

3 Recovery against Environmental Action

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3.1 Autogenic Self-Healing

Autogenic self-healing has been defined in chapter 1 as a self-healing process where the recovery process uses materials components that could also be present when not specifically designed for self-healing (own generic materials).

3.1.1 Causes of Autogenic Self-Healing

An example of recovery against environmental action through self-healing is the width of a through-crack that diminishes with time. The cause for this is manifold. One can distinguish between physical, chemical, and mechanical causes [3.1], see also Fig. 3.1.

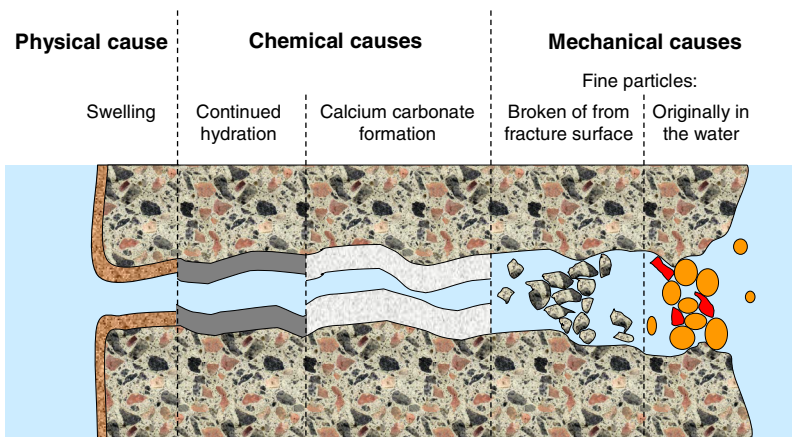


Fig. 3.1 Different causes that can lead to autogenic self-healing

The physical cause is due to swelling of hydrated cement paste (HCP) near the crack faces. This phenomenon occurs when water is absorbed by HCP and reaches the space between the constituents of HCP. When the effect of swelling is estimated it turns out that this is marginal and does cause a reduction of the fluid flow by less than 10%.

There are two chemical processes. The first is the continued hydration of cement which has not reacted hitherto due to lack of water. When new reaction products are formed they grow into the free space of the crack. The hydration products occupy about double the space of the original cement grain. The continued hydration cannot be responsible for a complete self-closing of a crack, but, assuming a small crack width of 0.1 mm and assuming a simultaneous action of swelling and hydration, one can get self-healing. With larger crack width the influence becomes minor.

The second chemical process is the formation of calcium carbonate and the growth of crystals on the crack faces. Calcium ions from the pore water of concrete (Ca^{2+}) react with the carbonate ions of the water in the crack (CO_3^{2-}) and combine to CaCO_3 which precipitates in the crack. The reaction depends on temperature, pH, and concentration of the reactants. The mechanism has been investigated by Edvardsen in great detail [3.1]. It has been shown that this mechanism gives by far the most important contribution to self-healing.

There are two mechanical causes which are contributing to self-healing, i.e. the presence of fine particles in the water which leaks through the crack, and the fracturing of small concrete particles from the crack faces. However, these effects are of minor importance.

The above mentioned causes of self-healing are influenced by the concrete, by the water in the crack, and by the crack geometry. Table 3.1 shows the influencing factors schematically.

Table 3.1 Influencing factors on self-healing [3.1]

Concrete	Water	Crack
Type of cement	Pressure	Width
Type of additions	Pressure gradient	Length
Type of aggregate	Hardness	Through-crack vs. bending crack
Age	pH	Branched crack
	Temperature	Accumulated crack

Concrete is a compound of matrix and aggregates. The matrix is composed of HCP, additions, and the very fine material of the aggregates. When cement hydrates calcium silicate hydrates (CSH), aluminates, and calcium hydroxide are formed. Calcium hydroxide is soluble in water and supplies the calcium ions which are necessary for the formation of calcium carbonate in the crack. (CSH is almost insoluble and will only supply calcium ions when the ions from calcium hydroxide are exhausted.) The supply of calcium ions from the HCP is the larger the more clinker is in the cement, i.e. Portland cement is the richest.

In composite cements, a part of calcium hydroxide is used for the formation of hydration products from a second main constituent like fly ash or pozzolanas. The

same is true for blast furnace slag cement which uses calcium hydroxide as an activator. The effects of such increasing demands of calcium hydroxide over time are shown in Fig. 3.2. One can see that the amount is larger for Portland cement than for blast furnace cement and that fly ash reduces the amount of calcium hydroxide with ongoing hydration.

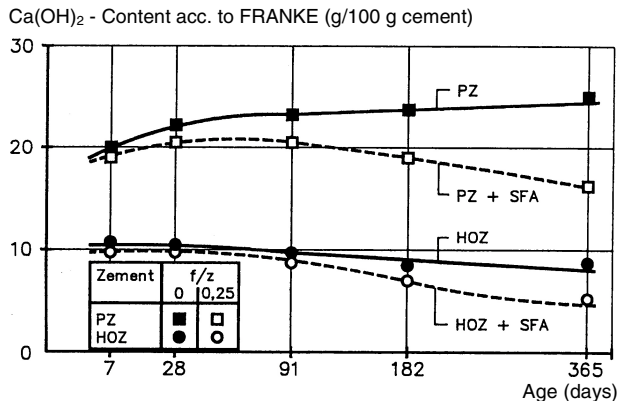


Fig. 3.2 Amount of calcium hydroxide as function of age and type of cement [3.2]. PZ = Portland cement, HOZ = blast furnace cement, SFA = fly ash.

As the type of aggregate is concerned limestone aggregates may support self-healing. Furthermore, the shape of the aggregate grains and the grading curve can influence the crack geometry. The influence of the aggregate size is exemplified in Fig. 3.3. For a given crack width w_m which is measured on the surface, the responsible opening for the flow of water is larger in case of small aggregates than with large aggregates. In concrete with a continuous grading of aggregates the situation is more complex but the same principle still holds.

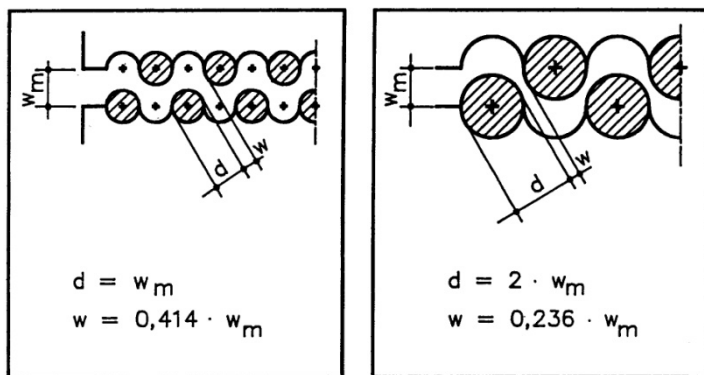


Fig. 3.3 Idealised cracks in concrete with different aggregate size [3.4]

Water in the crack is the most important factor for autogenic self-healing. Without water there is no self-healing because water is needed for the chemical reaction and for the transport of fine particles. If carbon dioxide is present in water there is always a certain equilibrium between bound CO_2 , half-bound CO_2 , and free CO_2 which can be divided into complementary CO_2 and surplus CO_2 . The surplus CO_2 is responsible for concrete attack where calcium carbonate dissolves to soluble calcium bicarbonate, whereas the complementary CO_2 leads to the formation of calcium carbonate from calcium hydroxide. There are several classifications of the aggressiveness of CO_2 on concrete (see for instance [3.3]).

The calcium carbonate grows on the crack surface as shown in Fig. 3.4. The calcite crystals (CaCO_3) form a dense layer which blocks the flow of water. The complete theory on crystal growth can be found in [3.5].

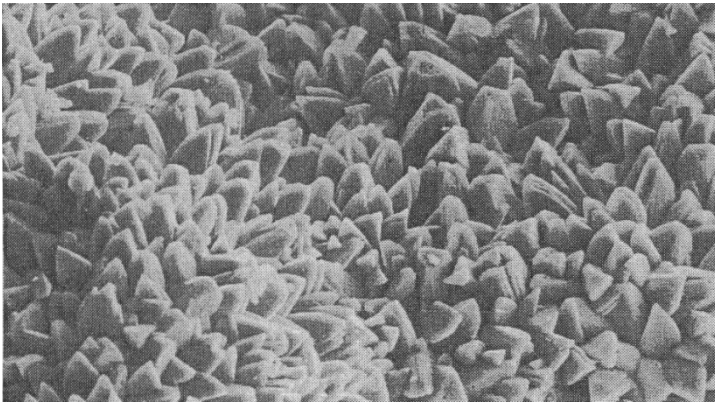


Fig. 3.4 Calcite crystals on a crack face after opening the crack [3.1]

3.1.2 Experimental Evidence on Autogenic Self-Healing

The self-healing phenomenon has been experienced since long time. Edvardsen performed a literature survey which will be used in the following paragraphs in short [3.1]. The first observation has been made by the French Academy of Science in 1836. Early publications mention the effect of self-healing [3.6-3.13]. Loving reported on self-healing of cracked concrete tubes mentioning the deposition of calcium carbonate in the crack [3.14]. Wagner [3.15] carried out experiments on the cement lining in steel pipes and found that cracks of 0.05 mm healed completely after 60 h. Cracks of 0.1 mm healed only after 1 year. Lauer and Slate [3.16] and Hannant and Keer [3.17] investigated the tensile strength of cracked hydrated cement paste (HCP) stored in water. They could show that 1 d old HCP regained about 25 % of its original strength whereas 28 d old HCP got only 7 % back. After 2 years moist storage [3.17] found even 50 % recovery. The authors concluded that continued hydration was the cause for the recovery. The influence of concrete age, ambient conditions, type of cement and aggregates, and

concrete composition have been studied by [3.18]. They concluded from the tests that the moisture supply is the most important factor for self-healing. They confirmed the conclusion by Lauer and Slate that the age at first cracking is also essential. The investigations by Clear [3.19] on cracked concrete cubes showed no influence of type of cement. He found that the smaller the initial flow was, the better the self-healing effect was. He found also that a slow increase of the pressure gradient during water exposure has a positive effect. As cause of self-healing, blocking of the crack by fine particles and the precipitation of calcium carbonate are assumed. Ripphausen [3.20] performed tests on reinforced concrete blocks. A main result was that the initial flow could be reduced to 1 to 10 % after 3 days. The most important parameters were found to be crack width and water pressure gradient. Meichsner [3.21] postulated two sources of self-healing. The first is the quick blocking of the crack by fine particles and the subsequent cause is the forming of calcium carbonate on the crack faces. Coarse aggregates in concrete yield a rougher crack surface and therefore a better self-healing effect. With increasing pressure gradient, the time until complete sealing becomes longer.

Edvardsen [3.1] performed systematic water permeability tests on small specimens with a single tensile through-crack. A typical result is shown in Fig. 3.5. The striking result is that the flow of water comes to standstill after 300 to 700 h.

The type of cement had a marginal influence. Several test series have been carried out with variation of water source (hardness), type of addition in the concrete, type of aggregate, pressure gradient, and crack width. It turned out that the first three parameters had hardly an effect on the results. The influence of the crack width is illustrated by Fig. 3.6. A 0.1 mm crack shows a flow reduction by

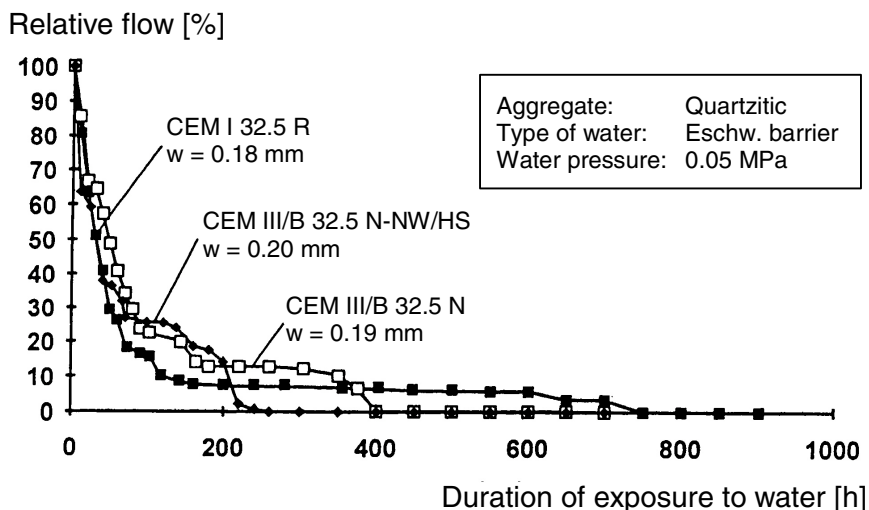


Fig. 3.5 Flow relative to initial flow vs. duration of exposure to water [3.1]. CEM I = OPC, CEM III = BFSC.

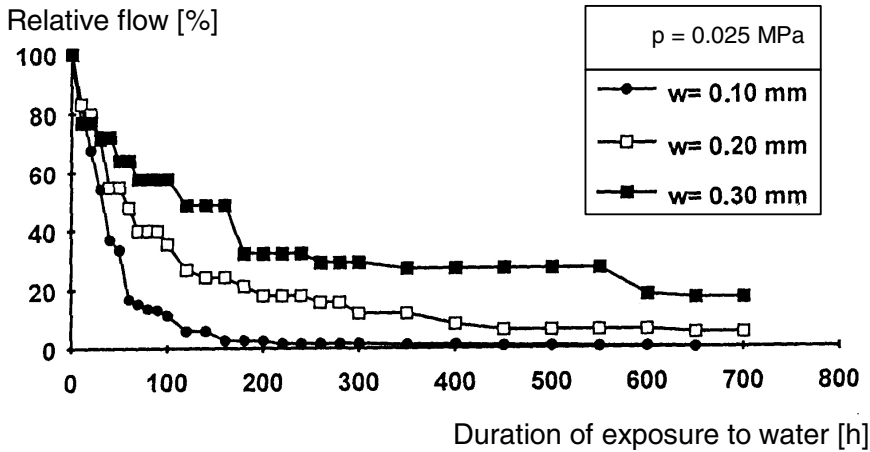


Fig. 3.6 Influence of crack width on flow relative to initial flow [3.1]

about 90 % after 100 h, the 0.2 mm crack a reduction by 60 % and the 0.3 mm crack a reduction by 40 %. After 300 h, the 0.1 mm crack is blocked whereas the 0.2 mm crack has a leakage rate of 10 % and the 0.3 mm crack of a little less than 40 %. After 700 h, the leakage rate of the 0.2 mm crack is almost zero whereas the 0.3 mm crack has still a leakage rate of about 20 %. However, one should also look to the initial flow which is given in Fig. 3.7. The initial flow can be approximated by Poiseuille's third power law and it depends on the pressure gradient. Evaluating Fig. 3.6 and Fig. 3.7 together one can postulate that the reduction of flow in absolute sense is strongest for the larger crack width.

Edvardsen explains the self-healing effect by crystallisation of CaCO_3 in the cracks. X-ray diffraction experiments from crack faces show CaCO_3 as the main element. The author presents also a model governing the nucleation of calcium carbonate based on two different crystal growth processes. In the initial phase of water exposure, the kinetics of crystal growth is surface controlled (reactions with crystals on the crack surfaces) and later it is diffusion controlled (reactions with ion diffusing through the calcium carbonate layer already crystallized). The author shows that blocking of flow path by broken concrete particles is of less importance [3.22].

The influence of temperature between 20 and 80 °C and crack width between 0.05 and 0.15 mm on self-healing has been investigated by Reinhardt and Jooss [3.23]. Fig. 3.8 shows the results for high-strength concrete ($f_c = 93$ MPa) with a constant crack width of 0.05 mm. The pressure gradient was 1 MPa/m over a specimen which was 50 mm thick. It is clearly visible that those specimens which were installed at 80 °C show the most efficient self-healing behaviour. After 75 h, the 20 °C specimen had a flow rate of 30 % of the initial flow rate, the 50 °C specimen about 10 % and the 80 °C specimen merely 3 %. After 330 h, the flow rate in all specimens went asymptotically to zero, i.e. complete sealing. Similar

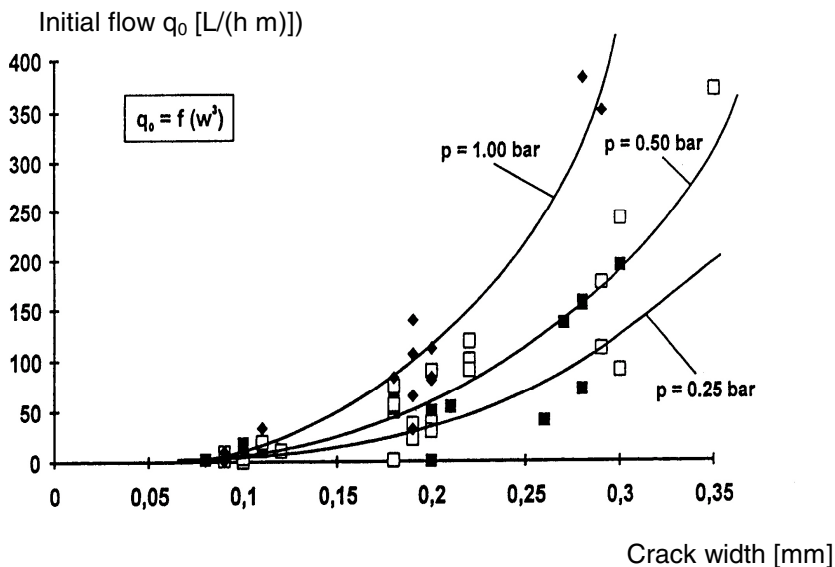


Fig. 3.7 Initial flow through cracks of various widths for 1 m length [3.1]

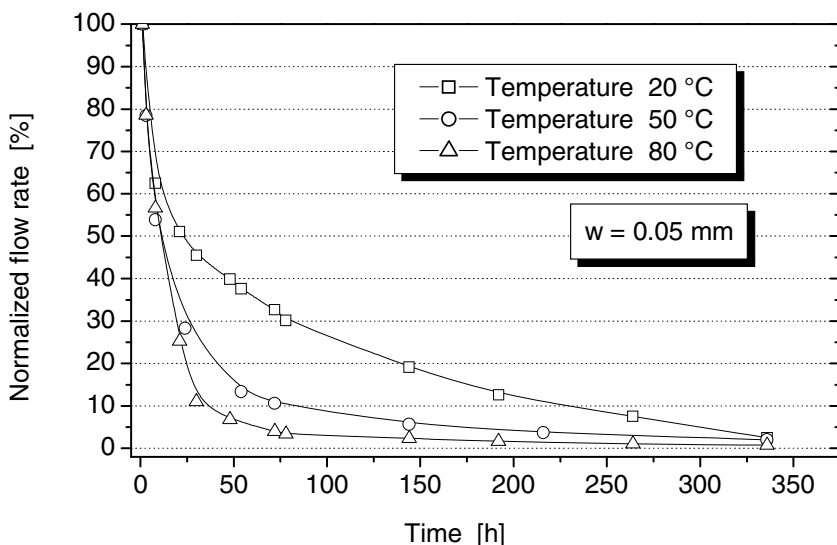


Fig. 3.8 Decrease of normalized flow rate of high-strength concrete at various temperatures [3.23]

graphs could also be drawn for 0.10 and 0.15 mm crack width. However, it should be kept in mind that the absolute flow is about 3 times higher for the 80 °C water than for the 20 °C water since the viscosity of water drops from 1.00 mPa s to 0.35 mPa s at the same time.

