Title:

Bioconcrete: next generation of self-healing concrete

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Abstract

Concrete is one of the most widely used construction materials, and has a high tendency to form cracks. These cracks lead to significant reduction in concrete service life and high replacement costs. Although it is not possible to prevent crack formation, various types of techniques are in place to heal the cracks. It has been shown that some of the current concrete treatment methods such as the application of chemicals and polymers are a source of health and environmental risks, and more importantly, they are effective only in the short-term. Thus, treatment methods that are environmentally friendly and long-lasting are in high demand. A microbial self-healing approach is distinguished by its potential for long-lasting, rapid, and active crack repair, while also being environmentally friendly. Furthermore, the microbial self-healing approach prevails the other treatment techniques due to the efficient bonding capacity and compatibility with concrete compositions. This study provides an overview of the microbial approaches to produce calcium carbonate ($CaCO_3$). Prospective challenges in microbial crack treatment are discussed and recommendations are also given for areas of future research.

Introduction

Concrete as one of the most commonly used construction materials, plays an indispensable role in many fields. It has been widely used in the construction of buildings, dams, storage tanks, sea-ports, roads, bridges, tunnels, subways and other infrastructures. Concrete is mainly a combination of water, aggregate (coarse and fine), and cement. Cement is the most important part of the concrete material. It binds the aggregates and fills the voids between coarse and fine particles. High compressive strength, availability, durability, as well as compatible behaviour with reinforcement bars, low price, simple preparation and possibility of casting in desired shapes and sizes make concrete the material of choice for many applications.

Despite concrete's advantages, it has a high tendency to form cracks allowing aggressive chemicals to penetrate into the structure. Cracks are one of the main cause of concrete deterioration and decrease in durability. Cracks can be formed in both plastic and hardened states. Formwork movement, plastic settlement, and plastic shrinkage due to rapid loss of water from the concrete surface result in crack formation during the plastic state. Whereas, weathering, drying shrinkage, thermal stress, error in design and detailing, chemical reaction, constant overload, and external load contribute to crack formation in hardened state [1-5]. Moreover, concrete structures suffer from relatively low tensile strength and ductility. To address low tensile strength and ductility, concrete is usually reinforced with embedded steel bars. Reinforcement bars have positive effect on cracks width restriction by controlling plastic shrinkage, however they cannot prevent crack formation. Although cracks may not endanger concrete strength in early age, undoubtedly, their formation can be a serious risk to concrete lifespan in the long term [5-11]. Annually, considerable budget is allocated for repair of existing cementitious structures in many countries worldwide [12, 13]. The direct cost of cracks repair and maintenance has been estimated at \$147 per m^3 of concrete, despite the fact that concrete production cost ranges between \$65 to \$80 per m^3 [14]. Therefore, preventive approaches to restrain and terminate crack formation at early stage are crucial.

Treatments of cracks and pores in concrete are generally divided into passive and active treatments. Passive treatments can only heal the surface cracks, whereas active methods can fill both interior and exterior cracks. To enhance the durability and also prevent penetration of aggressive materials into concrete, passive treatments can be done by means of external coatings such as application of chemical mixtures and polymers. In passive treatments, since cracks are detected, sealants will be either injected or sprayed into the cracks [8, 15]. These sealers usually comprise chemical materials such as epoxy resins, chlorinated rubbers, waxes, polyurethane, acrylics, and siloxane. Although passive treatments are applicable to many existing concrete structures, they have various limitations which hinder their usage. Some of the limitations in the use of chemical sealers are poor weather resistance, moisture sensitivity, low heat resistance, unsustainability, poor bonding with concrete, susceptibility to degradation and delamination with age, and different thermal expansion coefficient between concrete and sealers [9, 16-18].

Active treatment techniques which are also known as self-healing techniques can operate independently in different conditions regardless of the crack position. They also have the ability of immediate activation upon crack formation, sealing the crack. A self-healing mechanism in concrete can be established through three main strategies: (i) autogenous healing; (ii) encapsulation of polymeric material; (iii) microbial production of calcium carbonate [19]. An ideal treatment should have quality, long shelf life, pervasiveness, and the ability to heal cracks repeatedly on unlimited number of times [20].

Autogenous healing is the natural process of repairing concrete cracks that can occur in the presence of moisture or water. Autogenous healing fills cracks through hydration of un-hydrated cement particles or carbonation of dissolved calcium hydroxide [21, 22]. Hydration of calcium oxide produces calcium hydroxide, which can react with carbon dioxide present in the atmosphere. As can be seen from Eqs. 1 and 2 these reactions result in production of calcium carbonate [23]. Due to abundance in nature and compatibility with cementitious compositions, calcium carbonate is one of the most useful and versatile fillers to plug the voids, porosities and cracks in concrete.

$$CaO + H_2O \rightarrow Ca(OH)_2 \tag{1}$$

$$Ca(OH)_2 + CO_2 \rightarrow CaCO_3 \; + \; H_2O \tag{2}$$

Success of autogenous healing depends strongly on factors such as presence of water or humidity in the surrounding environment, amount of un-hydrated cement, and concrete matrix composition [24-26]. Moreover, it has been noted that only cracks ranging from 0.1 to 0.3 mm can be filled through autogenous healing [27-31]. A practical way to improve autogenous healing is to reduce water to cement (w/c) ratio. However, increasing cement portion to reduce w/c ratio has an adverse effect on shrinkage and workability, and demands more cement production.

