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Bio-Based Self-Healing Concrete: From Research to Field Application

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Abstract Cracks are intrinsic concrete characteristics. However, cracking can endanger the durability of a structure, because it eases the ingress of aggressive gasses and liquids. Traditional practices tackle the problem by applying manual repair. Scientists inspired by nature have created self-healing concrete able to self-repair as a result of the metabolic activity of bacteria. Various research groups have studied bio-based self-healing concepts over the last decade. Although the metabolic pathways of different bacteria can vary, the principle is essentially the same: a bio-based healing agent is incorporated into fresh concrete and when a crack appears in hardened concrete the bacteria become active, precipitate limestone and seal the open crack. Bio-based self-healing concrete technology targets the recovery of the original performance of concrete by regaining water tightness lost by cracking. Along these lines, bio-based repair systems have also been developed to protect existing structures by applying materials that are more concrete-compatible and environmentally friendly than existing repair materials. All these innovative concepts have shown promising results in laboratory-scale tests. Steps have been taken towards the first full-scale outdoor applications, which will prove the functionality of this new technology.

Keywords Bacteria · Calcium carbonate precipitation · Concrete · Crack sealing · Repair systems

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1 Introduction

In the past decade, several research groups worldwide have addressed the durability-related problem of micro-crack formation in traditional concrete by developing bacteria-based self-healing concrete and repair systems [1]. Early age crack formation in concrete is known to occur as a result of temperature effects and shrinkage of the setting concrete, as well as loading of steel-reinforced structures. Although micro-crack formation does not pose an immediate threat to the strength and integrity of the structure, it does typically lead to problems with water tightness and durability, particularly in wet or moist environments. Networks of micro-cracks can expose the embedded reinforcement to the outside environment with, as a consequence, premature corrosion that necessitates costly manual maintenance and repair actions.

Limestone and other types of mineral formation in concrete can be beneficial and durable, as these are concrete-compatible materials. Filling up (i.e. sealing) of cracks makes concrete water tight and at the same time protects the embedded steel reinforcement from detrimental compounds such as chlorides and other corrosion-stimulating ions that otherwise migrate rapidly through micro-cracks. One of the main targets of applying bacteria that stimulate limestone deposition is, therefore, the recovery of decreasing functional concrete properties such as water tightness and strength. Self-healing of concrete therefore relates to the regain of a certain functional property, which could be water tightness, strength, porosity or aesthetics. In order to enhance the natural, or autogenous, healing capacity of concrete, a specific ‘self-healing agent’ in the form of encapsulated bacteria and required mineral precursor compounds or chemicals is added to the concrete mixture. This enhanced ‘autonomous’ healing should not only reduce manual maintenance and repair costs but also extend the service lifetime of constructions.

Several specific species of bacteria are known to precipitate calcium carbonate and other inorganic minerals in the direct vicinity of the cells. This process, however, is strongly dependent on environmental conditions. It can therefore be stated that bacteria, by employing specific metabolic pathways, change environmental conditions in such a way that precipitation of inorganic minerals is

increased. The designation of 'limestone-producing bacteria' in relation to a functional trait is strictly speaking incorrect, because metabolically driven limestone precipitation by bacteria is always a result of a specific combination of metabolic pathway, activity and physico-chemical environmental conditions. A specific bacterium can thus be 'limestone-producing' in one environment but not in another.

The potential application of limestone deposition enhanced by bacteria for improving durability aspects of concrete has been investigated using specific types of bacteria employing different metabolic pathways. A common characteristic of the various bacterial metabolic pathways is that they result in supersaturation of calcium carbonate in solution, resulting in precipitation of calcium carbonate. Although bacteria usually only change environmental calcium ion concentration to a minor extent, several metabolic pathways strongly affect the environmental concentration of carbonate ions and thereby the saturation state of calcium carbonate in solution. Typical bacterial metabolic pathways that increase the carbonate ion concentration and related calcium carbonate saturation in solution are the hydrolysis of urea, oxidation of organic compounds using oxygen under aerobic conditions and oxidation of organic compounds using nitrate under anaerobic conditions. This chapter describes these bacterial metabolic routes and their applicability for enhancing the autogenous self-healing capacity of concrete.

2 Autogenous Healing

Concrete has a natural or autogenous ability to seal micro-cracks, regain water tightness and greatly reduce chemically driven degradation phenomena. Autogenous healing upon contact with water is primarily attributed to three processes: swelling of the cement matrix, hydration of unhydrated cement particles and precipitation of calcium carbonate [2, 3].

The most significant of these is the precipitation of calcium carbonate ($CaCO_3$), which is formed as a result of carbon dioxide (CO_2) reacting with calcium hydroxide [$Ca(OH)_2$] according to Eq. (1):

$$CO_2 + Ca(OH)_2 \rightarrow CaCO_3 + H_2O \quad (1)$$

Occurrence of precipitation depends on the solubility product of calcium carbonate, which in turn depends on the temperature, ionic strength, composition, pH and carbon dioxide partial pressure of water in the crack [2]. Primarily, the amount of precipitate and, hence, crack healing potential depends on the amount of calcium and carbonate ions available in the cracks.

The autogenous healing ability of concrete was first reported by the French Academy of Science in 1836 [8]. Numerous studies have followed since then [e.g. 2, 4–6]; however, studies quantifying this ability are still relatively few [2, 5, 7–9]. Of the techniques employed to quantify the autogenous healing capacity of cementitious materials, water permeability has received the most attention. Clear [8] was the first to quantify the autogenous healing ability of cementitious materials

through permeability measurements, demonstrating how cracks narrower than 200 μm healed even under continuous water flow. Edvardsen [2] in a later study examined the autogenous healing ability of cracked concrete specimens in freshwater through visual observation and permeability measurements. She found that approximately 50% of the specimens with crack widths of 0.2 mm (mean value) healed completely during 7 weeks of exposure. She also noted that the highest healing rate occurred within the first 3–5 days after submersion. Reinhardt and Jooss [5] investigated the relationship between the initial crack width, temperature and autogenous healing potential. They revealed that the flow rate for specimens with cracks of 50 μm wide incubated at 20°C, 50°C or 80°C was almost reduced to zero after 14 days, whereas for cracks 100 μm wide at 20°C it was ~5%, and for those of 150 μm it was ~15% of the initial flow. Van Tittelboom et al. [9] investigated the influence of blast furnace slag (BFS) and fly ash (FA) binders on the autogenous healing capacity of continuously submerged cementitious specimens. They showed that the series containing these binders had 3–400 times greater flow reduction than those containing only Portland clinker. This was attributed to the latent hydraulic properties of these binders, which caused a lower reaction rate and thus a higher availability of unhydrated material for further hydration (Fig. 1). When, however, series containing these binders were exposed to wet–dry cycles, where carbonation-driven carbonate precipitation was expected to be the main contributor to healing, the series with BFS and FA binders healed visually less than ordinary Portland cement (OPC) specimens. The latter phenomenon was attributed to calcium hydroxide consumption by the binders, resulting in less carbonation-driven calcium carbonate precipitation as a result of calcium hydroxide limitation.

In a recent study, Palin et al. [7] visually quantified the autogenous healing ability of cracked OPC (CEM I) and BFS cement (CEM III/B) specimens submerged in fresh water and seawater. After 56 days, BFS cement specimens in

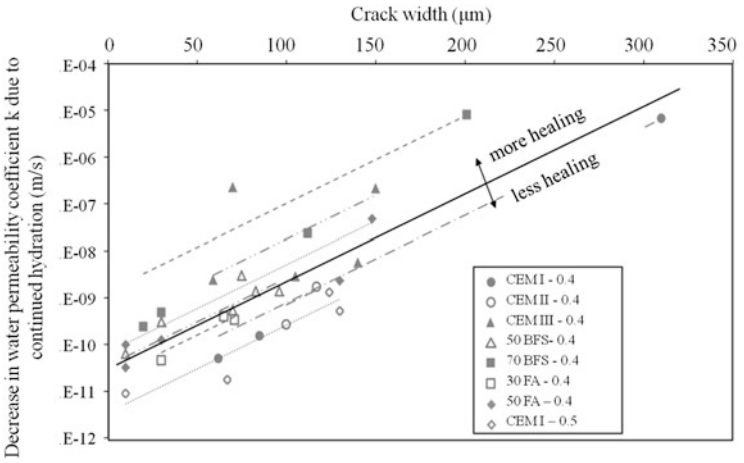

Fig. 1 Difference in water permeability as a result of autogenous crack healing for different mixes [9]. Codes *30*, *50* and *70* in the legend refer to the percentage of BFS or FA in the binder; *0.4* and *0.5* refer to the water/cement ratio of the specimens

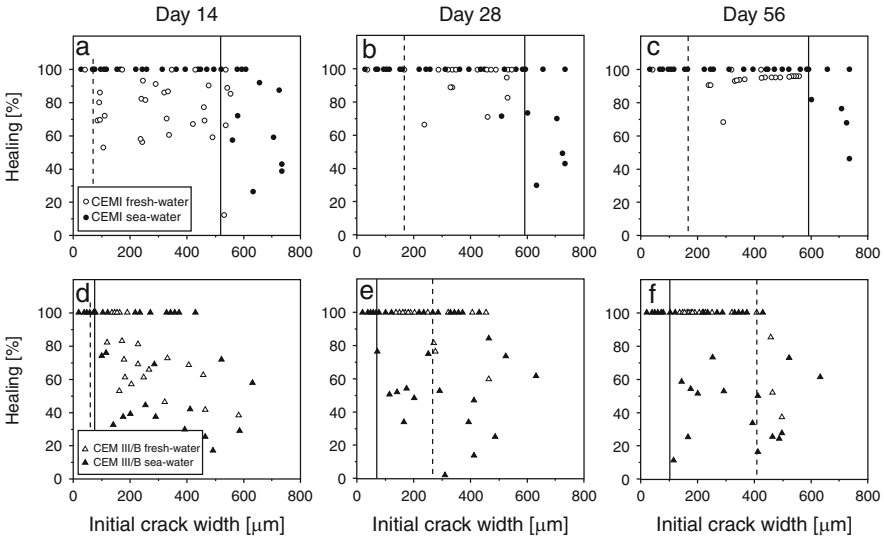

Fig. 2 Crack healing percentage as a function of the initial crack width for (**a**–**c**) CEM I-based specimens in fresh water and seawater and (**d**–**f**) CEM III/B in fresh water and seawater after (**a**, **d**) 14, (**b**, **e**) 28 and (**c**, **f**) 56 days submersion. *Unbroken* and *broken vertical lines* mark 100% visual crack closure (up to the largest crack width) for specimens submerged in seawater and fresh water, respectively [7]

seawater healed 100% of cracks up to 100 μm width, whereas for OPC specimens healing was 100% for cracks up to 600 μm. In fresh water, blast furnace slag cement specimens healed 100% of cracks up to 400 μm, whereas OPC specimens healed 100% of cracks up to 170 μm (Fig. 2). However, although OPC specimens submerged in seawater displayed the greatest autogenous healing capacity, they also developed an unacceptable loss in compressive strength. Observed differences in healing and strength loss phenomena were attributed to higher amounts of calcium hydroxide present in OPC mortars and the presence of magnesium ions in seawater, respectively. Seawater contains high concentrations of calcium and magnesium ions, which at high pH precipitate to yield hydroxide- and carbonate-based minerals, giving seawater-submerged specimens a greater healing potential. However, exchange of calcium ions for magnesium ions results in formation of magnesium-silicate hydrates, which are substantially weaker than calcium-silicate hydrates, explaining the loss of strength of seawater-incubated OPC specimens.

3 Autonomous Healing

The main healing product in bacteria-based self-healing concrete is biogenic $CaCO_3$. Under suitable conditions, most bacteria can mediate the formation of $CaCO_3$. This part reviews bacteria-based self-healing systems that have been or

are being investigated by research groups at Ghent University and Delft University of Technology.

