Self-healing of cracked concrete: A bacterial approach

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ABSTRACT: Crack occurrence in reinforced concrete should be minimized for both durability and economical reasons as crack repair is costly. Autogenous repair, or self-healing, of concrete would save a substantial amount of money, as manual inspection and crack repair could be minimized. Thus, a reliable self-healing mechanism for concrete would not only result in more durable structures, but would also be beneficial for the global economy. This study exploited the potential to apply calcite-precipitating bacteria as a crack-healing agent in concrete. The potential of different species to precipitate calcite, produce endospores, survive concrete-production, and heal cracks by sealing them with calcite was investigated. Furthermore, the mechanical properties of 'bacterial concrete' were tested. ESEM studies showed that alkali-resistant spore-forming bacteria embedded in the concrete matrix can precipitate substantial amounts of calcite. The bacterial approach thus seems a highly promising mechanism to mediate self-healing in concrete structures.

1 INTRODUCTION

Cracks can occur in concrete structures due to multiple reasons such as autogenous shrinkage, freeze-thaw reactions, mechanical compressive- and tensile forces. Although micro-cracks do not necessarily result in significant strength loss of concrete, the ingress of water and other reactive chemicals such as chloride and water may pose a thread to the steel reinforcement as these strongly enhance its corrosion rate. Thus for durability reasons and potential repair costs, crack occurrence should be minimized or, alternatively, occurring cracks should ideally be healed directly after formation by an autonomous repair mechanism. Different autonomous repair systems are feasible. One such a self-healing mechanism could involve secondary hydration reactions of still present but not fully reacted cement particles. Although a high percentage of non-reacted cement particles within its matrix may result in a concrete with a substantial self-healing capacity, the material characteristics of the initial concrete structure may not be satisfactorily as it may be more brittle and initially weaker as wanted. Another self-healing mechanism could be based on the addition of a self-healing agent that would make up a part of the concrete matrix without or insignificantly affecting its structural and mechanical characteristics. In this study the potential of bacteria to act as a self-healing agent in concrete is investigated. Although the idea to use bacteria and integrate them in the concrete matrix may seem odd at first, it is not from a microbiological viewpoint. Bacteria naturally occur virtually everywhere on earth, not only on its surface but also deep within, e.g. in sediment and rock at a depth of more than 1 km (Jorgensen & D'Hondt 2006). Various species of so-called extremophilic bacteria, i.e. bacteria that love the extreme, are found in highly desiccated environments such as deserts (Dorn & Oberlander 1981; DeLaTorre et al 2003), but also inside rocks (Fajardo-Cavazos & Nicholson 2006) and even in ultra-basic environments (Pedersen et al 2004; Sleep et al 2004) which can be considered homologous to the internal concrete environment. Typical for many desiccation- and/or alkali-resistant bacterial species is their ability to form endospores. These specialized cells are characterized by an extremely low metabolic activity, are known to be able to resist high mechanically- and chemically induced stresses (Sagripanti & Bonifacino 1996) and are viable for periods up to 200 years (Schlegel 1993). In some previously published studies the application of bacteria for cleaning of concrete surfaces (DeGraef et al 2005) and strength improvement of cement-sand mortar (Ghosh et al 2005) was reported. Furthermore, in some studies the crack-healing potential by mineral-precipitating bacteria on degraded limestone (Dick et al 2006) and ornamental stone surfaces (Rodriguez-Navarro et al 2003) as well as on concrete surfaces (Bang et al 2001; Ramachandran et al 2001) was investigated and reported. Although promising results were re-

ported, the major drawback of the latter studies was that the bacteria and compounds needed for mineral precipitation could only be applied externally on the surface of the structures after crack-formation had occurred. This methodological necessity was mainly due to the limited lifetime (hours to a few days) of the (urease-based) enzymatic activity and/or viability of the applied bacterial species. In the present study the application of alkali-resistant spore-forming bacteria to enhance the self-healing capacity of concrete is investigated. Tensile- and compressive strength characteristics of reference (no bacteria added) and bacterial concrete are quantified. Furthermore, the viability of bacteria immobilization in concrete is quantified and, finally, calcite precipitation potential of bacterial concrete is demonstrated by ESEM analysis.