Encapsulation of polymeric material is another type of active treatment. This method can contribute to filling cracks by conversion of healing agent to foam in the presence of moisture. Although releasing chemicals from incorporated hollow fibres inside concrete can fill the cracks [32], these materials do not behave the same as concrete compositions in many conditions and in some cases they cause to extend the existing cracks. In addition, this technique requires capsules which can easily be mixed with concrete and can survive in concrete matrix. More importantly, the embedded capsules have to protect the healing agent for a long period of time and must not influence the concrete workability and mechanical properties. These requirements make encapsulation method a difficult practice for commercial self-healing concrete application.

Due to the drawbacks of existing treatments, alternative innovative active treatment methods are in demand. Recently, biotechnological approaches have attracted researchers' attention as a promising way to address the issues associated with active and passive treatments. Biological healing process is based on the production of calcium carbonate through biomineralization. Successful implementation of this innovative treatment method will result in a longer lifespan of concrete structures as well as significant reduction in cement production and structural replacement.

Biomineralization

Biomineralization refers to the process of mineral formation by living organisms which is a widespread phenomenon in nature [33]. Biomineralization can be accomplished through biologically induced mineralization process. Biologically induced mineralization usually occurs in an open environment as an uncontrolled consequence of microbial metabolic activity [34]. In this process biominerals are formed through reaction of metabolic products generated by microorganisms with the surrounding environment. Bacterial structure and a schematic diagram of calcium carbonate production are shown in Fig. 1. Mineral precipitation occurs by successful attachment of the positively charged ions to the negatively charged microbial cell walls. Biologically induced mineralization usually occurs in an anaerobic environment or at oxic–anoxic boundary. Its effectiveness highly depends on the concentration of dissolved inorganic carbon, nucleation site, pH, temperature and Hartree energy (E_h) [35, 36]. Among widespread production of minerals through biomineralization, precipitation of calcium carbonate has drawn interest due to the efficient bonding capacity and compatibility with concrete compositions.

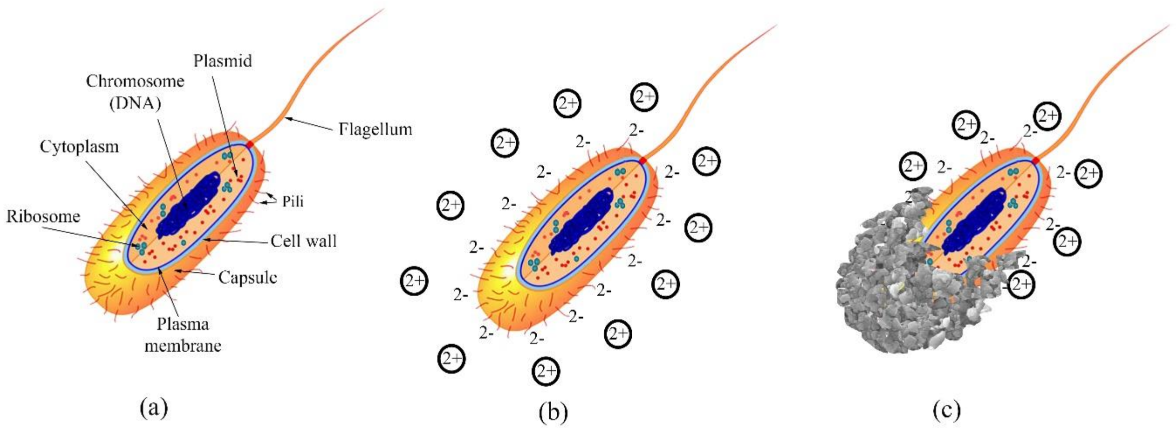

Fig. 1 (a) Bacteria structure; (b) Negative charged cell wall and presence of positive charged ions; (c) Biomineral production by means of binding ions to cell wall

Calcium carbonate precipitation

It is known that microorganisms, specifically bacteria, are able to produce a wide range of minerals such as carbonates, sulphides, silicates, and phosphates [37]. Calcium carbonate is one of the most suitable fillers for concrete due to high compatibility with cementitious compositions. Calcium carbonate can be precipitated through biologically induced mineralization process in the presence of a calcium source. In this process carbonate is produced by microorganisms extracellularly through two metabolic pathways namely autotrophic and heterotrophic.

Autotrophic pathway

Autotrophic pathway happens in the presence of carbon dioxide for which microbes convert carbon dioxide to carbonate through three distinct ways, namely (i) non-methylotrophic methanogenesis (by Methanogenic archaea); (ii) oxygenic photosynthesis (by *Cyanobacteria*), and (iii) anoxygenic photosynthesis (by Purple bacteria [38].

