A significant review on the performance of microbial concrete in comportment of diverse nutrients

Gouthami Patnaik Palter, Misbah Syeda, Kanaka Durga Sambhana, Pothisraju Malasani, Venkata Giridhar Poosarla

Abstract
Concrete is substantially a prerequisite material being used but as the year’s pass, the concrete structures due to external load application might be subjected to the inevitable crack formation that can degrade their durability and strength. The addition of bacteria and the supplementary calcium source creates a pervious layer over concrete fissures similar to calcite precipitation in sealing pores and micro-cracks in the concrete. This review exemplifies the usage of several species of calcite-precipitating, alkali-resistant Bacillus bacteria as crack healing agents and nutrients added for bacteria sustainability in the concrete and mortar at diverse age periods. Various strategies have been proposed to endow self-healing in concrete in past decades. This review summarizes the effect of micro-capsules, hydrogels, cellulose fiber, polymers, mineral admixtures and bacteria type when employed in cementitious materials. This study exuviates light on the advantages of bio minerals produced via bacteria metabolism that improves mechanical properties, durability parameters and microstructure behaviour. It can be summarized that the inclusion of bacteria in concrete and mortar improves its properties resulting in crack healing, making it more sustainable and reducing maintenance cost. Furthermore, research can be a promising investigation into the longevity of the bacteria for its extensive practical outcome-based application.

Keywords: bio-mineralization, self-healing concrete, microbial CaCO₃, strength properties, microstructure analysis

1. Introduction
Concrete is an inherent construction material used in most infrastructures worldwide at a large scale. A structure’s service life is typically 50 years; however, it can be increased to 100 years in intricate structures. In this regard, materials performance in durability aspects needs to ensure their realistic alimony (Roig-Flores et al. 2021). Despite its benefits, it is sometimes prone to cracking because of shrinking, thermal stress, chemical reactions, incessant overload, and external load in the hardened state and causes detrimental effects on the life span of the structures in the long term. When exposed to water, CO₂, and chemicals from the environment, crack formation reduces the structure’s lifetime and strength and impairs the reinforcement (Prasad and Lakshmi 2018). It is crucial to cease the cracks in the advanced stage. Various crack repair techniques have been developed, but they were expended at the high cost and life span of the structures anticipated to be around 15 years and hazardous to the human environment (De Belie and Wang 2016; Van Breugel 2007). Cracks can be treated in two-way classification passive and active methods (Seifan et al. 2016). In the former case, surface cracks can be healed by providing external coatings either by injecting or spraying but due to poor resistance to heat, sensitivity to moisture, differential coefficient of thermal expansion, and it is limited (De Muynck et al. 2008a; Qian et al. 2015; Wang et al. 2012a). The latter method, regardless of crack position, can be treated with the aid of a technique termed a self-healing approach for a cement-based matrix, as shown in (Figure 1). In an autogenous process, concrete itself can self-heal crack after several weeks and months for a maximum closure of crack width up to 150 μm, as seen in (Figure 2), calcium carbonate is generated when unhydrated cement particles are hydrated in the presence of moisture seen in the (Eq. (1), (2)) (Hearn 1998). However, these micro-cracks do not influence the strength of the structure but will impart to material porosity and permeability (Jonkers et al. 2010). The autogenous healing process can be ameliorated by bringing down the water/cement (w/c) ratio, but simultaneously cement content comes into demand, reflecting on the workability and shrinkage properties (Seifan et al. 2016). The maximum crack healing was reported to be 0.30 mm during one year and about 0.45 mm when immersed in CO₂ water for 90 days (Suleiman and Nehdi 2018; Yıldırım et al. 2018). Additionally, reinforcing bars implanted in
concrete can slow the rate of fracture propagation but cannot prevent crack initiation, which has an adverse effect on the structure’s lifespan and maintenance costs (Seifan 2018).

\[
\begin{align*}
\text{CaO} + \text{H}_2\text{O} & \rightarrow \text{Ca(OH)}_2 \\
\text{Ca(OH)}_2 + \text{CO}_2 & \rightarrow \text{CaCO}_3 + \text{H}_2\text{O} 
\end{align*}
\]

Figure 1 Hierarchy of self-healing strategies for cementitious based material (Muhammad et al 2016).