2 METHODS

2.1 *Cultivation of alkali-resistant spore-forming bacteria*

Four strains of alkaliphilic spore-forming bacteria were purchased from DSMZ (German Collection of Microorganisms and Cell Cultures), Braunschweig, Germany: *Sporosarcina pasteurii* DSM 33; *Bacillus cohnii* DSM 6307; *Bacillus halodurans* DSM 497 and *Bacillus pseudofirmus* DSM 8715 and cultivated according to the suppliers recommendations (medium DSMZ-2 for *S.pasteurii* and DSMZ-31 for the others).

Endospore-forming potential was determined in mineral medium. This medium contained per liter of Milli-Q ultra pure water: 0.2g NH_4Cl, 0.02g KH_2PO_4, 0.225g $CaCl_2$, 0.2g KCl, 0.2g $MgCl_2.6H_2O$, 1 ml per liter trace elements solution SL12B, 0.1g yeast extract, 6.45g citric acid trisodium salt and 8.4g sodium bicarbonate. The pH of this medium was 9.2. Aerobic batch cultures were incubated in 2-l Erlenmeyer flasks on a shaker table at 150 rpm. Growth was monitored by microscopy and cell numbers and percentage of sporulating cells were quantified by microscopy using a Burger-Turk counting chamber.

2.2 *Preparation and strength characteristics of bacterial concrete*

Concrete bars with and without (control) added bacteria were prepared for tensile- and compressive strength determination. The aim of these tests was to check whether the strength of the concrete was not negatively affected by the bacteria. Firstly, for the preparation of bacterial concrete, a dense culture of *S.pasteurii* was obtained after growth in medium DSMZ-2. Total cell number was quantified by microscopy using a Burger-Turk counting chamber. Subsequently, cells were washed twice by centrifugation (20 min x 10000g) and resuspension of the cell pellet in tap water. Washed cells were finally resuspended in a 20-ml aliquot of tap water. This cell suspension was applied as part of the needed water for concrete bar preparation.

Concrete bars for tensile- and compressive strength determination were prepared as follows. Two sets (bacterial concrete and control concrete without bacteria) of nine bars each (bar dimensions 16 x 4 x 4 cm) were made using ordinary portland cement (ENCI CEMI 32.5R), a water-cement ratio of 0.5 and aggregate composition (sand and gravel) as listed in Table 1. The bars were initially cured for 24 hours in plastic foil-sealed molds at room temperature, subsequently uncased and further cured in tap water-filled separate plastic containers at room temperature. Subsets of three bars each were tested for flexural tensile- and compressive strength after 3, 7 and 28 days curing following the procedure according to EN 196-1 Standard Norm.

Table 1. Cement, water and aggregate composition needed for the production of 9 concrete bars of dimensions 16 x 4 x 4 cm used for tensile- and compressive strength characterization of bacterial- and control (no bacteria added) concrete. For bacterial concrete, the 20-ml cell suspension was part of the total water volume needed.

Compound / Aggregate size (mm):	Weight (g):
Cement (ENCI CEM I 32.5)	1170
Water	585
Aggregate size fraction:	
4 - 8	1685
2 - 4	1133
1 - 2	848
0.5 - 1	848
0.25 - 0.5	730
0.125 - 0.25	396

