American Journal of Engineering Research (AJER) **2022**

American Journal of Engineering Research (AJER)
e-ISSN: 2320-0847 p-ISSN : 2320-0936
Volume-11, Issue-06, pp-182-201
www.ajer.org
Research Paper Open Access

The effect of self-healing by bio- precipitation by bacteria on the properties Cement based Materials-A review

Mariam Farouk Ghazy[1], AlsaeedAbdelsalam Maaty[2] and GhadaR.Mohamed[3]
[1,2]Prof, Structural Engineering Dept., Faculty of Engineering, Tanta University, Tanta, Egypt
[3]MSc, Student, Faculty of Engineering, Tanta University, Tanta, Egypt

ABSTRACT : *The crack formation is not good for structural health. There are plenty of conventional methods to fill in the cracks and remediate structure damage, but most of these methods are tedious, expensive and/or hazardous to environment. This led to the pursuit of an effective and healthy remediation technique which would be effectively filling up cracks without deteriorating our environment. Biological agents have been used to repair concrete cracks, making concrete constructions more serviceable. This self-healing technique displayed efficient monitoring of structural health, detects damage, and expeditiously filled in the gap. The method is not only safe from the environment point of view but provides extra durability and longevity to the structure. This study aims to investigate about the self-healing capacity and the mechanism involved in the process. Bacillus bacteria have the ability to decrease structural failure rates, operation cost, and environmental consequences. This paper discusses the types of bacteria used in concrete and how they can be utilized as healing agents, and a brief overview of the various properties of concrete that change when bacteria are applied. The freshly composed micro-cracks in concrete can be filled up by the continual hydration process. Concrete mixtures containing urea-producing bacteria from the genus Bacillus are combined with the calcium source to seal the newly formed micro fractures via* $CaCO_3$ *precipitation. The bacterial concentrations were tuned for improved outcomes in improving the pore structure in concrete. The use of bacteria can increase the strength and durability properties of concrete.*

KEYWORDS *bacteria; bio cementation; construction; microbially induced calcium carbonate precipitation.*

Date of Submission: 12-06-2022 Date of acceptance: 27-06-2022

I. INTRODUCTION

Concrete is one of the most extensively utilized construction materials. Concrete cracks are unavoidable since it is weak in tension and strong in compression. When cracks appear in concrete, it can shorten the life of the structure. To repair the cracks, there are a variety of methods available but they are extremely expensive [1-3]. Many concrete structures, especially infrastructure, will eventually deteriorate and degrade over time. This is due to water penetration, which reduces the concrete's efficiency [4]. Some cracks are not apparent and so cannot be reached. Cracks grow in size and number as a result of material expansion, contraction, and permeation. Thus, infrastructure inspection and maintenance techniques are becoming increasingly common. It may be challenging to implement continuous inspection and maintenance in large-scale infrastructures due to the significant amount of funds needed to use it [5].

Numerous research publications on using MICP to improve the mechanical properties of construction materials have been published. Concrete fractures have a negative impact on structural performance in terms of service life [6]. Microbially induced calcium carbonate precipitation (MICP) is a process in which microorganisms, especially bacteria, are given appropriate substrates and therefore promote the formation of calcium carbonate ($CaCO_3$) crystals. The $CaCO_3$ formed is very useful in coating surfaces and binding different particles together [7]. Most recent studies on MICP focus on consolidation of sand and soil, self-healing concrete and crack sealing [8-9], and removal of heavy metals/ions from water [10-11]. Integration of technology across industries, including construction and others, will make the application of MICP easier. It is absolutely necessary for there to be bacteria present for MICP to occur. The bacteria produce the enzymes urease and carbonic anhydrase required to convert suitable compounds into carbonate ions [12]. These activities affect the environment, allowing $CaCO_3$ to precipitate in the presence of calcium ions. The surface charges of bacterial cells attract calcium ions, and the cells then serve as $CaCO_3$ crystal precipitation sites. Extracellular polymeric substances (EPS) are produced by some bacteria and can help the MICP process. The MICP potential of several microorganisms has been investigated. Because they are frequently placed in difficult conditions with high alkalinity, lack of nutrients, and high compressive force. Bacteria must have high cell availability and enzyme activity. $CaCO_3$ crystal morphologies (calcite, vaterite, and aragonite) are affected by bacterial strain and medium composition, affecting the stability and strength of structures formed [14]. The potential of bacteria as a concrete self-healing agent has been examined [15-16]. This was supported by numerous other researchers [17-20]. It was shown that applying isolated bacterial cultures and mixed cultures to fractured concrete efficiently sealed all cracks [21]. This was related to the bacteria's metabolic activities precipitating calcium carbonate [22].

Injecting a bacterial culture into the surface of the concrete can trigger it to begin the process of self-healing [23]. In addition to this, a bacterial culture was sprayed over the surface of the broken concrete in the parking garage [24]. As a consequence of this, the water permeability was significantly improved as a result of the self-healing. Compressive strength, on the other hand, did not significantly regain its previous levels. [25-37] .Numerous studies were carried out with the purpose of improving the compressive strength and durability properties of cement mortar and concrete by using microorganisms. When bacteria was mixed with admixtures in concrete, the result was a further improvement in the material's compressive strength [27]. Besides, compressive strength was reported to have increased by 36% [38]. Up to now, many researches are still ongoing, where bacteria are being applied in concrete purpose fully for more strength and durability improvement.

II. CLASSIFICATION OF BACTERIA

Different researchers around the world have tried a variety of bacterial strains for MICP. In the next sections [39], we will cover some of the commonly used bacterial strains that have been effectively utilized for MICP.

a. TYPES OF BACTERIA

i. SPOROSARCINAPASTEURII (BACILLUS PASTEURII)

Sporosarcinapasteurii, previously known as Bacillus pasteurii is the most commonly used bacterium for studying MICP due to its high urease activity. It is a non-pathogenic bacterial strain. Urease catalyzes the hydrolysis of urea to form ammonia and carbonic acid as shown in Equations (1) and (2).

$$CO\,(NH_2)_2 + H_2O \longrightarrow NH_2COOH + NH_3 \quad (1)$$

$$NH_2COOH + H_2O \longleftrightarrow NH_3 + H_2CO_3 \quad (2)$$

Ammonia then forms ammonium and hydroxide ions in water (Equation (3)) [8].

$$NH_3 + H_2O \longleftrightarrow NH_4^+ + OH^- \quad (3)$$

Carbonic acid also forms bicarbonate and hydrogen ions in water (Equation (4)) [8].

$$H_2CO_3 \leftrightarrow HCO^{3-} + H^+ \quad (4)$$

Formation of hydroxide ions causes pH to increase and shifts the bicarbonate equilibrium. This causes the formation of carbonate ions. The overall equation becomes as shown below (Equation (5)) [8].

$$HCO_3^- + H^+ + 2NH_4^+ + 2OH^- \leftrightarrow CO_3^{2-} + 2NH_4^+ + 2H_2O \quad (5)$$

In the presence of calcium ions, calcium carbonate crystals can be precipitated as shown in Equation (6) [8]

$$Ca^{2+} + CO_3^{2-} \leftrightarrow CaCO_3 \quad (6)$$

Table (1, 2) shows some studies on sand and soil improvements using MICP with Sporosarcinapasteurii.

Table (1): MICP with Sporosarcinapasteurii for sand and soil improvement.

Ingredients	Structure and Properties after MICP	Reference
Sand, clay	Increased tensile strength (40.8 kPa) and compressibility, decreased permeability (0.53×10^{-7}) m/s	[40]
Sand, metakaolin, OPC	OPC-MICP has best properties with UCS 1.2 MPa, water	[41]
Sand, PVA fiber	Highest UCS 1.6 MPa, highest splitting tensile strength 440 kPa, lowest permeability 1.05×10^{-5}m/s	[42]
Sand	Highest CS 3.29 MPa	[43]
Desert Aeolian sand	Highest UCS 18 MPa, lowest permeability $0.92*10^{8}$m/s	[44]
Poorly graded course sand	UCS 525 kPa	[45]

Table (2): Construction materials made by MICP with Sporosarcinapasteurii.

Materials	Structure and Properties after MICP	Reference
Bio-mortar	Highest UCS 43 MPa, lowest water absorption 2.54%	[46]
Bio-mortar	Highest CS 54/70 MPa at 7/28 days curing	[47]
Bio-mortar with superplasticizers	Crack width healed 0.36 mm	[48]
Bio-cement	CS 42 MPa, water absorption 24%	[49]
Bio-mortar	Crack width healed 0.41 mm, water adsorption restored 95%,	[50]

Bio-mortar	Crack width healed 0.27 mm	[51]
Bio-mortar with fiber and zeolite as bacteria carriers	CS 70/100 MPa at 7/270 days	[52]
Geo-polymer	Self-healing observed in 1 month old sample	[53]

ii. BACILLUS SPHAERICUS

Bacillus sphaericus, now known as Lysinibacillussphaericus, is an aerobic Gram-positive, mesophilic, rod-shaped bacteria found in soil and water. Aeration with adequate oxygen improved the MICP of Bacillus sphaericus and Bacillus licheniformis, according to Seifan et al [54]. The bacterium could tolerate pH levels as high as 12. Higher pH increased MICP and resulted in smaller crystals. Their research found that at pH 9–10, more vaterite was created, while at pH 11–12, more calcite was formed.

Table (3): Materials produced using MICP with Bacillus sphaericus.

Materials	Structure and Properties after MICP	Reference
Concrete with fly ash	Highest CS 32.5 MPa, highest tensile strength 4.1 MPa, highest flexure strength 3.5 MPa	[55]
Concrete with fly ash	CS 30–40 MPa, tensile strength 2.9–5.0 MPa	[56]
Bonding repair mortar	Highest slant shear strength 17 MPa	[57]
Industrial ceramic aggregates (treatment)	Water absorption 6–16%, weight gained 3–7%	[58]

Zhang et al. [59] obtained Bacillus sp. Strain H4 from a mangrove conservation area in Shenzhen Bay, China, and then used the bacteria in combination with oxygen-releasing tablets to create a self-healing system. Another study [60] reported that increasing nitrate and calcium concentrations inhibited the MICP process. Because this bacteria strain cannot survive high alkalinity, the surrounding pH must be kept between 9.5 and 11. Achal and Pan [61] studied the effects of calcium supply on the MICP process of Bacillus sp. CR2 isolated from mine tailing soil in Urumqi, Xinjiang, China. Lv et al. [62] investigated the stability of vaterite produced by Lysinibacillus sp. GW-2 isolated from soil in China's Nanjing Botanical Garden. They studied the formation and transitions of several $CaCO_3$ crystals. They reported that organic materials allowed vaterite to remain stable without changing morphology. Lee et al. [63] identified Lysinibacillus sp. YS11 can generate spores, EPS, and biofilms. Only in aerobic settings with enough aeration could the bacteria exhibit MICP. Micro-bacterium sp. GM-1 isolated from active sludge was tested for MICP potential by Xu et al. [64]. They reported that urea concentration was the most significant factor for the MICP and calcite was the dominant crystals formed. All results are shown in table (3).

iii. BACILLUS HALODURANS

The results of the tests revealed that the inclusion of bacteria significantly improves strength and durability. Concrete's compressive strength is increased with the inclusion of Bacillus halodurans bacteria [65].

iv. BACILLUS SUBTILIS

Bacillus subtilis is a Gram-positive, aerobic, rod-shaped bacteria that is widely found in soil, water, and plants. It can produce a variety of metabolites and has a high potential for industrial uses [66–67]. It is not ureolytic, however research have revealed that it contains functional urease that can be activated using specialized processes. Perito et al [68] discovered that a solution containing dead Bacillus subtilis cells could precipitate $CaCO_3$. The advantage is that dead cells can withstand high temperatures (up to 100 degrees Celsius). Furthermore, because calcite is only generated on the dead cell wall, the MICP process has the ability to be controlled. It was utilized to cure stones and an Angara Church wall by the writers. After treatment, water adsorption was reduced by 16.7 % on laboratory stones and 6.8 %on Angara Church. In addition to $CaCO_3$, Bacillus subtilis has been shown to create phosphates when appropriate chemicals are applied, resulting in bio-sandstone with a compressive strength of 2.1 MPa [69].

Table 4 lists some of the projects completed. According to the reports discussed above, Bacillus subtilis is activated through specific procedures. Its use in MICP processes ensures that concrete with significant compressive strength is established. Certain parameters can also be used to control the MICP processes.

Table (4): Materials produced using MICP with Bacillus subtilis.