3.1 Enzymatic Hydrolysis of Urea

3.1.1 Laboratory Work

Enzymatic hydrolysis of urea is a process that results in formation of carbonate ions, and thus increases the potential for calcium carbonate precipitation.

The biogenic, urease-driven, reaction rate of urea hydrolysis is approximately 1,014 times faster than the chemical (non-urease driven) rate [10]. Equation 2 shows the overall reaction of enzymatic urea hydrolysis:

$$CO(NH_2)_2 + 2H_2O \rightleftarrows 2NH_4^+ + CO_3^{2-} \quad (2)$$

The amount of calcium carbonate formed by the reaction of available calcium ions with produced carbonate ions is directly dependent on the amount of urea decomposed during urea hydrolysis. Urease-active bacteria can be used to increase autogenous healing rates of concrete as a result of the potentially enhanced rate of calcium carbonate formation. To obtain truly autonomous healing of concrete, both bacteria and nutrients required for bacteria-driven calcium carbonate formation should be introduced to the concrete mixture. Therefore, spore-forming bacteria instead of vegetative cells are selected, because only the former can survive direct incorporation into the concrete matrix. Spores can stay dormant inside concrete and become activated when concrete cracks if water, oxygen and nutrients are present, and other environmental conditions such as temperature and pH are at values compatible with their metabolic capacity. Wang and colleagues of Ghent University used *Bacillus sphaericus*, an alkali-resistant spore-forming ureolytic strain initially applied for consolidation of building materials [11].

Ghent University studies revealed that the concentration of bacteria should be higher than 10^6 cells/mL to obtain a considerable amount of calcium carbonate precipitation. Apart from bacterial numbers, the concentrations of urea and calcium ions (Ca^{2+}) also greatly influence the yield of $CaCO_3$ precipitation. Theoretically, ureolytic bacteria can continue to hydrolyse urea as long as the urease enzyme remains active. Recommended concentrations of both urea and calcium source are 0.5 M [12]. Wang and colleagues also found that yeast extract ($\geq$2 g/L) is an essential nutrient as it stimulates germination of spores, a requirement for urease-driven formation of calcium carbonate. Because working with pure cultures is relatively expensive, a more economical way of producing ureolytic bacterial suspensions was developed at Ghent University. A new selective process named CERUP (cyclic enriched ureolytic powder) was developed to obtain a ureolytic microbial community [13]. It was estimated that the production cost of CERUP was about 40 times less than that of pure cultures [14].

Encapsulation of bacteria is considered of crucial importance in a bacteria-based self-healing system as mechanical forces during concrete mixing can damage the bacterial spores. Sorption of bacterial cells onto diatomaceous earth (DE) provides a protective effect for bacteria in high pH cement slurry [15]. Furthermore, DE has no negative effect on the mechanical properties of mortar and may even show pozzolanic activity. The concept of impermeable microcapsules containing bacterial spores has been patented [16]. The capsules change properties from humid to dry state, allowing them to withstand forces during concrete mixing, but break when cracking occurs [17]. Another idea for encapsulation is to use hydrogels as carrier. Hydrogels act not only as protective carrier for bacterial spores during mixing and hydration, but also as water reservoir for spore germination and bacterial activity when cracking occurs. In normal humidity conditions, hydrogels can absorb moisture and retain it for bacterial use, which is beneficial for realistic self-healing. Wang et al. [18] observed substantial and fast crack closure: a crack of 0.5 mm was completely closed within 1–2 weeks (Fig. 3). However, a drastic loss in strength (as much as 50%) occurred after addition of bio-hydrogels, which indicated incompatibility between the hydrogels and concrete matrix. More compatible hydrogels have now been developed [19], as well as modified alginate- based hydrogels [20].

Besides bacteria, the nutrients and deposition agents (urea and calcium source) are also essential elements for biogenic precipitation and should also be

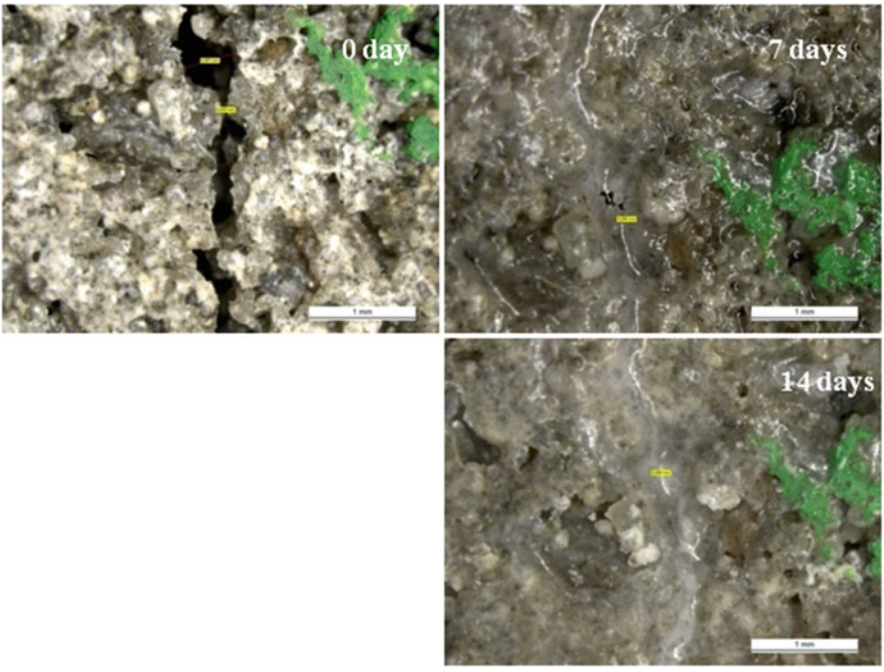

Fig. 3 Crack closure process in specimen with hydrogel-encapsulated *Bacillus sphaericus* spores (the initial widest part is 507 μm) [18]

incorporated into concrete in advance. The latter compounds, however, do not necessarily need to be encapsulated and therefore can be added directly to the concrete mixture. In subsequent studies it was found that, although urea and calcium source have no negative effect on concrete strength development, yeast extract and other organic compounds do. Encapsulation of yeast extract should therefore be considered in future studies.

In most studies, evaluation of self-healing efficiency was based on crack sealing and a decrease in water permeability. Realistic cracks in test specimens were made by three-point bending, splitting or tensile loading. The sealing of a crack can increase water tightness of the structure and possibly prevent corrosive substances from penetrating into the matrix.

Wang et al. [18] observed substantial and fast crack closure; for example, a crack of 0.5 mm was completely closed within 1–2 weeks (Fig. 3).

In addition to light microscopy, which only provides information on the surface part of the crack, X-ray computed micro-tomography (X-ray μCT) has proved to be useful in providing a three-dimensional (3D) deep view inside the crack to quantitatively establish the amount and distribution of internal precipitates. Figure 4

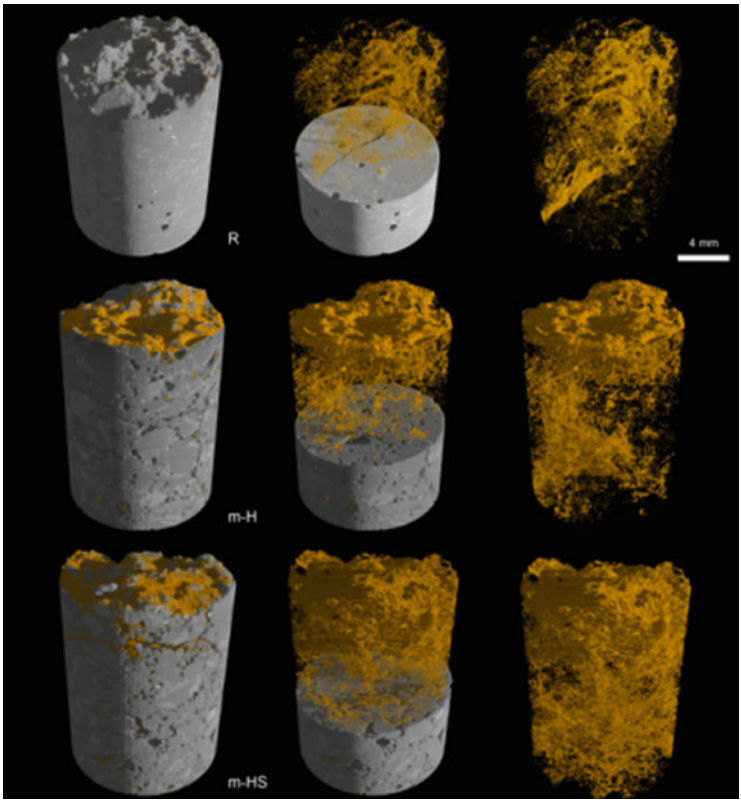

Fig. 4 3D view of the spatial distribution of healing products (in *yellow*) in reference specimens (*R*), in specimens with pure hydrogel (*m-H*) and in specimens with bacteria-loaded hydrogel (*m-HS*) [21]

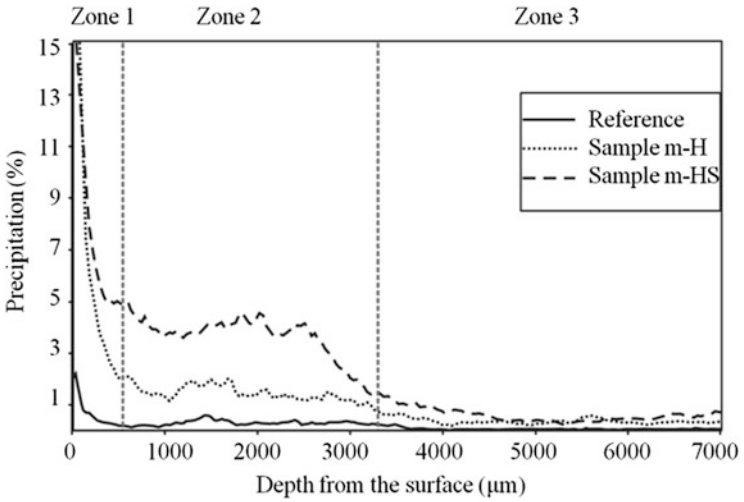

Fig. 5 Quantification of precipitation as a function of depth of the specimen. Three different zones were distinguished: surface, subsurface and internal [21]

shows a 3D view of the healing products inside reference (R) specimens and specimens with (m-HS) and without (m-H) hydrogel-encapsulated bacteria. Precipitates appeared mostly distributed in the surface layer, but not in the deeper part of the crack in the specimens containing bacteria-hydrogels. For the R specimen, the small amount of precipitate was attributed to autogenous healing. A higher amounts of precipitation was found in the specimen containing hydrogel. The amount of precipitate as a function of the depth inside the specimen was calculated and is shown in Fig. 5. For all specimens, the volume ratio of the precipitation was much higher in the surface layer (zone 1) than in the deeper zones 2 and 3. The m-HS and m-H specimens exhibited similar amounts of precipitate in the surface layer, around 8% (volume ratio). However, the former showed more precipitation in the subsurface layer than the latter (about 3% versus 1%), generated from the bacterial activity.

