Enhanced self-healing capacity in cementitious materials by use of encapsulated carbonate precipitating bacteria: from proof-of-concept to reality

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Abstract

In this study, two bacteria-based self-healing systems were developed for the proof-of-concept and approach to a realistic self-healing. A self-healing system with glass capillaries and silica sol gel carried bacterial cells was first built. The bio-$CaCO_3$ formed in-situ (in silica gel) after glass capillaries breakage preliminarily showed the feasibility of this system. The investigation on the self-healing efficiency demonstrated that the water permeability was decreased by about two orders of magnitude due to self-healing. However, practical application of this system was limited by the use of the un-mixable and expensive glass capillaries. A second self-healing system therefore was built in order to approach a realistic self-healing, by using hydrogel encapsulated bacteria. Great superiority in healing efficiency was obtained in this system. A maximum crack width of 0.5 mm could be healed within 7 days in the specimens of the bacterial series; while the maximum crack width can be healed in other series was in the range of 0.2~0.3 mm. Water permeability was greatly decreased (68%) in the bacterial series.

Originality

Two items in the development of a microbial based strategy for self-healing concrete are of utmost importance. One is the microbial source; the other is a suitable bacterial carrier. In previous research, it was found that Bacillus sphaericus was the most suitable strain due to its spore-forming property and alkali-resistant nature. Therefore, this strain was also used in this study. Due to the harsh environment in concrete, encapsulation of bacteria is preferable before incorporation into the matrix. The bacteria carrier should, on the one hand protect bacteria during the mixing and hardening stage; on the other hand not inhibit bacterial carbonate productivity upon cracking.

Silica gel used in this research can be formed in situ in the cracks and embed bacteria inside to protect them from the high pH in the surroundings. Moreover, it is also a kind of filling material to block the cracks. However, silica gel has low water retention capacity. Therefore, it easily becomes dry in normal humidity conditions, which is not good for long term bacterial activity. Besides, dry silica gel is really fragile and prone to cracking, resulting in further permeability problems in concrete. Compared with silica gel, hydrogel has the distinct advantages of high water absorption and retention capacity, which greatly benefits for long term bacterial activity, and hence an enhanced healing efficiency. Furthermore, some hydrogels have high moisture uptake capacity, which is really promising to achieve a realistic self-healing for practical application.

Keywords: *self-healing; crack; $CaCO_3$; bacteria; carrier*

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1. Introduction

In the last decade, self-healing concrete has gained more and more attention from researchers and civil engineers because of its great potential to reduce the high maintenance and repair cost of concrete infrastructure. Due to the limited autogenous healing capacity of concrete itself, additives are needed to enhance the self-healing properties. These healing agents are pre-added into the concrete during the casting and are expected to play their role (heal cracks) when cracking occurs. Various strategies are being investigated for this aim, such as incorporation of encapsulated polymer solutions, addition of superabsorbent polymers, etc. A promising strategy is the microbial-based self-healing strategy, which has the distinct features of environmental friendliness, long-term viability and possibly low cost.

The microbial-based strategy is mainly based on the microbial induced carbonate precipitation process. Bacteria can produce or induce the formation of calcium carbonate under suitable conditions. This biogenic precipitation is natural and compatible with the building materials. In this research, *Bacillus sphaericus* was used. This strain 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 environment. The aim of this study is to use this bio-$CaCO_3$ to heal concrete cracks autonomously. Due to the high pH in concrete and the small pore sizes, bacteria cannot be added directly. Hence, an immobilization process in or on a protective carrier was needed.

For proof of concept, a self-healing system with glass capillaries and silica sol gel carried bacterial cells was built. Silica gel is an often used carrier for microorganisms, like bacterial cells, yeast and algae, because of the good properties of mechanical, thermal and photochemical stability, biological inertness, and suitable matrix porosity for the transmission of molecules and ions (Soltmann et al. 2003; 2008). In previous research, silica sol gel immobilized bacteria were investigated for manual crack healing. The silica sol and bacterial suspension were injected into the simulated cracks (Van Tittelboom et al. 2010). Differently, in this study, to mimic a real self-healing, the healing agents were first encapsulated in the glass capillaries, which were then incorporated into the specimens during casting. The glass capillaries will break upon cracking and healing agents come out into the cracks. Silica sol forms gel in situ and bacterial cells are embedded in the gel matrix. The embedded bacteria induce the precipitation of $CaCO_3$ with the existence of urea and Ca source, hence heal the cracks. More details on this system can be found in Wang et al. (2012).

