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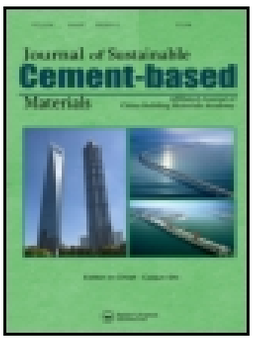

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Effects of biocementation on some properties of cement-based materials incorporating *Bacillus Species* bacteria – a review

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There is a growing need in the construction industry to improve transfer and durability aspects of Portland pozzolana cement. Ureolytic bacteria have recently emerged as potential micro-organisms well known for precipitation of calcium carbonate through microbiologically induced calcite precipitation (MICP) process. MICP process has emerged as a viable mechanism for improvement of the PPC performance. This paper presents an in-depth discussion on the effects of *Bacillus pseudofirmus, Bacillus sphaericus, Sporosarcina pasteurii, Bacillus cereus, Bacillus megaterium and Bacillus subtilis on* some selected physico-mechanical properties of cement-based materials. These properties include standard consistency, setting time, compressive strength, water absorptivity, porosity and chloride ingress. The influence of pH, temperature and various bacteria nutrient requirements on optimum MICP process is also presented. In conclusion, benefits and drawbacks on the use of MICP has been discussed. MICP as a potential technique for improvement of physico-mechanical properties as well as repair of cracked cement-based structures has been discussed.

Keywords: alkaliphilic; bacteria; biocementation; calcite precipitation; cement; microbially mediated reactions

1. Introduction

Cement is generally described as a material with adhesive and cohesive properties which makes it capable of bonding mineral fragments together [1–3]. The cementing or bonding action of calcareous cements is attained through a chemical reaction involving lime or lime compounds [4]. Mortar/concrete show self-healing freshly formed microcracks capacity. This property could be attributed to the presence of non-hydrated excess cement particles in the matrix, which undergoes delayed or secondary hydration upon reacting with ingress water.

A lot of concrete structures suffer deterioration and degradation due to the presence of such cracks in due course of time [5]. This is due to penetration of water into the concrete/mortar which has an adverse effect on the efficiency of the concrete [6]. One of such causes for deteriorations due to the formation of cracks at macro and micro levels which create the path for water ingress, dissolved particles in fluid sand unwanted acidic gases [5]. As

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a result, unwanted materials and other substances penetrate into the concrete/mortar, thus affecting the reinforcement and durability [7]. Few cracks formed will be at a micro level, hence are invisible and difficult to access. The expansion, contraction, and permeation of materials cause an increase in both size and number of cracks [4,8].

Currently, huge monies are required to repair cracks and maintain concrete/mortar structures. To decrease the susceptibility of crack development, organic solvents and synthetic polymers, hollow fibres, micro encapsulation, epoxy, resins, epoxy mortars among other synthetic fillers are applied [8]. To control structural decay, water repellants and stone consolidating synthetic/mineral admixtures are currently being applied. This may result in the formation of incompatible and often harmful surface films [8,9]. All these methods are expensive and have proven to be nonsatisfactory. Therefore, a sustainable, cheaper, alternative green and clean methodology, the use of a biological self-healing repair technique is necessary for saving enormous structures from damage [10].

Microbiologically induced calcite precipitation (MICP) can be achieved extracellular either through heterotrophic processes or autotrophic processes. Both of these processes follow the pathways of urea decomposition, oxidation of organic acids, or nitrate reduction [11,12]. However, the pathway that has been studied most is probably the ureolysis, which is the decomposition of urea by bacterial urease enzyme. This could be attributed to the fact that ureolytic bacteria are anaerobic and water grown and as such they grow well inside the mortar/concrete matrix without supply of oxygen. They can also thrive for long as endospores until favorable conditions and the presence of food is available [7]. Favourable condition includes future cracks formation where the bacteria get activated and through a suitable pathway deposits calcium carbonate which fills the cracks [13].

