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and studies of biomineralization

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Regulation and Applications of Microbial Nanomineral Interfaces

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Abstract

Microorganisms are the earliest, most diverse, and most abundant life forms on Earth, with the most comprehensive metabolic capabilities. Throughout the co-evolutionary process spanning over three billion years with the Earth, microorganisms have played an extremely important role in mineralization. On one hand, through their mineralization activities, they drive the chemical cycling of elements on Earth and participate in the transformation of terrestrial environments. On the other hand, many microorganisms use the minerals they produce to perceive external signals, respond to environmental changes, or adapt to extreme conditions. The microbe-mineral interface is the smallest site on Earth where substances and energy exchange occurs, serving as a crucial model for understanding theories of biomineralization, geomagnetism, and other aspects of geochemistry or Earth biology. However, naturally occurring microbe-mineral interfaces have a limited range of structures, greatly constraining their potential applications.

This study employed a multidisciplinary approach to functionalize and modify microbe-mineral interfaces, constructing composite interfaces such as microbe/palladium-gold alloy/reduced graphene oxide. This innovation resolved the challenge of balancing the catalytic activity and stability of nanocatalysts, offering new options for fuel cell electrocatalysts. Furthermore, by creating interfaces like

microbe/gold-platinum nanozymes and microbe/Prussian blue, it enhanced the targeted killing ability of nanodrugs against tumors while reducing their toxicity to normal cells. This achievement realized tumor chemodynamic and immunotherapy, potentially providing a precision medicine solution for cancer treatment.

References

- Liu, J.#; Zheng, Y.#; Hong, Z.; Cai, K.; Zhao, F.*; Han, H.* Microbial synthesis of highly dispersed PdAu alloy for enhanced electrocatalysis. *Sci. Adv.* 2016, 2, e1600858.
- Li, X.#; Liu, J.#; Zhang, W.; Wu, Y.; Li, J.; Foda, M. F.; Han, H.* Biogenic Hybrid Nanosheets Activated Photothermal Therapy and Promoted Anti-PD-L1 Efficacy for Synergetic Antitumor Strategy. *ACS Appl. Mater. interfaces* 2020, 12, 29122-29132.
- Zhang, W.; Liu, J.*; Li, X.; Zheng, Y.; Chen, L.; Wang, D.; Foda, M. F.; Ma, Z.; Zhao, Y.*; Han, H.* Precise Chemodynamic Therapy of Cancer by Trifunctional Bacterium-Based Nanozymes. *ACS Nano* 2021, 15, 19321-19333.
- Deng, Z.#; Liu, J.#; Xi, M.; Wang, C.; Fang, H.; Wu, X.; Zhang, C.; Sun, G.; Zhang, Y.; Shen, L.; et al. Biogenic platinum nanoparticles on bacterial fragments for enhanced radiotherapy to boost antitumor immunity. *Nano Today* 2022, 47, 101656.
- Wang, D.#; Liu, J.#; Wang, C.; Zhang, W.; Yang, G.; Chen, Y.; Zhang, X.; Wu, Y.; Gu, L.; Chen, H.; et al. Microbial synthesis of Prussian blue for potentiating checkpoint blockade immunotherapy. *Nat. Commun.* 2023, 14, 2943.

The migration and mineralization process of microorganisms on Fe and Mn in oceanic ferromanganese nodules

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Abstract

Ferromanganese (Fe-Mn) nodules are being widely explored for their significant economic potential. It was found that the iron and manganese minerals in polymetallic nodules are not only formed by pure physical mineralization, but also include the biologically processes driven by microorganisms (biomineralization). Microorganisms can promote the oxidation of ferrous ions or regulate the pH value of the environment, resulting in the oxidation of ferrous ions by oxygen; Microbes can also cause anaerobic oxidation of ferrous ions; Both dissolved and non-dissolved trivalent iron ions can be reduced by microorganisms to ferrous iron. Similar to the oxidation process of ferrous iron, the reduction reactions of these trivalent iron ions include both enzymatic and non-enzymatic reactions. Microorganisms can greatly increase the rate of manganese oxidation. Like the biological oxidation process of iron, the biological oxidation of manganese can also be divided into enzymatic and non-enzymatic processes. Rock lacquer, iron-manganese nodules, and some sedimentary manganese deposits are all believed to be related to microbial oxidation of manganese. These studies indicate that biomineralization has a great contribution to the mineralization of ferromanganese nodules, and provide a more comprehensive and accurate understanding of biogenetic process of deep-sea ferromanganese nodules.

Key words: ferromanganese nodules; biomineralization; microbial diversity

Structural and chemical characteristics of siderite produced by iron-reducing bacteria *Shewanella oneidensis* MR-4

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Abstract

Dissimilatory iron-reducing bacteria can use organic matter or H₂ as an electron donor to reduce Fe(III) and mineralize extracellularly to form Fe(II) minerals, such as siderite (FeCO₃) and vivianite (Fe₃(PO₄)₂·8H₂O), or mixed-valence iron minerals, such as magnetite (Fe₃O₄) and green rust ([Fe(II)_{1-x}Fe(III)_x(OH)₂]^{x+}·[(x/n)Aⁿ⁻, mH₂O]^{x-}). The extracellular mineralization processes induced by dissimilatory iron-reducing bacteria can directly or indirectly affect the redox cycle of Fe and degradation of organic pollutants in groundwater, soil and sediment systems (Han et al., 2020). Moreover, it may have played an important role in the biogeochemical cycle of Fe in the early Earth, such as the deposition of iron formations in the Precambrian. A large number of studies

have shown that the magnetite and siderite in iron formations may be related to the metabolic activities of dissimilatory iron-reducing bacteria (Han et al., 2021; Heimann et al., 2010; Johnson et al., 2008; Li et al., 2011). However, compared with magnetite, the process and mechanism of siderite mineralization by dissimilatory iron-reducing bacteria remain unclear. In this study, dissimilatory iron-reducing bacterium *Shewanella oneidensis* MR-4 was cultured with ferrihydrite as an iron source. By measuring the protein concentration, Fe concentration and pH value of the culture system, we quantitatively analyzed the growth of microorganisms and changes in the chemical properties of the system during the reduction process of *S. oneidensis* MR-4. Scanning electron microscopy-Raman combined systems and transmission electron microscopy methods were

used to monitor the formation process of magnetite and siderite for 54 days. Finally, a model for extracellular biomineralization of siderite by the dissimilatory iron-reducing bacterium *S. oneidensis* MR-4 was proposed.

References

- Han, X., Tomaszewski, E.J., Sorwat, J., Pan, Y., Kappler, A., Byrne, J.M., 2020. Effect of Microbial Biomass and Humic Acids on Abiotic and Biotic Magnetite Formation. *Environ. Sci. Technol.* 54, 4121-4130.
- Han, X., Tomaszewski, E.J., Schoenberg, R., Konhauser, K.O., Amor, M., Pan, Y., Warter, V., Kappler, A., Byrne, J.M., 2021. Using Zn and Ni behavior during magnetite precipitation in banded iron formations to determine its biological or abiotic origin. *Earth Planet. Sci. Lett.* 568, 117052.
- Heimann, A., Johnson, C.M., Beard, B.L., Valley, J.W., Roden, E.E., Spicuzza, M.J., Beukes, N.J., 2010. Fe, C, and O isotope compositions of banded iron formation carbonates demonstrate a major role for dissimilatory iron reduction in ~2.5 Ga marine environments. *Earth Planet. Sci. Lett.* 294, 8-18.
- Johnson, C.M., Beard, B.L., Klein, C., Beukes, N.J., Roden, E.E., 2008. Iron isotopes constrain biologic and abiologic processes in banded iron formation genesis. *Geochim. Cosmochim. Acta* 72, 151-169.
- Li, Y.-L., Konhauser, K.O., Cole, D.R., Phelps, T.J., 2011. Mineral ecophysiological data provide growing evidence for microbial activity in banded-iron formations. *Geology* 39, 707-710.

Interaction between magnetotactic bacteria and environment

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Abstract

Microorganisms drive biogeochemical cycling in the Earth System. In addition to having versatile metabolisms, some microbes have evolved biomineralization capabilities, through which various minerals form either extra- or intra-cellularly. Microbial metabolism and biomineralization interact with many geological and geophysical processes from molecular to planetary scales, and have shaped Earth's surface and impacted geochemical cycles over the past four billion years.

Magnetotactic bacteria (MTB) are phylogenetically and morphologically diverse prokaryotes that tend to live at or just below the oxic-anoxic transition zone (OATZ) in aquatic environments. They can produce intracellularly size-tailored and morphologically defined nanocrystals of magnetite (Fe_3O_4) or/and greigite (Fe_3S_4) each enveloped by a lipid bilayer membrane called a magnetosome. Within most MTB, these magnetic particles are organized into a chain or chains that enable cells to swim along geomagnetic field lines, a unique behavior initially called magnetotaxis. Although many microorganisms produce minerals, magnetotactic bacteria (MTB) are among the most intriguing because they (1) have versatile metabolic abilities, (2) can biomineralize no less than 10 known minerals besides magnetite and greigite, (3) take advantage of magnetotaxis efficiently driving the geochemical cycling of elements such as C, O, N, P, S, Fe, and Si, and (4) may originate during the Archean, therefore, coevolving with the surface environment for a long time in geological history.

In this study, we mineralogically and chemically identified the biominerals in environmental MTB cells using the advanced electron microscopic and microspectroscopic technologies. The link between taxonomy, morphology, genomics, mineralogy, and stoichiometry of environmental MTB were further established on single-cell level by correlatively fluorescence *in situ* hybridization and scanning electron microscope method. Finally, we proposed a concept model for MTB interacting with diverse aquatic environments.