Materials	Structure and Properties after MICP	Reference
Bio-mortar	Highest CS 50 MPa, lowest water absorption 5.5% Crack width healed up to 1.2 mm	[70]
Bio-concrete	Highest CS 44 MPa Self-healing observed	[68]
Sand column (mixture of Sand column S. pasteurii)	UCS 1.69 MPa, permeability 1.06×10^{-5} m/s	[71]

v. BACILLUS MEGATERIUM

Bacillus megaterium is a rod-shaped Gram-positive bacterium. [72] It is the largest Bacillus species. Before Bacillus subtilis was developed, it was utilized as a model organism for many studies. It is also ureolytic, therefore its MICP potential is being investigated. Bacillus megaterium solution was employed by Dhami et al. [73] to treat sand columns with grain sizes ranging from 0.2 mm to 1.5 mm. In their previous investigation [74], the authors attempted to adjust medium for Bacillus megaterium SS3 in order to get the best MICP performance. They discovered that glucose and peptone are the bacteria's top carbon and nitrogen sources. They also discovered that glucose, urea, and $NaHCO_3$ all improved MICP performance significantly. $CaCO_3$ production was increased by 70% using the improved material. Table 5 lists some of the projects completed.

Table (5): Materials produced using MICP with Bacillus megaterium.

Materials	Structure and Properties after MICP	Reference
Treat sand column with varying grain size	Up to 30% $CaCO_3$ formation	[74]
Bio-concrete with recycled aggregates, Nano silica	Water absorption 5%, void volume 10%	[75]
Bio-mortar	Highest CS 36 MPa, permeability 5×10^{-5} m/s	[76]

vi. BACILLUS PSEUDOFIRMUS

Sharma et al. [77] used a Bacillus pseudofirmus solution including calcium lactate as a calcium salt, calcium nitrate as an inorganic salt, and yeast extract as a nutrient to heal autogenously mended mortar that had been left for a year. Sharma et al. [77] produced Bacillus pseudofirmus DSM 8715 spores. The spores were utilized to produce mortar and to treat concrete cracks via injection. This bacteria strain has great spore formation and germination properties, however it requires sufficient essential such as alanine, inosine, or NaCl. This bacterium's MICP generated calcite and aragonite. Table 6 lists some of the projects completed.

Table (6): Performance of MICP by various bacteria in making concrete and mortar.

Bacteria (Initial Concentration)	Other Additives	Performance	Reference
Bacillus sphaericus (Not provided)	Fly ash	Compressive strength = 32.5 MPa	[78]
Bacillus sp. CT5 (OD_{600} = 0.5)		Compressive strength = 46.0 MPa Water penetration = 14.2 mm	[79]
Bacillus subtilis (10^3–10^7 cell/mL)		Compressive strength = 54.0 MPa Water adsorption = 4% Crack width healed = 1.2 mm	[70]
Lysinibacillus sp. I13	Fly ash	Compressive strength = 33.6 MPa *Able to heal cracks but no exact values provided	[80]
Bacillus cohnii (5.2 * 10^8 cell/mL)	Expanded pearlite	Crack width healed = 0.8 m^{i}m	[81]
Bacillus sphaericus (10^5 cell/mL)	Fly ash	Compressive strength = 40.4 MPa	[82]
Bacillus cereus (5 * 10^8cfu/mL)	Metakaolin	Compressive strength = 40.2 MPa	[83]
Bacillus aerius (10^5 cell/mL)	Cement baghouse filter dust	Compressive strength = 36.3 MPa Water adsorption = 1.2%	[84]
Bacillus aerius (10^5 cell/mL)	Rice husk ash	Compressive strength = 35.0 MPa Water adsorption = 1.1%	[85]
Bacillus mucilaginous (10^8–10^9 cell/mL)		Crack width healed = 0.5 mm Water permeability = 0.8*10^7 m/s	[86]
Bacillus megaterium (OD_{600} = 1.5)		Compressive strength = 35.0 MPa	[87]
Pseudomonas aeruginos	Granular activated carbon	Crack width healed = 0.5 mm	[88]

b. PRECIPITATION OF BACTERIA

i. CALCIUM CARBONATE PRECIPITATION

Concrete microorganisms were utilized to enhance the concrete's overall performance. Precipitation of bacterial calcium carbonate ($CaCO_3$) was hypothesized to be a consequence of basic metabolic processes such as urea hydrolysis. In this study, calcium carbonate-precipitating ureolytic bacteria were isolated and their urease activity was evaluated based on the generation of urease [89].

ii. DOLOMITE PRECIPITATION

Yu et al. [41] used the Sporosarcinapasteurii injection technique to treat loose quartz sand in columns. Instead of calcium salts, magnesium chloride ($MgCl_2$) was used, resulting in the precipitation of magnesium carbonates. Ruan et al. [90] treated fractures in reactive magnesium cement with Sporosarcinapasteurii isolated from activated sludge. After two treatment cycles, cracks wider than 0.15 mm were entirely repaired by the formation of magnesium carbonate on their surfaces. They observed that urea content had no effect on the healing process, but did affect pH and carbonate morphology.

iii. SILICA PRECIPITATION

Ghosh et al. [91] evaluate the effects of a facultative anaerobic hot spring bacteria on the microstructure of a cement–sand mortar.

c. NUTRIENTS OF BACTERIA

i. CALCIUM CHLORIDE AND CALCIUM ACETATE

As alternate calcium sources, the usage of calcium chloride ($CaCl_2.2H_2O$) and calcium acetate ($CaCH_3COOH$)-2.H_2O was examined. XRD analysis was conducted to see what other material was generated besides $CaCO_3$ [92].

ii. PEPTONE, CALCIUM LACTATE AND CALCIUM GLUTAMATE

Three different organic compounds, peptone, calcium lactate, and calcium glutamate, which could be used as energy and carbon sources by both bacterial strains, were evaluated for their potential application as bio-mineral chemical precursors in concrete [93].

iii. CALCIUM FORMATE AND CALCIUM NITRATE

After 72 hours of incubation in a minimum nutrient solution with a pH range of 9.5–10 including calcium Formate ($Ca(HCOO)_2$) and calcium nitrate ($Ca(NO_3)2$), bacterial cells that were recovered from mortar specimens that had been cured for 28 days showed evidence of nitrate reduction activity [120]. It was discovered that the presence of alkali-resistant bacteria in a solution containing calcium Formate increased the compressive strength of cement mortar after 28 days of curing [94].

iv. LACTOSE MOTHER LIQUOR

LML has the potential to be a more effective supply of nutrients for the growth of bacteria as well as for the precipitation of calcite. The use of LML rather than normal media not only leads to reducing costs in the remediation of cracks and fissures in structures caused by bacteria, but it also operates as environmentally friendly technology that helps prevent environmental pollution [95].

v. CORN STEEP LIQUOR

Many researches have been conducted to improve Sporosarcinapasteurii MICP so that better materials can be produced at lower costs. Various nutrients and calcium salts had different effects on Sporosarcinapasteurii when it was used to produce mortar, according to a study by Amiri and Bundur [47]. The calcite-producing bacterial strain AKKR5 was used in a study to cure concrete concentration of 10^5 cells/mL [84] [96].

vi. GLUCOSE AND SODIUM SILICATE

The calcium source is only from the cement paste. The carbon source is glucose (D (+) Glucose Monohydrate, Boom, The Netherlands). To evaluate the influence of solution pH on bacterial activity, 148 g/L glucose solutions were produced in either demineralized water (neutral pH) or water-glass (alkaline pH) [97].

vii. UREA

The role of microbial urease in calcite precipitation was investigated using a recombinant Escherichia coli HB101 plasmid harboring the Bacillus pasteurii urease plasmid, pBU11. Although the precipitation level of calcite by E. coli HB101 (pBU11) was not as high as that of B. pasteurii, it was significant [98].

viii. YEAST EXTRACT AND STARCH

Although concrete with a high self-healing (crack-healing) capacity is wanted, the addition of healing agents such as bacteria and/or (organic) chemical compounds to the paste may lead to an undesirable reduction in strength properties [99].

III. EFFECTS OF BACTERIA ON CONCRETE PROPERTIES

a. PROPERTIES OF THE FRESH CONCRETE MIXTURE

Fresh bacterial concrete's workability is an important property that needs to be looked into in depth. When bacteria are added to concrete, they are usually mixed with a suitable nutrient that provides them the food they need to grow. On the one hand, nutrients are essential for bacterial growth as long as there is water and moisture. On the other hand, this additive may also change some of the properties of fresh concrete. According to studies done previously, bacteria are often added to normal concrete mixes as an additional agent, while the mix composition stays the same as for the reference concrete. From the authors' point of view, this concept could be a concern because the properties of fresh concrete could change. Uncertainty remains as to whether the water demand and setting time of a concrete mixture containing a constant concentration of bacteria would abruptly alter or remain steady. So, a comprehensive review, like the one below, is decided to look into the problems with workability caused by the addition of bacteria and nutrients.

Vijay et al. [100] studied the combination effect of Bacillus subtilis bacteria spore powder and calcium lactate on the workability of two concrete mixes, i.e., normal concrete and basalt-fiber reinforced concrete. The concentration of bacteria was fixed at 10^5cfu/mL (cfu = cell or colony-forming unit) with the purpose of improving the strength of concrete, and calcium lactate was used as nutrient source for bacteria at the dosage of 0.5% by weight of cement. Both bacteria and nutrient were added to the normal mix while maintaining the volume of aggregates and no superplasticizer was used in the mix design. Coarse aggregate with the nominal size of 20 mm and fine aggregate were used. The results showed that the addition of Bacillus subtilis together with calcium lactate increased the slump value of normal concrete. The workability of bacterial concrete improved due to the role of calcium lactate as a retarding agent in the concrete that may increase the setting time and fluidity of the concrete. Furthermore, basalt fibers were introduced in the bacterial concrete mix and the slump value was nearly the same as for the normal concrete without bacteria. Due to the internal friction between fibers and cement, the traditional bacterial concrete is more workable than the fiber-reinforced bacterial concrete. Siddique et al. [101] added isolated ureolytic bacteria Bacillus aerius strain AKKR5 at a concentration of 10^5cfu/mL to a standard concrete mixture without incorporating a nutrient for bacterial growth. In this investigation, the w/c and aggregate-to-binder (a/b) ratios were held constant at 0.50 and 4.44, respectively, and no superplasticizer was used. The mix design called for coarse aggregate with a maximum size of 12.5 mm and a fineness modulus (FM) of 6.38 and fine aggregate with an FM of 2.58. The results demonstrated that the slump value of bacterial concrete did not differ from that of the control concrete. Mohammed et al. [102] also discovered a similar outcome. The addition of Tryptone Soy Broth (TSB)-cultured iron-resisting bacteria had no effect on the fresh density and slump value of CEM I concretes. On the other hand, a contrasting behavior was found with CEM III concretes, which exhibited a slight decrease in unit weight and a 9 % decrease in slump value. Nonetheless, all concrete mixtures attained a medium workability, which was categorized as S3 by EN 12350-2:2009.

Ameri et al. [103] used slump flow, V-funnel, U-flow, and L-box tests to compare self-compacting concrete (SCC) mixtures with different concentration of Bacillus pasteurii cells. Calcium lactate was chosen as the source of nutrients, and 5 %by weight of cementitious was added to the mixtures.

To obtain comparative results, reference concrete was made with the same composition as bacterial concrete with RHA as secondary cementitious material (SCM), only without adding bacteria and nutrients. It is worth noting that for all workability tests, all mixes showed identical results despite different bacteria concentrations. For instance, the slump flows of reference concrete and bacteria concretes with cell concentrations of 10^3, 10^5, and 10^7 cells/mL were 680, 684, 689 and 690 mm. This result contradicts the findings of Vijayet al. [100] concerning the effect of bacteria and nutrients on workability. Fahimizadeh et al. [104] produced intriguing research that can be used to comprehend the effect of nutrients and microorganisms with nutrients in concrete applications.

b. PROPERTIES OF THE HARDENED CONCRETE

The percentage changes in mechanical and durability properties compared to the control concrete without bacteria, nutrients, and SCMs are expressed. The performance of concrete embedded with various genus of bacteria and suitable nutrient(s) often shows an improvement in mechanical qualities due to calcite precipitation. In fact, it should be observed that no changes to the mix designs were made to enable the addition of bacteria-based healing agents.