Due to the negative-charged bacterial cell wall and the high surface area to volume ratio of the bacteria, the bacteria draw cations from the surrounding micro environment, including calcium ions and deposit on their cell surface [14]. The Ca^{2+} reacts with the carbonate ions leading to the precipitation of calcium carbonate at the cell surface. This way, bacteria cell wall acts as the nucleation site for cement hydration [15]. Through MICP, calcium carbonate is deposited in cement mortar/concrete. Such deposits have recently emerged as promising binders for protecting and consolidating various building materials. The deposits enhance nucleation of cement hydration on early cement compressive and flexural strength and as well improve reactivity of pozzolana and other noncementitious material. Furthermore, the microbial biocementation increases resistivity of the resultant mortar/concrete to aggressive media [16]. Due to the 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/mortar [16]. This innovative treatment makes these microbial treated concrete/mortars to act as intelligent systems that are different from the ordinary prepared cement structures [17,18]. These smart structures have self-sensing and self-healing properties toward external factors such as change in temperature, pH, stress, humidity, and concrete/mortar pore solution chemistry [12].

Bacillus species is a common soil bacterium, can continuously precipitate calcite under favourable conditions, is completely harmless to human beings and should be able to withstand a wide range of temperature and pH. They precipitate inorganic crystals hence the healing of the cracks takes place in the concrete/mortar [12]. Despite the various concrete/mortar advantages, it has a high tendency to form

cracks allowing aggressive substances to penetrate into the concrete/mortar structure. In concrete/mortar structures, cracking is a common phenomenon due to relatively low tensile strength [3]. Concrete/mortar cracking may be caused by concrete/mortar internal/chemical processes as a result of plastic shrinkage/settlement. Plastic shrinkage/settlement cracks occur mostly in freshly prepared structures when the rate of water loss through evaporation exceeds the rate at which water is reaching the cement matrix [7]. High tensile stresses can result either from external loads or imposed deformations due to temperature gradients which cause differential volume variations and against structure rigidity, tensions arise causing cracking. Micro cracks may also develop due to cement hydration heat which results in internal stresses. At times, micro-cracks develop due to continuous vibration of machine in later works in the same or adjacent cement structure. Other causes of cracks include long-term shrinkage and creep as a result to constant loads over time on concrete/mortar structures [2,19].

Cracks are one of the main causes of concrete/mortar deterioration and a decrease in durability. The presence of cracks results in increased porosity and permeability. The permeability of the mortar/concrete is dependent on the porosity and on the connectivity of the pores. The more open the pore structure of the mortar/concrete, the more vulnerable the material is to degradation mechanisms [20]. Cracks provide pathways for the penetration of potentially aggressive substances in the mortar/concrete, possibly causing damage [21]. Treatment of cracks and pores in concrete are generally divided into passive and active treatments. Passive treatments can only heal the surface cracks, while active treatments can heal both interior and exterior cracks. In passive treatment, sealants are sprayed or injected into the cracks [22,23]. The sealants comprise of chemical materials such as epoxy resins, chlorinated rubbers, polyurethane, acrylics, and siloxanes. Passive treatments have proven to have many limitations which hinders their usage [24].

Active treatment techniques also referred to as self-healing techniques have the ability to operate independently in different conditions regardless of the crack position. Once the crack is formed, they activate immediately and seal the crack. The suitable treatment of concrete/mortar cracks should have quality, long shelf-life, pervasiveness, and the ability to repeatedly heal the cracks on unlimited number of times [25]. Self-healing mechanism can be established either through autogenous healing, encapsulation of polymeric materials or through microbial production of calcium carbonates [26].

Recently, biomineralization approaches have attracted the researcher's attention as a novel way to address the durability issues related to active and passive treatments. Due to the 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 mortar/concrete [27]. This innovative treatment makes these microbial treated cement/mortars to act as intelligent systems that are different from the ordinary prepared cement structures [17,18]. These smart structures have self-sensing and self-healing properties toward external factors such as change in temperature, pH, stress, humidity, and concrete/mortar pore solution chemistry. MICP is a relatively green and sustainable improvement structural technique [28,29].