Modeling the crystallographic transformation in shells of *Mytilus galloprovincialis* during diagenesis through thermal simulation

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Abstract

Biominerals of hard parts are widely used as paleoenvironmental archives, with proxies including elemental and isotopic composition, microstructure, and crystallography. Considering fossil materials, the choice and application of the individual proxies depend on their preservational potential. As of now, this preservational potential has not been adequately compared within a controlled experiment in bivalves, which are one of the commonly used fossils for paleoenvironmental reconstructions. We used cultured specimens of *Mytilus galloprovincialis* in a well-controlled thermal experiment to explore how elevated temperatures affect different proxies, including the isotopic composition, microstructure, and crystallography of the aragonitic and calcitic parts of the same shell. The results suggest that increasing temperatures lead first to changes in oxygen isotope composition (in aragonite and calcite), then modifications of the aragonite phase, as well as texture, nacre microstructure and grain size of aragonite crystals. Calcite-

composed microstructure and the calcite texture seem to be the most resilient to the temperature increases. The results of this work provide a reference to choose appropriate proxies for paleoenvironmental reconstructions.

Key words: Crystallography; Ciominerals; Electron Back-Scattered Diffraction; Preservational potential; Stable isotopes

Co-removal of ultra-high concentrations of calcium and ferric ions by *Virgibacillus dokdonensis* in hypersaline wastewater

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Abstract

Biom mineralization widely applied in wastewater treatment is due to its advantages compared to traditional methods. In this study, four methods with *Virgibacillus dokdonensis* WLR-1 bacteria were used to remove calcium and ferric ions in wastewater containing 5,000 mg/L of sodium chloride. The results showed that calcium and ferric ions were biomineralized into calcite and vaterite containing Fe(II) and Fe(III). Bacterial growth was inhibited by higher concentration of calcium ion whether ferric ions were present or not, and promoted by ferric ion at certain calcium ion

concentrations (Fig. 1a). The great decrease in carbonic anhydrase activity at higher calcium concentrations resulted in lower concentrations of bicarbonate and carbonate ions, and then leading to a great decrease in pH values. The contents of protein, polysaccharides, DNA and humic acid-like substances (Fig. 1b and Figs. 2a-2d) in the extracellular polymers increased in the presence of calcium/ferric/calcium+ferric ions, promoting to remove calcium and ferric ions. Nanominerals (Figs. 2e-2h) and denser distribution of calcium and iron on the cell surface and inside the cell revealed the other removal paths of these ions.

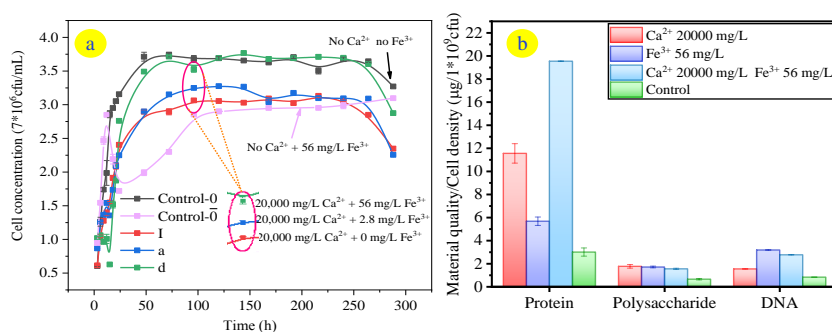


Fig. 1: Cell concentrations and EPS components at different conditions

With increasing ferric ion concentrations, calcium ion precipitation ratio reached bacteria 51%-57% (50%-52%), 16%-29% (<10%-23%), 8%-20% (4%-21%), 37%-56% (28%-45%) at 20,000 mg/L (28,000 mg/L) of calcium ion under the effect of free bacteria, bacteria immobilized by sodium alginate, immobilized by activated carbon fiber and periodically adding bacteria and activated carbon fiber for about 14 days, suggesting that adding free bacteria was the best method to remove higher concentrations of calcium ion; ferric ion precipitation ratio reached 64%-84%, 60%-88%, 86%-92% and 91%-98% for 158 h, suggesting that periodically adding bacteria and activated carbon fiber was the best method in ferric ion removal.

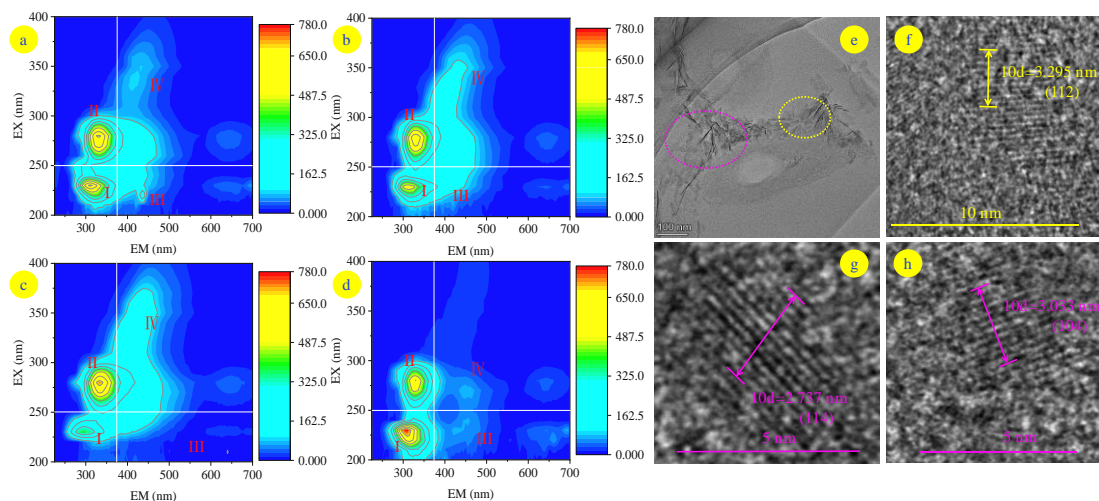


Fig. 2: Three-dimensional fluorescence spectra of EPS from WLR-1 bacteria at different conditions (a, Ca^{2+} 20,000 mg/L; b, Fe^{3+} 56 mg/L; c, Ca^{2+} 20,000 mg/L/ Fe^{3+} 56 mg/L; d, without $\text{Ca}^{2+}/\text{Fe}^{3+}$) and HRTEM and SAED analyses of biominerals on the cell surface of bacteria cultured in medium with 28000 mg/L of Ca^{2+} and 5.6 mg/L of Fe^{3+} (e, Morphology of the biomineral on the cell surface; f, an enlarged view of some part in a yellow circle in figure a; g and h, HRTEM analyses of the area marked by a pink circle in figure a)

Selecting bacteria that can survive at ultra-high concentrations of calcium and iron ions in hypersaline environment and then using the bacteria to remove such ultra-high concentrations of calcium and iron ions is a challenging problem. This study is beneficial to the application of biomineralization in the removal of ultra-high concentrations of calcium and ferric ions in hypersaline wastewater.

References

- Han, Z.Z., Li, D., Zhao, H., Yan, H.X., Li, P.Y., 2017a. Precipitation of carbonate minerals induced by the halophilic *Chromohalobacter Israelensis* under high salt concentrations: implications for natural environments. *Minerals* 7 (6), 95.
- Hasanzadeh, R., Abbasi Souraki, B., Pendashteh, A., Khayati, G., Ahmadun, F.R., 2020. Application of isolated halophilic microorganisms suspended and immobilized on walnut shell as biocarrier for treatment of oilfield produced water. *J. Hazard. Mater.* 400, 123197.
- Joss, A., Baenninger, C., Foa, P., Koepke, S., Krauss, M., McArdell, C.S., et al., 2011. Water reuse: >90% water yield in MBR/RO through concentrate recycling and CO_2 addition as scaling control. *Water Res.* 45, 6141–6151.

The roles of microbes in Cambrian animal skeletonization

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Abstract

The evolutionary mechanism behind the Cambrian animal skeletonization is a complex and incompletely understood process involving various environmental, ecological, and biological factors. Oxygen levels, predatory pressure, and changing seawater chemistry have often been proposed as drivers of this innovation, yet the influence of microbial selection pressures has been largely overlooked. This study

presents evidence that calcified micromollusks from the Cambrian Terreneuvian of southwestern Mongolia developed advanced tubule systems as an evolutionary response to intensive microbial attacks in a microbial-dominated ecosystem. These high-density tubules, comprising approximately 35% of shell volume, represent significant advances in shell calcification, enabling micromollusks to cope with microborings inflicted by cyanobacteria euendoliths. This anti-microboring mechanism plays a vital role in maintaining the health and ecological adaptability of nascent calcified mollusks within seafloor microbial mat environments. This study highlights the role of microbial selection pressures in shaping the shell calcification of early Cambrian mollusks, emphasizing the importance of considering animal-microbial interactions in understanding the origin and evolution of animal skeletons during the Cambrian Explosion.

Keywords: Cambrian Explosion; Animal-microbe interaction; Biomineralization; Mongolia

Microbial mineralization in the human urinary system promotes the formation of urinary stones

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Abstract

Urinary stones in the human body are mostly considered to be the result of mineral crystallization caused by ion level imbalance. In recent years, some studies have shown that there is a spatial binding relationship between microorganisms and urinary stones. However, it is unclear whether there is a causal relationship between the two. Here we explore whether bacteria in the human urinary system can promote the formation of stones through microbial-induced mineralization. In this study, the mineralogy and microbiology of human urinary stones and the corresponding urine composition were explored for 60 samples from patients in the Affiliated Hospital of Qingdao University. Subsequently, microbial mineralization experiments were carried out in artificial urine using bacteria that were screened from urinary stones. This study found that the main mineral components of human urinary stones are mainly whewellite/weddellite, struvite

and uric acid. The whewellite/weddellite stones have an obvious layered structure. The bacteria-sized holes and mineralized envelopes were found inside the whewellite/weddellite and struvite stones. And the dominant bacteria at genus level in the urinary stones are chiefly *Pseudomonas*, *Shigella* and *Neisseria*. The bacterium *Shigella dysenteriae* JS3 was isolated from the urinary stones, which can induce minerals constituting human urinary stones under simulated urine conditions. It was interesting to find that the characteristics of mineral precipitates from artificial urine, induced by the *S. dysenteriae* JS3, are similar to those of human urinary stones, and they are clearly different from equivalent minerals precipitated abiotically. These findings indicate that microbial mineralization in the human urinary system promotes the formation of urinary stones, providing new insights into the nature, origin and potential treatment human urinary stones.