Hence, the autogenous healing method is a naturally occurring process. It is intractable to anticipate or rely on as they depend heavily on environmental conditions and occur only in water. To surmount the above drawbacks, the invention of an autonomous healing mechanism came into existence. In this system, methods comprising expansive agents, encapsulation, and bacteria (microorganisms) have been pioneered by several researchers (Qian et al 2009). Various self-healing (autonomous) approaches have been adopted, that could spring up the encapsulation method by carrying out the self-healing mechanism using polymeric materials (Kessler et al 2003; Muhammad et al 2016; White et al 2001). The micro- or macro-encapsulated polymers/minerals, bacteria-based system, and other designed agents are to be combined directly with the cement matrix or implanted in an encapsulation system throughout the autonomous healing process (Han and Xing 2017). This mechanism can heal the crack of about 300 μm, or even up to 1 mm at a faster time span minimum of 1 day up to 4 weeks. A cementitious composite specimen cement: slag: sand) (1:0.67:0.67) using urea-formaldehyde/epoxy resin microcapsule was designed (Dong et al 2013). In this, different particle sizes of microcapsule were considered (132μm, 180μm, and 230μm), and for recovery of strength at 60 % pre-loading, maximum self-healing efficiency ascends to 6.11, 9.63, and 10.23 %, respectively. A major concern was the semi-permanent stability when encapsulated polymers are used as the shell has inescapable permeability. Delay in hydration reaction can also depict a significant crack healing property when there is presence of high binder mixtures but the limitation of the crack width is less than 0.2 mm (Li and Yang 2007). Additionally, higher the binder content more is the emissions of CO₂ (Wiktor and Jonkers 2011). To reduce all the issues mentioned earlier, currently, one mechanism is being considered: application of bacterial spores (encapsulated or not) or mineral additives.
Altogether, around 200 studies are being made using a bacteria-based system on the durability aspects but limited to a short-term period (De Belie et al 2019). Novel methods like biotechnology and nanotechnology are employed to improve concrete’s durability and other qualities. With the aid of the biotechnology approach, microbially-induced calcite precipitation (MICP) produced due to the addition of bio-cultures (bacteria) became an assuring path to encounter the consequences pertinent to passive and active treatments (Zhang et al 2023). In this mechanism, cracks with efficient self-healing by mineral-precipitation was observed by spraying or applying the bacteria-based mixtures into the cracks (Bang et al 2001; De Muynck et al 2008a; De Muynck et al 2008b; Ramachandran et al 2001). This modality of repair cannot be assorted as truly self-healing hence, applying the microbes in the concrete matrix has overreached various other processes due to its benefits eco-friendly, durable and economical (Jonkers and Schlangen 2008; Jonkers 2007; Jonkers et al 2010; Vijay and Murmu 2020). This study was initiated on assessing the self-healing of minor damages in a human body. This led to the development of bio-cement or bio-influenced self-healing concrete, which serves as a practical healing agent to stop crack growth (Elkateeb et al 2021). This review aims to describe the state-of-the-art mechanisms/strategies involved in producing MICP through bacteria metabolism. Also, it discusses the bacteria effect on concrete properties elaborately.

2. Biological agents: Metabolic conversions of organic acid using bacterial and enzymatic ureolysis

Microscopically small, unicellular rod-shaped bacteria with a diameter of around 0.5 µm and a length of 1 µm are found in low-nutrient settings (Beveridge 1981). Hence, they have a larger surface to volume ratio; thereby larger contact area can be developed to perform interactions within the environmental surroundings (Ghosh et al 2009). Microorganisms, particularly bacteria, have a geochemical activity responsible for the deposition of minerals like carbonates, phosphates, oxides, sulphates, etc., to a great degree (Wang et al 2016). Inhomogeneous materials made of organic and inorganic compounds, such as carbonate, phosphate, oxalate, silica, iron, or sulphur-containing minerals, with non-uniform distributions are created through the process of biomineralization by reflecting the environment (Skinner and Jahnren 2003). It has been discovered to be the most promising method, also known as self-healing concrete or bio concrete (Seifan et al 2016). This includes incorporating a unique self-healing mechanism into the concrete in which bacteria aids in mineral production swiftly closes newly created cracks, reducing concrete permeability, improving durability, and preserving embedded steel reinforcement from corrosion (Bashir et al 2015). The influence of microorganisms in mortar or concrete has become immense potential in research. This naturally occurring, pollution-free mineral precipitation created by the bacterial metabolism is highly sought-after (Ramakrishnan 2007). Mineralization is the result of the ongoing metabolic activity of microbes and reaction with their generated metabolic products with an adjoining environment (Afifudin et al 2011). The underpinning metabolic theory of bacterial crack healing is that the bacteria themselves work largely as a catalyst, converting a precursor component into suitable filler material. This compound now serves as a bio-cement and healing the crack. With various metabolic routes, several microorganisms can obstruct the synthesis of calcium carbonate, as seen in (Table 1), such as photosynthesis, sulphates reduction, urea hydrolysis or denitrification, and for these pathways to start, bacteria need nutrients. The process in which the self-healing mechanism develops in the concrete by MICP is the bacterial respiration process to produce CO₂ or urea hydrolysis by ureolytic bacteria or reduction of nitrates (Roig-Flores et al 2021; Soysal et al 2020). In the first mechanism, bacteria convert the precursor by acting as a catalyst to calcium carbonate, a hard filler material. These materials are formed as seen in (Eq. (3)) and seal the formed cracks (Rajczakowska 2019).
\[
\text{Ca}_3(\text{H}_2\text{O}_2)_2 + 7 \text{O}_2 \rightarrow \text{CaCO}_3 + 5 \text{CO}_2 + 5\text{H}_2\text{O} \quad \text{(respiration of bacteria)} \quad (3)
\]

