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Use of silica gel or polyurethane immobilized bacteria for self-healing concrete

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ABSTRACT

Cracks in concrete are the main reason for a decreased service life of concrete structures. It is therefore more advisable and economical to restrict the development of early age small cracks the moment they appear, than to repair them after they have developed to large cracks. A promising way is to pre-add healing agents to the concrete to heal early age cracks when they appear, i.e. the so-called self-healing approach. In addition to the more commonly studied polymeric healing materials, bacterial $CaCO_3$ precipitation also has the potential to be used for self-healing. It is more compatible with the concrete matrix and it is environment friendly. However, bacterial activity decreases a lot in the high pH (>12) environment inside concrete. In this research, the possibility to use silica gel or polyurethane as the carrier for protecting the bacteria was investigated. Experimental results show that silica gel immobilized bacteria exhibited a higher activity than polyurethane immobilized bacteria, and hence, more $CaCO_3$ precipitated in silica gel (25% by mass) than in polyurethane (11% by mass) based on thermogravimetric analysis. However, cracked mortar specimens healed by polyurethane immobilized bacteria had a higher strength regain (60%) and lower water permeability coefficient (10^{-10}–10^{-11} m/s), compared with specimens healed by silica gel immobilized bacteria which showed a strength regain of only 5% and a water permeability coefficient of 10^{-7}–10^{-9} m/s. The results indicated that polyurethane has more potential to be used as a bacterial carrier for self-healing of concrete cracks.

1. Introduction

Concrete is the one of the most popular construction materials. However, it is quite vulnerable to cracking because of its inherent heterogeneity and the non-ideal service environments. Since cracks provide an easy path for water and other aggressive substances like Cl^- and SO_4^{2-} to penetrate inside the concrete matrix, they should be repaired in time to prolong the service life of concrete structures. Generally, the normal repair methods follow the procedure of monitoring, detecting and repairing. The repair work will be performed after the cracks are discovered. Repair agents are applied from the outside and penetrate into the cracks. This technology is quite suitable for repairing large cracks. For small and deep cracks, it will be difficult for healing agents to reach the inner part. Therefore, an alternative repair method by means of a self-healing process is being strived for. Healing agents are incorporated into the concrete matrix during casting. When cracks appear, healing agents will be released from within the concrete and flow into cracks to seal the cracks from the inside to the outside. A self-healing method is especially useful to repair deep-micro cracks and it can restrain early-age cracks to develop to large cracks.

Self-healing properties in concrete may be obtained by different methodologies, such as secondary hydration of unhydrated cement, addition of fibers, and encapsulation of polymers [1–5]. Another alternative self-healing material is bacterially produced calcium carbonate [6–9]. Compared with the healing agents like expanded additives and polymers, the proposed bio-mineral ($CaCO_3$) is more compatible with the concrete matrix and more environmentally friendly. Most bacteria are able to induce carbonate precipitation under suitable conditions [10–13]. In general, there are three mechanisms associated with bio-carbonate precipitation. One is the dissimilatory sulfate reduction carried out by sulfate reducing bacteria under anoxic conditions. The second is the degradation of organic acids. Another pathway is related to the nitrogen cycle, in particular the degradation of urea by ureolytic bacteria [14]. Among the three pathways to precipitate $CaCO_3$, decomposition of urea by ureolytic bacteria is easier to operate and control [15,16].

In our previous research, *Bacillus sphaericus* was found to be able to precipitate calcium carbonate ($CaCO_3$) on its cell constituents and in its micro-environment by decomposition of urea ($CO(NH_2)_2$) into ammonium (NH_4^+) and carbonate (CO_3^{2-}). The latter subsequently promotes the microbial deposition of $CaCO_3$ in a calcium rich

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environment. Through this process, the bacterial cell is coated with a layer of $CaCO_3$. The aim of this study is to use this bio-$CaCO_3$ to heal concrete cracks autonomously. The problem is that bacterial cells cannot be added to cement specimens directly. On one hand, bacterial activity decreases a lot in the high pH (>12) environment as present in concrete. On the other hand, bacterial cells might be destroyed during the process of hydration. Jonkers et al. indicated that bacteria did not survive due to the decreasing of pore diameters during the hydration of the cement materials [17]. Therefore, a suitable carrier is necessary to immobilize bacteria and to protect them from the harsh environment in concrete. In this work, silica gel and polyurethane were used as the carrier for the bacteria.

