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Application of expanded perlite encapsulated bacteria and growth media for self-healing concrete

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HIGHLIGHTS

- The crack healing ability of spores and nutrients encapsulated separately was proven.
- Crack healing requires an appropriate ratio of spores to calcium salt.
- A minimum number of bacterial spores are required for the healing process.
- Addition of growth components to the media results in greater calcite precipitation.

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ABSTRACT

Self-healing concrete based on calcium carbonate precipitation induced through bacterial activity has been investigated in recent years by teams around the world. For the first time, optimisation of the self-healing performance was considered in terms of the number of bacterial spores required, the concentration and composition of nutrients and precursors, and whether a two-component system was likely to efficiently produce self-healing in concrete. This information is required if efficient and cost-effective self-healing systems based on bacterial activity are to be implemented. For this research, coated expanded perlite was used to immobilise bacterial spores and encapsulate nutrients as two separate components for self-healing concrete. Self-healing capacity was evaluated by imaging and by initial surface absorption of water. The results indicated that healing could be achieved when coated expanded perlite containing self-healing agents was used as a 20% replacement of fine aggregate and if a suitable ratio of spores to calcium acetate was provided. This research is the first to show that self-healing is not simply a requirement of having sufficient healing compounds (e.g. calcium acetate) but that a minimal number of bacterial spores are also required to ensure that sufficient cells take part in the healing process.

1. Introduction

The effect of some water-borne ions (e.g. chlorides) on the durability of reinforced concrete is well documented, and cracked concrete has been shown to be more susceptible to permeation of these deleterious ions. Consequently, research is being undertaken in an attempt to develop concrete that can self-heal cracks; potentially reducing repair and maintenance costs on key infrastructure [1–3]. One approach to autonomic self-healing is the utilization of microbiologically induced calcite precipitation (MICP). This approach utilises the metabolic activity of bacteria and biomineral precursors embedded within the concrete to form an inorganic compound as a healing material. This is usually calcium carbonate, typically in the form of calcite but sometimes as vaterite [4,5]. This healing material can precipitate in small cracks soon after they form and it has the potential to limit the permeation of water and dissolved ions. Thereby the life of concrete structures can be extended without the need for manual intervention; which can be both costly and dangerous, particularly for structures with poor access.

While there have been a number of studies into the feasibility of using MICP for self-healing in concrete, there have not been studies on optimising the self-healing performance through consideration of the number of bacterial spores required, the concentration and composition of nutrients and precursors or whether a two-component system is likely to efficiently produce self-healing in concrete.

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There are three key pathways for delivering a MICP healing process: (i) enzymatic hydrolysis of urea [6], (ii) dissimilation of nitrates [7] and (iii) aerobic metabolic conversion of calcium salts [8]. In the aerobic metabolic conversion pathway healing occurs because the bacteria induce the oxidation of an organic calcium salt (precursor), for example calcium acetate or calcium lactate, to calcium carbonate under favourable conditions. These include environmental conditions for the bacteria to thrive (appropriate temperatures, pH and other environmental factors), and the presence of water, oxygen and nutrients for the bacteria to grow. The by-products of the conversion of calcium acetate to calcium carbonate are carbon dioxide and water, both of which are compatible with concrete (Eq. (1)). Furthermore, a weak carbonic acid may form in the presence of carbon dioxide and water that can lead to carbonation of calcium hydroxide within the concrete (Eq. (2)). This leads to a form of enhanced autogenous healing as the carbonated molecule is larger than the uncarbonated version [9].

$$Ca(C_4H_6O_4) + 4O_2 \rightarrow CaCO_3 + 3CO_2 + 3H_2O \quad (1)$$

$$3CO_2 + 3Ca(OH)_2 \rightarrow 3CaCO_3 + 3H_2O \quad (2)$$

Most bacteria-based self-healing concrete systems require spores to be immobilised and separated from any germination triggers, normally via encapsulation, prior to their addition to concrete. This also overcomes concerns with their viability in the aggressive conditions that occur in hydrating concrete [5,8]. Three standard approaches to immobilization have been studied: (i) encapsulation in porous solids, (ii) microencapsulation in gels [10], and (iii) use of pellets and flakes [11].

In addition to the encapsulation of the spores, the extra components required for self-healing (the calcium precursor) and to aid germination of the spores and growth of the cells (usually just yeast extract) need to be included into the concrete. In many self-healing systems, these extra components are added directly to the concrete during the mixing process: this is partly due to difficulties with encapsulating water-soluble compounds [10]. When added directly to concrete these compounds may affect the setting and hardening of concrete, but there has been little consensus on this to date. Reviews of these factors are provided in Paine [12] and De Belie et al. [13] but these do not include an in-depth investigation into how the compounds influence fresh or hardened concrete properties or how they affect the self-healing efficiency.