One of the main issues in introducing bacteria into concrete mixes is the limited cell viability when the bacteria are directly incorporated to the fresh concrete due to the concrete’s high pH and the shear forces occurring during the mixing stage. To address this issue, Seifan et al. [105] developed immobilized bacteria cells using magnetic iron oxide nanoparticles to overcome this issue (IONs). Bacillus sphaericus was chosen to be the microorganism cultured in calcium chloride, urea, and yeast extract. The healing agent was added to their normal concrete mix, and no superplasticizer was used. At 7 and 28 days, the compressive strength of bacterial concrete was increased by 43% and 15%, respectively, as compared to the reference concrete. Reddy et al. [106] discovered that a cell concentration of 10^5 cells/mL given the highest improvement in mechanical properties of bacterial concrete based on Bacillus sphaericus. The addition of a bacterial agent, on the other hand, led in more free shrinkage due to the chemical reaction of nutrients during cement hydration. Vijay et al. [100] and Khaliq et al. [107] investigated use of Bacillus subtilis with calcium lactate as a nutrition source in concrete applications.

The compressive strength of concrete with Bacillus subtilis at a cell concentration of 10^5 cells/mL is at least 20% higher than that of concrete without bacteria after 28 days. According to the microstructure findings, the strength increase was caused by the formation of calcite, which filled in the voids.

i. MECHANICAL PROPERTIES

1. COMPRESSIVE STRENGTH

Concrete compressive strength has been linked to the long-term durability of concrete constructions. Due to increased sensitivity to loads and pressure, inadequate compressibility can result in crack formation. Such cracks then facilitate in the degradation of the concrete structure by increasing the exposure of the cement matrix and reinforcements to corrosive agents (similar to high-porosity concrete structures). As a result, the compressive strength of experimental concrete samples containing additives is commonly evaluated in order to determine the impact of additives on structural strength and concrete durability. Mors and Junkers [108] demonstrated that the direct addition of 6×10^8 cells/mL B. pseudofirmus spores retards the initial compressive strength of the cement sample. Initially, the compressive strength of biotic mortars was 60% lower than that of abiotic mortars. Their strengths became identical after 14 days due to bacterial precipitate ion and remained unchanged over the next 56 days. Mondal and Ghosh [70] used concrete to incorporate 10^3, 10^5, and 10^7 cells/mLof B. subtilis spores. After 3 days, all bacterial samples had higher compressive strengths than control specimens, which continued up to 28 days. During this time, the highest gain in compressive strength was observed in samples containing 10^5 cells/mL. Khaliq and Ehsan [107] incorporated 2.8×10^8 cells/mL of B. subtilis and Ca–L spores either directly or after LWA or GNP immobilized ion and reported improved compressive strengths in bacterial samples compared to control specimens after 3 d and up to 28 d, resulting in a maximum compressive strength increase of 12 % with LWA-immobilized bacteria and 9.8 %with graphite nanoparticle (GNP)- Furthermore, the interaction between bacteria and carrier surface may affect healing rate, create nucleation sites, and promote biofilm formation, which may affect MICP in a manner similar to the impact of soil particles on $CaCO_3$ precipitate ion caused by soil bacteria [109].

B. subtilis in vitro MICP evaluation using Ca–A demonstrated that B. subtilis promotes $CaCO_3$ precipitation by biofilm formation [110]. Prior study has also shown the importance of biofilm formation for MICP by B. subtilis [110] and B. cohnii [111]. Schwantes-Cezario [110] investigated B. subtilis in vitro $CaCO_3$ precipitation using Ca–A (0.25 %s w/w).They reported that B. subtilis promotes $CaCO_3$ precipitation by biofilm formation and associated the rise in pH with biofilm development. Hence, attention to bacteria-carrier interactions can lead to beneficial discoveries, especially when the healing agent is a biofilm forming bacteria. Such an approach may focus on factors affecting biofilm development, including water availability [112] The reported improved compressive strength upon the direct addition of healing agents by Khaliq and Ehsan [107] differs from the findings of Jonkers et al. [113] the latter suggests that the retarding effect of bacterial spores on the compressive strength of concrete may be species-dependent. This suggestion is possible because both studies used Ca–L as the primary nutrient source and comparable spore concentrations. Moreover, the data indicates a slight positive impact of 10% by Ca–L on compressive strength .The comparison between the findings of Khaliq and Ehsan [107] and those of Ramachandran and Ramakrishnan [114] (who directly incorporated 7.2×10^7 $cell/cm^{-3}$ of ureolytic bacteria Bacillus pasteurii and found no compressive strength improvement) suggests that B. subtilis is a better choice for direct addition in terms of compressive strength. However, the direct addition of healing agents to concrete can be disadvantageous considering the structural integrity of concrete and self-healing efficiency.

Luo and Qian [94] compared the capacity for self-healing of a system. It is composed of non-ureolytic bacterial spores, Ca–L, Ca–N, and Ca–F. The authors reported the effect of bacteria/Ca–F (1 %w/w) on the compressive strength of cement samples, specifically a decrease in compressive strength with increasing bacteria/Ca–F concentration. The reported compressive strength results of bacteria/Ca–L also revealed the effect of various calcium substrate concentrations on compressive strength. The best performance was obtained when 3 % w/w of bacteria/Ca–L was added to cement samples, resulting in an initial decrease of compressive strength; however, after 10 days, their compressibility surpassed that of the control samples. Bacteria containing Ca–N decreased the compressive strength as Ca–N levels increased. According to reports, the pore size distribution was dependent on the calcium substrate used. Zhang et al. [46] studied the impacts of calcium source on MICP formation by Sporosarcinapasteurii in mortar samples. Calcium acetate, calcium chloride, and calcium nitrate were tested as calcium sources. The amount of $CaCO_3$ formed and the amount of water absorbed by the sand column were unaffected by the type of calcium sources tested. However, calcium acetate samples had the highest UCS and tensile strength. The pore structure of these samples was also smaller and more uniformly distributed. 88 % aragonite and 12 %calcite formed in samples treated with calcium acetate, while only calcite formed in those treated with the other two calcium salts. Amira and Bandura [47] compared the effects of various nutrients and calcium salts on the formation of mortar samples by Sporosarcinapasteurii using MICP. Similar bacterial growth was observed in both corn steep liquor (CSL) and yeast extract, but CSL had cells with a lower surface charge. Both nutrients increased the setting time, but CSL had a lesser effect than yeast extract. After 28 days, CSL also produced more $CaCO_3$ than yeast extract. However, yeast extract sample had a higher compressive strength than CSL sample. The authors also stated that the variable solubility of calcium salts affects the $CaCO_3$ crystal morphology.

Since the cost of the nutrient source can be up to 60 % of the total cost, it is evident that using cheaper alternatives can reduce the cost of produce bio cement.Yoosathaporn et al. [115] used chicken manure effluent as an alternative source of nutrients for Sporosarcinapasteurii to make bio cement cubes in this direction. The bio cement had a 30.27 % higher compressive strength, a 5.38 %higher density, 3.2 % more voids, and slightly higher water absorption than normal cement. Calcite and vaterite were also observed to have been formed. The authors said that chicken manure effluent could make more than two times as much urease as nutrient broth and was 88.2% cheaper. Hao et al. [116] used a Bacillus sp. strain isolated from soil sample from Perth, Australia for MICP surface treatment of polypropylene before making fiber reinforced cementitious composites. Compressive strength of the composites was decreased by 6.9% but energy adsorption capacity increased by 69.3%. Seifan et al. [79] investigated the effects of a variety of variables on the MICP of several bacteria. They identified that the significant factors were bacteria species, bacteria concentration, yeast extract, $CaCl_2$, urea, and agitation speed. A high concentration of bacteria improved MICP. More than 3 g/L of yeast extract greatly decreased MICP. $CaCl_2$ is better to calcium lactate, calcium nitrate, and calcium acetate, according to studies. Too low or high Ca^{2+} concentration will reduce MICP.

In ordinary grade concrete (M_{20}) the compressive strength is increased up to 20.85% at 28 days by addition of Bacillus halodurans bacteria when compared to conventional concrete. The split tensile strength is increased up to 23.60% at 28 days by addition of Bacillus halodurans bacteria when compared to conventional concrete. Also the flexural strength is increased up to 10.08% at 28 days by addition of Bacillus halodurans bacteria when compared to conventional concrete [117]. Li et al. [83] used MICP from Bacillus cereus NS_4 to make mortar containing metakaolin. The bio-sample has greater compressive strength and lower permeability than normal mortar. The addition of 25% mass metakaolin increased compressive strength and permeability in compared to 0% or 50% mass metakaolin.

Zhu et al. [118] reported that Bacillus cereus can be used to remove nickel from soil on a large scale. Their study revealed that the concentration of nickel in the soil went down from 400 mg/kg to 38 mg/kg. After the nickel was treated, most of it was bound to carbonate. The study showed that MICP by Bacillus cereus could be a possible alternative for large-scale remediation of metal-contaminated soil.

Ghosh et al. [119] describe a method for improving the strength of cement–sand mortar through microbiologically induced mineral precipitation. Various cell concentrations of a thermophilic anaerobic microorganism are incorporated with the mixing water. The study showed that the addition of approximately 10^5 cell/ml of mixing water increased the compressive strength of cement mortar by 25% after 28 days. As shown by scanning electron microscopy, the increase in strength results from the growth of filler material within the pores of the cement–sand matrix.

Rizwan et al. [120] used two effective microorganism consortiums containing yeast, lactic acid bacteria, and photosynthetic bacteria to make bio cement. The addition of the consortia solution sped up the cement paste's setting time. In addition, super plasticizer was required for this method. The water absorption of the bio cement was lower than that of the control sample. The highest reported compressive strength for bio cement was 89 MPa. Analysis revealed that the effective microorganisms mainly produced calcite and some wollastonite. Concrete obtained from Bacillus flexus produced significant results as compared to normal concrete. It showed a considerable increase in compressive (over 40%), flexural (over 30%) and split tensile (over 10%) strength. X-ray diffraction (XRD) results confirmed the impressive ureolytic properties of B. flexus. [121]. Vashisht et al. [122] isolated Lysinibacillus sp. from alluvial soil and sewage samples collected from various locations in the Indian district of Solan and then used the bacteria to make self-healing concrete. The compressive strength of self-healing concrete was 34, 6% higher than that of normal concrete. The authors claimed that their concrete was better at self-healing than Bacillus megaterium-made concrete. Siddique et al. [84] isolated ureolytic bacteria Bacillus aerius strain AKKR5 from marble sludge to make bacterial concrete with cement baghouse filter dust replacing up to 30% of ordinary Portland cement. Bacteria concrete without cement baghouse filter dust had 10% higher compressive strength than normal concrete. In another study, the same group [85] also used the Bacillus aerius strain AKKR5 to make bacterial concrete with rice husk ash replacing up to 20% of ordinary Portland cement. Best bacterial concrete properties were obtained using 10% rice husk ash with 14.7% higher compressive strength than normal concrete, 0.8% water absorption, 1.5% porosity, and very low to moderate chloride permeability. Jonkers and Schlangen [16] reported that mortar compression resistance could be reduced by 10% when adding bacteria and organic bio-mineral precursor compounds into cement pastes. However, bacterial mortar compression resistance improved during 28-days bio-calcification due to the significant precipitation of calcium carbonate by bacteria. The 28-day compressive strength of the bacterial mortar was found to be compatible with that of cement mortar. Lactose mother liquor, an industrial effluent from the dairy industry, is a good source of nutrients that can support the growth and urease activity of the bacteria [117].

2. FLEXURAL STRENGTH

The bending of concrete structure due to external forces often lead to crack formation because concrete has low flexural strength (usually 10%–20% of its compressive strength). Accordingly, the impact of concrete self-healing systems on flexural strength has been assessed as a self-healing benchmark. In an earlier study, Xu and Yao [123] assessed the effectiveness of 10^7 cells/mL B. cohnii spores and Ca–L or Ca–Glut as a surface treatment to improve healing. In addition, they assessed the effect of the healing system on the flexural strength of mortar when it was applied as a surface treatment or incorporated as a self-healing system. They reported no adverse effects on flexural strength due to individual system components when incorporated into mortars, even noting a slight improvement in the case of Ca–L addition in an earlier study [124]. After 28 days, surface-treated mortars had greater flexural strength than cement mortars healing agents. The flexural strength of the samples containing Ca–Glut was double that of the control samples, whereas the samples containing spores and Ca–L did poorly. The higher flexural strength recovery of samples that contain Ca–Glut was attributed to the bacteria's higher $CaCO_3$ conversion of Ca–Glut. This resulted in a larger and denser transition zone between the biologically deposited mineral layer and cement matrix (Fig. 1) [123] forming a strong bond between the deposition layer and cement matrix. The flexural strength results were confirmed using grid nano-indentation, and the hardness and modulus of the mortar, the outer precipitates, and the transition zone of different specimens were measured. The highest values of modulus and hardness were observed in the transition zone of the sample with Ca–Glut, and the lowest measurements were observed in the transition zone of the Ca–L sample.

