mediterraneus*, there was progressive enrichment in  $^{40}\text{Ca}$  from culture media to biological material to the carbonate minerals precipitated (Krause et al., 2012). These authors concluded that there was a two-step calcium isotope fractionation, with calcium isotope fractionation during the initial association of calcium with the bacterial biofilm, and further calcium isotope fractionation with the precipitation of dolomite.

In contrast, the role of phototrophic microorganisms, cyanobacteria and their metabolites in particular, in the process of the accumulation of dolomitic rocks, is poorly studied. Zaitseva et al. (2006) showed that the presence of cyanobacteria *Microcolius chthonoplastes* tend to slow down the precipitation of magnesium carbonates due to the formation of exometabolites with a chelating effect. The presence of cyanobacteria in the light or in the darkness considerably altered the structure of the sediment, the size of carbonate crystals, and their shape. Where photosynthesis was present, huntite ( $\text{Mg}_3\text{Ca}(\text{CO}_3)_4$ ), a possible forerunner of dolomite) was clearly observed whereas in the dark protodolomite was formed in association with a broad range of magnesium calcites, as a result of the crystallization of the carbonate sediment. Other experiments with cyanobacteria *Microcoleus chthonoplastes* reached the same conclusions (Ushatinskaya et al., 2006).

The study of the dynamics of transformation of Ca-Mg carbonate under the conditions of a soda lake showed that the activity of various organisms of a cyanobacterial mat resulted in a restructuring of the initial carbonate; its mineralogical composition changed depending on the conditions of the mat. In Mg calcites, which are formed under low-temperature conditions, a cation adjustment can occur with the formation of dolomite domains (Zaitseva et al., 2007).

The study of the natural variation of Mg and Ca stable isotopes of carbonates in carbonate skeletons of perforate foraminifera and reef coral together with Mg/Ca ratios (Chang et al., 2004) showed that in reef coral, the controlling factor of carbonate biomineralization was principally inorganic, physicochemical crystal growth from an aqueous solution was close to seawater in composition. In foraminifera, the processes of nucleation and crystallization were similar and Ca isotopes could not be sensitive enough to distinguish various mechanisms of  $\text{Ca}^{2+}$  transport within the cells of different phyla. The basic question as to whether biomineralization of foraminifera is a simple inorganic process or proceeds under biological control cannot be determined on the basis of Ca isotope fractionation alone.



### 3 Mg carbonates

The calcium carbonate mineral precipitation is by far the most studied microbially mediated carbonation reaction that has been documented in most surface environments such as oceans, lakes, soils, caves, deserts, and groundwater. However, cyanobacteria- and diatom associated magnesium carbonate precipitation has also been documented in several alkaline aquatic environments. Magnesium carbonate precipitation, like calcium carbonate formation, is strongly affected by pH and alkalinity, although, the biogeochemical conditions required for magnesium carbonate precipitation are not fully understood.

#### 3.1 Formation of different Mg carbonates

According to Power et al. (2007), who performed laboratory experiments to compare abiotic and biotic carbonate precipitation under controlled conditions and characterize the newly formed precipitates, the nature of the precipitated mineral is different depending on whether or not cyanobacteria were present. Indeed, the results of the laboratory experiments suggest that cyanobacteria promote carbonate formation at a faster rate and, more importantly, of a different mineralogy than the abiotic systems. In association with experimental biofilms, dypingite ( $\text{Mg}_5(\text{CO}_3)_4(\text{OH})_2 \cdot 5(\text{H}_2\text{O})$ ) was the primary precipitate. The abiotic controls precipitated only nesquehonite ( $\text{Mg}(\text{HCO}_3)(\text{OH}) \cdot 2(\text{H}_2\text{O})$ ). In the abiotic control, the addition of NaOH caused the precipitation of dypingite rosettes similar to those associated with the biofilms. However, nesquehonite continued to be the dominant precipitate favored under abiotic conditions, even at higher pH values. This suggests that pH within the experimental biofilms must have been at least one pH unit greater than the final bulk solution pH. Furthermore, it strongly indicates that the hydroxyl ions produced during photosynthesis promote precipitation of dypingite.