2.3 *Viability of concrete-immobilized spores*

The viability (ability to germinate) of spores of the alkaliphilic bacterial species *B.cohnii*, *B.halodurans* and *B.pseudofirmus* immobilized in cement stone was determined. Cultures of the respective species were firstly grown in mineral medium (see above). These cultures were washed twice by centrifugation (20 min x 10000g) and resuspension of the cell pellet in tap water after the number of spores formed was quantified by microscopy. Obtained spore suspensions were divided in two parts, one part was stored in a fridge at 4ºC and served as non-concrete immobilized control for determination of spore viability during storage (see below), and one part was used for cement stone sample preparation. For the latter, the spore suspension was used as part of the make up water, and bacterial and control (no bacterial spores added) cement stone specimen were pre-

pared. Number of spores added to cement stone as determined by microscopic counting was 10^9 cm^{-3}. Ordinary portland cement (ENCI CEMI 32.5R) and a water-cement ratio of 0.5 was used for the preparation of cement stone disks (4 cm diameter, 1 cm height), cast in plastic vials closed with a plastic lid. After 24 hours curing at room temperature, disks were further cured in tap water at room temperature. Disks were removed from the plastic vial molds after ten days curing, chipped to pieces with a chisel and further crushed to powder using a robust pharmaceutical stone mortar. Powdered cement stone (1.84 g representing 1 cm^3, containing 10^9 spores) was subsequently slurried and diluted 10-fold by addition of 9 volumes sterile mineral medium. In parallel, the endospore-containing cell suspensions which were kept in a fridge at 4ºC were diluted to a spore density of 10^9 ml^{-1} and also 10-fold diluted by addition of 9 ml sterile mineral medium. Cement stone slurries and original spore suspensions were further homogenized by three cycles of vigorous mixing at 2500 rpm and 20 seconds ultrasonic treatment in a Branson 1210, 47 KHz, 80 Watts Ultrasonic bath. Number of viable spores in cement stone slurries and spore suspensions was estimated according to the Most Probable Number (MPN) dilution technique. For this procedure, 8 x 12 wells sterile microtiter plates were filled with mineral medium, 180 µl per well. Four consecutive wells of the first row were inoculated with 20-µl slurry or control cell suspension aliquots, and these were subsequently serially diluted in ten-fold dilution steps up to the 10^{11} dilution level, leaving the last (12^{th}) row as non-inoculated control to check for medium contamination. Thus, each cement stone slurry and corresponding control cell suspension was serially diluted in four parallel series. During the following incubation period at room temperature, growth occurred in the lower but not in the higher dilution levels due to dilution-to-extinction of the viable cells present in the samples. Growth could easily be determined visually due to increased turbidity of positive wells during the following 2-weeks incubation period. Viable number of cells in cement stone slurries and their corresponding spore suspensions was calculated from the number of positive wells using the MPN computer program of Clarke and Owens (1983).

2.4 *Calcite precipitation potential of bacterial concrete*

Chips of 10 days cured cement stone samples (see under 2.3) were incubated in rich medium (yeast-extract and peptone based medium) after pasteurizing for 30 min at 70ºC. Pasteurization of bacterial and control cement stone chips before incubation was done to inactivate bacteria that potentially came into contact with the cement stone samples during curing period or non-sterile handling of the cement stone samples and chips after curing. As bacterial endospores are not killed by the pasteurization procedure, this treatment ensured that potential differences between control and bacterial concrete samples after incubation were mediated by added bacteria and not by accidentally introduced contaminants. Rich medium contained 5 g peptone, 3 gram yeast extract and 8.4g sodium bicarbonate and had a pH of 8.6. Individual chips were incubated aerobically in 100-ml medium aliquots on a shaker table at 100 rpm at 25ºC for 12 days. Chips were rinsed with tap water after incubated and stored wet in closed plastic vials until ESEM analysis, what was done within two days after incubation without any further treatment. Chips were mounted on a 1-cm^2 metal support and kept in place with adhesive tape and observed with a Philips XL30 Series Environmental Scanning Electron Microscope.

3 RESULTS

3.1 *Cultivation of alkali-resistant spore-forming bacteria*

Three out of four strains produced copious spores in mineral medium, except for *S.pasteurii*, which did not grow in this medium. Spore production was considerably less in rich yeast extract- and peptone-containing medium. Percentage of cells with endospores was quantified by microscopic counting, and amounted to 75, 50 and 25% for *B.cohnii*, *B.halodurans* and *B.pseudofirmus* respectively.

