MICROSCOPY AND COMPUTED MICRO-TOMOGRAPHY FOR EVALUATION OF MICROBIAL SELF-HEALING IN CONCRETE

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Abstract

Autonomous self-healing of materials, implies that the material is adapted in such a way that damage is repaired automatically. To obtain self-healing in concrete, several smart mechanisms can be introduced, such as activators or fibres to stimulate autogenous healing, encapsulated (pre)polymers or bacteria. The technology of microbial self-healing makes use of bacteria that promote the precipitation of calcium carbonate when they make contact with the appropriate nutrients and water. In order to survive the high alkalinity in the concrete matrix and the reducing pore sizes due to the ongoing cement hydration, bacterial cells or spores are applied, protected by a carrier material or encapsulation system. Crack formation in the concrete acts hereby as a trigger to break the capsules and activate the bacteria. The precipitated bacterial calcium carbonate will fill the crack and restore the water tightness of the concrete element.

The most obvious technique to evaluate the self-healing efficiency is a visual examination of the crack filling. Optical microscopy allows to evaluate crack filling at the concrete surface, whereas computed micro-tomography also allows to visualize the complete crack internally in a non-destructive way. The current study gives an overview of crack healing ratios and maximum healed crack widths for various microbial based self-healing strategies. Incorporation of *Bacillus sphaericus* cells, protected by diatomaceous earth, allowed healing of 0.17 mm wide cracks in mortar specimens in 40 days time. *B. sphaericus* spores, protected by melamine based microcapsules, were able to heal cracks of almost 1 mm in three weeks time. Encapsulation of spores in hydrogels resulted in healing of 0.5 mm cracks within one week. Computed micro-tomography enabled quantification of the amount of deposited calcium carbonate. Also crack filling by polymeric carrier materials for bacteria, such as polyurethane, could be evaluated with this technique.

KEYWORDS: *microbial* $CaCO_3$, crack, quantification, healing ratio

1 INTRODUCTION

Microbial-based self-healing concrete is regarded as a promising material to enhance the durability and sustainability of concrete structures. The principle is that mineral precipitating bacteria, more specifically carbonate precipitating bacteria, together with nutrients, are added into the concrete mix. When cracks appear, bacteria in the crack zone are expected to be activated and precipitate $CaCO_3$ to in-situ heal the cracks. Due to the harsh environment inside concrete, encapsulation of bacteria is preferable. In this research, three carriers were selected, diatomaceous earth, microcapsules and hydrogel, to immobilize bacteria and investigate the self-healing efficiency in cementitious specimens with incorporated biological healing agents.

Diatomaceous earth (DE) is a natural soft siliceous sedimentation. It is highly porous, chemically stable, and inert. DE has been mainly used as filtration agent and functional filler for paints and plastics, and as filling material for concrete. It is also a widely used bacterial carrier. The microcapsules used in this study are impermeable, resistant to the high pH of concrete, and humidity sensitive. They are flexible in high humidity (like in water) and become fragile in dry state. This implies that the capsules can withstand the forces during concrete mixing and can be easily broken upon cracking. Hydrogels are hydrophilic gels which have high water absorption capacity and can retain a large amount of water or aqueous solution in their network without dissolving. The water absorbed will be released slowly, which will be of benefit for bacterial activities and stimulate $CaCO_3$ precipitation.

During self-healing, $CaCO_3$ precipitation can be induced by a bacterial and/or autogenic process. It is difficult to differentiate the biogenic-$CaCO_3$ from abiotic-$CaCO_3$. In order to examine the healing effect from bio-precipitation, the crack filling was investigated qualitatively and quantitatively by use of light microscopy analysis on the specimens with and without bacteria added. Furthermore, to obtain a view of the healing products deep inside the specimens, a high resolution X-ray computed microtomography (X-ray μCT) was used. X-ray μCT is a non-destructive technique, which generates three-dimensional (3D) images by combining a series of cross-sectional images. It provides information (visualization and quantification) about the internal structure of the matrix. X-ray μCT has become a frequently used technique in material research [1-3].

