Remediation of Historic Buildings and Patrimony by Bacterially Induced Mineralization

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Abstract Laboratory experiments were conducted to protect and consolidate historic architectural heritages by bacterially induced carbonate mineralization on the surface of samples of marble and concrete. Some properties of samples and mineral, such as the composition and growth of the mineral deposited on samples, porosity or pore size distribution of samples, the efficiency of protection, the bond behavior between the deposited mineral and substratum, were analyzed by X-ray diffraction, scanning electron microscopy, mercury intrusion porosimetry and ultrasonic test. The results show that the phases of mineral crystal are calcite and vaterite, and the calcium source has an effect on the phase of calcium carbonate mineralization and precipitation. Bacteria act as nucleation sites in the course of precipitation of the mineral crystallization, and the crystal is deposited uniformly on the surface and subsurface of the matrix. The precipitation has no significant effect on the pore size distribution of the matrix, but results in a decrease of porosity, and mineral crystals are strongly attached to the substratum. Bacterial mineralization for remediation of historic buildings can be an ecological and novel alternative to traditional techniques.

Keywords: Bacterium, biomineralization, bioremediation, bioreinforcement, calcium carbonate, historic buildings.

Introduction

Bacteria are small, prokaryotic, microorganism that are ubiquitous in terrestrial and aquatic habitats. Some of them can cause deterioration to construction materials such as stone and concrete by the weathering action of various physical, chemical and biological damage factors at the object site (Warscheid 2000, Gaylarde 2003). Actually, some of them are also capable of remediation of historic buildings. Biodeposition consolidating and/or protecting efficacy are of great worth, because bacteria induce carbonate cementation to a depth of several hundred micrometers ($\geq 500 \mu$m). Furthermore, no plugging or blocking of pores takes place during this cementation (Rodríguez-Navarro 2003). Bacterially induced carbonate mineralization has been proposed as a novel method on corrosion protection of construction materials (Qian 2009, De Muynck 2010). However, the efficacy in bacterially mediated bioremediation of historic architectural heritages can be less well known.

The aim of this study is to determine the efficacy of the application of Sporosarcina pasteurii induced carbonate mineralization on the consolidation of marble and concrete samples. Laboratory experiments were conducted to protect and consolidate samples of marble and concrete. The composition and growth of the mineral deposited on samples, porosity or pore size distribution of samples, the efficiency of protection, the bond behavior between the deposited mineral and substratum, were analyzed by X-ray diffraction, scanning electron microscopy, mercury intrusion porosimetry and ultrasonic test, respectively. This paper will offer an insight into bioremediation of historic buildings in practice.
Materials and Methods

**Bacterial Strain and Culture Media** The microorganism used throughout the study was Sporosarcina pasteurii (strain number 11859 provided by American Type Culture Collection(ATCC)). The diameter of the rotundity spore is about 0.5~1.5 μm. For inoculum preparation, Sporosarcina pasteurii was precultured in liquid medium NH4-YE (ATCC Medium 1376 broth: Tris±HCl, 130 mM (pH 9.0); (NH4)2SO4, 10.0 g; yeast extract, 20.0 g; and distilled water, 1 L; to which 1.5% agar was added to obtain a solid medium for the stock culture.). Individual ingredients were autoclaved separately and mixed afterward to avoid precipitation. The pH of the medium was adjusted to 8.6. Precipitation experiments were carried out in liquid medium (Urea±Ca2+) containing the following L-1 of glass distilled water: nutrient broth (Bacto), 3.00 g; urea, 20.00 g; NH4Cl, 10.00 g; and NaHCO3, 2.12 g. The pH of the medium was adjusted to 6.0 with 6 mol/L HCl prior to autoclaving for 20 min at 121°C. 10 ml of filter-sterilized solution containing 5.60 g CaCl2 or 7.96 g Ca(CH3COO)2 was added afterward to yield 50.4 mM Ca2+. The final pH of the medium was measured to be 8.0.

**Marble and Concrete Samples** Marble blocks were selected from Guangxi province and cut into 10 mm× 10 mm× 10 mm. Concrete samples were made by using ordinary Portland cement. The composition of the concrete mix: cement, 442Kg; sand, 473Kg; aggregates, 1280Kg; and water, 221Kg. Moulds with dimensions of 40 mm× 40 mm× 160 mm were used. After casting, all moulds were placed in an air-conditioned room with a temperature of 20 ±2°C and a relative humidity of more than 90% for a period of 24h. After demoulding, the specimens were cured for more than 28 days and cut into 10 mm× 10 mm× 10 mm.