The following two paragraphs are partly literally taken from [3.22]. Hearn [3.24, 3.25] carried out water permeability tests on mortar with 78 % hydrated clinker and 26 years old water-cured concrete with insignificant amount of unhydrated clinker. The author shows that without drying, especially on virgin never dried old concrete, there is no self-healing of cracks whereas drying results in an increasing initial permeability followed by self-healing. The author carried out chemical analyses and could find a phenomenon of dissolution-deposition, especially of CaCO_3 . Moreover, self-healing was completely absent where water was replaced by propan-2-ol as a permeant. That is the most convincing evidence that physical clogging is not responsible for the phenomenon since its viscosity is close to that of water. Flow reversal tests show that self-healing is permanent. Concerning continued hydration, it is important for early-age concrete but not for mature one.

Another type of experiments was carried out by Jacobsen *et al.* [3.26, 3.27]. It consisted of damaging concrete cubes by rapid freeze-thaw cycles, and then storing them in 20 °C warm water for 3 months. Two types of concrete at water-cement ratio of 0.4, one with silica fume and the other without silica fume, were tested. Deterioration and healing was measured by resonance frequency (converted to dynamic modulus of elasticity) and compressive strength. Freeze-thaw cycles led to loss in both properties. Self-healing gave a substantial recovery of the frequency, but only a small recovery in compressive strength. It was explained by SEM observations that the cracks were only partly filled with new products. Most of the crystals seen in the cracks were newly formed CSH. Portlandite (Ca(OH)_2) was also observed locally. The study demonstrates that self-healing is less apparent in concrete containing silica fume than in concrete without silica fume.

Tests on Portland limestone composite cement (PLC) in comparison to OPC have been performed in order to study the self-healing properties of cracks which were 0.07 to 0.14 mm wide [3.28]. The authors did not find a difference between OPC and PLC. The results showed that the sealing time varied between 20 h for crack width of 0.10 mm and 70 h for crack width of 0.13 mm. However, this is only a rough indication since the scatter was rather large.

Some researchers [3.29-3.41] try to stimulate the self-closing potential of cementitious materials by replacing part of the cement by fly ash or blast furnace slag. In their study, Van Tittelboom *et al.* [3.41] prepared mortar specimens, with 8 different mix compositions. Three different cement types were used: CEM I or Portland cement, containing 100% Portland clinker; CEM II/B-M or also called Portland composite cement, containing 65-80% Portland clinker and 20-35% of a mixture of blast furnace slag, fly ash and limestone; and CEM III/B or blast furnace cement, containing 20-35% Portland clinker and 65-80% blast furnace slag. For some of the mixtures made with ordinary Portland cement (CEM I), respectively 30 and 50% of cement weight was replaced by fly ash and in two other mixes 50 and 70% of cement weight was substituted by blast furnace slag. All mortar mixtures were made with a water-to-binder ratio of 0.4, except for one mixture where a water-to-binder ratio of 0.5 was used. In Table 3.2, the composition of each mix is shown.

Table 3.2 Composition of the mortar mixes giving weight of each constituent in gram [3.40]

	Composition					
	Sand	Cement			Fly ash	Blast furnace slag
		DIN 196-1	CEM I 52.5 N	CEM II/ B-M 32.5 N		
CEMI-0.4	1350	450				180
CEMI-0.4-30FA	1350	315			135	180
CEMI-0.4-50FA	1350	225			225	180
CEMI-0.4-50BFS	1350	225			225	180
CEMI-0.4-70BFS	1350	135			315	180
CEMII-0.4	1350		450			180
CEMIII-0.4	1350			450		180
CEMI-0.5	1350	450				225

When specimens were cured for 1 month and a half, they were fractured in a crack opening controlled splitting test. Subsequently, the crack width of each sample was measured at different locations, equally divided along the crack length. The average value was used to characterize the crack width of each specimen. The effect of the mortar composition on the self-healing potential was investigated by measuring the evolution in water permeability of the cracked specimens. A low pressure water permeability test setup was used. The drop in water level, due to water flow through the cracked specimen, was measured once a day and water was restored each time to the original level. Darcy's law was used to calculate the water permeability coefficient k .

During the test it was seen that the water flow through the specimens decreased. Van Tittelboom and De Belie [3.40] attributed the decrease during the first days of the test to disappearance of air bubbles present in the specimens. The ongoing decrease in water flow after 5 days from the beginning of the test was ascribed to crack-closing. For each specimen, the difference in the water permeability coefficient calculated at day 5 of the test and at the end of the test, being 14 days later, was calculated. In Fig. 3.9, the result is displayed.

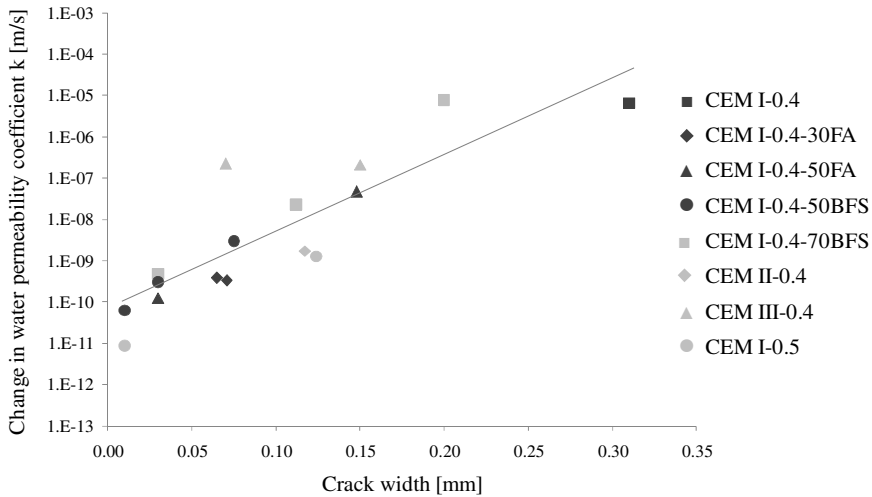


Fig. 3.9 Decrease in water permeability coefficient k [m/s] due to autogenous closing of the crack in specimens with different crack widths [mm] and with different mix compositions; the grey line indicates the mean change in water permeability [3.40]

Van Tittelboom and De Belie [3.40] determined that for increasing crack widths, the difference in water permeability coefficient and consequently also the self-healing potential increased. It was supposed that mixes with a higher amount of cement particles or a lower water-to-binder ratio would contain more unhydrated particles and thus result in a higher degree of self-healing. However, this was not clear in the results. No big difference in self-healing potential was found between samples with a water-to-binder ratio of 0.4 and 0.5. By contrast a clear difference in self-healing potential was noticed depending on the mix composition. The degree of self-healing was higher when a higher amount of slag was used. It was supposed that part of the slag had not yet been hydrated during curing of the mortar and that unhydrated particles reacted during the water permeability test. As blast furnace slag is a latent hydraulic component, it may have been activated upon contact with water and quicker in the presence of free lime. In the case Portland clinker was replaced by fly ash, no big decrease in water permeability was detected. It seemed that the alkalinity in the crack environment was high enough to cause a fast hydration of blast furnace slag in the presence of water. However, this was not the case for fly ash. Van Tittelboom and De Belie stated that this difference could also be caused by the fact that the reaction of fly ash is slower than the reaction of blast furnace slag. From these experiments Van Tittelboom and De Belie concluded that the self-healing potential of cementitious materials may be altered by changing the mix composition.

3.1.3 Limiting Crack Width for Autogenic Self-Healing

There are several hypotheses as the maximum crack width is concerned which can heal. Self-healing of bending cracks is more likely than of through-cracks. Ter Heide [3.38] postulated that a through-crack can never heal completely because of lack-of-fit of the crack faces. However, several other investigators have performed experiments on tensile cracks which were made by uniaxial pre-cracking or splitting. In these cases, a through-crack develops. They have found that such cracks can heal completely. However, one should consider the age and the composition of the concrete, the ambient environment, and the water supply. It turned out that young concrete heals faster, and that concrete with silica fume shows minor self-healing. There must always be water available and carbon dioxide because the main cause of self-healing seems to be the formation and precipitation of calcium carbonate.

Jacobsen *et al.* [3.42] made a survey and came to the conclusion that a through-crack, which is the most dangerous in water-retaining structures, of 0.2 mm can heal completely. The same value is supported by Meichsner [3.43]. Edvardsen [3.1] found that a crack of 0.3 mm can still heal. In her design proposal, she differentiates between water head (hydraulic gradient), crack width, and occurring movement of the crack [3.5, 3.44]. The proposal is shown in Table 3.3.

Table 3.3 Permissible crack width for self-healing [3.5, 3.44]

Hydraulic gradient, m/m	Design crack width, mm	
	With 10 % movement	With 10 to 20 % movement
40	0.10 to 0.15	0.10
25	0.15 to 0.20	0.10 to 0.15
15	0.20 to 0.25	0.15 to 0.20

A crack movement of 10 % means that the crack may change its width by 10 % due to alternate loading, temperature, creep, or shrinkage/swelling of the structural element. Design crack width is a terminus technicus from DIN 1045-1 [3.45] and means the crack width which is calculated in the structural analysis. It is a probabilistic value with a certain distribution function. This value can be smaller or greater in real structures.

3.2 Autonomic Self-Healing

Autonomic self-healing has been defined in chapter 1 as a self-healing process where the the recovery process uses materials components that would otherwise not be found in the material (engineered additions).

3.2.1 Superabsorbent Polymers

Crack sealing can also be enhanced by using superabsorbent polymers (SAPs). These cross-linked polymers have the ability to absorb a significant amount of liquid from the surrounding environment (up to 500 times their own weight) and to retain the liquid within their structure without dissolving (Fig. 3.10).

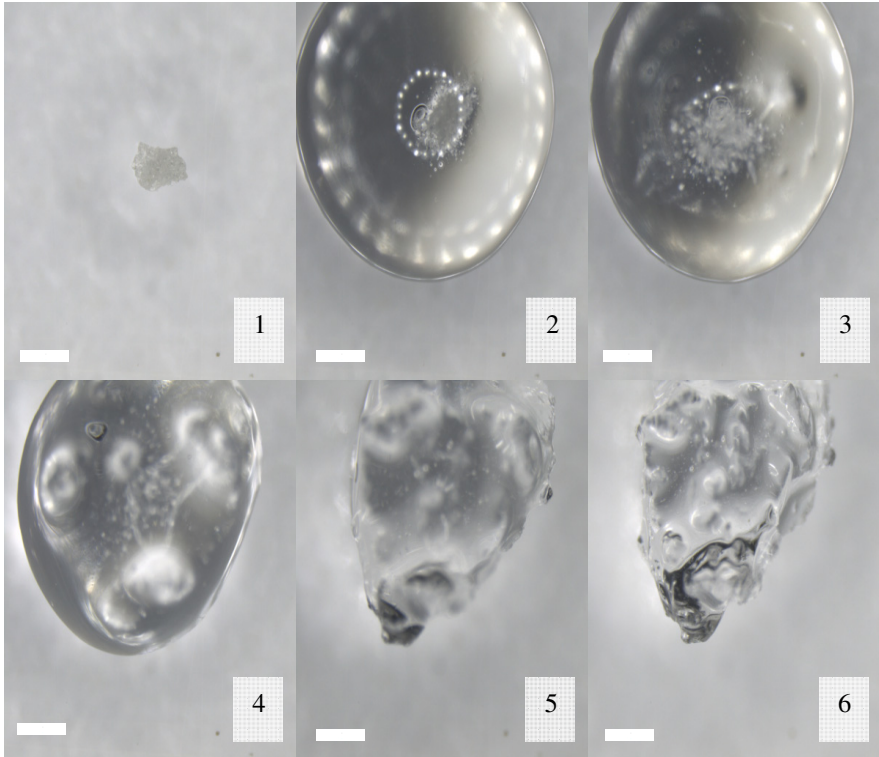


Fig. 3.10 Absorption of water by a SAP particle. The scale bars on the bottom right have a length of 500 μm . [3.46]

Lee *et al.* [3.47] used superabsorbent polymers to obtain self-sealing properties. In his research, Lee used SAPs based on partially neutralized acrylates and acrylate/acrylamide copolymers and proved the sealing potential in different solutions between glass plates [3.47]. Cement pastes were also studied and the flow rate of a dilute sodium chloride solution through cracked specimens was measured (Fig. 3.11) [3.48]. The cumulative flow through specimens containing SAPs (xSy: x m% of cement weight of SAP y) was lower than for control specimens and specimens with 4-5 m% of SAPs by weight of cement (specimens 5S2 and 4S3) showed even a reduction up to 80% of the cumulative flow. When

cracking thus occurs, superabsorbent polymers are exposed to the humid environment and swell. In this way, the crack is blocked and the permeability is reduced.

Tsuji *et al.* [3.49] also investigated the effect of SAPs on the leakage of water through cracks. They found a decrease of the amount of water leaked through a crack in a mortar specimen containing 0.5 m% of cement weight of SAPs.

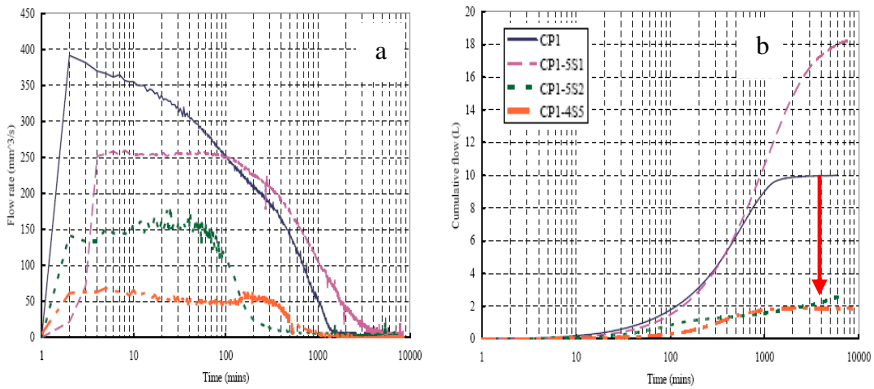


Fig. 3.11 Flow rate (a) and cumulative flow (b) for specimens with a crack width of 200 μm [3.48]

Snoeck *et al.* [3.50] visualized water permeation by means of neutron radiography. Small specimens, 10 mm in diameter and 20 mm high with a crack width of 150 μm , were subjected to permeability tests and the change in water head was monitored in time. Fig. 3.12 shows the water permeability in time of the cracked samples without SAPs (0-CRA) and the cracked samples containing 1 m% of SAP B (1B-CRA) and 1 m% of SAP C (1C-CRA). It is clearly visible that all fluid has permeated through the samples containing no SAPs after 40 s. Samples containing SAPs are able to block the crack due to swelling of the SAP particles at the crack faces.

The water head on specimens without SAPs decreases rapidly. The water head on specimens with SAPs, however, did barely change in time. This proves the self-sealing effect of SAPs in cementitious materials.

The ability of three types of superabsorbent polymers (Table 3.4) to promote self-sealing and self-healing was investigated in water permeability tests by Snoeck *et al.* [3.46] (Fig. 3.13). Cracked specimens without SAP have a final k value of $1 \cdot 10^{-5}$ m/s. Specimens containing SAPs, however, show a decrease in permeability. This is mainly due to the swelling effect of SAPs and the blockage of the crack by SAPs. The SAP B particles are able to seal the crack more effectively in comparison to SAP A and SAP C. The difference noticed between

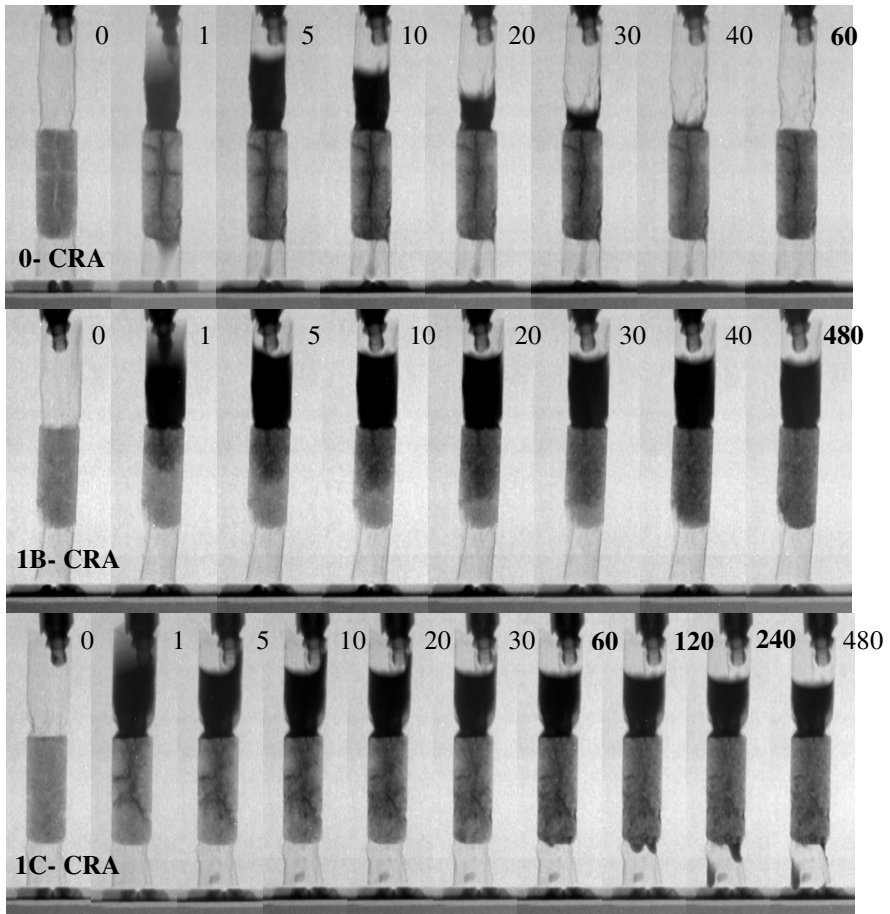


Fig. 3.12 Water permeability of the samples without SAP and samples containing 1 m% SAP B and SAP C. In the upper right corner, the time is mentioned in seconds. [3.50]

SAP A and SAP B is due to the smaller particle size of SAP A. The small size is inappropriate for sealing as the small particles cannot bridge the crack as their expanding diameter does not reach over the total crack and water may flow in between the swollen particles. The difference noticed between SAP B and SAP C is due to a lower absorption capacity of SAP C (Table 3.4). As the particles swell less, they result in less crack sealing. SAP B in an amount of 1 m% relative to the cement weight provided the highest decrease in permeability over time. A higher amount of SAP B caused a coarser matrix (due to the formation of macro pores) and lower strength and thus more pathways for water to migrate.