**Table 1** Different metabolic pathways of bacterial calcium carbonate precipitation (De Belie and Wang 2016).

<table>
<thead>
<tr>
<th>Autotrophic bacteria</th>
<th>Heterotrophic bacteria</th>
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<tbody>
<tr>
<td>Non-Methylo trophic methanogenesis</td>
<td>Assimilatory pathways</td>
</tr>
<tr>
<td>Urea decomposition</td>
<td>Dissimilatory pathways</td>
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<tr>
<td>Oxidation of organic carbon</td>
<td>Aerobic Process</td>
</tr>
<tr>
<td>Respiration</td>
<td>e-acceptor</td>
</tr>
<tr>
<td>Methane oxidation</td>
<td>CH\textsubscript{4} / O\textsubscript{2}</td>
</tr>
<tr>
<td></td>
<td>Anaerobic Process</td>
</tr>
<tr>
<td></td>
<td>NO\textsubscript{3} reduction</td>
</tr>
<tr>
<td></td>
<td>SO\textsubscript{4}^2\textsuperscript{-}</td>
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</tbody>
</table>

Carbon dioxide and calcium hydroxide react to produce calcium carbonate, which is more in quantity in this process when compared to autogenous healing techniques. This process of oxidation of organic salt compounds under aerobic conditions leads to carbon dioxide production, further producing calcium carbonate in an alkaline environment (Sierra-Beltran et al 2014; Wiktor and Jonkers 2011). In the second mechanism, microorganisms give urease enzyme during the microbial urease movement, which catalyzes urea to ammonium and carbonates NH\textsubscript{4}\textsuperscript{+} and HCO\textsubscript{3}\textsuperscript{-} (Murari and Kaur 2021; Vijay and Murmu 2020). One mole of urea is hydrolyzed intracranial to a mole of NH\textsubscript{3} and carbonate each. The constitution of NH\textsubscript{3} mol and H\textsubscript{2}CO\textsubscript{3} mol takes place through impulsive hydrolysis of carbamate as seen in (Eq. (4), (5)). These substances also generate bimolecular ammonium and hydroxide ions, as well as unimolecular bicarbonate ions, as seen in (Eq. (6), (7)). Bicarbonate equilibrium gets shifted as a rise in pH leads to the formation of carbonate ions (Eq. (8)). Once the supersaturation level is attained in the presence of calcium ions, calcium carbonate precipitation is formed, as seen in (Eq. (9)). A schematic diagram for the structure of the bacteria and precipitation of calcium carbonate are shown in (Figure 3). Microbial cell walls are negatively charged, and it attracts positively charged ions of the environment such as Ca\textsuperscript{2+}, facilitating the mineral precipitation. This could be understood from (Eq. (10), (11)) (Seifan et al 2016). Hence, the second mechanism is not suitable as it releases excess ammonium, which is detrimental to the concrete environment through the leaching action of calcium hydroxide analogous to acid attack (Singh and Gupta 2020; Soysal et al 2020). In the third mechanism, bacteria respire nitrogen instead of oxygen. During this denitrification, there will be a metabolic conversion of the organic salts, thereby producing carbonates that will produce calcium carbonate upon reaction with calcium ions.

**Figure 3** Formation of calcium carbonate on bacteria cell wall (a) Structure of bacteria (b) Presence of positive charged ions and negative charged cell wall (c) Binding ions to cell wall leading to bio mineral production (Seifan et al 2016; Vijay and Murmu 2020).

\[
\text{CO(NH}_2\text{)}_2 + \text{H}_2\text{O} \rightarrow \text{NH}_3\text{COOH} + \text{NH}_3 \quad (4)
\]

\[
\text{NH}_2\text{COOH} + \text{H}_2\text{O} \rightarrow \text{H}_2\text{CO}_3 + \text{NH}_3 \quad (5)
\]

\[
\text{H}_2\text{CO}_3 \rightarrow \text{HCO}_3^- + \text{H}^+ \quad (6)
\]
\begin{align}
2\text{NH}_3 + 2 \text{H}_2\text{O} & \leftrightarrow 2 \text{NH}_4^+ + 2 \text{OH}^- \\
\text{HCO}_3^- + \text{H}^+ + 2 \text{NH}_4^+ + 2 \text{OH}^- & \leftrightarrow \text{CO}_3^{2-} + 2 \text{NH}_4^+ + 2 \text{OH}^- + 2 \text{H}_2\text{O} \\
\text{Ca}^{2+} + \text{Cell} & \leftrightarrow \text{Cell} - \text{Ca}^{2+} \\
\text{Cell} - \text{Ca}^{2+} + \text{CO}_3^{2-} & \leftrightarrow \text{Cell} - \text{CaCO}_3
\end{align}