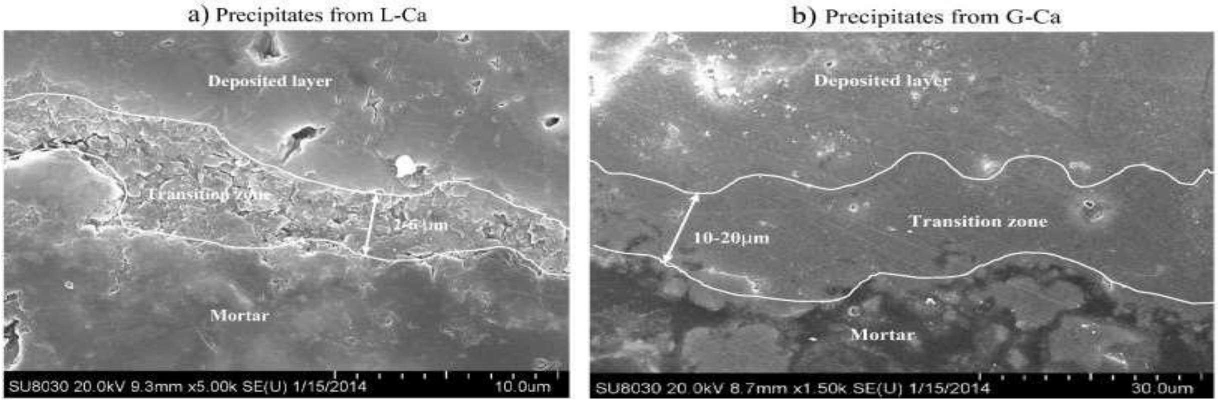


Fig.1. Scanning electron micrographs of transition zone in (a) Ca–L and (b) Ca–Glut specimens.

Sierra-Beltran et al. [125] impregnated LWA with Ca–L and yeast extract, and B. cohnii spores were added to a cement-based composite that hardens under strain (SHCC). The presence of bacteria improved flexural strength in comparison with the control specimen. In addition, the healing agents only slightly improved the mechanical properties of the SHCC with insignificant $CaCO_3$ precipitation as compared to the control samples. Inadequate nutrition was cited as a possible explanation for the lack of distinguishable $CaCO_3$ precipitates in the bacterial samples, and further study was suggested. Gao et al. [125] coated calcium alginate capsules containing B. pseudofirmus with chitosan and recorded a 3.78 % in flexural strength compared to that of the capsule-free control specimen; only 1.2% – 1.5% (with capsule/with cement) of the capsules were included, which reduced the expected strength reduction. Shanmuga Priya et al. [126] discovered that Bacillus sphaericus improved the compressive strength of high-strength concrete (60 MPa) and Zhang et al. [127] demonstrated that Bacillus halodurans improved the compressive strength of engineered cementitious composites.

3. DURABILITY

The impact of non-ureolytic biological self-healing systems on concrete durability has primarily been assessed by investigating the water permeability of structures. Non-ureolytic concrete self-healing systems perform this function through pore and void blocking as well as crack healing by $CaCO_3$ precipitation. Consequently, concrete durability against chemical and physical deterioration is enhanced.

a. POROSITY

Although fluids can enter concrete via several transport mechanisms, the most significant parameter for concrete degradation is the extent to which the cement matrix's interconnected pore system is further exposed to the environment through micro cracks. Therefore, limiting concrete exposure to corrosions by reducing concrete porosity has been a strategy to achieve more durable construction materials [128].

Mondal and Ghosh [70] incorporated 10^3, 10^5, and 10^7 cells/mL of B. subtilis spores into concrete and reported lower water absorption rates of 13%, 23%, and 27%, respectively; they also reported that the water penetration depths were reduced in comparison to those of corresponding abiotic control samples (Fig. 2). As stated in Section 4.1.1, the highest improvement in compressive strength was observed in specimens with 10^5 cells/mL, suggesting a discrepancy between compressive strength and porosity measurements. The discrepancy was attributed to higher surface $CaCO_3$ precipitation in conjunction with a higher bacterial concentration. This prevented the early entry of water and oxygen into the cement matrix, which could have impeded hydration and bacterial activity inside the matrix. The difference observed between improvements in porosity and compressive strength as a result of different bacterial concentrations has practical importance for applications in different environments or under different load-induced pressure.

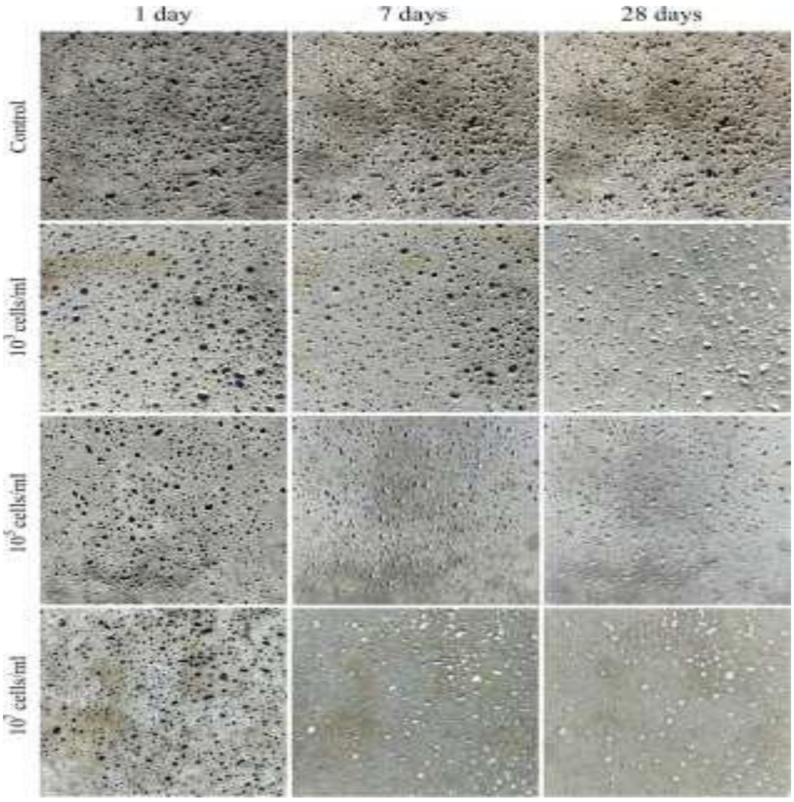


Fig.2. Surface porosity changes over 28 d with different bacterial concentrations. Each image represents 50 cm × 50 cm section.

Luo and Qian [94] compared the self-healing capacity of a system composed of non-ureolytic bacterial spores and Ca–L, Ca–N, or Ca–F; they reported that the pore size distribution was associated with the calcium substrate used. The control specimen primarily had pores in the range of 10–100 nm, whereas the addition of self-healing agents shifted the pore size distribution to larger pores in the range 100–1000 nm. In addition to non-ureolytic bacteria incorporation into concrete structures, these bacteria have also been investigated in terms of surface treatment. Xu et al. [111] reported a reduction of approximately 50% in the water absorption capacity of cement mortars surface-coated with B. cohnii after two weeks of incubation in nutrient media supplied with Ca–L or Ca–Glut. The reduction was attributed to pore blocking by bacterial $CaCO_3$ precipitation. Additionally, MICP by cyanobacteria Synechococcus PCC8806 was investigated in a concrete powder solution (pH 11.7) [129].

Some commercially available products can be used to make a surface impermeable, but due to disadvantages such as incompatibility between the protective layer and the underlying layer due to differences in thermal expansion coefficient or disintegration of the protective layer over time and the need for constant maintenance, they are not used effectively [130-131].

The calcite precipitation resulted in a slight decrease in porosity and water absorption by capillarity [132], which was reduced about 60 % from the limestone [133]. Precipitation of bacteria on the stone surface was confirmed and resulted in a reduction in permeability without affecting the stone's aesthetic appearance, leading to the conclusion that biological mortars or cement could be used to affix broken statue pieces and fill small cavities on limestone surfaces [134].

Later reported precipitation of calcite by Bacillus sphaericus was effective in reducing the water absorption rate of limestone [135].

There were research that provided alternatives for building materials. Using calcite precipitating bacteria increased the resistance of concrete towards alkali, sulphate, freeze-thaw attack, and drying shrinkage [100]. While studying the durability effect of Bacillus sphaericus on mortars, a significant decrease in water absorption compared to untreated specimens (a decrease of 45, 43, and 24 %with increasing w/c) and a 19 % decrease in the chloride migration coefficient were observed, resulting in a decrease in water absorption and gas permeability due to the limited penetration of the bacteria into the porous matrix and the precipitation of calcium carbonate [136].

Bacillus sphaericus was used to prepare mortars for testing, and after 168 hours, the mortars with bacterial cells absorbed nearly six times less water than the control cubes.

Compared to untreated mortars, the presence of bacteria resulted in a considerable reduction in water absorption. The surface permeability decreased due to the deposition of a layer of calcium carbonate crystals [38]. The water impermeability test was performed on 150-mm concrete cubes prepared with Bacillus pasteurii bacteria, nutrients, corn steep liquor, urea as a substrate, and calcium chloride as a calcium source [137]. Nosouhian et al. [138] MICP by Sporosarcinapasteurii for concrete surface treatment increases the durability of concrete exposed to sulphate conditions.

b. MICROBIAL CONCRETE AND CORROSION

The corrosion of steel and reinforcing bars is undesirable factor causing early deterioration of concrete constructions worldwide [84]. Corrosion and permeability go together, higher the permeability, corrosion would be more and vice versa. The ingress of moisture, carbon dioxide and chloride ions through the concrete to the steel surface cause initiates corrosion. Chloride induced corrosion of reinforcing steel is one of the most pressing problems worldwide that the construction industry is facing today. The corrosion products of iron oxides/hydroxides expose the reinforcement to direct environmental attack that results in accelerated deterioration of the structure [25]. The solution to prevent corrosion can be achieved by closing the paths of ingress to improve the life of the reinforced concrete (RC) structures.

MICP reduces the permeability of concrete and can effectively reduce corrosion in reinforced concrete by precipitating calcite into a protective layer. However, there is few research on MICP in corrosion prevention of RC structure. Such research was mainly reported by researchers and performed detailed investigation leading to positive impact of MICP in the RC corrosion prevention [139] Researchers determined the effect of MICP by preparing the RC specimens with bacterial cells (Bacillus sphaericus) and induced corrosion by applying a constant anodic potential of 40 V for 7 days. There was visible calcite precipitation on bacterially treated RC specimens.

Researchers concluded that the formation of calcite might facilitate the passive protective film around the steel and operate as a corrosion resistant by stopping the transport process in such samples, hence reducing the mass loss of reinforcing bars due to MICP and increasing their durability [139-141].

c. CHLORIDE ION PERMEABILITY

In reinforced concrete, chloride ion diffusion can harm the reinforcement. One of the major mechanisms of deterioration affecting the long-term performance of building structures is chloride-induced corrosion. S. pasteurii successfully utilized calcium nitrate as an efficient calcium source. [140]. Further, as calcium chloride results in massive production of ammonia, increasing the risk of reinforcement corrosion. Xu et al. [111] compared the ability of calcium lactate and calcium glutamate to precipitate calcium carbonate induced by B. cohnii and observed that calcium glutamate precipitated a thicker $CaCO_3$ layer than calcium lactate [61] [124] [136] [142].

d. CRACK HEALING

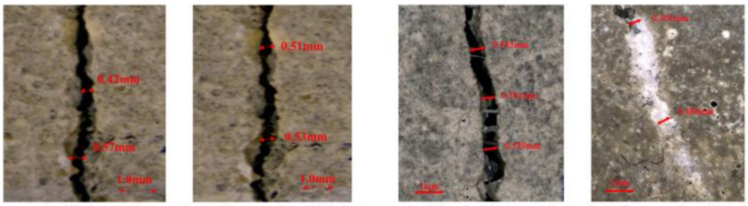
Crack healing by non-ureolytic bacterial concrete self-healing systems can forestall micro cracks from propagating and coalescing into larger cracks, thus preventing the entry of deleterious environmental elements into the structure. The efficiency of non-ureolytic biological concrete crack healing has been assessed by monitoring the crack width over time, the visual and chemical analyses of healing agents, and the changes in water flow and absorption through the cracks.