Non-methylotrophic methanogenesis pathway converts carbon dioxide and hydrogen to methane (Eq. 3). Accordingly, anaerobic oxidation of methane by electron acceptors such as sulfate as shown in Eq. 4 results in the production of bicarbonate [39]. Produced carbonate will then result in calcium carbonate precipitation in the presence of calcium ions as it is shown in Eq. 5. This pathway is more common in marine sediments.

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \tag{3}$$

$$CH_4 + SO_4^{2-} \rightarrow HCO_3^- + HS^- + H_2O \tag{4}$$

$$Ca^{2+} + 2HCO_3^- \leftrightarrow CaCO_3 + CO_2 + H_2O \tag{5}$$

Photosynthesis process is also an autotrophic pathway to produce calcium carbonate in the presence of calcium ions. There are two groups of photosynthetic bacteria namely oxygenic and anoxygenic photosynthetic bacteria. Oxygenic and anoxygenic photosynthesizing organisms utilize different types of electron donors to produce methanal. As shown in Eq. 6, water acts as an electron donor in oxygenic photosynthesis. In anoxygenic photosynthesis, however, hydrogen sulphide (H_2S) acts as an electron donor in the redox reaction (Eq. 7) and therefore oxygen is not generated [40, 41]. Removal of carbon dioxide during microbial photosynthesis from bicarbonate solutions results in carbonate production [42]. This phenomenon leads in localised increase in pH and finally calcium carbonate precipitation in the presence of calcium ions [36]. Summary of photosynthesis chemical reactions for calcium carbonate production are listed in Eqs. 6-9.

$$CO_2 + H_2O \xrightarrow{Oxygenic\ Photosynthesis} (CH_2O) + O_2 \tag{6}$$

$$CO_2 + 2H_2S + H_2O \xrightarrow{Anoxygenic\ Photosynthesis} (CH_2O) + 2\mathrm{S} + 2H_2O \tag{7}$$

$$2HCO_3^- \leftrightarrow CO_2 + CO_3^{2-} + H_2O \tag{8}$$

$$CO_3^{2-} + H_2O \leftrightarrow HCO_3^- + OH^- \tag{9}$$

Despite the possibility of calcium precipitation through photosynthesis, this method is only possible in the presence of carbon dioxide in the surrounding environment. This indicates that photosynthesis pathway can only be used in the areas that concrete structure is exposed to carbon dioxide and light.

Heterotrophic pathway

Microbial communities may precipitate crystals as a result of their growth in different natural habitats. Crystal formation is attributed to the medium composition used to growth heterotrophic bacteria, and is a common phenomenon in nature. Heterotrophic growth of different genera of bacteria such as *Bacillus*, *Arthrobacter,* and *Rhodococcus* species on organic acid salts (acetate, lactate, citrate, succinate, oxalate, malate, and glyoxylate) results in production of carbonate minerals. These bacteria use organic compounds as a source of energy. Based on the salts and carbon sources present in the medium, these bacteria are able to produce various crystals such as calcium carbonate and magnesium carbonate. Chemical reactions to form calcium carbonate in the presence of calcium acetate as a source of low molecular weight acid and calcium ion are listed in Eqs. 10-12 [43].

$$CH_3COO^- + 2O_2 \xrightarrow{Heterotrophic\ bacteria} 2CO_2 + H_2O + OH^- \quad (10)$$

$$2CO_2 + OH^- \rightarrow CO_2 + HCO_3^- \quad (11)$$

$$2HCO_3^- + Ca^{2+} \rightarrow CaCO_3 + CO_2 + H_2O \quad (12)$$

Calcium carbonate precipitation through utilization of organic acid has been widely documented in different substrate environments, including caves (walls, ceilings, and speleothems), marines, lakes, and soils. It was noted that utilization of heterotrophic bacterial communities (*Arthrobacter* and *Rhodococcus*) isolated from stalactite in the cave could produce calcium carbonate in the presence of calcium acetate [44, 45]. Moreover, the contribution of *Arthrobacter* and *Rhodococcus* species isolated from polar environments on precipitation of calcium carbonate crystal with calcium citrate and calcium acetate as carbon source has been extensively investigated [46]. Cacchio et al. [47] did another conceptual research and it was found that *Bacillus* and *Arthrobacter* species are capable of precipitating calcium carbonate under alkaline carbonate medium. The viability of these microbes in a concrete matrix will be discussed in a following section.

The presence of organic acid as the sole source of carbon and energy is the most significant advantage of this pathway. It is also worth noting that the cell surface properties of bacteria (as nucleation sites), proteins, and extracellular polymeric substances (EPS) have crucial effect on the morphology and mineralogy of produced calcium carbonate. Therefore, different morphologies of calcium carbonate such as calcite (rhombohedra crystal), vaterite (hexagonal crystal) or aragonite (needle-like crystal) can be precipitated based on chemical properties of bacteria cell wall.