3.1.2 Upscale Application

Most of the laboratory-scale research was performed on small cylinders ($h = 20$ mm; $d = 80$ mm) or prisms ($40 \times 40 \times 160$ mm^3 or $30 \times 30 \times 360$ mm^3). Recently, an upscaled bacteria-based self-healing system has been developed in which ureolytic mixed self-protected bacterial cultures (CERUP) can be produced at the 50 L scale [13]. This allowed casting of a concrete beam of $150 \times 250 \times 3{,}000$ mm^3, into which was incorporated 3% (per cement weight) of CERUP. Multiple cracks were created by four-point bending. To facilitate the water permeability test (Fig. 6), the beam was loaded in the upward direction (Fig. 7) and

Fig. 6 Test setup for measuring water permeability

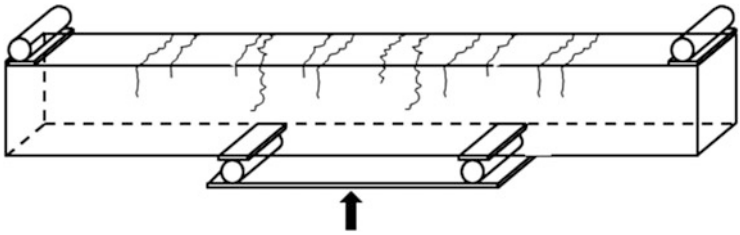

Fig. 7 Scheme of four-point loading on the beam

subjected to cyclic water supply instead of full immersion to mimic realistic conditions. An automatic water-spray system was installed above the beam, which sprayed water for 1 min four times per day over 6 weeks. It was found that less sealing was obtained in the large-scale beam than in the smaller prisms. The average crack filling ratio was only around 24% (for the R beam 20%), which was much lower than obtained in the small specimens (40%) using the same mixed culture [14]. The recovery of water permeability after healing was not significant. At this point, it can be confirmed that, in bacteria-based self-healing systems, the concrete should have good water retention properties, which can be realized by embedding functional materials such as hydrogels.

3.2 Oxidation of Organic Carbon

3.2.1 Expanded Clay Particle System

Laboratory Work

Biological calcium carbonate precipitation and carbon dioxide production can occur during the degradation of organic compounds, where bacteria act as catalysts. This biochemical reaction (via organic carbon oxidation) is the principle of bio-based healing in concrete.

Healing agents can be defined as the chemical/biochemical components that, upon crack formation, are activated to seal a crack. In the case of bio-based self-healing concrete using organic carbon oxidation (as developed in Delft University of Technology), the healing agent consists of alkaliphilic bacterial spores of the genus *Bacillus*, calcium lactate and yeast extract. For protection and immobilization of the healing agent during concrete mixing, all components are dissolved in water and are impregnated via vacuum application into lightweight aggregates (LWA). The healing agent solution contains bacteria spores (10^{11} spores/mL), calcium lactate (200 g/L) and yeast extract (4 g/L) and is incorporated into expanded clay particles (Liapor 1/4 mm; (Liapor GmbH, Germany) via impregnation under vacuum.

The $CaCO_3$ crystals that are formed during the healing process can seal open concrete cracks as a result of the biological conversion of the available organic carbon source; for example, in the case of calcium lactate, $Ca(C_3H_5O_3)_2$, the reaction producing calcium carbonate is the following [22]:

$$Ca(C_3H_5O_3)_2 + 6O_2 \rightarrow CaCO_3 + 5CO_2 + 5H_2O \quad (3)$$

According to the metabolic reaction shown in Eq. (3), one equivalent of calcium lactate is converted to one equivalent of $CaCO_3$, five equivalents CO_2 and five equivalents of H_2O. If the above reaction takes place in concrete, the produced carbon dioxide reacts further with the calcium hydroxide present, as cement hydration product, to produce another five equivalents of calcium carbonate [23]:

$$5CO_2 + 5Ca(OH)_2 \rightarrow 5CaCO_3 + 5H_2O \quad (4)$$

Presumably, Eqs. (3) and (4) result in the production of six equivalents of $CaCO_3$ from one equivalent of calcium lactate. It has been reported that biochemical $CaCO_3$ production can improve autogenous healing by efficiently sealing cracks of up to 460 μm [23].

Three significant aspects of bio-based self-healing concrete are examined and analyzed below:

- Influence of the healing agent on the compressive strength of concrete
- Crack sealing efficiency of the healing agent

Table 1 Mortar mix designs

Mixture	CEM I (kg/m^3)	Water (kg/m^3)	0.125/1 mm sand (kg/m^3)	1/4 mm sand (kg/m^3)	1/4 mm LWA (kg/m^3)
REF	463	231.5	855	825	0
CTRL	463	231.5	855	0	257
BAC	463	231.5	855	0	280[a]

[a]Includes the weight of impregnated healing agent in pores of the LWA

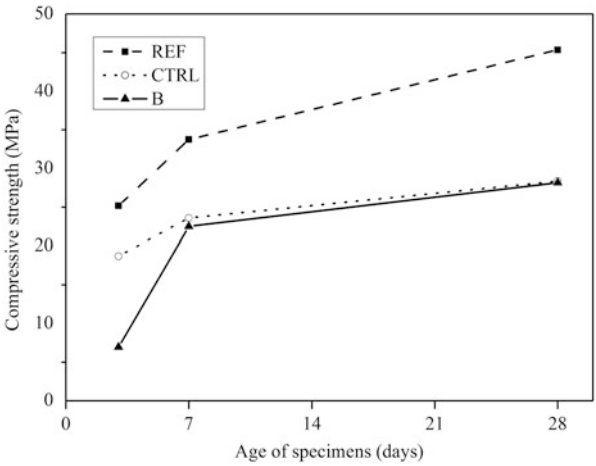

Fig. 8 Compressive strength for REF, CTRL and B specimens (see Table 1 for descriptions)

- Validation that crack sealing in bio-based concrete originates from bacterial activity

Experiments were conducted on three types of mortar mixtures: a reference mixture (REF) with normal weight aggregates (sand fraction 0.125/4.0 mm); a control mixture (CTRL) in which normal-weight sand fraction 1/4 mm was substituted by unloaded LWA; and a mixture with loaded LWA (B). Mix designs are presented in Table 1.

Investigation of the influence of the healing agent on the compressive strength of mortar was conducted on prisms ($40 \times 40 \times 160$ mm). All specimens were demoulded 24 h after casting and kept in a room with standard temperature ($20 \pm 2°C$) and $> 90\%$ relative humidity for 28 days. Compressive tests were performed on 28-day-old specimens. The results (Fig. 8) revealed that replacement of sand with LWA leads to substantial reduction in compressive strength, as expected. Furthermore, the healing agent seems to delay the development of compressive strength of mortar (at the age of 3 days), but has no significant effect after 7 days.

The crack-sealing ability of mortar is of prime importance in addition to its strength characteristics. In many studies, light microscopy images taken before and after healing treatment are used as a tool to quantify the visual sealing efficiency of

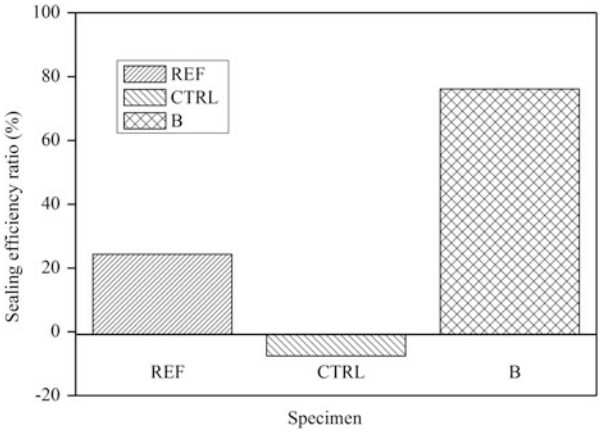

Fig. 9 Average sealing efficiency ratio for REF, CTRL and B specimens (see Table 1 for descriptions)

a certain healing agent. However, visual crack closure does not always leads to reliable conclusions about the functional effectiveness of the healing agent. Thus, a crack permeability test has been designed. The test was performed on steel-reinforced prismatic specimens ($40 \times 40 \times 160$ mm) modified with a hole (diameter 5 mm) in the middle and similar holes along their length. A crack of approximately 350 µm was introduced to the specimen via three-point bending. Following crack creation, the specimen was placed horizontally in a plastic bucket and subjected to 12-h wet–dry cycles for 28 days. The crack permeability test was performed on the specimen before and after water submersion, as described in the literature [24].

After test completion, the sealing efficiency ratio (SER) was calculated according to a reported method [24]. This ratio indicates the amount of healing that has taken place inside the crack during the 28 days of healing treatment. Figure 9 presents the average SER values calculated after completion of crack permeability tests via water flow. The graph shows that there is a noticeable difference in crack sealing between specimens with and without healing agent. Specimen B exhibited more than three times higher sealing performance than REF and CTRL specimens, as a result of the presence of the bio-based healing agent.

Additional tests were conducted to confirm that the improved sealing performance of specimens containing bacteria can be attributed to the bio-based system. The bacterial activity was traced by oxygen concentration measurements on 3-month-old specimens submerged in carbonate–bicarbonate buffer solution (0.1 M, pH 10.5, 20 ± 2°C, in a 4-L tank). For this test, the microsensor Oxy50M (Pyro-Science, Germany) was used. The microsensor measured the oxygen concentration in vertical steps of 50 µm, from 5 mm above the specimen down to the surface. Typical oxygen microprofile measurements for REF, CTRL and B samples are displayed in Fig. 10. The profiles of specimens without bacteria were similar (i.e. oxygen concentration was almost stable along the measuring height). The profile of the bacteria-containing specimen, however, differed. The oxygen concentration was constant until approximately 1 mm above the surface of the specimen, where it started to decrease. Oxygen consumption near the surface of the

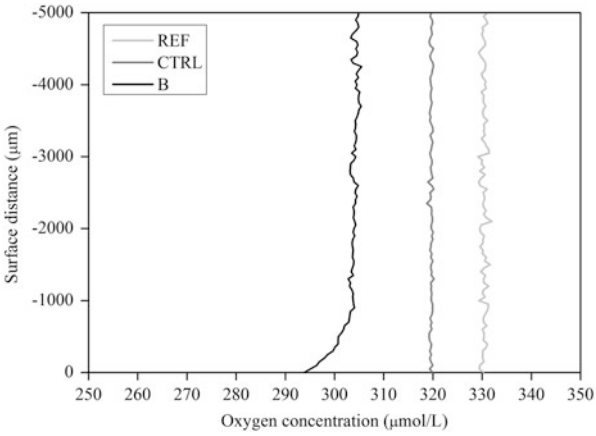

Fig. 10 Oxygen profile measurements for REF, CTRL and B specimens (see Table 1 for descriptions)

specimen provides evidence that metabolically active bacteria (germinated bacterial spores) were degrading the available organic carbon inside the mortar.

Test results revealed that the compressive strength of mortar specimens containing the bio-based system was considerably reduced (approximately 37%), caused by replacement of normal weight sand by LWA. Therefore, this material can be used where a lightweight structure is needed, or as an external layer on a structure of normal weight. Despite the lower strength performance, this self-healing material exhibited a significantly improved crack sealing performance when exposed to wet–dry cycles, compared with mortars without healing agent. Consequently, it is safe to state that bio-based self-healing concrete with LWA is suitable for preventing micro-cracking-related durability problems in structures.

Field Application

The first field application of bio-based self-healing concrete with LWA and natural fibres took place on July 2014 in the Andean highlands in Ecuador, South America. In this region the local economy is based on agriculture, for which a constant water supply is necessary. For this reason, about 100 years ago several canals were dug in the soil to transport water from melting ice and snow to the valleys. Because of infiltration of water through the walls (soil) the yield was very low. In 2011, the farmers who owned the canals and the local authorities cast concrete linings in the canals to improve this situation. Unfortunately, the concrete started cracking soon after casting, resulting in a large amount of leakage that put the sustainability of the system in danger. Bio-based self-healing concrete with LWA was proposed as a measure to improve the sustainability and performance of the irrigation system.