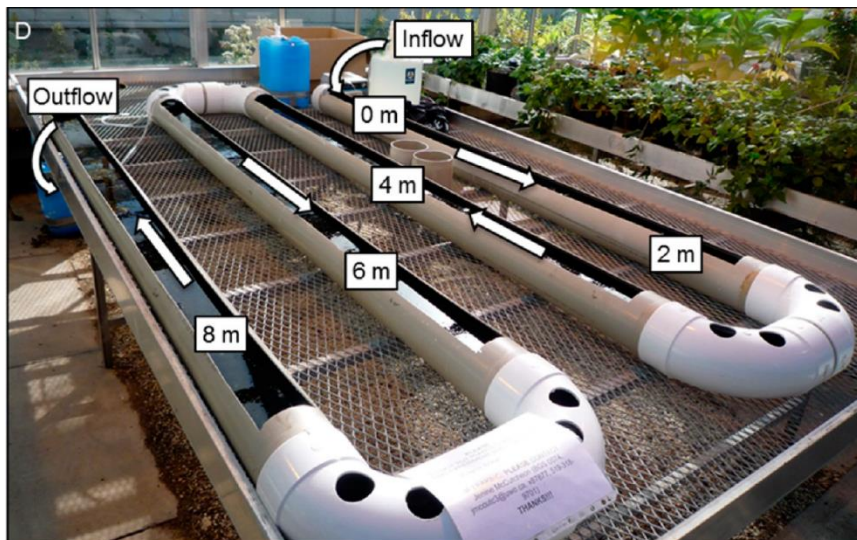
Other studies indicate no difference between Mg carbonates formed under biotic or abiotic conditions. As an example, Mavromatis et al. (2012) performed Mg carbonates precipitation laboratory experiments in batch reactors in presence and absence of the cyanobacteria *Gloeocapsa* sp. under various conditions: stirring and air bubbling, continuous light, darkness, day/night cycle. All experiments yielded similar bulk precipitation rates of hydrous Mg carbonates nesquehonite and dypingite, although the pH of the reactive fluids was 0.5–1.0 units higher in biotic experiments compared to abiotic experiments. Similarly, retrieved  $\Delta^{26}\text{Mg}_{\text{solid-liquid}}$  values obtained from abiotic experiments are identical within uncertainty of those obtained in the presence of *Gloeocapsa* sp. Taken together, these observations indicate that the presence of cyanobacteria affects neither the rates nor the Mg isotopic fractionation of the precipitated hydrous magnesium carbonates in natural systems. This conclusion suggests that Mg isotopic composition of magnesium carbonate may not

be indicative of either the role of bacteria or the fluid composition of past ecosystems.

In addition to the formation of dypingite and nesquehonite, biomineralization of hydromagnesite ( $\text{Mg}_5(\text{CO}_3)_4(\text{OH})_2 \cdot 4(\text{H}_2\text{O})$ ) is also possible. For example, Shirokova et al. (2013) performed batch laboratory experiments in presence of cyanobacteria *Synechococcus* sp. isolated from stromatolites of Salda Lake (Turkey), which resulted in the precipitation of dypingite and hydromagnesite similar to the natural hydromagnesite of stromatolites. Rates of precipitation were similar to that reported for dypingite precipitation in the presence of *Gloeocapsa* sp. during stirred, non-bubbled experiments of Mavromatis et al. (2012). In control experiments, no precipitation was observed.

### 3.2 Experimental reactors used to reproduce natural settings

Although numerous laboratory experiments were realized in closed (batch) reactors, alternative types of reactors may be used to study the biomineralization in various conditions in order to better approach the natural environments. For instance, McCutcheon et al. (2014) used a plug flow bioreactor constructed from a 15 cm diameter polyvinyl chloride pipe to study the hydromagnesite precipitation in presence of sediments covered with a microbial mat, composed mainly of filamentous cyanobacteria and diatoms, from an alkaline wetland. The gravity driven flow-through channel had a volume, length, and surface area of 155 L, 10 m, and 1.5 m<sup>2</sup>, respectively (Figure 3). The bioreactor was located in a greenhouse which maintained a minimum air temperature of 18 °C and received at least 14 h of light/day through combined natural and artificial lighting. The microbial mat sample was layered onto the sediments, all of which was then submerged below 100 L of growth medium (5 cm depth). After 8 weeks, this medium was replaced by a feedstock solution containing 41.07 mM of Mg and 82.4 mM of NaHCO<sub>3</sub>, added at the 0 m position at a rate of 5 L/day. The pH and dissolved oxygen (DO) trends indicated that mineral precipitation occurred at the inflow to the bioreactor. This observation was strongly complemented by the large decrease in conductivity, alkalinity, and dissolved magnesium concentration during the carbonation phase. There was a drop in DO during the first week of the carbonation phase, which may be due to the onset of carbonate mineral precipitation slowing photosynthesis by shielding the mats from sunlight. The precipitated mineral was identified as hydromagnesite. The mineralization values were used to determine the rate of carbon storage in the precipitated hydromagnesite. The maximum rate of hydromagnesite precipitation achieved within the upper 2 m of the bioreactor during the first week was 13.2 g/day. The average rate for the first 2 m of the bioreactor was 8.0 g/day over the course of the experiment.



