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Biomineralized cement-based materials: Impact of inoculating vegetative bacterial cells on hydration and strength

Zeynep Basaran Bundur [1], Mary Jo Kirisits, Raissa Douglas Ferron *

Department of Civil, Architectural, and Environmental Engineering, The University of Texas at Austin, 301 East Dean Keeton Street, C1748, Austin, TX 78712, USA

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ABSTRACT

Biomineralization in cement-based materials has become a point of interest in recent years due to the possibility that such an approach could be used to develop a self-healing cement-based system. The objective of this study was to investigate the impact of vegetative cells of *Sporosarcina pasteurii* on the hydration kinetics and compressive strength of cement-based materials. The hydration kinetics were greatly influenced when a bacterial solution consisting of urea-yeast extract nutrient medium and vegetative cells was used to prepare bacterial cement pastes; specifically, severe retardation was observed. In addition, an increase in calcium carbonate precipitation, particularly calcite, occurred within the bacterial pastes. Furthermore, after the first day of hydration, the bacterial mortar displayed compressive strength that was similar to or greater than the compressive strength of the neat mortar.

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1. Introduction

Recent research in the field of cement-based materials suggests the possibility of developing a smart, cement-based material that is capable of self-healing by leveraging the metabolic activity of microorganisms to provide biomineralization [1,2]. Biomineralization is a biochemical process in which microorganisms stimulate the formation of minerals such as carbonates [3]. Microbial-induced calcium carbonate precipitation (MICCP) is an example of a biomineralization process, and the resultant precipitates from MICCP could serve to bind particles (e.g., sand and gravel) together to form a composite material and/or seal and plug cracks in concrete. Similar to the healing process that occurs in cracked bones, a "bio-calcified" concrete would autogenously heal the cracks formed in the matrix phase via the precipitation of carbonate minerals. The limited research involving biomineralization in cement-based systems has shown promising results (e.g., sealing of cracks, recovery of toughness, and increases in compressive strength [4–6]) and suggests that biomineralization can significantly reduce permeability by filling cracks on the surface of concrete.

Biomineralization involves a series of biochemical reactions by microorganisms. Microorganisms can exist in either a viable (vegetative or endospore[2]) state or dead state. Dead microorganisms are no longer metabolically active and thus would not contribute to the continued production of urease for biomineralization; the urease enzyme catalyzes the decomposition of urea into ammonia and carbon dioxide, thereby providing an increased pH and carbonates for $CaCO_3(s)$ precipitation. However, since free urease can induce calcium carbonate precipitation [7], it is possible that urease activity might continue even after the bacteria die. Additionally, it should be noted that it is currently unclear as to whether dead microorganisms might passively influence biomineralization by serving as nucleation sites for precipitation. Vegetative cells are alive and metabolically active; thus, vegetative cells can continue to produce urease, which can lead to carbonate production and biomineralization. However, vegetative cells are more sensitive to environmental stress than are endospores. An endospore is a metabolically inactive structure that is resistant to nutrient depletion, desiccation, and extreme temperatures [8], and it enables a bacterium to remain dormant for extended periods [8]. When exposed to a suitable environment, the endospores can return to the vegetative state, produce urease, and induce precipitation. Since endospores are not metabolically active, they should not precipitate calcium carbonate via the traditional pathways of MICCP in which precipitation results from a chain of bacterially mediated metabolic reactions.

A key issue that must be addressed for the development of a biomineralized cement-based system for healing internal cracks is how the microorganisms will be introduced into the cement-based mixture. There are several methods of incorporating bacteria into cement-based materials. Perhaps the simplest approach is to suspend vegetative bacterial cells in the mixing water; yet it also is possible to replace the mixing water with a bacterial culture consisting of vegetative cells and a nutrient medium. The nutrient medium is comprised of carbon, nitrogen, phosphorus, trace elements, and vitamins, which the microorganisms need for metabolic activity. We regard both of these approaches

* Corresponding author. Tel.: +1 512 232 2691; fax: +1 512 471 7259.
E-mail addresses: zeynep.basaran@ozyegin.edu.tr (Z. Basaran Bundur), kirisits@utexas.edu (M.J. Kirisits), rferron@mail.utexas.edu (R.D. Ferron).

[1] Current Affiliation: Ozyegin University, Department of Civil Engineering, Cekmekoy, 34987 Istanbul, Turkey. Tel.: +90 2165649453.

[2] Only some microorganisms have the ability to form endospores.

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(i.e., suspending bacterial cells in mixing water or replacing the mixing water with a bacterial culture) as "vegetative inoculation" approaches. Achal et al. [9] inoculated vegetative *Bacillus megaterium* cells in nutrient broth to mortar and concrete; an increase in compressive strength and a decrease in permeability were observed in both systems. Ghosh et al. [10,11] suspended *Shewanella* in the mixing water of mortar. An increase in the compressive strength of the mortar was reported, and this was postulated to be due to a decrease in the porosity of the system due to the microorganisms. Neither of these studies investigated the influence of the inoculated microorganisms on hydration kinetics.

Another method of incorporating bacteria into cement-based materials is to encapsulate the microorganisms, synthetically or naturally, prior to adding them to the concrete [2,12–14]. This approach protects the microorganisms from the high pH environment of the cement paste and is intended to maintain the viability of the microorganism for an extended time. The "synthetic encapsulation" method consists of immobilizing the bacteria in a protective barrier (e.g., porous aggregates, polymeric membrane) [2,12,13]. The "natural encapsulation" method consists of introducing the bacteria in the form of endospores [14]. *Bacillus* and *Sporosarcina* species can produce endospores when they are exposed to restricted environmental conditions [15,16]. Jonkers et al. incorporated *Bacillus cohnii* endospores in mortar (by suspending the endospores in the mixing water) and determined that the endospores remained viable for approximately 4 months [14]. Wiktor and Jonkers [13] performed a procedure that consisted of encapsulating *Bacillus alkalinitrilicus* endospores and calcium lactate in lightweight organics. The authors stated that the endospores remained viable for an extended time frame, though no specific duration was reported [13]. However, a priori encapsulation of the microorganisms might be unnecessary, especially if the bacteria can form endospores. With the proper selection of bacteria and nutrient medium, microorganisms added via the vegetative inoculation approach also might be able to survive for extended periods.