3. Enforcement of the bacteria in different self-healing approaches and nutrient types

Various alkali-resisting spoil-forming bacteria are useful for self-healing: \textit{Bacillus pasteurii}, \textit{Bacillus subtilis}, \textit{Sporosarcina pasteurii}, \textit{Enterococcus faecalis}, \textit{Bacillus sphaericus}, \textit{Bacillus cereus}, \textit{Bacillus lentus}, \textit{Bacillus megaterium} (El Enshasy et al 2020). An intrinsic bio-culture (bacteria) material acting as a self-healing agent is applied into the cement matrix to circumvent the cracks (Vijay and Murmu 2020; Zawad et al 2021). Cracks in concrete can be healed in three primary ways. The first is autogenous healing, and the second is polymeric encapsulation (Van Tittelboom and De Belie 2013). The third is microbial production of calcium carbonate by biomineralization. MICP develops an environment that produces mineral calcium carbonate at a specified pH, ion concentration, and nucleation site (Hoffmann et al 2021). In an autonomous self-healing process, incorporation of the bacteria can be done in two techniques, as mentioned above, direct and encapsulation. Either of these methods envisages the capability of self-healing performance and the specimen's compressive strength. In the direct method, either the bacteria spores instead of living cells or bacterial solution will be added into the concrete and mortar during mixing along with the bacteria, organic mineral precursors or the carriers are incorporated to enhance the efficiency of self-healing property and also the bacterial survival (Tang and Xu 2021). Adopting spores directly into the mixture can resist the mechanical and chemical stresses induced, but the longevity and survival of spores are highly concerned, especially in an abrasive environment, as they will be crushed to death at an increasing age of concrete. To avoid this, bacteria must be protected to remain viable in the concrete or mortar, and this can be done by immobilizing the spores, nutrients, calcium-based sources, either organic or inorganic carriers like lightweight aggregates (LWA), expanded clay (EC), expanded perlite (EP), diatomaceous earth (DE), graphite nanoplatelets (GNA), glycerol (GL), metakaolin, granular activated carbon, zeolite. In the latter method, impregnation of carriers in bacteria solution and then encapsulating a polymer-based coating layer will be done (Erşan et al 2015a). In this microencapsulation process, bacteria spores are immobilized with silica gel, polyurethane (PU), polymeric membrane, microcapsules, hydrogel, and then to provide better protection to the bacteria, as shown in (Figure 4) and they are encapsulated in a glass tube (Bundur et al 2017; Khaliq and Ehsan 2016). Based on thermogravimetric analysis (TGA), when concrete is embedded with bacteria encapsulated with silica gel produces more amount of CaCO\textsubscript{3} precipitation compared to PU (Wang et al 2012b). But most of these encapsulated materials were highly-priced excepting DE, EC (Silva et al 2015; Wang et al 2012a). It has been determined to what extent self-healing concrete contains organic precursors calcium lactate and calcium glutamate and non-ureolytic bacteria (Xu and Yao 2014). When Bacterial spores were applied directly to the cement matrix, the viable duration lasted up to 4 months, but as the crack closure potential increased, its application reached out to be anticipated (Jonkers et al 2010). Under concealed marine and tidal environmental circumstances, the activity of the bio-concrete made with \textit{Halobacillus halophilus} bacteria and carriers such as calcium lactate and EP was examined (Khan et al 2021). The proposed bio-mineral CaCO\textsubscript{3} is more congenial with the concrete matrix and eco-friendly compared with expanded additives and polymer healing agents (Wang et al 2012b). \textit{Bacillus subtilis}, \textit{B. sphaericus}, \textit{B. pasteurii}, \textit{B. cohnii}, and \textit{B. megaterium} are among the \textit{Bacillus} bacteria that can withstand extremely alkaline conditions. \textit{Diaphorobacter nitroreducens} and \textit{B. pseudofirmus} are the candidates mostly used for the metabolic conversion of organic acids and denitrification (Erşan et al 2015b; Jonkers et al 2010). The nutrients added to concrete before mixing are calcium-based sources to ensure the alkaline condition. Most of the studies adopted calcium lactate and nitrate, and apart from the two mentioned, calcium acetate, calcium chloride, and calcium formate are also used (Kevin Paine 2016). The nutrients like urea and yeast extract (YE) also assist in spore germination when added to the concrete mix and offer a source for developing bacterial cells, significantly influencing the self-healing efficiency and concrete characteristics (Rajczakowska 2019). Water penetrates through cracks in concrete buildings and activates bacterium spores, which subsequently feed on minerals like calcium lactate. Insoluble limestone is the transformed form of the soluble calcium lactate being produced due to the oxygen deficit (Prasad and Lakshmi 2018). This limestone hardens over time, filling up the fractures in the damaged surface. Calcium chloride was not found to be optimal due to chloride, but calcium lactate was seen to produce more calcium ions, thus increasing the strength of concrete (Rajczakowska 2019). Due to the abrasive environmental conditions in cement matrix, there is a negative stimulus towards the bacterial survival. The competent way to conserve the microbial activity of bacteria is to protect it (Tang and Xu 2021). Some self-healing bacteria can tolerate extreme conditions and survive up to 200 years in concrete under a dormant state (Jonkers 2007).
4. Overview of the subsisting research on the effect of bacteria on properties and performance of concrete

