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# An Up-To-Date Review of Microbially-induced Carbonate Precipitation Process and its Applications

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## Abstract

Microbially-induced carbonate precipitation (MICP) is a natural process wherein microbes alter the environment and cause the creation of carbonate minerals. MICP is quicker than typical mineralization owing to the involvement of microbial enzymes. It is economical, sustainable, and environmentally friendly. MICP has many applications, including reinforcing soil and building materials, mending concrete cracks, capturing $CO_2$, and producing bio-composites. This review seeks to understand the physiology of the MICP process, along with its applications in sustainable construction. Research progress made in this area over the past one decade is lucidly presented. Focus is placed on bio-concrete, which through microbial self-healing, effectively combats concrete's vulnerability to cracking in a durable and practical fashion. Use of the ureolytic bacterium, *Lysinibacillus sphaericus* is explored in the context of self-healing concrete formulation, with focus on its merits over other microbial species with carbonate precipitating potential.

*Keywords:* Microbially-induced carbonate precipitation; self-healing; bio-concrete; *Lysinibacillus sphaericus*

## Introduction

Microbially-induced calcite Precipitation (MICP) is a biomineralization process in which microorganisms, typically certain types of bacteria, facilitate the precipitation of calcium carbonate ($CaCO_3$) minerals from calcium-rich solutions. This method is environmentally friendly and sustainable in terms of cost-effectiveness. Several bacterial species that are capable of carbonate precipitation in natural environments (such as *Sporosarcina pasteurii*, *Bacillus megaterium*, etc.) have been extensively studied. Using enzymes such as urease, these bacteria manipulate their environment leading to calcite precipitation. MICP is seen as a novel technology that comes under the framework of sustainable development goals. Two of the best-known applications of MICP are Bioaugmentation (which involves introducing ureolytic bacteria like *Sporosarcina pasteurii* or *Bacillus species* into soil, concrete, or other matrices where calcium carbonate precipitation is desired) and Biogrouting (which creates calcium carbonate cementation, improving soil cohesion and load-bearing capacity for applications like slope stabilization and foundation reinforcement). This review focuses on one prime application of the MICP process i.e. in the preparation of bio- concrete (also known as self-healing concrete or microbial concrete), a product which is expected to revolutionize tomorrow's construction industry. The uses and applications of the MICP process continue to evolve and expand as researchers explore new ways to leverage the potential of microbial carbonate precipitation in diverse fields.

## Microbially-induced carbonate precipitation: the underlying mechanism

Selective cementation has great significance in various fields like petroleum, civil, and geological engineering where microorganisms have been shown to remediate cracks in natural environments. Microbially-induced Carbonate Precipitation (MICP) utilizes different metabolic pathways:

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1. **Urea Hydrolysis**: Bac[4]teria break down urea, releasing carbonate ions and raising the pH, which encourages the precipitation of calcium ions. This method is commonly employed in MICP [3]for bio- concrete development.
2. **Denitrification**: Denitrifying bacteria use nitrate instead of oxygen, resulting in nitrate reduction to nitrite and creating an alkaline environment favorable for calcium carbonate precipitation. Denitrification is beneficial in low-oxygen conditions like concrete cracks.
3. **Dissimilatory Sulfate Reduction**: Sulfate-reducing bacteria metabolize sulfate ions, generating sulfide ions that can react with calcium ions to form calcium carbonate. While less commonly used, dissimilatory sulfate reduction offers another potential pathway for MICP.

Although these three pathways are in use, urea hydrolysis has been widely employed due to its high energy efficiency, low cost, controllable reaction process, and direct separation and harvest procedure. Kinetic studies have shown that the rate of calcite precipitation depends on the concentration of bacterial cells, the ionic strength of the medium, and the pH.