The sulphur cycle and the nitrogen cycle are other mechanisms of producing calcium carbonate. Sulphur cycle follows by dissimilatory reduction of sulphate. In this process, calcium carbonate is produced if calcium source, organic matter, and sulphate are present in the medium. The increase in pH as a result of degasification of hydrogen sulphide shifts the reaction towards precipitation of calcium carbonate [38]. Production of calcium carbonate through reducing calcium sulfate ($CaSO_4$) to calcium sulphide (CaS) by sulfate reducing bacteria is shown in Eqs. 13-16 [48].

$$CaSO_4 + 2(CH_2O) \rightarrow CaS + 2CO_2 + 2H_2O \quad (13)$$

$$CaS + 2H_2O \rightarrow Ca(OH)_2 + H_2S \quad (14)$$

$$CO_2 + H_2O \rightarrow H_2CO_3 \quad (15)$$

$$Ca(OH)_2 + H_2CO_3 \rightarrow CaCO_3 + 2H_2O \quad (16)$$

Production of carbonate or bicarbonate through nitrogen cycle can be established through three main pathways namely (i) urea or uric acid degradation (ureolysis), (ii) ammonification of amino acids, and (iii) dissimilatory nitrate reduction [39, 49]. As a result of the nitrogen cycle, calcium carbonate is precipitated upon the presence of sufficient calcium ion in the medium [38]. The following sections will describe the calcium carbonate production through nitrogen cycle in concrete.

Precipitation of calcium carbonate in concrete matrix

Microorganisms such as *Bacillus spharecus* and *Bacillus peusturii* are able to produce biominerals through metabolic reaction in the presence of calcium source (see Table 1) [15]. These urease positive microorganisms are involved in the nitrogen cycle and can produce calcium carbonate through urea hydrolysis [50-52]. Fundamental reactions to induce calcium carbonate precipitation are shown in Eqs. 17 and 18 [53].

$$Ca^{2+} + CO_3^{2-} \leftrightarrow CaCO_3 \quad (17)$$

$$Ca^{2+} + 2HCO_3^{-} \leftrightarrow CaCO_3 + CO_2 + H_2O \quad (18)$$

Microbial metabolic activities lead to an increase of carbonate concentration and pH [8, 9, 54]. Increase in pH facilitates transformation of carbon dioxide to carbonate [26]. These metabolic conversions promote calcium carbonate precipitation (mostly in the stable form of calcite that is abundant in nature) which plays the role of barrier and blocks ingress of corrosive chemicals into cracks [53, 55]. Through urease activity in the presence of bacteria, one mole carbamic acid (NH_2COOH) and one mole ammonia (NH_3) are produced from urea hydrolysis (Eq. 19). As can be seen from Eq. 20, carbamic acid hydrolysis produces one mole carbonic acid (H_2CO_3) and one mole of extra ammonium simultaneously.

$$CO(NH_2)_2 + H_2O \xrightarrow{Microorganism} NH_2COOH + NH_3 \quad (19)$$

$$NH_2COOH + H_2O \rightarrow NH_3 + H_2CO_3 \quad (20)$$

According to Eqs. 21 and 22, reaction of hydroxide ion (which is already produced from reaction of water and ammonia) and carbonic acid produces carbonate (CO_3^{2-}) [56]. As can be seen in Eq. 23, positively charged calcium ions can then bind to the negatively charged bacterial cell.

$$2NH_3 + 2H_2O \rightarrow 2NH_4^{+} + 2OH^{-} \quad (21)$$

$$2OH^{-} + H_2CO_3 \rightarrow CO_3^{2-} + 2H_2O \quad (22)$$

$$Ca^{2+} + Cell \rightarrow Cell - Ca^{2+} \quad (23)$$

$$Cell - Ca^{2+} + CO_3^{2-} \rightarrow Cell - CaCO_3 \quad (24)$$

To complete the last reaction (Eq. 24), calcium ion can be provided either by internal sources that are available in the cement structure or by adding chemicals such as calcium chloride, calcium nitrate or calcium lactate externally [57]. Utilization of calcium chloride as a calcium source may cause chloride ion attack and consequently degradation of reinforcement bars. Thus, application of calcium nitrate or calcium lactate is recommended. Precipitation of calcium carbonate crystals by *B. sphaericus* and *B. subtilis* are shown in Fig. 2.

Although this approach has proven to be successful, there are still some drawbacks that are needed to be addressed. Production of ammonium ions (NH_4^+) through ureolytic activity results in nitrogen oxides emission into the atmosphere. It is estimated that remediation of one m^2 of concrete needs 10 g/L of urea which produces 4.7 g of nitrogen. This amount is about one-third of the nitrogen that is produced by each person every day [58]. Furthermore, presence of excessive ammonium in the concrete matrix increases the risk of salt damage by conversion to nitric acid. Hence, an optimization to find required amount of urea is beneficial to avoid excessive ammonium emission.

