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Assessment of cyanobacterial species for carbonate precipitation on mortar surface under different conditions

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ABSTRACT

Microbial carbonate precipitation (MCP) has the potential to restore concrete, contributing to a longer lifespan of construction. The MCP process starts with microbes attaching to the damaged substrate, and completes with carbonate precipitation. Tremendous studies have focused on ureolytic bacteria, while this research investigated the function of cyanobacteria by monitoring the chemical variation of solution, morphological and spectroscopic changes of mortar surfaces. By comparing the biomineralization mediated by three cyanobacterial species in experimental solutions and on mortar surfaces, this study concludes that *Synechocystis* PCC6803 exerted the highest impact in carbonate precipitation under both conditions. Environmental factors such as viability of cells, UV exposure and light intensity modified the MCP processes of *Syn.* PCC6803 and *Synechococcus* LS0519, but not *Synechococcus* PCC8806. UV-killed *Syn.* PCC6803 brought about the highest rate of carbonate precipitation.

1. Introduction

Microbial carbonate precipitation (MCP) has been vastly investigated due to its high application potential in heavy metal remediation, carbon sequestration and construction restoration (Zhu and Dittrich, 2016). As the most widely used construction material in the world (Aïtcin, 2000), concrete was studied under various aspects in order to enhance its performance and durability. MCP offers one of the solutions to improve its compressive strength and prevent damage from water penetration (De Belie and De Muynck, 2009; Ramakrishnan et al., 1998). MCP can be applied in concrete restoration by producing self-healing bio-concrete, cementing and recycling concrete aggregates, and repairing micro-cracks in existing concrete constructions (Grabiec et al., 2012; Jonkers et al., 2010; Van Tittelboom et al., 2010). In each case, the interface of the concrete, microbes and carbonate precipitates is where the MCP starts to exert an impact on the performance of concrete. This study focuses on the MCP on surfaces of mortar, which is composed of cement, sand and water, and is similar to concrete, which contains gravel as an additional component.

A variety of microorganisms are capable of inducing carbonate precipitation through physiological activities such as photosynthesis, ureolysis, denitrification, and sulfate reduction, or by serving as a template for crystal nucleation (Zhu and Dittrich, 2016). A considerable amount of research on MCP technology focuses on ureolytic bacteria for construction restoration and on cyanobacteria for carbon sequestration. The fact that cyanobacteria contribute to the deposition of carbonate during Earth history, especially at end of Proterozoic (Altermann et al., 2006) is one of the arguments for exploring the potential of cyanobacteria for MCP application.

Microbial carbonate precipitation, as an example of biomineralization processes that can occur through biologically-controlled, -induced or -influenced mechanisms (Trichet and Défarge, 1995; Weiner and Dove, 2003). When organisms actively control and govern all stages of mineral formation, the mineral is a product of a biologically controlled process. In many cases a mineral formed in this process serves for a specific purpose of organisms, such as bones, shell, teeth (e.g., Addadi et al., 2006). When an organism's metabolism leads to conditionsfavorable to the precipitation of a specific mineral, a process of mineral formation is called biologically induced mineralization (e.g., De Muynck et al., 2010). Lastly, biologically influenced biomineralizaton or organomineralization is a process of mineral formation by templating on organic by-products of organism activity (e.g., Trichet and Défarge, 1995).

Since the capability of carbonate precipitation by different microbial species varies greatly (Lee et al., 2004), it remains unclear which is the most suitable one for concrete restoration. Most studies on the carbonate precipitation by numerous cyanobacterial species were done with a certain calcium concentration (up to 20 mM), which generally ranges from fresh to marine water (Bundeleva et al., 2014; Obst et al., 2009; Yates and Robbins, 1995). In practical application, however,

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higher calcium concentration works more effectively. For example, a calcium concentration of 25–450 mM was frequently used in the studies of MCP for concrete restoration (Achal et al., 2011; De Muynck et al., 2008; Qiu et al., 2014). The variations in calcium concentrations challenge the choice of a specific species and treating conditions for concrete restoration. Additionally, most of the experiments were done under well-controlled laboratory conditions, which may not be applicable in practice. For example, light is not naturally available underground, which blocks photosynthesis. In many applications, legislation may limit the use of living bacteria for fear of ecological harm (HealthCanada).

This study aims to compare the carbonate precipitation capacity of three cyanobacterial species under both low and high calcium concentrations, as well as under broad environmental conditions to bridge their calcification potential from experimental solution to practical conditions in mortar. To gain insights into the roles of metabolic activities such as photosynthesis of cells, different conditions of light regime such as with illumination, under darkness and UV-killed pretreatment were applied. The interfaces between the mortar, cells and precipitates reflect the initial impacts of MCP, and therefore were examined under all conditions.

2. Experimental methods

2.1. Cyanobacterial species and culture conditions

This study investigated two freshwater cyanobacterial species *Synechocystis* sp. PCC6803 (*Syn.* PCC6803) and *Synechococcus* sp. LS0519 (*Syn.* LS0519), and a marine species *Synechococcus* sp. PCC8806 (*Syn.* PCC8806). *Syn.* PCC6803 is a model cyanobacterial species that has been well studied, and its entire genome sequence has been revealed. *Syn.* PCC8806 is a marine species living in high-saline environments. Compared to these phycocyanin-containing species, *Syn.* LS0519 contains phycoerythrin, reflecting their habitat at a lower light intensity or deeper underwater layers (Croce and van Amerongen, 2014; Stomp et al., 2007). *Syn.* PCC8806 and *Syn.* PCC6803 were received from Pasteur Culture Collection, and *Syn.* LS0519 was isolated from Lake Superior (Ivanikova et al., 2008).