Immobilization of bacteria, either intrinsic or extrinsic, would lead to the precipitation of calcium carbonate. Though it is a prolonged process in the presence of high pH value of surrounding cement matrix, once it indurates to the alkali environment, it tries to develop cell growth. When bacteria cells receive sufficient nutrients and oxygen, they get activated, thereby plugging the pores, leading to lesser porosity and permeability in concrete or cement mortar. Later, the spores will retrieve to the dormant state or form endospores when all the pores in the matrix are filled. This microbiologically induced self-healing mechanism influences the healing capacity of the concrete and triggers the mechanical properties and the performance of the bio concrete (Khaudiyal et al. 2022). In order to protect the bacterial spores from crushing or shearing, protective carriers must be induced to ensure the viability of the microbes in the concrete (Tang and Xu 2021). Compressive strength is the most widely tested parameter. Indirect tensile tests were also studied, i.e., flexure and split tensile. The significant durability aspects covered are the rapid chloride permeability test (RCPPT) and water absorption. SEM images were analyzed by many researchers apart from X-Ray diffraction (XRD).

5. Effect of biocementation on concrete properties, durability parameters

Optimization of bio concrete properties for M30 grade concrete by the response of calcium lactate (CL) content (0.22-2.18 g/L) using response surface methodology (RSM) was investigated (Abo Sabah et al. 2021). In this study, *B. sphaericus* was isolated from fresh urine and was sub-cultured. With an increase in CL and age of curing period, compressive strength, split tensile strength, and flexural strength increased proportionally. On the 28th day, specimen denoting 2.18 *B. sphaericus* the compressive, split and flexural strength increments were 13.4, 18.5 and 2.2%, respectively. At 23.4 days of curing period and CL content (2.18 g/L), the operating parameters for improving the compressive, split and flexural strength were 43.51 vs 43.43 MPa, 3.19 vs 3.19 MPa, 6.93 vs 5.50 respectively, and water absorption was 7.55 vs 7.55 mm. Cement mortar cubes specimens (50.8 x 50.8 x 50.8 mm) were prepared, one being control and the other containing biomass *B. pasteurii, Pseudomonas aeruginosa* (live or dead) suspended in (saline, phosphate solution) with different concentrations of 7.6x10^5, 7.6x10^6, 7.6x10^7 cells/cm^3 (Ramachandran et al 2001). Compressive strength of mortar cubes in phosphate solution at different cell concentrations gave higher strengths at 28 days than cubes in saline condition and control. Live cell forms showed enhanced strength of 65% compared to lower concentrations. *Sporosarcina pasteurii* PTCC 1645 bacterial concentration of 10^7 cells/mL was used to prepare a concrete mix of M25 grade (Parastegari et al 2019). Three curing mediums were considered, fresh-water, urea-calcium lactate, and seawater. Specimens cured in urea-calcium lactate solution possess better performance than the rest two. The addition of bacteria to mixing water at 5% air-entrained concrete (AEC) resulted in 25% of electrical resistivity, and the chloride ion penetration has reduced to 28% by the addition of bacteria with nutrients in water mixing concrete. *B. sphaericus* LMG of 10^7 cells/mL was used in the mortar specimens with varying w/c ratios 0.5, 0.7. Because the specimen is porous, the development of calcium carbonate crystals on the surface reduced the water absorption by 65–90%. The carbonation rate and resistance to chloride penetration decreased to around 25-30% and 10-40%, respectively (De Muynck et al 2008b). The study also denoted that bio deposition of calcite was in line with the surface treatment. It was recommended to further research on the surface treatment method in distinct environmental conditions. *B. Subtilis* JC3 suspension (10^8 cells/mL) was procured and sample concentrations of 10^5, 10^6, and 10^6 cells/mL had been derived (Meera and Subha 2016). A concrete mix of M20 grade cube specimens (150 X 150 X 150 mm) was prepared. The substantial increment was observed at 42% and 63% in compressive and tensile strength, respectively, at 28 days for 10^5 cells/mL concentration. Water absorption and percentage weight loss due to acid and chloride attack after 90
days contributed to diminishing growth of 17, 44, and 58%, respectively, for the same concentration. The effect of bacterium *S. pasteurii* in fly ash (FA) concrete (M20 grade) as a replacement in cement at 10, 20, and 30% for cell concentrations of $10^2$, $10^3$, and $10^7$ cells/mL was investigated (Chahal et al 2012a). Compressive strength has increased to 22% for 0% FA, and there was a reduction in water absorption, about four times, i.e., 26% for 10% FA and chloride penetration has shown charge reduction to 762 C (in coulomb) for 10% FA at 28 days at an optimum concentration of $10^3$ cells/mL. A study showed that aerobic *S. pasteurii* with an optical density (OD) (0.5, 1.0, and 1.5) was incorporated into the cement mortar (CM), and there was an escalation of compressive strength of 33% for CM specimens with 1 OD than 1.5 OD and control specimens at 28 days of curing (Abo-El-Enein et al 2013). Water absorption at all concentrations of OD resulted in lesser values. The relative water permeability of the reference concrete specimen at an age period of 56 days before and after 28 days of healing was 82.2%, and for spore and microbial groups, it was 71.8 and 4.23%, respectively. The initial and final setting time of the microbial group (235 and 490 min) was the smallest due to calcium nitrate, which accelerates the hydration of cement. The compressive strength of the spore group has shown a consistent growth compared to the microbial group at 14 and 28 days due to a lack of the grain strength of the agents in line with the curing period (Zheng et al 2020). Table 2 provides summarized outcome on bacteria types, concentration and their effect on the properties of concrete. Two bacterial strains, namely, *B. pseudofirmus* and *D. nitroreducens* were considered, and calcium alginate hydrogel was encapsulated into the bacterial suspension at varying dosages (0, 0.5, 1.5, and 3.0%) along with (38 mm) polymers, calcium nitrate (2% by cement weight) and concrete beams were casted (Soysal et al 2020). Specimens with *B. pseudofirmus* of 0.5%, for *D. nitroreducens* of 0.5% yielded greater strength compared to rest similar to the control samples. Significantly, modulus of elasticity did not result in much variation for all dosages. Stiffness recovery was maximum depicted for the control specimen consisting 3% micro-capsule and DN 3.0% post being exposed to wet-dry cycles (in absence of bacteria).