To address the drawbacks associated with ammonium ions production through ureolysis pathway, metabolic conversion of organic compound (organic acid salt) to calcium carbonate has been proposed [11, 51, 52, 59]. In this approach, aerobic oxidation of organic acids leads to production of carbon dioxide which results in carbonate production in an alkaline environment. Existence of a calcium source as cation leads to the production of calcium carbonate [60]. Metabolic conversion of calcium lactate to calcium carbonate in the presence of oxygen is shown in Eq. 25 [51].

$$CaC_6H_{10}O_6 + 6O_2 \xrightarrow[conversion]{Metabolic} CaCO_3 + 5CO_2 + 5H_2O \tag{25}$$

Reaction between the produced water and carbon dioxide from Eq. 25 and the available calcium oxide in the concrete matrix contributes to the increase of autogenous healing [5, 24, 61]. Compared to ureolysis pathway, this metabolic conversion is more sustainable due to absence of ammonium. Moreover, oxygen consumption by bacteria and formation of calcium carbonate to avoid aggressive penetration into concrete would prevent the corrosion of reinforcement bars. Although high concentrations of calcium source are required for calcite production [62], this may result in accumulation of high level of salts in concrete matrix. Therefore, the concentration of calcium source is required to be optimized in order to reduce cost, prevent salt formation, and obtain maximum calcium carbonate production. Compatibility with concrete composition, protection of reinforcement bars and high calcium carbonate production are among the advantages of this method.

Another pathway to produce minerals is known as dissimilatory nitrate reduction. Denitrification defines as a respiratory process that results in reduction of nitrate (NO_3^-) to nitrite (NO_2^-), nitric oxide (NO), nitrous oxide (N_2O), and nitrogen gas (N_2). Minerals are precipitated through oxidation of organic compounds by the reduction of nitrate (NO_3^-) via denitrifying bacteria. The most significant attribute of this approach is its application in anaerobic zones. The microorganisms that are involved in denitrification process are facultative anaerobes; mainly *Denitrobacillus*, *Thiobacillus*, *Alcaligenes*, *Pseuodomonas*, *Spirillum*, *Achromobacter*, and *Micrococcus* species [60]. As a consequence of organic compound denitrification, carbon dioxide, water, and nitrogen are produced (Eq. 26). According to Eq. 27 an increase in pH due to consumption of H^+ during the denitrification process results in carbonate or bicarbonate production [63]. The final reaction of calcium source and carbonate results in precipitation of calcium carbonate (Eq. 28).

$$Organic\ compound + a\ NO_3^- + b\ H^+ \xrightarrow{Denitrification} c\ CO_2 + d\ H_2O + e\ N_2 \tag{26}$$

$$CO_2 + 2OH^- \rightarrow CO_3^{2-} + H_2O \tag{27}$$

$$Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3 \tag{28}$$

Production of calcium carbonate via denitrification process in concrete is not well-developed and needs further research to elucidate. In comparison to the ureolytic approach, this mechanism can be also applied in soil and agricultural research. However, studies on soil improvement properties have illustrated that efficacy of ureolysis is higher than denitrification approach in respect to the production of calcium carbonate [64, 65]. Urea hydrolysis occurs in short period of time. Therefore, calcium carbonate precipitation through ureolysis pathway is the fastest approach among calcium carbonate biomineralization processes [58].

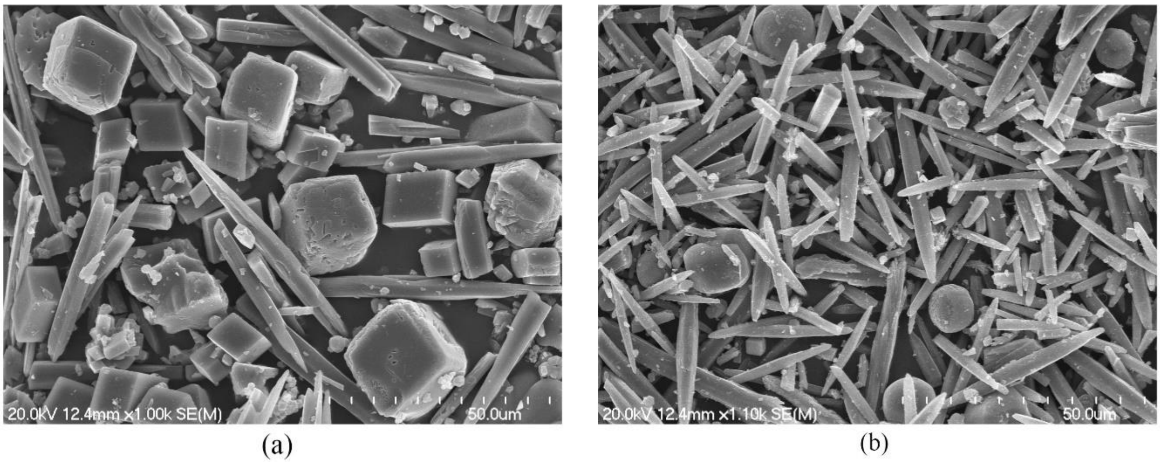

Fig. 2 (a) SEM micrographs of calcite precipitation by *B. sphaericus*; (b) SEM micrographs of calcite precipitation by *B. subtilis*

Due to the fact that biomineralization of calcium carbonate is slow, application of nutrients which may accelerate the biomineralization process are in demand. Moreover, selection of low risk bacteria with high capability of calcium carbonate precipitation, enzyme activity and growth rate are preferred. However, bacterial overgrowth may lead to production of uncontrolled superficial biofilm and uneven surface [66]. Therefore, optimum amount of nutrients and inoculum size are needed to be optimized in order to prevent overgrowth of bacteria as well as maximal precipitation. An overview of microbial strains and nutrients which have been used to produce calcium carbonate in concrete matrix are listed in Table 1.