The concrete mix was designed taking into account the materials available in Ecuador, the strength and performance demands for the concrete linings and the building practice in the highlands in Ecuador. This mix included gravel with maximum size of 10 mm, sand, LWA with and without healing agent and natural

fibres. To ensure the sustainability of the self-healing concrete a fibre indigenous to Ecuador, Abaca, was chosen. This fibre has been successfully studied in Ecuador as reinforcement for mortar to improve the structural behaviour of houses under seismic forces [25].

In the laboratory, the mechanical properties of the concrete mix as well as the healing capacity were evaluated. The average compressive strength was 30 MPa for the mix with healing agent and 26 MPa for the mix without healing agent [26]. Specimens pre-cracked by means of three-point bending tests and placed to heal in contact with water for 6 weeks exhibited crack sealing.

In Ecuador, the concrete mix design was adjusted to the properties of the materials available at the site. A section of canal that did not have concrete lining was chosen for this field application. First, the water supply was closed. Then, the local farmers who are the owners and users of the canal cleaned the canal section of dirt and vegetation. The framework was prepared while the LWA was impregnated with bacteria and nutrients in the field.

Three linear meters of concrete linings with bacteria and 3 m without bacteria were cast. About 110 L of concrete were prepared at the time. The temperature during the casting was around 5°C. This portion of canal is located at about 2,900 m above sea level.

The framework was removed after 3 days. Two days later, the water flow through the canal was reopened. Now, a year after casting, the concrete linings show no signs of cracking or deterioration (as reported from the last inspection).

3.2.2 Condensed Powder System

Laboratory Work

Although proof of concept was shown for healing agent components contained in LWA, the application range may be limited because of incorporation of volumes of expanded clay, which influence the mixture design. In order to extend the applicability range, the volume of added healing agent was reduced by increasing the content of efficient healing agent component in particles. For this reason, scalability of production is an important factor to take into account. A way of producing scalable particles almost fully consisting of active ingredients is by roller-compacting powders to sheets, with subsequent milling to flakes that are in the size range of the sand fraction (1–4 mm) [27]. A typical property of these flakes is solubility in water, which is beneficial for matrix cracking and water ingress, dispersing the healing agent in the crack volume. It is challenging, however, to retain particle integrity during the wet mixing stage of mortar or concrete production to prevent premature incorporation of nutrients in the cementitious matrix. Partial particle dissolution can be prevented by application of a protective barrier around the soluble particle, in the form of a coating. The coating material can be inorganic (e.g. cement paste or geopolymer) or organic (e.g. calcium cross-linked polyvinyl alcohol alginate or lactic acid derivatives) (Fig. 11) [28–30]. To retain

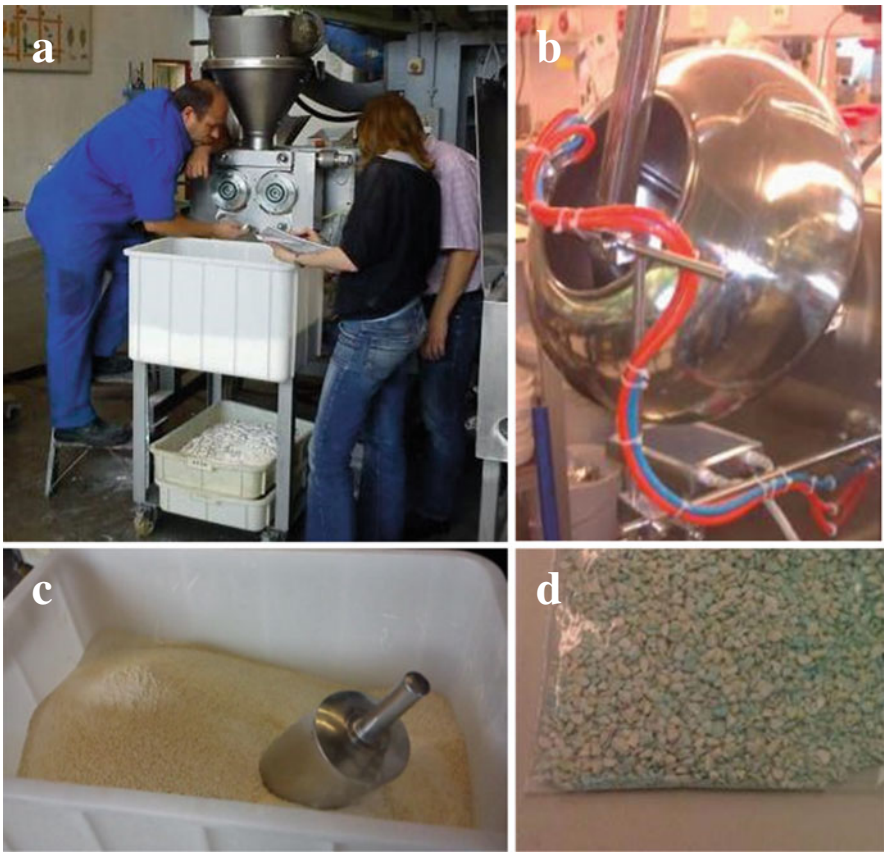

Fig. 11 (**a**) Roller compaction of powders, (**b**) coating of particles, (**c**) uncoated powders and (**d**) coated powders

equal active healing agent content in the mortar or concrete mixture, the addition of particles decreased from 30% by volume for LWA to 1% for the proposed flakes.

Important characteristics of flakes containing healing agent are survivability during the mixing stage, limited influence on mortar or concrete hardening properties, nutrient availability over time and ability to enable regain of crack water tightness [31]. Negligible influence on fresh mixture consistency was seen from flow tests, but some delay in strength development indicated interaction of healing agent particles and cementitious matrix (Fig. 12). After 7 days of hardening, the influence on strength development became negligible. The effect on strength is expected to be caused by partial loss of soluble core material near the particle surface, which could be amplified by increased surface area. Decreasing the surface area, by increasing particle size or homogeneity towards a spherical shape, should limit the superficial material loss and therefore further reduce the effect on strength development. Visual evidence of particle presence upon wet sawing of hardened

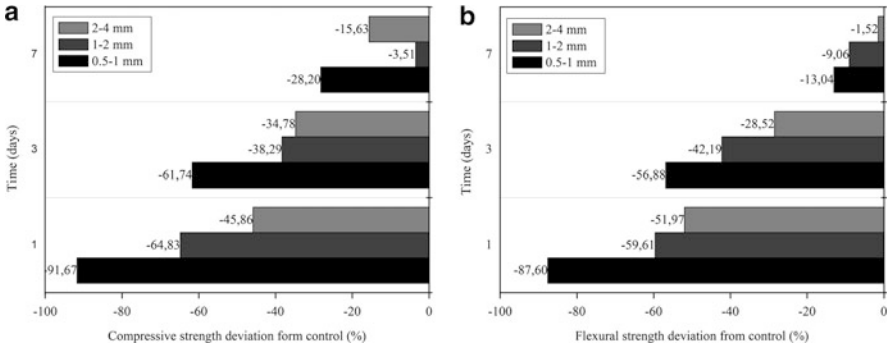

Fig. 12 (**a**) Flexural and (**b**) compressive strength deviation from control mortar after addition of healing agent flakes of various sizes

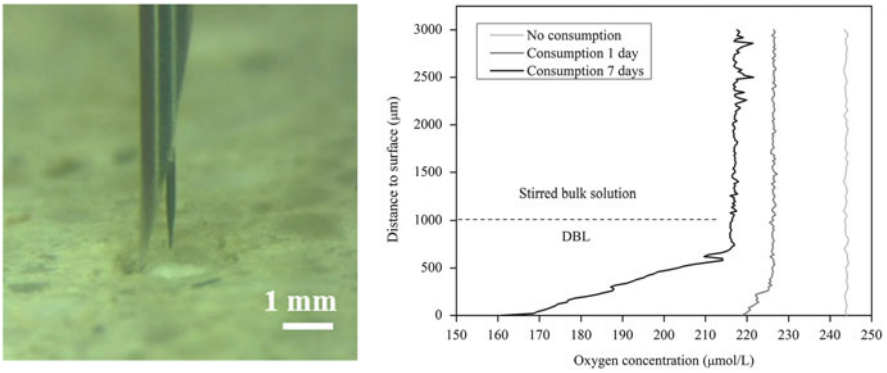

Fig. 13 *Left:* Oxygen microsensor approaching the surface of the mortar slice. *Right:* Profile measurement for surface oxygen consumption in time, showing a decrease in oxygen concentration in time towards the surface. *DBL* diffusive boundary layer

mortar specimens confirmed that particle retrieval was possible from hardened matrices and, therefore, that particles survived the mixing process.

In order to indicate bacterial activation and nutrient conversion, mortar slices with exposed healing agent flakes were immersed in water in a closed vial or stirred in an open system to measure the decrease in oxygen concentration, as evidence for bacterial metabolic activity (Fig. 13). Externally visible progressive mineral formation in a water-immersed cracked mortar specimen indicated crack closure. More important than visual mineral formation is confirmation of functionality regain, for which water tightness tests were performed. Half-cube mortar specimens were split to a crack width above the autogenous healing capacity (>400 µm). After determination of the initial water flow rate through the cracks, specimens were either stored in a 95% relative humidity room or placed under water. Water leakage was measured at intervals, until crack water tightness was restored. To indicate the possibility for multiple healing events, after drying of the specimens, the water leakage was checked and cracks were reopened and again placed in a water bath or humidity

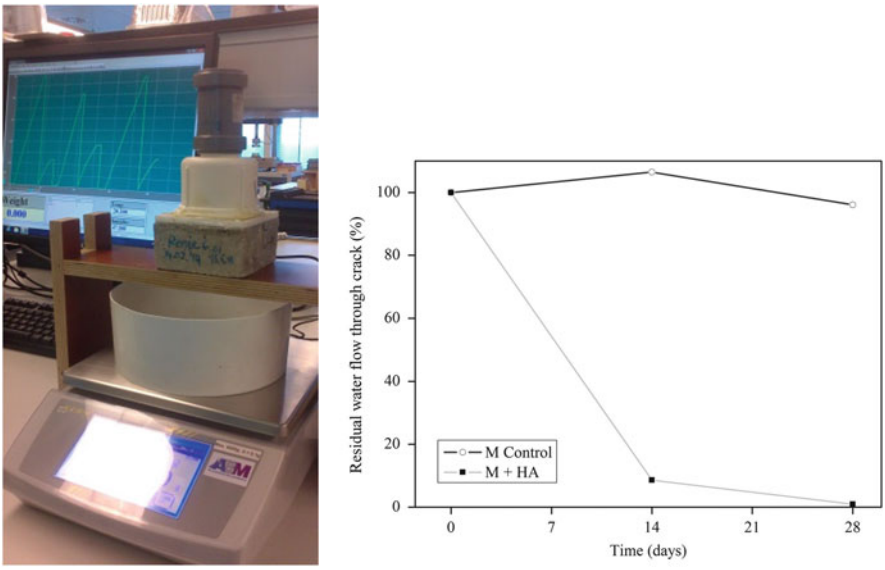

Fig. 14 *Left:* Set-up for the water leakage test. *Right:* Residual water flow through cracks at various water curing times. *M* control mortar, M+HA mortar with healing agent flakes

room. For specimens with similar crack widths, significantly higher recovery of water tightness was found in mortar containing healing agent flakes (Fig. 14).