**Fig. 3.** The bioreactor inoculated with the Atlin microbial mats from McCutcheon et al. (2014). The feedstock solution was added to one end of the system and flowed passively through the bioreactor (arrows) and out a valve in the other end. Sampling points are marked in meters from the start of the channel.

Overall, such medium-scale laboratory mesocosms operated under controlled external (T, light,  $p\text{CO}_2$ ) and internal (pH,  $\Omega$ , salts, organic matter) parameters, ideally in a flow-through biofilm-bearing systems, are the best suited to study the governing factors and mineral precipitation rates at the environments that can be reasonably well extrapolated to contemporary natural settings. In order to use these laboratory facilities to reproduce Precambrian environments (high water temperature and high  $p\text{CO}_2$ ), a number of adjustments have to be implemented. The main shortcoming of any biofilm-like cyanobacterial culture is that sizable gradients of pH, Ca(Mg) and  $\Omega$  in the vicinity of biofilm surface, at the scale of  $\mu\text{m}$  to mm, have to be monitored; ideally, this monitoring should include a diel pattern with high temporal resolution.

### 3.3 Mg carbonates precipitation with fermentative bacteria

As for Ca carbonates, the biomineralization of Mg carbonates can occur in the presence of other microorganisms than cyanobacteria. For example, Sanz Montero et al. (2019) showed the potential of the fermentative bacteria *Desemzia incerta* to induce the carbonate precipitation in both liquid and solid media. Geochemical modeling indicated that the culture medium was undersaturated with most carbonate minerals except for dolomite and magnesite which were slightly supersaturated. Hydromagnesite and nesquehonite should not precipitate from this fluid. After 15 days

of incubation at 30 °C, mineral precipitation was observed in both solid and liquid culture experiments. The mineralogical analysis of the precipitates indicated the presence of a variety of high Mg-carbonates in all the samples. The monohydrated Ca-carbonate, monohydrocalcite ( $\text{CaCO}_3 \cdot \text{H}_2\text{O}$ ), is also present in the experiments using liquid medium, whilst minor amounts of Mg-calcite are recorded only in the solid culture. The most abundant minerals are the hydrous Mg-carbonates formed by a dypingite-hydromagnesite assemblage that always occurs associated with dolomite and most rarely with Mg-calcite and magnesite. Nesquehonite is formed in the solid culture experiment. In contrast, huntite was only detected in liquid medium. The microorganisms and the EPS embedding the carbonate crystals contained  $\text{Mg}^{2+}$  and to a lesser extent  $\text{Ca}^{2+}$  and other cations. The release of Mg and Ca through the bacteriolysis of the biomass may significantly increase the saturation levels inside the mats, favoring the precipitation of Mg-enriched phases (Cabestrero et al., 2018). The creation of micro-environments around the bacterial cells through the alteration of carbonate alkalinity and  $\text{Mg}^{2+}$  availability, and providing nucleation sites, led to the precipitation of the Mg-rich carbonates (Pontoizeau et al., 1997; García Del Cura et al., 2014). In addition, the organic ligands on microbial cells may cause partial dehydration of the adsorbed  $\text{Mg}^{2+}$  that further facilitate the formation of Mg-bearing minerals by attracting the counter ions (Power et al., 2009; 2017).

#### ***4 Efficiency of phototrophic bacteria to precipitate calcium and magnesium carbonates for ex-situ carbon sequestration***

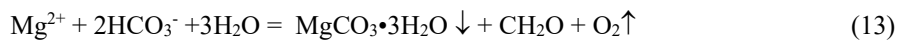
Based on batch laboratory experiments performed by our group on cyanobacterially-induced precipitation of Mg and Ca carbonates, we analyzed the ratio between sequestered metal cation or inorganic carbon (precipitated in the form of carbonate minerals) and the amount of sequestered organic carbon, *via* an increase in microbial biomass due to cell growth. This allowed assessing the efficiency of biological C sequestration *via* cyanobacterial biomineralization under controlled laboratory conditions.