**Biomineralization Experiments** Biomineralization experiments were conducted in liquid media under constant shaking and stationary conditions. One small samples per test tube was used in experiments with shaking, whereas the other per Erlenmeyer flask was used in stationary experiments. Cell concentrations were determined by viable cell counting on NH4-YE plates. The culture was incubated on a shaker (with constant shaking 160 rpm) for 24 h at 30°C, which is the optimal duration for Sporosarcina pasteurii to reach a density of $2.8 \times 10^7$cfu/mL during the exponential growth. Tubes and Erlenmeyer flasks were sealed with kraftpaper-covered cotton plugs. Liquid media were sterilized by autoclaving for 20 min at 121°C. Samples of marble and concrete were placed in Urea±Ca2+ culture media (10 ml of culture medium in each test tube; 100 ml of culture medium in each Erlenmeyer flask) and inoculated with 0.2 ml (test tubes) and 2 ml (Erlenmeyer flasks) of Sporosarcina pasteurii inoculum culture. Test tubes were incubated at 30 °C with constant shaking (100 rpm) using a rotary shaker. Erlenmeyer flasks were incubated at 30°C in stationary condition. Control experiments identical to those indicated above were carried out without bacterial inoculation. The same procedure was used to check contamination of inoculated samples. A minimum of three samples were run in each experiment.

**Analyses and Measurements** The diffractometer, a model D/max255 0VB3+/PC X-ray diffraction (XRD), was used to determine the mineralogy of deposit crystal. Biodeposition samples were collected, and dried for 12 h in an 80°C oven. Texture and penetration depth of biodeposition on the samples substratum were observed using scanning electron microscopy (SEM, with a model SPA-300HV). Samples were gold coated prior to observation. Samples were collected, and dried for 24 h in an 37°C oven in a dark and dust-protected environment. Changes in samples porosity and pore size distribution following biomineralization were analyzed using mercury intrusion porosimetry (MIP, with a Micromeritics AutoPore IV9500 device). Samples were dried overnight in a oven at 80°C prior to MIP analysis. The samples were sonicated in deionized water for a duration of 5 min, five times in succession, using a 40 kHz ultrasonic bath (with a model KQ-5200DB numerical control ultrasonic device). Samples were rinsed three times with distilled water and collected, dried to constant weight in an 105°C oven, and weighed after each 5-min sonication cycle. SEM was used to analyze the final appearance of the stone surfaces. Samples of the same size that were not subjected to biomineralization were used as controls. One set of control samples was subjected to sonication, while another set was not sonicated. The latter samples were immersed...
in distilled water to estimate weight loss not due to sonication. A set of biomineralized samples was used to estimate weight loss due to dissolution.

**Results**

**XRD Analysis** For bacterium Sporosarcina pasteurii and culture medium urea-Ca2+, bacterially induced mineralization crystal are calcite and vaterite. Calcium source has effect on the phase of calcium carbonate precipitation. Calcite was the main phase in culture medium urea-CaCl2 (Fig. 1a). Vaterite was the main phase in culture medium urea-Ca(CH3COO)2 (Fig. 1b).

Figure 1: X-ray diffraction (XRD) patterns of bacterially induced carbonate mineralization

(a) CaCl2 acted as calcium source    (b) Ca(CH3COO)2 acted as calcium source

**SEM Analysis** Details of bacterially induced carbonate mineralization under SEM are depicted in Fig. 2, Calcite (Cc) crystals are indicated by arrows, and bacterial cells (bb) and vaterite (Vat) crystals are also indicated. Calcite was the main phase in culture medium urea-CaCl2 (Fig. 2a), Vaterite was the main phase in culture medium urea-Ca(CH3COO)2 (Fig. 2b). The presence of crystal associated with the bacteria suggests that bacteria served as nucleation sites during the mineralization process.