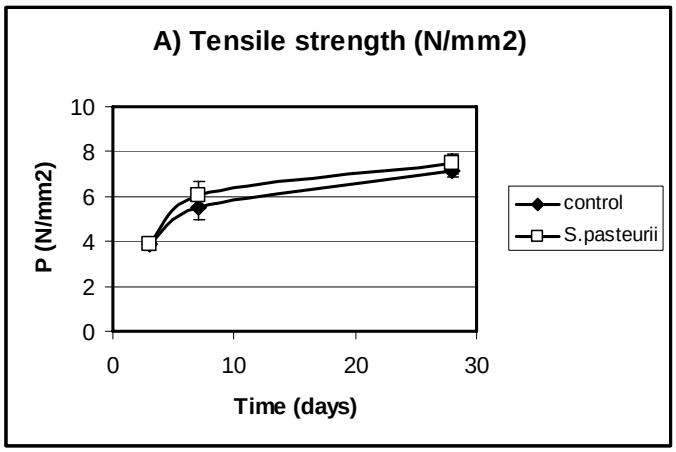
3.2 *Strength characteristics of bacterial concrete*

The 20-ml washed cell suspension of the *S.pasteurii* culture used for the making of bacterial concrete bars contained $3.48*10^{12}$ cells, what resulted in a final density of $1.14*10^9$ cells cm^{-3} concrete. As the average volume of an *S.pasteurii* cell equals about 2.5 μm^3, the total cell volume amounts to 0.3% of the bacterial concrete volume. Tensile- and compressive strength tests after 3, 7 and 28 days curing revealed no significant difference between control- and bacterial concrete (Fig 1).

3.3 *Viability of cement stone-immobilized spores*

The number of viable spores in cement stone samples after 10 days curing as well as original spore suspensions, both with a spore density of 10^9 cm^{-3}, were estimated (Table 2). Results revealed that about one percent of the spores in the spore suspensions (10^7 ml^{-1}) could be retrieved as viable (Table 2). The number of viable spores in the corresponding cement stone samples appeared significantly lower, i.e. between 10^5 and 10^6 cm^{-3}. Compared to spore

suspensions, estimated viable spores in cement stone slurries amounted to 1.9, 7.0 and 2.0% for *B.halodurans*, *B.pseudofirmus* and *B.cohnii* respectively. Number of viable bacteria in control cement stone samples (no bacterial spores added) and tap water used for concrete sample preparation were below detection limit (<500 cells cm^{-3}).



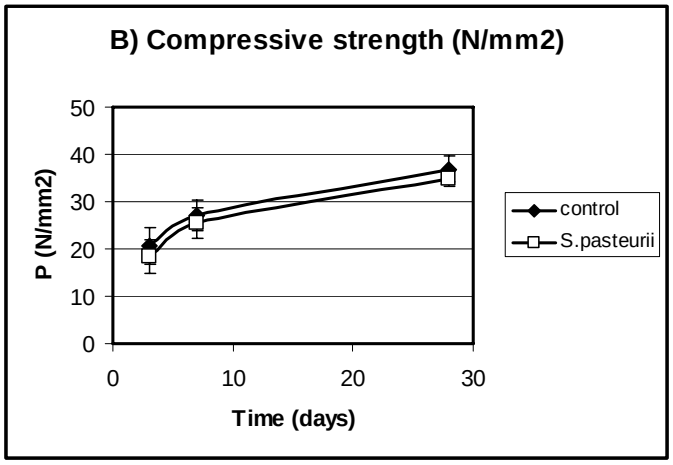


Figure 1. Flexural tensile- (A) and compressive- (B) strength testing, after 3, 7 and 28 days curing, revealed no significant difference between control- and bacterial concrete. The latter contained $1.14*10^9$ *S.pasteurii* cells cm^{-3} concrete.

