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# Use of pre-wetted lightweight fine expanded shale aggregates as internal nutrient reservoirs for microorganisms in bio-mineralized mortar

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ABSTRACT

Interest in developing bio-based self-healing cement-based materials has gained broader attention in the concrete community. One of challenges in developing bio-based self-healing cement-based materials is that cell death or insufficient metabolic activity might occur when the cells are inoculated to the cement paste. This paper investigates the use of internal nutrient reservoirs via pre-wetted lightweight fine expanded shale aggregates to improve cell viability in mortar. Incorporation of internal nutrient reservoirs resulted in an increase in the vegetative cells remaining without any substantial loss in strength. These results pave the way to develop a self-healing and self-curing concrete with an extended service life.



## 1. Introduction

Portland cement-based materials are complex composite materials that can be susceptible to cracking due to internal stresses caused by various mechanisms (e.g., mechanical loading, humidity variations, and temperature changes). Not only do cracks reduce the strength of portland cement-based materials, but they also provide entry to water and aggressive agents that affect the structural integrity of the composite. However, recent research suggests that it might be possible to develop smart, cement-based composites that are capable of self-healing by using microorganisms to induce biomineralization [1–5]. Biomineralization involves a series of biochemical reactions in which microorganisms trigger mineral precipitation [6–8]. Microbial-induced calcium carbonate precipitation (MICCP) is an example of a biomineralization process, and the precipitates from MICCP can be used to bind particles (e.g., sand and gravel) to form a composite material [9,10] and/or seal cracks in concrete [4,11–13]. Inspired by the healing process that occurs in cracked bones, the goal is for MICCP to autogenously repair cracks that occur in cement-based matrices. Previous research regarding biomineralization in cement-based systems has shown promising results (e.g., sealing of cracks, recovery of toughness, and increases in compressive strength [1,6,8]) and suggests that biomineralization can significantly reduce permeability by filling cracks on the surface of concrete.

One of the main challenges in biomineralization applications in cement-based materials is the restrictive environment (e.g., high pH, lack of moisture, low nutrient concentrations), which might result in cell death or dormancy. Viable microorganisms can exist in either a vegetative or endospore[1] state. Endospores are metabolically inactive structures that are resistant to nutrient depletion, desiccation, and extreme temperatures. Sporulation enables a bacterium to remain dormant for extended periods [14], but when the endospores are exposed to suitable environments, they can return to the vegetative state and resume metabolic activities. Vegetative cells are alive and metabolically active; however, vegetative cells are more sensitive to environmental stress than are endospores. Dead microorganisms are not metabolically active, but it has been suggested that they might passively influence biomineralization by serving as nucleation sites for precipitation [15].

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[1] Only some types of microorganisms have the ability to form endospores.

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Concerns about microbial survival in cement-based materials have led researchers to propose various techniques to encapsulate the microorganisms prior to inoculating them into the cement paste matrix [12,16–18]. Wiktor and Jonkers [12] proposed the encapsulation of *Bacillus alkalinitrilicus* endospores and calcium lactate-yeast extract solution within clay particles, but this approach resulted in a 50% decrease in compressive strength of the cement-based material as compared to the control sample that did not contain encapsulated endospores [4]. Alternatively, Wang et al. [19] suggested suspending *Bacillus sphaericus* endospores in hydrogels to create bio-based hydrogels; even though cracks were sealed in the presence of the endospores, the influence of bio-based hydrogels on concrete performance and the moisture uptake capacity of bio-based hydrogels are unclear [19]. On a related note, Wang et al. studied the impact of polyurethane membranes and silica gel as protective carriers to immobilize *B. sphaericus* cells and found that polyurethane foams yielded higher strength regain than did silica gel after cracks were sealed [20]. It was estimated that the extra costs associated with using a polyurethane immobilization approach would increase the cost of the concrete by 7–28% [20]. However, with proper selection of microorganism, nutrients, and inoculation approach, vegetative microorganisms can survive in cement-based composites without encapsulation [21,22]. Literature on bacterial concrete reports viability of up to 4 months when endospores were suspended in mixing water [23]. However, Bundur et al. [21] inoculated vegetative bacteria in growth medium to cement paste and observed that approximately 42% of the total viable cells remaining were vegetative even after 1 year. Additionally, in the mortars containing these inoculated bacteria an increase in the $CaCO_3$ content of the cement paste matrix and an increase in the compressive strength of the mortar were observed as compared to the mortars not containing the inoculated bacteria. Prior to the work of Bundur et al. [21] the longest time period reported in the literature for viability of inoculated vegetative cells was 28 days by Achal et al. [24], with approximately 1% of the initial inoculated *Bacillus megaterium* cells remained viable.