Figure 2: Scanning electron microscope (SEM) micrographs of bacterially induced carbonate mineralization

(a) CaCl2    (b) Ca(CH3COO)2
Figure 3: SEM micrographs of bacterially induced carbonate precipitation on concrete samples for 48 h

Micrograph of bacterially induced carbonate precipitation on concrete samples for 48 h is depicted in Fig. 3. Calcite (Cc) crystals are indicated by arrows, bacterial cells (bb) crystals are also indicated. Representative image of control concrete samples is depicted in Fig. 3a. Concrete sample subjected to biomineralization in the culture medium urea-CaCl$_2$ is depicted in Fig. 3b, showing bacterial cells (bb) and calcite (Cc) crystals newly formed calcite layer on the surface of concrete samples. Bacterial cells fully enclosed by precipitation crystals can be observed in samples treated with inoculated. These crystals are observed on the surface of concrete samples.

Details of of bacterially induced carbonate precipitation on marle samples for 48 h is depicted in Fig. 4. Representative image of control marble samples is depicted in Fig. 4a. Marble sample subjected to biomineralization in the culture medium urea-CaCl$_2$ is depicted in Fig. 4b. Bacterial cells fully enclosed by precipitation crystals can be also observed in samples treated with inoculated. These crystals are observed on the surface of concrete samples.

Figure 4: SEM micrographs of bacterially induced carbonate precipitation on marble samples for 48 h

Figure 5: SEM micrographs of concrete samples treated by ultrasonic
Fig. 5 shows an SEM micrograph of concrete samples treated by ultrasonic. Detail of a sample showing a partially removed deposit which covered the newly formed calcite cement. Carbonated bacterial cells in samples cultivated in culture medium and subjected to sonication were also preserved. Concrete (Ct) fragments are indicated by arrows, gravel aggregate (gravle) are also indicated. Fig. 6 shows an SEM micrograph of marble samples treated by ultrasonic. Marble (Fr) fragments are indicated by arrows, calcite split (Sp) are also indicated. Sonication induced significant damage in the controls. However, samples subjected to bacterially induced mineralization showed much less damage, and the newly formed carbonate grains were not removed in detectable amounts.

![SEM micrographs](image)

(a) Marble sample  
(b) Marble sample

*Figure 6: SEM micrographs of marble samples treated by ultrasonic*

**Porosimetry Analysis** Mercury intrusion porosimetry curves of the cumulative intrusion represent porosity and curves of log of differential intrusion vs the pore diameter represent pore size distribution. Details of changes in pore size distribution of biodeposit and control concrete samples are depicted in Fig. 7a, those of biodeposit and control marble samples are depicted in Fig. 7b. Fig. 7 shows no significant changes in pore size distribution when comparing treated and untreated (control) samples. However, biodeposition results in porosity of concrete samples decrease by 15.3% and marble samples decrease by 22.2%.

![Porosimetry Curves](image)

(a) Concrete (in Urea-CaCl₂)  
(b) Marble (in Urea-CaCl₂)

*Figure 7: Mercury intrusion porosimetry curves of porosity and pore size distribution*

**Ultrasonic Treatment** Two control sets were tested: One set of control samples was subjected to sonication (control sonicated), while another set was not sonicated. The latter samples were immersed in distilled water to estimate weight loss not due to sonication (nonsonicated, e.g., calcite dissolution). Samples subjected to biodeposition showed weight loss values between those of sonicated controls and non-sonicated controls (controls immersed in deionized water but not sonicated.). Fig. 8 also shows evidence of a weight loss reduction immersed in water but not submitted to sonication. Sonication caused significant damage to samples of controls, which demonstrated the dramatic effect of the test. However, samples subjected to bacterially induced mineralization showed much less damage, and the deposit grains were not removed in detectable amounts.
amounts (Fig. 5, Fig. 6). SEM observations and weight loss measurements of sonicated samples demonstrate the strong adhesion between the samples and the deposit grains, as well as the positive consolidating and/or protecting effect of the biodeposition.

![Graph](image_url)

(a) Concrete (Urea-CaCl$_2$)  (b) Marble (Urea-CaCl$_2$)

*Figure 8: Mass loss ($\Delta W$) vs ultrasonication time of samples*

**Conclusions**

Bacterially induced mineralization crystal are calcite and vaterite. The crystal deposit uniformly on the surface and subsurface of samples. mineral crystal are strongly attached to the substratum, biodeposition as an alternative surface treatment effectively improve construction materials such as marble and concrete.

For bacterium Sporosarcina pasteurii, biodeposition has no significant effect on pore size distribution of samples, but results in porosity of marble and concrete decrease by 22.2% and 15.3%, respectively.

Bacterially induced bioremediation of historic buildings can be an ecological and novel alternative for traditional ones.

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**References**