Table 3.4 Mean diameter of SAP A, SAP B and SAP C particles [μm] ($n=50$), absorption capacity of SAP [g fluid/g SAP] ($n=3$) in de-ionised water; tap water and cement slurry with standard deviations. [3.46, 3.50]

Method	SAP A	SAP B	SAP C
Diameter	100 ± 22	477 ± 53	420 ± 148
de-ionised water (pH=6.5)	305.0 ± 3.7	283.2 ± 2.4	10.86 ± 0.16
tap water (pH= 6.8)	163.9 ± 1.2	148.9 ± 0.9	9.27 ± 0.22
cement slurry (pH=12.8)	61.0 ± 1.0	58.4 ± 1.7	7.42 ± 0.15

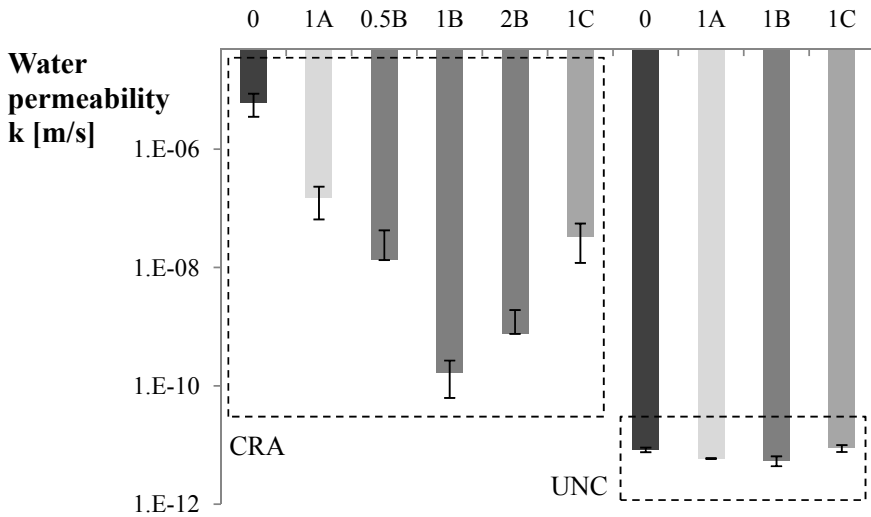


Fig. 3.13 Water permeability k [m/s] at 30 days of the cracked (CRA) and un-cracked (UNC) specimens in the low-pressure water permeability test set-up (age of 37 days). The used code is xy (x m% of SAP y). The error bars show the standard deviation. (redrawn after [3.46, 3.51])

The swelling reaction not only seals the crack from intruding substances at first, but can also stimulate and enhance autogenic self-healing due to the release of their absorbed water into the cementitious matrix. Kim and Schlangen [3.52] and Snoeck *et al.* [3.46, 3.51] showed that superabsorbent polymers can contribute to the internal healing of a crack. The latter researchers conducted a thermo gravimetric analysis (TGA) to determine the composition of the white crystallization found in a crack after conducting permeability tests [3.46]. TGA showed that the crystals consisted of CaCO_3 and washed out hydration products. SAP particles were not found in the TGA analysis, supporting the conclusion that SAPs effectively seal the crack without dissolving or degrading.

3.2.2 Protection against Carbonation of Blast Furnace Slag Mortar

Sisomphon *et al.* [3.53, 3.54] made use of expanded clay particles impregnated with a sodium monofluorophosphate (Na_2FPO_3 , Na-MFP) solution and coated with a slag containing cement paste layer to protect blast furnace slag mortar against carbonation (Fig. 3.14 I and II). When gaseous CO_2 diffuses into the matrix, carbonation attack will damage the microstructure and increase the

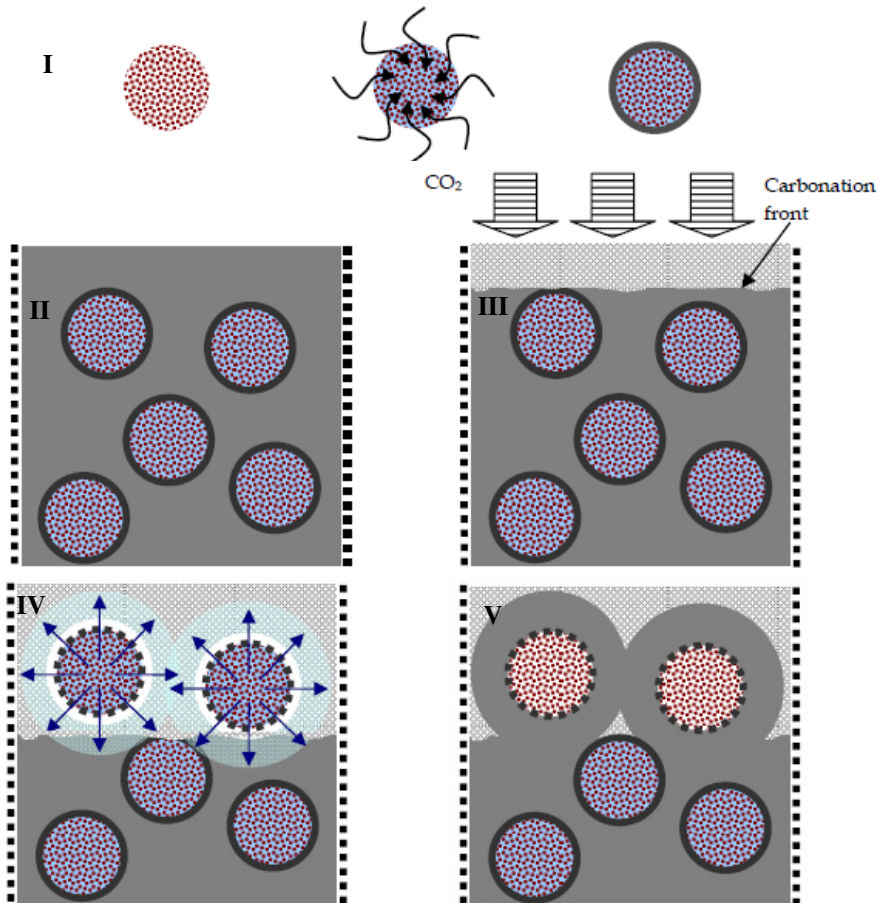


Fig. 3.14 (I) LWAs impregnated with Na-MFP solution is prepared under vacuum. Then, the particles are encapsulated in a cement paste layer. (II) Encapsulated LWA embedded in the matrix. (III) Carbonation coarsens the matrix porosity. (IV) Carbonation damages the coating layer. Na-MFP is released to the matrix. (V) Healing takes place. The microstructure of the matrix is improved. [3.53]

porosity of both the matrix and the coating of the expanded clay particles (Fig. 3.14 III). With the presence of moisture, the Na_2FPO_3 solution diffuses into the degraded matrix to form phosphate and fluoride ions (Fig. 3.14 IV). These ions react with hydration and carbonation products to stable amorphous formations analogous to apatite resulting in a stronger and denser matrix and an improved frost scaling durability of the carbonated blast furnace slag matrix (Fig. 3.14 V).

3.2.3 Corrosion Prevention

Dry and Corsaw [3.55] developed a technique to prevent corrosion of steel reinforcement. Corrosion of steel reinforcement is usually caused by chloride ions that penetrate in the concrete. Chloride sources include de-icing salts and marine waters. Methods to protect the concrete reinforcing steel against corrosion include the use of water repellent agents, such as silanes or siloxanes, additives such as microsilica and corrosion inhibitors such as calcium nitrite. Water repellent agents are sometimes unsuccessful because of their inability to protect the concrete if cracking occurs after treatment. In some structures containing microsilica, full-depth cracking has occurred due to high autogenous and drying shrinkage. Concretes containing calcium nitrite have generally performed well, however the set time of concrete may be affected [3.55].

The system proposed by Dry and Corsaw [3.55] involves the use of coated, hollow, porous (40%) polypropylene fibers (400 μm) filled with calcium nitrite. The fibers were filled by placing them in a solution of calcium nitrite into a vacuum flask and using vacuum saturation techniques to draw the calcium nitrite into the fibers. Because the used fibers were porous, they were treated with a coating to maintain the content. The coating controls the time-release of the calcium nitrite, ideally by responding to the environment of the pore solution. Polyol, one of the two types of coating used by Dry and Corsaw [3.55], breaks down in the presence of chloride ions. At the moment chloride ions penetrate, the steel reinforcement is endangered but at the same time the polyol coating will dissolve and the released calcium nitrite will protect the reinforcement. The second coating, paraffin wax, was applied to another set of fibers to simulate a mechanical release response. In this case the rebar should be heated to melt the wax and release the embedded calcium nitrite. After they were coated, the polypropylene fibers were wound around the reinforcement bars of the test samples. Both methods of response were investigated in this study. The efficiency was compared with specimens without any corrosion inhibitor and specimens with freely mixed calcium nitrite.

A plexiglass dam was built on each sample and sealed with silicone caulk to allow salt water to be ponded as shown in Fig. 3.15. The samples were ponded 1 week with a salt water solution that contained 1.321% sodium chloride and then allowed to dry 1 week. The voltage drop across a 100-ohm resistor, which was connected externally between the anode and the cathode, was measured weekly. Systems were compared by electrochemical measurements on the basis of the time to initiation of corrosion and the amount of corrosion occurring over time.

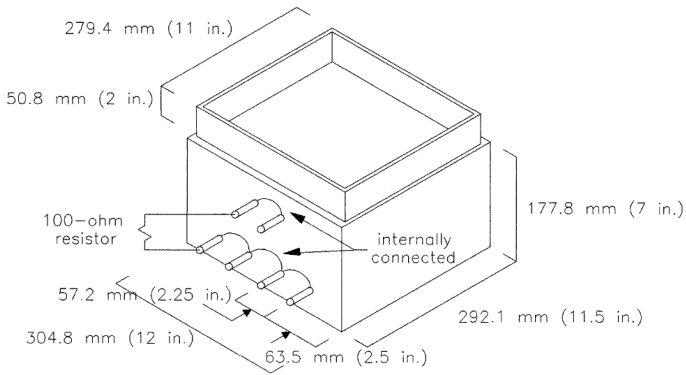


Fig. 3.15 Setup to subject samples to salt solution and measure rebar corrosion [3.55]

The corrosion condition rating and expected time until the initiation of corrosion show that the time-release method delayed the onset and severity of corrosion, as shown in Fig. 3.16. Both the control group and the group with freely mixed calcium nitrite had much higher ratings than the other groups. In comparison both groups of fiber samples had almost less than half the rating of the control and freely mixed group.

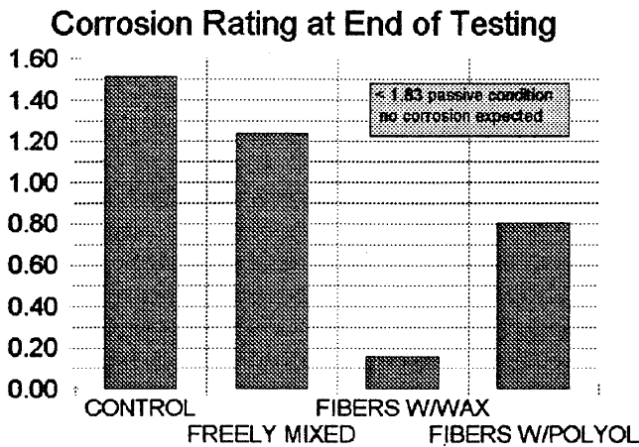


Fig. 3.16 Corrosion rating measured for each of the different test series [3.55]

3.2.4 Textile-Reinforced Concrete

Textile-reinforced concrete (TRC) is a high-performance, cement-based composite consisting of fine-grained concrete and textile reinforcement, the latter usually consisting of glass or carbon multi-filament yarns. When subjected to tensile

loading, TRC shows pronounced strain hardening behaviour accompanied by multiple cracking. Mechtcherine and Lieboldt [3.56] investigated transport of water and gases through cracked TRC at chosen strain levels under uniaxial tensile loading and the subsequent self-healing behaviour of the TRC specimens.

The matrix composition was developed with an eye to the requirements of workability, good bonding to the textile reinforcement, durability, and other considerations [3.57]. Table 3.5 shows the composition of the matrix used for the production of TRC specimens.

Table 3.5 Mix proportions of the TRC matrix

Material	Mass ratio	Mix proportion
	[-]	[kg/m ³]
CEM III B 32.5 N-NW/HS/NA	2.0	550
Fly ash	0.9	248
Micro silica suspension (solid:water = 1:1)	0.2	55
Fine sand 0/1	4.0	1101
Water	0.9	248
Super plasticizer	0.03	8

Four different types of biaxial textile reinforcements made of alkali-resistant glass (AR glass) and one made of carbon were used as reinforcement for the specimens in this investigation, see Table 3.6. Variable parameters were the fineness of the yarns and the presence or absence of a coating. A polymer coating composed of styrene-butadiene was used to improve the bond between the textile and the surrounding finely grained concrete matrix as well as the bond between the outer and inner filaments in the yarn.

Table 3.6 Textile reinforcements

Material		Alkali-resistant glass (AR glass)				Carbon
		1280	2400	1280	2400	
Yarn fineness	[tex] ¹	1280	2400	1280	2400	800
Filament diameter	[µm]	14	27	14	27	7
Number of filaments per yarn	[-]	3200	1600	3200	1600	12000
Yarn spacing	[mm]	7.2	7.2	7.2	7.2	7.2
Mass/area	[g/m ²]	364	676	374	707	257
Content of polymer coating	[% by mass]	-	-	2.7	4.6	9.8

¹tex = g/km

Rectangular plates 600 mm long, 100 mm wide, and 14 mm thick were produced with four textile layers. The plates were removed from the mould at a concrete age of two days and then stored in water up to an age of 7 days. Then the plates were stored in a climate-controlled room at 20 °C and 65% RH until testing or further preparation.

The in-situ permeation tests were performed at a specimen age of 56 days or slightly higher. At this age the hydration of binder components was to a large extent completed. A special permeation cell was developed which excluded any significant hindrance of the longitudinal deformation of the enclosed TRC specimen by the permeation cell, see Fig. 3.17.

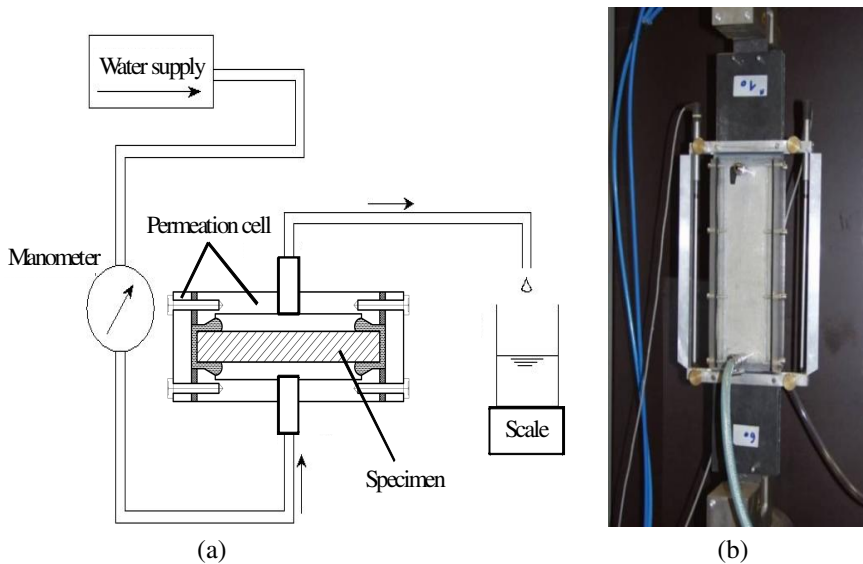


Fig. 3.17 In-situ permeability measurements: a) schematic of the test set-up, b) testing under tensile load [3.56]

During the test, the specimen was exposed to constant water pressure on one surface, while the opposite surface was subjected to atmospheric pressure. The water amount passing through the specimen was measured and recorded automatically using a high precision balance. The effect of the strain on the volume flow rate in the quasi-stationary state is shown in Fig. 3.18 for TRC made of different textiles. The transport rates increased hyper-proportionally at strain levels above 0.2%. As expected, the tests on the specimens with finer crack patterns (large number of crack and accordingly smaller crack width for the same stain level) exhibited lower flow rates.

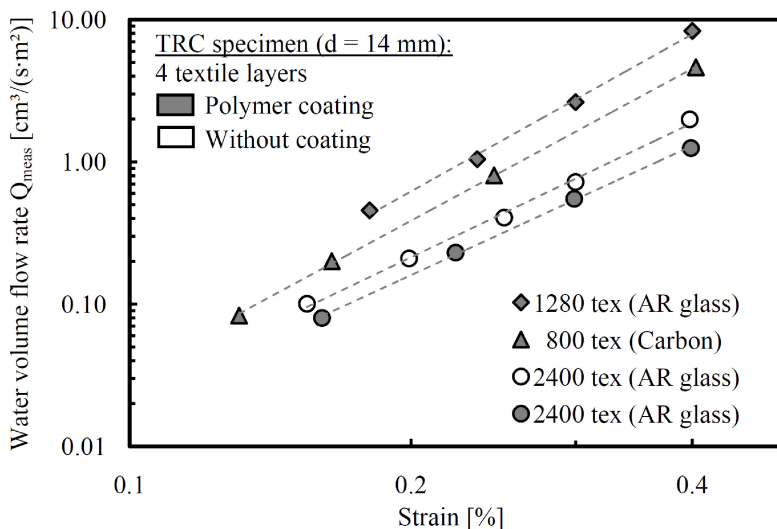


Fig. 3.18 Effect of the imposed strain level on the specific water flow rate for TRC with different textile reinforcements, induced pressure 100 kPa [3.56]

Following the in-situ water permeation measurements, the samples were left in the permeation cell. In order to investigate the effect of self-healing on the transport properties of TRC, further water permeation measurements were carried out in the unloaded, saturated state of specimens at time intervals of 7 days. Fig. 3.19 shows the time-dependent reduction of the transport rates of samples with different reinforcement textiles. After storing the cracked specimens in water for 14 days, the flow rate was reduced to less than 50% of the value obtained on the same specimen before the water storing. No measurable volume flow occurred after continued water exposure of 21 to 35 days, the particular duration depending on the type of the textile reinforcement used. The samples containing uncoated AR glass yarns with low yarn fineness (1280 tex) needed a longer time until a negligible volume flow was reached, which can be traced back to the initially wider cracks in these specimens. The specimens containing coated carbon textile layers showed the fastest self-healing, which could be expected since they had the finest cracks.

The reduction in the flow rate resulted mainly from the formation of new crystal structures in the fine cracks and additional hydration of the previously non-reacted material as well as from the swelling of cement gel (primarily C-S-H) due to additional water intake. In order to eliminate the influence of swelling, a further oxygen permeability test was performed after water storage of up to 35 days. Since the specimens were dried before the oxygen permeation test, the effect of swelling of the cement matrix could be excluded. The verification should be apparent from the comparison of the oxygen permeability measured before the first loading cycle (crack-free samples) and after the second loading cycle

(cracked samples) with the results of the additional tests on the dried specimens after long-term water storage. Oxygen permeation was reduced to a minimum; the flow rates were similar to the reference measurement data of the crack-free samples before the first loading. Hence, the obvious explanations for the measured decrease in the gas volume flow rate are the self-healing of fine cracks and further hydration of the cement matrix.