Because of concerns over the effect of the additional components on cement hydration, Wiktor and Jonkers [14] encapsulated calcium lactate (6% by mass of aggregate) and yeast extract (less than 0.1% by mass of aggregate) along with the spores in 1–4 mm expanded clay aggregates in order to eliminate as much as possible any effect on early-age properties. Impregnation of *Bacillus alkalinitriculus* spores was carried out twice under vacuum. It was shown that upon cracking these encapsulated particles were capable of providing healing in mortars. There was no significant effect on setting, which demonstrates that there was no leaching of detrimental compounds from the expanded clay aggregates. More recent research has used expanded perlite to immobilize spores of *Bacillus cohnii*. The volumes used were microscopically measured to be 3.6×10^9 cell/ml [15]. Calcium lactate (8 g/l) and yeast extract (1 g/l) were sprayed onto the surface of the particles but were not encapsulated or prevented from interfering with hydration reactions.

Diatomaceous earth, a fine porous powder, has also been considered as a carrier for bacterial spores for self-healing concrete applications [16]. However, the spores were found to sorb on the surface of the particles and not within the powder itself, while the bacteria were shown to maintain their ureolytic activity. In other work, Erşan et al. [7] investigated expanded clay particles of 0.5–2 mm in size as carriers of bacterial cells to precipitate calcium carbonate from conversion of calcium nitrate and calcium formate. In this case, impregnation of the particles was carried out under vacuum saturation. The resulting particles contained approximately 10% by mass of cells of *Diaphorobacter nitroreducens* or *Pseudomonas aeruginosa* and 0.15 M sodium chloride. No additional protection was considered necessary to prevent leaching of the cells from the particles. It is worth noting that using sodium chloride as a precursor may raise concerns relating to corrosion of reinforcement in concrete because of an increase in chloride ions.

Essential to self-healing concrete is a requirement for a sufficient quantity of Ca^{2+} to be available in the concrete to enable sufficient calcium carbonate to form and fill cracks. Conversion of the soluble calcium precursor to relatively insoluble calcium carbonate relies on the presence of bacterial cells. Because cells grow and multiply it has been considered that healing can be generated initially in the presence of relatively few cells. However, it has been shown that the spore concentration necessary to deliver calcium carbonate precipitation needs to be greater than 4×10^7 spores/ml [17]. Interestingly, it was suggested that the required spore concentration may be independent of calcium precursor content.

Any method that includes encapsulation of spores, precursors and nutrients in the same capsule can create a problem with ensuring that germination of the spores does not occur within the aggregate. Therefore a dual approach in which the spores and other ingredients are encapsulated separately has potential benefit.

In this study, expanded perlite (EP) was used as a carrier of spores, precursor and essential nutrients. Differently from earlier work, the (i) spores and (ii) the precursor and nutrients were encapsulated separately. Upon cracking of concrete, both the EP containing spores and the EP containing the precursor and nutrients in the crack zone were expected to break and allow the two to come together, provided that there was sufficient water to carry the water-soluble precursor and nutrients to the spores, or vice versa. Spores would then germinate and precipitate calcium carbonate crystals to heal the cracks. The aim of this research was to demonstrate the suitability of a two-component encapsulated system and ascertain the necessary ratio of spores to precursor needed to ensure healing. In order to fully understand the processes involved, microbiological experiments were undertaken to determine spore germination and carbonate productivity.

2. Materials and methods

2.1. Bacterial strain

Bacillus pseudofirmus DSM 8715 (German collection of microorganisms and cell cultures (DSMZ)) was used in this study. Living cells were routinely cultured on buffered lysogeny broth (LB) which contained 100 ml/l Na-sesquicarbonate to achieve pH 9.5. Spores were prepared in a sporulation media [8] and incubated at 30 °C on an orbital shaker for 72 h. Spores were harvested by centrifugation at 10,000 rpm for 15 min. Spore formation was confirmed by phase contrast microscopy. The spore pellet was washed three times with a chilled 10 mM Tris HCl buffer, pH 9.5. The spore pellet was then freeze dried to obtain a spore powder and stored in a desiccator prior to use.

2.2. Growth media

Three growth media were investigated (Table 1). GM1 consisted of a multi-component media based on initial microbiological studies and was selected to maximise as much as possible the germination of bacterial spores, growth of bacterial cells, precipitation of calcite and sporulation of bacteria [5]. GM2 consisted of just three