6. Microstructure

Table 3 and Figure 5 presents major outcomes on self-healing effect by incorporating various types of bacteria, nutrients/carriers. Mortar specimens containing *B. pasteurii* were suspended in a phosphate solution and cured in urea-CaCl$_2$ solution; the ratio of calcite to sand components was 0.3 (Ramachandran et al 2001).

![Figure 5 Stereomicroscopic images of crack-healing process in control mortar specimen before (a) and after 100 days healing (c), in biochemical agent-based specimen before (b) and after 100 days healing (d) (Wiktor and Jonkers 2011).](https://www.malque.pub/ojs/index.php/msj)
The SEM and Energy-dispersive X-ray analyzer (EDAX) presented morphology and size of calcite crystals for control and (CM) with an OD (0.5, 1.0, 1.5) specimens (Abo-El-Enein et al. 2013). They ensured calcium carbonate (CC) precipitation of 0.5 OD as spherical calcite crystals smaller than 1.0 OD bacteria cells of rod-shaped crystals. 1.5 OD cells have displayed amorphous calcite and a small amount of spherical calcite crystals. Morphology characteristics were determined by using Bacillus mucilaginosus L3 which has vegetative cells (10^3 cells/mL), spores (1.0x10^{10} cfu/g) as shown in (Figure 6). Concrete cylindrical specimens of size 100 X 50 mm were prepared using sulfoaluminate cement (SC), water reducer, calcium nitrate, sand, water, and microbial self-healing agents. When subjected to a loading 0.1-0.2 kN/s under electrohydrodynamic pressure testing machine width of the cracks created, these specimens were observed to be around 0.3-0.5 mm. Different curing age periods of (7, 14, 28, and 56) days, the morphology and calcite precipitated at the mouth of the crack was analysed prior and later to 28 days of healing time using SEM, energy-dispersive spectrometer (EDS), and X-ray diffraction. The area repair ratio was below 10% for the reference group of all ages. As the spores are not viable unless they are protected, the precipitation gradually decreases in the spore group. The ratio has shown 93% at an age period of 7 and 14 days. Beyond 14 days, the spores are dead, and the ratio falls below 30%. In the microbial group, the precipitation was observed with the increase in age period, and the ratio was 90% throughout. Lysinibacillus boronitolerans YS11 optimum proliferation was arrived (1x10^8 cfu/mL) using the components at 1% of rice bran, malt, corn syrup, and ammonium sulfate each (Ryu et al 2020). The bacterial sporulation was investigated, and the highest observed with Fe^{3+} compared to Ca^{2+}, Mn^{2+} divalent cations have been chosen as the promoter. Initially, the width of the crack on average was observed to be 0.34 mm, after 28 days, in the case of control mortar cubes, a crack of 0.11 mm was healed, and for cubes with nutrients 0.15 mm. After 7 days itself, mortar cubes containing spore powder plus nutrient healed completely.