2. Isolation and characterization of calcite precipitating bacteria

Calcium precipitating bacteria grow and survive in alkaline medium. The medium described by Oren [30] is the most commonly used general method for the

isolation of alkaliphiles. Isolated strains were examined for colony, cell morphologies, and motility [30]. Colonial morphologies of the isolates were described using standard microbiological criteria, with special emphasis on pigmentation, colour, shape, size, and form. The Gram staining technique was used to categorize the isolates into Gram positive and Gram negative [31].

The MICPbacteria upon isolation were characterized. The characterization of the isolates was either morphological, physiochemical, and/or molecular [32]. Various studies have suggested the use of Christensen's medium (Urea agar base) to screen and detect urease-producing bacteria [32,33]. The MICP bacteria were primarily grouped according to their morphological characteristics, substrate utilization, endospore stain reaction, catalase, and oxidase tests performed by standard methods [34,35]. It can be inferred that the specific urease activity of the ureolytic bacteria strains serve as a good urea hydrolytic agent, potentially useful for MICP applications.

2.1. *Calcite precipitating* **Bacillus** *bacteria*

Different studies have focused on different types of MICP bacteria feeding on different

Table 1. Mechanism of MICP reactions.

Mechanism of Precipitation	Microorganism	Nutrients	Embedment in concrete / mortar	Reference
Bacterial metabolic conversion of organic acid	*B. pseudofirmus*	Calcium lactate, calcium glutamate, yeast extract, and peptone	Direct	Jonkers et al. [17]
	B. pseudofirmus	Calcium lactate, calcium acetate, yeast extract, and peptone	Direct	
Ureolysis	*B. sphaericus*	Urea, calcium nitrate, and yeast extract	Encapsulated	Wang [23]
	B. sphaericus	Urea and calcium chloride	Direct	Achal et al. [19]
	B. sphaericus	Urea, calcium chloride, calcium nitrate, and yeast extract	Encapsulated	Van Tittelboom and De Belie (2013)
	Sporosarcina pasteurii,	Urea and calcium chloride	Direct	Park et al. [61]
	Sporosarcina pasteurii,	Urea, calcium chloride, and nutrient broth	Direct	Maheshwaran et al. [58]
Denitrification	*B. sphaericus*	Urea, calcium formate, calcium nitrate, and yeast extract	Encapsulated	Ersan et al. [63]

types of nutrients and metabolic products used for growing these calcifying microorganisms [16,36]. In the scope of this review paper, the bacteria species, the bacterial precipitation mechanism, the bacterial nutrients and the mode of their embedment into concrete/mortar is summarized in Table 1. (X-ray Diffraction), XRD analysis is employed by most researchers to determine the chemical composition of the precipitate that occurs during MICP [37]. Bacterial calcite precipitate is quantified by the use of XRD analysis and visualized by the use of scanning electron microscopy (SEM). XRD is used to determine the crystallinity of a compound present in a sample. This method allows the identification of various compounds present in the sample using X-ray diffraction. This method may also be used to further understand the influence of bacteria in forming the cement hydration products. Most researchers used the combination of SEM with one or more electron-dispersive X-ray instrument, energy dispersion spectroscopy or energy disperse X-ray analyzer to determine and quantify the chemical constituents.

Figure 1 shows some microscopic images of the crack surfaces immediately after inducing the cracks and after immersing in water for 28 days (a) Control sample (CN) and (b) SHM sample (Encapsulated light weight aggregates impregnated with sodium silicate mortar sample [14]. Crack surfaces of the SHM sample were sealed completely within 28 days exhibiting a significant decrease in the crack depth than the CN sample. Alghamri and co-workers, observed reduction in crack depth to an average of 80% on the SHM sample compared to 21% reduction on the CN sample. This could be attributed to dense calcium carbonate depositions from the healing products, aided by the ongoing hydration of the cement grains and precipitated concrete fragments. The higher silica content

