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Viability of bacterial spores and crack healing in bacteria-containing geopolymer

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HIGHLIGHTS

- Direct addition of bacteria without encapsulation into geopolymer mix.
- Bacterial spores remained viable in the metakaolin-based geopolymer.
- Cracks in bacteria-containing geopolymer were sealed with $CaCO_3$ after conditioning.

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ABSTRACT

Geopolymer is an emerging alternative green binder to Portland cement. Geopolymer is often more brittle and thus it is highly desirable to impart self-healing into geopolymer. Unlike Portland cement concrete, self-healing through hydration of cement, and leaching and carbonation of hydration products are not feasible in geopolymer. Microbially induced carbonate precipitation (MICP)-enabled sealing is therefore a potential way to engage self-healing in geopolymer. Using *Sporosarcina pasteurii* as a model MICP bacterium, this paper investigated viability of bacterial spores in a metakaolin-based geopolymer and crack healing in bacteria-containing geopolymer. Spores of *S. pasteurii* were added into geopolymer mix directly without encapsulation or immobilization. Results showed that bacterial spores did not leak out from the geopolymer matrix and the spores remained viable in the metakaolin-based geopolymer. Cracks in bacteria-containing geopolymer were sealed with $CaCO_3$ after conditioning in precipitation medium for 3 days, which suggests bacterial spores remain viable. The microstructure of metakaolin-based geopolymer is controlled by Si/Al, Na/Al, and H_2O/Na_2O molar ratio and less depend on age, which allows direct addition of bacteria into geopolymer mix without encapsulation or immobilization to engage MCP-induced self-healing in geopolymer.

1. Introduction

Concrete is the most used construction material. In field applications, mechanically- and/or environmentally-induced cracks are almost inevitable for concrete structures. Self-healing of cracks, therefore, is desired to ease the maintenance and repair of concrete infrastructure. It has been reported that concrete is capable of autogenously healing cracks through hydration of cement, and leaching and carbonation of hydration products (e.g., $Ca(OH)_2$) in the presence of water and CO_2 [1]. Recent studies indicate that such mechanism is only capable of healing cracks up to 150 μm in width depending on mix composition [2,3]. In reality, however, cracks in concrete can be as wide as several hundred microns, which makes self-healing of concrete difficult.

Several engineering approaches have been attempted to promote self-healing of concrete. Micro-fibers were used to control crack width in concrete [2,3] while expansive chemical agents were added in concrete to allow expansion of crack surfaces and thus reducing crack width [4–6]. Another approach used bacteria that can withstand the alkaline environment (pH = 12–13 [7]) in concrete to induce microbial carbonate precipitation (MCP) for crack sealing [8–10]. The bacterial spores can be activated in desirable liquid environment and create local alkaline environment in its vicinity. The cell wall is negatively charged and attracts Ca^{2+} for nucleation [11]. Such chemical environment effectively promotes the formation of $CaCO_3$. Jonkers et al. [8] were the first to study the viability of *Bacillus* spores in concrete for self-healing purpose. The results showed that viability of spores reduced drastically with increasing age of concrete, as hydration of cement is a continuous process which reduces the pore size of cement paste and crushes the spores.

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To overcome this problem, several researchers suggested encapsulation or immobilization of spores in a protective matrix prior to addition to the concrete mixture. Various soft or porous materials such as polyurethane foam [12], porous glass beads, Siran™ [13], diatomaceous earth [14], porous expanded clay particles [9], silica sol-gels [15,16], hydrogel [17,18], lightweight aggregates [19] have been used for encapsulation of bacterial spores. This approach indeed protects spores from harsh concrete environment. It has been reported that cracks up to several hundred microns can be completely sealed [9] and noticeable mechanical recovery is also noticed [20] for MCP self-healing concrete. However, inclusion of these soft or porous materials reduces concrete strength [18].

Geopolymer is an emerging alternative green binder to Portland cement [21–23]. Alkaline solutions are used to activate minerals of geo-origin (e.g., metakaolin) or industrial by-products (e.g., fly ash) that contain silicate and aluminate, forming three-dimensional polymeric structures that sustain mechanical loading. It has been demonstrated that many geopolymers possess better performance of fire-resistance [24] and heavy metal immobilization [25] than concrete. However, geopolymer is often more brittle and thus it is highly desirable to impart self-healing into geopolymer. Unlike concrete, self-healing through hydration of cement, and leaching and carbonation of hydration products are not feasible in geopolymer, as geopolymer does not contain unhydrated cement or dissolvable Ca^{2+} to be leached out [22]. MCP-induced sealing is therefore a potential way to engage self-healing in geopolymer.

This paper investigated viability of bacteria in a metakaolin-based geopolymer and crack healing in bacteria-containing geopolymer. Bacterial spores of *Sporosarcina pasteurii* were added into geopolymer mix directly without encapsulation or immobilization. Leakage and viability of bacterial spores in geopolymer were examined. Healing of cracks was investigated by conditioning pre-cracked bacteria-containing geopolymer in a precipitation medium. Morphology and chemical composition of healing products were characterized by Field Emission Scanning Electron Microscopy (FE-SEM), and Energy Dispersive Spectroscopy (EDS) and Thermo-Gravimetric Analysis (TGA), respectively.