Due to the limitations in the first system (un-mixable and expensive glass capillaries, extra human interference, etc.), it is not possible for practical application. For an improvement, hydrogels were investigated as the bacterial carrier. Hydrogel has high water absorption and retention capacity, which would facilitate bacterial activity and greatly reduce the water supply frequency. One special hydrogel, superabsorbent polymer (SAP) is being investigated to self-close concrete cracks (Lee et al.2010; Snoeck et al. 2012a, 2012b). It has been proven that SAPs can block the cracks and facilitate the autogenous healing because the water released from SAPs can be used for continuation of hydration and carbonates precipitation or other healing processes. Therefore, it could be expected that the use of hydrogel immobilized bio-agents brings the research closer to a realistic self-healing. More details on this system can be found in Wang (2013). The aim of the current paper is to compare the self-healing efficiency of the two above-mentioned methods for bacterial self-healing.

2. Experimental

2.1. Materials

2.1.1 Bacterial strain

The bacterial strain used in the experiments was *Bacillus sphaericus* LMG 22557 (Belgian coordinated collection of microorganisms, Ghent). This strain has a high urease activity (40 mM urea hydrolyzed.$OD^{-1}.h^{-1}$), long survival time (Wang et al. 2010) and can produce $CaCO_3$ in a simple and controllable way (Dick et al. 2006).

2.1.2 Bacterial carriers

Levasil®200/30% sol, with a specific surface area of 200 m^2/g and a solid content of 30% was used together with glass capillaries (length = 40mm, inner diameter = 3mm) to encapsulate bacteria.

A modified hydrogel, which was synthesized based on the commercial Pluronic®F-127 (Sigma Aldrich), was also used to encapsulate bacteria.

2.2. Methods

2.2.1 Cultivation of the bacteria

2.2.1.1 Cultivation of vegetative cells

B. sphaericus was cultivated in growth medium that consisted of yeast extract and urea. The yeast extract medium was first autoclaved and the filter sterilized urea solution was added. The final concentrations of yeast extract and urea were 20g/L. The pH of the medium was 7. The cultures were incubated at 28˚C on the shaker at 100rpm for 24h. The living cells were harvested by centrifugation (7000 r/min, 7min, Eppendorf MiniSpin, Hamburg, Germany) and were resuspended in sterile saline solution (NaCl, 8.5g/L). The concentration of the cells in the suspension was about 10^9 cells/mL.

2.1.1.2 Cultivation of bacterial spores

The sporulation medium used for cultivation of *B. sphaericus* spores was MBS liquid medium (Kalfon et al. 1983): $MgSO_4.7H_2O$ (0.3g/L), $MnSO_4$ (0.02g/L), $Fe_2(SO_4)_3$ (0.02g/L), $ZnSO_4.7H_2O$ (0.02g/L), $CaCl_2$ (0.2g/L), Tryptose (10g/L), Yeast extract (2g/L). The pH was 7.4 (if not, it was adjusted by using 1M HCl or NaOH). Mature spores were transferred as inocula (1%) into MBS medium. The cultures were incubated at 28 ˚C on a shaker at 100rpm for 14~28 days till more than 90% of the cells were spores. The spores were then harvested by centrifuging the culture for 7min. The centrifuged spores were resuspended in the sterile saline solution. Subsequently, the suspension of the spores was subjected to pasteurization to minimize the amount of vegetative cells in the culture. The concentration of the spores in the suspension was about 10^9 spores/mL.

2.2.2 Self-healing system 1: glass capillary + silica sol + B. sphaericus cells

2.2.2.1 Encapsulation

Glass capillaries with a length of 40 mm and an inner diameter of 3 mm were used to carry the healing agents. First, two glass tubes were glued together and one of their ends was sealed by an adhesive (Schnellklebstoff X 60, HBM). Then the healing agents were injected into each tube from the other end, which was sealed afterwards. One capillary was filled with the mixture of bacterial suspension (BS) and silica sol (SS). The volume ratio between BS and SS was 2:3. The other capillary was injected with the deposition medium (DM) which consisted of equimolar (0.33M) urea and Ca-nitrate. Pure silica sol and dead bacterial cells (obtained by autoclaving at 120 ˚C for 20 min) were used as controls. The three sets of the glass capillaries containing different healing agents are shown in Fig.1.