2 MATERIALS AND METHODS

2.1 Bacterial strain and cultivation

Bacillus sphaericus LMG 22557 (Belgian coordinated collections of microorganisms, Ghent) was used in this study. This strain has a high urease activity (40 mM urea hydrolyzed.$OD^{-1}.h^{-1}$), long survival time and can produce $CaCO_3$ in a controllable way [4].

B. sphaericus was cultivated in a sterile growth medium that consisted of yeast extract (20g/L) and urea (20 g/L). The cultures were incubated at 28℃ on a 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.

B. sphaericus spores were cultivated in the liquid minimal basal salts (MBS) medium [5]. Mature spores were transferred as inocula (1%) into MBS medium. The cultures were incubated (28 ℃, 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 Mortar specimens with diatomaceous earth (DE) immobilized bacteria

2.2.1 Immobilization of bacteria into/onto DE

DE particles had sizes ranging from 4μm to 20μm with large amount of tiny pores (0.1μm to 0.5μm) on the surface. The bacterial suspension (BS, 10^9 cells/mL)) was mixed with DE powders (20%, w/v); the mixture was then put on a shaker (100 rpm, 28°C) for 1h to make bacterial cells attach to the DE surface.

2.2.2 Preparation of mortar specimens

Two series of mortar specimens (40mm x 40mm x 160mm) were made with a water to cement ratio of 0.5 and a sand to cement ratio of 3 (Table 1).

Table 1 Components of mortar specimens in each series

Series	*Cement (g)*	*Sand (g)*	*Water (g)*	*DE(g)*	*BS (mL)*	*Nutrients (g)*
DE	450	1350	225	22.5	0	35
DE+BS	450	1350	112.5	22.5	112.5	35

Nutrients included 1.25g yeast extract, 11.25g urea and 22.5g $Ca(NO_3)_2.4H_2O$

Reinforcement was added to the mortar specimens to control the crack width. A 10 mm mortar layer was first added into the moulds. After this layer was compacted by vibration, two reinforcements (D = 2mm, L = 140 mm) were placed on top of it. Afterwards, the moulds were completely filled with mortar and vibrated. All moulds were put in a climate room (20°C, > 95%RH) for 24h. After demoulding, the mortar specimens were placed in the same climate room.

2.2.3 Creation of cracks and incubation of cracked specimens

After 14 days, specimens were taken out of the moist room and cracks were created by a crack width controlled three-point bending test. Crack width was measured by a linear variable differential transformer (LVDT) which was attached at the bottom of the specimens. The final crack width created ranged from 0.15-0.17mm. Afterwards, three cracked specimens were immersed in water and three were immersed in a deposition medium (DM, made of urea and $Ca(NO_3)_2$, 0.2M) for 40 days.

2.3 Mortar specimens with microencapsulated bacteria

2.3.1 Encapsulation of bacterial spores into microcapsules

The bacterial spores were encapsulated in melamine based microcapsules following a patented poly-condensation reaction based microencapsulation process (Patent WO 2010/142401) [6]. The size of the microcapsules was about 5μm. After encapsulation, an emulsion consisting of microencapsulated spores and water was obtained. The concentration of the spores in the microcapsules was around 10^9 cells/g microcapsules (dry weight).

2.3.2 Preparation of mortar specimens

Two series of specimens (30mm x 30mm x 360mm, including a rebar r_{ϕ}=6mm) were made and the composition is shown in Table 2. Group NC and NCS are the specimens with nutrients and the microcapsules which were (NCS) or were not (NC) loaded with spores. The specimens were stored in the same moist room.