<table>
<thead>
<tr>
<th>Bio-culture used</th>
<th>Bacterial cell concentration</th>
<th>Major studies on the impact of concrete properties/ microstructure</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus megaterium</td>
<td>30x10^6 cfu/mL</td>
<td>Compressive strength improved by 24% for the highest grade (50 MPa) bacterial concrete. It was found that concrete was filled by the mineral calcite precipitation was 38.76% and had less porosity.</td>
<td>(Andalib et al 2016)</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>2.8x10^6 cells/mL</td>
<td>12% increase in compressive strength was found when incorporated along with LWA carrier in the concrete mix. Calcium carbonate formation is mainly due to bacteria and calcium lactate.</td>
<td>(Khalil and Ehsan 2016)</td>
</tr>
<tr>
<td>Bacillus subtilis HU58</td>
<td>10^9 cfu/g</td>
<td>Compressive strength was increased to 14% when bacteria were incorporated with diatomite pellets.</td>
<td>(Huynh et al 2017)</td>
</tr>
<tr>
<td>Bacillus pasteurii</td>
<td>5x10^6 cells/mL</td>
<td>Compressive strength of 9.16% increment was found at 10SF20BC optimal mixing material ratio. 9.49% was found to be the highest calcium carbonate precipitate at same optimal mix.</td>
<td>(Metwally et al 2020)</td>
</tr>
<tr>
<td>Spore-forming alkali-resistant bacteria</td>
<td>10^6 cells/mL</td>
<td>The white crystals at the crack surface showed lamellar closed morphology.</td>
<td>(Luo et al 2015)</td>
</tr>
<tr>
<td>Bacillus pasteurii</td>
<td>-</td>
<td>Bacterial concrete casted using LWA, calcium lactate and cured in urea+ calcium acetate gave a large amount of precipitate at outer edges than at centre.</td>
<td>(Chen et al 2019)</td>
</tr>
<tr>
<td>Bacillus Sp. BY1</td>
<td>10^6 CFU/mL</td>
<td>Compressive strength increased by 9.5%, 9.9%, and 16.4% with addition of calcium formate, calcium acetate, and calcium lactate respectively. The crystals formed were rhombohedral, rugged structures usual shape of calcite formed in the presence of calcium formate or calcium lactate. Polyform resulted in 95% calcite by weight for calcium formate, 78.61% by weight for calcium acetate, and 94.26% by weight for calcium lactate.</td>
<td>(Jeong et al 2019)</td>
</tr>
<tr>
<td>Bacillus sphaericus</td>
<td>-</td>
<td>Bacterial concrete with 20 mL solution and at 8% micronized biomass silica (MBS) gave 13.53% higher compressive strength, 15.46% lower water absorption and 15.46% lower water sorptivity.</td>
<td>(Priya et al 2019)</td>
</tr>
<tr>
<td>S. pasteurii</td>
<td>10^6 cells/mL</td>
<td>Concrete with 10% fly ash concrete gave minimum of 3.25% water absorption. Capacity of chloride ingress in fly ash concretes decreased with increase in bacterial concentration, maximum reduction in chloride ions was observed with 10^6 cells/mL for all 0, 10, 20, and 30% fly ash concretes. Compressive strength of fly ash concrete increased up to 10^5 cells/mL, and then there was reduction in the strength at 10^6 cells/mL cement concentration.</td>
<td>(Chahal et al 2012b)</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>10^7 cells/mL</td>
<td>Application of bacteria and steel fibers in the LWAC cured in a urea-calcium lactate environment was found to decrease its water absorption significantly by 13.1% and also gave the highest reduction in the chloride penetration by 20.5%.</td>
<td>(Salmasi and Mostofinejad 2020)</td>
</tr>
<tr>
<td>S. pasteurii and B. subtilis</td>
<td>10^7 and 10^6 cells/mL</td>
<td>Decrease in water absorption of 19.5% in specimens containing silica fume and S. pasteurii in the mix and cured in the urea-calcium lactate solution compared to the specimens prepared without bacteria and cured in tap water. S. pasteurii had a greater effect than B. subtilis on the resistance against penetration when specimens cured in the urea–calcium chloride and urea–calcium lactate media.</td>
<td>(Tayebari and Mostofinejad 2019)</td>
</tr>
<tr>
<td>Method of application;</td>
<td>Type of micro-</td>
<td>Nutrient</td>
<td>Carriers</td>
</tr>
<tr>
<td>------------------------</td>
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</tr>
<tr>
<td>Immobilisation; Bacteria metabolic conversion of organic solid</td>
<td><em>Bacillus alkalinitrilicus</em></td>
<td>YE and calcium lactate</td>
<td>LWA, EC</td>
</tr>
<tr>
<td>Direct; ureolytic activity</td>
<td><em>Bacillus sp.</em></td>
<td>Nutrient broth (NB), Urea, calcium chloride</td>
<td>When stimulated a crack of maximum width 3mm and depth 27.2mm, on 28th day maximum depth of 27.2mm was filled</td>
</tr>
<tr>
<td>Immobilization and passive; Bacteria metabolic activity</td>
<td><em>Bacillus subtilis</em> M9</td>
<td>Beef extract, peptone, sodium carbonate, calcium lactate</td>
<td>Polyvinyl alcohol fibers (PVA)</td>
</tr>
<tr>
<td>Direct and Immobilisation; Bacteria metabolic conversion of organic solid</td>
<td><em>Halobacillus halophilus</em></td>
<td>Calcium lactate, calcium acetate, magnesium acetate</td>
<td>LWA, EP, GL</td>
</tr>
<tr>
<td>Direct; Bacteria metabolic activity</td>
<td><em>Bacillus mucilaginosus</em></td>
<td>Calcium nitrate, magnesium sulfate, potassium chloride, YE, ammonium sulfate</td>
<td>-</td>
</tr>
<tr>
<td>Direct; ureolytic activity</td>
<td><em>B. pasteurii, B. sphaericus</em> and <em>D. salina</em> alga</td>
<td>Calcium lactate powder, beef extract, peptone, magnesium sulfate, Urea</td>
<td>-</td>
</tr>
<tr>
<td>Immobilization; Denitrification and ureolytic activity</td>
<td><em>Bacillus sphaericus, Diaphorobacter nitroreducens</em></td>
<td>Calcium nitrate, Calcium formate, Urea, YE</td>
<td>DE, EC, metakaolin, zeolite, granular activated carbon</td>
</tr>
<tr>
<td>Immobilization; Bacteria metabolic activity</td>
<td><em>Bacillus subtilis</em> 168</td>
<td>Calcium lactate, peptone, NaCl, YE</td>
<td>Cellulose fiber</td>
</tr>
<tr>
<td>Microencapsulation process</td>
<td><em>Bacillus sphaericus</em></td>
<td>Calcium nitrate, YE, Urea</td>
<td>Micro-capsule</td>
</tr>
<tr>
<td>Encapsulation; Denitrification and metabolic conversion of organic acids</td>
<td><em>Diaphorobacter nitroreducens</em> and <em>Bacillus pseudofirmus</em></td>
<td>Calcium nitrate</td>
<td>Calcium alginate hydrogel beads, polymers</td>
</tr>
<tr>
<td>Encapsulation; Urea hydrolysis</td>
<td><em>Lysinibacillus sphaericus</em></td>
<td>Calcium, nutrient urea (stored in capsule)</td>
<td>A numerical model developed to predict the evolution of calcite. Finite element method (FEM) was adopted to solve the differential equations. After 70 days, crack width of 0.4 mm was healed whilst predicted healing of crack at the time of 42 days was reaching to 58%.</td>
</tr>
</tbody>
</table>
7. Considerations and practical applications

