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Influence of concrete-related environmental stressors on biomineralizing bacteria used in self-healing concrete

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ABSTRACT

Biogenic calcium carbonate precipitation has been identified as a way to improve durability and remediate cracks in cement-based materials. However, conditions that occur in cement-based materials (e.g., elevated temperature, nutrient depletion, and high pH) might impede microbial-induced calcium carbonate precipitation (MICCP) in these systems. Thus, the effects of heat, nutrient depletion, and pH treatments, all designed to mimic conditions in fresh cement paste, on the ureolytic bacterium *Sporosarcina pasteurii* were examined; specifically, impacts to bacterial viability, ability to hydrolyze urea, and surface charge were assessed. Viability and urea hydrolysis were most impacted by exposure to extreme temperature (55 °C) and extreme pH (13.6), but the impact was greatly reduced with exposure to milder temperature (45 °C) and pH (12.9) conditions. The zeta potential of *S. pasteurii* cells, which was used to approximate surface charge, was minimally affected by all tested conditions; the negative surface charge of *S. pasteurii* cells suggested that the cells might serve as heterogeneous nucleation sites for calcium carbonate precipitation. This was supported by the observation of substantially higher calcite concentrations in bacterial pastes containing viable cells or cells inactivated by autoclaving as compared to cell-free pastes. These new findings provide important insight as to how the conditions within cement paste could influence the viability of microorganisms and MICCP within cement-based materials. Overall, this study shows the importance of bacteria, whether viable or not, as potential heterogeneous nucleation sites for MICCP in cement-based materials.

1. Introduction

Biomineralization, the process by which organisms stimulate the formation of inorganic minerals, has been used to improve the properties of porous construction materials [13–15,39]; biomineralization can increase the cohesion and stiffness of soil [15,51], reduce the permeability of portland cement concrete [1,2,14], and remediate cracks in concrete [2,9,47]. One way that biomineralization is commonly achieved is through ureolytic bacteria; these bacteria possess the urease enzyme, which catalyzes the hydrolysis of urea and triggers a series of chemical reactions that results in microbial-induced calcium carbonate precipitation (MICCP). Urea hydrolysis produces ammonia and carbon dioxide, causing the precipitation of calcium carbonate in the presence of dissolved calcium [35,41,44]. The availability of dissolved calcium is critical for MICCP, making the calcium-rich pore solutions intrinsic to cement-based systems [11,19] chemically well-suited for this process.

Interest in MICCP to improve the durability of portland cement concrete (primarily by reducing permeability via pore filling) has increased rapidly over the last decade. Portland cement concrete (herein called concrete) consists of sand and gravel embedded in a cement paste matrix. Volumetric changes in the cement paste matrix often lead to the formation of cracks in concrete; these cracks increase the permeability of the concrete and can impact the structural integrity if left unattended. However, in a self-repairing bioconcrete, these cracks could be repaired autogenously through the calcium carbonate precipitation induced by microorganisms [28,29,32,37,47,48]. However, one challenge for long-term use of MICCP in concrete is to ensure that the bacteria remain viable during the service life. In bacterial cement paste, where vegetative cells of the bacterium *Sporosarcina pasteurii* (previously *Bacillus pasteurii*) were added to cement paste at a concentration of 2×10^6 most probable number per milliliter (MPN/mL), Basaran [9] reported that 9×10^3 MPN/mL (0.4% of the original inoculum) remained viable after 28 days. Similarly, Jonkers and Schlangen

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[28] found that 0.12% of *Bacillus cohnii* spores (1.15×10^6 spores/cm^3 out of 10^9 spores/cm^3 in the original inoculum), 0.01% *Bacillus halodurans* spores (1.07×10^5 spores/cm^3 out of 10^9 spores/cm^3 in the original inoculum) and 0.06% *Bacillus pseudofirmus* spores (5.62×10^5 spores/cm^3 out of 10^9 spores/cm^3 in the original inoculum) survived 10 days after inoculation to cement paste. Understanding the factors contributing to bacterial inactivation (i.e., death) is essential for industrial application of biomineralization in cement-based materials. Bacterial inactivation in cement-based systems might be attributable to sudden environmental changes during the mixing process, including, but not limited to, an increase in temperature due to heat generated by cement hydration, lack of nutrients, and an increase in pH.

Previous studies have explored ways to prolong the viability of microorganisms in these systems, such as encapsulating or immobilizing cells [28,48], but little attention has been given to understanding how vegetative cells are affected by environmental conditions in cement-based systems. Furthermore, the role of inactivated cells and endospores (i.e., bacteria in a dormant state) in MICCP is uncertain. It has been shown that free urease, isolated from bacteria, can induce $CaCO_3$ precipitation [7]; however, it has not been conclusively determined whether urease activity can persist in microorganisms that have been inactivated. It has also been proposed that inactivated cells might passively aid in calcium carbonate precipitation by serving as nucleation sites for calcium carbonate precipitation [10]. To address these uncertainties and to provide insight regarding the persistence of vegetative *S. pasteurii* in concrete, we investigated the effect of conditions (e.g., heat, nutrient depletion, and high pH) designed to mimic those present in cement-based systems on the viability, urea hydrolysis, and zeta potential of vegetative *S. pasteurii*.