The term silica sol is derived from silicic acid sol. Silica sols are colloidal dispersions of silicic acid in water. Silica gel is a popular carrier for microorganisms, like bacterial cells, yeast and algae, because it has good properties of mechanical, thermal and photochemical stability, biological inertness (not a food source for bacteria), and suitable matrix porosity for the transmission of molecules and ions [18,19]. In our previous work, silica gel immobilized bacteria were used to manually heal cracks in concrete. The mixture made of silica sol and bacterial suspension (containing bacterial cells and NaCl) was injected into simulated cracks by a syringe. When gel formation (caused by high concentrations of Na^+ and Cl^-) began, the injection was repeated several times until the crack was completely filled. After silica sol became a gel, the specimens were immersed into the medium consisting of urea and Ca^{2+} and then precipitation of $CaCO_3$ occurred. Water permeability of the specimens decreased about 3 orders of magnitude after this treatment [20]. In contrast to the previous work, in the current study the immobilized bacteria together with nutrients and other agents, encapsulated in glass tubes, were incorporated into the specimens during casting. When cracking occurs, the glass tubes will break and healing agents will flow out into the cracks. Silica gel forms in situ when silica sol meets with Ca^{2+} from the concrete matrix and from the healing agent pre-incorporated inside the specimens. At the same time, bacterial cells are immobilized into the silica gel. When bacteria meet with urea and Ca^{2+}, $CaCO_3$ precipitates.

Polyurethane (PU) is widely used as a waterproof material. PU with immobilized bacteria has already been used to repair concrete cracks [21]. In 2001 Bang et al. first used PU foam to immobilize bacteria for manual repairing of concrete cracks. The PU foam, containing bacterial cells, was cut into equal-sized pieces. Afterwards, PU foam strips were placed into simulated cracks of mortar specimens. The specimens were then incubated in a urea-$CaCl_2$ medium at room temperature. As a result of $CaCO_3$ precipitation, the 7d compressive strength of the cracked specimens remediated by PU immobilized bacteria was increased by 12% compared with the ones only remediated with PU. Different from the method described above, in which PU foam with immobilized bacteria was applied externally (pre-formed and placed into the cracks manually), in this work bacteria and PU prepolymer were applied internally (to heal cracks from the inside). PU foam should form in the crack automatically when cracking occurs and bacteria are incorporated inside the foam at the same time. The aim of this work was to investigate the potential use of silica gel or polyurethane immobilized bacteria to bring about self-healing concrete.

2. Materials and methods

2.1. Bacterial strain

The bacterial strain used in the experiments was *B. sphaericus* LMG 22,557 (Belgian coordinated collection of microorganisms, Ghent). This strain has a high urease activity (40 mM urea hydrolyzed. $OD^{-1}\,h^{-1}$), long survival time [22] and can produce $CaCO_3$ in a simple and controllable way [23].

The medium used to grow *B. sphaericus* consisted of yeast extract and urea. The yeast extract medium was first autoclaved for 20 min at 120 °C and the urea solution was added which was sterilized by means of filtration through a sterile 0.22 μm Milipore filter (Millipore, USA). The final concentrations of yeast extract and urea were 20 g/L. Cultures were incubated at 28 °C on a shaker at 100 rpm for 24 h. Bacterial cells were harvested by centrifuging (7000 r/min, 7 min, Eppendorf MiniSpin, Hamburg, Germany) the 24 h-old grown culture and the cells were resuspended in saline solution (NaCl, 8.5 g/L). The concentration of bacterial cells was 10^9 cells/mL.