The objective of this work was to investigate the effects of vegetatively inoculated *Sporosarcina pasteurii* cells on the properties of cement paste, specifically hydration kinetics and compressive strength. *S. pasteurii* is an endospore-forming, alkaliphilic bacterium and is the most commonly used microorganism for biomineralization applications in cement-based materials [1,4,17]. Results of this study will provide a better understanding of the influence of vegetatively inoculated *S. pasteurii* cells on cement-based properties and provide insight regarding the role of different nutrients on hydration kinetics.

2. Materials and methods

2.1. Bacterial culture

To keep the *S. pasteurii* in an active metabolic state, carbon, nitrogen, and other nutrients are required. In MICCP applications, urea is commonly used as a nitrogen source, and yeast extract is commonly used as a carbon source for *S. pasteurii* [9]. *S. pasteurii* (ATCC 6453) was grown in Urea-Yeast Extract (UYE) medium, which contains 0.13 M Tris base, 10 g urea, and 20 g yeast extract per liter of distilled deionized water (DDI). When a solid nutrient medium was required, 20 g of agar was added per liter of liquid UYE medium. The pH of the UYE medium was adjusted to 9 via the addition of hydrochloric acid.

S. pasteurii cells were grown in batch culture aerobically at 30 °C with shaking in 600 mL of UYE medium. Over time, aliquots were removed for optical density measurements and viable plate counts. Optical density at 600 nm (OD_{600}) was measured with a Bio-Tek Synergy HT microplate reader (Winooski, VT, United States). Viable plate counts were conducted by plating onto UYE agar medium and incubating at 30 °C; colony forming units (CFUs) were counted after 3 days. A correlation between OD_{600} and CFU/mL was developed, and OD_{600} was used thereafter for routine monitoring of cell concentration.

The *S. pasteurii* inocula for cement paste/mortar were grown from freezer-stock in 1000-mL batches of UYE medium (pH 9) at 30 °C with shaking. Mid-exponential phase cells (10^6–10^7 CFU/mL) were used for the paste/mortar inocula, which required approximately 12–15 h of growth (see Fig. 1). Generally, the pastes and mortars containing the bacterial culture were prepared 30 to 45 min after the bacterial cells reached the desired concentration in the UYE medium (10^6–10^7 CFU/mL).

2.2. Cement

Texas Lehigh Type I/II (Buda, TX) Portland cement was used for all cement paste mixtures, and Table 1 shows the mass percentage distribution of oxides. A Mastersizer 2000 particle size analyzer equipped with a Hydro MU 200 wet dispersion unit (Malvern, Worcestershire, United Kingdom) and a sonicator were used to determine the particle size distribution (PSD) of cement. A refractive index (RI) and absorption value of 1.7 and 1.0, respectively, were used for the cement. The RI value was obtained from current literature [18], and the absorption value was chosen such that the residual weighted PSD calculated by the Mastersizer software was less than 1 [19]. A specific gravity value of 3.15 was assumed for the cement [18]. Approximately 0.1 g of cement powder was dispersed in isopropyl alcohol (RI: 1.39), which was sonicated for 30 s prior to the PSD measurement. The resulting PSD is presented in Fig. 2. The d_{50} value (where 50% of the total particle powder volume consists of particles with an effective diameter less than the d_{50} value) was 23.2 μm.

2.3. Aggregates

Colorado River sand with a fineness modulus of 2.37 was used. The PSD for Colorado River sand was determined according to ASTM C136 Standard Test Method for Sieve Analysis for Fine and Coarse Aggregate [21]. ASTM C128-07 Standard Test Method for Density, Relative Density (Specific Gravity) and Absorption of Fine Aggregate [22] was used to determine the absorption coefficient of the sand. The absorption capacity of the sand was 0.65%, and the specific gravity was 2.62. Fig. 3 shows the PSD for the sand.

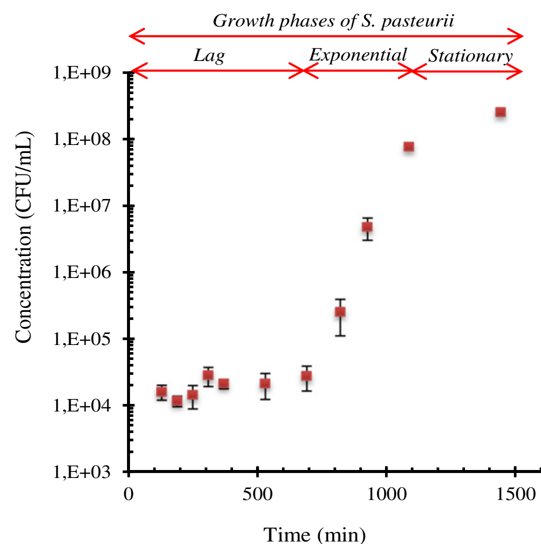

Fig. 1. Representative batch growth curve (cell concentration vs. time) for ATCC 6453 *S. pasteurii* grown in UYE Medium (pH 9) at 30 °C. Error bars represent the standard deviation (based on triplicate viable plate counts).

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