2. Materials and methods

2.1. Cement

Texas Lehigh (Buda, TX) portland cement conforming to ASTM C150 Type I and Type II specifications [5] was used for all cement paste mixtures. X-ray Fluorescence (XRF) was performed using a Bruker S4 EXPLORER (Madison, WI, United States) to determine the mass composition of oxides (Table 1).

2.2. Microorganism and media

S. pasteurii, a bacterium commonly used in MICCP applications, was selected for this work. This bacterium is alkaliphilic and ureolytic [3,7,21,24,44,50]. American Type Culture Collection (ATCC) 6453 *S. pasteurii* was grown in Urea-Yeast Extract (UYE) medium [52]. UYE medium contains 15.75 g/L Tris base, 20 g/L yeast extract, and 10 g/L urea, and the pH was adjusted to 9 with hydrochloric acid. *S. pasteurii* was grown in batch in UYE medium at 30 °C with shaking. Absorbance at 600 nm (OD_{600}), which is directly correlated to cell concentration, was monitored during growth using a BIO-TEK Synergy HT spectrophotometer (Winooski, VT, United States) until OD_{600} = 0.8 was reached. Then, the bacteria were subjected to various treatments designed to mimic the conditions in cement-based systems (Table 2).

2.3. High alkalinity (HA) solution

The HA solution contained 17.94 g/L KOH and 5.24 g/L NaOH [36], and it was used to simulate the high alkalinity environment that is inherent to the pore solution of cement paste. A Thermo Scientific™ Orion™ 720A pH probe (Waltham, MA, United States) was used to measure the pH of the HA solution, with a pH of approximately 13.6. The pH values of fresh cement pastes range from approximately 12.5–13.5 [42]; thus, the HA solution represents an extreme pH for a fresh paste. For some experiments, the HA solution was modified to include nutrients (N); HA+N solution consisted of 20 g/L yeast extract and 10 g/L urea in HA solution.

Table 1
Type I/II portland cement oxide composition; LOI: Loss on ignition.

Oxides	% (w/w) composition
CaO	63.63
SiO_2	20.14
Al_2O_3	5.42
Fe_2O_3	2.47
MgO	1.32
SO_3	3.09
Na_2O	0.17
K_2O	0.95
LOI	2.82

2.4. Cement extract (CE) solution

To serve as a complement to the HA solution, CE solution was prepared by mixing 350 g of cement with 2 L of deionized water and continuously stirring the suspension for one hour with a magnetic stirrer [17,38]. Then, the suspension was vacuum-filtered with Grade 1 qualitative filter paper. The resulting CE solution had a pH of approximately 12.9. The CE solution was used to examine the effects of a more realistic, milder pH as compared to that of the HA solution, and it also provided a more accurate depiction of the chemical composition of an actual cement paste pore solution than does the HA solution. For some experiments, the CE solution was modified to include nutrients; CE+N solution consisted of 20 g/L yeast extract and 10 g/L urea in CE solution.

2.5. Treatment conditions

Batch cultures of *S. pasteurii* were grown as described in Section 2.2, and the impact of nine treatment conditions on *S. pasteurii* from those cultures was explored (Table 2). For some treatment conditions, the cells were harvested from UYE medium by centrifugation at 7500*g* for 10 min and resuspended in a treatment solution (S, HA, HA+N, CE, CE+N); however, for the remaining treatment conditions, the cells remained in their original UYE growth medium during treatment (U, HT-45, HT-55, A). Each treatment condition was applied to triplicate 100-mL samples, and all treatments were applied for four hours except for the autoclaving treatment. The untreated (U) sample set served as the control, and it represents live, vegetative *S. pasteurii* cells. The other eight sample sets were treated to examine how conditions designed to mimic those that occur in cement-based systems would impact the viability, urea hydrolysis, and zeta potential of *S. pasteurii*. Additional information about the treatments is provided as follows:

- Heat-treatment (HT) was chosen to elucidate the effect of heat generated by cement hydration on *S. pasteurii*. The maximum allowable temperature in concrete is commonly specified as 57 °C, and the typical temperature rise for concrete using Type I/II cement can range up to 30 °C above the placement temperature [22]. Thus, the HT-55 samples were submerged in a water bath at 55 °C to simulate conditions in concrete designed to approach the conventional maximum allowable concrete temperature of 57 °C. HT-45 represents a milder heat-treatment, with those samples being submerged in a water bath at 45 °C.