Wiktor and Jonkers [143] impregnated ECPs with Ca–L, yeast extract, and 1 $\times 10^5$ cells/mLB.alkalinitrilicus spores and observed the healing of up to 0.46 mm cracks after 100 d of curing and up to 0.18 mm crack healing in control samples attributable to the autogenous self-healing of cement-based structures. Khaliq and Ehsan [107] introduced 2.8×10^8 cells/mL of B. subtilis spores and Ca–L directly or immobilized in LWA or GNP into the mortar. Crack healing was measured on pre-cracked samples 3–28 days after preparation. In samples pre-cracked after 3 and 7 days, the highest crack healing was observed after 28 days in mortar specimens containing GNPs, with both values slightly exceeding 0.8 mm, followed by samples containing LWA, with crack healing of approximately 0.6 mm.

Khaliq and Ehsan [107] suggest that GNP particles cannot protect the bacteria from the dense concrete. The visual analysis of the microstructure of different specimens by Khaliq and Ehsan [107] showed more abundant $CaCO_3$ crystal formation in samples with higher crack closure rates in agreement with previous reports on bio-concrete crack healing [124]. Xu and Yao [123] investigated the healing of cracks in concrete containing bacterial spores, Ca–L, or Ca–Glut, or cured in a solution containing healing agents. The samples with healing agents in the curing solution had higher spore germination and required fewer nutrients. In contrast, spore germination was slower and required more nutrients in the specimen containing embedded healing agents. The self-healing systems successfully healed 0.4mm-wide cracks. The authors reported the better performance of Ca–Glut than that of Ca–L in terms of crack healing efficiency and regained post-healing flexural strength. They attributed this finding to the higher $CaCO_3$ precipitation rates with Ca–Glut, denser crystal structure (especially in the transition zone), and micrometer-long $CaCO_3$ layer connecting the cement surface to the main body of $CaCO_3$ precipitates (Fig. 3). These findings led to the conclusion that Ca–Glut in MICP-based concrete self-healing systems were applicable. Tziviloglou et al. [112] investigated the effect of an immobilized healing system that contains Bacillus spores, Ca–L, and yeast extract. The analysis of the cracks by field-emission scanning electron microscopy (FE-SEM) revealed larger $CaCO_3$ precipitates in the bacterial specimens than in the control samples after 28 days, with the imprint of bacterial cells discernible on the crystal surfaces. That's a well feature of bacterial bio-mineralization.

Tziviloglou et al. [112], who only observed the imprints after 28 d the imprints disappeared after 56 d. This difference may have arisen from the various self-healing efficiencies of systems or may have resulted from the different curing Conditions employed.

Gao et al. [144], who induced millimeter-wide cracks in concrete specimens with chitosan-coated calcium alginate capsules containing B. pseudofirmus spores, reported that a 1 mm wide crack completely healed in 28 days. Zhang et al. [145] investigated the self-healing potential of mixed bacterial cultures in concrete. They examined AE, FA, and AN consortiums, which were chosen for their ability to precipitate $CaCO_3$ with Ca–L as the primary nutrition source. To assess the effect of these three consortia on concrete, ECPs were impregnated with each consortium and appropriate nutrients: for AE, 8 g·L−1 of Ca–L and 1 g·L−1 of yeast extract; for FA and AN, 4 g·L−1 of Ca–L, 4 g·L−1 of Ca–N, and 1 g·L−1 of yeast extract. The ECP containing each consortium was added to separate concrete samples, whereas the control sample did not contain added bacteria. Cracks ranging from 0.1 to 0.9 mm were produced in the samples and re-examined after28 d of curing. Erşan et al. [146] produced self-protected granules containing denitrifying bacteria and organic matter Ca–F, Ca–N, and dry granules were added to the cement mixture. $CaCO_3$ precipitation was observed to repair approximately 90% of 0.5 mm cracks in 28 days. Erşan et al. [88] continued by immobilizing the denitrifying bacteria in ECP and granulated activated carbon (GAC) particles. The authors reported that the maximum widths of fully healed cracks are approximately 370 and 480 μm after 28 and 56 d of incubation, respectively. Comparison of self-healing effect are shown in (Fig. 4). Luo et al. [147] used some unknown spore forming alkali resistant bacteria to make self-healing concrete. The self-healing capability was 85% for crack width less than 0.3 mm, 50–70% for 0.3 mm–0.5 mm, and less than 30% for up to 0.8 mm within 20 days. Good healing was observed up to 28 days but then decreased greatly at 60 days to 90 days. They also reported that cracked concrete needed to be immersed in water to achieve good healing as self-healing at atmosphere with 90% relative humidity was quite low. In another study, the same group [148] reported crack healing of up to 0.48 mm wide cracks within 80 days and water permeability reduction up to 96% within 28 days of self-healing. Calcite was detected in all cases .Qian et al. [149] compared the performance of bacteria formed calcite and phosphate on sheet glass surfaces. The author concluded that bio-calcite was better to other samples in terms of the intensity of interface interactions, the strength per mass, and the interfacial bonding strength. Mors and Jonkers [108] reported that the addition of a bacterial healing agent has insignificant effect on concrete strength but increased the self-healing capability to three times higher than normal concrete. They also proposed a method for its applications to reduce environmental impact and costs. All results are shown in table (7).



a. Initial crack b. After curing 28 days c. The initial crack d. After curing 28 days

Fig.3. Comparison of self-healing effect [150]

Table (7): Non-ureolytic bacterial concrete self-healing systems

Healing agent	Most effective treatment	Improved characteristics	Curing days	Reference
B. alkalinitrilicus	ECP carrying Ca–L (w/w ECP) and yeast extract	0.46 mm crack healing, 60% crack healing improvement compared to the control. Bacterial survival up to 100 d.	100	[143]
B. cohnii	Direct addition of bacteria and Ca–G	50% decrease in capillary water absorption with *B. cohnii* With both Ca–L and Ca–G. Total $CaCO_3$ precipitation (mol·L^{-1}): Ca–L: 0.081, Ca–G: 0.092 (from 0.2 mol·L^{-1}).	40	[111]
B. cohnii	Direct addition of bacteria and Ca–L	0.40 mm crack healed. Change in flexural strength (MPa):Ca–L: +7%, Ca–G: no sig. difference	28	[123]
B. cohnii	LWA-immobilized healing agents	Presence of bacteria lead to reduced delamination, improved flexural strength and deflection capacity.	56	[125]
alkaliphilic bacteria	Bacteria with Ca–L	Ca–Land bacteria improved compressive strength as 1%–3% of cement weight, highest improvement in 3% samples.Ca–F and spores improve compressive strength, but the improvement did not correlate with 0.5% added.	28	[94]
B. subtilis	LWA-immobilized bacteria and Ca–L	GNP resulted in better crack healing at an early age, but LWA offered more long-term protection and sustained crack healing. Both carry improved compressive strength compared to controls (LWA 12%; GNP 9.8%). 0.81 mm max. crack healed with GNP, 0.60 mm with LWA	28	[107]
D. nitroreducens **P. aeruginosa**	*D.nitroreducens* with Ca–F immobilized in GNP	Maximum width of fully healed cracks as approximately 370 µm in 28 d and 480 µm in 56 d of incubation.	28	[88]
B. subtilis	10^5 cells/mL for water absorbance reduction and compressive strength improvement,10^7cells /mL for crack healing	Compressive strength increased by 15%, 27%, and 19%, respectively. Water penetration depth reduced by 23%, 30%, and 53%, respectively. Maximum healed crack width was 0.6, 0.9, and 1.2 mm.	28	[70]
3 mixed bacterial cultures	AE mixture with salt mix.	The highest crack healing was reported by the AE mixture (0.36 mm), followed by the AN mixture (0.33 mm) and the	28	[145]

		FA mixture (0.28 mm).		
B. subtilis, B. megaterium (ureolytic), their mixture	*B.subtilis*for compressive strength and *B. megaterium* for tensile strength	Improved 28 d compressive strength by 14.36% (*B.subtilis*), 22.58% (*B. megaterium*) and 15.86% (mixture). Improved tensile strength by 25.3% (*B. subtilis*), 18.29% (*B.megaterium*) and 19.51% (mixture) compared to control.	28	[151]

IV. MORPHOLOGICAL AND CHEMICAL EVIDENCE OF BIO-CEMENTED CONSTRUCTION MATERIALS

Literature studies on the morphology of scanning electron micrographs (SEMs) of bio-cemented construction materials provided an in-depth understanding on the binding mechanism of the particles within the materials. On the other hand, a review of chemical compositions of bio-cemented construction materials provided information on the mechanism of MICP to solidify them. In this regard, literature discoveries from X-ray diffraction (XRD) and energy dispersive X-ray (EDX) of materials are important for providing evidence on the chemical constituents that characterize their bio-cementation process. Detailed analyses and discussions of the literature related to these aspects are presented in this section.

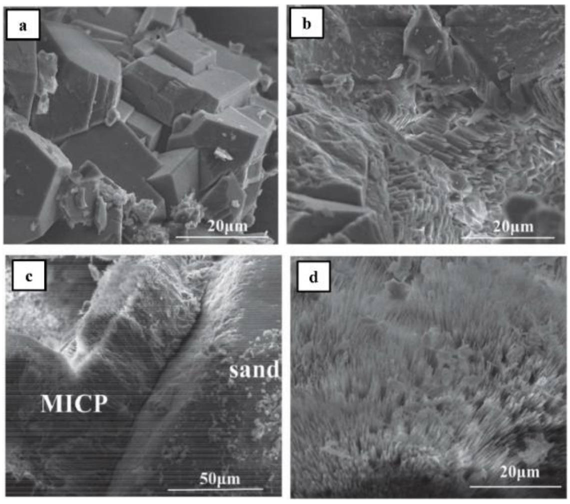


Fig.4. Microstructures of microbial mortars treated with three types of calcium source and three pumped batches: (a) chloride sample; (b) nitrate sample; (c,d) acetate sample

V. APPLICATION OF BIO-CEMENTATION IN CONSTRUCTION PRACTICE

In construction practice, Lors et al. [152] discovered that B. pseudofirmus could induce calcium carbonate precipitation, which offered an eco-friendly solution for repairing concrete wall micro-cracks in a 500 kPa nuclear structure. The ability of bacteria to survive in a highly alkaline concrete environment during the formation of calcium carbonate crystals in concrete suggests that the presence of bacteria had no negative impact on the hydration reaction [153]. By spraying a bacterial suspension of bio-precipitation over the concrete walls, micro-cracks were sealed with calcium carbonate. Due to exposure to high pressure, the repaired surfaces of the concrete walls were observed for minimal deformation. Additional laboratory findings from the work of Lors et al. [152] revealed that precipitated calcium carbonate in the micro-cracks of the concrete could tolerate a 450 kPa air flow. This showed that the bacteria-induced calcium carbonate was extremely pressure-resistant and chemically compatible with concrete in a highly alkaline environment. The method of spraying bio-precipitation is viable for the repair and sustained rehabilitation of concrete structures, it can be determined.

a. BIO-SANDSTONE APPLICATION

A new MICP method for cementing loose sands to produce structural materials known as bio-sandstone was proposed, in which sand (either mixed with bacterial culture or later injected directly in the column) was plugged through a plastic column and the cementation fluid (consisting of nutrient media with urea and calcium source) was injected or dropped at a specific rate in the column in a gravity free flow direction. Nutrients are required for bacterial growth and metabolism in order for MICP to take place, and they can be transferred through sandstone cores [154].

Sand porosity was reduced as a result of bacterial sand consolidation. In sand consolidated by Bacillus pasteurii, porosity and permeability were reduced by up to 50% and 90%, respectively. This reduction could be attributed to calcite deposition in the column [154]. When $CaCl_2$ was employed as a calcium source for bio-sandstone, the sand column of sizes 32.10 and 18.40 mm had good compressive strength, measuring up to 2 MPa.