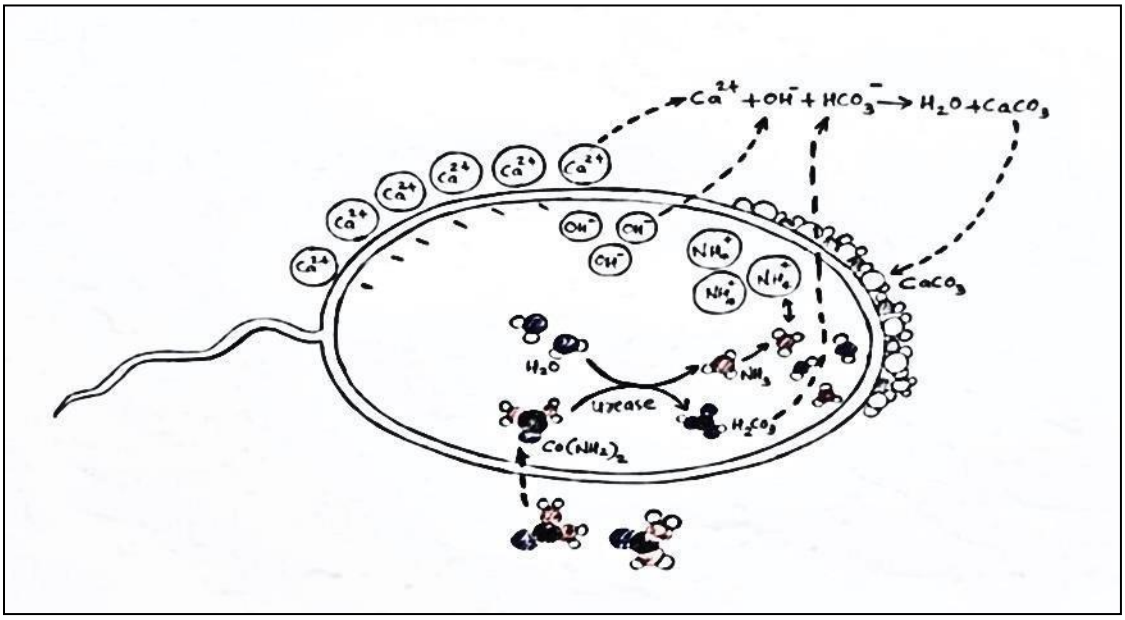


Fig 1 **. Biochemical mechanism of MICP**

Urea ($NH_2CONH_2$) hydrolyzed by urease enzyme produced by the bacteria results in the production of ammonium ions ($NH_4^+$) and bicarbonate ($H_2CO_3$):

$$NH_2\text{-}CO\text{-}NH_2 + H_2O \rightarrow 2NH^{+} + H_2CO_3$$

The bicarbonate formed is then reduced to carbonate ions:

$$H_2CO_3 \rightarrow CO^{2-} + 2H^+$$

Microorganisms with a net negative cell surface charge can attract and bind cations, including calcium ions ($Ca^{2+}$), from their surrounding environment.

$$Cells + Ca^{2+} \rightarrow Cells\text{-}Ca^{2+}$$

The bound calcium ions ($Ca^{2+}$) on the cell surface react with the carbonate ions ($CO_3^{2-}$) available in the vicinity to form solid calcium carbonate (calcite) as a precipitate at the nucleation site:

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$$\text{Cells-}Ca^{2+} + CO_3^{2-} \rightarrow \text{Cells-}CaCO_3 \quad (4)$$

This process plays a crucial role in biomineralization and can have various environmental and engineering applications.

## Current trends and methodologies in self-healing concrete

The self-healing concrete field is rapidly advancing with innovative methods to improve structure durability and sustainability. It has the potential to extend the structure life span and reduce maintenance costs, thus offering promising prospects for the future of infrastructure. Various microorganisms have been utilized in recent studies on bio-concrete to investigate its self-healing properties. These studies focused on exploring the effectiveness of different bacteria and their biomineralization capabilities. Comparative studies on some notable microorganisms such as *Bacillus subtilis, Brevibacillus sp., Bacillus megaterium and Microvirga sp.* led to the conclusion that *B. subtilis*- based bio-concrete yielded the highest compressive strength of 50.37 $N/mm^2$ . (Vanjinathan, 2023).