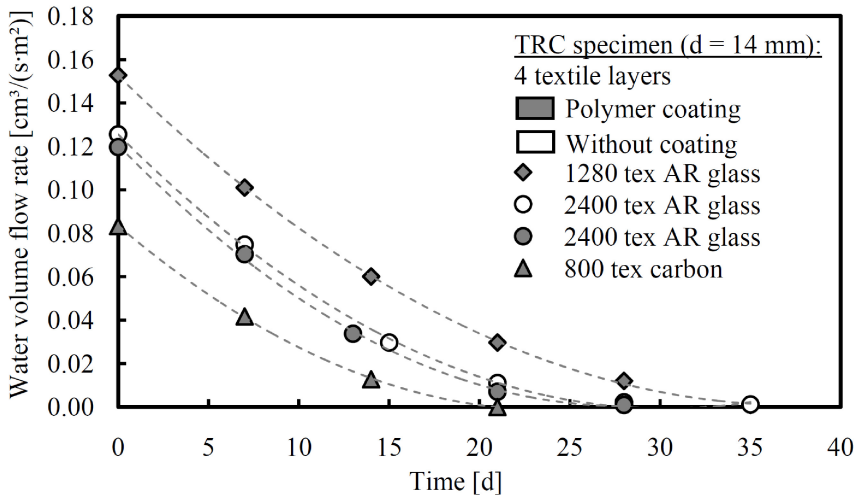


Fig. 3.19 Time-dependent reduction of the water transport trough cracked TRC in unloaded state [3.56]

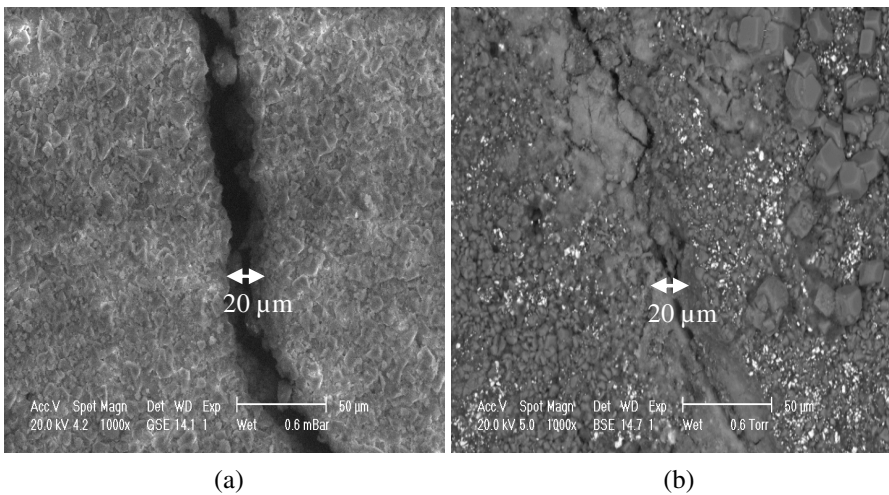


Fig. 3.20 Self-healing effect: a) crack before and b) after water exposure [3.56]

Using an ESEM (Environmental Scanning Electron Microscope), additional investigations of cracks before and after exposure to water revealed the formation of new crystal structures which closed the cracks to transport, cf. Fig. 3.20. Fig. 3.20a shows the original crack with a width of 20 μm before water contact. Fig. 3.20b illustrates the condition of a similar crack after the water permeability tests with duration of 21 to 35 days, indeed largely closed by deposits of calcium carbonate (calcite).

3.2.5 Microbially Induced Carbonate Precipitation

In the last decades, microbially induced carbonate precipitation (MICP) has been investigated as an environment friendly method for surface consolidation and protection of decayed ornamental stone and concrete. The method has been first applied to form a compatible and highly coherent carbonate precipitate on limestone. Later, this technique has been explored for the improvement of the durability of cementitious materials. Furthermore, the same principle has also been used for the generation of a biological binder. An in-depth overview on the use of MICP has recently been published [3.56] and has been used as a basis for the first part of this chapter, to provide a deeper insight into the principles and important parameters of MICP. Recent advances indicate the potential use of this technique for the remediation of cracks in building materials, strength improvement and self-healing of cementitious materials.

3.2.5.1 The Principles of Microbially Induced Carbonate Precipitation (MICP)

Like other biomineralization processes, calcium carbonate (CaCO_3) precipitation can occur by two different mechanisms: biologically controlled, or induced [3.59]. In biologically controlled mineralization, the organism controls the process, i.e. nucleation and growth of the mineral particles, to a high degree. However, calcium carbonate production by bacteria is generally regarded as “induced”, as the type of mineral produced is largely dependent on the environmental conditions [3.60] and no specialized structures or specific molecular mechanism are thought to be involved [3.61]. Different types of bacteria, as well as abiotic factors (salinity and composition of the medium) seem to contribute in a variety of ways to calcium carbonate precipitation in a wide range of different environments [3.62, 3.63].

Calcium carbonate precipitation is a rather straightforward chemical process governed mainly by four key factors [3.64]:

- 1) the calcium concentration,
- 2) the concentration of dissolved inorganic carbon (DIC),
- 3) the pH and
- 4) the availability of nucleation sites.

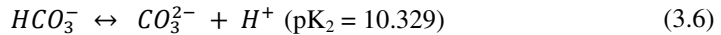
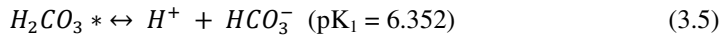
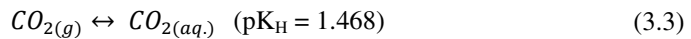
CaCO₃ precipitation requires sufficient calcium and carbonate ions so that the ion activity product (IAP) exceeds the solubility constant (K_{so}) (Eq. (3.1) and (3.2)).



$$\Omega = \frac{(Ca^{2+}) \cdot (CO_3^{2-})}{K_{so}} \quad (3.2)$$

with K_{so calcite, 25°C} = 4.8 x 10⁻⁹.

The concentration of carbonate ions is related to the concentration of DIC and the pH of a given aquatic system. In addition, the concentration of DIC depends on several environmental parameters such as temperature, salinity and the partial pressure of carbon dioxide (for systems exposed to the atmosphere). The equilibrium reactions and constants governing the dissolution of CO₂ in aqueous media (25°C and 1 atm) are given in equations (3.3) to (3.6) [3.65].



With H₂CO₃* = CO_{2(aq.)} + H₂CO₃

Microorganisms can influence precipitation by altering almost any of the precipitation parameters described above, either separately or in various combinations with one another [3.64]. However, the primary role has been ascribed to their ability to create an alkaline environment through various physiological activities. Both autotrophic and heterotrophic pathways are involved in the creation of such an alkaline environment (for an extensive review, see Castanier *et al.* [3.66]. A first heterotrophic pathway involves the sulphur cycle, in particular the dissimilatory sulphate reduction, which is carried out by sulphate reducing bacteria under anoxic conditions. A second heterotrophic pathway involves the nitrogen cycle, and more specifically, (1) the oxidative deamination of amino acids in aerobiosis, (2) the dissimilatory reduction of nitrate in anaerobiosis or microaerophily and (3) the degradation of urea or uric acid in aerobiosis. Another microbial process that leads to an increase of both the pH and the concentration of dissolved inorganic carbon is the utilization of organic acids [3.67], a process which has been commonly used in microbial carbonate precipitation experiments. The precipitation pathways described above are general in nature, which accounts for the common occurrence of microbial carbonate precipitation and validates the statement by Boquet *et al.* [3.68]) that under suitable conditions, most bacteria are capable of inducing carbonate precipitation.

Besides changes induced in the macro-environment, bacteria have also been reported to influence calcium carbonate precipitation by acting as sites of nucleation or calcium enrichment [3.69]. Due to the presence of several negatively charged groups on the cell wall, at a neutral pH, positively charged metal ions can be bound on bacterial surfaces [3.70, 3.71]. Such bound metal ions (e.g. calcium) may subsequently react with anions (e.g. carbonate) to form an insoluble salt (e.g. calcium carbonate). The anion (e.g. carbonate) in this reaction may be a product of the bacterial metabolism, or it may have an abiotic origin [3.71].

The evidence of microbial involvement in carbonate precipitation has subsequently led to the exploration of this process in a variety of fields. A first series of applications is situated in the field of bioremediation. In addition to conventional bioremediation strategies which rely on the biodegradation of organic pollutants [3.72, 3.73], the use of MICP has been proposed for the removal of metal ions. Applications include the treatment of groundwater contaminated with heavy metals [3.74] and radionucleotides [3.75], the removal of calcium from wastewater [3.76, 3.77]. Another series of applications aims at modifying the properties of soil, i.e. for the enhancement of oil recovery from oil reservoirs [3.78], plugging [3.79] and strengthening of sand columns [3.80, 3.81]. Moreover, microbially induced precipitation has been investigated for its potential to improve the durability of construction materials such as limestone and cementitious materials. The latter can be divided into processes for the deposition of a protective surface layer with consolidating and/or waterproofing properties i.e. biodeposition, and processes for the generation of a biologically induced binder, i.e. biocementation.

3.2.5.2 Biodeposition

Boquet *et al.* [3.68] were among the first to demonstrate the ability of soil bacteria to precipitate calcium carbonate under laboratory conditions. Among the organisms tested, several *Bacillus* strains (incl. *Bacillus cereus*) and *Pseudomonas aeruginosa* were observed to form crystals. Adolphe *et al.* [3.82] were among the first to consider the use of microbially induced carbonate precipitation (MICP) for the protection of ornamental stone. They applied for a patent regarding the use of calcinogenic bacteria on stone surfaces. Since the best performance was obtained with *B. cereus*, which could also be easily produced on an industrial scale, this organism was selected for *in situ* applications [3.83]. The nutritional medium was designed to stimulate the production of carbonate through the nitrogen cycle metabolic pathways. More specifically, the media contain a source of proteins for the oxidative deamination of amino acids in aerobiosis and a source of nitrate for the dissimilatory reduction of nitrate in anaerobiosis or microaerophily. In addition, a fungicide was added to prevent the unwanted growth of fungi present on the stone, or deposited from the air [3.84]. The proposed *in situ* treatment consists of first spraying the entire surface to be protected with a suitable bacterial suspension culture. Subsequently, the deposited culture is fed daily or every two

days (about five applications in total) with the suitable medium in order to create a surficial calcareous coating [3.85]. The first application *in situ* was carried out in 1993 in Thouars on the tower of the Saint Médard Church, consisting of Tuffeau limestone. The presence of the biocalcin decreased the water absorption rate to a significant extent (5 times) while retaining the permeability for gas. Furthermore, no influence on the aesthetic appearance could be observed [3.85].

The promising results of this so called Calcite Bioconcept technique encouraged different research groups to evaluate alternative approaches for the biomediated carbonate precipitation on limestone. These approaches can be mainly divided into those falling within and those falling outside the specifications of the patent by Adolphe *et al.* [3.82], i.e. the application of calcinogenic bacteria to a stone surface. The first series of approaches, those falling within the patent specifications, are characterized by the use of different microorganisms, metabolic pathways or delivery systems to overcome some of the potential limitations of the Calcite Bioconcept technique. In the second series of approaches, no microorganisms are applied to the surface. These approaches can be divided into studies where inducing macromolecules are supplied to the stone together with a supersaturated solution of calcium carbonate and studies which obtain carbonate precipitation by the microbiota inhabiting the stone. In the latter, only nutrients are added to the stone.

3.2.5.2.1 *Biodeposition through the Application of Calcinogenic Bacteria to a Stone Surface*

At the University of Granada, Rodriguez-Navarro *et al.* [3.86] proposed the use of *Myxococcus xanthus* for the creation of a consolidating carbonate matrix in the porous system of limestone. For the production of carbonate ions, the authors proposed a medium containing a pancreatic digest of casein as the nitrogen source. Also, the effect of a phosphate buffer on the carbonate production was investigated. The authors observed carbonate cementation to a depth of several hundred micrometers ($> 500 \mu\text{m}$) without the occurrence of any plugging or blocking of the pores.

At Ghent University, Dick *et al.* [3.87] proposed the microbial hydrolysis of urea as a strategy to obtain a restoring and protective calcite layer on degraded limestone. The hydrolysis of urea presents several advantages over the other carbonate generating pathways, as it can be easily controlled and it has the potential to produce high amounts of carbonate within a short period of time. The hydrolysis of urea is catalyzed by means of urease. As a consequence, urea is degraded to carbonate and ammonium, resulting in an increase of the pH and carbonate concentration in the bacterial environment [3.88]. As calcium ions are bound to the cell wall as a result of the negative charge of the latter, this can result in the formation of crystals on the bacterial cell. A schematic overview, of the ureolytic carbonate precipitation occurring at the microbial cell wall is given in Fig. 3.21.

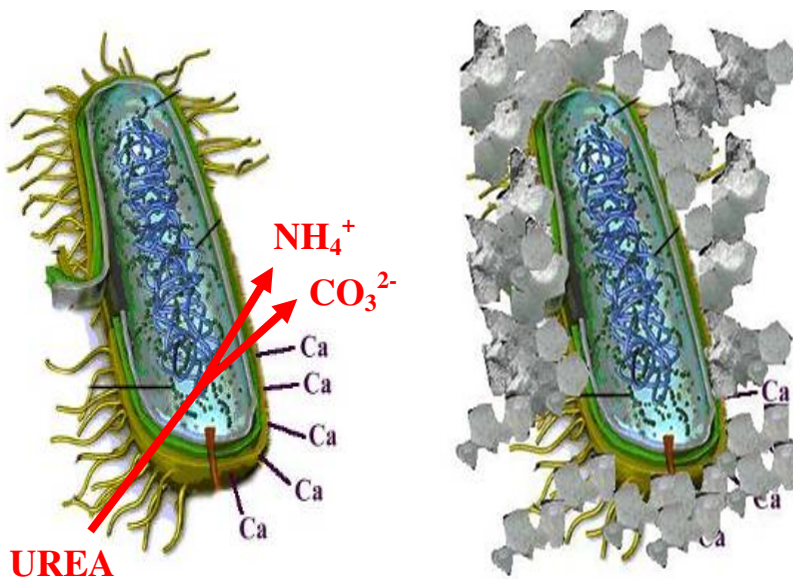


Fig. 3.21 Principle of CaCO_3 precipitation by ureolytic bacteria (after 3.64)

First, Dick *et al.* [3.87] identified the microbial key factors which contribute to the performance of the biodeposition treatment. For the evaluation of the performance, the authors investigated the water absorption rate of treated and untreated Euville limestone. Of the parameters that were screened (calcite deposition on limestone cubes, pH increase, urea degrading capacity, EPS production, biofilm formation, ζ -potential and deposition of dense crystal layers), the ζ -potential proved to be the factor with the greatest predictive power for good limestone restoration, reflecting the effect on the initial water absorption rate. The ζ -potential is a measure of the potential of the electric layer at the surface of the cells, and is therefore an important parameter in the adhesion and surface colonization by bacteria. Due to the positive ζ -potential of the limestone, bacteria with a highly negative ζ -potential will be more easily retained. The second important key factor was the specific urea degradation rate. Bacteria with a high initial specific urea degradation rate show a high affinity for urea. This allows for a high substrate turnover for a limited amount of cells.

Although urease activity is widespread among different groups of microorganisms, it was mainly microorganisms closely related to the *Bacillus sphaericus* group which were shown to proliferate and express the urease gene under the given cultivation conditions [3.77]. From the screening procedure described above, 2 strains of *B. sphaericus* were selected for further experiments. These strains were shown to decrease the initial water absorption rate by approximately 50%.

As an extension to the above mentioned study, De Muynck *et al.* [3.89, 3.90] investigated the biodeposition with *B. sphaericus* as a surface treatment for cementitious materials (Portland cement mortar) with different porosities. All experiments were performed at 28°C under non-sterile conditions. The mortar specimens were immersed for 24 hours in a one day old culture of *B. sphaericus* containing *ca.* 10^7 cells.mL⁻¹, after which they were transferred to fresh medium containing a calcium source. The specimens were removed from the solution after three days. The authors demonstrated that the biodeposition treatment resulted in an increased resistance of mortar specimens towards carbonation, chloride penetration and freezing and thawing, especially for more porous mortars with higher water to cement ratios (w/c). Moreover, the biodeposition treatment showed a similar protection towards degradation processes as some of the conventional surface treatments under investigation (silanes, siloxanes, silicates and acrylates). The biodeposition treatment on cementitious materials can be regarded as a coating system, since carbonate precipitation is mainly a surface phenomenon due to the limited penetration of the bacteria in the porous matrix. From thin section analyses, it was observed that the majority of the surface was covered with a layer of crystals with thicknesses within the range of 10-40 µm, in which often larger crystals (up to 110 µm) could be found. The morphology of the crystals was observed to be highly dependent on the medium composition. In the event calcium chloride was used as the calcium source, rhombohedral carbonate crystals were obtained (Fig. 3.22). In the presence of calcium acetate, spherulitic crystals were observed. However, no differences in the protective effect were observed between biodeposition treatments with a different calcium source.

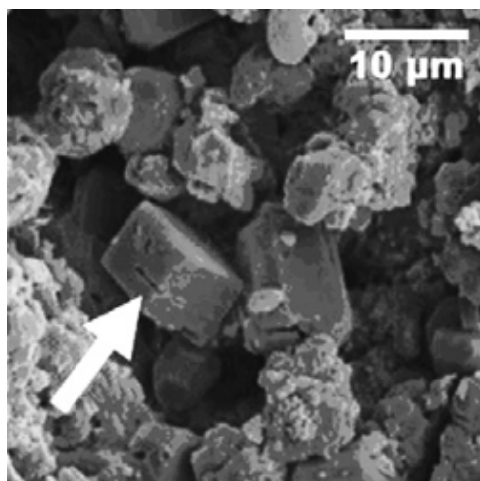


Fig. 3.22 Scanning electron micrographs of rhombohedral CaCO₃ crystals on the surface of mortar specimens treated with *Bacillus sphaericus* and a nutrient medium consisting of urea and calcium chloride

The Biobrush (BIOremediation for Building Restoration of the Urban Stone Heritage) project consortium introduced the use of Carbogel into the field of biodeposition. According to the consortium, these delivery systems could be used to control the possible harmful side effects of bacteria to stone. Within the framework of the Biobrush project regarding biodeposition, bacteria isolated from a stream in Somerset (UK), and bacteria from culture collections that had been reported to have calcifying activity, were screened for their ability to deposit calcite. From the 10 isolates that were retained and assessed for their ability to deposit calcite on stone surfaces, *Pseudomonas putida* was chosen for further study in field trials. In these field trials, bacteria were applied to the stone by brushing. Subsequently, the bacteria were covered with moistened Japanese paper, above which a 1-1.5 cm thick layer of Carbogel prepared with modified B4 medium was applied. Tris-HCl buffer was added to the Carbogel to adjust the low pH of this carrier. Finally, the gel was covered with a polyethylene sheet. As a result of this treatment a decrease of the water absorption and open porosity by 1% and 5% respectively was obtained. In order for this treatment to be effective as a consolidant, a 2 week treatment was observed to be necessary.