2.2. Survival test of the bacteria

In this experiment it was tested how long the bacteria can remain viable and sustain high urease activity. Batches of 2 mL bacterial solution (10^9 cells/mL, same as in the Section 2.1) were added into a sterile vial (12.5 mm (diameter) × 46 mm (height), VWR). The vials were then closed tightly and put in the incubator at 28 °C. At certain time intervals, three vials were taken out from the incubator. Bacteria of each vial were inoculated into 100 mL sterile deposition medium (yeast extract 20 g/L, urea 20 g/L and $Ca(NO_3)_2.4H_2O$ 79 g/L). The media were then put on the shaker (28 °C, 100 rpm) for three days. The amount of urea decomposed by bacteria after three days was calculated based on the total ammonium nitrogen (TAN) measured in the deposition medium. Since 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, and hence the amount of $CaCO_3$ precipitation. TAN concentrations were measured calorimetrically by the method of Nessler [24].

2.3. Activity of bacteria after being immobilized into silica gel and polyurethane

2.3.1. Immobilization of bacteria

Immobilization of bacteria into silica gel: Levasil® 200/30% sol, with a specific surface area of 200 m^2/g and a solid content of 30% was used to embed bacterial cells. Two concentrations of saline solutions were used. The saline solution with 8.5 g/L NaCl was used to re-suspend centrifuged bacteria. Another saline solution with 60 g/L NaCl (represented as HS) was used to make silica sol become silica gel. Silica sol, bacterial suspension (BS, 10^9 cells/mL) and HS were mixed together with the volume ratio 5:1:4. About 2 h later, the silica sol became gel and bacterial cells were thus incorporated inside the gel.

Immobilization of bacteria into polyurethane: A two-component polyurethane (MEYCO MP 355 1 K, BASF), represented as PU, was also used to encapsulate bacterial cells. The volume ratio of component A of PU (polyurethane prepolymer, PU A), component B of PU (accelerator, PU B) and bacterial suspension (BS, 10^9 cells/mL) was 5:0.5:1. About 15 min after mixing of the three components, PU foam formed and the bacterial cells were embedded inside the foam.

At the same time, silica sol and PU were also combined with dead bacteria (dead bacteria were obtained by autoclaving living cells at 120 °C for 20 min) and prepared without bacteria as a control.

2.3.2. Bacterial activity after immobilization

The bacterial activity was evaluated by bacterial ureolytic activity (ability to decompose urea) and carbonatogenesis activity (ability to precipitate $CaCO_3$). The bacterial ureolytic activity was expressed as the amount of the urea decomposed by bacteria in the urea solution (20 g/L), which was determined by measuring the conductivity of the urea solution. One mole of urea ($CO(NH_2)_2$) produces 2 mol of NH_4^+ and 1 mol of CO_3^{2-}. Therefore, the more urea is decomposed, the higher the conductivity of the urea solution will be. The relationship between urea decomposed and conductivity is shown in the following equation [14]:

$$\text{Urea decomposed (mM)} = \text{conductivity (ms cm}^{-1}) \times 9.6 \quad (1)$$

The bacterial carbonatogenesis activity was determined by the decomposition of urea in the deposition medium (DM) consisting of 20 g/L urea and 79 g/L $Ca(NO_3)_2.4H_2O$. The amount of $CaCO_3$ precipitated by bacteria can be indicated by the quantity of urea decomposed in the deposition medium. The more urea decomposed, the more $CaCO_3$ formed. Since the deposition medium consisted of urea and $Ca(NO_3)_2$, the increase of CO_3^{2-} because of urea decomposition and the decrease of Ca^{2+} and CO_3^{2-} because of the formation of $CaCO_3$ would make it difficult to relate conductivity with the amount of urea decomposed. Therefore, the decomposed urea was calculated also by measuring the TAN values in the deposition medium.

Living bacteria and dead bacteria, after being immobilized into silica gel and PU, were immersed into 50 mL urea solution and 50 mL deposition medium, separately. Besides, as the first control, the same amount of free bacterial cells (un-immobilized) was also added to the same urea solution and deposition medium. As the second control, silica gel and PU without bacteria were also immersed into the same media. The experiments were done in triplicate. The conductivity of the urea solution was measured every 24 h for 4 days. The TAN value was measured after the different series of silica gel and PU were immersed into the deposition medium for 3 days.

One week later, the original urea solutions, which were used to immerse SG and PU immobilized bacteria, were poured out and 50 mL new urea solutions (also 20 g/L) were added to the same SG and PU immobilized bacteria. The conductivity values of the new solutions were measured to investigate whether the immobilized bacteria still had urease activity after being in silica gel and PU for one week.