##### **4.1 Mg hydrous carbonate precipitation in the presence of *Gloeocapsa* sp. cyanobacteria**

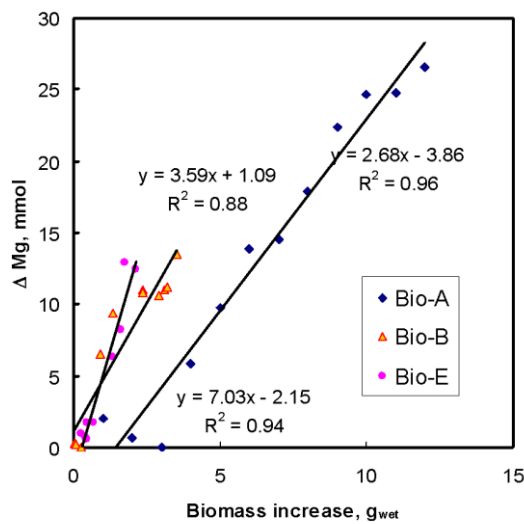
Mavromatis et al. (2012) studied the precipitation of Mg carbonate in presence of cyanobacteria *Gloeocapsa* sp. The mass of precipitated hydrous magnesium carbonate is plotted as a function of the measured biomass present in the reactor fluid in Figure 4. The lines drawn in this figure, consistent with the results of all experiments containing biomass, can be described by:

$$\text{Mg}_{\text{precipitated}} (\text{mmol}) = (2.7 - 7.0) \times \text{Biomass}_{\text{produced}} (\text{g}_{\text{wet}}), \quad r^2 = 0.96-0.88 \quad (12)$$

Converting this relationship into molar scale and considering that the ratio of wet to dry biomass of *Gloeocapsa* sp. is  $10 \pm 2$  and that the proportion of carbon in dry biomass is 50% yield the molar inorganic Mg to organic C ratio in reaction product of 0.54 to 1.4 depending on stirring/bubbling regime and the status of *Gloeocapsa* sp. bacteria. This value is compatible with the theoretical Mg/C<sub>org</sub> ratio of 1 for nesquehonite as first precipitating phase during cyanobacterial photosynthesis:



Note that the theoretical value Mg/C<sub>org</sub> in case of dypingite or hydromagnesite formation is equal to 1.25.



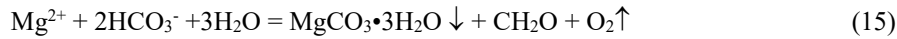
**Fig. 4.** Plot of Mg removed (mmol) as a function of absolute biomass increase during *Gloeocapsa* sp. growth and hydrous Mg carbonate precipitation. Data from Mavromatis et al. (2012).

#### 4.2. Mg hydrous carbonate precipitation in the presence of *Synechococcus* PCC sp. cyanobacteria separated from the lake Salda hydromagnesite stromatolites

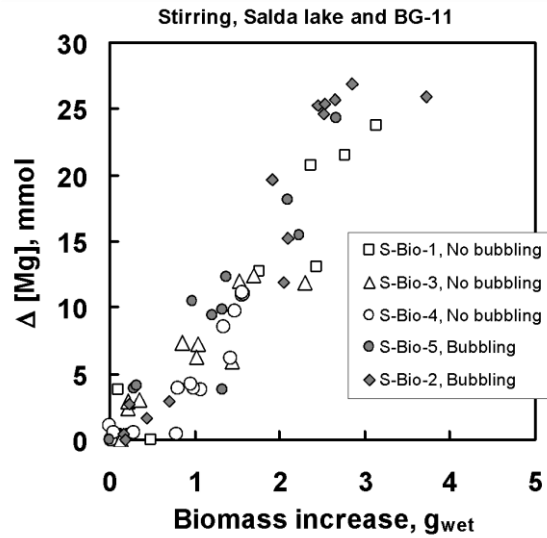
The mass of precipitated hydrous magnesium carbonate is plotted as a function of the measured biomass present in the reactor fluid in Figure 5. The line drawn in this figure, consistent with all the results of all experiments containing biomass, can be described by:

$$\text{Mg}_{\text{precipitated}} (\text{mmol}) = (7.1 \pm 0.9) \times \text{Biomass}_{\text{produced}} (\text{g}_{\text{wet}}), \quad r^2 = 0.93-0.84 \quad (14)$$