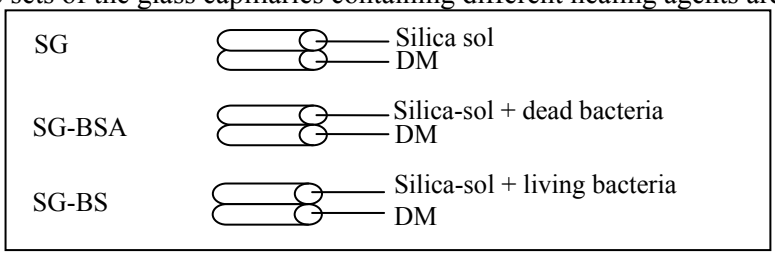


Fig.1 Scheme of the glass capillaries with different healing agents

2.2.2.2 Mortar specimens with healing agents

Cylinders (Φ = 78 mm, H = 22 mm) were made with a water to cement ratio of 0.5 and sand to cement ratio of 3 by using ordinary Portland cement CEM I 52.5N and standard sand (DIN EN 196-1). Firstly, a mortar layer (about 10mm) was brought into the moulds. After vibration, two steel fibers (Φ = 1 mm, L = 50 mm) and three sets of glass capillaries were put on top of the layer. Subsequently, the moulds were completely filled with mortar and vibrated. After casting, the moulds were put in an air-conditioned room (20°C, > 95%RH) for 24h. The specimens were then demoulded and placed in the same room. After two weeks, the cylinders were taken out and subjected to crack creation. The detailed description of the methodology can be found in previous research (Wang et al. 2010). During cracking, the healing agents gradually flew out and silica sol became silica gel (after about 3 hours). The cracked cylinders were then immersed into water for 7 days.

Additionally, extra sets of glass capillaries, which were the same as the ones used in the cylinders, were also stored under identical conditions. After two weeks, they were broken manually and the

healing agents flowed into a beaker. In the beaker, silica gel formed with or without bacteria, urea and Ca^{2+} incorporated inside. The beakers were put into the same room. One week later, samples from different beakers were taken out and subjected to different characterization methods.

2.2.3 Self-healing system 2: hydrogel + B.spaericus spores

2.2.3.1 Encapsulation

Bacterial spores suspension (10^9 cells/mL) and the bio-reagents (yeast extract, urea and Ca-nitrate) were first mixed with a 20% w/w polymer solution (modified Pluronic®F-127) for 2 min and subsequently, the initiator (Irgacure 2959) was also added to the solution. The mixture was degassed and mixed for another 5min, which was then subjected to UV radiation for 1h. The formed hydrogel sheet was subjected to freeze grinding (IKA Yellowline A10 Analytical Grinder) and freeze drying (Christ Alpha 2-4 LSC, Germany) to obtain the dry powders for experimental use.

Three types of hydrogels, pure hydrogel (H), hydrogel encapsulated with bio-reagents (HR) and hydrogel encapsulated with bacterial spores and bio-reagents (HSR), were made for the incorporation into the mortar specimens.

2.2.3.2 Mortar specimens with healing agents

The cement and sand used were the same as in the section 2.2.2.2. The water to cement ratio was 0.5 and sand to cement ratio was 3. The addition dosages of the hydrogels were 2% (H) and 5% (HR and HSR) of cement by weight. Four series of mortar specimens were made and added with the hydrogels made in section 2.2.3.1, represented by R (without hydrogels), m-H, m-HR, m-HSR, respectively.

Two types of specimens were made: prisms (30 mm x 30 mm x 360 mm) with a reinforcement (Φ = 6 mm, L = 660 mm) in the center and cylinders (Φ = 78 mm, H = 22 mm). After 28 days in the same air-conditioned room, multiple cracks and single crack were created in the prisms and cylinders, respectively. The cracked specimens were subjected to wet-dry cycles (1h in water, 11h in 60%RH).

Additionally, the same hydrogels as the ones used in the specimens were immersed in water for 7 days to investigate whether there will be precipitation formed in/on the hydrogel matrix or not.

2.2.4 Characterization of precipitation

2.2.4.1 Thermogravimetric analysis (TGA)

TGA was used to verify the formation of $CaCO_3$ in/on the bacterial carriers. Samples of silica gel were ground into powders and samples of bio-blended hydrogel were cut into small pieces to fit for the pan of the TGA instrument (SDT 2960 Simultaneous DSC-TGA). The temperature increased from room temperature to 1000 ˚C at a speed of 10˚C/min in argon atmosphere. The weight loss of the samples during the process of heating was recorded and shown in a weight-temperature graph.