Key words: Human Urinary Stones; *Shigella dysenteriae* JS3; Whewellite; Struvite; Extracellular Polymeric Substances (EPS)

Mechanism and application of the biomineralization of calcium and manganese ions induced by halophilic bacteria in hypersaline environment

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Abstract

The composition of microbiolites is complex, including calcium carbonate components such as calcite, aragonite, aragonite, etc. and some manganese-containing microbiolites such as rhodochrosite minerals (Marín-Ortega et al., 2023). Researchers have conducted in-depth studies on the formation mechanism of microbiolites (Lan et al., 2022), but most of them used to induce the mineral formation were focused on cyanobacteria. Therefore, in this study, moderate halophilic bacteria were used to carry out mineralization of calcium carbonate and rhodochrosite in the laboratory, and the physiological and biochemical characteristics of halophilic bacteria, intracellular and

extracellular minerals, changes in the concentrations of calcium and manganese ions were studied. This study is expected to provide some reference for the formation mechanism of microbialites containing calcium and manganese, and has certain application potential in the bio-removal of calcium and manganese in hypersaline wastewater.

Halovibrio mesolongii HMY2 bacteria can grow at 5%-30% NaCl concentration, and the optimal salinity is 10%, similar to the salinity of the environment that microbialite was formed. In the biomineralization process of calcium and manganese ions, HMY2 bacteria can release carbonic anhydrase, resulting in a higher concentration of carbonate and bicarbonate anions and then promoting the pH of the system to rise to about 8.7. The presence of calcium and manganese ions can inhibit the pH rise and decrease the carbonic anhydrase activity and the concentration of carbonate and bicarbonate anions, not conducive to the occurrence of biomineralization. The contents of polysaccharides, proteins, DNA (Fig. 1a) and organic acids such as humic acid and fulvic acid increased in EPS (Fig. 1b), which can provide nucleation sites for calcium-manganese ions to form carbonate minerals; adding buffer to calcium/manganese system could significantly increase the activity of carbonic anhydrase ($p < 0.01$). These conditions are more conducive to biomineralization and bio-removal of calcium and manganese ions.

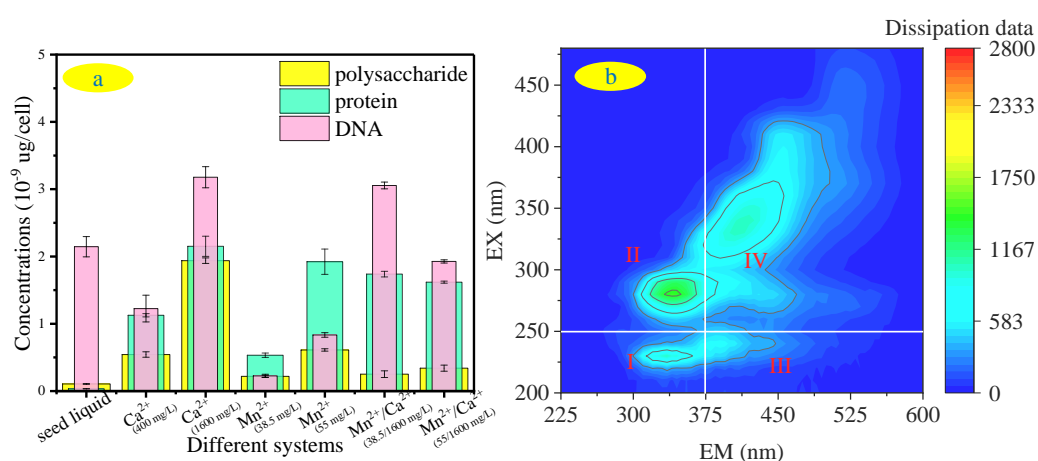


Fig. 1: Changes in polysaccharide, protein and DNA components (a) and 3D-EEM analysis of EPS components (b).

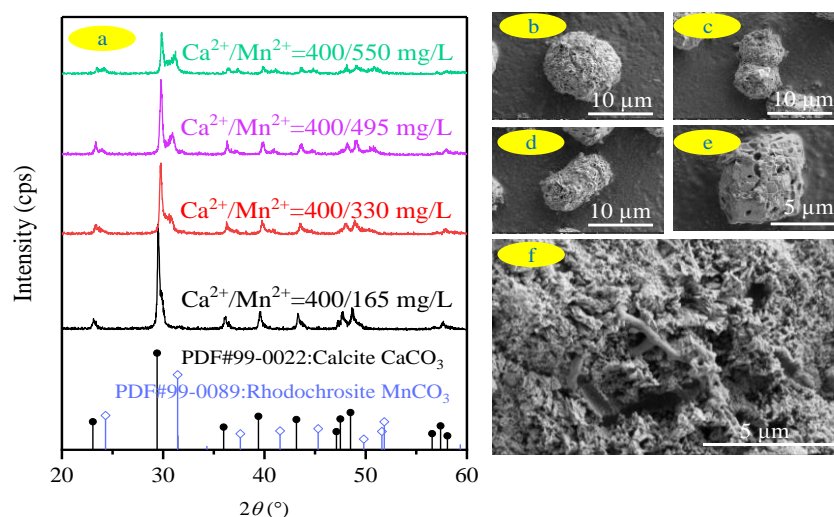


Fig. 2: XRD and SEM analyses of calcite and rhodochrosite

Under the action of HMY2 bacteria, calcium and manganese ions were biomineralized into calcite (CaCO_3) and rhodochrosite (MnCO_3), respectively (Fig. 2a). The minerals have abundant morphology (spherical, dumbbell, rod-shaped) (Figs. 2b-2f), organic functional groups (C-O-C, C=O, C-OH), organic elements (N, P, S), protein secondary structure (β -fold, α -helix, 3_{10} -helix and β -turn) and chemical bonds (O-C=O, N-C=O, P=O). The content of O-C=O and N-C=O bonds in the mineral increases with increasing Ca/Mn/Ca+Mn ion concentrations. The above results confirm the biogenesis of calcite and rhodochrosite. There are manganese ions in calcite and calcium ions in rhodochrosite in the mixed system, suggesting the inter-doping of ions. Calcite and rhodochrosite crystals were found both outside and inside the cell. Therefore, the mineralization process includes four paths: the solution system, the cell surface, the intracellular mineralization and ion doping. The removal ratios of calcium and manganese ions are 90.9% and 96.5%, respectively, and the concentrations of calcium and manganese ions after being treated satisfy the Chinese National Standard (GB/T15454-2009, $\text{Ca}^{2+} < 200 \text{ mg/L}$; GB3838-2002, $\text{Mn}^{2+} < 2 \text{ mg/L}$).

The study provides a theoretical reference for the in-depth understanding of the formation mechanism of microbialites, but also provides an environmentally friendly and economical biological method for the removal of calcium and manganese ions in wastewater.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (42072136, 41972108, 42106144); Natural Science Foundation of Shandong Province (ZR2020MC041, ZR2020QD089); National College Students' Innovation and Entrepreneurship Training Program (202210424110); Foreign visiting scholar funded by Shandong Provincial government.

References

- Marín-Ortega, S., Àngels Calvo i Torras, M. and Iglesias-Campos, M.Á. (2023) Microbially induced calcium carbonate precipitation in fossil consolidation treatments: Preliminary results inducing exogenous *Myxococcus xanthus* bacteria in a miocene *Cheirogaster richardi* specimen. *Heliyon*, e17597.
- Lan, C., Xu, Z., Chen, H., Yang, W., Lu, C., Li, P. and Zou, H. (2022) Paleooceanographic reconstruction of the Ediacaran Dengying Formation, Sichuan Basin, Southwest China: Implications for the origin of Precambrian microbial carbonates. *Journal of Asian Earth Sciences* 236, 105340.
- Giovanella, P., Vieira, G.A.L., Ramos Otero, I.V., Pais Pellizzer, E., de Jesus Fontes, B. and Sette, L.D. (2020) Metal and organic pollutants bioremediation by extremophile microorganisms. *Journal of Hazardous Materials* 382, 121024.

Study on the biomineralization mechanism of calcium and barium ions induced by halophilic bacteria and the application in the treatment of oilfield hypersaline wastewater

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Abstract

The oilfield wastewater contains a large number of ions, including calcium ions and barium ions. Calcium ions are a common scale-forming ion, which can cause blockage of equipment pipes and bring great inconvenience to production and life. Barium ion is a kind of heavy metal ion, which can cause protein denaturation and destroy various enzymes in the body. If oilfield wastewater is not properly treated and directly reinjected or discharged, which will inevitably cause great harm to human beings, animals and plants (Zakaria et al., 2020). In this study, a lower-cost and environmentally friendly microbial mineralization method was used to achieve the

purpose of calcium and barium ion removal, and provide a basis for further exploration of the deposition mechanism of microbialites.

The optimal salinity (sodium chloride) of the halophilic bacterium *Bacillus licheniformis* SRB2 is 3%, and calcium, barium and calcium+barium can inhibit carbonic anhydrase activity (Fig. 1), resulting in a significant decrease in the concentrations of carbonate and bicarbonate anions and pH. These conditions are not conducive to the mineralization and removal of calcium and barium ions in hypersaline wastewater from oilfields (Krejci et al., 2011). EPS is one of the ideal nucleation sites for carbonate mineral deposition. 3D-EEM result showed that the higher the concentration of calcium and barium ions, the more humic acid and fulvic acid (III and IV regions) would appear (Figs. 2a-2g), suggesting a sharp increase in the number of carboxyl groups. The contents of protein, polysaccharide and DNA in EPS also increased significantly with increasing concentrations of calcium and barium ions (Fig. 2h). These conditions are conducive to attracting more calcium and barium ions, thus promoting the biommineralization of these ions and then removing these ions.