3.2.5.2.2 *Biodeposition without the External Application of Micro-Organisms*

Tiano *et al.* [3.91] proposed the use of natural and synthetic polypeptides to control the growth of calcite crystals in the pores. The first suggestions in this direction already date from the time at which the Calcite Bioconcept treatment was developed. Tiano *et al.* [3.92, 3.93] proposed the use of organic matrix macromolecules (OMM) extracted from *Mytilus californianus* shells to induce the precipitation of calcium carbonate within the pores of the stone. The organic matrix was shown to produce a more relevant and durable carbonate precipitation compared to the single use of calcium chloride or hydroxide. This precipitation resulted in a slight decrease in porosity and water absorption by capillarity [3.93]. Tiano *et al.* [3.94] proposed the alternative use of acid functionalized proteins such as polyaspartic acid. Calcium and carbonate ions for crystal growth were supplied by means of an ammonium carbonate and calcium chloride solution or a saturated solution of bicarbonate, and were supplemented in some cases by calcite nanoparticles, in order to maintain a saturated carbonate solution in the pore over a prolonged period. Proteins, calcium ions and nanoparticles were introduced in the stone by means of spraying. According to the authors, the method is most suitable for the use on marble statues and objects of high aesthetic value where conservation is required with the minimum change in the chemistry of the object. Field test results, however, indicate that the effects of the treatment with bio-inducing macromolecules (BIM) were rather small [3.94].

The European Bioreinforce (BIOmediated calcite precipitation for monumental stones REINFORCEment) project consortium demonstrated the ability of autoclaved cells and cell fragments to induce calcite crystallization in liquid media. After application of dead cells and *Bacillus* cell fragments (BCF) to stone surfaces, a slight decrease in the water absorption was noticed; the effect was more pronounced on high porosity stones such as Tuffeau. Again, this method

only appeared to be useful for very delicate small calcareous stone objects, rather than for a monumental façade [3.95].

Jimenez-Lopez *et al.* [3.96] proposed the application of a culture medium, able to activate the calcinogenic bacteria from the microbial community of the stone, as a more user friendly method for the *in situ* consolidation of ornamental stone. In addition to their work on decayed limestone fragments [3.96, 3.97], this technique was recently proposed for the treatment of new stones used for replacement purposes [3.98]. Some of the identified bacteria in the stone, *Pseudomonas* and *Bacillus*, had already been reported to produce calcium carbonate both in laboratory conditions and in nature. From these findings, the authors proposed the use of bacto-casitone as a way to activate the calcinogenic bacteria from the stone microbial community. Bacto-casitone is a source of carbon and nitrogen, which favours alkalisation due to the oxidative deamination of amino acids. Gonzalez Muñoz *et al.* [3.99] applied for a new patent for the protection and reinforcement of construction and ornamental materials by means of the application of an activator medium able to induce the formation of calcium carbonate. Due to the time required for the activation of the microbial community, Jimenez-Lopez *et al.* [3.96] proposed the additional use of *M. xanthus* for those restoration interventions in which time is an issue and fast formation of calcium carbonate is required. Very recently, the application of an activator medium has been successfully applied *in situ* on calcarenite stone (Monasterio de San Jeronimo and Hospital Real, Granada) [3.100]. Results showed surface strengthening of the stone without alteration of its appearance.

3.2.5.3 Biocementation: Biological Mortar

Besides the deposition of a layer of carbonate on the surface of building materials, MICP has also been used for the generation of binder-based materials. Initial developments were mainly situated in the field of geotechnical engineering, i.e. plugging, strengthening and improvement of soils [3.78, 3.79, 3.81, 3.101]. Recent advances, however, indicate the potential use of this technique for the remediation of cracks in building materials, strength improvement and self-healing of cementitious materials.

A biological mortar refers to a mixture of bacteria, finely ground aggregates (limestone) and a nutritional medium containing a calcium salt. The term biological refers to the microbial origin of the binder, i.e. microbiologically produced calcium carbonate, which cements the aggregates together. The aim of the biological mortars is to avoid some of the problems related to chemical and physical incompatibilities of commonly used repair mortars with the underlying material, especially in the case of brittle materials [3.84, 3.85, 3.102]. The optimization of the mortar composition encompassed the dosage and composition of the three main components, i.e. limestone powder, nutrients and bacterial paste. The biological mortars necessitated the use of larger amounts of bacteria and as a result the composition of the nutrient medium had to be altered. Best results were obtained with one part of bacterial paste (containing 10^9 cells.mL⁻¹), one part of nutritional medium and two parts of limestone powder. Limestone powder with a

granulometry between 40 and 160 μm was observed to be the most suited. The technique has already been successfully tested on a small scale on sculptures of the Amiens Cathedral and on a portal of the church of Argenton-Château (France). Visual observations two years after the treatment indicated a satisfactory appearance of the repaired zones [3.84, 3.85].

3.2.5.4 Microbiological Remediation of Cracks in Concrete

In the recovery of heavy oil from oil fields, where water is more readily removed than the viscous oil, the ability to selectively plug porous rock to focus pumping energy in oil rich zones is highly desirable. For this purpose, the use of a microbial mineral plugging system based on the precipitation of carbonates was suggested and investigated by, amongst others, Ferris and Stehmeier [3.79], Zhong and Islam [3.101], Whiffin [3.103], Kucharski *et al.*, [3.104]. Zhong and Islam [3.101] used the consolidation of sand mixtures for the remediation of cracks in granite. Cracks in granite were packed with a mixture of bacteria, nutrients and a filler material. Among the different materials that were mixed with *S. pasteurii*, a silica fume (10%) and sand (90%) mixture lead to the highest compressive strength and lowest permeability. As a further extension to this research, Ramachandran *et al.* [3.105] investigated the microbiological remediation of cracks in concrete. The authors proposed MICP as an effective way to seal cracks. The appearance of cracks and fissures is an inevitable phenomenon during the ageing process of concrete structures upon exposure to weather changes. If left untreated, cracks tend to expand further and eventually lead to costly repair. Specimens with cracks filled with bacteria, nutrients and sand demonstrated a significant increase in compressive strength and stiffness values when compared with those without cells. Similar conclusions were obtained by Achal [3.106] for crack remediation with a mixture of sand and *Bacillus sp.* (strain CT-5 which they isolated from cement). The presence of calcite was, however, limited to the surface areas of the crack. The authors attributed this to the fact that *S. pasteurii* grows more actively in the presence of oxygen. Still, the highly alkaline pH (12-13) of concrete was a major hindering factor to the growth of the moderate alkaliphile *S. pasteurii*, whose growth optimum is around a pH of nine. In order to protect the cells from the high pH, Day *et al.* [3.107] investigated the effect of different filler materials on the effectiveness of the crack remediation. Beams treated with bacteria and polyurethane showed a higher improvement in stiffness compared to filler materials such as lime, silica, fly ash and sand. According to the authors, the porous nature of the polyurethane minimizes transfer limitations to substrates and supports the growth of bacteria more efficiently than other filling materials, enabling an accumulation of calcite in deeper areas of the crack. No differences could be observed between the overall performances of free or polyurethane immobilized cells in the precipitation of carbonate [3.108]. In addition to this research, Bachmeier *et al.* [3.109] investigated the precipitation of calcium carbonate with the urease enzyme immobilized on polyurethane. The

immobilization was shown to protect the enzyme from environmental changes, as the immobilized urease retained higher enzymatic activities at high temperatures and in the presence of high concentrations of pronase. While the rate of calcite precipitation of the immobilized enzyme was slower compared to that of the free enzyme, lower concentrations of the former were needed to obtain the theoretical maximum precipitation in a period of 24 hours. Although the authors mentioned ongoing research on the use of immobilized urease in the remediation of surface cracks in concrete, to our knowledge no published results are available at the moment.

As an extension to research of the Ghent University Group regarding biodeposition on cementitious materials, the use of microbially induced carbonate precipitation was further investigated for the repair of cracks in concrete [3.110, 3.111]. Both standardised cracks with a depth of 10 mm or 20 mm and a width of 0.3 mm, and realistic cracks were examined. For the biological crack healing, the *Bacillus sphaericus* strain LMG 222 57 was chosen because of its optimal CaCO_3 precipitation capabilities [3.87]. To protect the bacteria from the strong alkaline environment in concrete, the bacteria were, for some of the treatments, immobilised in silica gel (Levasil[®]200/30% sol, with a specific surface area of 200 m^2/g and a solids content of 30%). The bacteria were suspended in a salt solution and then added to the silica sol. The obtained suspension was brought into the cracks by means of a syringe where the salt triggered the transformation of the silica sol into a silica gel. After treatment of the cracks, samples were placed in an equimolar urea-calcium solution, which enhanced the formation of carbonate crystals inside the pores of the silica gel and resulted in sealing of the crack. Samples were removed from the solution after 3 days. Treatments with *Bacillus sphaericus* in silica gel and provided with a calcium source appeared to be effective in healing realistic cracks of 0.01 to 0.9 mm wide (Fig. 3.23). As a result, a decrease of the water permeability, similar to that obtained with traditional epoxy injections, was observed. However, it was seen that the decrease in water flow was also obtained if autoclaved bacteria were used instead of active bacteria. This corroborates that the greater part of the decrease in water permeability is attributed to crack filling by the sol-gel matrix. TGA analysis on the crack repair material indicated that only in the case of active bacteria CaCO_3 crystals were present (Fig. 3.24). Precipitation of these crystals inside the gel-matrix may enhance the durability of this repair material. When only bacteria were used, without immobilization in silica gel, no CaCO_3 precipitation could be observed. Treatment with sol-gel, with or without successive immersion in BS + CaCl_2 , resulted in cracking of the sol-gel matrix. Efficiency of the biological treatment was also evaluated by means of ultrasonic transmission measurements and visual examination [3.111]. Crack treatment with *Bacillus sphaericus*, immobilized in silica gel, resulted in an increase in ultrasonic pulse velocity, indicating that crack bridging was obtained.

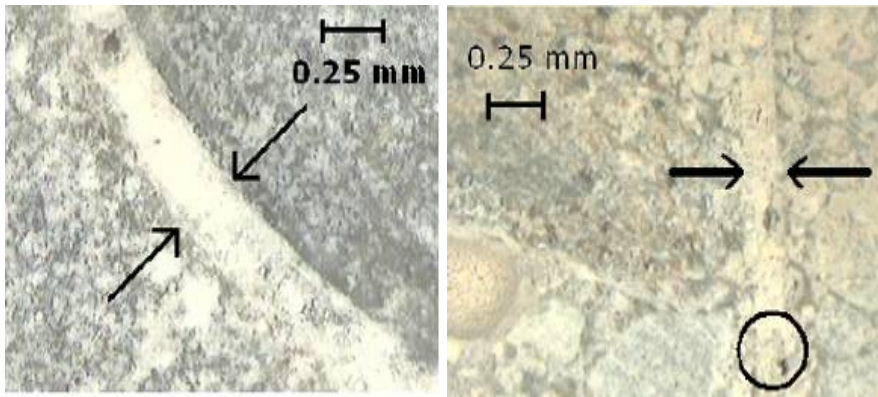


Fig. 3.23 Visual evaluation of crack repair by *Bacillus sphaericus* in sol-gel; left: surface, right: cross section; the crack tip is encircled; arrows indicate the crack edges (Source: [3.110])

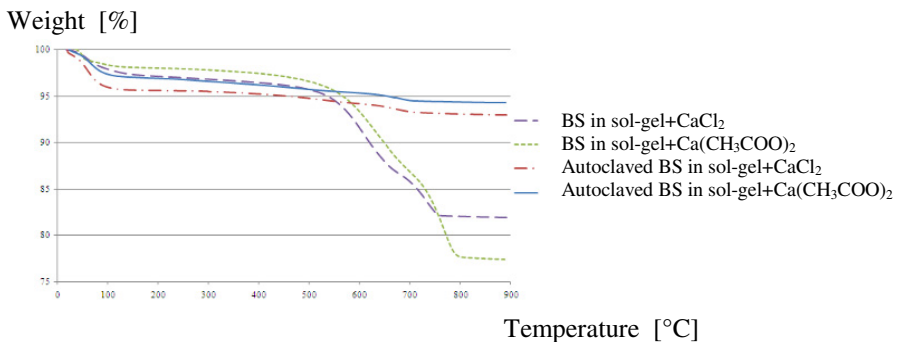


Fig. 3.24 TGA results for different crack repair materials containing active or autoclaved *Bacillus sphaericus* (BS) bacteria, showing the reduction in weight versus temperature increase (Source: [3.111])

3.2.5.5 Bacterial Concrete

Besides external application of bacteria in the case of remediation of cracks, microorganisms have also been applied in the concrete mixture. Until now, research has mainly focused on the consequences of this addition on the material properties of concrete, i.e. strength and durability.

Ramachandran *et al.* [3.113] investigated the use of microbiologically induced mineral precipitation for the improvement of the compressive strength of Portland cement mortar cubes. This study identified the effect of the buffer solution and type and amount of microorganisms, i.e. *S. pasteurii* and *P. aeruginosa*, used. Furthermore, in order to study the effect of the biomass, the influence of both living and dead cells was investigated. Before addition to the mortar mixture,

bacteria were centrifuged and washed twice. The final pellets were then suspended in either saline or phosphate buffer, which was subsequently added to the mixture. After demolding, the mortar specimens were stored in a solution containing urea and calcium chloride for seven days. Subsequently, the specimens were cured in air until the measurement of the compressive strength.

At lower concentrations, the presence of *S. pasteurii* was shown to increase the compressive strength of mortar cubes. While the 28-day compressive strength of the control cubes amounted to about 55 ± 1 MPa, specimens treated with 10^3 cells.cm⁻³ had a compressive strength of about 65 ± 1 MPa. The contribution of *P. aeruginosa* to the strength was found to be insignificant. From the X-Ray diffraction (XRD) analysis, no significant increased amounts of calcite could be found in mortar specimens treated with bacteria. This could be attributed to the inhibition of the microorganisms by the high pH and the lack of oxygen inside the mortar mixture. The overall increase of strength, therefore, resulted from the presence of an adequate amount of organic substances in the matrix due to the microbial biomass. However, an increase of the biomass, as dead cells in particular, resulted in a decreased strength. According to the authors, this could be attributed to the disintegration of the organic matter with time, making the matrix more porous [3.113].

Ramakrishnan *et al.* [3.113] investigated the effect of this technique on the durability of concrete. The presence of bacteria was observed to increase the resistance of concrete towards alkali, sulfate, freeze thaw attack and drying shrinkage; the effect being more pronounced with increasing concentrations of bacterial cells. The authors attributed this to the presence of a calcite layer on the surface, as confirmed by XRD analysis, lowering the permeability of the specimens. The best results were obtained with the phosphate buffer.

Achal *et al.* [3.114] added a grown culture of *Sporosarcina pasteurii* (concentration corresponding to an optical density OD₆₀₀ of 1.0) to cement mortar, and cured in a medium containing lactose mother liquor, urea, CaCl₂ and NaCl. They found an increase in 28 days compressive strength of 17% compared to a control without bacteria or medium. When using *Bacillus sp.* (CT-5) and curing in a medium containing corn steep liquor, they even obtained a 44% increase in 28 days compressive strength. Curing in the media without adding bacteria into the mortar, did not result in strength enhancement [3.106].

Ghosh *et al.* [3.115] demonstrated the positive effect of the addition of *Shewanella* on the compressive strength of mortar specimens. Contrary to the aforementioned research, these authors did not intend mineral precipitation, as these specimens were cured in air and not in a nutrient containing medium. An increase of 25% of the 28 day compressive strength was obtained for a cell concentration of about 10^5 cells.mL⁻¹ and a water-to-cement ratio of 0.4. For these samples, the presence of a fibrous material inside the pores could be noticed. As a result, a modification of the pore size distribution was observed. The positive effect of the addition of *Shewanella* improved with increasing curing times. For a concentration of 10^5 cells.mL⁻¹, an increase of the compressive strength of 17% and 25% was observed after 7 and 28 days, respectively. However, no increase of

the compressive strength was observed with additions of *Escherichia coli* to the mortar mixture. This led the authors to suggest that the choice of the microorganism plays an important role in the improvement of the compressive strength. More specifically, the production of EPS by the bacteria seemed to be of importance. In a subsequent study Ghosh *et al.* [3.116] attributed strength improvement of bacteria-amended mortar specimens to the formation of new phases of silicates (Gehlenite) within the mortar matrix. This phenomenon was apparently triggered by a novel protein released by mortar embedded bacteria. Also the addition of only the protein to mortar mixtures was found to result in substantial improvement of mortar strength.

3.2.5.6 Self-Healing Concrete Using Bacteria

3.2.5.6.1 Self-Healing Concrete with Bacteria in Porous Aggregates

As part of a larger research program at TUDelft on the development of novel self-healing materials [3.117] the application of bacteria as self-healing agent in concrete was considered. In preliminary studies Jonkers [3.118] and Jonkers and Schlangen [3.119] added various species of alkali-resistant spore-forming bacteria (*Bacillus pseudofirmus* DSM 8715 and *B. cohnii* DSM 6307) to mortar mixtures and observed substantially higher production of mineral precipitates on crack surfaces of bacteria-based specimens compared to control ones (Fig. 3.25).

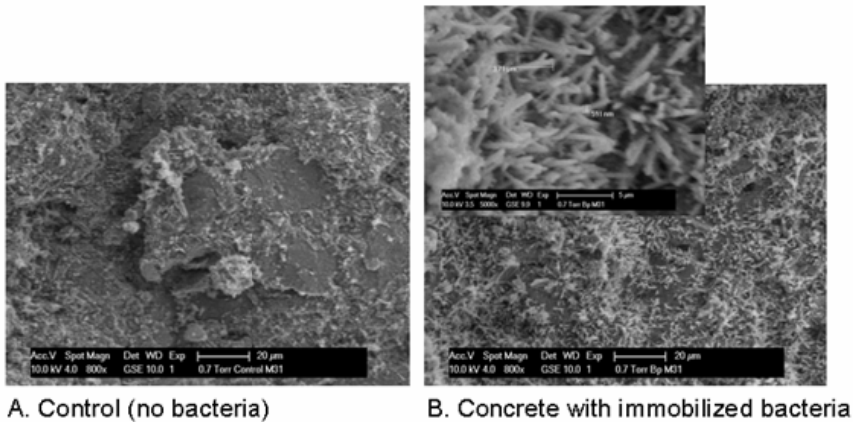


Fig. 3.25 Cement stone samples, which were cured for 10 days and subsequently further incubated in yeast extract- and peptone-containing medium. (A) Control (cement stone without added bacteria) and (B) cement stone containing 10^9 cm^{-3} spores of *B. pseudofirmus*. The inset in Figure 2B ($\times 5000$ magnification) shows a close up of the massive calcite-like crystals formed on the specimen surface.