Syn. PCC6803 were cultivated with BG11 medium as batch cultures, while *Syn.* LS0519 and *Syn.* PCC8806 were cultured in 2.5 L chemostats with Z medium and ASN-III medium, respectively (Supporting Information, SI). The temperature was maintained at 25 °C in all experiments.

2.2. Cell preparation for biomineralization experiments

Cells were harvested from the chemostats or Erlenmeyer flasks at the stationary growth stage by centrifuging at 7200 rpm for 15 min at 20 °C. The supernatant was decanted and the cell pellet was re-suspended in a 10 ml sterilized 0.1 M $NaNO_3$ solution. This procedure was repeated three times to wash the cells. After the last wash, the cell pellet was suspended in 15 ml sterile de-ionized water.

The cell concentration was determined by optical density (OD) measured by GENESYS 5 UV–Vis at a wavelength of 650 nm and the relation between the cell number and OD reading was established by counting the cells under epifluorescence microscopy with an R^2 larger than 0.98 (see the details in the Supplementary Information, SI).

2.3. Mortar preparation

In this study, mortar is used to represent concrete materials. Mortar cuboids in a size of 30 × 30 × 15 mm were prepared with SAKRETE® Sand Mix and formed in a customer-built equipment. After 24 h, they were demolded, and cured for 28 days under a humid atmosphere (90% R.H., 20 °C). After curing, the mortar cuboids were cut evenly into 8 with a height of 6 mm. They were washed with acetone in ultrasound for 5 min, and followed by a wash with deionized water. Prior to experiments, each face of the mortar cuboids was exposed to UV light for one hour to sterilize the surface.

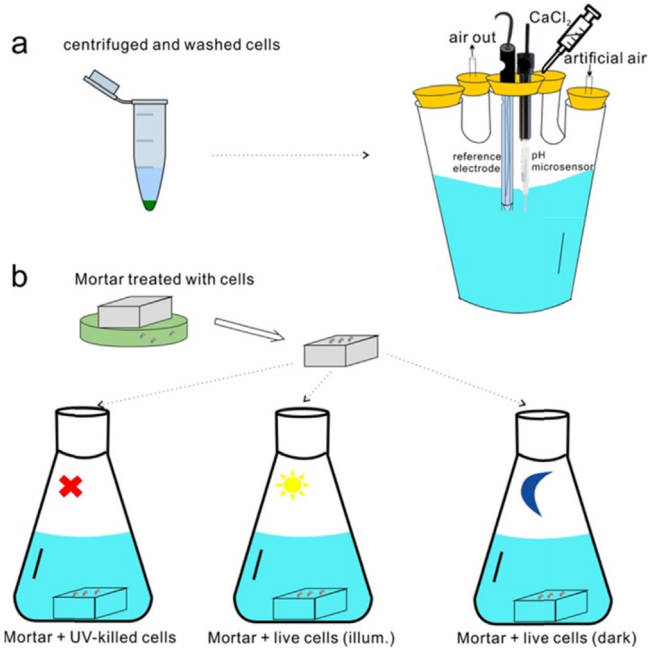

Fig. 1. The setup of experiments A and B.

2.4. Experiments A setup: MCP in solution

Experiments A were carried out with *Syn.* PCC6803, *Syn.* LS0519, and *Syn.* PCC8806 in 750 ml five-necked flasks (Fig. 1a). Experiments were done with a large volume solution (700 ml) in order to minimize the influence of the successive sampling that decreases the amount of the experimental solution. Stock solutions, sodium bicarbonate and calcium chloride were prepared from chemical reagents (ACS reagent, Sigma Aldrich) using deionized water. A 630 ml $NaHCO_3$ solution, previously filtered through a 0.2 µm membrane, was introduced to a five-necked flask and equilibrated with air until the pH was stable. A 70 ml $CaCl_2$ solution was added drop-wise through a sterile 0.2 µm filter using a syringe into the continuously stirred bicarbonate solution. The mixture of the stock solutions made the bicarbonate and calcium concentration in the experimental solution 2 and 4.2 mM, respectively. The calcium concentration, 4.2 mM, is within the range of that in the fresh or marine water applied in many experiments (Obst et al., 2009; Yates and Robbins, 1995), while other studies used a calcium concentration up to 20 mM (Bundeleva et al., 2014). Once pH was stable, the cell suspension was added with a syringe to reach a concentration of approximately 10^7 cells L^{-1}. An abiotic control without cell addition was carried out, of which the speciation and the saturation state was determined by PHREEQC. The saturation index $\Omega_{aragonite}$ was 4.9 and $\Omega_{calcite}$ was 6.9. The changes of pH were monitored with pH microelectrodes (Unisense, Denmark). Sample aliquots (10 ml) were taken by sterile syringes after the addition of cells at 0, 2, 4, 8, 20, 22, 26 and 30 h. Aliquots were filtered through 0.2 µm Nuclepore polycarbonate membrane filters. Filtered solution was collected for measurements of aqueous calcium concentration. The solution for calcium concentration analysis was immediately acidified with 10% (v/v) HNO_3. The filters were rinsed with 2 ml deionized water to remove salt and then stored in petri-dishes for microscopic and spectroscopic examination. All experiments were conducted in duplicates over a 30-hour duration. Sterility was ensured throughout the experiments by using sterile pipettes, needles, flasks and solution.