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Application of hydrogel encapsulated carbonate precipitating bacteria for approaching a realistic self-healing in concrete

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HIGHLIGHTS

- Hydrogel was used as the bacterial carrier in concrete for self-healing cracks.
- Hydrogel acts as water reservoirs for continuous crack healing (autogenic-, biogenic-).
- Carbonate precipitating bacteria can precipitate $CaCO_3$ in/on hydrogel.
- Crack of a width 0.5 mm can be completely healed in the specimens with bio-hydrogels embedded.
- Bacterial based self-healing is a potential solution for sustainable development of concrete.

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ABSTRACT

Bacterial-based self-healing is a promising solution for sustainable concrete maintenance. In this study, bacterial spores were first encapsulated into hydrogels and then were incorporated into specimens to investigate their healing efficiency. The precipitation of $CaCO_3$ by hydrogel-encapsulated spores was demonstrated by Thermogravimetric analysis (TGA). The mortar specimens with hydrogel-encapsulated spores, showed a distinct self-healing superiority: the maximum healed crack width was about 0.5 mm and the water permeability was decreased by 68% in average. Other specimens in non-bacterial series had maximum healed crack width of 0–0.3 mm and the average water permeability was decreased by 15–55% only.

1. Introduction

Carbonate precipitate induced by bacteria has been regarded as an environmental-friendly and economic material which has a promising potential for a wide range of engineering applications. For instance, microbial $CaCO_3$ is being investigated for soil treatment [1–4], remediation for heavy metals and radionuclides [5–8], sand cementation [9–11], surface protection of building materials [12–17], etc. Recently, researchers started applying microbial $CaCO_3$ for self-healing of concrete cracks, which is a possible solution to reduce the high maintenance and repair costs of concrete infrastructures [18]. Carbonate-precipitating bacteria are added into concrete during the process of mixing. When cracks appear, bacteria in the crack zone will be activated and precipitate $CaCO_3$ to in situ heal the cracks. Considering the alkaline environment inside concrete and the requirement of long shelf life of the healing agents, alkaliphilic/alkali-tolerant spore-forming strain should be used. It is generally known that spores, the dormant state of living cells, are much more resistant to heat, radiation and various chemicals, and have much longer survival time than living cells, from several years to hundreds of years [19–21]. Living cells (vegetative cells) are the bacteria in active state while spores are metabolically inert. When growth conditions are limited or in harsh environments, some type of bacterial vegetative cells can sporulate into spores to withstand the deleterious conditions. Spores can also germinate and form vegetative cells when conditions for growth become favorable. In this research, *Bacillus sphaericus*, a ureolytic, alkali-tolerant spore-forming strain was used. The remarkable characteristic of this strain is its high productivity of calcium carbonate precipitation by the pathway of urea hydrolysis [13,22].

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It should be noted that there might be a risk that the bacterial cells or spores will be damaged during the stage of mixing and cement hydration. This is because the cement-based matrix gradually becomes a dense structure during the ongoing hydration. Most of the pores are smaller than 0.5 μm in size, while the size of bacteria is in the range of 1–3 μm. Hence, it is possible that bacteria would be squeezed when the pores become smaller and smaller. Additionally, the mechanical forces during mixing could also damage the bacteria. Therefore, encapsulation of bacteria before addition is preferable. Suitable carriers should have a 'shell' function to protect bacteria, no hindering effect on bacterial carbonate precipitation (upon cracking) and no/limited negative effect on the concrete matrix. Jonkers et al. have applied porous aggregates to immobilize bacterial spores and relevant bio-reagents [23]. Healing superiority in the specimens with bio-aggregates was observed after 40 days. The maximum crack width healed reached 0.46 mm, which was almost two times that in the reference specimens. In our previous research, we developed a system in which glass capillaries were used to encapsulate bacteria and filling materials (silica sol gel) and found that the water permeability in the bacterial series was 2–3 orders of magnitude lower than the non-bacteria series [24]. Diatomaceous earth was also explored to immobilize bacteria for self-healing. Cracks up to a width of 0.17 mm were completely healed in the specimens incorporated with in diatomaceous earth immobilized bacteria, while no crack healing was observed in the non-bacterial series [25].

Sufficient water supply is the determining factor to obtain a good healing efficiency in the bacteria-based healing system, because water is an essential element for bacterial activity. In the above-mentioned self-healing systems, crack healing was achieved in fully immersed conditions. However, full submersion is not feasible in many practical cases. To obtain sufficient water for bacterial activity and autogenous healing (if possible) without human interference, is of crucial importance for a realistic self-healing mechanism. Hydrogels may fulfill this task. Hydrogels are hydrophilic gels which have networks of polymer chains and water is the dispersion medium [26]. They have a high water absorption capacity and can retain a large amount of water or aqueous solution in the network without dissolving. The water absorbed would be released slowly to the surroundings. Therefore, two benefits would be expected from the combined use of hydrogel and microbial $CaCO_3$ for self-healing: (1) Hydrogel can be used as the carrier for the protection of bacterial spores during the process of mixing and hydration; (2) Swollen hydrogel can be used as the water reservoir for spores germination and bacterial activity when cracking occurs, and hence, facilitate the precipitation of $CaCO_3$.

The study consisted of two consecutive steps. Firstly, a 'proof of concept' step, to verify whether bacterial spores were still viable or not after encapsulation. Subsequently, the hydrogel encapsulated spores (bio-hydrogels) were applied into mortar specimens and the self-healing efficiency of the specimens was investigated.