Field Application

To check performance under outdoor conditions, flakes containing healing agent were added to a commercially available repair mortar. A 16 m^2 square part of a wall was divided into four quarters. For the top square, half the surface was removed until the reinforcement appeared; for the bottom half, the surface was removed until behind the reinforcement and the reinforcement was cleaned. Reference mortar, without healing agent, was applied on the left half of the square by spraying, whereas mortar containing healing agent was applied to the right half (Fig. 15, left). Observations were obtained on practicality, workability, consistency and visual appearance of the healing agent complemented mixture throughout application by spraying. Surface speckling indicated the locations of agent particles (Fig. 15, right); the stain intensity reduced over time. A first visual comparison of the superficial speckle pattern with laboratory cube specimens indicated homogenous spread of particles throughout the matrix. It would be interesting to visualize particle distribution over the depth of the sprayed mortar patch, as particles could have the tendency to settle predominantly at the surface as a result of rebound action at jetting. Because crack closing ability is mainly needed in the concrete covering the steel, a preferential distribution of particles towards the surface creates

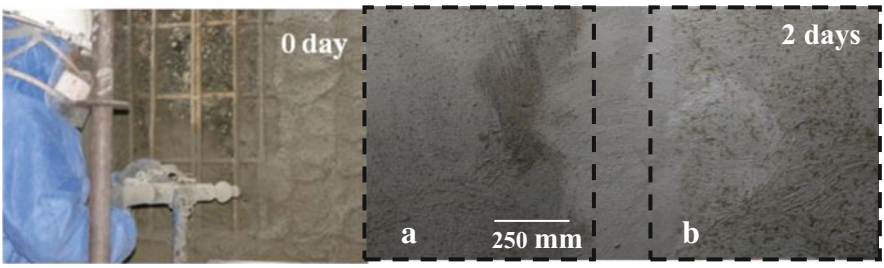

Fig. 15 *Left*: Spray application of commercial ready-mix mortar complemented with healing agent flakes. *Right*: During hardening, locations of healing agent particles are indicated by formation of speckles (**b**) compared with control (**a**)

an efficient use of healing agent particles. For an equal healing capacity, the amount of particles could therefore potentially be reduced, limiting the total cost added to that of the base mortar. Effective healing agent particle distribution and functional crack sealing capacity for regain of water tightness will be quantified in further outdoor applications.

3.3 Anoxic Oxidation of Organic Carbon

Microbial urea hydrolysis and aerobic oxidation of organic carbon require oxygen to initiate and maintain microbial activity, respectively. As a result of the poor solubility of oxygen in water (~9 mg/L) and its related deficiency in the deeper parts of the crack, healing efficiency through either of the processes is mostly inhibited with depth. Moreover, aerobic oxidation of organic carbon is known for its relatively low $CaCO_3$ yield [32], and urea hydrolysis is well known for its ammonia production, which is toxic for the environment [33, 34]. These issues have prompted study of alternative pathways in several applications of microbially induced $CaCO_3$ precipitation (MICP). Anoxic oxidation of organic carbon consumes nitrate (NO_3^-) or nitrite (NO_2^-) ions, as electron acceptor, and does not produce any toxic by-products. The process is called nitrate reduction and has been proposed for soil consolidation and Ca^{2+} removal from industrial wastewater [32, 35, 36]. Because of its highly negative standard Gibbs free energy (ΔG°) [Eq. (5)], nitrate reduction can be expected to dominate in the presence of NO_3^- and organic carbon, under oxygen-limited conditions, and generate carbonate (CO_3^{2-}) and bicarbonate (HCO_3^-) ions, which are necessary for $CaCO_3$ precipitation [Eq. (5)]:

$$5HCOO^- + 2NO_3^- \leftrightarrow N_2 + 3HCO_3^- + 2CO_3^{2-} + H_2O \quad \Delta G^\circ = -1153 \text{ kJ/mole} \tag{5}$$

MICP studies aiming at sand and soil consolidation through nitrate reduction have revealed that $CaCO_3$ precipitation rates achieved through NO_3^- reduction are not adequate for in situ application [32, 35, 37]. Indeed, under optimum conditions, MICP rates achieved through denitrification are 100–1,000 times lower than those achieved through ureolysis [36]. Yet, the concrete environment is at far from optimum conditions. Apart from its alkaline state, in concrete cracks oxygen and nutrients are limited and are the main factors affecting the germination and growth rate of the used bacteria. $CaCO_3$ precipitation rate mainly depends on the initial bacteria concentration, growth rate and specific metabolic activity of each bacterium in the relevant environment. Therefore, the condition in concrete cracks favours denitrifying bacteria rather than aerobic bacterial cultures. Bacteria that are able to grow on NO_3^- under nutrient-limited conditions could supersede the MICP rate and self-healing performance of aerobic bacteria.

Generally, concrete properties are affected by the addition of bacteria, nutrients and the protective carriers necessary to develop microbial self-healing concrete. If the appropriate bacteria are chosen to minimize the necessary modifications, application of the self-healing technology becomes more feasible. Anoxic oxidation of carbon sources as a self-healing mechanism requires three main components:

- Organic carbon source
- NOx (NO_3^- and/or NO_2^-) as electron acceptor
- Appropriate bacteria for enzymatic oxidation

There are already certain concrete admixtures, calcium formate and calcium nitrate, that can serve as carbon source and electron acceptor, respectively. Calcium formate, $Ca(HCOO)_2$, is used as an accelerator (0.5–4% w/w cement) and calcium nitrate, $Ca(NO_3)_2$, is used as set accelerator, strength enhancer or anti-freeze admixture (0.2–4% w/w cement) in concrete [38, 39]. Using this information, at Ghent University, microbial strains that can grow on these admixtures were selected and investigated for MICP [36]. Among the selected strains, *Diaphorobacter nitroreducens*, a NO_3^- reducing vegetative bacterial strain, appeared to grow on formate and nitrate salts with a higher growth rate than the NO_3^- reducing *Bacillus* species (Fig. 16).

The difference in growth performance was attributed to the absence of yeast extract, which is an essential nutrient for germination of spores. Ability to grow in the absence of trace elements and nutrients such as vitamins and yeast extract makes *D. nitroreducens* advantageous over currently studied strains. Furthermore, a $CaCO_3$ precipitation yield of 7 g $CaCO_3$/g NO_3-N per day could be achieved, which is distinctive among the reported MICP studies [36]. Moreover, by using starvation-resilient bacteria, repetitive $CaCO_3$ precipitation could be achieved. If the growth of the microbial culture is limited (due to the absence of yeast extract, oxygen, etc.) precipitation around the cell membrane kills bacterial cells and decreases the rate of precipitation [32, 40]. The resilience of *D. nitroreducens* against starvation enabled repetitive $CaCO_3$ precipitation to be achieved from a single inoculum, with a constant precipitation rate of 0.3–0.5 mM $CaCO_3$/h [36]. The bacterial strain could survive when nutrient diffusion was limited as a result of mineral formation

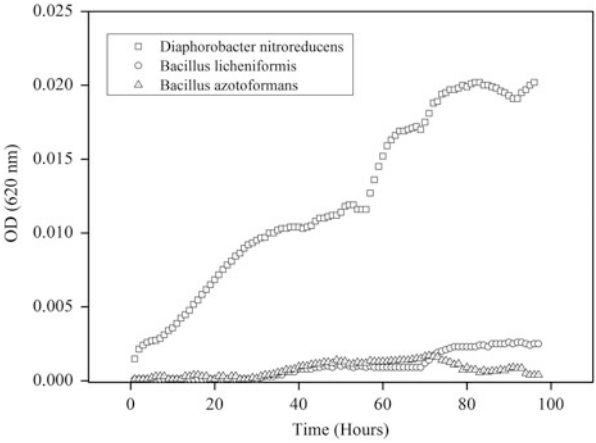

Fig. 16 Difference in growth of different NO_3^- reducing species under minimal nutrient conditions, lacking yeast extract and trace elements

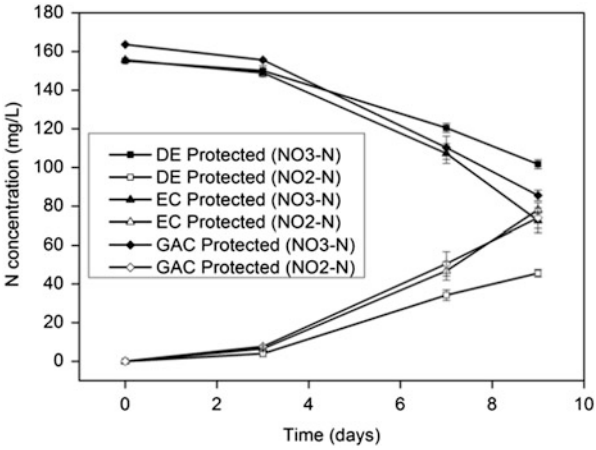

Fig. 17 Resuscitation performance of protected *Diaphorobacter nitroreducens* cells after 28 days in mortar. *DE* diatomaceous earth powder, *EC* expanded clay particles, *GAC* granular activated carbon particles ($n = 3$)

around the cell membrane, and created new cells that enabled repetitive $CaCO_3$ precipitation. Survival experiments showed that *D. nitroreducens* can survive at pH 13 with the aid of protective carriers [41]. Nitrate reduction activity of the bacteria could be recovered in 48–96 h following a pH decrease to below 10. Survival tests were further conducted in mortar environments. Prior to addition to mortar, bacterial cells (0.5% w/w cement) were incorporated into diatomaceous earth, expanded clay particles or granular activated carbon (5% w/w cement). These protective carriers enabled bacterial cells to survive in mortar environments. Bacterial cells extracted from the 28-day cured mortar specimens revealed nitrate reduction activity after 72 h of incubation (Fig. 17) in a minimal nutrient solution (pH 9.5–10) containing $Ca(HCOO)_2$ and $Ca(NO_3)_2$.

In a recent study, various types of commercially available protective carriers and nutrients were investigated for their influence on mortar setting and strength [42]. Results indicated that although diatomaceous earth provided protection for

NO_3^- reducing vegetative strains, it significantly decreased the setting time when combined with the respective nutrients, $Ca(HCOO)_2$ and $Ca(NO_3)_2$, necessary for self-healing [41, 42]. Therefore, diatomaceous earth was suggested not to be used as a protective carrier for denitrifying microorganisms in the development of self-healing concrete. By contrast, promising results were achieved when either expanded clay particles or granular activated carbon particles were used as protective carriers for vegetative bacterial strains. These porous carriers did not significantly change the setting and strength properties of mortar [42].

Recently, the self-healing performance of mortar specimens containing *D. nitroreducens* has been investigated. Bacterial cells (0.5% w/w cement) were loaded into 0.5–2 mm expanded clay particles (5% w/w cement) and added to the mortar during mixing. As nutrient source, concrete admixtures $Ca(HCOO)_2$ (2% w/w cement) and $Ca(NO_3)_2$ (3% w/w cement) were dissolved in water and added during mixing. The amounts used were in the range of suggested literature values for concrete use [38, 39]. Mortar specimens composed of sand, cement and water in the ratio 3:1:0.5 ($30 \times 30 \times 360$ mm) with embedded steel reinforcement (diameter 6 mm) were cured under temperature- and humidity-controlled conditions (20°C and relative humidity >90%) for 28 days. After the curing period, specimens were submitted to direct tensile load applied to the reinforcement, and multiple cracks (crack width range 100–500 μm) were achieved. Upon formation of cracks, mortar prisms were immersed in tap water for 28 days. Self-healing performance of the mortars was observed through visual crack analysis using a stereomicroscope (Leica S8 APO). At the end of 4 weeks treatment in wet conditions, specimens with bacteria could completely close the cracks up to 350 μm (Fig. 18).

At the end of the healing period, capillary sorption tests were conducted to check the water tightness of the healed crack (235 ± 35 μm original crack width). Mortar prisms containing healing agents absorbed 51% less water than the reference specimen in the first 24 h (Fig. 19).