3.4 *Calcite precipitation potential of bacterial concrete*

ESEM analysis revealed that bacterial cement stone, in contrast to control cement stone samples, precipitated substantial amounts of calcite-like crystals on its surface when incubated in peptone- and yeast extract containing medium. An example is shown in Figure 3, depicting control and *B.pseudofirmus* endospore-containing cement stone pieces incubated for 12 days.

4 DISCUSSION AND CONCLUSIONS

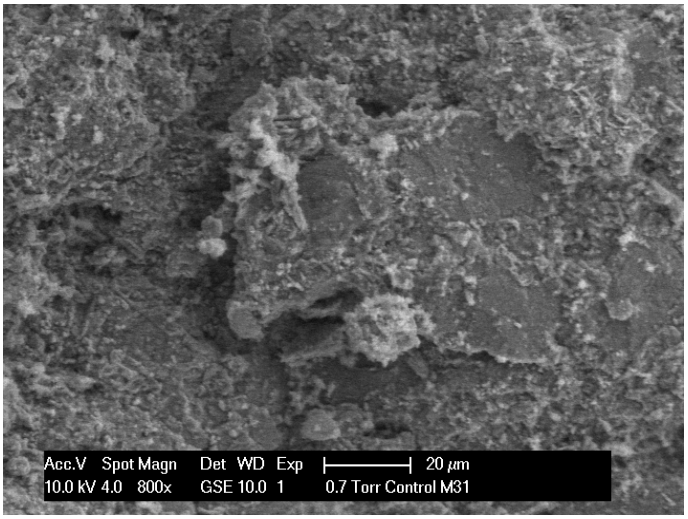
Application of self-healing concrete, i.e. concrete that is able to repair, seal or plug newly formed cracks autogenously, will not only result in more durable structures but will also save a significant amount of money as labor intensive check and repair can be minimized. In this study we investigated the potential of bacteria-mediated calcium carbonate production as a possible healing mechanism. In contrast to some previous studies where bacteria were externally applied for concrete and monument crack repair (Bang et al. 2001; Ramachandran et al. 2001; Dick et al. 2006; Rodriguez-Navarro et al. 2003), we here incorporated bacterial spores, i.e. dormant or resting cells, in the concrete matrix. The results of our study are promising. The estimated number of viable spores retrieved from young cement stone, i.e. after ten days curing, was between 1.9 and 7.0% of the number of viable spores present in the original spore suspension used for the preparation of cement stone samples.

Table 2. Estimate of number of viable (cultivable) bacterial spores in spore suspension and cement stone in which spores of respective bacterial species were immobilized. In brackets: confidence interval; In square brackets: percentage of number in spore suspension

Spore suspension and tap water (control):	Number cm^{-3}:
B.cohnii	5.73 E7 (1.76-18.58)
B.halodurans	5.63 E6 (1.74-18.17)
B.pseudofirmus	7.98 E6 (2.63-24.24)
Tap water (control)	< 500
Cement stone samples:	
B.cohnii	1.15 E6 (3.80-34.80) [2.0]
B.halodurans	1.07 E5 (0.36-3.20) [1.9]
B.pseudofirmus	5.62 E5 (1.74-18.14) [7.0]
Control	< 500

These numbers are substantial, considering the mechanical forces (grinding) needed to liberate and suspend the cement stone-immobilized bacterial spores. Moreover, even if the percentages retrieved reflect truly viable spores, absolute numbers are still high, i.e. between 1.7 and $7.5*10^7$ spores cm^{-3} cement stone, realizing that one viable cell is theoretically enough to start microbial growth and calcite precipitation, providing that suitable conditions prevail. Incubation experiments with 10-days cured cement stone samples demonstrated the mineral precipitation potential of bacterial concrete. We hypothesize that the actual bacterial mineral precipitation mechanism is as follows. Once into contact with copious amounts of water and growth sub-

strates (yeast extract and peptone), bacterial endospores germinate and start to produce CO_2 due to metabolic turnover of growth substrates. CO_2, what can locally reach high concentrations due to rapid metabolic conversion of organic compounds, will chemically react with $Ca(OH)_2$ produced from C_2S and C_3S hydration reactions. The $Ca(OH)_2$ that leaks out of the concrete's pore system reacts with CO_2 and precipitates as calcite or any other calcium carbonate based mineral. The calcite-like crystals found on the surface of bacterial- but not on the surface of control cement stone samples support this hypothesis.



