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Application of bacteria in concrete: a critical review

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Abstract

Microbially induced carbonate precipitation has been tested over more than a decade as a technique to enhance concrete properties. Mainly bacteria following the pathways of urea decomposition, oxidation of organic acids, or nitrate reduction have been studied for this purpose. For bacteria mixed into fresh concrete, it is difficult to prove that they actively contribute to calcium carbonate precipitation and the effects on concrete strength are variable. Application of bacteria for surface consolidation has been shown to reduce water absorption and increase durability. Microbial self-healing of cracks in concrete shows promising results at the laboratory scale. Especially the use of self-protected mixed cultures opens perspectives for practical application. However, their self-healing efficiency needs to be further proven in larger concrete elements, and under non-ideal conditions. The use of denitrifying cultures for concurrent self-healing and production of corrosion inhibiting nitrites is a promising new strategy.

Keywords: Micro-organisms; Bacteria; Microbial Calcium carbonate; Bioconsolidation; Self-healing

1 Introduction

Over the last decade, the application of bacteria for construction purposes has become a topic of research worldwide. Just like towards the human body micro-organisms can have pathogenic or probiotic effects, for building materials their presence can be devastating or useful [1]. Maybe nowadays, biodeterioration is still better recognized than possible positive effects micro-organisms can have on construction materials [2-4]. Micro-organisms can cause mechanical erosion and internal pressures by growth of hyphae, as well as chemical solution, precipitation and alteration of mineral phases. Organic and inorganic acids produced by micro-organisms can induce extensive leaching effects on concrete. Certain acids, like sulfuric acid produced by Thiobacilli bacteria in sewer systems, have an additional aggressive effect. In this case the involved sulfates cause expansive ettringite and gypsum formation. Biofilms, extracellular polymeric substances excreted by micro-organisms to protect themselves, can cause discoloration and scaling of surfaces by inducing changes in water transport properties. According to a US estimation, the contribution of microorganisms to the deterioration of materials as a whole may be in the range of 30% [5].

It comes therefore as no surprise that engineers question the beneficial effects micro-organisms may have on building materials. Nevertheless, many studies now provide proof for these positive effects, but some challenges still remain before microbial techniques would be used at a larger scale in practical applications.

2 Microbially induced calcium carbonate precipitation

The positive effect that has been studied by far the most, is the Microbially Induced $CaCO_3$ precipitation (MICP). MICP in civil engineering has been studied mainly for application in the fields of surface protection of natural stone, crack remediation in concrete and soil improvement. Also strength enhancement by mixing bacteria into concrete has been investigated. For more information on carbonate precipitation in construction materials the reviews of Castanier et al. [6] and De Muynck et al. [7] can be consulted. More recent information is included in an updated paper [8]. Many bacteria can mediate the formation of calcium carbonate according to various metabolic pathways if given suitable conditions (Table 1). It seems that calcium carbonate, precipitated in heterotrophic processes, is more abundant than that from autotrophic processes. Autotrophs are organisms that produce complex organic compounds, such as carbohydrates, from simple substances, generally using energy from light (photosynthesis) or chemical reactions (chemosynthesis); whereas heterotrophs are organisms that cannot fix carbon to form their own organic compounds and need organic carbon sources for growth. The microbially produced calcium carbonate forms a mineral layer that covers the bacterial cells (Fig. 1).

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This layer can grow to such extent that it can fill pores and cracks in porous materials and glue sand particles together.

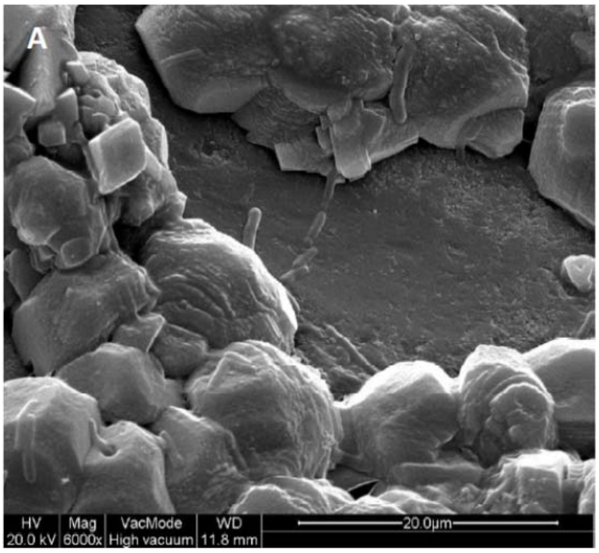

Figure 1. SEM micrograph of bacterial $CaCO_3$ precipitation on concrete samples (courtesy of Lien Standaert).