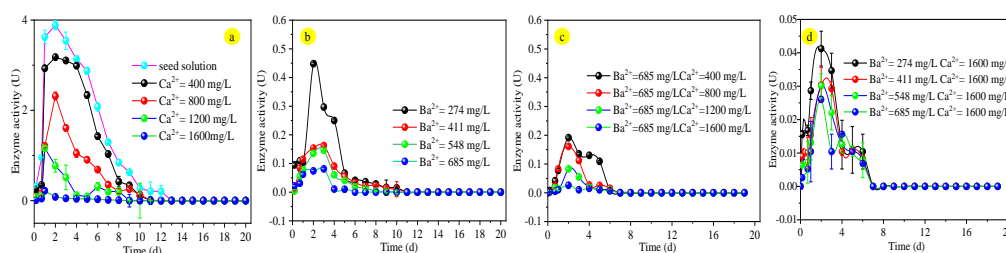


Fig. 1: Changes of carbonic anhydrase activity

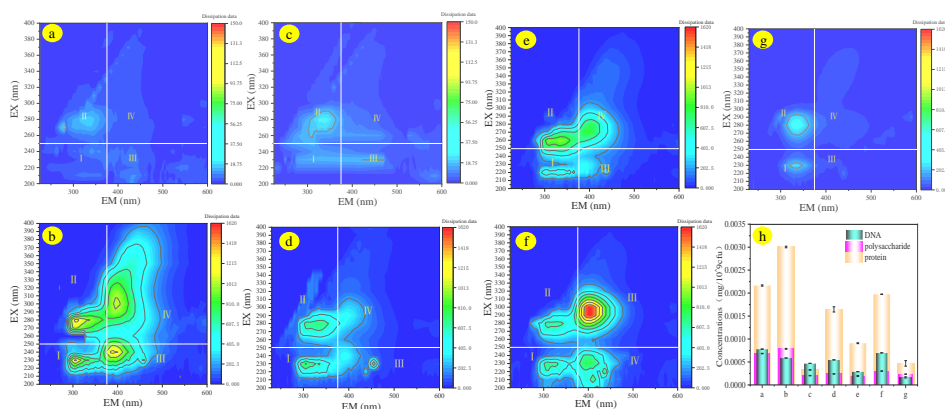


Fig. 2: 3D-EEM analyses of EPS and components of polysaccharide, protein and DNA in EPS.

The mineralized products were characterized by SEM-EDS, FTIR, XRD, XPS, etc. It was found that calcium ions were mineralized to calcite and aragonite, and barium

ions were mineralized to barium carbonate. The morphologies of these minerals are diverse and have organic elements such as nitrogen, phosphorus and sulfur. Organic matters such as protein and carboxylic acids exist in minerals, and the content increases with increasing concentrations of calcium and barium ions. These features further confirm that the minerals are biotic. The removal process of calcium and barium ion includes four pathways: biomineralization in the fluid system, cell surface biomineralization, intracellular biomineralization and ion inter-doping biomineralization. The concentration of barium ions in calcium carbonate increased significantly with increasing barium ion concentration ($p < 0.01$). On 20th day, the sedimentation ratios of calcium ion were 75.41%-93.07% (calcium) and 75.41%-81.93% (calcium+barium), and 91.26%-96.27% (barium) and 85.55%-92.16% (calcium+barium) as for barium ion, meeting the standards of China's industrial wastewater discharge GB/T15454-2009 (calcium ion < 200 mg/L) and GB8978-1996 (barium ion < 1 mg/L).

This study maybe provides a in-depth understanding of biomineralization mechanism, and also conducive to the exploration of the deposition mechanism of barium-bearing microbialites, meanwhile has great application potential in the removal of calcium and barium ions in oilfield wastewater.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (42072136, 41972108, 42106144); Natural Science Foundation of Shandong Province (ZR2020MC041, ZR2020QD089); National College Students' Innovation and Entrepreneurship Training Program (202210424110); Foreign visiting scholar funded by Shandong Provincial government.

References

- Krejci, M.R., Wasserman, B., Finney, L., McNulty, I., Legnini, D., Vogt, S. and Joester, D. 2011. Selectivity in biomineralization of barium and strontium. *J Struct Biol* 176(2), 192-202.
- Zakaria, K., Salem, A.A. and Ramzi, M. 2020. Cost-effective and eco-friendly organophosphorus-based inhibitors for mineral scaling in Egyptian oil reservoirs: Theoretical, experimental and quantum chemical studies. *Journal of Petroleum Science and Engineering* 195.

Biom mineralization mechanism of struvite for nitrogen and phosphorus removal based on magnesium supplied by dolomite and its application

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Abstract

Eutrophic water is caused by excessive nutrient content in water body, mainly pointed to high contents of nitrogen and phosphorus. Common eutrophic water bodies have lower magnesium content, and additional magnesium source reagents need to be added to the water body to remove nitrogen and phosphorus. Some studies have reported that soluble magnesium reagents are used to provide Mg^{2+} , such as $MgCl_2$ and $MgSO_4$, resulting in expensive production costs and secondary pollution ^[1]. In order to better remove nitrogen and phosphorus and reduce production costs, biom mineralization has been paid more attention by researchers. Through the growth and metabolism of

bacteria, ammonium, magnesium and phosphate ions in the wastewater reach a saturation state, and then struvite minerals (a slow-release fertilizer often used in agriculture) were formed to achieve an aim of the removal of nitrogen and phosphorus and resource recycling [2]. It is an economic and environmentally friendly method usually used in some cleaner production processes.

This study used dolomite as a cheap and efficient magnesium source, and *Bacillus licheniformis* ZXT was selected as the strain to induce the formation of hydroxyapatite and struvite at different magnesium-nitrogen-phosphorus ratios (0.6:1:1, 1:1:1 and 1.4:1:1). In this study, the reasons for pH increase were explained by measuring the pH increase caused by bacteria, NH_4^+ , alkaline phosphatase activity (ALP) [3]. The actual pH can reach 9.12 (Fig. 1), and the pH caused by NH_4^+ can be 8.84 (<9.12), further indicating that NH_3 is not the only factor resulting in the pH increase. ALP removes phosphate groups from nucleic acids and proteins through dephosphorylation to generate phosphate and hydroxyl groups to increase the pH of the system. The pH caused by the presence of phosphate can reach 10.79 (>9.12), indicating that pH increase was also closely related to ALP activity (Fig. 1). Compared to the liquid seed, contents of humic acids in EPS of bacteria at different magnesium-nitrogen-phosphorus ratios (0.6:1:1, 1:1:1 and 1.4:1:1) were higher (Fig. 2), beneficial to the adsorption of Mg^{2+} , NH_4^+ and PO_4^{3-} ions and then promoting the formation of struvite.

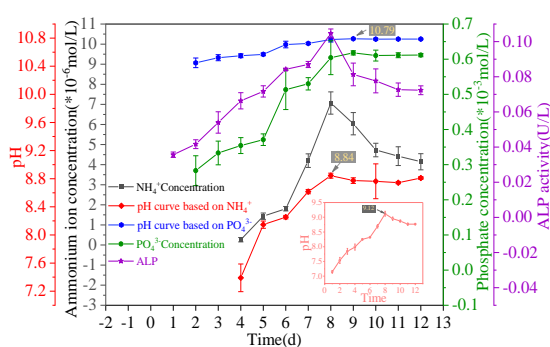


Fig. 1. The reason for pH increase under the action of *B. licheniformis* ZXT strain.

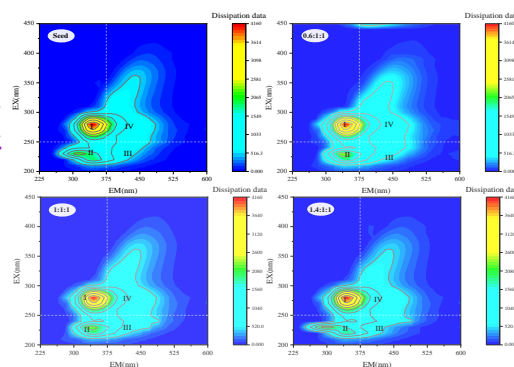


Fig. 2. Humic acid content in EPS of *B. licheniformis* ZXT strain.

The hydrolysate of dolomite was added into the culture systems and the magnesium-nitrogen-phosphorus ratios were set as 0.6:1:1, 1:1:1 and 1.4:1:1. With incubation time increasing from 6 to 15 days, the minerals were changed from hydroxyapatite (0.6:1:1 and 1:1:1) and brushite (1.4:1:1) (Fig. 3a) to struvite (0.6:1:1, 1:1:1 and 1.4:1:1) (Fig. 3b). The presence of Ca^{2+} inhibited the removal of Mg^{2+} and promoted the removal of NH_4^+ and PO_4^{3-} ions. The removal ratios of NH_4^+ , PO_4^{3-} , Mg^{2+}

and Ca^{2+} ions were 99.7%-100%, 98.8%-99.4%, 28.5%-73.3%, and 63.3%-72.7%, respectively. When the ratio of magnesium to nitrogen to phosphorus reaches 1.4:1:1 during the biomineralization process, it is not easy to remove Mg^{2+} and Ca^{2+} ions. The European Union (EU) defines the critical total P concentration of non-eutrophic lakes as < 0.01 mg/L, and has reduced the permissible P concentration in discharged wastewater from 2 mg/L to 1 mg/L to 0.1 mg/L [4]. Similarly, the permissible $\text{NH}_4^+\text{-N}$ concentration was reduced to 1 mg/L [5]. In this study, $\text{NH}_4^+\text{-N}$ concentration reached 0.1-0.9 mg/L, P concentration was 0.1-0.2 mg/L, satisfying the requirements of discharged wastewater.

This study proved the feasibility of using dolomite to supply magnesium in the process of microbial recovery of nitrogen and phosphorus, and was beneficial to further understanding the mechanism of struvite biomineralization. At the same time, the struvite generated by biomineralization is a slow-release fertilizer, reaching an aim of resource recovery. This study also supplied an economic and environmentally friendly method to bio-remove and recycle the $\text{NH}_4^+\text{-N}$ and $\text{PO}_4^{3-}\text{-P}$ from eutrophic water.