Table 1 Overview of microorganisms and nutrients which have been used to produce calcium carbonate in concrete matrix

	Microorganism	Nutrient	Embedment in concrete	References
Bacterial metabolic conversion of organic acid	*Bacillus pseudofirmus*	Calcium lactate, calcium glutamate, yeast extract, and peptone	Direct	[67]
	Bacillus pseudofirmus *B. cohnii*	Calcium lactate, calcium acetate, yeast extract, and peptone	Direct	[51]
	B. cohnii	Calcium lactate and yeast extract	Immobilized	[59]
	Bacillus alkalinitrilicus	Calcium lactate and yeast extract	Immobilized	[52]
Ureolysis	*Bacillus sphaericus*	Urea, calcium nitrate, and yeast extract	Immobilized	[68]
	Bacillus sphaericus	Urea and calcium chloride	Direct	[4]
	Bacillus sphaericus	Urea, calcium nitrate, and yeast extract	Immobilized	[69]
	Bacillus sphaericus	Urea and alcium chloride	Direct	[5]
	Bacillus sphaericus	Urea, calcium nitrate, and yeast extract	Immobilized	[70]
	Bacillus sphaericus	Urea, calcium chloride, calcium nitrate, and yeast extract	Immobilized	[9]
	Bacillus sphaericus	Urea, calcium nitrate, and yeast extract	Immobilized	[26]
	Bacillus sphaericus	Urea, calcium chloride, and calcium acetate	-	[17]
	Bacillus sphaericus	Urea, calcium nitrate, and yeast extract	Immobilized	[15]
	S. pasteurii *Pseudomonas aeruginosa*	Urea and calcium chloride	Direct	[71]
	Bacillus sphaericus *S. pasteurii*	Urea and calcium acetate	Direct	[72]
	S. pasteurii	Urea and calcium chloride	Immobilized	[73]
	S. pasteurii	Urea, caclium nitrate, and calcium chloride	-	[74]
	S. pasteurii	Urea and calcium nitrate	-	[75]
	S. pasteurii	Urea and calcium chloride	Immobilized	[76]
	S. pasteurii *Bacillus cereus*	Urea, nutrient broth, and calcium chloride	Direct	[77]
	Bacillus amyloliquedaciens	Urea, calcium acetate yeast extract, and glucose	-	[78]
	Sporosarcina soli *Bacillus massiliensis* *Arthrobacter crystallopoietes* *Lysinibacillus fusiformis*	Urea and calcium chloride	Direct	[79]
Denitrification	*Diaphorobacter nitroreducens* *Bacillus sphaericus*	Urea, calcium formate, calcium nitrate, and yeast extract	Immobilized	[80]

Embedment of microorganism in concrete matrix

Healing agent (bacteria and nutrients) can be inserted in concrete matrix through vascular network or can be directly mixed during concrete preparation. Vascular method has been inspired by the structure of human bone. Bone consists of two parts. The outer layer is the cortical bone which is compact and the inner spongy layer is the trabecular bone. As shown in Fig. 3 a, vascular technique supplies healing agent from outside of structure by using distributed vascular networks which have been already embedded in matrix during concrete preparation. As cracks appear, healing agent moves through vessel due to pressure gradient between agent source and cracks positions. Dry [32] proposed a self-healing mechanism in which the interior and exterior concrete parts were joined via single or multiple hallow vascular fibres. In another investigation, Sangadji and Schlangen [81] simulated vascular networks with cylindrical concrete which its core and outer parts were porous and compact, respectively. The porous core distributes the healing agent through concrete matrix and it can be activated as the crack appears in the structure outer part. Vascular network method seems to be impractical due to several shortcomings. Firstly, healing agent should have constant viscosity throughout the concrete's service life to help it flow easily as well as to prevent leakage under environmental circumstances [24]. If the amount of released healing agent is more than the crack capacity, it causes aesthetic issues. Secondly, it would be difficult to distribute vessels homogenously throughout the structure. Thirdly, incorporation of vascular system in concrete may decrease the bond between concrete compositions and consequently leads to structural delamination.

Bacteria and nutrients can be also embedded directly in concrete matrix during the concrete preparation and casting as shown in Fig. 3 b. In this process, healing agents dissolve in water and then the mixture is added to cement and sand. Alkaliphilic bacteria such as *Bacillus* species can tolerate the extreme concrete environment and therefore they are the most attractive species for bio self-healing concrete. Studies illustrate that these thick membrane spore forming bacteria can survive without nutrients up to hundreds of years [82]. Moreover, dormant endospores are able to withstand environmental changes or chemicals as well as ultraviolet radiations and mechanical stresses [11, 24, 83].

However direct incorporation of microorganism into construction materials such as concrete dramatically influences the microbial metabolic activity. High pH (i.e >11) and dry condition of concrete even make bacteria vulnerable to death [15]. Jonkers et al. [51] incorporated *B. cohnii* spores directly into the concrete matrix. The number of viable cells in the concrete specimen were investigated after curing ages of 9, 22, 42, and 153 days. Although the number of viable bacteria cells in concrete matrix was approximately constant up to 9 days, it dramatically decreased after 22 and 42 days by 80% and 90%, respectively. These results indicate that the bacteria cells could be viable for up to 4 months (135 days) in concrete structure. Therefore, to help bacteria remain alive in harsh conditions for a longer period of time, incorporation of immobilized spore former bacteria is essential [84].