The pathway that has been studied most for engineering purposes is probably the decomposition of urea by bacteria, with the aid of the bacterial urease enzyme. This results in the production of ammonium (NH_4^+), dissolved inorganic carbon (DIC), and an increase in pH favoring $CaCO_3$ precipitation. Furthermore, due to the negative charges on the bacterial cell wall and the high surface to volume ratio of bacteria, the bacterial surface has a high capability of binding cations and serves as an ideal nucleation site. The bacterially catalyzed urea hydrolysis is approximately 10^{14} times faster than the un-catalyzed rate [9]. Especially *Bacillus pasteurii* and *Bacillus sphaericus* have been studied for this purpose. A downside of urea hydrolysis especially for the use in soil improvement, is the byproduct ammonia, that needs to be removed by flushing.

A pathway which does not produce this byproduct is the oxidation of organic acids, where promising results have been obtained with *Bacillus cohnii* [10, 11]. In alkaline environments, organic compounds are degraded into CO_2 and H_2O. In high pH environment, CO_2 transforms to CO_3^{2-} and in the presence of Ca^{2+}, $CaCO_3$ can be formed. Biological reduction of NO_3^- in the denitrification pathway also generates CO_3^{2-} and HCO_3^- ions, which are necessary for $CaCO_3$ precipitation.

3 Selection of bacteria

The selection of the most suitable bacteria for MICP is mostly based on their carbonate yield. Bacteria are mainly isolated from natural carbonate producing environments, such as calcareous stones [12], calcareous sludge [13], calcareous soil [14], etc. Castanier et al. [6] obtained the best performance with *B. cereus* (through oxidative deamination of amino acids), which showed a carbonate yield of 0.6 g $CaCO_3$ per g organic matter. This organism was then further used *in situ* for consolidation of limestone monuments [15]. With *B. cohnii* living cells (10^8 cells/mL) Wang [16] found over 15 d an acetate degradation of 7.5 g/L, leading to about 25 g/L $CaCO_3$ production (1.7 g/(L.d)) with the highest production in the first two days. In the same study [16], it was found that *B. sphaericus* vegetative cells, can precipitate 60 g/L $CaCO_3$ within one day at optimal conditions, illustrating the higher carbonate precipitation yield of the ureolytic pathway. De Muynck et al. [17] have shown that between various ureolytic strains even at lower temperatures of 10°C the mesophilic *Bacillus sphaericus* produced a higher amount of carbonate in the shortest amount of time than other more cold resistant strains like *Sporosarcina psychrophila*. Ersan et al. [18] have investigated MICP through denitrification, in a minimal-nutrient environment. In this study, $CaCO_3$ precipitation performances of two newly isolated resilient strains *Diaphorobacter nitroreducens* and *Pseudomonas aeruginosa* were optimized and the repetitive $CaCO_3$ precipitation of a single inoculum was investigated. $CaCO_3$ precipitation yields of 14.1 g to 18.9 g $CaCO_3$/g NO_3-N were achieved in 2 d.

Nevertheless, a straight comparison is difficult to make, since there are many different factors governing the $CaCO_3$ precipitation yield, that need to be optimized and that furthermore interact with each other [8], such as bacterial cell concentration, concentration of urea, organic acids or nitrate (for the above mentioned pathways), and calcium concentration.

4 Microbial $CaCO_3$ for strength improvement

The literature on strength improvements obtained by mixing bacteria into concrete shows variable results. Several authors have mentioned increases of 28 d compressive strength with 9 to 25% when mixing bacterial cells into mortar or concrete [19-21]. Others mention both positive and negative effects, depending on the bacterial strain, cell concentration or concrete age [22-23].