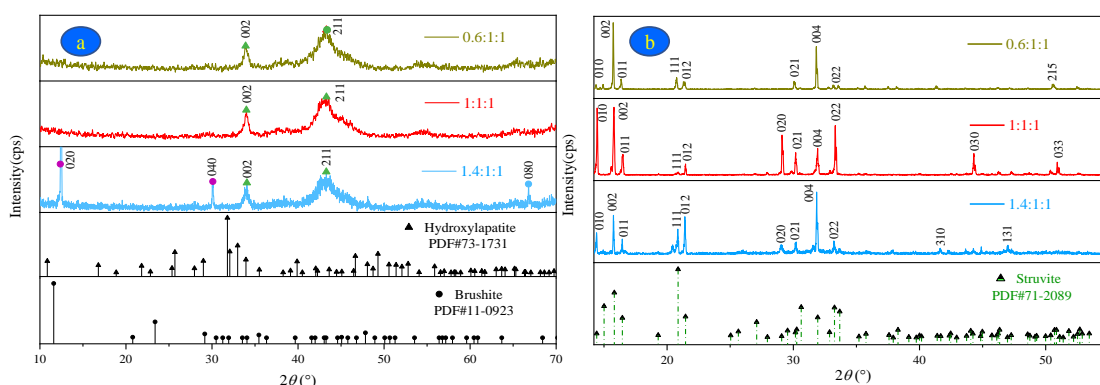


Fig. 3. XRD analyses of minerals induced by *B. licheniformis* ZXT cultured at magnesium-nitrogen-phosphorus ratios of 0.6:1:1, 1:1:1 and 1.4:1:1, respectively.

Acknowledgements

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References

- Labry, C., Delmas, D., Herbland, A., 2005. Phytoplankton and bacterial alkaline phosphatase activities in relation to phosphate and DOP availability within the Gironde plume waters (Bay of Biscay). *J. Exp. Mar. Biol. Ecol.* 318 (2): 213-225.
- K. Suguna, M. Thenmozhi, C. Sekar, 2012. Growth, spectral, structural and mechanical properties of struvite crystal grown in presence of sodium fluoride. *Bull. Mater. Sci.* 35: 701-706.

- M. Quintana, E. Sanchez, M.F. Colmenarejo, J. Barrera, G. Garcia, R. Borja, 2005. Kinetics of phosphorus removal and struvite formation by the utilization of by-product of magnesium oxide production, *Chem. Eng. J.* 111(1): 45-52.
- Li, S., Zhang, Q., Yin, C., Chen, J., Yang, X., Wang, S., 2021. Tuning microscopic structure of Al-based metal-organic frameworks by changing organic linkers for efficient phosphorus removal. *J. Clean. Prod.* 292.
- Guida, S., Conzelmann, L., Remy, C., Vale, P., Jefferson, B., Soares, A., 2021. Resilience and life cycle assessment of ion exchange process for ammonium removal from municipal wastewater. *Sci. Total Environ.* 783, 146834.

Effect of magnesium and ferric ions on the biomineralization of calcium carbonate

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Abstract

The interaction between microorganism and mineral is a kind of geological activity which occurs widely in natural environment, and the dissolution and precipitation of mineral are almost inseparable from the action of microorganism. The researchers found that magnesium and iron are contained in natural minerals. Researchers have conducted in-depth studies on the biomineralization of calcium carbonate induced by microorganisms, but the effects of trace elements such as magnesium and iron on the microbial induction of calcium carbonate are often ignored. Therefore, this study intends to carry out cyanobacteria-induced calcium carbonate mineralization in Ca^{2+} ion system, $\text{Ca}^{2+}+\text{Mg}^{2+}$ mixed system and $\text{Ca}^{2+}+\text{Mg}^{2+}+\text{Fe}^{3+}$ mixed system, in order to explore the mineralization mechanism of Mg^{2+} and Fe^{3+} affecting calcium carbonate

minerals, and at the same time provide a certain reference for the treatment of oil field wastewater containing Ca^{2+} , Mg^{2+} and Fe^{3+} ions.

In the system containing different concentrations of Ca^{2+} ions, the bacterial concentration was maximum at 800 mg/L of Ca^{2+} ion, indicating that the optimum Ca^{2+} concentration for the growth of cyanobacteria was 800 mg/L. *Synechocystis* sp. PCC 6803 can release carbonic anhydrase, and its activity decreased with increasing concentrations of Ca^{2+} ions, and the maximum value of carbonic anhydrase activity was 4.18 U/mL at 800 mg/L of Ca^{2+} ions (Fig. 1a), much higher than those under the action of Fe^{3+} (3.28-3.83 U/mL) (Fig. 1b) and Mg^{2+} (1.21-1.62 U/mL) ions (Fig. 1c), suggesting that Ca^{2+} , $\text{Ca}^{2+}+\text{Fe}^{3+}$, and $\text{Ca}^{2+}+\text{Mg}^{2+}$ ions could inhibit the carbonic anhydrase activity. Carbonic anhydrase activity also decreased with increasing Mg^{2+} ion concentrations at 800 mg/L of Ca^{2+} ion (Fig. 1c), and the maximum value was 1.36 U/mL at 1440 mg/L of Mg^{2+} ion and 800 mg/L of Ca^{2+} (Fig. 1c), much lower than those (1.71, 1.90, and 2.32 U/mL) when additionally adding Fe^{3+} ions on the basis of Ca^{2+} and Mg^{2+} ions (Fig. 1d), suggesting that Fe^{3+} ions could increase carbonic anhydrase activity in this system ($\text{Ca}^{2+}+\text{Mg}^{2+}+\text{Fe}^{3+}$). Accordingly, the concentrations of CO_3^{2-} and HCO_3^- anions decreased with increasing Ca^{2+} (Fig. 2a) and Mg^{2+} (fixed Ca^{2+}) (Fig. 2c), increased with increasing Fe^{3+} ion concentrations (Figs. 2b and 2d). Therefore, the presence of Fe^{3+} was beneficial to the biomineralization of carbonate minerals in such systems.

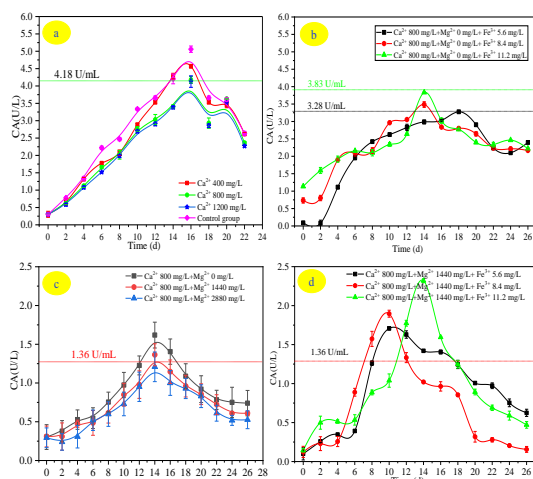


Fig. 1. CA activities in different conditions. a, only Ca^{2+} ; b, $\text{Ca}^{2+} + \text{Fe}^{3+}$; c, $\text{Ca}^{2+} + \text{Mg}^{2+}$; d, $\text{Ca}^{2+} + \text{Mg}^{2+} + \text{Fe}^{3+}$.

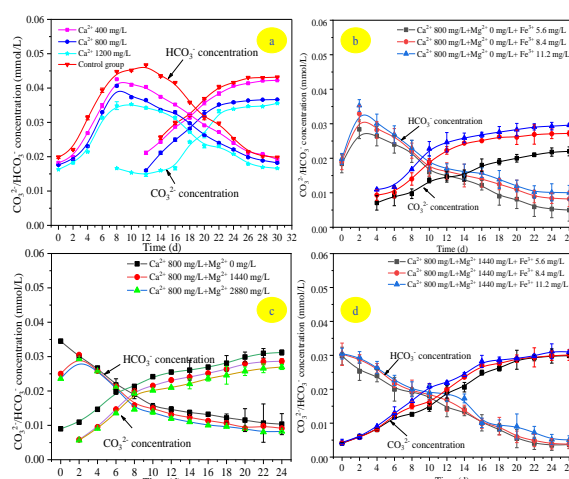


Fig. 2. Concentrations of HCO_3^- and CO_3^{2-} ions in different conditions. a, only Ca^{2+} ; b, $\text{Ca}^{2+} + \text{Fe}^{3+}$; c, $\text{Ca}^{2+} + \text{Mg}^{2+}$; d, $\text{Ca}^{2+} + \text{Mg}^{2+} + \text{Fe}^{3+}$.

EPS components were also analyzed by 3D-EEM and the results showed that the contents of humic acid and fulvic acid are elevated in EPS of *Synechocystis* sp. PCC

6803 in environments containing Ca^{2+} , $\text{Ca}^{2+}+\text{Mg}^{2+}$, $\text{Ca}^{2+}+\text{Fe}^{3+}$ and $\text{Ca}^{2+}+\text{Mg}^{2+}+\text{Fe}^{3+}$ compared to the liquid seed (Figs. 3a1-3a7). The contents of DNA, polysaccharide and protein in EPS also increased in environments as mentioned above (Figs. 3a8). These organic matter could be beneficial to the adsorption of Ca^{2+} , Mg^{2+} and Fe^{3+} ions, promoting the biomineralization of these ions.