2.2.4.2 SEM

The morphology of the precipitation was studied in a FEI QUANTA 200F SEM at accelerating voltage of 20 kV. Secondary electron imaging (SEI) was used for electron micrography. Samples were completely dried in the oven at 50 ˚C for 2 days and were gold coated before examination.

2.2.5 Evaluation of self-healing efficiency

2.2.5.1 Visualization of crack filling

Crack filling in the specimens was visualized by light microscope (Leica S8 APO, Switzerland).

2.2.5.2 Water permeability

The cracked cylinders were subjected to a water permeability test after the incubation periods. The detailed description of the test procedure and calculation of the water permeability coefficient (k) can be seen in the previous research (Wang et al. 2012).

3. Results and Discussion

3.1. Characterization of the precipitation

3.1.1 TGA

$CaCO_3$ decomposes into CaO and CO_2 in the range of 650-800˚C, which will cause a weight loss due to the release of CO_2. No weight loss in this temperature range was observed on the TGA graphs of the samples from SG or SG+BSA (graphs not shown), which indicated that no $CaCO_3$ formed after the glass capillaries were broken. While for the samples taken from SG+BS, obvious weight decrease was seen in the temperature range of 650˚C to 750˚C (shown in Fig. 2 (a), marked by the circle). Therefore,

it can be concluded that no $CaCO_3$ will precipitate if there are no living bacteria in the glass capillaries. Dead bacteria have lost the ureolytic activity, and hence, cannot precipitate $CaCO_3$.

In the systems with hydrogels, no $CaCO_3$ formed in the pure hydrogels or the ones only loaded with bio-reagents (TGA graphs not shown). Only the ones encapsulated with both bacteria and bio-reagents had weight loss in the range of 650˚C to 750˚C (shown in Fig. 2 (b)), indicating the existence of $CaCO_3$. Therefore, it can be demonstrated that hydrogel encapsulated bacterial spores still had ureolytic activity; they can decompose urea and precipitate $CaCO_3$ in or on the hydrogel matrix.

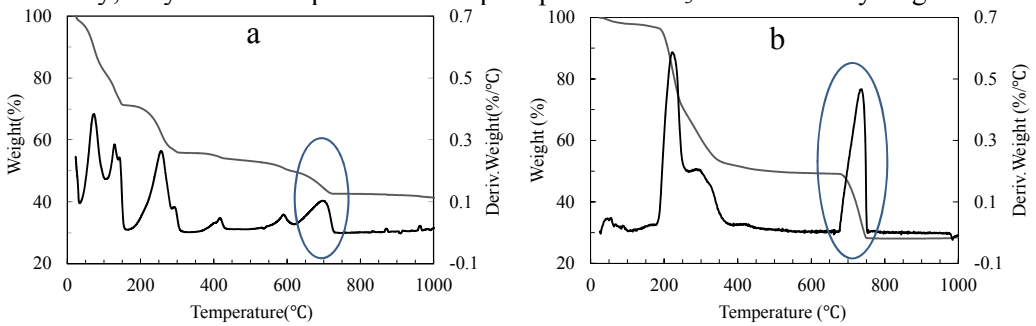


Fig.2 TGA graphs of thel samples (a: the reaction products after the glass capillaries (containing SG+BS) were broken; b: HSR pieces after immersion in water for 7d; the circles mark the decomposition of $CaCO_3$)

3.1.2 SEM

$CaCO_3$ precipitation from silica gel immobilized bacteria had a cubic shape. The particle size was about 100 μm (Fig.2 (a)). It is difficult to draw a conclusion whether the particles were on the surface or inside the matrix since the samples were ground into powders. However, from a direct observation of the whole piece before grinding (picture not shown), quite some precipitation distributed on the surface or in the surface layers. Much more precipitation was found with the hydrogels immobilized bacteria. The hydrogel piece was almost completely covered by the precipitation layer (Fig.2(b)). The particles also showed a similar cubic shape and gather together.