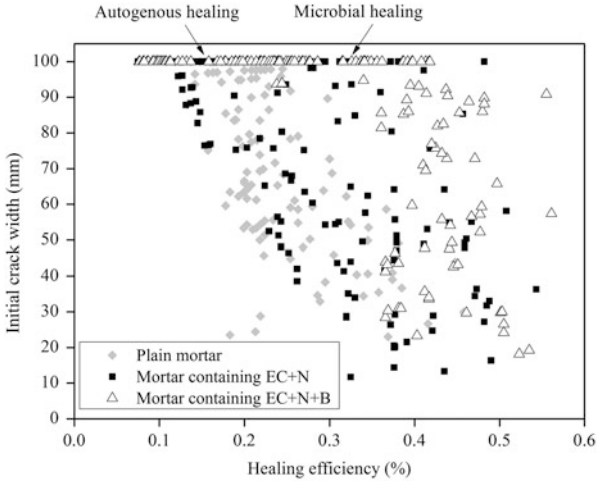

Fig. 18 Self-healing capability of mortar specimens with and without *Diaphorobacter nitroreducens*. *EC* expanded clay particles, *N* nutrients $Ca(HCOO)_2$ + $Ca(NO_3)_2$, *B* *Diaphorobacter nitroreducens*)

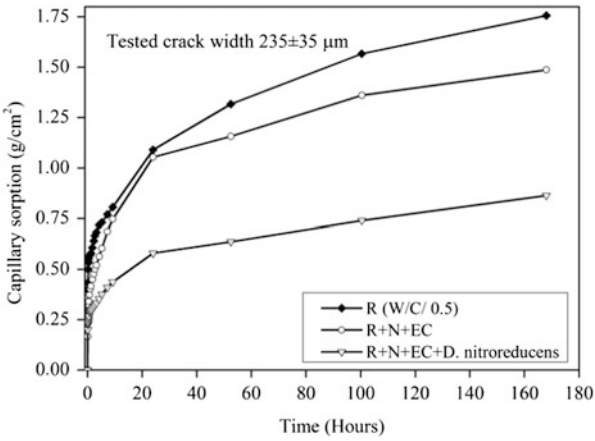

Fig. 19 Microbial self-healing through NO_3^- reduction provides higher water tightness than autogenous healing. *R* plain mortar (0.5 water-to-cement ratio), *EC* expanded clay particles, *N* nutrients Ca $(HCOO)_2$ + $Ca(NO_3)_2$

Elaborating visual quantification of the crack healing to 3D crack closure is important for clear distinction between autogenous and autonomous healing [7]. Therefore, it is necessary to investigate reported NO_3^- reducing strains in terms of their actual precipitation performance inside the crack and the stability of healing product under pressure, which is an on-going research topic.

It has been indicated that implementation of axenic bacterial cultures can be expensive for applications in situ [43]. Silva et al. [14] revealed that use of self-encapsulated non-axenic cultures decreases the cost while providing comparable performance. Therefore (following the proof of concept with denitrifying axenic cultures), non-axenic denitrifying cultures that have comparable resilience and performance to axenic cultures have been developed for microbial self-healing concrete through NO_3^- reduction.

From economic point of view, it is essential to optimize the amount of bacteria and nutrients necessary for efficient self-healing of concrete cracks. Therefore, various combinations of bacterial healing agents and nutrients should be tested in concrete and optimized according to the needs of the concrete industry.

4 Repair Systems

4.1 Repair with Ureolytic Bacteria

4.1.1 Microbial $CaCO_3$ for Surface Protection

Because many current surface protection treatments are not satisfactory, in recent years researchers have focused on the application of bacterial calcium carbonate precipitation for surface consolidation and protection.

For research on the laboratory scale, specimens were immersed in bacterial culture or/and deposition media. The immersion time varied from 3 to 30 days

depending on the bacterial activity. The formed $CaCO_3$ layer decreased the water permeability, and showed a strong cohesion and adhesion with the matrix. This was confirmed by ultrasonic measurements and drilling resistance tests on limestone. In the latter tests, the resistance the drill bit experiences while drilling a small hole is a measure of the consolidation.

For on-site use, application by immersion is not easy to perform and has to be replaced by other techniques such as brushing or spraying. The procedure generally contains the following steps:

- Removal of loose particles from the original surface
- Spraying the bacterial culture
- Spraying the deposition medium

The second and the third steps can be repeated several times depending on the matrix type, bacterial concentration, etc. The first on-site application (50 m^2) was carried out in 1993 on the limestone of the Saint Medard Church in Thouars, France. The formed biocalcin reduced water absorption (1:5 ratio), yet the stone retained its gas permeability and aesthetic surface [44]. Tiano et al. [45] and Jroundi et al. [46] also applied bio-mineralization for on-site conservation of limestone surfaces. Jroundi applied only a nutritious medium to stimulate the carbonatogenesis activity of bacteria inhabiting the decayed calcarenite stones of San Jeronimo Monastery, Lisbon. Subsequently, bio-$CaCO_3$ crystals formed in situ, resulting in significant strengthening. To apply this in-situ bio-treatment, it is necessary to have preliminary knowledge about the microbial community inhabiting the matrix, and then the culture medium should be carefully chosen. However, it is easier and faster to import external bacterial strains whose high carbonatogenesity has been testified. De Muynck et al. [47] have been able to homogeneously strengthen limestone up to depths of 30 mm with similar or better performance than traditional surface treatments (such as ethyl silicates). The bio-deposition treatment is solvent free and entails both a significant protective and consolidating effect after two spray applications within the same day. By optimizing the concentrations of the urease and carbonate precursor solutions, they were able to lower the cost of the treatment to within the range of traditional consolidants. Furthermore, the replacement of carbonate precursors with silica or calcium carbonate nanoparticles presents a promising strategy for decreasing the amount of by-products, but needs optimization prior to being used in practice. A patent application was submitted (EP12155132.9 and PCT/EP2013/052783) with respect to the modified bio-deposition procedure.

Several aspects should be highlighted when applying a bio-treatment to a surface:

- Culture medium composition, culture conditions and bacterial type should not cause further damage to the building materials. One should be able to repeat the treatment.
- The amount of nutrients and deposition medium supplied should be balanced with the amount of bacteria, because the remains of nutrients and salts could

facilitate extra growth of other microbes on the surface and cause unpredicted damage to matrix materials.

- Spore-forming bacteria were suggested not to be used on the surface by many researchers [48, 49]. The germination of spores and uncontrolled growth pose a potential risk for the environment. However, other researchers such as Le Metayer-Levrel [44] have stated that no increase in microbial activity or changes in the autochthonous microbiota were observed immediately or 4 years after the treatment with *Bacillus cereus*, a spore-forming strain. Therefore, with a carefully controlled culture and medium supply, the risk could be greatly reduced.
- Bacterial strains applied should not be pathogenic.

Until now, the on-site application of bio-treatment is mainly used on historical building materials such as porous calcareous stones, but not on cementitious materials. Nevertheless, promising results have been obtained with bio-treatment of cement-based materials at the laboratory scale and in small on-site tests [50–55]. In most studies, the specimens were immersed in bacterial culture and deposition media. Compared with porous limestones, cement/concrete specimens are denser with a lower porosity, resulting in less retention of bio-agents in the surface layer after spraying or brushing. Also for limestone, the pore structure has an influence on the penetration depth and bio-deposition treatment efficiency, as shown by De Muynck et al. [56]. It was found that the largest amount of bacterial precipitation occurred in stones with a large amount of macropores, which is because absorption of bacterial cells (1–4 μm) occurs in pores with dimensions of 4–20 μm. De Muynck et al. (Fig. 20) have shown that the surface deposition of calcium carbonate crystals on mortar decreased the water absorption by 65–90% depending on the porosity of the specimens. As a consequence, the carbonation rate and chloride migration decreased by about 25–30% and 10–40%, respectively. An increased resistance to freezing and thawing was also noticed. The results obtained

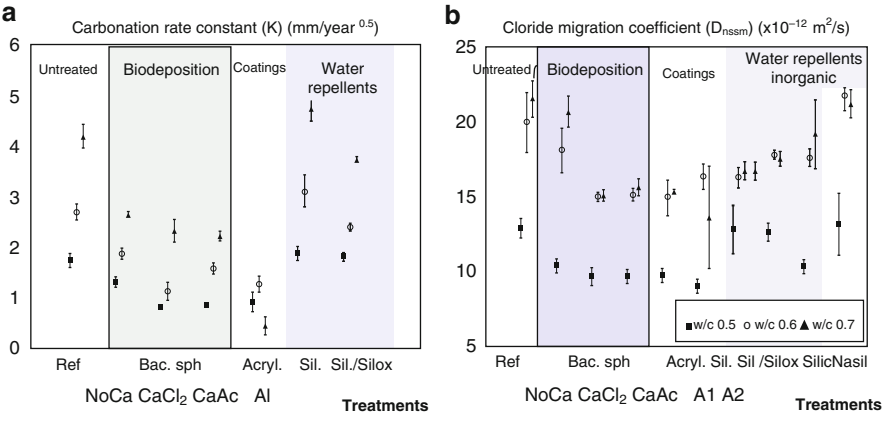

Fig. 20 (**a**) Carbonation rate constant and (**b**) chloride migration coefficient for mortar with different water-to-cement ratios, with different surface treatments [51]

with the bio-deposition treatment were similar to those obtained with conventional surface treatments (e.g. acrylate, silane, siloxane, silicone, silicate).

4.1.2 Microbial $CaCO_3$ for Crack Repair

Microbial $CaCO_3$ is also a promising material for repair of cracks in concrete. Bang et al. were among the first researchers to investigate the bacterial remediation of concrete cracks [57–59]. The bacterium they used was *Bacillus pasteurii*, a spore-forming strain with high urease activity and endurance in extreme environments. Artificial cracks with a constant width of 3.175 mm but with depths ranging from 3.175 to 25.4 mm were made on the specimens artificially by saw cutting. Pure microbial $CaCO_3$ is not able to seal such wide cracks completely. Therefore, Ramachandran et al. [58] applied a mixture of bacterial cells, nutrients and sands to the artificial cracks, followed by immersion of the specimens in urea–$CaCl_2$ medium for 28 days. The experimental results showed that specimens with cracks filled with bio-mixture demonstrated a significant increase in compressive strength compared with those without bacteria, from 10% to 27% depending on the bacterial amount added [58]. Scanning electron microscopy (SEM) showed that dense crystals embedded with bacterial cells mainly occurred close to the surface area of the crack. Because the bacterium *B. pasteurii* has higher activity in the presence of oxygen, the authors attributed this result to the limited oxygen availability in the deeper zones of the cracks. To induce precipitation throughout the cracks, Bang et al. [57] tried to repair cracks with *B. pasteurii* immobilized on polyurethane (PU) open cell foam. An obvious increase in the compressive strength was observed in the specimens with bacteria compared with those without bacteria, especially after 7 days. However, because there was no bonding between PU strips and the crack wall, PU together with MICP only functioned as filling material to seal the cracks.

De Belie et al. [60] and Van Tittelboom et al. [61] (Fig. 21) utilized silica sol–gel as filling material and as protective carrier for bacteria in the high pH environment in concrete. Bacterial cells were well mixed with silica sol and injected into the cracks manually, after which they were immersed in a solution of urea and calcium chloride, calcium nitrate or calcium acetate. The efficiency of this repair technique was compared with traditional repair techniques such as epoxy injection and application of grout mortar. Crack closure was shown by ultrasonic pulse velocity, microscopic investigation and water permeability tests. Crack sealing by means of this biological treatment resulted in a decrease in water permeability. However, the greater part of the decrease in water permeability was attributed to crack filling by the sol–gel matrix. Thermogravimetric analysis (TGA) of the crack repair material showed the presence of $CaCO_3$ crystals only in the case of active bacteria. Precipitation of these crystals inside the gel matrix could enhance the durability of this repair material.