Table 2 Composition of the specimens in each series

	Cement (g)	*Sand (g)*	*Water (g)*	*Nutrients (g)*	*Microcapsule emulsion (g)*	*Dry weight of Microcapsules (g)*	*Bacterial spores*
NC	450	1350	201.4	57.84	26.1	13.5	N
NCS	450	1350	192.8	57.84	34.7	13.5	Y

Nutrients included yeast extract, urea and $Ca(NO_3)_2.4H_2O$. The addition ratio was 0.85%, 4% and 8% of cement by weight. $Ca(NO_3)_2.4H_2O$ contains 30.5wt% water. Therefore the amount of mixing water was reduced by the amount of the water in $Ca(NO_3)_2.4H_2O$ and the amount of water in the microcapsule emulsion. The last column shows whether bacteria were present (Y) or not (N).

2.3.3 Creation of cracks and incubation of cracked specimens

28 days after casting, the specimens were subjected to a tensile test to create multiple cracks (Amsler 100, SZDU 230, Switzerland). A uniaxial tensile load was applied to the specimen at a speed of 0.01mm/s under stroke control. The loading was stopped at the point where the average crack width in the specimen reached 150 μm. The cracked specimens were subjected to five incubation conditions (for 8 weeks): 1) 20℃, > 95%RH; 2) immersion in water; 3) immersion in the deposition medium (DM); 4) wet-dry cycles with water; 5) wet-dry cycles with DM. During one wet-dry cycle, the specimens were immersed in water/DM for 16 h and were then exposed to air for 8h. The incubation of 2), 3), 4) and 5) was performed in an air-conditioned room (20℃, 60%RH).

2.4 Mortar specimens with hydrogel encapsulated bacteria

2.4.1 Encapsulation of bacterial spores in hydrogel

Bacterial spores suspension (10^9 cells/mL) was first mixed with a 20% w/w polymer solution (modified Pluronic®F-127). Then the initiator (Irgacure 2959) was also added to the solution. The mixture was degassed, mixed for 5min, and then subjected to UV radiation for 1h. A hydrogel sheet was formed, which was then subjected to freeze grinding and freeze drying to obtain the dry powders.

2.4.2 Preparation of mortar specimens

Three series of mortar specimens were made (R, H and HS). The reference specimens (R) only contained nutrients (the dosage can be seen in Table 2). The specimens with nutrients and pure hydrogels or spores loaded hydrogels were represented as H or HS, respectively. The addition of hydrogels was 2% of cement by mass. The size of the specimens were the same as those in 2.3.2. After casting, the specimens were stored in the same moist room.

2.4.3 Creation of cracks and incubation of cracked specimens

28 days after casting, the prisms were subjected to multiple cracking (similar as in 2.3.3). The cracked specimens were subjected to two incubation conditions (for 4 weeks): 1) 20℃, > 95%RH; 2) wet-dry cycles with water, 1h in water and 11h exposed to air (20℃ , 60%RH).

2.5 Visualization and quantification of crack filling by light microscopy

The cracked specimens were investigated under the light microscope (Leica S8 APO, Switzerland) once a week during the period of incubation to monitor the crack healing. For multiple cracking, the crack healing efficiency was evaluated by the absolute amount of healed crack area (for the ones with microcapsules) or the healing ratio which is the ratio between the healed crack width to the initial crack width (for specimens with hydrogels). Both crack area and crack widths were analyzed by the Leica software (LAS).

2.6 Quantification of crack healing by 3D X-ray μ-CT

The X-ray tomography images of cracked small cylinders (Φ=8mm, h=10mm) were scanned before and after the incubation under wet-dry cycles. The scanning was done by the Center for X-ray Tomography of Ghent University (UGCT). The images were analysed by use of the reconstruction software package Octopus and the 3D analysis software package Morpho+ and VGStudio. In order to obtain a 3D distribution of the healing products, digital image subtraction was performed on the scans taken before and after the healing, resulting in a differential volume.

In addition, X-ray μ-CT was also applied to visualize the crack healing in specimens which contained glass capillary encapsulated polyurethane, which was used as the bacterial carrier in previous research [7].