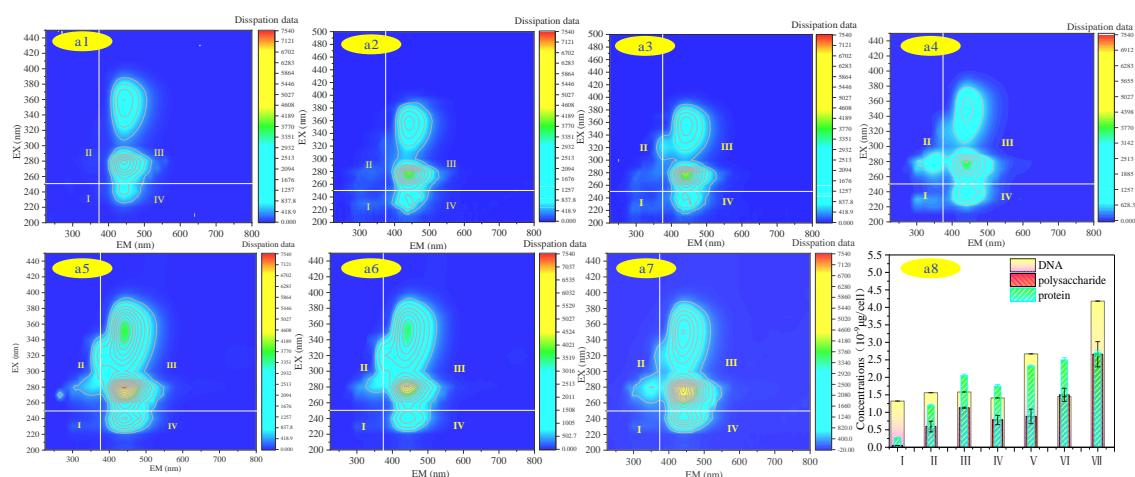


Fig. 3. 3D-EEM analyses of EPS and contents of DNA, Polysaccharide, protein in EPS. a1, liquid seed; a2, 800 mg/L Ca^{2+} ; a3, 800 mg/L Ca^{2+} + 2880 mg/L Mg^{2+} ; a4, 800 mg/L Ca^{2+} + 0 mg/L Mg^{2+} + 5.6 mg/L Fe^{3+} ; a5, 800 mg/L Ca^{2+} + 0 mg/L Mg^{2+} + 11.2 mg/L Fe^{3+} ; a6, 800 mg/L Ca^{2+} + 1440 mg/L Mg^{2+} + 5.6 mg/L Fe^{3+} ; a7, 800 mg/L Ca^{2+} + 1440 mg/L Mg^{2+} + 11.2 mg/L Fe^{3+} ; a8, contents of DNA, polysaccharide and protein in EPS (I-VII, the conditions same as a1-a7).

The minerals obtained in system containing only Ca^{2+} ions (Fig. 4a) and a mixture of Ca^{2+} and Fe^{3+} ions (Fig. 4c) were only calcites, and calcite, Mg-rich calcite and aragonite in system containing Ca^{2+} and Mg^{2+} ions (Fig. 4b). The minerals were Mg-rich calcite and aragonite in system containing 800 mg/L of Ca^{2+} and 1440 mg/L of Mg^{2+} (Fig. 4b), after adding different concentrations of Fe^{3+} ions, the minerals were calcite (Fig. 4d), indicating that Fe^{3+} ions contributed to the formation of calcium carbonate with better stability. The morphology of minerals was observed by SEM and the result showed that the minerals were long rod-like, spherical and cuboid, and the EDS result showed that elements N, P and S were present. FTIR results revealed that some organic functional groups were present in the minerals besides the protein secondary structures. The XPS results showed that organic acids, protein, and phospholipid existed in the mineral surface. All these results proved that these minerals were biotic.

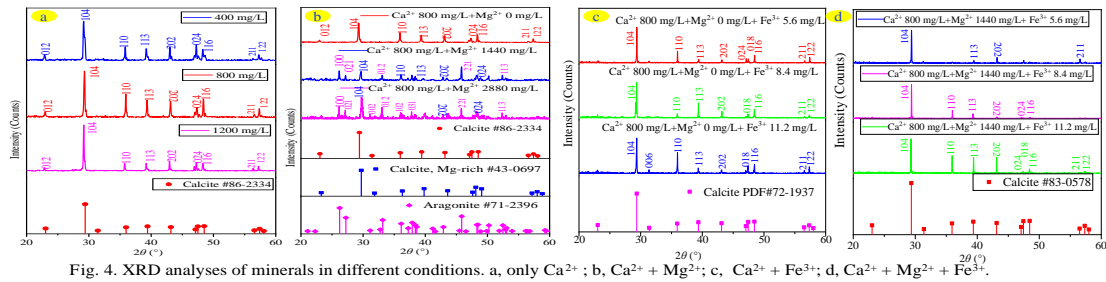


Fig. 4. XRD analyses of minerals in different conditions. a, only Ca^{2+} ; b, $\text{Ca}^{2+} + \text{Mg}^{2+}$; c, $\text{Ca}^{2+} + \text{Fe}^{3+}$; d, $\text{Ca}^{2+} + \text{Mg}^{2+} + \text{Fe}^{3+}$.

The removal ratios of Ca^{2+} ions were 59.3%-75.1% in system containing only Ca^{2+} ions, higher than 54.6%-70.1% in system containing $\text{Ca}^{2+} + \text{Mg}^{2+}$ ions, suggesting that Mg^{2+} ions inhibited the sedimentation of Ca^{2+} ions. The extracellular minerals on the cell surface and the intracellular minerals were further analyzed by HRTEM-SAED and the results were well consistent with the XRD results. Intracellular mineralization is also a way of the bio-removal of different kinds of metal ions^[1]. In systems containing Ca^{2+} , Mg^{2+} and Fe^{3+} , the removal ratios of Ca^{2+} , Fe^{3+} , and Mg^{2+} were 58.3%-78.5%, 34.5%-80.4% and 39.4%-78.8%, respectively. Adding Fe^{3+} ions promoted the sedimentation of Ca^{2+} , and the promotion effect became more obvious with increasing Fe^{3+} ion concentrations. It can be seen from FTIR result that the protein secondary structures becomes more and more abundant with increasing Mg^{2+} and Fe^{3+} concentrations. Maybe more nucleation sites could be supplied with more abundant protein secondary structures, beneficial to the biomineralization of carbonate minerals^[2]. The precipitation ratio of Ca^{2+} ion is the highest in system containing Ca^{2+} and Fe^{3+} ions, maybe due to the release of a large number of humic acids by bacteria (Fig. 3), which can act as a medium between bacteria and minerals, affect the electron transfer between microorganisms and extracellular electron acceptors, and promote the formation of minerals^[2].

This study maybe provides some reference for further understanding the mechanism of Mg^{2+} and Fe^{3+} ions affecting the precipitation of calcium carbonate minerals, and also has great application potential in the bio-removal of Ca^{2+} , Mg^{2+} and Fe^{3+} ions from oilfield hypersaline wastewater.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (42372135, 42072136, 41972108, 42106144); Natural Science Foundation of Shandong Province (ZR2023MD063, ZR2020MC041,

ZR2020QD089); National College Students' Innovation and Entrepreneurship Training Program (202210424110); Foreign visiting scholar funded by Shandong Provincial government.

References

Qin, W., Wang, C., Ma, Y., et al. Microbe-mediated extracellular and intracellular mineralization: environmental, industrial, and biotechnological applications. *Adv. Mater.* 2020, 32(22): 1-12.

Pal, A., Paul, A.K. Microbial extracellular polymeric substances: central elements in heavy metal bioremediation. *Indian. J. Microbiol.* 2008, 48 (1): 49-64.

Biom mineralization of calcium, magnesium and ferric ions induced by halophile *Bacillus subtilis* in hypersaline environment and its application

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Abstract

At present, the research on formation mechanism of microbiolite has obtained rich results through the simulation experiments in laboratory, but mainly focused on using cyanobacteria to induce the formation of calcium carbonate minerals such as calcite, vaterite and aragonite, etc. Few researchers have explored the effect of Mg^{2+} and Fe^{3+} ions on the formation of calcium carbonate under the action of bacteria. The purpose of this study is to use *Bacillus subtilis* YXY-1 to induce the sedimentation of carbonate minerals containing Ca^{2+} , Mg^{2+} and Fe^{3+} , so as to provide a reference for further understanding the influence of Mg^{2+} and Fe^{3+} ions on the mineralization of calcium carbonate, and also provide an environmentally friendly method for the treatment of

oilfield wastewater containing Ca^{2+} , Mg^{2+} and Fe^{3+} ions.

Carbonic anhydrase plays an important role in biomineralization⁰. Carbonic anhydrase activity decreased sharply when Ca^{2+} ion concentrations increased from 1,000 to 9,000 mg/L, revealing that higher concentrations of Ca^{2+} inhibited CA activity (Fig. 1a); CO_3^{2-} and HCO_3^- concentrations also decreased with increasing Ca^{2+} ion concentrations, and CO_3^{2-} ion concentration decreased to zero at certain Ca^{2+} ion concentrations (Fig. 1b). It can be concluded that higher concentrations of Ca^{2+} would inhibit the biomineralization. At 1,000 mg/L of Ca^{2+} ion concentration, CA activity (Fig. 1c) and CO_3^{2-} and HCO_3^- concentrations (Fig. 1d) rised with increasing Fe^{3+} ion concentrations, suggesting that the presence of Fe^{3+} ions would be beneficial to the biomineralization of calcium carbonate. Through the electrostatic interaction, the negative-charged EPS adsorbed metal ions easily and crystals formed once saturation reached⁰, and finally metal ions were biomineralized into solid minerals. The content of protein and DNA in EPS increased to tolerate the adverse environment, beneficial to the adsorption of metal ions.