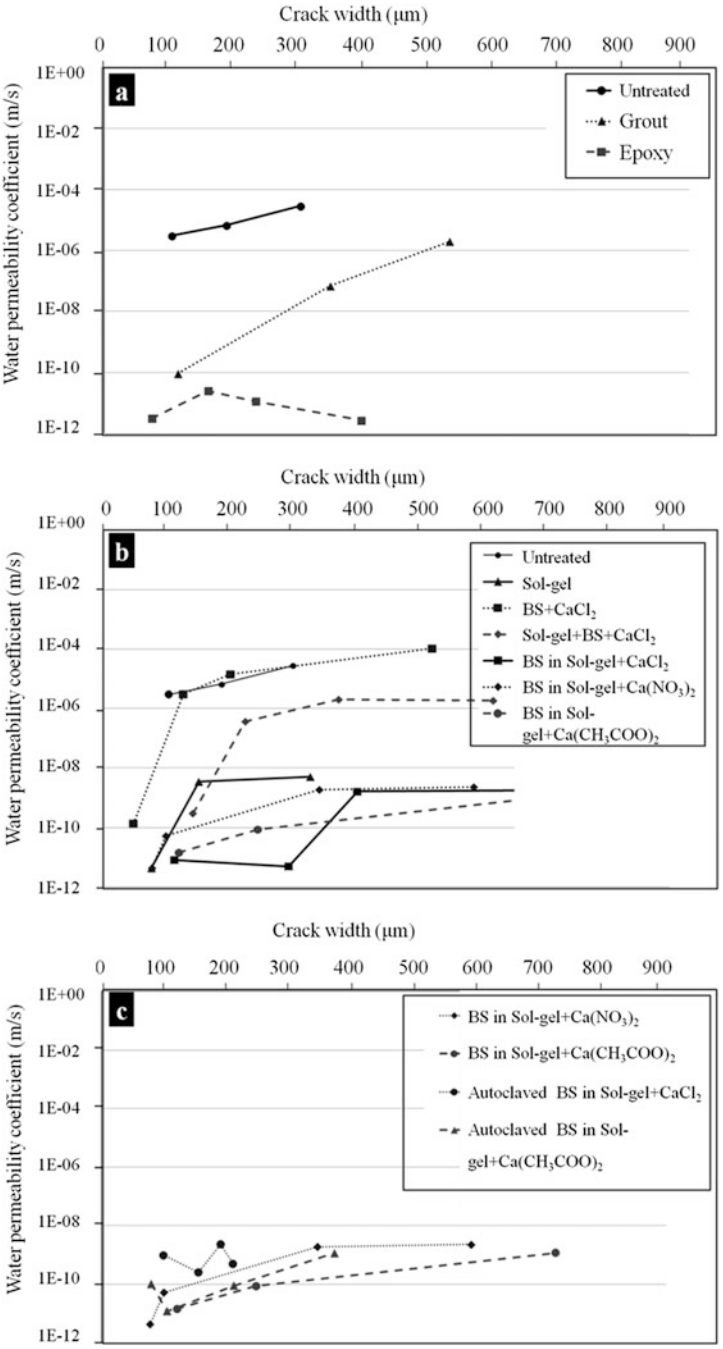

Fig. 21 Water permeability coefficient k (m/s) versus crack width (μm). (**a**) Untreated specimens and specimens treated with traditional repair techniques. (**b**) Untreated specimens and specimens treated with bacteria-based repair techniques. (**c**) Comparison of specimens treated with living and autoclaved *Bacillus sphaericus* [61]

4.1.3 Microbial $CaCO_3$ for Mechanical Strengthening

In addition to surface protection or crack healing, applying bacteria internally could also improve the mechanical properties and durability of concrete. Achal et al. [62, 63] applied *B. pasteurii* and *Bacillus megaterium* (urease +), both at a concentration of 107 cells/mL, and the 28-day compressive strength was increased by 17% and 21%, respectively. The durability of the specimens was also improved as a result of decreased water permeability. An increased pull-out strength of rebars in reinforced concrete after MICP was also reported [55]. Park et al. [64] isolated different calcite-forming bacterial strains from concrete structures and found that only one of the considered strains contributed to an increase (by 9%) in the 28-day compressive strength, whereas a decrease of 17% was observed in specimens with *B. pasteurii*. This is in contradiction to the results of Achal et al. [62]. Basaran [65] mixed vegetative *Sporosarcina pasteurii* cells in a urea–yeast extract medium with cement. An increase in calcium carbonate precipitation, reduction in porosity (24% at 28 days) and increase in compressive strength occurred when the bacterial medium was used. Viable *S. pasteurii* cells were found in hardened cement paste specimens as old as 330 days, with approximately 2% viability retention. About 50% of the viable cells detected were defined as vegetative cells, which could be metabolically active. This is the longest survival period recorded for microorganisms in cement-based materials without any encapsulation.

Overall, according to the authors of this section, two main mechanisms could be related to bacterial strengthening of concrete.

Formation of MICP as a Result of Bacterial Urease Activity

The formation of calcite inside the matrix could be related to urease activity inside both alive and dead bacterial cells. From the results of bacterial viability inside mortar specimens, it was found that the average number of viable bacteria decreased from 6×10^6 to 4.5×10^4 after 7 days, which means that most bacteria died. Bacterial growth was greatly inhibited, as shown by the similar cell numbers at 7 days and 28 days [63]. On the other hand, additional mortar strength increased slowly from 7 to 28 days. Urease remains in dead cells, but because of the high pH inside the specimens, urease activity is dramatically decreased, resulting in an extremely low calcite formation rate, which might be the reason for measurable strength increase only after 28 days. A high concentration of bacterial cells would cause extra voids inside concrete, which cannot be completely compensated for by the formation of calcite. Therefore, there would be a threshold value of bacterial cells.

New Silica Phase Introduced by Enzymes from Special Bacterial Strains

Ghosh et al. [66] investigated the interaction between bacteria and mortar matrix at the microscale. Mercury porosimetry confirmed the modification in pore size distribution resulting from the addition of microorganisms. They attributed the modification of pore properties to the newly formed silicate phase, which was induced by a silica-related enzyme excreted from bacteria. At higher cell concentrations, matrix integrity could be disrupted by excessive bacterial activity and thus result in a decrease in compressive strength of the mortar. A control experiment was carried out by adding the isolated protein (from the bacterium) to mortar specimens. An obvious increase in compressive strength was observed. The protein was characterized and found to dissociate silica from silica-rich substances and to form new silica phases. This resulted in enhanced coherence between sand particles and cement matrix at the microscale and, hence, in increased strength.

To summarize, use of calcite-forming bacteria on surfaces to improve surface properties and, hence, durability is a promising and convincing technique. However, to apply bacteria internally with the aim of increasing strength is more complex and dependent on bacterial strain. Whether bacterial cells simply remain in the matrix as organic fillers or make an active contribution still needs further exploration.

4.1.4 Microbial Repair Mortars

For the repair of (limestone) monuments, mortars made of Euville stone powder and traditional ethylsilicates, colloidal $Ca(OH)_2$ nanoparticles, amorphous silicon dioxide (SiO_2) nanoparticles, or *Bacillus sphaericus* in combination with nutrients have been investigated [67]. The procedure used by the Royal Institute for Cultural Heritage in Belgium was adopted for evaluation of the consolidation performance of loose grains of two different fractions (diameter, $d \leq 1.25$ mm and $1.5 \leq d \leq 3$ mm). According to this procedure, the consolidative action is determined from the weight of grains cemented compared with the total weight of grains present.

Overall, the cohesion of loose grains in Flacon tubes was better for the fine stone powders than for the coarse powders. The SiO_2 nanoparticles performed better than $Ca(OH)_2$ nanoparticles. Specimens treated only with bacteria absorbed water vapour after storage at 20°C and 65% relative humidity, giving rise to the loss of initial cohesion between grains. Treatment with SiO_2 nanoparticles in combination with *B. sphaericus* (Fig. 22), however, maintained cohesion and showed superiority over the traditional ethylsilicate consolidant.

The preparation of microbial repair mortars shows similarity to soil improvement techniques in that bacterial suspension and cementation media are injected from the top. Bio-$CaCO_3$ forms in situ in the pore space of the sand matrix and, hence, cements the sand particles. Using MICP from urea hydrolysis for soil improvement has been applied successfully at a scale of 1 m^3 and the first field

Fig. 22 Consolidation of Euville stone powder in Falcon tubes, using SiO_2 nanoparticles in combination with *Bacillus sphaericus*

test at a large scale (100 m^3) has also been performed, showing very promising efficiency [32]. These studies demonstrate the feasibility of in-situ soil improvement by MICP from bacterial urea hydrolysis.

4.2 Oxidation of Organic Carbon

4.2.1 Liquid Repair Systems

The repair system developed at the Technical University of Delft consists of concrete-compatible bacteria [23] and feed, which produces calcite-based minerals that decrease concrete porosity. This system is composed of two solutions:

- Solution A: Sodium-silicate (alkaline buffer), sodium-gluconate (carbon source for bacteria growth), alkaliphilic bacterial spores
- Solution B: Calcium nitrate (nitrate source for denitrification when oxygen is depleted and calcium for $CaCO_3$ precipitation), alkaliphilic bacterial spores

The silicate-based compound, sodium silicate, ensures an alkaline pH in the system and the formation of a gel inside the crack. Although not very strong, this gel allows rapid sealing of the crack (within a few hours) and an optimum environment for bacteria to precipitate $CaCO_3$. By the time the gel becomes too weak, a substantial amount of $CaCO_3$ has been precipitated to seal the crack.

Example of Application in a Parking Garage

The test location was a two-storey underground parking garage. The parking deck was suffering from cracking, resulting in leakage of the structure. Some cracks seem to have been previously injected with cementitious grout but were still leaky. For the new repair campaign, the bacteria-based liquid system developed at the Delft University of Technology was selected as repair material over other traditional repair methods such as epoxy injection. Besides lower cost, this material requires significantly shorter time for application than other methods, one day rather than several weeks. The system was hereby tested for the first time on a large scale. The surface area to be treated was around 2,000 m^2.

Figure 23 gives an overview of the pilot test, and the various treatments and tests performed. The treatment of the parking deck with the bacteria-based repair system took place on 18th October 2014. Seven weeks later (9th December 2014), a leakage test was performed on three cracks, selected randomly on the parking deck. Two cores were drilled for analysis to look for any evidence of biomineral formation. Because the tested cracks were still leaky, a second treatment was carried out on one crack on 17th December 2014. This time, as the treatment was performed in winter time, bacteria that grow at low temperatures (4–8°C) were added to the repair system. Crack impregnation was carried out manually with sprayers. Seven weeks later (10th February 2015), a second leakage test was carried out on the same cracks as previously tested on 9th December 2014. Two cores from the crack treated with cold temperature bacteria were drilled again for analysis.