Converting this relationship into molar scale and considering that the ratio of wet to dry biomass of *Synechococcus* sp. is  $8 \pm 2$  and that the proportion of carbon in dry biomass is 50% yield the molar inorganic Mg to organic C ratio in reaction product of  $1.4 \pm 0.2$ . This value is compatible with the theoretical Mg/C<sub>org</sub> ratio of 1 for nesquehonite as first precipitating phase during cyanobacterial photosynthesis:



The amount of precipitated hydrous Mg carbonate and biomass production were highly correlated ( $r^2 \sim 0.9$ ) in our laboratory experiments which was consistent with theoretical mineral yield during the biomass production (see Figure 5). Moreover, the mineral yield due to microbial photosynthesis and, thus, mineral precipitation rates, were very similar among different experiments despite the fact that the experiments were performed in various distinct fluids, durations, biomass concentration, and air bubbling regimes. Given the large variety of investigated conditions, we suggest that the linear relationship shown in Figure 5 (Eqn. 14) can be used for the quantitative prediction ( $\pm 25\%$ ) of the amount of hydrous Mg carbonate precipitation in the presence of photosynthesizing cyanobacteria.



**Fig. 5.** Relationship between the amount of precipitated magnesium (mmol) and the increase of the biomass ( $g_{wet}$ ) in different biotic experiments ( $r^2 = 0.84 - 0.93$ ) performed by Shirokova et al. (2013).

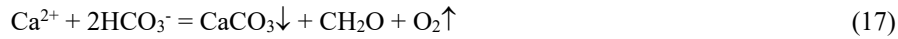
#### 4.3. Calcium carbonate precipitation in the presence of *Gloeocapsa* sp. cyanobacteria

Results of the work of Bundeleva et al. (2014) allowed establishing a direct relationship between the amount of precipitated calcium and the amount of biomass produced during culture growth (Figure 6). Quantitative analysis of the amount of precipitated mineral (mole of Ca removed from solution) as a function of biomass production yielded a clear relationship with a slope of 2.0 following the relationship:

$$Ca_{precipitated} \text{ (mmol)} = (2.0 \pm 0.2) \times Biomass_{produced} \text{ (g}_{wet}) \quad (16)$$

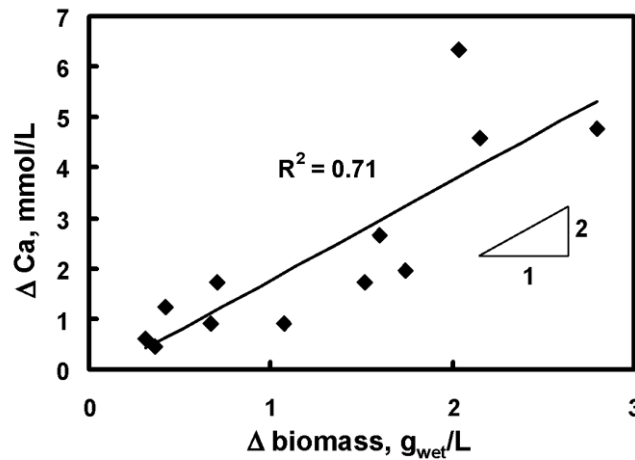
Converting this relationship into molar scale and taking into account that the ratio of wet to dry biomass of *Gloeocapsa* sp. is  $10 \pm 2$  and that the proportion of carbon in dry biomass is 50% yield the molar inorganic Ca to organic C ratio in the

reaction product of  $0.48 \pm 0.12$ . This value is two times smaller than the theoretical  $\text{Ca}/\text{C}_{\text{org}}$  ratio corresponding to  $\text{CaCO}_3$  precipitation due to photosynthesis:



Several factors may be responsible for the smaller amount of sequestered  $\text{CaCO}_3$  compared to the reaction stoichiometry such as *i*) inhibition of calcite nucleation by the cell exometabolites allowing the solution to maintain elevated supersaturation degree, *ii*) decreased heterogeneous crystallization rate on already-formed nucleus due to surface passivation by dissolved organic matter and EPS, and *iii*) enhanced production of extracellular polymeric substances by bacteria that lead to increase the organic matter yield without massive  $\text{CaCO}_3$  precipitation.