Bacterial protection through encapsulation or by protective materials such as diatomaceous earth, hydrogel and porous expanded clay particles has been the aim of some articles [15, 52]. Encapsulation of healing agent (Fig. 3 c) into tubular or ball-shaped capsules helps to increase the viability of bacteria for a long period of time. Microcapsules resist mechanical forces during the concrete preparation process. Healing process will commence when the capsule ruptures upon crack formation. Capsule preparation and mixing with aggregate as well as the empty space remaining after the capsule activation are the significant challenges of encapsulation technique. The effect of embedding healing agent in tubular glasses on crack treatment has been recently investigated [69]. Alkaline solutions have detrimental impact on silicate materials like glass. Due to the fact that cement based composites are highly alkaline, glass tube (vessel) wall dissolves and consequently glass corrosion occurs. Capsule tolerance will be enhanced during mixing by capsule radius reduction or by increasing capsule wall thickness. However, decreasing radius to thickness ratio may cause the capsules to be restricted from activating as crack appears. Hence, utilization of capsules that become brittle with age is recommended [85]. If a ceramic can remain intact during mixing and acts properly in a harsh environment, it can overcome glass capsule's shortcomings. Increasing the amount of incorporated capsules will lead to a large surface area. This may diminish cohesiveness between binders, which eventually decreases the workability of concrete [24, 86]. It was found that filling pores by microcapsules can cause creation of larger pores. Lower mechanical properties were noted in microcapsules incorporated samples as compared to those without microcapsules. This can be attributed to the spaces which appear after microcapsules rupture [26]. Thus, it can be concluded that encapsulation efficiency strictly depends on capsules size, their properties and distribution throughout the concrete matrix. Therefore, immobilization of bacteria into hydrogel, silica gel, zeolite, expanded clay, granular activated carbon, and metakaolin can address the encapsulation shortcomings [80]. Immobilization of bacteria in hydrogel has been explored and the viability of embedded bacteria in silica gels has been observed under harsh conditions [68, 83]. It was found swollen hydrogel can provide extra water supply to enhance efficiency of calcium carbonate precipitation to fill the crack width up to 0.5 mm. In another investigation, bacteria were immobilized into silica gel and polyurethane [69]. The protection of bacteria and algae by silica gels was identified to retain their enzymatic activity [87]. Thus, to obtain a satisfactory bio self-healing mechanism to heal the concrete cracks, investigation of those protective particles which can preserve bacteria for longer periods are in demand.

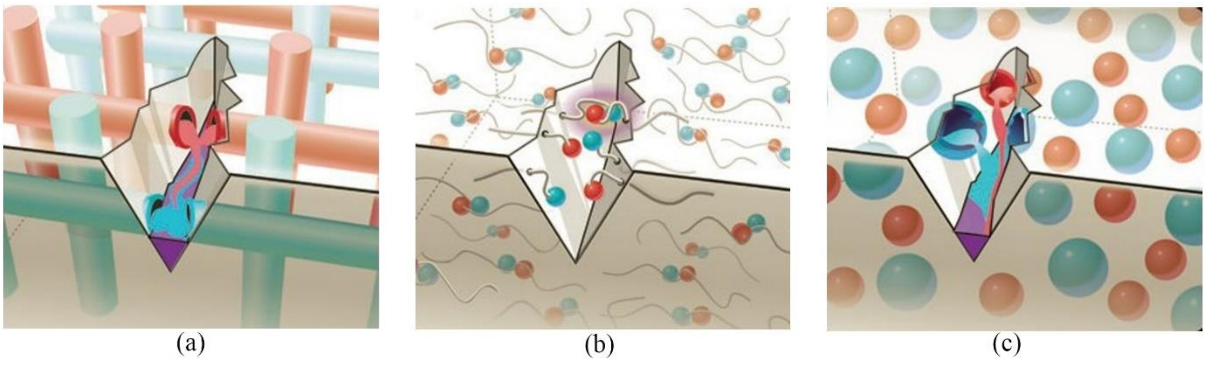

Fig. 3 Three main self-healing types: (a) vascular; (b) mixing with other ingredients; (c) encapsulation [88]

Performance of bio-concrete

The most significant attributes of concrete are compressive strength and durability. The influence of biomineralization on these attributes needs to be evaluated. Crack, pore size, and their distribution have adverse impacts on concrete properties and consequently service life of concrete structures. The durability of concrete can be improved by reducing absorption, permeability, and diffusion as the major mechanisms for transportation of fluids and gasses into concrete [8]. The influence of bio based healing agents on permeability and water absorption of concrete has been reported by several studies. As can be seen from Table 2, permeability and water absorption of concrete structures have been decreased by the presence of bio based agents. Wang et al. [69] studied the influence of calcium carbonate precipitation on permeability by incorporation of immobilized *Bacillus sphaericus* cells. It was found that the permeability of specimen with polyurethane immobilized bacteria decreases by six times as compared to specimens without bacteria. Moreover, effectiveness of immobilized *Bacillus sphaericus* in diatomaceous earth on water absorption was reported. The results indicated that the water absorption in specimen with immobilized bacteria was 50% of those specimen without bacteria [15]. Achal et al. [5] noted that application of *Bacillus sphaericus* caused the concrete to be watertight. The permeation test showed that the coefficient of water absorption in treated specimens were six times less than control specimens over a period of 168 hours. This observed phenomena can be related to the presence of newly formed calcium carbonate as a result of bacterial metabolism. Based on the literature, the biological approach can substantially increase the durability of concrete structure by sealing cracks and cavities in sustainable manner.