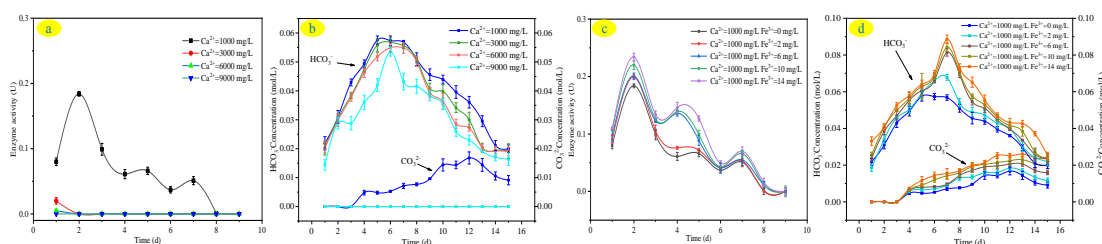


Fig. 1: Changes in CA activity and concentrations of CO_3^{2-} and HCO_3^- anions in different systems

At 1,000 mg/L of Ca^{2+} ion, the mineral induced by *Bacillus subtilis* YXY-1 was calcite, and at 3,000, 6,000 and 9,000 mg/L of Ca^{2+} , the mineral was a mixture of calcite and vaterite (Fig. 2a). At 1,000 mg/L of Ca^{2+} ion, all the minerals obtained in the experimental groups were calcite with Fe^{3+} ion concentrations increasing from 0 through 2, 6 and 10 to 14 mg/L (Fig. 2b). Calcite, aragonite and monohydrocalcite were formed in the system containing Ca^{2+} and Mg^{2+} (Fig. 2c), and calcite and aragonite in system with Ca^{2+} , Fe^{3+} and Mg^{2+} . XRD results show that all the minerals are calcium carbonate, no iron-bearing minerals were found. However, TEM results about the intracellular biomineralization show that Fe_2O_3 (yellow font) and FeO (white font) can be formed (Fig. 3). The porosity of minerals and the bacteria in some pores prove the biogenesis of minerals. Many organic functional groups were also found from FTIR results, including C=O from proteins, C-H of alkanes, and C-O-C from ethers. Several

protein secondary structures can also be found in these minerals, including β -sheet, α -helix, 3_{10} helix, and β -turn. Compared to the system without Fe^{3+} ions, the precipitation ratios of Ca^{2+} ion concentration increased from 60% to 70% under the action of Fe^{3+} ions, suggesting that Fe^{3+} ions promote the sedimentation of Ca^{2+} ions. With increasing Mg^{2+} ion concentrations, the precipitation ratios of Ca^{2+} and Fe^{3+} ions decreased, revealing that Mg^{2+} ions inhibit the sedimentation of Ca^{2+} and Fe^{3+} ions.

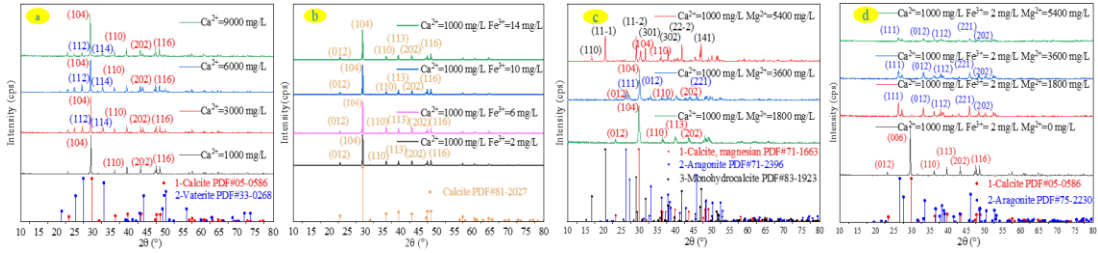


Fig. 2: XRD analyses of minerals in different systems.

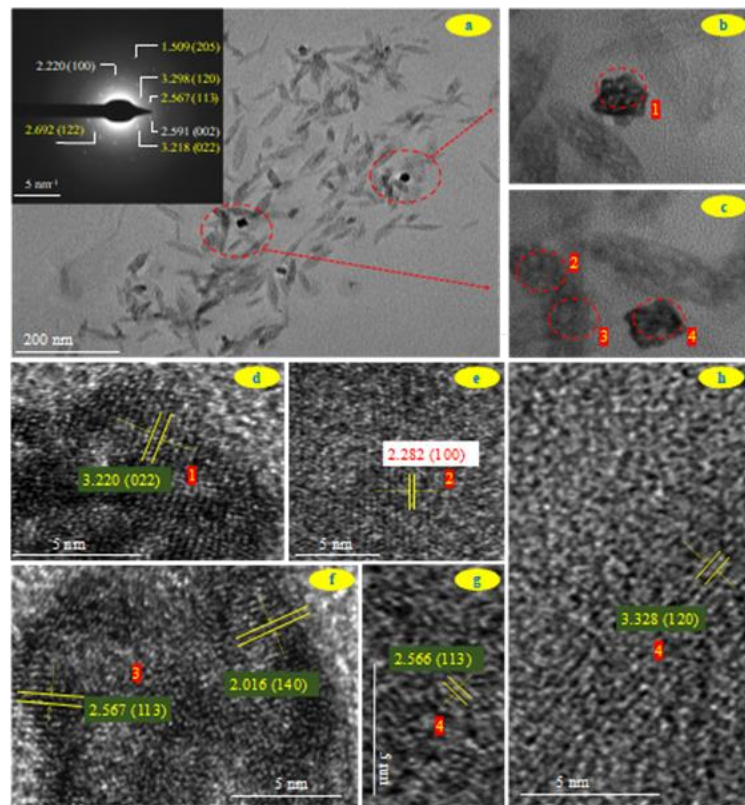


Fig. 3: HRTEM-SAED analyses of bacteria in a system containing Ca^{2+} and Fe^{3+} ions.

This study is helpful to further understand the mechanism of Fe^{3+} and Mg^{2+} ions affecting the biomineralization of Ca^{2+} ion, and helps to explore the formation mechanism of microbiolites containing iron and magnesium, and also provides some references for the bio-removal of Ca^{2+} and Mg^{2+} ions in hypersaline wastewater.

Acknowledgements

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References

- Zhuang, D.; Yan, H.; Tucker, M.E.; Zhao, H.; Han, Z.; Zhao, Y.; Sun, B.; Li, D.; Pan, J.; Zhao, Y.; Meng, R.; Shan, G.; Zhang, X.; Tang, R. Calcite precipitation induced by *Bacillus cereus* MRR2 cultured at different Ca^{2+} concentrations: Further insights into biotic and abiotic calcite. *Chemical Geology*, 2018, 500: 64-87.
- Muynck, W.; Belie, N.; Verstraete, W. Microbial carbonate precipitation in construction materials: A review. *Ecological Engineering*, 2010, 36(2): 118-136.

Co-removal of calcium and zinc ions from hypersaline oilfield wastewater by *Lysinibacillus xylanilyticus* bacteria

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Abstract

In the manufacturing industry, wastewater treatment has always been a challenging issue, especially when it involves wastewater containing heavy metal ions. High concentrations of calcium ions in wastewater can cause scaling in pipes, leading to equipment damage. Zinc ions often enter the food chain and accumulate in organisms, posing serious risks to human health and industrial-agricultural activities when higher than certain concentrations. The remediation method of microbially induced calcium carbonate precipitation (MICP) has gained widespread attention. During the MICP process, bacteria produce carbonic anhydrase to release HCO_3^{2-} and CO_3^{2-} , which effectively induces the formation of carbonate precipitates⁰. This study focuses on the biomineralization mechanism and application of *Lysinibacillus xylanilyticus* DB1-12

bacteria in the precipitation of Ca^{2+} and Zn^{2+} ions from the hypersaline wastewater with varying Zn/Ca ratios (0 , 2×10^{-3} , 4×10^{-3} , 6×10^{-3} , 8×10^{-3}).

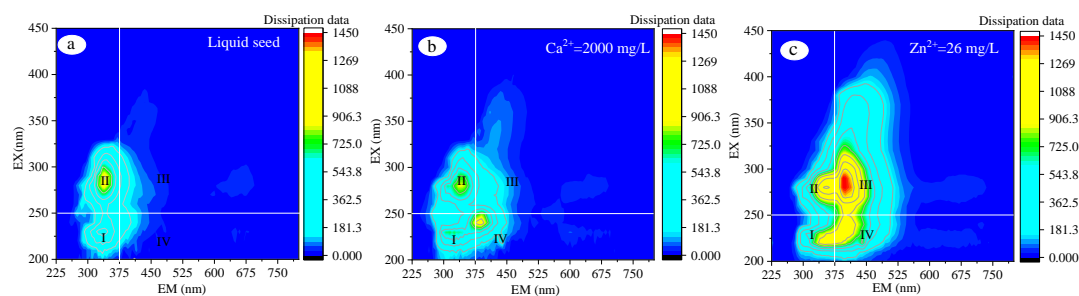


Fig. 1: 3D-EEM analyses of EPS from DB1-12 bacteria after 17 days of cultivation in different environments (a, liquid seed; b, 2,000 mg/L of Ca^{2+} ion; c, 26 mg/L of Zn^{2+} ion).

During the biomineralization process, the pH of the system gradually increases from an initial value of 6.0 to around 8.2 and then decreases. As the concentration of Zn^{2+} increases, the pH increases. The concentration of bicarbonate (HCO_3^-) initially increases within the first 72 hours and then decreases, stabilizing at 312 hours. The trends of pH and bicarbonate concentration increases with increasing Zn^{2+} ion concentrations. The activity of carbonic anhydrase in the biomineralization system increases with the increasing Zn^{2+} ion concentrations, indicating that the addition of Zn^{2+} ions promotes the activity of carbonic anhydrase. The presence of carbonic anhydrase plays a key role in the pH increase of the biomineralization system⁰. Compared to the liquid seed, the contents of fulvic acid and humic acid in the extracellular polymeric substances (EPS) sharply increased in the fluid containing Ca^{2+} and Zn^{2+} ions (Fig. 1). These organic acids can provide electrons for the metal ions in the biomineralization system, facilitating the adsorption of Ca^{2+} and Zn^{2+} ions.

The minerals induced by DB1-12 bacteria were characterized by XRD, FTIR, SEM-EDS and XPS to analyze their mineral properties, organic composition, morphology, and elemental composition. XRD results showed that the induced mineral was calcite when the Zn/Ca ratio was 0, while a mixture of calcite and vaterite at other Zn/Ca ratios (Fig. 2). With increasing Zn^{2+} ion concentrations, FWHM ($^\circ$) of calcite (104) increased from 0.124 to 0.177, and FWHM ($^\circ$) of vaterite (101) increased from 0.108 to 0.206, suggesting that the crystallinity of the formed minerals decreases after the addition of Zn^{2+} ions. Maybe the hydration radius of Zn^{2+} ions is smaller than that of Ca^{2+} ions, resulting in Zn^{2+} ions replacing Ca^{2+} ions and affecting the crystallinity of calcium carbonate. The minerals were in a shape of ball, dumbbell, and short rod, etc (Fig. 3), and contain organic elements such as N, P and S. FTIR results revealed that

the minerals contained abundant protein secondary structures and functional groups, which have a significant influence on mineral nucleation. XPS analysis revealed that proteins, organic acids and phospholipids were present in the mineral surface, provided further evidence of bacterial involvement in mineral formation. The Ca^{2+} ion precipitation ratios reaches 84.25%-89.81% on the 15th day under the action of immobilized bacteria, much higher than the 66.16%-79.05% for free bacteria. The presence of Zn^{2+} ions inhibits the precipitation of Ca^{2+} ions. The Zn^{2+} ion precipitation ratios ranges from 96.81% to 100%, exceeding 87.38%-100% under the effect of free bacteria. Ca^{2+} ion concentration could decrease to 200-315 mg/L, and Zn^{2+} ion concentration 0-0.83 mg/L, satisfying the the national standard GB/T15454-2009 (Ca^{2+} , 200 mg/L) and GB 8978-1996 (Zn^{2+} , 2 mg/L).