Noteworthy that the measured apparent bulk rates of  $\text{CaCO}_3$  precipitation due to *Gloeocapsa* sp. photosynthesis (0.01-0.100 mM/hr, Bundeleva et al., 2014) are similar or higher than those reported for capsule-free cyanobacteria *Synechococcus* sp. and *Planktothrix* sp. (0.03-0.04 mM/hr, Martinez et al., 2010), aerobic ureolithic bacteria (0.01 mM/hr, Micthel and Ferris, 2005) and anoxygenic phototrophic bacteria (0.005-0.13 mM/hr, Bundeleva et al., 2012).



**Fig. 6.** Relationship between the amount of precipitated Ca (mM/L) and increase of the biomass (g<sub>wet</sub>/L) in biotic experiments with *Gloeocapsa* sp. in nutrient solution. Experimental conditions:  $\Omega$  initial = 15-150; pH = 8.6-9.6.



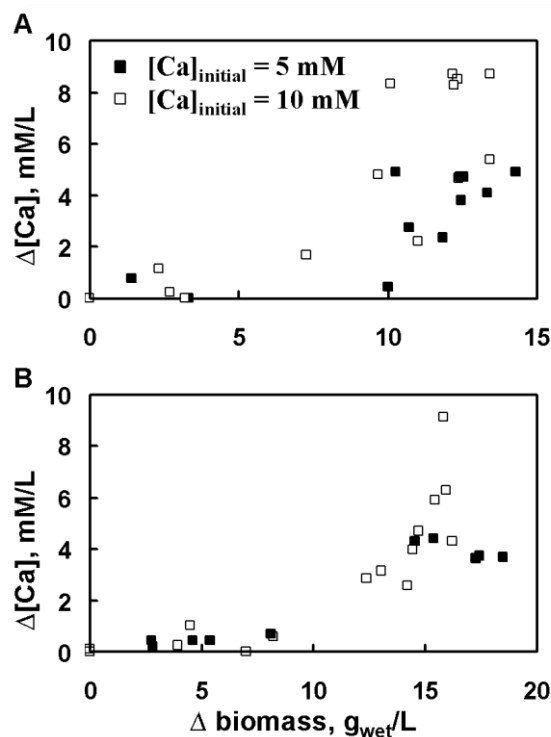
#### 4.4. Calcium carbonate precipitation in the presence of anoxygenic phototrophic bacteria (APB)

For APB, a clear correlation between the absolute increase of bacterial biomass and the amount of precipitated  $\text{CaCO}_3$  for both bacterial species was observed (Bundeleva et al., 2012). This dependence for *Rhodovulum steppense* A-20s and *Rhodovulum* sp. S-17-65 can be represented by the equations (18) and (19) respectively:

$$\text{Ca}_{\text{precipitated}} (\text{mol}) = (1.5 \pm 0.5) \times \text{Biomass}_{\text{produced}} (\text{g}_{\text{wet}}), \quad r^2 = 0.80 \pm 0.10 \quad (18)$$

$$\text{Ca}_{\text{precipitated}} (\text{mol}) = (0.5 \pm 0.3) \times \text{Biomass}_{\text{produced}} (\text{g}_{\text{wet}}), \quad r^2 = 0.85 \pm 0.15 \quad (19)$$

The slope of dependence ranges from 1 to 2 and 0.2 to 0.8 for A-20s and S-17-65, respectively depending on the  $\text{CaCl}_2$  and DIC concentrations (Figure 7). Converting this relationship into molar scale and taking into account that the ratio of wet to dry biomass of *R. steppense* A-20s and *Rhodovulum* sp. S-17-65 is 11.7 and 16.1, respectively, and that the proportion of carbon in dry biomass is 50% yield the molar inorganic Ca to organic C ratio in the reaction product of  $0.45 \pm 0.15$  and  $0.2 \pm 0.1$  for A-20s and S-17-65 strains, respectively.



**Fig. 7.** Relationship between the amount of precipitated Ca (mM/L) and increase of the biomass (g<sub>wet</sub>/L) in biotic experiments with *Rhodovulum steppense* A-20s (A) and *Rhodovulum* sp. S-17-65 (B) in nutrient solution. Initial experimental conditions: [Ca] = 5 and 10 mM; [DIC] = 5-20 mM. Data from Bundeleva et al. (2012).