Table 1. Different metabolic pathways of bacterial calcium carbonate precipitation [8].

Autotrophic bacteria	Heterotrophic bacteria				
non-methylotrophic methanogenesis	Assimilatory pathways	Dissimilatory pathways			
	Urea decomposition	Oxidation of organic carbon			
anoxygenic photosynthesis		Aerobic		Anaerobic	
	Ammonification of amino-acids	Process	e^- acceptor	Process	e^- acceptor
oxygenic photosynthesis		Respiration	O_2	NO_x reduction	NO_3^-/NO_2^-
		Methane oxidation	CH_4/O_2	Sulfate reduction	SO_4^{2-}

Hereby, it is very difficult to prove the actual influence of living bacteria. Inside the concrete matrix, due to the lack of oxygen, high pH and limited space availability, activity of (aerobic) bacteria would be greatly inhibited.

Achal et al. [24] noticed that the average number of viable bacteria decreased from $6{\cdot}10^6$ to $4.5{\cdot}10^4$ after 7 d, which means most bacteria died and bacterial growth was greatly inhibited since there was no difference in cell numbers at 7 d and 28 d.

Basaran [21] did find viable *S. pasteurii* cells in hardened cement paste samples that were as old as 330 d with approximately 2% viability retention, and 50% of the viable cells detected were defined as vegetative cells, which could be metabolically active. Nevertheless, it is difficult to prove that the bacteria would actively contribute to calcium carbonate precipitation at the high pH of 13 inside the concrete matrix.

The measured increase in calcite content, occurring within cement paste when the bacterial medium was used [21], could also be due to the nucleation effect of the bacterial cell walls or to the urease enzyme remaining active even in the dead cells.

Another issue is that bacteria will need nutrients to become active. These nutrients are added to the mortar mix or provided by immersion of the mortar samples into nutrient solutions. The nutrients could also affect the mortar strength. Wang et al. [25] investigated the influence of calcium nitrate (as calcium source) and yeast extract (as nutrient) on the cement hydration with isothermal calorimetry. It was found that Ca-nitrate can accelerate cement hydration, whereas yeast extract (0.85% versus cement weight) significantly delayed the hydration and resulted in a lower hydration degree at 7 d of age and a reduced compressive strength at 3 months.

5 Microbial $CaCO_3$ for surface consolidation

The first on-site application of the bioconsolidation treatment was carried out in 1993 on an area of 50 m^2 of the tower of the Saint Medard Church in Thouars [26]. Tiano et al. [27] and Jroundi et al. [28] also applied biomineralization for on-site conservation of decayed stones. De Muynck et al. [29] measured the strengthening effect of biodeposition on Maastricht limestone by means of the drilling resistance measurement system (DRMS Cordless SINT Technology, Italy). They were able to homogeneously strengthen limestone up to depths of 30 mm with a similar or better performance than that of traditional surface treatments such as ethyl silicates. The biodeposition treatment is solvent free and entailed both a significant protective and consolidating effect after two spray applications within the same day: the drilling resistance was more than doubled. By optimizing the concentrations of the urease and carbonate precursor solutions, the reseachers were able to lower the cost of the treatment within the range of that of traditional consolidants. However, one point of concern by practitioners is the use of hygroscopic salts (calcium chloride, acetate, formate, …) as a calcium source for the bioprecipitation, which makes them fear for later damage to the stone.

Until now, the on-site bioconsolidation has been mainly restricted to porous calcareous stones of cultural heritage buildings. Nevertheless, several researchers have successfully applied the MICP treatment on cement-based materials in the laboratory and in small on site tests [20, 30-33]. Compared with porous limestone, concrete is denser, which results in less retention of bacteria in the surface layer. De Muynck et al. [31] have shown that the surface deposition of calcium carbonate crystals on mortar decreased the water absorption with 65 to 90% depending on the porosity of the specimens. As a consequence, the carbonation rate and chloride migration decreased by about 25–30% and 10–40% respectively. An increased resistance towards freezing and thawing was also noticed. The results obtained with the biodeposition treatment were similar as those obtained with conventional surface treatments under consideration (acrylate, silane, siloxane, silicone, silicate).