A: Control

B: + Bacteria

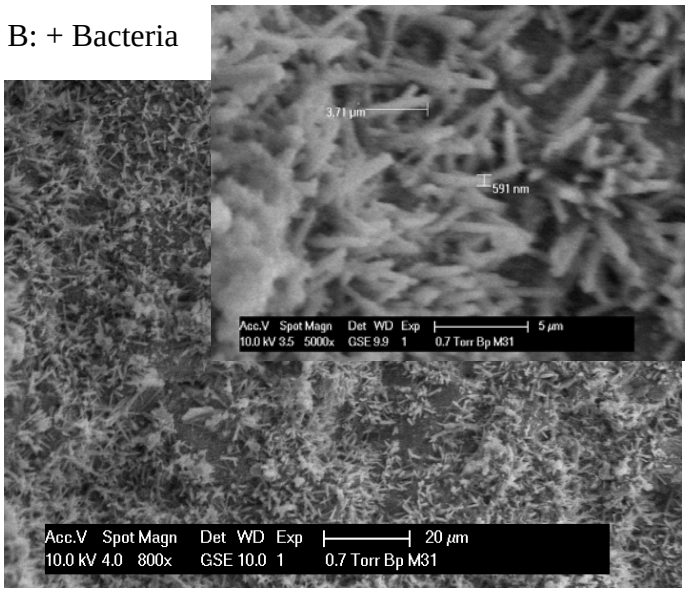


Figure 2. Concrete samples incubated in yeast extract- and peptone-containing medium. A: Control (concrete with no bacteria added) and B: Concrete containing 10^9 cm^{-3} *B.pseudofirmus* endospores. The inset in Figure 2B (5000x magnification) shows a close up of the massive calcite-like crystals formed on the concrete surface.

The experiments done in this study show that alkaliphilic endospore-forming bacteria integrated in the concrete matrix can actively precipitate calcium carbonate minerals. Water, needed for the activation of endospores, can enter the concrete structure through freshly formed cracks. Furthermore, for mineral precipitation, active cells need an organic substrate that can metabolically be converted to inorganic carbon what can subsequently precipitate with free calcium to calcium carbonate. Free calcium is usually present in the concrete matrix, but organic carbon is not. In the present experiments organic carbon was applied externally as a part of the incubation medium, while ideally it should also be part of the concrete matrix.

In that case only external water is needed to activate the concrete-immobilized bacetria which can then convert organic carbon present in the concrete matrix to calcium carbonate and by doing so seal freshly formed cracks. We currently investigate which specific kind of organic compounds are suitable to include in the concrete matrix. This is certainly not trivial as such compounds should be a suitable food source for bacteria as well as be compatible with concrete. Certain classes of organic compounds are less- or not suitable at all, e.g. compounds such as carbohydrate derivatives that are known to inhibit the setting of concrete even at low concentrations. We furthermore presently investigate the long-term viability and potential possibilities to increase the viability of concrete immobilized endospores to ensure long-lasting bacterially enhanced self-healing. Other ongoing investigations address the possible decrease in concrete permeability and the change of mechanical characteristics of healed cracked concrete due to bacterial calcite precipitation.

To conclude we can state that the bacterial approach has potential to contribute to the self-healing capacity of concrete. We have shown that bacteria incorporated in high numbers (10^9 cm^{-3}) do not affect concrete strength, that a substantial number of added bacteria remain viable and, moreover, that these viable bacteria can precipitate calcium carbonate needed to seal or heal freshly formed cracks.

5 ACKNOWLEDGEMENTS

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