In contrast to the literature on durability, there are contradictory results available in regards to the influence of bio based healing agents on concrete strength. It was reported that application of encapsulated *Bacillus sphaericus* in mortar results in the decrease of compressive strength by 15% to 34% [26], whereas utilization of *Bacillus sphaericus* in cube mortar increased compressive strength in 7 and 28 days [4]. Although bio based agent had a positive influence on compressive strength for the cell concentration of 5×10^6 cells/mm^3, the mortar experienced reduction in compressive strength for higher cell concentration (5×10^8 cells/mm^3). Bang et al. [76] studied the effect of *Sporosarcina pasteurii* on compressive strength of mortar specimen for 7 and 28 days. It was found that highest concentration of immobilized *Sporosarcina pasteurii* on porous glass beads can substantially increase the compressive strength of the mortar specimen by 24%. Moreover, compressive strength improved with the increase of cell concentration from 6.1×10^7 cells/cm^3 to 3.1×10^9 cells/cm^3. Furthermore, Erşan et al. [80] reported the effect of immobilized ureulytic and denitrifying bacteria into protective materials on compressive strength. Their study indicated that application of *Bacillus sphaericus* in concrete decreased compressive strength in 7 and 28 days by 63% and 60%, respectively. Although utilization of denitrifying bacterium (*Diaphorobacter nitroreducens*) caused the reduction in compressive strength for both 7 and 28 days, immobilization of *Diaphorobacter nitroreducens* in expanded clay and granular activated carbon marginally enhanced compressive strength. However, immobilization of *Bacillus sphaericus* in metakaolin and zeolite had adverse impact on compressive strength. These contradictory results may be attributed to brittleness of the produced calcium carbonate. In addition, use of different culture medium and nutrients as well as environmental conditions may have resulted in these variations. Apart from surface cracks, the biomineralization process can plug the porosities and voids inside the concrete matrix. Therefore, application of microorganisms that are able to produce smaller bio-minerals may address the contradictory results for compressive strength.

Table 2 Effect of microbial agent on compressive strength, permeability and water absorption

Microorganism	Effect on compressive strength		Effect on durability		References
	Effect	Time	Permeability	Water absorption	
Bacillus sphaericus	N	28 days	P	-	[26]
	N	90 days			
	P	3 days	-	P	[5]
	P	7 days			
	P	21 days			
	-	-	-	P	[15]
	P	7 days	P	-	[4]
	P	28 days			
	-	-	P	-	[68]
	-	-	P	-	[69]
S. pasteurii	P	7 days	-	-	[76]
	P	28 days			
	P	28 days	-	P	[7]
Bacillus cohnii	P	7 days	-	-	[59]
	P	28 days			
	P	56 days			
Bacillus pseudofirmus	N	3 days	-	-	[51]
	N	7 days			
	N	28 days			
Diaphorobacter nitroreducens	N	7 days	-	-	[80]
	N	28 days			

N: negative effect; P: positive effect

Conclusion and prospectives

Application of bio self-healing approach commends itself over existing treatment methods due to efficient bonding capacity, compatibility with concrete compositions, and sustainability. It is capable of filling deep micro cracks as well as restricting crack development. This can reduce inspection labour and maintenance costs [51, 69]. Moreover, it reduces carbon dioxide emission due to the decrease of cement production [16, 17, 89]. Reduction in porosity of structure, rendering the concrete watertight, good compatibility between precipitated calcium carbonate and concrete compositions and favourable thermal expansion are the other advantages of this method. Bio self-healing treatment provides safer, more sustainable, more long-standing, and more economical construction materials. Therefore, mixing healing agent with cement and other materials during casting makes this method a promising technique as compared to the conventional treatment approaches.

For early future industrial application, several critical challenges must be addressed. Despite the recent progresses in designing protocols for bio based self-healing concrete, the existing studies are still suffering from the lack of numerical simulation to reduce experimental costs and time [90, 91]. In addition, feasibility of using healing agent during mixing and activity of bacteria in hardened concrete for a long period of time needs more investigation. Bond coherence between filler and crack edge is another desired criterion that should be considered to avoid new crack formation. Apart from concrete robustness via bio based healing approach, bio concrete production cost is another challenge. There is a need of more investigation into the reduction of associated costs;

namely bacteria, nutrients and labor. For sure strategies to increase bio self-healing efficiency and reduce costs will encourage contractors to use bio concretes as the material of choice in the early future.

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Conflict of interest

The authors declare that they have no competing interests.

Ethics

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