Integration of the microbial biomass either by immobilization or encapsulation way and their viability, the modality has been explicated comprehensively. The scope for visualizing metabolic calcite production was intensively studied under numerous applications, but distinct investigations on the survivability of bacteria in real life need to be examined. The addition of LWA has also implemented various protection strategies, GNP, micro-capsules, hydrogels etc., to safeguard the bio-culture against the pressure or forces endorsed in the concrete (Tang and Xu 2021; Wang et al 2014a). Their suitability in versatile environments and their effect on concrete properties like stiffness, modulus of elasticity, stress-strain behavior, shrinkage, and corrosion are to be assessed heretofore to achieve microbial self-healing concrete. All the tests performed in the lab affirmed promising output in the wide-scale range to date, but when we adapt to massive concrete structures its elements necessitate analyzing at longer service life and marine environmental conditions. Further study may also focus on choosing certain routes or processes, carriers, and nutrients that can enhance the resilience and self-healing capability of the microbial concrete as well as creating a theoretical model simulation to reduce the expense and time. Fatherly, to surmount the cost-effectiveness in production cost of bacterial concrete an inexpensive industrial waste with a high protein content can be adopted as the nutrient ingredients (Stanaszek-Tomal 2020).

8. Conclusion

This paper aims to stipulate an overview of the various types of bacteria or micro-organisms mostly used as self-healing agents, their metabolic function, different types of nutrients to enhance bacterial cell growth, and carrier’s requisite for acting as protective cover aids in healing cracks in concrete. This review elucidates the self-healing mechanisms or strategies, their pros and cons, and different incorporating bacteria into the cement matrix. The study has reviewed various properties, including compressive strength, water absorption, split tensile strength, sorptivity, chloride ion penetration, water permeability, and microstructure analysis using SEM, EDS, TGA, XRD, and EDAX. Imbibing the bio-based material in concrete has reduced to very much extent the durability parameters, enhanced the mechanical properties, and the efficiency of self-healing has given a remarkable outcome. Compared to other species, a few of the ureolytic bacteria like Bacillus subtilis, B. pasteurii, and B. sphaericus exhibited an increasing trend in compressive, flexural, and split tensile strengths. Application of the microbes in the concrete, under mechanism of bacterial metabolic conversion, crack healing of 0.46 mm was significant post 100 days of curing. On subsidiary, in micro-encapsulation process, for a combination of micro-capule and nutrients maximum crack width of 970 μm was healed but on other hand it rendered adverse effect on degree of hydration and compressive strength loss up to 47% after 90 days. But taking utmost care during selecting a suitable self-healing agent and preferable strategy to arrive at authentic self-healing in concrete is of great concern regarding prevailing favorable or unfavorable conditions. Furtherly, investigating the bacterial activity and its survivability at a more extended age period pertaining to concrete in a real-life time basis, its cost effectiveness is not yet discoursed. This microbial approach of self-healing concrete is proven to be the best way as the bacteria is harmless, eco-friendly and sustainable.

Ethical considerations

Not applicable.

Declaration of interest

The authors declare no conflicts of interest.

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Han N-X, Xing F (2017) A comprehensive review of the study and development of microcapsule based self- resilience systems for concrete structures at Shenzhen University. Mater 10.2. DOI: 10.3390/mat10010002