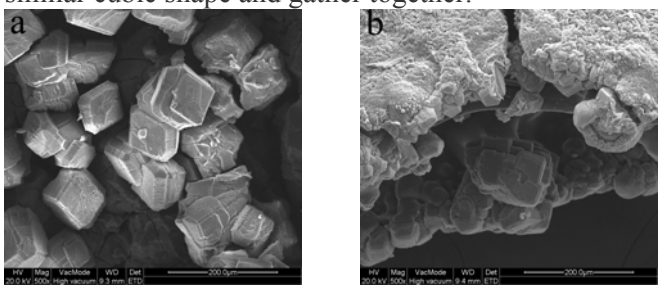


Fig.3 Scanning electron micrographs of $CaCO_3$ precipitation in/on silica gel (a) and hydrogel matrix (b)

3.2. Self-healing efficiency

3.2.1 Visualization of crack healing

3.2.1.1 Self-healing system 1: glass capillary + silica sol + B. sphaericus cells

No crack healing was observed in the R specimens after 7 days immersion in water.

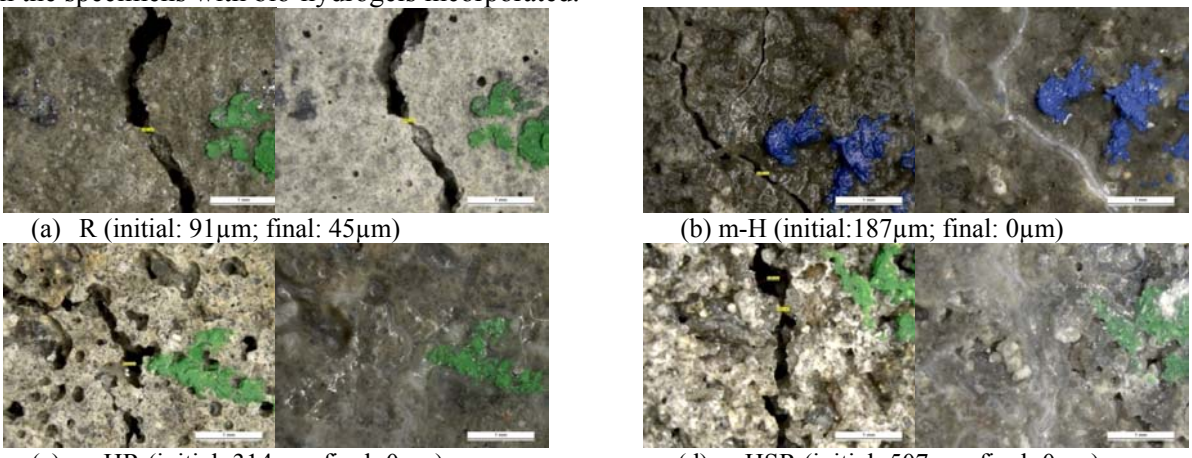
For the specimens in the series of SG and SG+BS, it was notice that some fluid came out during crack creation. After 3 to 4 hours, white gel was observed in the cracks of the specimens. Cracks were partly or completely filled. The amount of the filled part varied in different specimens. No difference was observed between the series of SG and SG+BS. However, after immersion in water, more white particles appeared on the gel surface which was in the cracks of the specimens in SG+BS series than that in SG series, which could be due to the formation of bio-$CaCO_3$.

3.2.1.2 Self-healing system 2: hydrogel + B.spaericus spores

Very limited amount of crack healing was observed in the prisms of the R series after the wet-dry cycles. The maximum healed crack part was only about 45μm (Fig.4(a)). While the specimens with hydrogels, pure or blended ones, both showed crack healing. The maximum crack widths healed in the specimens m-H, m-HR, m-HSR were about 0.18mm, 0.31mm and 0.5mm, respectively. Furthermore, it was found that the prisms with bio-hydrogels added, exhibited the superiority not only in the healed crack part but also the healing speed. The crack width of 0.5mm can be completely healed within 7d in

m-HSR, while other specimens (m-H and m-HR) needed about 21d to entirely heal their specific maximum crack width.

The crack healing in the specimens m-H and m-HR was due to the autogenous healing, which was enhanced by the long term contact with the water released from the hydrogels. The water absorbed by the hydrogel facilitated the autogenous healing and the bacterial activity, resulting in improved healing in the specimens with bio-hydrogels incorporated.