The formation of minerals was thought to be bacterially mediated due to metabolic conversion of dissolved organic compounds present in crack-ingress water to insoluble calcium carbonate-based minerals by embedded bacterial spores

after activation (germination). In order to obtain a truly self-healing material, in a follow-up study both bacterial spores and a suitable organic mineral precursor compound (calcium lactate) were added to the mortar mixture [3.120]. Although again profound mineral formation on crack surfaces was observed, the process appeared to be limited to very young (7 - 10 days) mortar specimens only (Fig. 3.26).

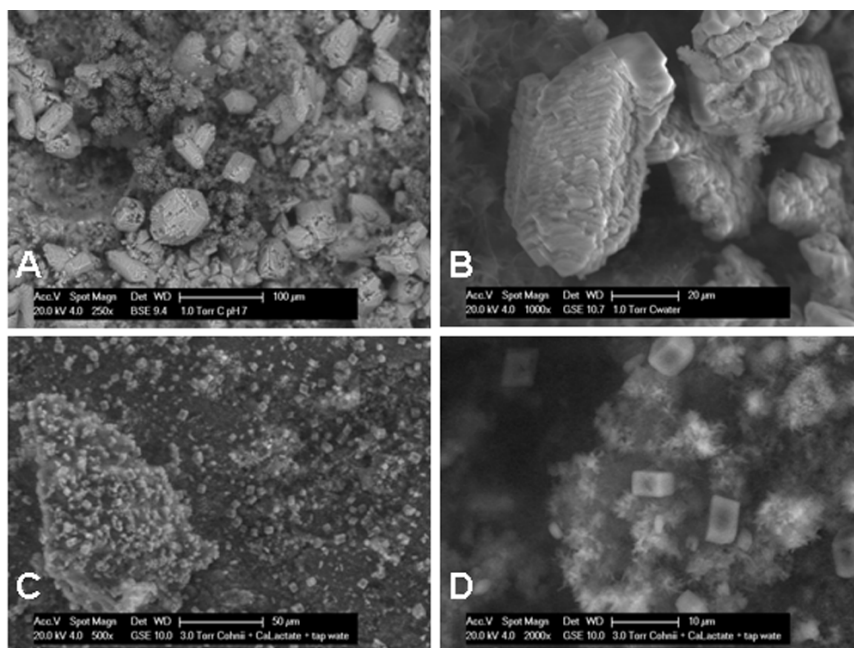


Fig. 3.26 Cement stone specimens with incorporated healing agent (*B. cohnii* spores plus calcium lactate), cracked after 7 (panel A: 250x and B: 1000x magnification) or 28 days curing (panel C: 500x and D: 2000x magnification). The relatively large (20-80 µm sized) mineral precipitates visible on crack surfaces of young specimens (A and B) are presumably due to bacterial conversion of calcium lactate to calcium carbonate. The small (2-5 µm sized) precipitates on crack surfaces of older specimens (C and D) resemble those produced by abiotic specimens (see C and D), larger bacterial precipitates are not produced here likely due to loss of viability of cement stone embedded bacterial spores.

This phenomenon was attributed in that study to the limited viability of bacterial spores when directly (unprotected) added to the mortar mixture. In currently running studies therefore, both bacteria and nutrients are immobilized in porous expanded clay particles, acting as healing agent reservoir particles, prior to addition to the concrete mixture (Fig. 3.27).

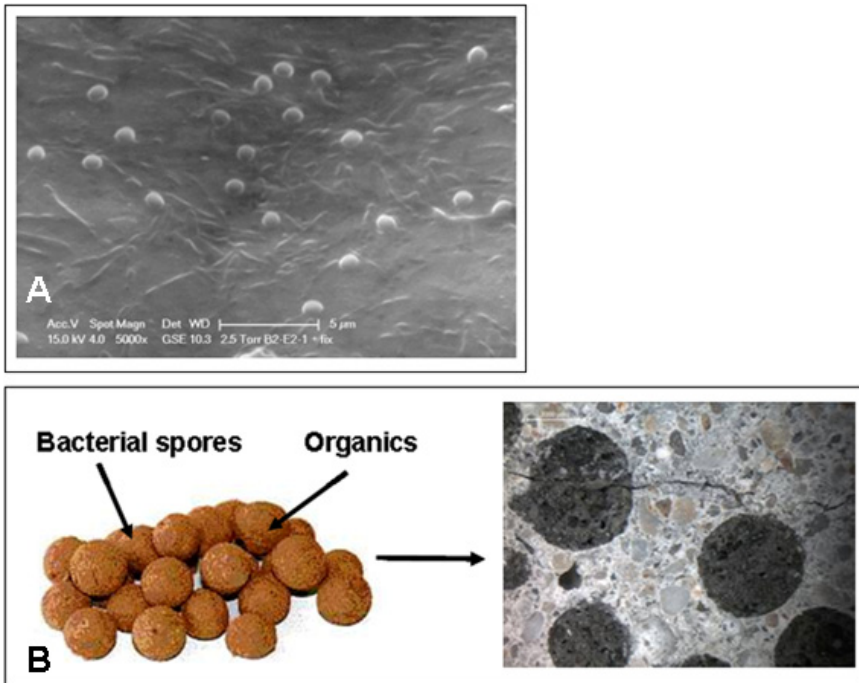


Fig. 3.27 A: ESEM photomicrograph (5000x magnification) of alkali-resistant spore forming bacterium (*Bacillus* strain B2-E2-1). Visible are active vegetative bacteria (rods) and spores (spheres), showing that spore diameter sizes are in the order of one micrometer; B: Self-healing admixture composed of expanded clay particles (left) loaded with bacterial spores and organic bio-mineral precursor compound (calcium lactate). When embedded in the concrete matrix (right) the loaded expanded clay particles represent internal reservoirs containing the two-component healing agent consisting of bacterial spores and a suitable bio-mineral precursor compound.

Results of these studies appear promising as viability of concrete embedded bacterial spores increased due to this treatment from days to several months, while compared to control specimens also significantly improved healing (plugging) of cracks was observed [3.121, 3.122, 3.123] (Fig. 3.28).

A similar approach was also followed by Wang [3.124] and Standaert [3.125] at Ghent University. Two kinds of porous aggregates, i.e. expanded clay (argex) and lava, were used to immobilize *Bacillus cohnii* spores. The carriers used for immobilizing or encapsulating should have a suitable strength, particle size and surface pore size. The suitable surface pore size for encapsulating bacteria is 1.0 to 100 μm , preferably between 1.0 and 25 μm [3.126]. Pore analysis indicated that the fraction of the pores within this size range was larger for argex particles than for lava particles, which means that argex is more suitable to immobilize bacteria. However, lava was shown to have a positive effect on compressive strength and tensile strength, whereas argex has a negative effect (Fig. 3.29). Furthermore, the influence of a range of nutrients on the strength of the matrix was investigated (Fig. 3.30).

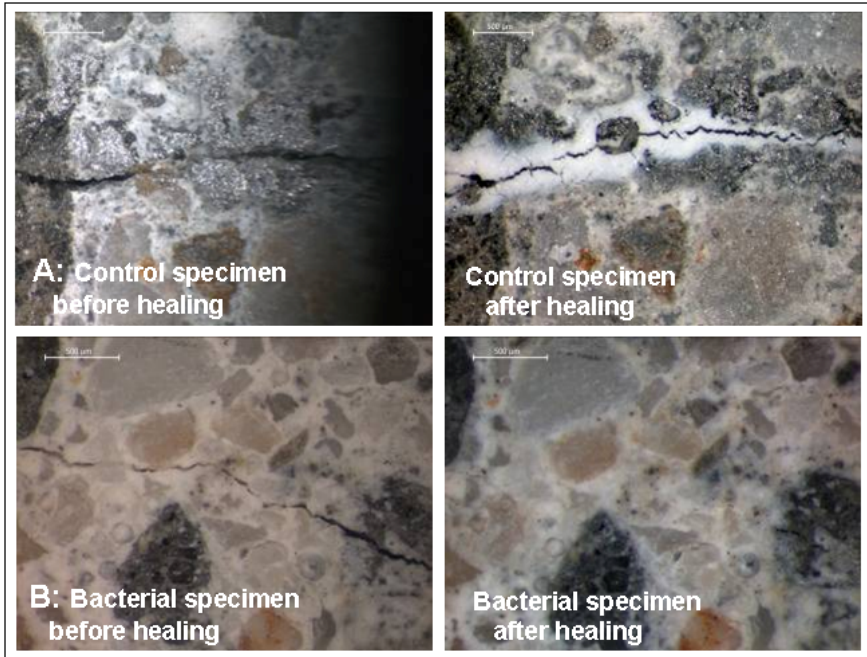


Fig. 3.28 Light microscopic images (40 times magnification) of pre-cracked control (A) and bacterial (B) concrete specimen before (left) and after (right) healing (2 weeks submersion in water). Mineral precipitation occurred predominantly near the crack rim in control but inside the crack in bacterial specimens. Efficient crack healing occurred in all six bacterial and two out of six control specimens.

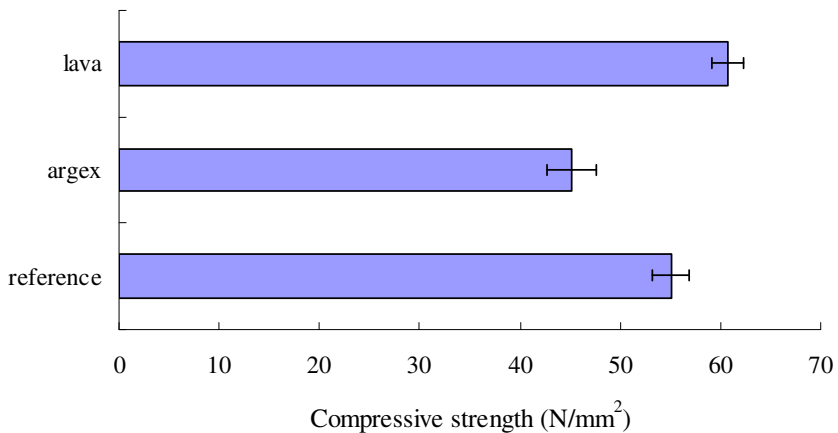


Fig. 3.29 Compressive strength at 28 days of mortar prisms ($160 \times 40 \times 40 \text{ mm}^3$) with different types of porous aggregates

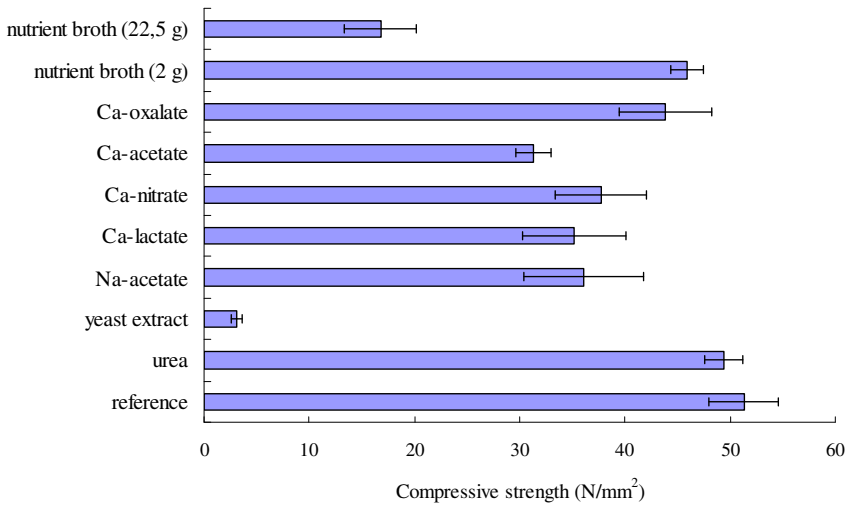


Fig. 3.30 Compressive strength at 28 days of mortar prisms ($160 \times 40 \times 40 \text{ mm}^3$) with different types of nutrients

To investigate the self-healing efficiency, the sand fraction 2/4 of mortar specimens was replaced by the same volume of porous aggregates. For each type of aggregate, 3 test series were prepared. The first one was the reference with aggregates that contained water, the second one was with aggregates that contained a solution of water and nutrients. The final series consisted of aggregates containing a spore suspension (*Bacillus cohnii*-spores) and nutrients. The expanded clay and lava aggregates were vacuum saturated with the solutions. For the third series, the spores and nutrients were added to separate aggregates. After saturation, the lava aggregates were provided with a cement coating to prevent leaching of the spores and nutrients. For each series, 4 cylinders (Ø : 76 mm, height: 20 mm) were made to investigate the evolution in water permeability. After 28 days, the prisms were subjected to a crack width controlled splitting test. The specimen was loaded until a crack-width of 0.4 mm was measured by an Linear Variable Displacement Transducer (resulting in a residual crack width after unloading of 0.1-0.2 mm). The cylinders were then positioned in a water permeability test facility as described by Aldea *et al.* [3.127]. The time of water flow through the cracked cylinders was measured daily and the water permeability coefficient was calculated according to Darcy's law.

A significant decline in the water permeability coefficient, in comparison with the reference and the specimens with only nutrients, was observed for specimens with spores and nutrients present in separate expanded clay particles. Precipitation was observed in the cracks of the specimens with nutrients and the specimens with nutrients and spores. This indicates that the precipitation might not be caused by the incorporated bacteria. Moreover, SEM analysis did not reveal differences in shape, size or degree of coverage of the precipitation noticed on the different

series. Also, the amount of CaCO_3 precipitation was not enough to completely heal the created cracks. SEM images of the specimens with lava saturated with nutrients and spores showed the presence of bacteria (Fig. 3.31). The bacteria appeared to be part of the crystals. This proves that the spores survived the immobilisation and germinated after crack formation.

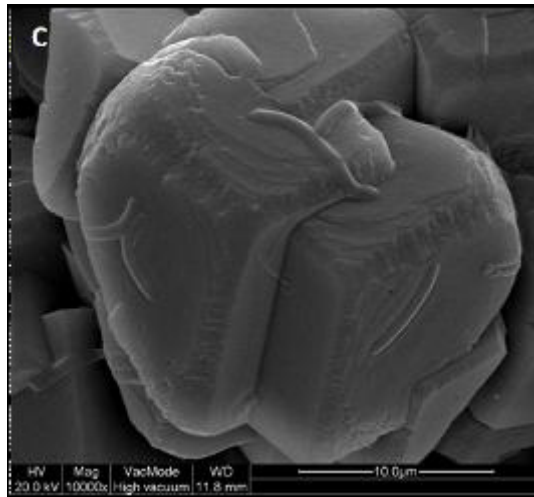


Fig. 3.31 Bacteria within CaCO_3 crystals formed in cracked mortar specimens containing *B. cohnii* in coated lava particles

3.2.5.6.2 Self-Healing Concrete with Bacteria in Diatomaceous Earth

The Ghent University group suggested the use of diatomaceous earth (DE) as the carrier for the bacteria [3.128, 3.129]. Diatomaceous earth (DE) is a natural soft siliceous sedimentary rock that is very easily crumbled into fine powders. It has a particle size ranging from less than $1\ \mu\text{m}$ to more than $1\ \text{mm}$, but typically 10 to $200\ \mu\text{m}$. The typical chemical composition of oven dried DE is 80% to 90% silica, with 2 to 4% alumina and 0.5% to 2% iron oxide. DE consists of fossilized remains of diatoms, which is a type of hard-shelled algae. Diatoms skeletons are highly porous, light in weight and chemically stable and inert. DE has been used in various fields. It is mainly used as filtration agent and functional fillers for paints and plastics. Furthermore, DE has been used before as a bacterial carrier, e.g. DE was used to incorporate non-pathogenic microbes inside a system for in-situ bioremediation of contaminated soil and ground water [3.130]. The aim of Wang *et al.* [3.128, 3.129] was to use DE as the protective carrier for the bacteria, which were added into the high pH cement environment of concrete as a self-healing agent.

First the activity of *Bacillus sphaericus* LMG 22557 (Belgian coordinated collection of microorganisms, Ghent) immobilized in DE, was tested in a cement slurry to mimic the high pH environment inside concrete. The pH of the suspension of Portland cement in water was about 12.5 after 1 day. Then a pre-mixture together with nutrients was added to the cement suspension. The pre-mixture was made of bacterial cells (concentration 10^8 cells/mL), water and DE. The nutrients were composed of urea and yeast extract. The ureolytic activity of the bacteria after immobilization into DE was evaluated by the decomposition of urea in the cement slurry. The amount of urea decomposed was measured by the determination of total ammonium nitrogen (TAN). TAN concentrations were measured calorimetrically by the method of Nessler [3.131]. The results showed that *B. sphaericus* bacteria immobilized in DE can decompose a significant amount of urea (on average 25 g/L), whereas almost no urea was decomposed in control slurries with DE but no bacteria, or with bacteria that were not immobilized on DE.

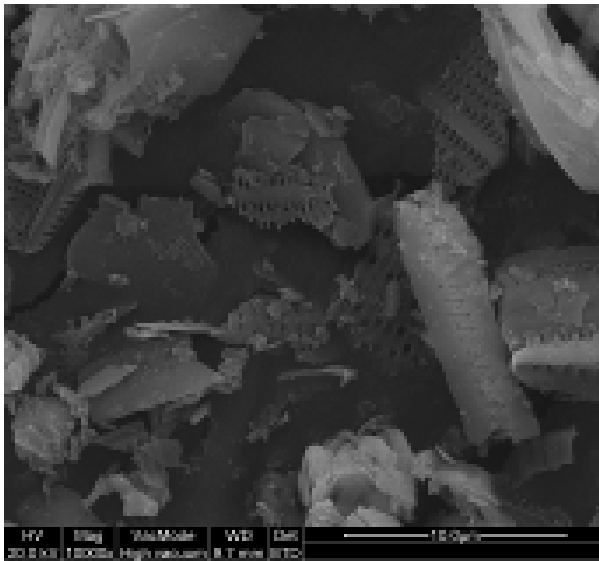


Fig. 3.32 Porous DE

Subsequently, mortar specimens (60 mm x 60 mm x 220 mm) with DE immobilized bacteria were prepared: half of the DE contained nutrients and the other half contained bacterial cells. As shown in Fig. 3.33, the addition of diatomaceous earth clearly improved the compressive strength of the mortar. It is thought that DE may function as a filler, and probably also as an internal curing aid. However, if the addition of DE is more than 5% of the cement by mass, the mortar paste becomes very dry and the workability decreases a lot.