6 Microbial $CaCO_3$ for self-healing of cracks in concrete

The principle of bacteria-based self-healing concrete is that carbonate precipitating bacteria are added into concrete during the mixing process. When cracking occurs, the bacteria will be activated to precipitate $CaCO_3$ to *in-situ* heal concrete cracks. This 'self-healing' property results in a recovery of water-tightness, and hence limits the penetration of corrosive substances into concrete structures and improves concrete durability. To apply bacteria for self-healing of concrete cracks, they should sustain their viability until crack formation. Therefore, researchers are generally proposing spores instead of vegetative cells for this application in view of the longer life-span [34]. As soluble Ca-source, calcium chloride should be avoided due to the corrosive effect on the reinforcement and calcium nitrate, calcium acetate, calcium formate or calcium lactate can be proposed. Addition of nutrients has a positive effect on the bio-precipitation process. Wang [16] found that yeast extract as nutrient for *B. sphaericus* accelerated spores germination and bioprecipitation, especially in an unfavorable environment, such as at low temperatures (10°C) and in presence of high concentrations of Ca^{2+}. This may be very important for practical application since the optimal temperature range for most of the bacterial processes is 20 to 30˚C, whereas lower temperatures that are relevant in many real-life situations retard the germination of spores and the growth of living cells.

To ensure bacterial survival during concrete mixing and hardening, encapsulation of bacteria in suitable carriers is preferable. To reduce the negative effect of the nutrients on the mechanical concrete properties, also encapsulation of the nutrients has been proposed.

Several encapsulation strategies have been tried for the bacteria (or nutrients): silica gel or polyurethane in glass capsules [35], expanded clay [36, 37], diatomaceous earth

[38], melamine formaldehyde based microcapsules [25, 39], synthetic or biobased superabsorbent polymers (hydrogels) [40-42]. The hydrogels have the double benefit of protecting the bacterial spores and of acting as water reservoir for spores germination and bacterial activity when cracking occurs. Recently, self-protected non-axenic mixed cultures have been tested [43]: Cyclic EnRiched Ureolytic Powder (CERUP), and Activated Compact Denitrifying Core (ACDC). CERUP is a ureolytic community protected by its high salt content and obtained from the further processing of side streams from vegetable industry. ACDC is a denitrifying microbial community protected by various bacterial partners and obtained in a sequential batch reactor by applying selective stress conditions. Silva [44] stated that non-axenic cultures have, due to the elimination of the need for sterile production conditions, much lower production costs when compared with axenic cultures (at least a factor 10 cheaper for CERUP versus production costs of *Bacillus sphaericus* or *Bacillus cohnii* spores). In the above mentioned references, generally cracks of up to 500 µm in width could be healed within 3 to 14 weeks (example in Figure 2).

Most types of encapsulation materials will negatively affect the compressive strength of concrete, especially if they are added in dosages exceeding 1% versus cement weight. Diatomaceous earth (DE) is an exception, since this has a positive effect on strength due to its pozzolanicity; but it cannot be considered as a real encapsulation technique since the bacteria are adsorbed on the DE instead of penetrating into its pores (which are too small for this purpose).

With regards to bacterial self-healing, several questions remain open for further study. The viability of the self-healing additive in time is yet unknown and will be different for each specific bacterial strain and protection method. Also it has not been proven whether spores that have germinated and actively contributed to crack healing, will sporulate again and remain in dormant state until their activity is once more needed. In addition, the self-healing efficiency needs to be proven for concrete elements, since until now most proof-of-concept tests were carried out in mortar specimens. When keeping the dosage of the bacterial additive constant relative to the cement weight when upscaling from mortar to concrete, this results in a significant dilution of the additive. However, when maintaining the same dosage in proportion to the total volume, an unacceptable strength decrease may result.

7 Bacterial self-healing to increase concrete durability and inhibit rebar corrosion

Cracks in cementitious materials have a negative influence on the durability in aggressive environments. With regard to chloride penetration, Maes [45] states that 10 µm is the critical crack width, above which cracks provide a preferential and fast way for chloride ingress. Based on chloride penetration tests in submersed conditions and service life modelling, Maes and De Belie [46] have found that the expected service life (based on corrosion initiation) of concrete structures containing cracks of 100-300 µm decreases to one fifth of the value expected for a sound structure. Taking into account the current self-healing efficiency of systems based on encapsulated polyurethane (so no bacterial self-healing), they estimated that the average service life could be doubled by introducing this self-healing technology. In case of perfect self-healing, the service life of a non-cracked structure could be approached. Similar durability tests and service life calculations for microbial self-healing concrete should be performed.