3 RESULTS AND DISCUSSION

3.1 Crack healing in the specimens with DE immobilized bacteria

The specimens with pure DE incorporated had very limited crack healing. Almost no precipitation was formed in the cracks for specimens immersed in water (Fig.1(a)), while the specimens immersed in DM showed a small amount of precipitation in the cracks and on the surface (Fig.1(b)). No cracks were completely healed. The specimens with DE immobilized bacteria incorporated showed improved crack healing. In this case, cracks in specimens immersed in water were partly healed (Fig.1(c)); and cracks in specimens immersed in DM were completely healed by the precipitation (Fig.1(d)). The improved healing in DM is due to the additional supply of urea and Ca^{2+} from the DM which allows the bacteria to produce more calcium carbonate. More information can be found in [8].

Theoretically, the healing agents are distributed all over the matrix. Therefore, the healing should be similar for different locations along the crack. However, it was noticed that only for some positions the cracks in the specimens of the bacterial series (immersed in water) had closed. Hence, to obtain more representative and comprehensive values for crack healing efficiency, in the following self-healing systems, multiple cracks were created to evaluate the self-healing efficiency.

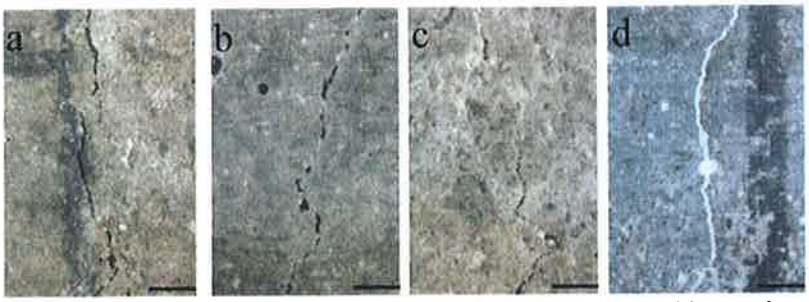


Figure 1. Light microscopy images of the cracks in different specimens (a and b: specimen with only DE, immersed in water and in DM, respectively; c and d: specimens with DE immobilized bacteria, immersed in water and in DM, respectively. The black bar indicates 1mm. The images were based on [8])

3.2 Crack healing in the specimens with microencapsulated spores

The initial crack area was different in all specimens (about 70-140 mm^2) and in none of the specimens all cracks were completely closed after healing (Fig.2). Therefore, the absolute amount of healed crack area in the specimens was calculated (details of crack area processing can be seen in [9]) and was used to evaluate the healing efficiency, which is also indicated in Fig.2. Crack healing was observed in all specimens except the ones stored at 95%RH, which indicated that the presence of water is an essential constituent for self-healing. When stored at other conditions, the specimen NC and NCS had a healed crack area ranging from $20mm^2$ to $40mm^2$ and $60mm^2$ to $80mm^2$, respectively. The maximum crack width healed in the specimen NCS was 970μm (Fig.3). The increased healing efficiency (at least 50%) was due to bacterial precipitation. It was found that the specimens subjected to wet-dry cycles with water as the immersion solution had the highest healing efficiency. During the wet stage, the specimens absorbed enough water which can keep the matrix in wet state during the dry stage. When the specimens were exposed to the atmosphere, more oxygen/CO_2 becomes available for the bacteria or for carbonation than in the case of continuous immersion. For the specimens with bacteria, a reduced immersion time could also decrease the escape of the bacteria from the crack surface and the leakage of the nutrients into the incubation solution. An optimal wet-dry cycle can promote the diffusion of nutrients from the internal matrix to the surficial cracking zone without leaching too much to the bulk solution and can provide sufficient available water for bacterial activities during the dry state.