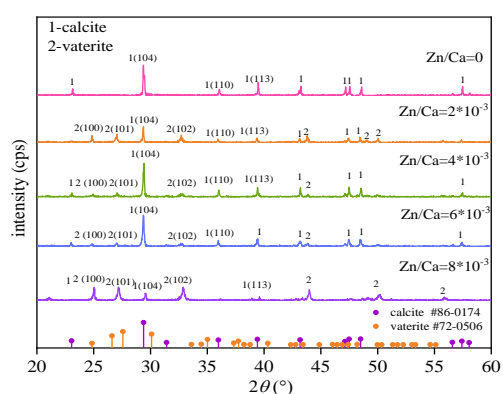


Fig. 2. XRD analyses of the minerals for 20 days of cultivation by DB1-12 bacteria (Ca^{2+} 2,000 mg/L, Zn^{2+} 6.5, 13, 20, 26 mg/L).

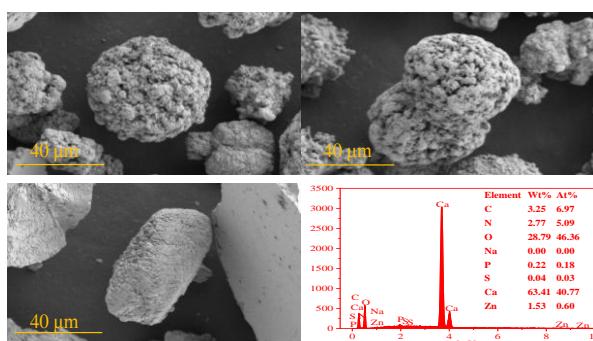


Fig. 3. SEM-EDS analyses of minerals.

This study was beneficial to deep understanding the significant role played by DB1-12 bacteria in the nucleation and growth of carbonate minerals under varying Zn/Ca ratios, and also had great application potential in the bio-removal of Ca^{2+} and Zn^{2+} ions in hypersaline oilfield wastewater.

Acknowledgements

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References

- Power, I.M.; Harrison, A.L.; Dipple, G.M. Accelerating mineral carbonation using carbonic anhydrase. *Environ. Sci. Technol.* 2016, 50: 2610-2618.
- Cai LX, Chu YM, Zhang GY. Mining and engineering of microbial carbonic anhydrases for biomimetic carbon dioxide sequestration. *Chin. J Biotech.* 2019, 35(1): 1-12.
- Pascual-Cebrian, E., Gotz, S., Bover-Arnal, T., Skelton, P.W., Gili, E., Salas, R., Stinnesbeck, W. Calcite/aragonite ratio fluctuations in Aptian rudist bivalves: Correlation with changing temperatures. *GEOLOGY.* 2016, 44: 135-138.

Proto-dolomite spherulites with heterogeneous interior
precipitated in brackish water cultivation of freshwater
cyanobacterium *Leptolyngbya boryana*

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Abstract

Primary dolomite can be formed through cyanobacterial calcification, yet the details and mechanisms of this process are not fully understood. In this study, a freshwater filamentous cyanobacterium, *Leptolyngbya boryana*, was cultured and domesticated in artificial freshwater and brackish solutions with various Mg/Ca ratios. The hydrochemistry, the extracellular polymeric substance (EPS) composition and the precipitates mineralogy in the medium were monitored. The results showed that the *Leptolyngbya boryana* induced proto-dolomite precipitation in brackish medium with salinity of 1.5‰ and Mg/Ca ratio of 5. The proto-dolomite in this study has a "double spherical" appearance and a hollow core, which may have originally been filled by the complex composed of EPS and amorphous Ca-Mg carbonate. With regard to elemental composition, the cyanobacterially-induced proto-dolomite is rich in calcium inside and magnesium-rich on the surface, and cyanobacterial organic matter is sealed inside the particles during spherulite growth. In this study, the accelerators for magnesium ions to enter the carbonate lattice mainly include extracellular acidic amino acids and polysaccharides. The changes of these promoters among different cultures were related

to the growth state of cyanobacteria under salinity stress. The polysaccharides concentration has a significant increasing in the dolomite-precipitating medium, indicating that it may be the main promoter of proto-dolomite precipitation and significantly increases the amount of magnesium ion precipitation. At the meantime, the amount of precipitated Ca^{2+} was suppressed by increasing salinity and Mg^{2+} , thus the proto-dolomite was precipitated in this shift process. This study can potentially provide a reference for explaining the dolomite (proto-dolomite) precipitation in aerobic brackish environment with cyanobacteria growth in.

Key words: Protodolomite; Freshwater Cyanobacteria; EPS; Salinity; Polysaccharides

Amorphous and Crystalline Carbonate Biomineralization in Cyanobacterial Biofilms Induced by *Synechocystis* sp. PCC6803 Cultured in CaCl₂–MgCl₂–SrCl₂ Mediums

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Abstract

Ca²⁺, Mg²⁺, and Sr²⁺ are elements with similar ionic and hydrochemical characteristics, yet the microbial mineralization behavior in their coexisting environments is seldomly explored. In this study, the cyanobacterium *Synechocystis* sp. PCC6803 was used to induce the precipitation of minerals in mediums with various Mg/Ca and Sr/Ca ratios (Ca²⁺ ¼ 0.01 M). The medium hydrochemistry, including cell density, solution pH, and alkalinity, was recorded periodically. The bioprecipitates were characterized with X-ray diffraction (XRD), scanning and transmission electron microscopy (SEM and TEM), Fourier Translation Infrared spectroscopy (FT-IR), and Thermal Gravimetric Analyzer (TGA). The results show the growth of *Synechocystis* sp. PCC6803 was inhibited by increasing ionic strength, although the final alkalinity and pH values of the medium were not affected. Two crystalline minerals, calcite (CaCO₃) and strontianite (SrCO₃) were precipitated in the mediums with low ionic strength; these were transformed from early amorphous precipitates by a dissolution and re-precipitation mechanism. The morphology of the precipitated cyanobacterial

strontianite changed from columnar to dumbbell shape and finally into a spherulite shape. High concentrations of Sr^{2+} , like Mg^{2+} , prolonged the stabilization of amorphous carbonate precipitates. The acidic amino acids (Glu and Asp) in the EPS of *Synechocystis* sp. PCC6803 cultured in mediums with high Sr^{2+} and high Mg^{2+} concentrations increased significantly, compared to those cultured in a medium with no Sr^{2+} or Mg^{2+} ($p < 0.005$) ions. The negative binding energy calculated by Density Functional Theory (DFT) on the binding between the two acidic amino acids (Glu and Asp) and ion- H_2O complexes ($\text{Ca}[\text{H}_2\text{O}]_6^{2+}$, $\text{Mg}[\text{H}_2\text{O}]_6^{2+}$, and $\text{Sr}[\text{H}_2\text{O}]_6^{2+}$), indicating that they are all thermodynamically-favored processes. Consequently, lower energy was needed in their subsequent precipitation and nucleation within the EPS of *Synechocystis* sp. PCC6803. This inferred process was also supported by the appearance of amorphous particles in the EPS of *Synechocystis* sp. PCC6803.

Sulfate-reducing bacteria-mediated pyrite formation in the Dachang tin polymetallic deposit, Guangxi

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Abstract

Mediation by sulfate-reducing bacteria (SRB) is thought to be the primary process responsible for the formation of pyrite (FeS_2) on Earth. The origin of the Dachang tin polymetallic ore field is related to the mineralization of submarine hydrothermal vents sedimentation. To investigate the existence of SRB in these ores, we carried out morphological, chemical, and isotopic analysis using light- and electron microscopy, Raman spectroscopy, and isotope ratio mass spectrometry. We have observed the trace characteristics of SRB in the metal sulfide ore of this ore field. These microfossils distributed near micro-hydrothermal vents are filamentous, tubular or spherical existed in pyrrhotite (FeS) matrix, with filamentous, tubular body walls and spherical bodies composed of pyrite (FeS_2). The content of C, N and O elements in the pyrite synthesized by SRB is high, and the biological spectral signal is obvious. The elemental composition information of organic matter is not observed in the peripheral pyrrhotite. It is speculated that these SRB living in submarine hydrothermal vents belong to a class of chemoautotrophic microbes, which can obtain energy and carbon from the vent hydrothermal fluid to meet the needs of life metabolism, and become the primary producers of submarine hydrothermal vents ecosystems. The contents of heavy isotopes ^{56}Fe and ^{34}S of pyrite synthesized by SRB are higher than those of pyrite, indicating that part of light Fe is lost and heavy S is increased during the conversion of FeS to pyrite (FeS_2). These heavy S are derived from the reduction of SO_4^{2-} in the vent hydrothermal fluid during SRB metabolic processes. The observation of the traces of

microbial presence, elements, and their isotopic composition shown by FeS₂ provide a new observation method for microbial research in submarine hydrothermal vents (in extreme environments), which will promote scientific research of submarine hydrothermal vent ecosystems in modern, geological, and historical periods. The traces characteristics of microbial presence, elements, and their isotopic composition shown by FeS₂ may constitute a novel approach for microbial research. May provide a new observation method for microbial research in submarine hydrothermal vents (in extreme environments), and may promote scientific research of submarine hydrothermal vent ecosystems and the exploration of extraterrestrial life.

Keywords: sulfate-reducing bacteria, hydrothermal vent, pyrrhotite, pyrite, microfossils