The favorable results of MICP on bio sandstone have prompted researchers to consider creating new materials. Researchers started with more complicated building materials like cement and concrete, studying the effect of MICP on permeability, strength, and other durability parameters.

b. USING MICP ON BRICKS

By percolating a S. pasteurii bacterial solution over sand, bio-bricks were made using MICP. Before the molds were sealed, the percolation process was repeated to ensure that the bricks were completely saturated. Bio-bricks have a maximum compressive strength of 2 MPa [155]. Kumar et al. [156] and Lambert and Randall [157] both used the same type of bacteria to make bio-bricks with a compressive strength of 4 MPa. Produced bio-bricks with a compressive strength of 2.7 MPa. It should be noted that the bio-brick tested by Lambert and Randall [157]. Was made of calcium phosphate and a urea-rich solution produced by a urine stabilization process. Bu et al. [158] used an immersion technique in which the sand was placed in a rigid full-contact mold and then immersed in a bio-cementation mixture for seven days to allow the MICP to occur. The bio-brick gained a compressive strength of 1.3MPa. The compressive strength of bio-bricks including waste materials such as fly ash and rice husk ash [158], and also recycled concrete aggregates [80], was investigated. The surface treatment by MICP on cement-stabilized rammed earth blocks increased compressive strength from 12 to 15 MPa, indicating a 25 % improvement in total compressive strength. [159]. in the study by Achal et al. [160], chromium slag was used to manufacture bricks with the surface treatment of Bacillus sp. CS8 ureolytic bacteria. The low water absorption of the treated bricks indicated that they have low permeability. It was also discovered that the chromium slag brick treated with bacteria could absorb water four times less than the control brick without bacteria. Using S. pasteurii, Raut et al. [161] studied bio-calcification technique to strengthen brick masonry. After bio-calcification of brick masonry with media optimized for urease production (OptU) for up to 28 days, water absorption was determined. Manzur et al. [162] examined MICP to determine the effectiveness of urease-positive bacteria in enhancing the water absorption efficiency of concrete. Concrete treatment periods of 24 and 48 hours of bacterial incubation were selected.

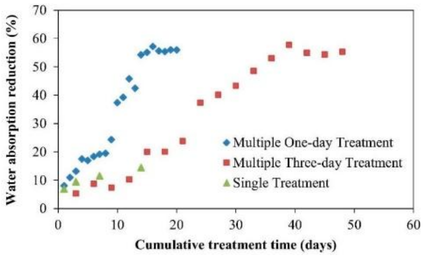


Fig.5. Reduction in water absorption in bricks with respect to different methods of treatment of $CaCO_3$

VI. CONCLUSIONS

In the light of various studies undertaken in the field of bacterial concrete, it can be safely assumed that the bacterial concrete is a path breaking technology which can be used in various aspects in the industry. This environment friendly, self-healing technique proves to be better than many conventional techniques present in the field. It is not only filling up gaps or cracks in the structure efficiently but is also assuring longevity and durability to the structure. The importance of this study is to understand the effect of the addition of healing agents on the behavior of concrete in the fresh and hardened state as well as in relation to its self-healing capacity. The effectiveness of microorganisms in inducing bio-cementation is dependent on the type of microorganism and the optimal conditions of microbially induced calcium carbonate precipitation.

1. The microbially induced calcium carbonate precipitation techniques show promising potential for applications in various fields such as construction, geo-technology, and nanotechnology
2. The addition of S. pasteurii and Bacillus subtilis species into concrete reduced chloride penetration in concrete which also enhances the declining tendency of concrete mass subjected to sulphate.
3. Calcium carbonate precipitated by microbially induced calcium carbonate precipitation served as a filler and cementing agent that minimized the porosity of construction materials. In addition to microbially induced calcium carbonate precipitation, the inclusion of additional materials such as rice husk ash, silica fume, fly ash, and chromium slag as partial cement substitutes in construction materials could further reduce their porosity. It was the reduction in porosity of construction materials by microbially induced calcium carbonate precipitation that led to the improvement of their mechanical properties.

4. Scanning electron micrographs and XRD results of bio-cemented construction materials from the literature studies revealed the presence of calcite, which is the most stable calcium carbonate mineral precipitated by microorganisms in the microbially induced calcium carbonate precipitation.
5. Various studies undertaken in the past or the ones ongoing have just improved our understanding and showed the possibilities the technique has.
6. Further research should be carried out to investigate the fire resistance of bio-cemented construction materials in order to establish a complete understanding of their fire durability.

REFERENCES

[1]. Luhar, S., Luhar, I. & Shaikh, F. U. A. A Review on the Performance Evaluation of Autonomous Self-Healing Bacterial Concrete: Mechanisms, Strength, Durability, and Microstructural Properties. Journal of Composites Science 6, 1–35 (2022).
[2]. Krishnapriya, S., Venkatesh Babu, D. L. & G., P. A. Isolation and identification of bacteria to improve the strength of concrete. 174, (Microbiological Research, 2015).
[3]. Wong, L. S., Oweida, A. F. M., Kong, S. Y., Iqbal, D. M. & Regunathan, P. The surface coating mechanism of polluted concrete by Candida ethanolica induced calcium carbonate mineralization. Construction and Building Materials 257, 119482 (2020).
[4]. Muhammad, N. Z., Keyvanfar, A., Majid, M. Z., Shafaghat, A. & Mirza, J. Waterproof performance of concrete: A critical review on implemented approaches. Construction and Building Materials 101, 80–90 (2015).
[5]. Aldea, C.-M., Shah, S. P. & Karr, A. Permeability of cracked concrete. Materials and Structures 32, 370–376 (1999).
[6]. T. Prozorov. Magnetic microbes: Bacterial magnetite biomineralization. Seminars in Cell and Developmental Biology 46, 36–43 (2015).
[7]. Huseien, G. F., Nehdi, M. L., Faridmehr, I. & Ghoshal, S. K. Smart Bio-Agents-Activated Sustainable Self-Healing Cementitious Materials: An All-Inclusive Overview on Progress, Benefits and Challenges. Sustainability (Switzerland) 14, (2022).
[8]. Vijay, K., Murmu, M. & Deo, S. V. Bacteria based self healing concrete – A review. Construction and Building Materials 152, 1008–1014 (2017).
[9]. Al-Salloum, Y., Hadi, S., Abbas, H., Almusallam, T. & Moslem, M. A. Bio-induction and bioremediation of cementitious composites using microbial mineral precipitation – A review. Construction and Building Materials 154, 857–876 (2017).
[10]. Mugwar, A. J. & Harbottle, M. J. Toxicity effects on metal sequestration by microbially-induced carbonate precipitation. Journal of Hazardous Materials 314, 237–248 (2016).
[11]. Yaqoob, Asim ALI Parveen, Tabassum Umar, Khalid Nasir, Mohammad Nasir, I. Role of Nanomaterials in the Treatment of waste water. Water 2020, 12, 495 (2020).
[12]. Iqbal, D. M., Wong, L. S. & Kong, S. Y. Bio-cementation in construction materials: A review. Materials 14, (2021).
[13]. Kang, C. H. & So, J. S. Heavy metal and antibiotic resistance of ureolytic bacteria and their immobilization of heavy metals. Ecological Engineering 97, 304–312 (2016).
[14]. Li, Q., Zhang, B., Ge, Q. & Yang, X. Calcium carbonate precipitation induced by calcifying bacteria in culture experiments: Influence of the medium on morphology and mineralogy. International Biodeterioration and Biodegradation 134, 83–92 (2018).
[15]. De Muynck, W., De Belie, N. & Verstraete, W. Microbial carbonate precipitation in construction materials: A review. Ecological Engineering 36, 118–136 (2010).
[16]. Jonkers, H. M. & Schlangen, E. Development of a bacteria-based self healing concrete. Proceedings of the International FIB Symposium 2008 - Tailor Made Concrete Structures: New Solutions for our Society 109 (2008). doi:10.1201/9781439828410.ch72
[17]. Vahabi, A., Ramezanianpour, A. A., Sharafi, H., Zahiri, H. S. & Vali, H. Calcium carbonate precipitation by strain Bacillus licheniformis AK01, newly isolated from loamy soil: A promising alternative for sealing cement-based materials. Journal of Basic Microbiology 55, 105–111 (2015).
[18]. Rao, M. V. S., Reddy, V. S., Hafsa, M., Veena, P. & Anusha, P. Bioengineered concrete - A sustainable self-healing construction material. Research Journal of Engineering Sciences 2, 45–51 (2013).
[19]. Irwan, J. M. & Othman, N. An overview of bioconcrete for structural repair. Applied Mechanics and Materials 389, 36–39 (2013).
[20]. Talaiekhozan, A., Fulazzaky, M. A., Keyvanfar, A., Andalib, R. & Zaimi, M. Identification of Gaps to Conduct a Study on Biological Self- healing Concrete. Journal of Environmental Treatment Techniques 1, 62–68 (2013).
[21]. Talaiekhozani, A., Keyvanfar, A., Andalib, R., Samadi, M. & Shafaghat, A. Application of Proteus mirabilis and Proteus vulgaris mixture to design self-healing concrete. Desalination and Water Treatment 52, 3623–3630 (2014).
[22]. Jonkers, H. M. & Schlangen, E. A two component bacteria-based self-healing concrete. Concrete Repair, Rehabilitation and Retrofitting II - Proceedings of the 2nd International Conference on Concrete Repair, Rehabilitation and Retrofitting, ICCRRR 119–120 (2009). doi:10.1201/9781439828403.ch27
[23]. Sangadji, S., Wiktor, V., Jonkers, H. & Schlangen, E. Injecting a Liquid Bacteria-Based Repair System To Make Porous Network Concrete Healed. Proceedings of the 4th International Conference on Self-Healing Materials, Ghent, Belgium. 118–122 (2013).
[24]. Wiktor, V. & Jonkers, H. M. Field performance of bacteria-based repair system: Pilot study in a parking garage. Case Studies in Construction Materials 2, 11–17 (2015).
[25]. Ghosh, P., Mandal, S., Chattopadhyay, B. D. D. & Pal, S. Use of microorganism to improve the strength of cement mortar. Cement and Concrete Research 35, 1980–1983 (2005).
[26]. S. Vempada, S. R. Strength enhancement of cement mortar using microorganisms–an experimental study. Int. J. Earth Sci. Eng. 04 06, 933–936 (2011).
[27]. Achal, V., Pan, X. & Özyurt, N. Improved strength and durability of fly ash-amended concrete by microbial calcite precipitation. Ecological Engineering 37, 554–559 (2011).
[28]. Abo-El-Enein, S. A., Ali, A. H., Talkhan, F. N. & Abdel-Gawwad, H. A. Application of microbial biocementation to improve the physico-mechanical properties of cement mortar. HBRC Journal 9, 36–40 (2013).
[29]. Gandhimathi, A. & Suji, D. Studies on the development of eco-friendly self-healing concrete - A green building concept. Nature Environment and Pollution Technology 14, 639–644 (2015).
[30]. Senthilkumar, V., Palanisamy, T. & Vijayakumar, V. N. Comparative studies on strength characteristics of microbial cement mortars. International Journal of ChemTech Research 6, 578–590 (2014).
[31]. Andalib, R. et al. Durability improvement assessment in different high strength bacterial structural concrete grades against different types of acids. Sadhana - Academy Proceedings in Engineering Sciences 39, 1509–1522 (2014).
[32]. Vekariya, M. S. & Pitroda, J. Bacterial Concrete: New Era for Construction Industry. International Journal of Engineering Trends and Technology 4, 4128–4137 (2013).

[33]. C.C. Gavimath, V.R. Hooli, J.D. Mallpur, A. B. P. Potential application of bacteria to improve the strength of cement concrete. International journal of advanced biotechnology and research, (2012).

[34]. Chahal, N., Siddique, R. & Rajor, A. Influence of bacteria on the compressive strength, water absorption and rapid chloride permeability of fly ash concrete. Construction and Building Materials 28, 351–356 (2012).

[35]. Pei, R., Liu, J., Wang, S. & Yang, M. Use of bacterial cell walls to improve the mechanical performance of concrete. Cement and Concrete Composites 39, 122–130 (2013).

[36]. Luhar, S. A Review Paper on Self Healing Concrete. Journal of Civil Engineering Research 5, 53–58 (2015).

[37]. Afifudin, H., Hamidah, S. M., Noor Hana, H. & Kartini, K. Microorganism precipitation in enhancing concrete properties. Applied Mechanics and Materials 99–100, 1157–1165 (2011).

[38]. Achal, V., Mukherjee, A. & Reddy, M. S. Microbial Concrete: Way to Enhance the Durability of Building Structures. Journal of Materials in Civil Engineering 23, 730–734 (2010).

[39]. Nosouhian, F., Mostofinejad, D. & Hasheminejad, H. Concrete Durability Improvement in a Sulfate Environment Using Bacteria. Journal of Materials in Civil Engineering 28, 04015064 (2016).

[40]. Cardoso, R., Pires, I., Duarte, S. O. D. & Monteiro, G. A. Effects of clay's chemical interactions on biocementation. Applied Clay Science 156, 96–103 (2018).

[41]. Yu, X., Qian, C. & Sun, L. The influence of the number of injections of bio-composite cement on the properties of bio-sandstone cemented by bio-composite cement. Construction and Building Materials 164, 682–687 (2018).

[42]. Choi, S. G., Wang, K. & Chu, J. Properties of biocemented, fiber reinforced sand. Construction and Building Materials 120, 623–629 (2016).