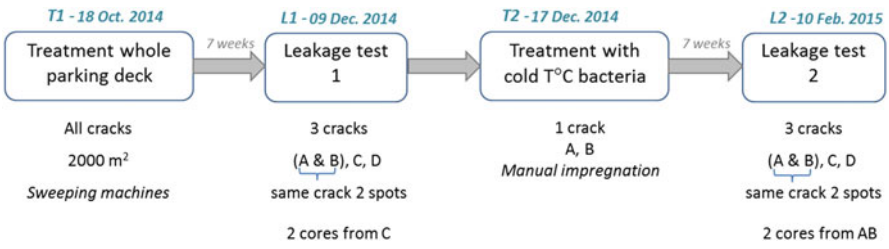

Fig. 23 Overview of treatments and leakage tests

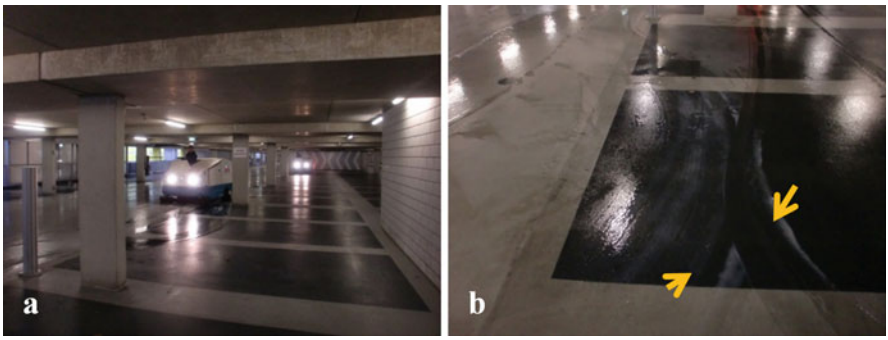

Fig. 24 (**a**) Application of solutions A and B (see text for composition) by the sweeping machines. (**b**) After the application/mixing of the two solutions. The *yellow arrows* indicate the gel that has formed at the surface of a crack

Preparation of the Bacteria-Based Repair System: 18th October 2014

The composition of the solution was calculated per bag of 25 kg of either sodium gluconate or calcium nitrate tetrahydrate for either 200 or 50 L of solution. Usually, 0.5 L of bacteria-based repair solution is used per square meter of surface to be treated. However, this amount can vary depending on the crack volume and the porosity of the substrate. Before the application, little was known about the crack volume, concrete porosity or dead volume of solution needed for the sweeping machine, so an excess quantity of powders were transported.

The buckets were first filled with 50 L warm water and the powders were added. The solutions were mixed with electric mixers until complete dissolution of the powders. pH was checked and the solutions were poured into the sweeping machines (solution A into machine A and solution B into machine B).

Sweeping machine B needed to deliver less solution than sweeping machine A in order to obtain the right mixing ratio of solution A to solution B. Because the amount of solution released by the machines could not be adapted, sweeping machine A went first and somewhat slower than sweeping machine B. The pH of the gel obtained after passage of the sweeping machines was checked to validate the mixing ratio (Fig. 24).

Leakage Test 1 (9th December 2014) and Core Analysis

Wooden frames were placed along the cracks to be tested and sealed with silicon. About 5 L of tap water was poured and left 15 min for saturation of the crack. The drops leaking through the crack were counted for 1 min; the count was performed six times to obtain an average value. Results showed that one crack was still leaking significantly, suggesting that the treatment might not have penetrated deep in the crack. However, it should be mentioned that no initial leaking test had been performed, and although the crack seemed to leak heavily, it might be less than before treatment.

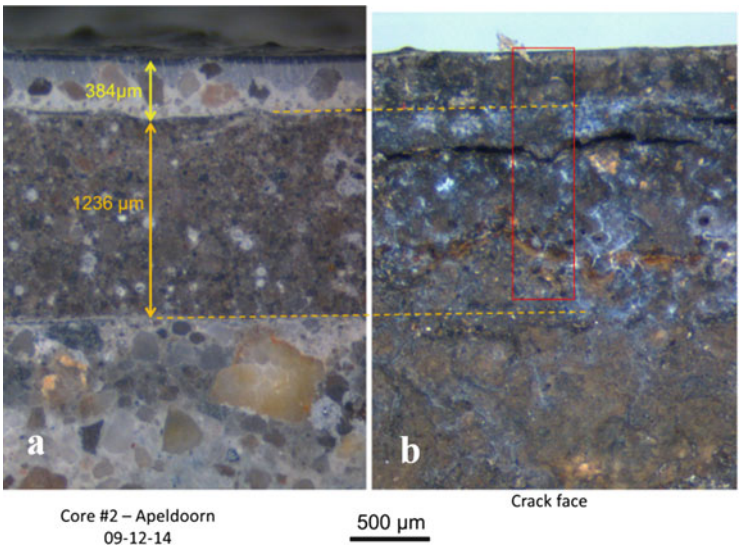

Fig. 25 Observation with stereomicroscope of the core: (**a**) outer part of the core and (**b**) crack face. The *red rectangle* indicates the area observed and analyzed with ESEM

Observation of the cores using stereomicroscopy (Fig. 25) and environmental scanning electron microscopy (ESEM) showed that the coating present on the parking deck was not well attached to the concrete, and so the repair solution might have penetrated along the delamination. Figure 25 shows a whitish colour along the crack surface, which might indicate the presence of the repair system. However, observations and elemental analysis with the ESEM did not show any significant differences in composition nor in morphology between these whitish and non-whitish areas, making it hard to draw any conclusion relating to the penetration depth of the repair system.

Second Treatment with Cold Temperature Bacteria: 17th December 2014

Based on the results from the leakage test, a second treatment with the repair system amended with bacteria adapted to cold temperatures was performed on 17th December 2014. One crack (AB) was impregnated manually with the repair system. Inspection from the level below showed that the repair solution was dripping through and thus had penetrated through the full length of the crack. The second leakage test (Fig. 26) was performed on crack AB 7 weeks after the second treatment, on 10th February 2015. Two cores were also drilled for analysis. Results of the leakage test were encouraging as the crack was leaking significantly less. Location A had 43% less leakage compared with the previous test and location B, which was dripping heavily, had 80% less leakage. Moreover, the leakage at location B only came from specific spots along the crack. Figure 27 shows ESEM observations of the core drilled after the second treatment. Calcium-based mineral (Fig. 27a) and distinctive morphologies, small rod-like imprints (Fig. 27b), were

Fig. 26 Second leakage test on crack AB

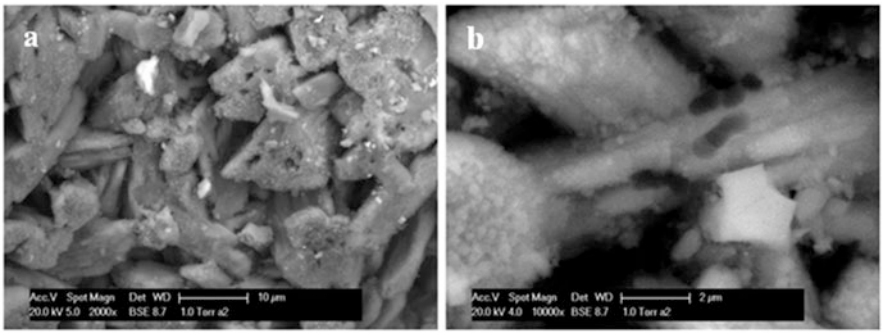

Fig. 27 ESEM observations from the core drilled along crack AB on 10th February 2015

observed along the core. The shape and size of these imprints were in good agreement with those of bacteria, suggesting that they were bacteria. This is a strong indication of the involvement of bacteria in the calcium carbonate formation, which suggests that the second treatment was successful.

4.2.2 Bio-Based Mortar for Patch Repair

Laboratory Scale Testing

Concrete patch repair systems frequently face durability-related problems because of a lack of compatibility between the repair material and the concrete substrate. The newly cast repair materials are subjected to differential shrinkage deformations, which lead to tensile stress development. Shrinkage deformations can be compensated for, but it is also possible to apply a material with a higher tensile capacity such as strain-hardening cement-based composites (SHCC). SHCC are

designed to have a large strain capacity because of the use of a low percentage of randomly distributed polymer fibres [68]. As a repair material, SHCC can carry more tensile load and accommodate larger tensile strain than other repair systems [69, 70]. The tensile strain translates into micro-cracking. To a certain extent these micro-cracks heal through autogenous healing, but to improve the healing capacity of SHCC a bacteria-based healing agent can be included into the mix design.

The healing agent consists of bacterial spores related to the species *Bacillus cohnii*, calcium lactate and yeast extract immobilized on LWA. The mortar mix includes CEM I, fly ash, limestone powder, LWA impregnated with the healing agent, poly(vinyl alcohol) fibres, water and superplasticizer.

The average compressive strength at 28 days of the SHCC with bacteria was 39.8 MPa and without bacteria 38.5 MPa, fulfilling the strength requirements for structural repair according to the EN 1504 standard. Under bending stress, both materials exhibited ductile behaviour and developed multiple cracks prior to failure [71]. When tested as a repair material, SHCC with healing agent showed reduced delamination with the concrete substrate, sufficient bond strength according to EN 1504 and improved flexural behaviour as a composite (together with the concrete substrate) compared with the mortar without healing agent [72].

To evaluate the healing capacity, specimens with and without bacteria were pre-cracked under bending stress, then unloaded and placed to cure under water. After a certain curing period the specimens were tested to failure. The results of these tests were compared with the results of tests conducted on specimens that had the same curing conditions but no pre-cracking. The healed specimens (with and without bacteria) had a higher strength than the reference specimens, which had no pre-cracking [71]. Same results were obtained even after 2 pre-cracking cycles [73]. Microscopic observation after the healing period showed calcium carbonate precipitates in the cracks in all specimens. Oxygen consumption measurements and Fourier transform infrared (FTIR) analysis of the precipitates showed bacterial activity in the SHCC with healing agent, but the lack of enhanced $CaCO_3$ precipitation could be attributed to limited amounts of feed applied [73].

In order to overcome this problem, more bacteria and food should be included in the mortar. For this purpose, particles as suggested in Sect. 3.2.2 were incorporated into the mortar mixture. The use of these particles (15 g/L of mortar) in combination with LWA are currently being tested both in the laboratory and in field applications.

Field Applications in the Netherlands

Since 2013, the mortar for concrete repair described above has been applied as patch repair in different locations in the Netherlands, under very diverse weather conditions. The first application took place during May 2013 in a garage exposed to the weather elements. In the structure to be repaired, the steel reinforcement showed signs of corrosion, which led to spalling of the concrete. During the week prior to application the temperature fluctuated around 20°C and only 1.3 mm of rainfall was registered; hence, the concrete was dry. The repair mortar was placed to

Fig. 28 Self-healing mortar applied in a tunnel in the Netherlands: (**a**) surface preparation and (**b**) monitoring for shrinkage and possible delamination

completely cover the reinforcement. After 1.5 years the patch repair is in good condition without signs of deterioration.

The repair mortar was also applied under extremely moist conditions in an underground parking garage where the retaining walls had suffered water leakage problems for months. Traces of previous repair materials were removed with a hisel prior to casting, including traces of bitumen. The mortar was cast in place and no curing compound was applied. A month after casting a very small amount of leakage was still spotted, but it has not led to spalling even after 6 months. Another indoor application took place in a tunnel that was built in the late 1930s. The concrete slab showed signs of steel bar corrosion and concrete spalling. A concrete patch of about $250 \times 500 \times 110$ mm^3 was removed and the exposed steel bars were cleaned of traces of corrosion (Fig. 28a). After casting the mortar, the patch was covered with a geotextile material for the first 7 days. The possible cracking or delamination as a result of restrained shrinkage was monitored for the following 2 months (Fig. 28b). No delamination occurred and only very small shrinkage cracks were observed.

5 Conclusions

Autogenous healing of concrete and mortar can be drastically enhanced by combining with limestone originating from bacterial activity. The bio-based self-healing agents described in this review use several bacterial strains that follow

different metabolic pathways; yet, in all systems the final healing product that fills the cracks is limestone. The selected bacterial strains are able to survive in the alkaline concrete environment. In order to obtain maximum protection during concrete mixing and to yield the highest survival potential for many years, encapsulation of the healing agent is of great importance, although it can affect the mechanical properties of the material.

The development of bio-based self-healing concrete aims to minimize durability problems related to cracking. Therefore, crack closure observations together with water permeability tests were conducted to assess the recovery of water tightness after healing treatment. The results proved to be very promising, thus upscaling processes and outdoor applications with bio-based self-healing concrete are currently being investigated.

In addition, the less innovative concept of concrete repair, but with novel technologies embracing environmentally friendly practices has been described. Repair systems with incorporated bacteria have been studied and applied in real structures suffering from cracking, spalling and other problems related to concrete ageing. Recent results revealed that there is definitely a market for these novel repair systems, which meet needs that conventional repair systems cannot.

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