Several researchers have focused on investigating the effects of different nutrient sources on the growth of microorganisms and, subsequently, their impact on the properties of bio-concrete. The use of corn steep liquor (an industrial by-product) as a nutrient source for cultivating *S. pasteurii* , a bacterium employed for remediating cracks and fissures in building materials and structures has yielded notable results. Urease activity and calcite production were significantly higher in the CSL-urea medium due to additional nitrogen sources. Calcite constituted 30.12% of sand samples in the CSL-urea medium. Incorporating *S. pasteurii* from the CSL-urea medium into mortar cubes led to a 35% increase in compressive strength. This approach holds the potential for enhancing the longevity and durability of construction materials and structures using industrial by-products like CSL (Achal, 2010).

Another study demonstrated the use of the dairy industry by-product, lactose mother liquor (LML), as a growth medium for *Sporosarcina pasteurii*, showcasing notable urease activity and calcite precipitation. Calcite constituted 24.0% of treated sand samples, with urease production reaching 353 U/ml in LML. Data analysis and conclusion emphasized the application of microbial calcite as a sealing agent for structural imperfections in both artificial and natural settings, with LML as a viable growth medium alternative (Achal 2008)

Investigations have been done on the impact of biomineralization in concrete by introducing ureolytic bacteria (*B. megaterium, B. pasteurii etc.*) without substrate and non-ureolytic bacteria (*B. cohnii*) without chemical feed. The research revealed a novel mineralization pathway that enhanced mechanical properties, reduced water absorption (by approximately 22%), decreased void volume (by around 24%), and lowered sulfate ion concentration at 180 days (by roughly 26%). Bacteria induced thick mineral deposition at the interfacial transition zone (ITZ) and accelerated the formation of hydrated products, resulting in a densified microstructure and improved macro properties of bacterial concrete. The results demonstrated a substantial enhancement of mechanical and durability properties due to the presence of bacteria in the concrete (Chaurasia, 2018)

Bacterial strain *Bacillus licheniformis AK01* isolated from loamy soils demonstrated significant calcite precipitation abilities. AK01 strain showed promising potential for improving concrete properties.The healing capability of AK01 was compared with three other competent bacteria: *Sporosarcina pasteurii DSM-33, Pseudomonas aeruginosa MA01*, and *Bacillus subtilis TRPC2*. The study evaluated the effects of these bacteria on compressive strength and water absorption in mortar specimens (Vahabiab, 2014).

The introduction of microorganisms into bioconcrete can be achieved through different methods, including percolation and direct mixing. These methods serve distinct purposes and can be chosen based on the specific goals and requirements of the bio-concrete project. Studies explored the use of microbiologically induced carbonate precipitation (MICP) for *in situ* soil reinforcement. Surface percolation was employed to immobilize bacteria along the entire length of a 1-meter sand column. The resulting biologically induced cementation exhibited homogeneity, and the efficiency of calcite crystal formation was linked to pore water content. Lower water content led to better strength, with crystals positioned at bridging points between sand grains. These findings suggest that optimizing conditions for crystal precipitation can reduce the cost of MICP technology, making it more feasible for large-scale applications (Chaurasia, 2018). Microorganisms are directly incorporated into the concrete mixture during the initial mixing stage, which ensures consistent microbial presence in the concrete.

A calcite-precipitating bacteria isolated from alluvial soil in Solan, India (identified *Lysinibacillus sp*. I13) is a promising strain. When incorporated into M20 concrete mixtures, *Lysinibacillus sp.* significantly enhanced

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compressive strength, showing a 1.5-fold increase compared to *Bacillus megaterium MTCC 1684* after 28 days of curing (Vashishta, 2017).

In the pursuit of self-healing concrete technologies, researchers explored the use of denitrifying bacterial cultures to induce calcium carbonate precipitation within cracks, blocking the ingress of harmful agents and preventing corrosion of steel reinforcement. This approach benefits from microbial nitrate reduction, which occurs when organic matter is oxidized using nitrate ($NO^{3-}$) instead of oxygen ($O_2$) as an electron acceptor. Notably, this process enables precipitation in oxygen-limited environments within concrete cracks. Additionally, nitrate reduction produces nitrite ($NO^{2-}$), a known corrosion inhibitor for steel in concrete. Using an in-house non-axenic culture called "activated compact denitrifying core" (ACDC), the passivation of steel in chloride-containing solutions was achieved, surpassing the performance of protected axenic cultures in various conditions. Researchers tested the use of two axenic $NO^{3-}$reducing cultures, *Pseudomonas aeruginosa* and *Diaphorobacter nitroreducens*, for microbial self-healing in concrete using denitrification. These bacteria were encapsulated within expanded clay particles (0.5-2mm) and tested on mortar specimens with cracks ranging from 100-500 µm under wet and wet/humid conditions (Ersan, 2013).