2. Materials and methods

2.1. Bacterial strain

Bacillus sphaericus LMG 22557 (Belgian Coordinated Collection of Microorganisms, Ghent) was used in this study. Living cells were grown in the sterile growth medium (YU medium) consisting of yeast extract (20 g/L) and urea (20 g/L). The pH of the medium was adjusted to 9.0. Original *B. sphaericus* spores were provided by Artechno Company (Liege, Belgium), which were used as the inoculums (1% v/v) in the liquid minimal basal salts (MBS) medium [27] to cultivate batches of *B. sphaericus* spores for experimental use. Mature spores were transferred as the inoculum (1% v/v) into the sterile MBS medium. The culture was incubated (28 °C, 100 rpm) for 14–28 days until more than 90% of the cells were spores. The spores were harvested by centrifuging the culture (7000 rpm, 4 °C) for 7 min. The centrifuged spores were resuspended in sterile saline solution (8.5 g/L NaCl). The suspension of the spores was subjected to a pasteurization process (20 min in a 80 °C water bath and then 5 min in an ice-water mixture to cool down). Afterwards, the spores suspension (about 10^9 spores/mL) was stored in a 4 °C fridge for future use.

2.2. Hydrogel

The hydrogels used were developed by the Polymer Chemistry and Biomaterials Group of Ghent University (PBM-UGent). They were synthesized based on commercial Pluronic® F-127 (Sigma Aldrich) which is a tri-block copolymer of poly (ethylene oxide) and poly (propylene oxide) (i.e. PEO–PPO–PEO). The OH-functionalities at the end of the chain were modified with methacrylate groups to introduce double bonds. The double bonds made it feasible to crosslink the Pluronic® F-127 bis-methacrylate (Pluronic®-BMA) in the presence of the initiator (Irgacure® 2959 upon applying UV irradiation. Irgacure® 2959 will then form free radicals which initiate the polymerization by reacting with the methacrylate moieties introduced [28]. The whole process of crosslinking includes initiation, propagation and termination. After crosslinking, a polymer network was formed enabling water absorption and retention within the crosslinked hydrogel network.

2.3. Encapsulation process

2.3.1. Procedures for synthesis of the pure hydrogel (H)

Firstly, the initiator was added to a 20% w/w polymer solution (2 g Pluronic®-BMA dry powders with 8 g water). The mixture was degassed and mixed (5 min, 500 rpm), and then was injected into a chamber made of two glass plates separated by a 1 mm thick silicone spacer. The glass plates were then clamped and subjected to UV irradiation for 1 h. After UV irradiation, the polymer solution in the chamber formed a gel-like sheet. The hydrogel sheet was removed from the chamber and subjected to freeze grinding (IKA Yellowline Analytical Grinder) and freeze drying (Christ Alpha 2-4 LSC, Germany) to obtain fine powders (in the range of 500 μm). In the present study, for each hydrogel sheet, 10 g polymer solution and 173.8 μL Irgacure® 2959 solution (8 g/L) were used. The pure hydrogel was represented as H.

2.3.2. Encapsulation of spores into the hydrogel (HS)

Bacterial spores suspension (10^9 cells/mL) was first mixed with the polymer solution and then the initiator was added to the mixture. The subsequent steps were similar to the procedure applied for the production of pure hydrogel sheets. 1 mL spores suspension (containing about 10^9 spores) was used for one hydrogel sheet. The total volume of one hydrogel sheet was about 10 mL. Hence, the concentration of the spores in the hydrogel sheet was about 10^8 spores/mL (10^9 spores per sheet). The hydrogel encapsulated with the spores was represented as HS.

2.3.3. Encapsulation of bio-reagents into the hydrogel (HA)

In this study, the term "bio-reagents" refers to the nutrient for bacteria (yeast extract) and the deposition agents (urea and calcium-nitrate). Powders of the bio-reagents were first added to the polymer solution. After they completely dissolved into the polymer solution, the initiator was then added. Next, the whole mixture was subjected to degassing while the subsequent steps were the same as described above for making pure hydrogel sheets. For each hydrogel sheet of 10 mL, 0.4 g yeast extract, 0.9 g urea and 1.2 g $Ca(NO_3)_2{\cdot}4H_2O$ were used. The hydrogel which was loaded with the bio-reagents was represented as HA.

2.3.4. Encapsulation of the spores together with the bio-reagents into the hydrogel (HAS)

Similarly, powders of the bio-reagents were first mixed well with the polymer solution. After that, the suspension of the spores and the initiator were added, and the whole mixture was immediately subjected to degassing to prevent the germination of the spores. The subsequent steps were the same as described above. The hydrogel encapsulated with both spores and the bio-reagents was represented as HAS. Each HAS sheet of 10 mL contained 1 mL spores suspension, 0.4 g yeast extract, 0.9 g urea and 1.2 g $Ca(NO_3)_2{\cdot}4H_2O$.

2.4. Viability of the encapsulated spores

A series of hydrogel sheets was made to investigate the viability of the spores after being immobilized into the hydrogels. The amount of urea decomposed by hydrogel-encapsulated spores was used as an index to evaluate the viability of the immobilized spores. The amount of urea decomposed was calculated based on the total ammonium nitrogen (TAN) measured in YU medium. One mole of urea ($CO(NH_2)_2$) produces 2 mol of NH_4^+. The amount of NH_4^+ can thus indicate the amount of urea decomposed. TAN concentrations were measured colorimetrically by Nessler method [29]. The following experiments were performed in triplicate ($n = 3$).