Several case studies described in this section demonstrate high potential of photosynthesizing microorganisms to effectively remove both organic (biomass) and inorganic (mineral) carbon during their metabolic activity.

The molar ratio of C<sub>inorganic</sub> to C<sub>organic</sub> removed from solution during biologically-induced Ca and Mg carbonate precipitation in the presence of cyanobacteria and anoxygenic phototrophic bacteria ranges from 0.2 to 1.4. The highest value (1 to 1.4), compatible with theoretical ratio of 1, is associated with Mg hydrous carbonate precipitation in the presence of *Synechococcus* sp. cyanobacteria.

We noted a remarkable similarity of the amount of inorganic carbon biomineralized by phototrophic bacteria per gram of organic carbon (in the form of biomass) produced (0.5 for *Gloeocapsa* sp.; 1.1 for *Synechococcus* sp.; 0.3-0.6 for anoxygenic phototrophs). This suggests some universal features of C sequestration by aquatic phototrophic bacteria. It follows that photosynthesizing microorganisms are capable of sequestering approximately equal amount of organic and inorganic carbon during their growth in Ca(Mg), HCO<sub>3</sub>-bearing media.

Moreover, the ratio of carbonate C to organic C produced by marine plankton coccolithophorids typically ranges from 0.8 to 0.9 and sizably decreases with an increase in  $p\text{CO}_2$  (Riebesell et al., 2000). This value should be explicitly considered during *i*) paleo-biogeochemical reconstructions and assessment of biota response to ocean acidification (Beaufort et al., 2011) and *ii*) quantitative modeling of *ex-situ* C sequestration (Gwenzi, 2019).

As perspectives of this research, we foresee a need of systematic assessment, using well elaborated experimental techniques, of the ratio between organic carbon produced by cells and inorganic carbon sequestered in the form of minerals, for a wide variety of photosynthesizing organisms of marine and freshwater environments. One should also consider combining a double culture of primary producers with heterotrophic bacteria. The coupled cyanobacteria-heterotrophic bacteria metabolism should allow a possibility of photosynthetically-produced  $\text{C}_{\text{org}}$  degradation by heterotrophic bacteria into dissolved  $\text{HCO}_3^-$  and  $\text{CO}_2$  that can be potentially transformed into mineral form thus doubling the efficiency of atmospheric  $\text{CO}_2$  sequestration by microorganisms.

An important step towards resolving a mystery of Mg-rich carbonate (dolomite, magnesite) formation in the past sedimentary environments in view of difficulties in production of such carbonates in the laboratory has been achieved via experimental modeling of non-equilibrium cyclic growth and replacement mechanism through precipitation and dissolution steps, reproducing possible lagoonal/playa environments (Hobbs and Xu, 2020). In this regard, it is important to note that both photosynthetically-induced pH and  $\text{CO}_3^{2-}$  concentration rise (during day time) and organic matter degradation by heterotrophs, thus maintaining high DIC concentration (especially during night time), are recognized as driving factors of calcification in microbial mats (i.e., Ludwig et al., 2005). It is therefore possible that alternating day/night cycles of microbial activity involving the dominance of cyanobacteria and heterotrophs may provide necessary cycling of solution saturation degree thus favoring anhydrous Mg-rich carbonate precipitation at ambient conditions.

## Conclusion

Experimental studies on the precipitation of calcium and magnesium carbonates in the presence of aquatic phototrophs (cyanobacteria) and heterotrophic bacteria allow a better understanding of the processes of formation of these minerals in natural environments. These processes are controlled by various parameters such as pH, concentrations of elements in solution, the presence of organic compounds, living or dead cells and their EPS. Studies under controlled laboratory conditions allow constraining the role of each of these parameters in the overall mechanisms. Subsequently, the use of reactors capable to mimic the natural environments and elaboration of more realistic models through the joint use of different strains of cyanobacteria should allow a better understanding of these mechanisms as a whole. The best

type of reactors to study microbial bio-carbonatation is flow-through (open system) biofilm plug flow reactors; however, these require high-resolution spatial and temporal monitoring. Quantitative biomineralization processes is essential for understand the formation of stromatolites in the past and also allows important applications for the society such as CO<sub>2</sub> sequestration, mine rehabilitation or building restoration.

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