[43]. Tang, Y., Lian, J., Xu, G., Yan, Y. & Xu, H. Effect of Cementation on Calcium Carbonate Precipitation of Loose Sand Resulting from Microbial Treatment. Transactions of Tianjin University 23, 547–554 (2017).

[44]. Duo, L. et al. Experimental investigation of solidifying desert aeolian sand using microbially induced calcite precipitation. Construction and Building Materials 172, 251–262 (2018).

[45]. Mahdi Maleki Kakelar,Sirous Ebrahimi, M. javad H. Improvement in soil grouting by biocementation through injection method Mahdi. Chemical Engineering Journal 11, 930–938 (2016).

[46]. Zhang, Y., Guo, H. X. & Cheng, X. H. Role of calcium sources in the strength and microstructure of microbial mortar. Construction and Building Materials 77, 160–167 (2015).

[47]. Amiri, A. & Bundur, Z. B. Use of corn-steep liquor as an alternative carbon source for biomineralization in cement-based materials and its impact on performance. Construction and Building Materials 165, 655–662 (2018).

[48]. 48. Yoosathaporn, S., Tiangburanatham, P., Bovonsombut, S., Chaipanich, A. & Pathom-aree, W. A cost effective cultivation medium for biocalcification of Bacillus pasteurii KCTC 3558 and its effect on cement cubes properties. Microbiological Research 186–187, 132–138 (2016).

[49]. Williams, S. L., Kirisits, M. J. & Ferron, R. D. Influence of concrete-related environmental stressors on biomineralizing bacteria used in self-healing concrete. Construction and Building Materials 139, 611–618 (2017).

[50]. Xu, J. & Wang, X. Self-healing of concrete cracks by use of bacteria-containing low alkali cementitious material. Construction and Building Materials 167, 1–14 (2018).

[51]. Xu, J., Wang, X. & Wang, B. Biochemical process of ureolysis-based microbial CaCO3 precipitation and its application in self-healing concrete. Applied Microbiology and Biotechnology 102, 3121–3132 (2018).

[52]. Bhaskar, S., Anwar Hossain, K. M., Lachemi, M., Wolfaardt, G. & Otini Kroukamp, M. Effect of self-healing on strength and durability of zeolite-immobilized bacterial cementitious mortar composites. Cement and Concrete Composites 82, 23–33 (2017).

[53]. Jadhav, U. U. et al. Viability of bacterial spores and crack healing in bacteria-containing geopolymer. Construction and Building Materials 169, 716–723 (2018).

[54]. Seifan, M., Samani, A. K. & Berenjian, A. New insights into the role of pH and aeration in the bacterial production of calcium carbonate (CaCO3). Applied Microbiology and Biotechnology 101, 3131–3142 (2017).

[55]. Cuzman, O. A., Rescic, S., Richter, K., Wittig, L. & Tiano, P. Sporosarcina pasteurii use in extreme alkaline conditions for recycling solid industrial wastes. Journal of Biotechnology 214, 49–56 (2015).

[56]. Kadapure, S. A., Kulkarni, G. S. & Prakash, K. B. A Laboratory Investigation on the Production of Sustainable Bacteria-Blended Fly Ash Concrete. Arabian Journal for Science and Engineering 42, 1039–1048 (2017).

[57]. Snoeck, D., Wang, J., Bentz, D. P. & De Belie, N. Applying a biodeposition layer to increase the bond of a repair mortar on a mortar substrate. Cement and Concrete Composites 86, 30–39 (2018).

[58]. García-González, J. et al. Quality improvement of mixed and ceramic recycled aggregates by biodeposition of calcium carbonate. Construction and Building Materials 154, 1015–1023 (2017).

[59]. Zhang, J. L. et al. A binary concrete crack self-healing system containing oxygen-releasing tablet and bacteria and its Ca2+-precipitation performance. Applied Microbiology and Biotechnology 100, 10295–10306 (2016).

[60]. Zhang, J. L. et al. Screening of bacteria for self-healing of concrete cracks and optimization of the microbial calcium precipitation process. Applied Microbiology and Biotechnology 100, 6661–6670 (2016).

[61]. Achal, V. & Pan, X. Influence of calcium sources on microbially induced calcium carbonate precipitation by Bacillus sp. CR2. Applied Biochemistry and Biotechnology 173, 307–317 (2014).

[62]. Lv, J. J., Ma, F., Li, F. C., Zhang, C. H. & Chen, J. N. Vaterite induced by Lysinibacillus sp. GW-2 strain and its stability. Journal of Structural Biology 200, 97–105 (2017).

[63]. 63. Lee, Y. S., Kim, H. J. & Park, W. Non-ureolytic calcium carbonate precipitation by Lysinibacillus sp. YS11 isolated from the rhizosphere of Miscanthus sacchariflorus. Journal of Microbiology 55, 440–447 (2017).

[64]. Xu, G. et al. Biomineralization of a calcifying ureolytic bacterium Microbacterium sp. GM-1. Electronic Journal of Biotechnology 25, 21–27 (2017).

[65]. Durga, C. S. S., Ruben, N., Chand, M. S. R. & Venkatesh, C. Evaluation of mechanical parameters of bacterial concrete. Annales de Chimie: Science des Materiaux 43, 395–399 (2019).

[66]. Yaqoob, A. A., Ibrahim, M. N. M. & Rodríguez-Couto, S. Development and modification of materials to build cost-effective anodes for microbial fuel cells (MFCs): An overview. Biochemical Engineering Journal 164, 107779 (2020).

[67]. Wang, T. et al. Natural products from Bacillus subtilis with antimicrobial properties. Chinese Journal of Chemical Engineering 23, 744–754 (2015).

[68]. Perito, B. et al. A Bacillus subtilis cell fraction (BCF) inducing calcium carbonate precipitation: Biotechnological perspectives for monumental stone reinforcement. Journal of Cultural Heritage 15, 345–351 (2014).

[69]. Yu, X., Qian, C., Xue, B. & Wang, X. The influence of standing time and content of the slurry on bio-sandstone cemented by biological phosphates. Construction and Building Materials 82, 167–172 (2015).

[70]. Mondal, S. & Ghosh, A. (Dey). Investigation into the optimal bacterial concentration for compressive strength enhancement of microbial concrete. Construction and Building Materials 183, 202–214 (2018).

[71]. Kalhori, H. & Bagherpour, R. Application of carbonate precipitating bacteria for improving properties and repairing cracks of shotcrete. Construction and Building Materials 148, 249–260 (2017).

[72]. Eppinger, M. et al. Genome sequences of the biotechnologically important Bacillus megaterium Strains QM B1551 and DSM319. Journal of Bacteriology 193, 4199–4213 (2011).

[73]. Dhami, N. K., Reddy, M. S. & Mukherjee, A. Significant indicators for biomineralisation in sand of varying grain sizes. Construction and Building Materials 104, 198–207 (2016).

[74]. Dhami, N. K., Mukherjee, A. & Reddy, M. S. Micrographical, minerological and nano-mechanical characterisation of microbial carbonates from urease and carbonic anhydrase producing bacteria. Ecological Engineering 94, 443–454 (2016).

[75]. Singh, L. P., Bisht, V., Aswathy, M. S., Chaurasia, L. & Gupta, S. Studies on performance enhancement of recycled aggregate by incorporating bio and nano materials. Construction and Building Materials 181, 217–226 (2018).

[76]. Kaur, G., Dhami, N. K., Goyal, S., Mukherjee, A. & Reddy, M. S. Utilization of carbon dioxide as an alternative to urea in biocementation. Construction and Building Materials 123, 527–533 (2016).

[77]. Sharma, T. K., Alazhari, M., Heath, A., Paine, K. & Cooper, R. M. Alkaliphilic Bacillus species show potential application in concrete crack repair by virtue of rapid spore production and germination then extracellular calcite formation. Journal of Applied Microbiology 122, 1233–1244 (2017).

[78]. Jagannathan, P., Satya Narayanan, K. S., Arunachalam, K. D. & Annamalai, S. K. Studies on the mechanical properties of bacterial concrete with two bacterial species. Materials Today: Proceedings 5, 8875–8879 (2018).

[79]. Seifan, M., Samani, A. K. & Berenjian, A. Induced calcium carbonate precipitation using Bacillus species. Applied Microbiology and Biotechnology 100, 9895–9906 (2016).

[80]. Saeedi Javadi, A., Badiee, H. & Sabermahani, M. Mechanical properties and durability of bio-blocks with recycled concrete aggregates. Construction and Building Materials 165, 859–865 (2018).

[81]. Zhang, J. et al. Immobilizing bacteria in expanded perlite for the crack self-healing in concrete. Construction and Building Materials 148, 610–617 (2017).

[82]. Shirakawa, M. A. et al. Susceptibility of biocalcite-modified fiber cement to biodeterioration. International Biodeterioration and Biodegradation 103, 215–220 (2015).

[83]. Li, M., Zhu, X., Mukherjee, A., Huang, M. & Achal, V. Biomineralization in metakaolin modified cement mortar to improve its strength with lowered cement content. Journal of Hazardous Materials 329, 178–184 (2017).

[84]. Siddique, R. et al. Influence of bacteria on compressive strength and permeation properties of concrete made with cement baghouse filter dust. Construction and Building Materials 106, 461–469 (2016).

[85]. Siddique, R. et al. Properties of bacterial rice husk ash concrete. Construction and Building Materials 121, 112–119 (2016).

[86]. Chen, H., Qian, C. & Huang, H. Self-healing cementitious materials based on bacteria and nutrients immobilized respectively. Construction and Building Materials 126, 297–303 (2016).

[87]. Dhami, N. K., Reddy, M. S. & Mukherjee, A. Synergistic role of bacterial urease and carbonic anhydrase in carbonate mineralization. Applied Biochemistry and Biotechnology 172, 2552–2561 (2014).

[88]. Erşan, Y. Ç., Hernandez-Sanabria, E., Boon, N. & De Belie, N. Enhanced crack closure performance of microbial mortar through nitrate reduction. Cement and Concrete Composites 70, 159–170 (2016).

[89]. Chahal, N., Rajor, A. & Siddique, R. Calcium carbonate precipitation by different bacterial strains. African Journal of Biotechnology 10, 8359–8372 (2011).

[90]. Ruan, S. et al. The use of microbial induced carbonate precipitation in healing cracks within reactive magnesia cement-based blends. Cement and Concrete Research 115, 176–188 (2019).

[91]. Ghosh, S., Biswas, M., Chattopadhyay, B. D. & Mandal, S. Microbial activity on the microstructure of bacteria modified mortar. Cement and Concrete Composites 31, 93–98 (2009).

[92]. Van Tittelboom, K., De Belie, N., De Muynck, W. and Verstraete, W. Use of Bacteria to Repair cracks in concrete", Cement and Concrete Research, 157–166 (2010).

[93]. onkers, H. M. and Schlangen, E. A two component bacteria-based self-healing concrete", Concrete Repair, Rehabilitation and Retrofitting II: 2nd International Conference on Concrete Repair, Rehabilitation and Retrofitting. 119–120 (2008).

[94]. Luo, M. & Qian, C. Influences of bacteria-based self-healing agents on cementitious materials hydration kinetics and compressive strength. Construction and Building Materials 121, 659–663 (2016).

[95]. Kashyap, V. N. & Radhakrishna. A study on effect of bacteria on cement composites. International Journal of Research in Engineering and Technology 356–360 (2013). doi:10.13140/2.1.3271.5207

[96]. Joshi, S., Goyal, S. & Reddy, M. S. Corn steep liquor as a nutritional source for biocementation and its impact on concrete structural properties. Journal of Industrial Microbiology and Biotechnology 45, 657–667 (2018).

[97]. Wiktor, V. and Jonkers, H.M. the Potential of Bio-Based Repair System to Increase the Durability of Repaired Concrete Structures. 1–8 (2012).

[98]. Bachmeier, K. L., Williams, A. E., Warmington, J. R. and Bang, S. S. Urease Activity in Microbiologically-Induced Calcite Precipitation. 171–181 (2002).

[99]. Nasser, Magdy Attia and M. A. Zahran, A. A. Self-Healing of Cracked Concrete with Bacterial Approach. Faculty of Engineering, El-Menoufia University 1–11

[100]. Vijay, K. & Murmu, M. Self-repairing of concrete cracks by using bacteria and basalt fiber. SN Applied Sciences 1, 1–10 (2019).

[101]. Siddique, R. et al. Effect of bacteria on strength, permeation characteristics and micro-structure of silica fume concrete. Construction and Building Materials 142, 92–100 (2017).

[102]. Hermawan, H., Minne, P., Serna, P. & Gruyaert, E. Understanding the impacts of healing agents on the properties of fresh and hardened self-healing concrete: A review. Processes 9, 1–33 (2021).