A key challenge in the development of bio-mortar/concrete is providing the inoculated microorganisms with access to water and nutrients. Replacing the mixing water used for concrete production with bacterial nutrient medium is one way to introduce the nutrients required by bacteria [25]. However, the water available to the bacteria from the nutrient medium will decrease during cement hydration. Thus, water to cement ratio (w/c) becomes a critical point in bio-mortar mixes. Most of the work in the literature use a w/c of 0.45 or above [21–23,26], however the w/c of concrete components is often lower than 0.40, especially in the case of high performance concrete mixes [27]. In such a case, the water and the nutrients to the bacteria will be even more limited in a lower w/c (<0.40) mix than it is in a higher w/c (>0.45) mix. A possible approach that can be used in a lower w/c mix is to provide internal nutrient reservoirs within the cement-based material that can release water and nutrients at later times for the bacteria; in essence, this would be leveraging the concept of internal curing to extend the viability of the bacteria. Internal curing is defined as "*the process by which the hydration of cement occurs because of the availability of additional internal water that is not part of the mixing water* [28]." This additional internal water is often provided via absorbent lightweight aggregates (LWA) or super absorbent polymers [29].

The aim of this study was to evaluate the potential for using pre-wetted lightweight fine expanded shale aggregates as internal nutrient reservoirs for the microorganisms. Instead of pre-wetting the LWA with water, the LWA were pre-wetted with bacterial nutrient medium. The metabolic state of the microorganisms was evaluated to determine whether these LWA could be used to extend the viability of the bacteria in mortar. Additionally, the compressive strength of the cement-based composite was evaluated to determine the effect of replacing a portion of the fine aggregates with pre-wetted lightweight fine expanded shale aggregates.

## 2. Materials and methods

### 2.1. Microorganism growth

*Sporosarcina pasteurii* (ATCC 6453) was grown in Urea-Yeast Extract (UYE) medium at pH 9. The UYE medium contains 0.13 M Tris base, 10 g urea, and 20 g yeast extract per liter of distilled deionized (DDI) water. Twenty grams of agar (per liter) were added to the liquid if a solid medium was required. The Tris base solution (0.13 M) was prepared in one liter of DDI water, and the pH was adjusted to 9 by adding 2 mL of hydrochloric acid. The Tris base solution was divided into two aliquots of 500 mL; urea was added to one aliquot, and yeast extract was added to the other aliquot. The solutions were autoclaved separately, cooled to 23 °C, and mixed together to obtain the final UYE medium.

*S. pasteurii* cells were grown aerobically in batch culture 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 (Winooski, VT, United States) microplate reader. Viable plate counts were conducted by serially diluting the samples, 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 determining the cell concentration. Further information regarding the procedure is described in Bundur et al. [21].

### 2.2. Aggregates

Colorado River sand and lightweight expanded shale aggregates were used as fine aggregates, and ASTM C128-15 [30] was used to determine the absorption coefficient of both aggregates. The Colorado River sand had a fineness modulus of 2.37, absorption capacity of 0.65%, and specific gravity of 2.62. The expanded shale aggregates had a fineness modulus of 0.92, absorption capacity of 24.2%, and specific gravity of 1.16. Fig. 1 shows the particle size distribution (PSD) for the aggregates, as determined by ASTM C136 [31]. Expanded shale aggregates that were retained between the #4 (4.75 mm) and #30 (600 μm) sieve was used in the mixes because greater variation in absorption capacity was seen in the finer size fractions (data not shown).

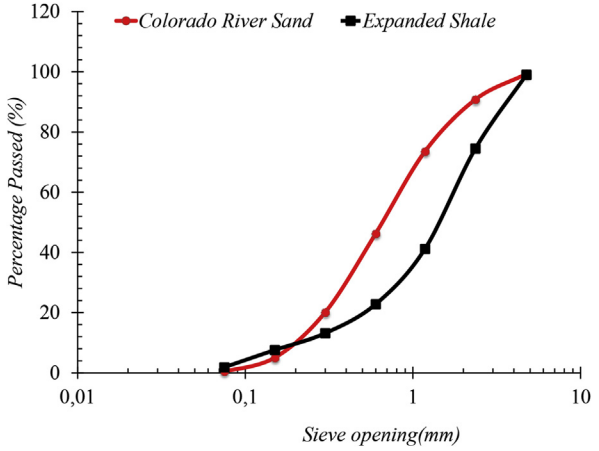


**Fig. 1.** Particle size distribution of Colorado River sand and expanded shale aggregates.