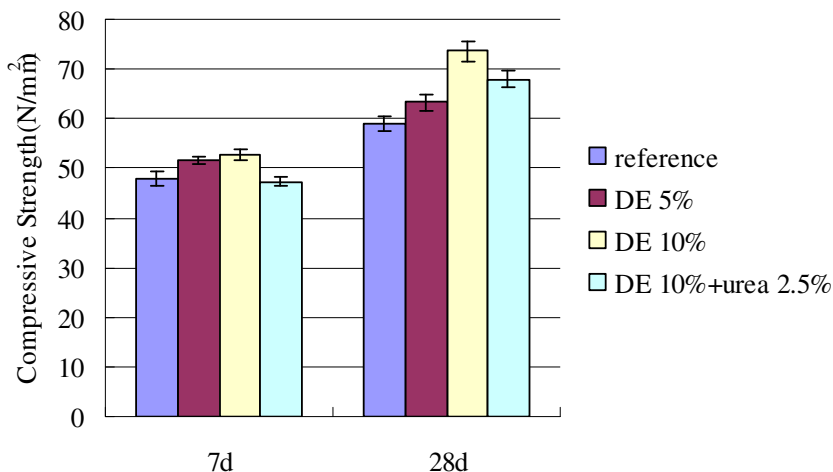


Fig. 3.33 Compressive strength of mortar with or without diatomaceous earth (DE)

After 7 days, the mortar specimens were taken out of the curing room and cracks were created by means of a crack width controlled three-point bending test. After unloading, the remaining crack width ranged from 0.15 mm to 0.17 mm. Afterwards, three of the cracked specimens were immersed into water and three were immersed into the deposition medium (made of urea and $\text{Ca}(\text{NO}_3)_2$, 0.2M) for 40 days. It was found that the cracks were filled with white crystals. Samples were taken from the crack wall for SEM and EDAX analysis. As shown in Fig. 3.34 (a) and (b), the particles appear in different shapes, with size ranging from 5 μm to 40 μm . EDAX results indicated that the particles are CaCO_3 .

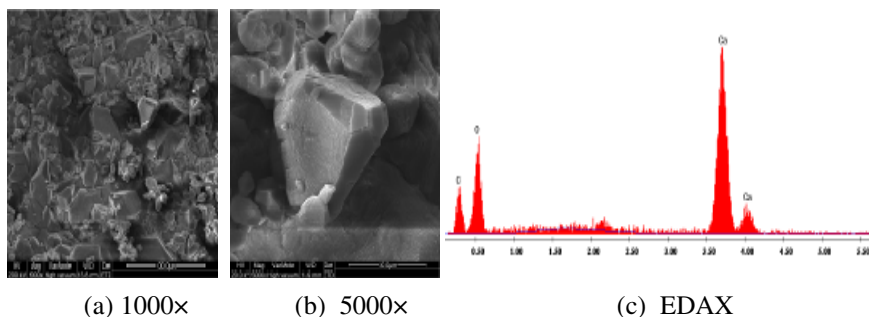


Fig. 3.34 CaCO_3 precipitation by DE immobilized bacteria in the cracks

A capillary water absorption test was performed to measure the water penetration resistance of the cracked mortar specimens with or without bacteria. The specimens were dried at 40 °C in an oven until weight changes were lower than 0.1% in 24 h intervals. Before the test, the specimens were coated with a

waterproof paint at four sides adjacent to the bottom surface where the crack existed. Also part of the bottom surface was coated with the paint except for an area of 40 mm x 20 mm around the crack. The coated specimens were immersed to a depth of 5 ± 1 mm in tap water and were weighed regularly. It appeared that the precipitation in the cracks profoundly decreased the water absorption of the cracked specimens. It can be seen from Fig. 3.35 that the speed of water absorption in the specimens without bacteria was much faster than in the ones with bacteria.

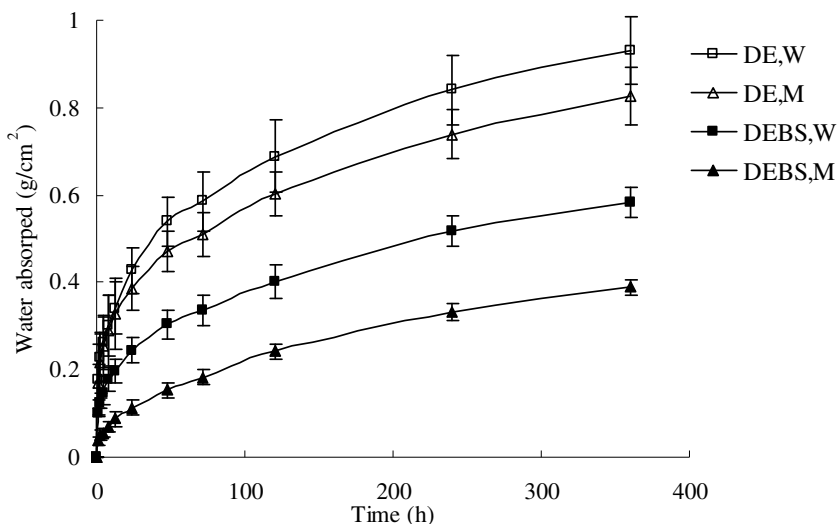


Fig. 3.35 Capillary water absorption of cracked (and self-healed) specimens as a function of time; all specimens contained diatomaceous earth (DE); BS refers to the specimens with bacteria; W refers to the previous incubation in water and M to incubation in urea and $\text{Ca}(\text{NO}_3)_2$ medium for 40 days (Source: [3.129])

3.2.5.6.3 Self-Healing Concrete with Bacteria in Tubular Capsules

The Ghent university team is also focussing on protection of the bacteria in tubular carrier materials in order to introduce them in a concrete matrix to obtain autonomous self-healing. As described in Wang *et al.* [3.132, 3.133] bacteria and PU were encapsulated in glass tubes with three compartments which were embedded inside mortar specimens. One compartment of the tubes contained the first component of the PU (prepolymer of PU). The second compartment was filled with the nutrients for bacterial growth, and a calcium source (this deposition medium contained yeast extract 20 g/L, urea 20 g/L and $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ 79 g/L) and the second component of the PU (accelerator). The last compartment was filled with the bacterial cells (*Bacillus sphaericus*, 10^8 cells/mL). The glass tubes were then placed in the tensile zone of mortar specimens (60 mm x 60 mm x 220 mm) and cracks were created in two weeks old specimens by means of a crack width controlled three-point bending test. When a crack appears in the mortar

matrix, the glass tubes break and all components mentioned above can flow into the crack and mix together. For the tubes with PU carrier material, first, polymerization of the filling agent (polyurethane) is initiated and through this process strength regain is obtained. In a second step, the bacteria, which are dispersed through the filling material can precipitate CaCO_3 crystals in the pores of the PU and through this, an additional regain in strength may be obtained. Different from the method of Bang *et al.* [3.108] described higher, in which PU foam with immobilized bacteria was pre-formed and placed into the cracks manually, in this research, PU foam is formed in the crack automatically upon crack appearance.

After performance of the bending test, all specimens were placed again into a curing room at a temperature of 20°C and a relative humidity of more than 90%. One week later, strength regain was measured by reloading the specimens. The self-healing efficiency was evaluated by comparing the peak load obtained during the first loading cycle and the reloading cycle. Considerable strength regain, on average 60%, was obtained in the case of specimens with incorporated PU and bacteria. However, there was no obvious difference in strength regain between specimens with living bacteria and specimens with dead bacteria. Therefore, the healing effect was mostly caused by the PU foam and not by the bacterially precipitated CaCO_3 . However, water permeability tests showed that the water permeability coefficient of the specimens with PU immobilized bacteria (10^{-8} to 10^{-11} m/s) was a little bit lower than for those with PU only (about 10^{-7} to 10^{-11} m/s).

TGA measurements indicated that CaCO_3 crystals were present in the cracks but that the amount of precipitation was quite limited. The main reason might be that there is insufficient water for microbial activity inside PU foam. This will hinder the contact among bacteria, urea and Ca^{2+} . Current research focuses on increasing the amount of bio- CaCO_3 precipitation in PU foam and on selection of other suitable carrier materials. First results indicate that increasing the amount of bacteria inside PU, enhances the precipitation of more CaCO_3 . Immobilization of the bacteria in PU did not lead to an obvious reduction in their ability to degrade urea. *B. sphaericus* immobilized into silica gel showed a somewhat higher activity than the same amount immobilized into PU.

Therefore as another alternative, the use of a two-component system was studied, existing of a tube with the bacteria suspended in silica sol, and a second tube with the deposition medium [3.124, 3.133]. The $\text{Ca}(\text{NO}_3)_2$ in the deposition medium also functions as activator for the silica gel formation. For the system with silica gel, this gel itself does not contribute to mechanical strength regain and only the precipitation of CaCO_3 crystals will allow regain in strength. For autonomously healed specimens with silica gel with or without bacteria, the water permeability coefficient of specimens was reduced much more in specimens with bacteria due to the bacterially induced CaCO_3 precipitation (10^{-6} m/s for specimens with silica gel only, to 10^{-6} - 10^{-9} m/s for specimens with silica gel and bacteria).

References

- 3.1. Edvardsen, C.K.: Water permeability and self-healing of through-cracks in concrete. DAFStb Bull 455 (1996) (in German)
- 3.2. Härtl, R.: Chemical binding of water during the hydration of portland cements and blast furnace slag cement bended with fly ash. In: 9th Intern. Congress on the Chemistry of Cement, New Dehli (1992)
- 3.3. Pistors, H.: Einwirkung von angreifenden Wässern auf Beton. Vom Wasser 30, 208–221 (1963)
- 3.4. Meichsner, H., Stelzner, S.: Über die Selbstdichtung von Rissen. *Betontechnik* 10(3), S.82–S.83 (1989)
- 3.5. Edvardsen, C.: Water permeability and autogenous healing of cracks in concrete. *ACI Materials J.* 96(4), 448–454 (1999)
- 3.6. Selbstheilung von Beton. *Beton und Eisen* 5/1935, S.96 (1935)
- 3.7. Von Emberger, F.: Neuere Bauwerke und Bauweisen aus Beton und Eisen. *Bauwerke III. Wasserbau. Zeitschrift des österr. Ingenieur- und Architekten-Vereins* Heft 24/1902, S.441–S.446 (1902)
- 3.8. Gilkey, H.J.: Die Selbstheilung von Beton und Mörtel. *American Society for Testing Materials*, Bd. 26, Teil ii, S. 470 und Bd. 29, Teil ii, S.593 (1926, 1929)
- 3.9. Maddocks, F.T.: Gebrochene Betonprüfkörper heilen und zeigen erhöhte Druckfestigkeit bei erneuter Prüfung, *California Highways*, p. S.11 (August 1925)
- 3.10. Merkle, G.: *Wasserdurchlässigkeit von Beton*. Springer, Berlin (1927)
- 3.11. Peckworth, H.F.: *Selbstheilung von Beton*. *Concrete Pipe Handbook*, Chicago, Kapitel XV (1965)
- 3.12. Soroker, V.M., Desov, A.J.: Selbstheilung von Beton, *constructeur de cement armé*, Bd. 18, Heft 197/1936, S.31. *Zement*, Bd. 25, Heft 30/1936, S.505 (1936)
- 3.13. Zschokke, B.: Über die Selbstdichtung von Betonblöcken bei den Wasserdurchlässigkeits-Proben. *Schweizerische Wasserwirtschaft* 2/1922, S.31–S.35 und 8/1923 (1923)
- 3.14. Loving, M.W.: Selbstheilung von Beton. *American Concrete Pipe Association, Bulletin* 13 (1936)
- 3.15. Wagner, E.F.: Autogenous healing of crack in cement-mortar linings for gray-iron and ductile-iron water pipe. *Journal of the American Water Works Association* 66(6), S.358–S.360 (1974)
- 3.16. Lauer, K.R., Slate, F.O.: Autogenous healing of cement paste. *Journal of the American Concrete Institute* 52(6), S.1083–S.1097 (1956)
- 3.17. Hannant, D.J., Keer, J.G.: Autogenous healing of thin cement based sheets. *Cement and Concrete Research* 13(3), S.357–S.365 (1983)
- 3.18. Dhir, R.K., Sangha, C.M., Munday, J.G.L.: Strength and deformation properties of autogenously healed mortars. *Journal of the ACI* (3), S.231–S.236 (1973)
- 3.19. Clear, C.A.: Leakage of cracks in concrete - summary of work to date. *Cement and concrete association* (1982) (Internal Publication)
- 3.20. Ripphausen, B.: Untersuchungen zur Wasserdurchlässigkeit und Sanierung von Stahlbetonbauteilen mit Trennrissen. *Dissertation RWTH Aachen* (1989)

- 3.21. Meichsner, H., Wuenschig, R.: Institut für Ingenieur- und Tiefbau: Die Selbstdichtung von Rissen in Beton- und Stahlbetonbauteilen. IRB-Verlag, Stuttgart (1991)
- 3.22. Granger, S., Loukili, A., Pijaudier-Cabot, G., Chanvillard, G.: Mechanical characterization of the self-healing effect of cracks in ultra high performance concrete (UHPC). In: Banthia, N. (ed.) *Construction Materials: Performance, Innovations and Structural Implications* (3rd Int. Conference), p. 9. The University of British Columbia and CD-ROM, Vancouver (2005)
- 3.23. Reinhardt, H.-W., Jooss, M.: Permeability and self-healing of cracked concrete as a function of temperature and crack width. *Cement and Concrete Research* 33, 981–985 (2003)
- 3.24. Hearn, N.: Self-healing property of concrete - Experimental evidence. *Materials and Structures* 30, 404–411 (1997)
- 3.25. Hearn, N.: Self-sealing, autogenous healing and continued hydration: what is the difference? *Materials and Structures* 31, 563–567 (1999)
- 3.26. Jacobsen, S., Sellevold, E.J.: Self-healing of high strength concrete after deterioration by freeze/thaw. *Cement and Concrete Research* 26(1), 55–62 (1996)
- 3.27. Jacobsen, S., Marchand, J., Hornain, H.: Sem observations of the microstructure of frost deteriorated and self-healed concrete. *Cement and Concrete Research* 25(8), 1781–1790 (1995)
- 3.28. Nanayakkara, S.M.A., Elakneshwaran, T.: Self-healing of Cracks in Concrete with Portland Limestone Cement. In: *Cement Combinations for Durable Concrete: Proceedings of the Int. Conference held at the University of Dundee, Scotland, UK, July 5-7*, pp. S.675–S.684. Telford, London (2005)
- 3.29. Termkhajornkit, P., et al.: Self-healing ability of fly ash-cement systems. *Cement and Concrete Composites* 31(3), 195–203 (2009)
- 3.30. Taniguchi, M., et al.: Self-healing of frost-damaged concrete incorporating fly ash. In: Bond, I., Varley, R. (eds.) *3rd International Conference on Self-healing Materials*, Bath, UK, pp. 48–49 (2011)
- 3.31. Na, S.H., et al.: Evaluation of self-healing effect of concrete using fly ash or ground granulated blast furnace slag. In: Bond, I., Varley, R. (eds.) *3rd International Conference on Self-healing Materials*, Bath, UK, pp. 125–126 (2011)
- 3.32. Haddad, R.H., Bsoul, M.A.: Self-healing of polypropylene fiber reinforced concrete; pozzolan effect, pp. 1412–1421 (1999)
- 3.33. Morimoto, T., et al.: Self-healing properties of Ultra High Performance Strain Hardening Cementitious Composites (UHP-SHCC). In: *4th International Conference on Construction Materials*, Nagoya, Japan, pp. 319–326 (2009)
- 3.34. Şahmaran, M., Li, V.C.: Durability properties of micro-cracked ECC containing high volumes fly ash. *Cement and Concrete Research* 39(11), 1033–1043 (2009)
- 3.35. Liu, X.-Y., et al.: Experimental study on self-healing performance of concrete. *Journal of Building Materials* (2) (2005)
- 3.36. Şahmaran, M., et al.: Self-healing of mechanically-loaded self consolidating concretes with high volumes of fly ash. *Cement and Concrete Composites* 30(10), 872–879 (2008)

- 3.37. Yang, E.-H., Yang, Y., Li, V.C.: Use of high volumes of fly ash to improve ECC mechanical properties and material greenness. *ACI Materials Journal* 104(6), 620–628 (2007)
- 3.38. ter Heide, N.: Crack healing in hydrating concrete. MSc thesis, Delft University of Technology, p. 128 (2005)
- 3.39. Liu, S., Zuo, M.: Influence of slag and fly ash on the self-healing ability of concrete. *Advanced Materials Research* 306-307, 1020–1023 (2011)
- 3.40. Van Tittelboom, K., De Belie, N.: Autogenous healing of cracks in cementitious materials with varying mix compositions. In: 2nd International Conference on Self-healing Materials, Chicago, USA, p. 160 (2009)
- 3.41. Van Tittelboom, K., et al.: Influence of mix composition on the extent of autogenous crack healing by continued hydration or calcium carbonate formation. *Construction and Building Materials* 37(0), 349–359 (2012)
- 3.42. Jacobsen, S., Marchand, J., Gerard, B.: Concrete Cracks I: Durability and Self-healing – a Review. In: Gjørsv, O.E., Sakai, K., Banthia, N. (eds.) *Concrete under Severe Conditions 2, CONSEC 1998: Environment and Loading: Proceedings of the 2nd International Conference*, Tromsø, Norway, June 21-24, pp. 217–231. E & FN Spon, London (1998)
- 3.43. Meichsner, H.: Über die Selbstdichtung von Trennrissen in Beton. *Beton-und Stahlbetonbau* 87(4), 95–99 (1992)
- 3.44. Edvardsen, C.: Self-healing of concrete cracks. *Concrete* 33 (April 1999)
- 3.45. DIN 1045-1, Concrete, reinforced and prestressed concrete structures – Part 1: Design, German Standardization Institute (July 2001)
- 3.46. Snoeck, D., et al.: Self-healing cementitious materials by the combination of microfibres and superabsorbent polymers. *Journal of Intelligent Material Systems and Structures* (2012)
- 3.47. Lee, H.X.D., et al.: Potential of superabsorbent polymer for self-sealing cracks in concrete. *Advances in Applied Ceramics* 109, 296–302 (2010)
- 3.48. Lee, H.X.D., et al.: Self-sealing cement-based materials using superabsorbent polymers. In: *International RILEM Conference on Use of Superabsorbent Polymers and Other New Additives in Concrete*, Lyngby, pp. 171–178 (2010)
- 3.49. Tsuji, M., et al.: Basic studies on simplified curing technique, and prevention of initial cracking and leakage of water through cracks of concrete by applying superabsorbent polymers as new concrete admixture. *Journal of the Society of Materials Science* 48, 1308–1315 (1999)
- 3.50. Snoeck, D., et al.: Visualization of water penetration in cementitious materials with superabsorbent polymers by means of neutron radiography. *Cement and Concrete Research* 42, 1113–1121 (2012)
- 3.51. Snoeck, D., et al.: The use of superabsorbent polymers as a crack sealing and crack healing mechanism in cementitious materials. In: *3rd International Conference on Concrete Repair, Rehabilitation and Retrofitting*, Cape Town, pp. 152–157 (2012)
- 3.52. Kim, J.S., Schlagen, E.: Super absorbent polymers to simulate self-healing in ECC. Presented at the 2nd International Symposium on Service Life Design for Infrastructures, Delft (2010)