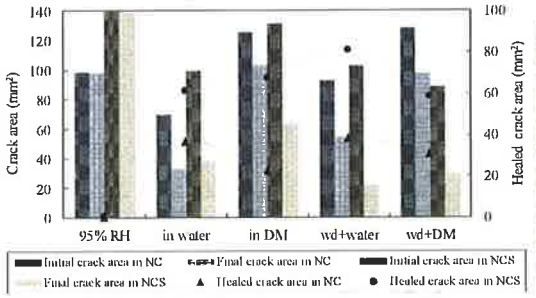


Figure 2. Crack healing in specimens NC and NCS in different conditions

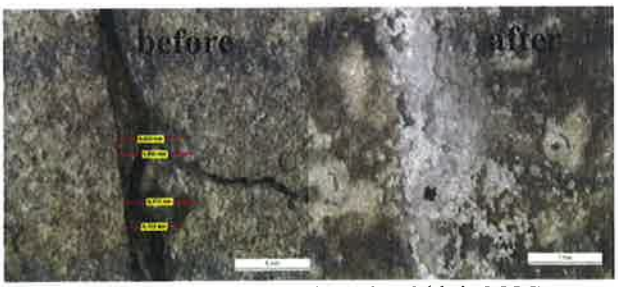


Figure 3. Maximum healed crack width in NCS (max. 850μm~970μm; white bar indicates 1mm)

Multiple crack healing efficiency, using healed crack area as the evaluation index, can give an overall estimation of the healing in the whole specimens. The disadvantage is that such an absolute value is dependent on the initial crack widths; while a relative value, such as healing ratio, is more representative

when comparing different specimens. Therefore, average crack healing ratio (in different crack widths ranges) was used to assess the multiple crack healing efficiency in the later self-healing system.

3.3 Crack healing in the specimens with hydrogel encapsulated spores

3.3.1 Light microscopy

All the specimens showed no obvious crack healing when they were stored at 95%RH. However, in wet-dry cycles, all specimens showed crack healing. The specimens with hydrogel encapsulated bacteria added, provided an obvious superiority in the healing ratio (Fig.4) and the maximum healed crack width. A maximum crack width of about 0.2mm and 0.5mm could be healed in the specimens H and HS, respectively (images not shown). The R specimen had no completely healed cracks. Hydrogels can absorb water during the wet stage and retain the water for continuous autogenous healing (secondary hydration, etc.) and bacterial activities (bio-precipitation), resulting in an enhanced healing efficiency in the specimens with hydrogels, especially in the ones with bio-hydrogels.

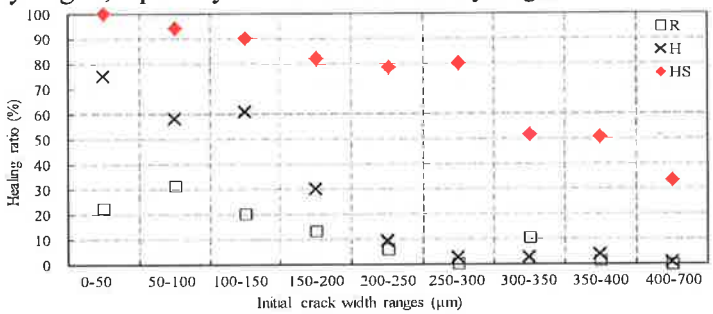


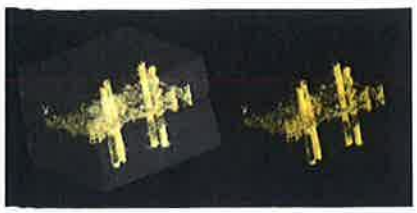
Figure 4. Average crack healing ratio for different crack widths of the specimens in wet-dry cycles.

3.3.2 3D X-ray CT

The distribution of the healing products through the whole matrix can be seen in Fig.5 (image of R is not shown). It can be seen that the precipitation was mostly distributed on the surface layer. Compared with the specimen H, the specimen HS had more precipitation distributed all over the matrix. The volume ratio of total precipitation was about 1.3% for the specimen H and 2.2% for HS, respectively. The increased amount of healing products was due to bacterial precipitation.