It is important to note that the selection of microorganisms, their stage, and the timing of their introduction should be carefully planned and optimized to achieve the desired results while considering factors like the concrete's curing conditions, environmental factors, and the form in which microbes are added (i.e. spore or vegetative form). The inclusion of *Sporosarcina pasteurii* vegetative cells in cement-based materials had several notable effects. It significantly delayed the hydration process, indicating an influence on the material's setting time. Moreover, the bacterial presence promoted increased precipitation of calcium carbonate, especially in the form of calcite. Most importantly, the compressive strength of the bacterial mortar matched or exceeded that of the regular mortar after just one day of hydration. These findings suggest the potential of *Sporosarcina pasteurii* in contributing to the self-healing properties of cement-based materials, thus enhancing their durability and strength (Bundur, 2014).

Another study explored the potential of certain alkali-resistant, spore-forming bacteria from the *Bacillus* genus to serve as self-healing agents in concrete. The research revealed that when bacterial spores were directly added to the cement paste mixture, they remained viable and active for an extended period of up to 4 months. This finding is significant because it demonstrates the potential for these microorganisms to survive in the harsh environment of cement. A noteworthy observation was the decrease in pore size diameter as the cement stone underwent setting. As pore widths reduced to less than 1 micron (the typical size of *Bacillus* spores), the spores' life span decreased (Jonkers, 2008).

Seminal work by several other research groups have been reviewed and the conclusions drawn from each of them are summarized in table 1.

MICP has multiple engineering applications in the fields of geotechnical, geological, hydraulic, geo- environmental and structural engineering, some of which are exemplified as follows:

**Geotechnical Engineering**

a. **Soil Stabilization**: MICP improves strength and stability of loose soils, thereby enhancing their load bearing capacity.
b. **Foundation Improvement**: In areas of challenging soil conditions, MICP can strengthen foundation soils, mitigate settlement issues and increase bearing capacity of foundations.
c. **Slope Stabilization**: MICP can stabilize slopes to prevent erosion, landslides and slope failures by enhancing its resistance to surface run off and rainfall.

**Geological Engineering**

a. **Mine Shaft and Tunnel Support**: By consolidation of surrounding geological materials, MICP can enhance the stability of mine shafts and tunnels.
b. **Erosion Control**: MICP can be employed to stabilize soil in erosion-prone areas, preventing soil loss due to water flow or wind erosion.
c. **Heritage Conservation***:* It helps preserve historical buildings and monuments by reinforcing deteriorating stone or mortar with calcium carbonate, maintaining their structural integrity.

**Hydraulic Engineering:**

a. **Seepage Control**: MICP can reduce soil permeability, effectively controlling seepage and ensuring the structural integrity of critical facilities such as dams and levees.

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b. **Riverbank and Coastal Protection**: It prevents erosion and offers protection against flooding by strengthening riverbanks and coastal structures (which includes shoreline protection, reef restoration and creation of artificial reefs).

**Geo-environmental Engineering:**

a. **Contaminated Soil Remediation**: It can improve the soil quality at contaminated sites and can immobilize heavy metals and contaminants in soil through calcite precipitation.
b. **Waste Containment**: It can be used to strengthen liners and caps of landfills and waste containment facilities by enhancing their integrity and prevent leachate migration.

**Structural Engineering:**

a. **Concrete Crack Repair:** MICP can repair cracks in concrete structures by introducing bacteria having calcium carbonate precipitation capabilities along with nutrient solution. This helps in self-healing of concrete and increases its service life.
b. **Structural Strengthening:** MICP can be used to enhance the durability and strength of existing concrete and masonry structures within cracks and walls.