- 3.53. Sisomphon, K., Copuroglu, O., Fraaij, A.: Application of encapsulated lightweight aggregate impregnated with sodium monofluorophosphate as a self-healing agent in blast furnace slag mortar. *Heron* 56(1/2), 13–32 (2011)
- 3.54. Sisomphon, K., Copuroglu, O., Fraaij, A.: Self-healing blast furnace slag mortar subjected to carbonation and frost salt scaling. In: White, S., Bond, I. (eds.) 2nd International Conference on Self-healing Materials, Chicago, USA, p. 159 (2009)
- 3.55. Dry, C.M., Corsaw, M.J.T.: A time-release technique for corrosion prevention. *Cement and Concrete Research* 28(8), 1133–1140 (1998)
- 3.56. Mechtcherine, V., Lieboldt, M.: Permeation of water and gases through cracked textile reinforced concrete. *Cement Concrete Comp.* 33, 725–734 (2011)
- 3.57. Butler, M., Mechtcherine, V., Hempel, S.: Experimental investigations on the durability of fibre-matrix interfaces in textile-reinforced concrete. *Cement and Concrete Composites* 31, 221–231 (2009)
- 3.58. De Muynck, W., Belie, N., Verstraete, W.: Microbial carbonate precipitation in construction materials: a review. *Ecological Engineering* 36(2), 118–136 (2010)
- 3.59. Lowenstan, H.A., Weiner, S.: On biomineralization. Oxford University Press, New York (1988)
- 3.60. Rivadeneyra, M.A., Delgado, R., del Moral, A., Ferrer, M.R., Ramos-Cormenzana, A.: Precipitation of calcium carbonate by *Vibrio* spp. from an inland saltern. *FEMS Microbiol. Ecol.* 13(3), 197–204 (1994)
- 3.61. Barabesi, C., Galizzi, A., Mastromei, G., Rossi, M., Tamburini, E., Perito, B.: *Bacillus subtilis* Gene Cluster Involved in Calcium Carbonate Biomineralization. *J. Bacteriol.* 189(1), 228–235 (2007)
- 3.62. Knorre, H., Krumbein, K.E.: Bacterial calcification. In: Riding, E.E., Awramik, S.M. (eds.) *Microbial Sediments*, pp. 25–31. Springer, Berlin (2000)
- 3.63. Rivadeneyra, M.A., Parraga, J., Delgado, R., Ramos-Cormenzana, A., Delgado, G.: Biomineralization of carbonates by *Halobacillus trueperi* in solid and liquid media with different salinities. *FEMS Microbiol. Ecol.* 48, 39–46 (2004)
- 3.64. Hammes, F., Verstraete, W.: Key roles of pH and calcium metabolism in microbial carbonate precipitation. *Rev. Environ. Sci. Biotechnol.* 1, 3–7 (2002)
- 3.65. Stumm, W., Morgan, J.J.: *Aquatic chemistry*, 2nd edn. John Wiley, New York (1981)
- 3.66. Castanier, S., Le Metayer-Levrel, G., Perthuisot, J.-P.: Ca-carbonates precipitation and limestone genesis – the microbiogeologist point of view. *Sediment. Geol.* 126(1-4), 9–23 (1999)
- 3.67. Braissant, O., Verrecchia, E., Aragno, M.: Is the contribution of bacteria to terrestrial carbon budget greatly underestimated? *Naturwissenschaften* 89(8), 366–370 (2002)
- 3.68. Boquet, E., Boronat, A., Ramos-Cormenzana, A.: Production of Calcite (Calcium Carbonate) Crystals by Soil Bacteria is a General Phenomenon. *Nature* 246(5434), 527–529 (1973)
- 3.69. Morita, R.: Calcite precipitation by marine bacteria. *Geomicrobiol. J.* 2, 63–82 (1980)
- 3.70. Douglas, S., Beveridge, T.J.: Mineral formation by bacteria in natural microbial communities. *FEMS Microbiol. Ecol.* 26(2), 79–88 (1998)

- 3.71. Ehrlich, H.L.: Geomicrobiology: its significance for geology. *Earth-Sci. Rev.* 45(1-2), 45–60 (1998)
- 3.72. Chaturvedi, S., Chandra, R., Rai, V.: Isolation and characterization of *Phragmites australis* (L.) rhizosphere bacteria from contaminated site for bioremediation of colored distillery effluent. *Ecol. Eng.* 27(3), 202–207 (2006)
- 3.73. Simon, M.A., Bonner, J.S., Page, C.A., Townsend, R.T., Mueller, D.C., Fuller, C.B., Autenrieth, R.L.: Evaluation of two commercial bioaugmentation products for enhanced removal of petroleum from a wetland. *Ecol. Eng.* 22(4-5), 263–277 (2004)
- 3.74. Warren, L.A., Maurice, P.A., Parmar, N., Ferris, F.G.: Microbially Mediated Calcium Carbonate Precipitation: Implications for Interpreting Calcite Precipitation and for Solid-Phase Capture of Inorganic Contaminants. *Geomicrobiol. J.* 18, 93–125 (2001)
- 3.75. Fujita, Y., Redden, G.D., Ingram, J.C., Cortez, M.M., Ferris, F.G., Smith, R.W.: Strontium incorporation into calcite generated by bacterial ureolysis. *Geochim. Cosmochim. Acta* 68(15), 3261–3270 (2004)
- 3.76. Hammes, F., Boon, N., de Villiers, J., Verstraete, W., Siciliano, S.D.: Strain-Specific Ureolytic Microbial Calcium Carbonate Precipitation. *Appl. Environ. Microbiol.* 69(8), 4901–4909 (2003)
- 3.77. Hammes, F., Seka, A., de Knijf, S., Verstraete, W.: A novel approach to calcium removal from calcium-rich industrial wastewater. *Water Res.* 37(3), 699–704 (2003)
- 3.78. Nemati, M., Voordouw, G.: Modification of porous media permeability, using calcium carbonate produced enzymatically in situ. *Enzyme Microb. Technol.* 33(5), 635–642 (2003)
- 3.79. Ferris, F.G., Stehmeier, L.G.: Bacteriogenic mineral plugging. USA Patent US5143155 (1992)
- 3.80. DeJong, J.T., Fritzges, M.B., Nusslein, K.: Microbially Induced Cementation to Control Sand Response to Undrained Shear. *J. Geotech. Geoenviron.* 132(11), 1381–1392 (2006)
- 3.81. Whiffin, V.S., van Paassen, L., Harkes, M.P.: Microbial Carbonate Precipitation as a Soil Improvement Technique. *Geomicrobiol. J.* 24, 417–423 (2007)
- 3.82. Adolphe, J.P., Loubière, J.F., Paradas, J., Soleilhavoup, F.: Procédé de traitement biologique d'une surface artificielle. European patent 90400G97.0 (after French patent 8903517, 1990)
- 3.83. Oriol, G.: La biomineralisation appliquée à la conservation du patrimoine: bilan de dix ans d'experimentation. *Restaurar la memoria*, Valladolid, Spain (2000)
- 3.84. Oriol, G., Vieweger, T., Loubiere, J.F.: Les mortiers biologiques: une solution pour la conservation de la sculpture monumentale en pierre. *Art Biology and Conservation*, Metropolitan Museum New York (2002)
- 3.85. Le Metayer-Levrel, G., Castanier, S., Oriol, G., Loubiere, J.F., Perthuisot, J.P.: Applications of bacterial carbonatogenesis to the protection and regeneration of limestones in buildings and historic patrimony. *Sediment. Geol.* 126(1-4), 25–34 (1999)

- 3.86. Rodriguez-Navarro, C., Rodriguez-Gallego, M., Ben Chekroun, K., Gonzalez-Munoz, M.T.: Conservation of Ornamental Stone by *Myxococcus xanthus*-Induced Carbonate Biomineralization. *Appl. Environ. Microbiol.* 69(4), 2182–2193 (2003)
- 3.87. Dick, J., De Windt, W., De Graef, B., Saveyn, H., Van der Meeren, P., De Belie, N., Verstraete, W.: Bio-deposition of a calcium carbonate layer on degraded limestone by *Bacillus* species. *Biodegradation* 17(4), 357–367 (2006)
- 3.88. Stocks-Fischer, S., Galinat, J.K., Bang, S.S.: Microbiological precipitation of CaCO_3 . *Soil Biol. Biochem.* 31(11), 1563–1571 (1999)
- 3.89. De Muynck, W., Cox, K., De Belie, N., Verstraete, W.: Bacterial carbonate precipitation as an alternative surface treatment for concrete. *Constr. Build. Mater.* 22(5), 875–885 (2008)
- 3.90. De Muynck, W., Debrouwer, D., De Belie, N., Verstraete, W.: Bacterial carbonate precipitation improves the durability of cementitious materials. *Cem. Concr. Res.* 38(7), 1005–1014 (2008)
- 3.91. Tiano, P., Biagiotti, L., Mastromei, G.: Bacterial bio-mediated calcite precipitation for monumental stones conservation: methods of evaluation. *J. Microbiol. Method* 36, 139–145 (1999)
- 3.92. Tiano, P., Addadi, L., Weiner, S.: Stone reinforcement by induction of calcite crystals using organic matrix macromolecules: feasibility study. In: 7th International Congress on Deterioration and Conservation of Stone, Lisbon, pp. 1317–1326 (1992)
- 3.93. Tiano, P.: Stone Reinforcement by Calcite Crystal Precipitation Induced by Organic Matrix Macro-molecules. *Stud. Conserv.* 40(3), 171–176 (1995)
- 3.94. Tiano, P., Cantisani, E., Sutherland, I., Paget, J.M.: Biomediated reinforcement of weathered calcareous stones. *J. Cult. Herit.* 7(1), 49–55 (2006)
- 3.95. Mastromei, G., Marvasi, M., Perito, B.: Studies on bacterial carbonate precipitation for stone conservation. In: Proc. of BioGeoCivil Engineering Conference, Delft, Netherlands, pp. 104–106 (2008)
- 3.96. Jimenez-Lopez, C., Rodriguez-Navarro, C., Piñar, G., Carrillo-Rosúa, F.J., Rodriguez-Gallego, M., Gonzalez-Muñoz, M.T.: Consolidation of degraded ornamental porous limestone by calcium carbonate precipitation induced by the microbiota inhabiting the stone. *Chemosphere* 68(10), 1929–1936 (2007)
- 3.97. Piñar, G., Jimenez-Lopez, C., Sterflinger, K., Etenauer, J., Jroundi, F., Fernandez-Vivas, A., Gonzalez-Muñoz, M.T.: Bacterial community dynamics during the application of a *Myxococcus xanthus*-inoculated culture medium used for consolidation of ornamental limestone. *Microb. Ecol.* 66, 15–28 (2010)
- 3.98. Jimenez-Lopez, C., Jroundi, F., Pascolini, C., Rodriguez-Navarro, C., Piñar-Larrubia, G., Rodriguez-Gallego, M., González-Muñoz, M.T.: Consolidation of quarry calcarenite by calcium carbonate precipitation induced by bacteria activated among the microbiota inhabiting the stone. *Int. Biodeterior. Biodegrad* 62(4), 352–363 (2008)
- 3.99. Gonzalez Muñoz, M.T., Rodriguez-Navarro, C., Jimenez-Lopez, C., Rodriguez-Gallego, M.: Method and product for protecting and reinforcing construction and ornamental materials. WO 2008/009771 A1 (2008)

- 3.100. Jroundi, F., Fernández-Vivas, A., Rodríguez-Navarro, C., Bedmar, E.J., González-Muñoz, M.T.: Bioconservation of deteriorated monumental calcarenite stone and identification of bacteria with carbonatogenic activity. *Microb. Ecol.* 60, 39–54 (2010)
- 3.101. Zhong, L., Islam, M.R.: A new microbial plugging process and its impact on fracture remediation. In: *Proc. of. Society of Petroleum Engineers. Annual Technical Conference*, Dallas, Texas, pp. 703–715 (1995)
- 3.102. Castanier, S.: Nouvelles compositions pour mortier biologique, procédé de recouvrement d’une surface ou de comblement d’une cavité à l’aide des compositions. French patent. No 95 05861 (1995)
- 3.103. Whiffin, V.S.: Microbial CaCO₃ precipitation for the production of Biocement. Ph.D thesis Murdoch University, Australia, p. 155 (2004), <http://wwwlib.murdoch.edu.au/adt/browse/view/adt-MU20041101.142604>
- 3.104. Kucharski, E.S., Cord-Ruwisch, R., Whiffin, V., Al-Thawadi, S.M.J.: Microbial biocementation. World intellectual property organization. WO 2006/066326 A1 (2006)
- 3.105. Ramachandran, S.K., Ramakrishnan, V., Bang, S.S.: Remediation of Concrete Using Micro-organisms. *ACI Mater. J.* 98, 3–9 (2001)
- 3.106. Achal, V.: Microbial remediation of defects in building materials and structures. PhD thesis, Thapar University, India (2010)
- 3.107. Day, J.L., Ramakrishnan, V., Bang, S.S.: Microbiologically induced sealant for concrete crack remediation. In: *Proc. of. 16th Engineering Mechanics Conference*, Seattle (2003)
- 3.108. Bang, S.S., Galinat, J.K., Ramakrishnan, V.: Calcite precipitation induced by polyurethane-immobilized *Sporosarcina pasteurii*. *Enzyme Microb. Technol.* 28(4-5), 404–409 (2001)
- 3.109. Bachmeier, K.L., Williams, A.E., Warmington, J.R., Bang, S.S.: Urease activity in microbiologically-induced calcite precipitation. *J. Biotechnol.* 93(2), 171–181 (2002)
- 3.110. De Belie, N., De Muynck, W.: Crack repair in concrete using biodeposition. In: Alexander, M.G., Beushausen, H.-D., Dehn, F., Moyo, P. (eds.) *Proceedings of the International Conference on Concrete Repair, Rehabilitation and Retrofitting (ICCRRR)*, Cape Town, South Africa, November 24–26, 2008, pp. 291–292 in abstract book; pp. 777–781 on CD-ROM. CRC Press, Boca Raton (2009) ISBN: 978-0-415-46850-3
- 3.111. Van Tittelboom, K., De Belie, N., De Muynck, W., Verstraete, W.: Use of bacteria to repair cracks in concrete. *Cement and Concrete Research* 40(1), 157–166 (2010)
- 3.112. De Belie, N.: Microorganisms versus stony materials: a love-hate relationship - Robert L’Hermite Medal lecture 2010. *Materials and Structures* 43(9), 1191–1202 (2010)
- 3.113. Ramakrishnan, S.K., Panchalan, R.K., Bang, S.S.: Improvement of concrete durability by bacterial mineral precipitation. In: *11th International Conference on Fracture*, Turin, Italy (2001)

- 3.114. Achal, V., Mukherjee, A., Basu, P.C., Reddy, M.S.: Lactose mother liquor as an alternative nutrient source for microbial concrete production by *Sporosarcina pasteurii*. *Journal of Industrial Microbiology and Biotechnology* 36, 433–438 (2009)
- 3.115. Ghosh, P., Mandal, S., Chattopadhyay, B.D., Pal, S.: Use of microorganism to improve the strength of cement mortar. *Cem. Concr. Res.* 35(10), 1980–1983 (2005)
- 3.116. Ghosh, S., Biswas, M., Chattopadhyay, B.D., Mandal, S.: Microbial activity on the micro-structure of bacteria modified mortar. *Cement & Concrete Composites* 31, 93–98 (2009)
- 3.117. Van der Zwaag, S., van Dijk, N.H., Jonkers, H.M., Mookhoek, S.D., Sloof, W.G.: Self-healing behaviour in engineering materials: bio-inspired but respecting their intrinsic character. *Philosophical Transactions of the Royal Society A-Mathematical Physical and Engineering Sciences* 367, 1689–1704 (2009)
- 3.118. Jonkers, H.M.: Self-healing concrete: a biological approach. In: van der Zwaag, S. (ed.) *Self-healing Materials: An Introduction*, pp. 195–204. Springer, The Netherlands (2007)
- 3.119. Jonkers, H.M., Schlangen, E.: Crack repair by concrete-immobilized bacteria. In: Schmetts, A.J.M., Van der Zwaag, S. (eds.) *Proc. of First International Conference on Self-healing Materials*, Noordwijk, The Netherlands, p. 7 (2007)
- 3.120. Jonkers, H.M., Thijssen, A., Muyzer, G., Copuroglu, O., Schlangen, E.: Application of bacteria as self-healing agent for the development of sustainable concrete. *Ecological Engineering* 36(2), 230–235 (2010)
- 3.121. Wiktor, V., Jonkers, H.M.: Self-healing of cracks in bacterial concrete. In: Van Breugel, K., Guang, Y., Yong, Y. (eds.) *Proc. 2nd Int. Symposium Service Life Design for Infrastructure*, Delft, The Netherlands, October 4–6, pp. 825–831 (2010)
- 3.122. Wiktor, V., Jonkers, H.M.: Quantification of crack-healing in novel bacteria-based self-healing concrete. *Cement and Concrete Composites* 33(7), 763–770 (2011)
- 3.123. Jonkers, H.M.: Bacteria-based self-healing concrete. *Heron* 56, 1–12 (2011)
- 3.124. Wang, J.: Self-healing concrete using microorganisms. In: *The 2nd CSC PhD Students Workshop*. Ghent, September 3, pp. 11–22 (2010)
- 3.125. Standaert, L.: *Zelfheling van beton met behulp van ingekapselde micro-organismen*. Masterproef, Gent, Faculteit Ingenieurswetenschappen (2010)
- 3.126. Samonin, V.V., Elikova, E.E.: A study of the Adsorption of bacterial cells on porous materials. *Microbiology* 7(6), 696–701 (2004)
- 3.127. Aldea, C.M., Ghandehari, M., Shah, S.P., Karr, A.: Combined effect of cracking and water permeability of concrete. In: *Fourteenth Engineering Mechanics Conference*. American Society of Civil Engineers (2000)
- 3.128. Wang, J., De Belie, N., Verstraete, W.: Self-healing of mortar by bacteria encapsulated in diato-maceous earth. In: Godbout, S., Potvin, L. (eds.) *Proceedings of the 7th International Symposium on Concrete for a Sustainable Agriculture (CSA)*, Quebec, September 18–21, pp. 17–24 (2011)
- 3.129. Wang, J.Y., De Belie, N., Verstraete, W.: Diatomaceous earth as a protective vehicle for bacteria applied for self-healing concrete. *Journal of Industrial Microbiology and Biotechnology* 39, 567–577 (2012)

- 3.130. Hunt, S.C.: Method and system for bioremediation of contaminated soil using inoculated diato-maceous earth. United States patent: US 005570973A (1996)
- 3.131. Ivanov, V.M., Figurovskaya, V.N., Barbalat Yu, A., Ershova, N.I.: Chromaticity characteristics of NH_2 , Hg_2 , I_3 and I_2 : molecular Iodine as a test form alternative to Nessler's reagent. *Journal of Analytical Chemistry* 60(7), 707–710 (2005)
- 3.132. Wang, J., Van Tittelboom, K., De Belie, N., Verstraete, W.: Potential of applying immobilized *Bacillus sphaericus* to heal cracks in concrete autonomously. In: Zachar, J., Claisse, P., Naik, T.R., Ganjian, E. (eds.) *Second International Conference on Sustainable Construction Materials and Technologies*, Ancona, Italy, May 31-June 3, vol. 3, pp. 1807–1818 (2010) ISBN 978-1-4507-1490-7
- 3.133. Wang, J., Van Tittelboom, K., De Belie, N., Verstraete, W.: Use of silica gel or polyurethane immobilized bacteria for self-healing concrete. *Construction and Building Materials* 26, 532–540 (2012)