Regarding rebar corrosion, it should be considered that microbial urea hydrolysis and aerobic oxidation of organic carbon require O_2 as final electron acceptor to initiate and/or to keep the microbial activity. This may result in oxygen consumption in the concrete, which may pose a limitation for rebar corrosion. Yet, for crack healing in concrete, when O_2 availability in the deeper parts of the cracks is limited, this will in turn inhibit the bacterial precipitation processes [47]. Moreover, $CaCO_3$ precipitation itself entraps the bacterial cells and may inhibit O_2 diffusion into cells [48]. These considerations have led to the investigation of nitrate reducing bacteria for self-healing. Biological NO_3^- reduction takes place during the microbial oxidation of organic matter by use of NO_3^- as an electron acceptor instead of O_2. Solubility of O_2 in water (9.1 mg/L at 20°C) is 102 to 105 times lower than for NO_3^- as electron acceptor. In the presence of calcium ions, NO_3^- reduction induces $CaCO_3$ precipitation. Our research has revealed that through NO_3^- reduction, even enhanced $CaCO_3$ precipitation performances could be achieved in nutrient-poor environments which makes the mechanism feasible for self-healing concrete [18, 49]. Nitrate reduction can also lead to the intermediate production of NO_2^- which is known as corrosion inhibitor. It will therefore protect the steel surface from corrosive substances during the healing period. It was shown that the non-axenic culture named “activated compact denitrifying core” (ACDC) induced passivation of steel in corrosive electrolyte solution (0.05 M NaCl) by producing 57 mM NO_2^- in 1 week [50]. Recently, accelerated corrosion experiments have been carried out on cracked (300 µm crack width) mortar specimens with an embedded steel rod subjected to 0.4 M chloride solution to mimic chloride concentrations in seawater (Fig. 2).

Figure 2. Micrographs of a self-healing (containing bacterial ACDC granules) mortar specimen with 300 µm wide crack before (left) and after (right) 28 d submersion in a 0.4 M chloride solution, showing complete crack healing (courtesy of Yusuf Çağatay Erşan).

These preliminary tests (data unpublished) revealed that microbial self-healing alone (with the ureolytic CERUP mixed cultures) could increase the time to corrosion initiation with a factor of 1.7, whereas the ACDC denitrifying cultures, due to the concurrent production of corrosion inhibitor, could increase the time to corrosion initiation with a factor of 2.4.

8 Conclusion

The concrete construction industry suffers from a negative image due to its high CO_2 footprint, use of resources and production of waste. The use of biological processes to improve concrete properties could help to change this public opinion. Over the last decade, researchers have focused their efforts on the application of microbially induced carbonate precipitation in concrete. Results related to strength improvements obtained by mixing bacteria into concrete are variable and the costs of a bacterial additive may offset the possible enhancements. Surface bioconsolidation treatments, as originally developed for porous limestone, may find an application in the concrete construction industry. The technique could in the future compete with conventional surface treatments to reduce water absorption and increase durability. Optimization of nutrient media and large scale demonstration may be needed before market introduction. Microbial self-healing of cracks in concrete shows promising results at the laboratory scale. Since the cost of the production of axenic cultures and subsequent encapsulation is considerable, for practical application the use of mixed self-protected cultures may be the way forward. Still, the efficiency of these new self-protected cultures needs to be further proven in larger concrete elements, at non-ideal temperatures, at high salt concentrations (like in marine environment) that would cause a considerable osmotic stress for the bacteria, at later ages of the concrete, etc. The use of denitrifying cultures for concurrent self-healing and production of corrosion inhibiting nitrites is hereby a promising new strategy. Good knowledge of the self-healing efficiency and its variability, will allow proper service life estimation. Only when this is available, contractors and owners could be convinced to apply these innovative technologies in practice.

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