Figure 5. 3D view of the spatial distribution of healing products (in yellow) in the sample H (a) and HS (b)

X-ray CT has also been used in earlier research, where bacteria were protected through encapsulation by brittle tubes [7]. In addition to the bacteria, the tubes contained a polyurethane based liquid healing agent. Upon crack formation and capsule breakage, both, the bacteria (and nutrients) and the polyurethane, were released into the crack. While the polyurethane reacted fast and filled the crack, bacterial activity started somewhat later. However, it resulted in pore filling of the polyurethane foam and thus additional crack sealing. In Figure 6 an X-ray CT render of a mortar sample with embedded



tubes is shown. It can be clearly seen that due to crack formation the tubes were emptied and the crack was filled with the polyurethane foam.

Figure 6. The specimen with embedded glass tubes containing polyurethane after cracking

4 CONCLUSIONS

In this study, by combined use of light microscopy and 3D X-ray μ-CT, crack healing was visualized and quantified, and an enhanced healing effect was proven in specimens in which immobilized bacteria had been incorporated. It can be seen that light microscopy is a useful tool for quantification of surficial crack healing and X-ray μ-CT is more powerful for characterization of the healing inside the specimen.

Among the three bacterial carriers used, microcapsules and hydrogels seem to provide superior crack healing efficiency in comparison with DE. However, a disadvantage is that they both caused some strength decline of the virgin specimens (data not shown) while DE had a rather positive effect on the strength. Also the incubation condition has a big effect on the self-healing behaviour. Without free water in the surroundings, no self-healing was observed in all the specimens. Wet-dry cycles can stimulate more self-healing than full immersion in the specimens with encapsulated bacteria. And by use of hydrogels, shorter wet periods would be sufficient to induce self-healing.

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REFERENCES

[1] Ketcham, R.A. and Carlson, W.D., 'Acquisition, optimization and interpretation of X-ray computed tomographic imagery: applications to the geosciences', *Comput. Geosci.* 27 (2001) 381-400.

[2] Cnudde, V., Boone, M., Dewanckele, J., Dierick, M., Van Hoorebeke, L., Jacobs, P., '3D characterization of sandstone by means of X-ray computed tomography', *Geosphere.*7 (2011) 54-61.

[3] Dewanckele, J., De Kock, T., Boone, M.A., Cnudde, V., Brabant, L., Boone, M.N., et al. '4D imaging and quantification of pore structure modifications inside natural building stones by means of high resolution X-ray CT', *Sci. Total. Environ.* 416 (2012) 436-448.

[4] Dick, J., De Windt, W., De Graef, B., Saveyn, H., Van der Meeren, P., De Belie, N. and Verstraete, W, 'Bio-deposition of a calcium carbonate layer on degraded limestone by Bacillus species', *Biodegradation.* 17(4)(2006) 357-367.

[5] Kalfon, A., Larget-Thiéry, I., Charles, J.F., De Barjac, H. 1983. 'Growth, sporulation and larvicidal activity of bacillus-sphaericus', *Eur J. App. Microbiol. Biotechnol.* 18 (1983) 168-173.

[6] WO 2010/142401. Microcapsules containing microorganisms. DEVAN CHEMICALS NV. Patent No. AU2010257855

[7] Wang, J., Van Tittelboom, K., De Belie, N., Verstraete, W, 'Use of silica gel or polyurethane immobilized bacteria for self-healing concrete', *Constr. Build. Mater.* 26 (2012) 532-540.

[8] Wang, J., De Belie, N., Verstraete, W, 'Diatomaceous earth as a protective vehicle for bacteria applied for self-healing concrete', *J. Ind. Microbiol. Biot.* 39 (2012) 567-577.

[9] Wang, J. Self-healing concrete by means of immobilized carbonate precipitating bacteria. PhD dissertation, 2013, Ghent University.