[103]. Ameri, F., Shoaei, P., Bahrami, N., Vaezi, M. & Ozbakkaloglu, T. Optimum rice husk ash content and bacterial concentration in self-compacting concrete. Construction and Building Materials 222, 796–813 (2019).

[104]. Fahimizadeh, M., Pasbakhsh, P., Mae, L. S., Tan, J. B. L. & Raman, R. K. S. Multifunctional, sustainable, and biological non-ureolytic self-healing systems for cement-based materials. Engineering (2022). doi:10.1016/j.eng.2021.11.016

[105]. Seifan, M. et al. Mechanical properties of bio self-healing concrete containing immobilized bacteria with iron oxide nanoparticles. Applied Microbiology and Biotechnology 102, 4489–4498 (2018).

[106]. Madhu Sudana Reddy, B. & Revathi, D. An experimental study on effect of Bacillus sphaericus bacteria in crack filling and strength enhancement of concrete. Materials Today: Proceedings 19, 803–809 (2019).

[107]. Khaliq, W. & Ehsan, M. B. Crack healing in concrete using various bio influenced self-healing techniques. Construction and Building Materials 102, 349–357 (2016).

[108]. Mors, R. M. & Jonkers, H. M. Feasibility of lactate derivative based agent as additive for concrete for regain of crack water tightness by bacterial metabolism. Industrial Crops and Products 106, 97–104 (2017).

[109]. Poorni, S. & Natarajan, K. A. Flocculation behaviour of hematite-kaolinite suspensions in presence of extracellular bacterial proteins and polysaccharides. Colloids and Surfaces B: Biointerfaces 114, 186–192 (2014).

[110]. Schwantes-Cezario, N. et al. Bioprecipitation of calcium carbonate induced by Bacillus subtilis isolated in Brazil. International Biodeterioration and Biodegradation 123, 200–205 (2017).

[111]. Xu, J., Yao, W. & Jiang, Z. Non-Ureolytic Bacterial Carbonate Precipitation as a Surface Treatment Strategy on Cementitious Materials. J. Mater. Civ. Eng. 26, 983–991 (2014).

[112]. Tziviloglou, E., Wiktor, V., Jonkers, H. M. & Schlangen, E. Bacteria-based self-healing concrete to increase liquid tightness of cracks. Construction and Building Materials 122, 118–125 (2016).

[113]. Henk M. Jonkers, H.M., Arjan Thijssen, A., Gerard Muyzer, G. Using bacteria to self-heal cracks in concrete. 2010 (2010).

[114]. Ramachandran, S. K., Ramakrishnan, V. & Bang, S. S. Remediation of concrete using micro-organisms. ACI Materials Journal 98, 3–9 (2001).

[115]. Van Paassen, L. A. et al. Scale up of BioGrout: A biological ground reinforcement method. Proceedings of the 17th International Conference on Soil Mechanics and Geotechnical Engineering: The Academia and Practice of Geotechnical Engineering 3, 2328–2333 (2009).

[116]. Hao, Y., Cheng, L., Hao, H. & Shahin, M. A. Enhancing fiber/matrix bonding in polypropylene fiber reinforced cementitious composites by microbially induced calcite precipitation pre-treatment. Cement and Concrete Composites 88, 1–7 (2018).

[117]. 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).

[118]. Zhu, X. et al. The large-scale process of microbial carbonate precipitation for nickel remediation from an industrial soil. Environmental Pollution 219, 149–155 (2016).

[119]. Achal, V., Mukherjee, A. & Reddy, M. S. Biocalcification by Sporosarcina pasteurii using corn steep liquor as the nutrient source. Industrial Biotechnology 6, 170–174 (2010).

[120]. Rizwan, S. A., Khan, H., Bier, T. A. & Adnan, F. Use of Effective Micro-organisms (EM) technology and self-compacting concrete (SCC) technology improved the response of cementitious systems. Construction and Building Materials 152, 642–650 (2017).

[121]. Rao, R., Kumar, U., Vokunnaya, S., Paul, P. & Orestis, I. Effect of Bacillus Flexus in Healing Concrete Structures. 7273–7280 (2015). doi:10.15680/IJIRSET.2015.0408106

[122]. Vashisht, R., Attri, S., Sharma, D., Shukla, A. & Goel, G. Monitoring biocalcification potential of Lysinibacillus sp. isolated from alluvial soils for improved compressive strength of concrete. Microbiological Research 207, 226–231 (2018).

[123]. Xu, J. & Yao, W. Multiscale mechanical quantification of self-healing concrete incorporating non-ureolytic bacteria-based healing agent. Cement and Concrete Research 64, 1–10 (2014).

[124]. Jonkers, H. M. Bacteria-based self-healing concrete. Heron 56, 5–16 (2011).

[125]. Sierra-Beltran, M. G., Jonkers, H. M. & Schlangen, E. Characterization of sustainable bio-based mortar for concrete repair. Construction and Building Materials 67, 344–352 (2014).

[126]. Shanmuga Priya, T., Ramesh, N., Agarwal, A., Bhusnur, S. & Chaudhary, K. Strength and durability characteristics of concrete made by micronized biomass silica and Bacteria-Bacillus sphaericus. Construction and Building Materials 226, 827–838 (2019).

[127]. Zhang, Z., Ding, Y. & Qian, S. Influence of bacterial incorporation on mechanical properties of engineered cementitious composites (ECC). Construction and Building Materials 196, 195–203 (2019).

[128]. Nolan, É. Basheer, P. A. M. & Long, A. E. Effects of three durability enhancing products on some physical properties of near surface concrete. Construction and Building Materials 9, 267–272 (1995).

[129]. Zhu, T., Paulo, C., Merroun, M. L. & Dittrich, M. Potential application of biomineralization by Synechococcus PCC8806 for concrete restoration. Ecological Engineering 82, 459–468 (2015).

[130]. Satyanarayana, T., Prakash, A. & Johri, B. N. Microorganisms in environmental management: Microbes and environment. Microorganisms in Environmental Management: Microbes and Environment 9789400722, 1–819 (2012).

[131]. Tiano, P., Addadi, L. and Weiner, S. No TitleStone Reinforcement by Induction of Calcite Crystals Using Organic Matrix Macromolecules. Proceedings of the 7th International Congress on Deterioration and Conservation of Stone, Lisbon, Portugal, Laboratório Nacional de Engenharia Civil 1317–1326 (1992).

[132]. Tiano, P. Stone Reinforcement by Calcite Crystal Precipitation Induced by Organic Matrix Macromolecules. Studies in Conservation 40, 171–176 (1995).

[133]. Tiano, P., Biagiotti, L. & Mastromei, G. Bacterial bio-mediated calcite precipitation for monumental stones conservation: Methods of evaluation. Journal of Microbiological Methods 36, 139–145 (1999).

[134]. Le Métayer-Levrel, G., Castanier, S., Orial, G., Loubière, J. F. & Perthuisot, J. P. Applications of bacterial carbonatogenesis to the protection and regeneration of limestones in buildings and historic patrimony. Sedimentary Geology 126, 25–34 (1999).

[135]. Dick, J. et al. Bio-deposition of a calcium carbonate layer on degraded limestone by Bacillus species. Biodegradation 17, 357–367 (2006).

[136]. De Muynck, W., Debrouwer, D., De Belie, N. & Verstraete, W. Bacterial carbonate precipitation improves the durability of cementitious materials. Cement and Concrete Research 38, 1005–1014 (2008).

[137]. Achal, V., Pan, X. & Zhang, D. Remediation of copper-contaminated soil by Kocuria flava CR1, based on microbially induced calcite precipitation. Ecological Engineering 37, 1601–1605 (2011).

[138]. Nosouhian, F., Mostofinejad, D. & Hasheminejad, H. Influence of biodeposition treatment on concrete durability in a sulphate environment. Biosystems Engineering 133, 141–152 (2015).

[139]. Charpe, A. U., Latkar, M. V. & Chakrabarti, T. Microbially assisted cementation – A biotechnological approach to improve mechanical properties of cement. Construction and Building Materials 135, 472–476 (2017).

[140]. Chunxiang, Q., Jianyun, W., Ruixing, W. & Liang, C. Corrosion protection of cement-based building materials by surface deposition of CaCO3 by Bacillus pasteurii. Materials Science and Engineering C 29, 1273–1280 (2009).

[141]. Achal, V., Mukherjee, A., Goyal, S. & Reddy, M. S. Corrosion prevention of reinforced concrete with microbial calcite precipitation . ACI Mater Corrosion Prevention of Reinforced Concrete with Microbial Calcite Precipitation. 157–164 (2012).

[142]. De Muynck, W., Cox, K., Belie, N. De & Verstraete, W. Bacterial carbonate precipitation as an alternative surface treatment for concrete. Construction and Building Materials 22, 875–885 (2008).

[143]. Wiktor, V. & Jonkers, H. M. Quantification of crack-healing in novel bacteria-based self-healing concrete. Cement and Concrete Composites 33, 763–770 (2011).

[144]. Gao, M. et al. Immobilized bacteria with pH-response hydrogel for self-healing of concrete. Journal of Environmental Management 261, 110225 (2020).

[145]. Zhang, J. et al. Microbial network of the carbonate precipitation process induced by microbial consortia and the potential application to crack healing in concrete. Scientific Reports 7, 1–10 (2017).

[146]. Ersan, Y. et al. Self-protected nitrate reducing culture for intrinsic repair of concrete cracks. Frontiers in Microbiology 6, (2015).

[147]. Luo, M., Qian, C. X. & Li, R. Y. Factors affecting crack repairing capacity of bacteria-based self-healing concrete. Construction and Building Materials 87, 1–7 (2015).

[148]. Luo, M., Qian, C., Li, R. & Rong, H. Efficiency of concrete crack-healing based on biological carbonate precipitation. Journal Wuhan University of Technology, Materials Science Edition 30, 1255–1259 (2015).

[149]. Qian, C., Yu, X. & Wang, X. A study on the cementation interface of bio-cement. Materials Characterization 136, 122–127 (2018).

[150]. Zhang, X. & Qian, C. Engineering application of microbial self-healing concrete in lock channel wall. Marine Georesources and Geotechnology 40, 96–103 (2022).

[151]. Nain, N. et al. Enhancement in strength parameters of concrete by application of Bacillus bacteria. Construction and Building Materials 202, 904–908 (2019).

[152]. Lors, C. et al. Bioprecipitation of a calcium carbonate – Biofilm composite on the surface of concrete for the maintenance of nuclear reactor enclosures. Construction and Building Materials 237, 117618 (2020).

[153]. Wong, L. S. Microbial cementation of ureolytic bacteria from the genus Bacillus: A review of the bacterial application on cement-based materials for cleaner production. Journal of Cleaner Production 93, 5–17 (2015).

[154]. Ramakrishnan, V., Bang, S. S. and Deo, K. S. A novel technique for repairing cracks in high performance concrete using bacteria. Proc. of the Int. Conf. on HPHSC 597–618 (1998).

[155]. Bernardi, D., Dejong, J. T., Montoya, B. M. & Martinez, B. C. Bio-bricks: Biologically cemented sandstone bricks. Construction and Building Materials 55, 462–469 (2014).

[156]. Prince Prakash Jeba Kumar, J. et al. In vitro synthesis of bio-brick using locally isolated marine ureolytic bacteria, a comparison with natural calcareous rock. Ecological Engineering 138, 97–105 (2019).

[157]. Lambert, S. E. & Randall, D. G. Manufacturing bio-bricks using microbial induced calcium carbonate precipitation and human urine. Water Research 160, 158–166 (2019).

[158]. Dhami, N. K., Reddy, M. S. & Mukherjee, A. Improvement in strength properties of ash bricks by bacterial calcite. Ecological Engineering 39, 31–35 (2012).

[159]. Porter, H., Blake, J., Dhami, N. K. & Mukherjee, A. Rammed earth blocks with improved multifunctional performance. Cement and Concrete Composites 92, 36–46 (2018).

[160]. Achal, V., Pan, X., Lee, D. J., Kumari, D. & Zhang, D. Remediation of Cr(VI) from chromium slag by biocementation. Chemosphere 93, 1352–1358 (2013).

[161]. Raut, S. H., Sarode, D. D. & Lele, S. S. Biocalcification using B. pasteurii for strengthening brick masonry civil engineering structures. World Journal of Microbiology and Biotechnology 30, 191–200 (2014).

[162]. Manzur, T. et al. Performance enhancement of brick aggregate concrete using microbiologically induced calcite precipitation. Case Studies in Construction Materials 11, (2019).