(a) R (initial: 91μm; final: 45μm) (b) m-H (initial:187μm; final: 0μm)

(c) m-HR (initial: 314μm; final: 0μm) (d) m-HSR (initial: 507μm; final: 0μm)

Fig.4 Maximum healed crack width in the prisms with and without hydrogels (or blended hydrogels)

3.2.2 Water permeability

As shown in Fig.5, the k values of the R specimens ranged from $4*10^{-6}$ to $7*10^{-6}$ m/s. The specimens with only silica gel were similar to the R ones and the final k value was about 10^{-6} m/s which indicated that silica gel in the crack had limited contribution to decrease the water permeability. The water permeability of the specimens with silica gel immobilized living bacteria (SG+BS) decreased about two orders of magnitude compared with the reference ones. The final k values of this series varied from 10^{-9} to 10^{-7} m/s. Adding living bacteria to silica gel decreased the water permeability because of the filling effect of $CaCO_3$ produced by bacteria.

The addition of hydrogels particles had a great influence on the properties of the mortar specimens, the strength, the microstructure, etc (data not shown). Therefore, the water transport properties also varied in different series of the specimens. Thus, the k-values of the specimens were not comparable with each other. Instead, the difference between initial and final k-values, that is, the decrease in k-values of each specific specimen after healing was meaningful. Regarding this, the water permeability of the specimens before and after the incubation was tested and the related k-values are shown in Fig.6. After the incubation (wet-dry cycles), the water permeability in all specimens was decreased. More decrease was found in the specimens with hydrogels, especially the ones with blended hydrogels. The largest reduction was in the specimens with bio-hydrogels (m-HSR), marked by the circle in Fig.6.

It can be concluded from the water permeability results of both self-healing systems that, bacterial $CaCO_3$ had a positive effect on decreasing the water permeability.

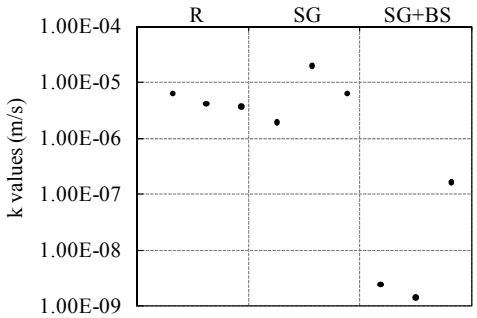


Fig.5 Water permeability of the cracked cylinders (with self-healing system 1 incorporated) after incubation

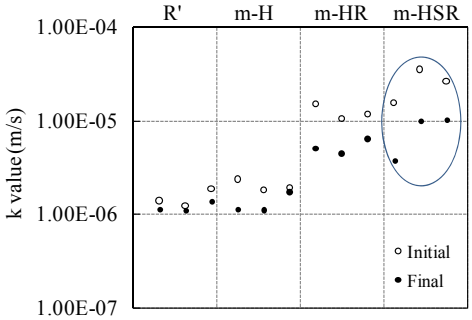


Fig.6 Water permeability of the cracked cylinders (with self-healing system 2 incorporated) before and after wet-dry cycles

3.3 Discussion of the two self-healing systems

Glass capillaries are a good option to carry a large volume of healing agents for a sufficient crack healing. They are easily broken upon cracking and release healing agents to assure an in-time healing. However, this advantage is also a disadvantage. Because they are too fragile, it is impossible to mix and cast them together with the mortar. Moreover, the expensive price of the glass capillaries will also hinder their use in practical situations. Besides, it was noticed that silica gel easily cracks if not under high humidity environment, which would cause further permeability problems in the specimens. Therefore, the cracked specimens always need to contact with water during the incubation and test periods. However, this is not realistic for the practical application.

Regarding the above mentioned problems, there are two distinct advantages of using hydrogel as the bacterial carrier for self-healing: 1) Hydrogel is mixable, it was used as the carrier for the protection of bacterial spores during the process of mixing and hydration; 2) Swollen hydrogel was used as the water reservoir for bacterial activity when cracking occurs, the formed bio-precipitation can enhance the crack healing; 3) Some hydrogels have a good moisture uptake capacity, which means that the bacteria-based self-healing system will also work under normal humidity conditions; the extra human interference (water supply) will be greatly reduced. This aspect is currently being further investigated in our research group.

4. Conclusions

Two bacterial-based self-healing systems were developed in this study based on different carriers used. Enhanced self-healing efficiency was obtained in both systems. For the aspect of the water permeability, the k-values were decreased about two orders of magnitude in bacterial series compared with reference ones in the first system. In the second system, the k-values in the specimens of the bacterial series had the largest reduction (68%) after healing. In view of the practical feasibility, the second self-healing system is more promising.

Acknowledgements

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