**Table 1: Review of contemporary literature on bacterial concrete**

| Sl. No. | Author (Year) | Microorganism(s) used | Key observations/Conclusions |
|---|---|---|---|
| 1 | Shannon Stocks-Fischer (1999) | *Bacillus pasteurii* | At the pH where calcium precipitation was favorable, the urease activity and its affinity to urea are significantly high. |
| 2 | V. Ramakrishnan (2000) | *Bacillus pasteurii*, *Sporosarcina* sp. | The chosen microorganism grew more efficiently in the presence of oxygen. Hence, microbial repair was more successful in surface cracks. |
| 3 | Sookie S. Banga (2001) | *Bacillus pasteurii ATCC 11859* | The effects on elastic modulus and tensile strength of polymer was studied, increased compressive strength of concrete cubes was also observed. |
| 4 | Qian Chunxiang (2008) | *Bacillus pasteurii* | Water penetration resistance of a bio-concrete specimen surface was greatly improved by adding $Ca^{2+}$ before urea. |
| 5 | Henk M. Jonkers (2008) | *Bacillus cohnii DSM 6307, Bacillus halodurans DSM 497 Bacillus pseudofirmus DSM 8715.* | Addition of Manganese to bio-concrete, induced the formation of bacterial spores. |
| 6 | Henk M. Jonkers (2008) | *Bacillus pseudofirmus DSM8715, B. cohnii DSM6307* | Bacterial spores remain viable for a period of 4 months when added to cement paste mixtures. |
| 7 | Varenyam Achal (2010) | *Sporosarcina pasteurii* | 35% increase compressive strength was observed. Urease activity and calcium production were also enhanced. |
| 8 | S.S. Bang (2010) | *Sporosarcina pasteurii ATCC 1832* | *S. pasteurii* cells were immobilized on porous glass beads. The overall performance increased with treatment by microbial calcite. |
| 9 | Mousumi Biswas (2010) | *Thermophilic bacteria BKH1* | Compressive and tensile strength of cement-paste and mortar increased. Durability and quality of concrete were also enhanced. |

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| Sl. No. | Author (Year) | Microorganism(s) used | Key observations/Conclusions |
|---|---|---|---|
| 10 | Varenyam Achal (2010) | *Bacillus sp. CT-5* | 36% increase compressive strength and enhanced durability of building materials. |
| 11 | J. Y. Wang (2011) | *Bacillus sphaericus* | Increased water penetration resistance of cracked specimens were observed. |
| 12 | Liang Cheng (2012) | *Ureolytic bacteria* | Homogeneous cementation was observed due to calcite crystal formation. |
| 13 | S.A. Abo-El-Enein (2012) | *S. pasteurii NCIMB 8841* | The amount of precipitated calcium carbonate, degree of crystallity and strength were increased. |
| 14 | Yusuf Cagatay Ersan (2013) | *Pseudomonas aeruginosa, Diaphorobacter nitroreducens* | It was found that the denitrification pathway is an effective and environment-friendly method for self-healing. |
| 15 | Ali Vahabiab (2014) | *Bacillus licheniformis AK01* | The compressive strength was improved to 15% and water absorption of mortar was reduced by 25%. |
| 16 | J. Y. Wang (2014) | *Bacillus sphaecus LMG 22557* | Hydrogel-encapsulated spores showed a high superiority in self-healing and water permeability decreased by 68%. |
| 17 | Wasim Khaliq (2015) | *Bacillus subtilis* | Bacterial immobilization in graphite nanoparticles gave significant enhancement in compressive strength on pre-cracked specimens. |
| 18 | Huaicheng Chen (2016) | *Bacillus mucilaginous L3 and Brewer's yeast JCS 05* | The use of ceramsite with immobilized bacteria and nutrients enhanced the flexural strength and self-healing power. |
| 19 | Jianyun Wang (2017) | *Bacillus sphaericus LMG 22257* | The bacterium had good tolerance and could survive at highly alkaline pH. The spores were able to germinate at low temperature and revived the ureolytic activity slowly. |
| 20 | Rajneesh Vashisht (2017) | *Lysinibacillus species* | Compressive strength increased by 1.5-fold along with higher calcite precipitation activity. |
| 21 | Jiaguang Zhang (2017) | *Bacillus cohnii* | Expanded perlite-immobilized bacteria were more efficient in crack healing than expanded clay-immobilized bacteria. |
| 22 | A F Alshalif (2018) | *Bacillus pasteurii, Pseudomonas aeruginosa, B. alkalinitrilicus, B. sphaericus, B. subtilis, Enterococcus faecalis, Shewanella sp. and S. pasteurii* | The resilience to survive in the harsh environment of concrete was confirmed from the extreme pH values of the samples taken. Self-healing process was also enhanced. |
| 23 | Leena Chaurasia (2018) | *Bacillus megaterium, Bacillus pasteurii, Bacillus cohnii* | Improved the mechanical properties, reduced water absorption and lowered sulfate ion concentration. |
| 24 | Yusuf Ç. Erşan, Nico Boon and Nele De Beli (2018) | *Aerobic heterotrophic bacteria (ureolytic strains)* | Calcium carbonate precipitation was enhanced, simultaneous corrosion inhibition was achieved. |
| 25 | Nuraiffa Syazwi Adzami, (2018) | *Bacillus sphaericus* | 100% pure calcite was produced by calcium carbonate precipitation using calcium nitrate. |
| 26 | Wiboonluk Pungrasmi (2019) | *Bacillus sphaericus LMG 22257* | Freeze drying had a high bacterial spore survival rate and high potential as a microencapsulation technique. |