2. Experimental program

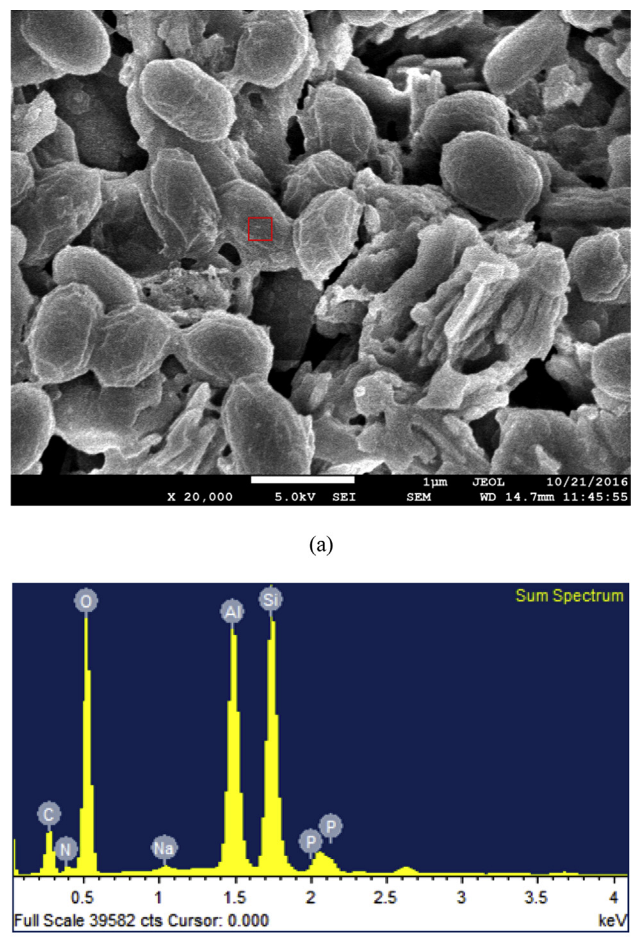
2.1. Materials

Analytical grade metakaolin, sodium silicate solution, distilled water, and *Sporosarcina pasteurii* DSM 33 were used to synthesize bacteria-containing geopolymer. The metakaolin is rich in SiO_2 and Al_2O_3 (Table 1) and easily participates in geopolymerization compared to other precursors such as fly ash because it is highly amorphous, and thus metakaolin system is often used as a model system for geopolymer study. Sodium silicate solution (extra pure, Merck) with 7.5–8.5% of Na_2O and 25.5–28.5% of SiO_2 by weight was used as the sole activator for geopolymer synthesis [26]. The *Sporosarcina pasteurii* DSM 33 was purchased from the German Collection of Microorganisms and Cell Cultures (DSMZ), Braunschweig, Germany.

2.2. Synthesis of bacteria-containing geopolymer

The bacterial cells were cultured in liquid media with composition of 20 g/l peptone, 5 g/l NaCl, and 20 g/l urea [27]. The cultures were incubated at 30 °C at 150 rpm. The alkaline mineral medium suggested by Jonkers et al. [8] (MilliQ ultra-pure water 0.2 g NH_4Cl, 0.02 g KH_2PO_4, 0.225 g $CaCl_2$, 0.2 g KCl, 0.2 g $MgCl_2{\cdot}6H_2O$, 0.01 g $MnSO_4{\cdot}2H_2O$, 1 ml trace element solution SL12B, 0.1 g yeast extract, 5.16 g citric acid trisodium salt, 4.2 g $NaHCO_3$ and 5.3 g Na_2CO_3 per liter, pH = 10) was used to induce sporulation. The cultures were grown for four weeks (until senescence) to get high number of spores. Several flasks were pooled after four weeks to collect the spores. The spores were collected by centrifugation at 10,000 rpm for 15 min and re-suspended in sterile saline solution (8.5 g/l NaCl). The process was repeated several times to wash the spores. The suspension of the spores was subjected to a pasteurization process (20 min in a water bath at 80 °C and then 5 min in an ice-water). These flasks contained varying concentrations of spore. Using these spores, about 10^9 spores/ml spore suspension was prepared to further apply for synthesis of bacterial spore containing geopolymer material. The spore suspension was then stored at 4 °C in a fridge for future use.

To prepare the bacteria-containing geopolymer, 400 g of spore suspension (about10^9 spores/ml) was first mixed thoroughly with



(a)

(b)

Fig. 1. (a) SEM microgram of fracture surface of geopolymer containing bacterial spores and (b) EDS of bacterial spores, i.e. the red square in (a).

Table 1
Chemical composition of metakaolin in the current study.

Oxides	SiO_2	Al_2O_3	Na_2O	K_2O	TiO_2	Fe_2O_3	CaO	MgO	P_2O_5	SO_3	LOI
Weight%	53.00	43.80	0.23	0.19	1.70	0.43	0.02	0.03	0.03	0.03	0.46