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| Sl. No. | Author (Year) | Microorganism(s) used | Key Observations/Conclusions |
|---|---|---|---|
| 27 | Atreyee Sarkara (2019) | *Alkaliphilic bacterium BKH4* | Due to the alkaliphilic nature, there was an enhancement in strength and durability. |
| 28 | B. Madhu Sudana Reddy (2019) | *Bacillus sphaericus* | Addition of the bacteria reduced the weakening (loss of strength) of concrete. |
| 29 | Z M Hussein (2019) | *Bacillus subtilis* | Deposition of calcium carbonate in the voids and subsequent closure of cracks were observed. |
| 30 | Minyoung Hong (2021) | *Bacillus miscanthi strain AK13* | The initial compressive strength of cement mortar was increased by the nitric acid treatment of oyster shell powder from which calcium nitrate was obtained. Increased bacterial survival and crack sealing were observed. |
| 31 | Adharsh Rajasekar(2021) | *Bacillus megaterium, Pseudomonas nitroreducens, Bacillus species, Bacillus licheniformis* | Good compressive strength was obtained. Reduced application cost and less environmental concern. |
| 32 | Shiren O. Ahmed (2021) | *Bacillus subtilis, Bacillus megaterium* | A novel concrete using selected Egyptian microorganisms had improved physical and mechanical properties. |
| 33 | Islam M. Riad (2022) | *Sporosarcina pasteurii DSM 33, Bacillus sphaericus DSM 396* | Compressive strength of concrete was tremendously increased, reduced chloride penetration, improved resistance to sulphate ingress. |
| 34 | Amal A. Nasser (2022) | *Bacillus pasteurii, Bacillus sphaericus* | Compressive strength increased by 28- 50% and so did the flexural strength. |
| 35 | Faisal Mahmood (2022) | *Bacillus subtilis* | Iron oxide particles were found to be ideal as carrier material to keep the cells alive until fracture formation. |
| 36 | J. Vanjinathan(2023) | *Bacillus subtilis, Brevibacillus sp., Bacillus megaterium, Microvirga sp.* | Higher compressive strength was observed in bio-concrete. |
| 37 | S. Udhaya (2023) | *Bacillus subtilis* | Flexural strength was found to be increased by 30%; tensile and compressive strength were also enhanced. |

## Conclusions

Microbially-induced Calcium Precipitation (MICP) represents a promising and sustainable approach for enhancing the durability and self-healing properties of concrete structures. Technologies based on MICP process utilize specially engineered microorganisms and metabolic processes to induce the precipitation of calcium carbonate, effectively sealing cracks and reinforcing concrete material. The efficiency of the self-healing process depends on various factors such as bacterial strain, nutrient source, environmental conditions, and crack width. Extensive research has been done on bio-concrete, aiming at understanding the multifaceted influences of various factors on its performance and exploring the avenues to enhance its efficiency. Aggregating this knowledge enables us to customize bio-concrete formulations for precise applications thus maximizing their self-healing effectiveness.

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