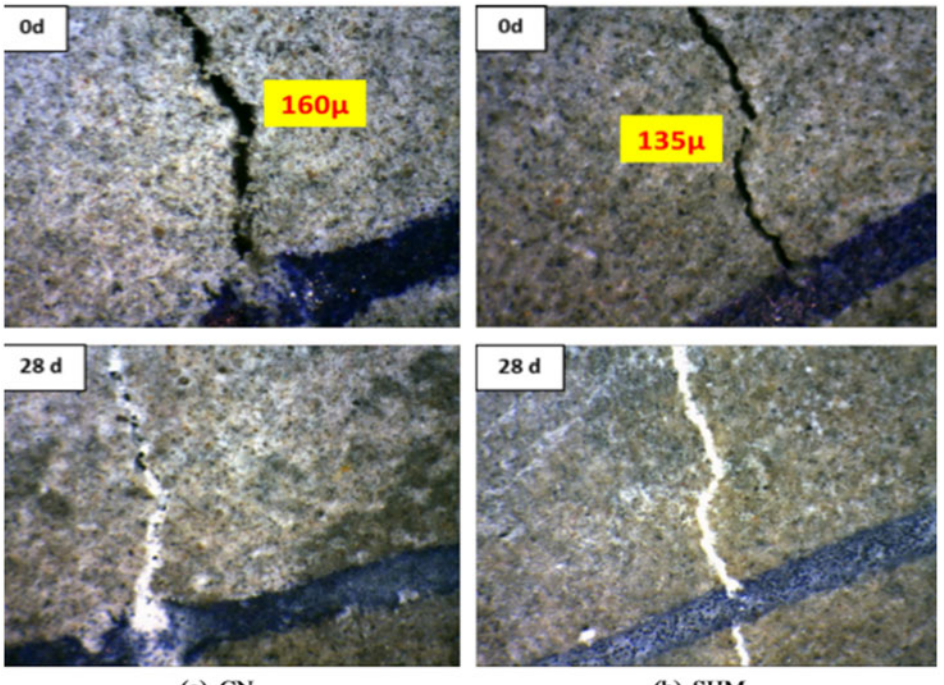

Figure 1. Microscopic images of the cracks surfaces immersed in water after 28 days of curing.

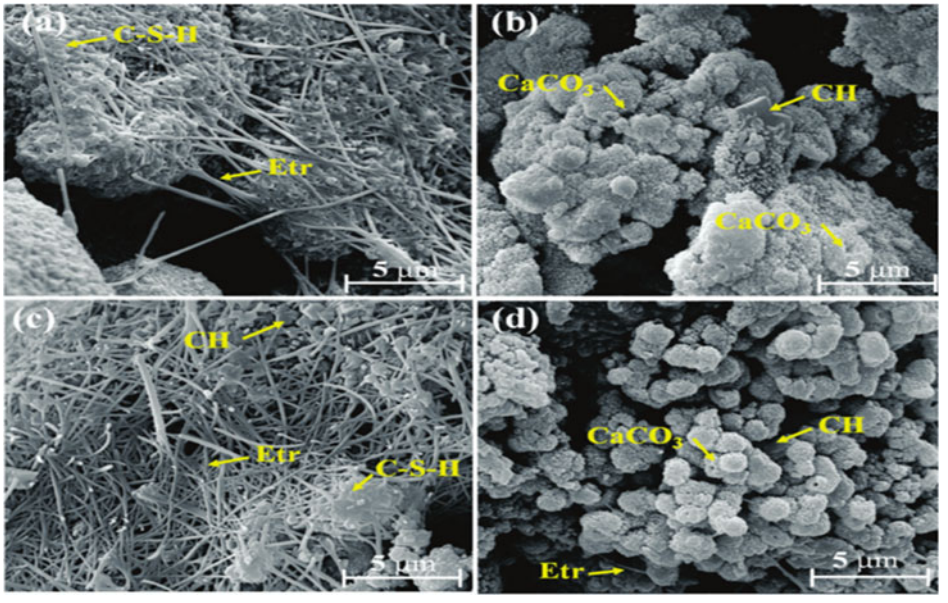

Figure 2. SEM of mortars after 7 days of curing at magnification of 12000x.

from sodium silicate in the SHM sample diffused into the crack and reacted with the calcium hydroxide from the cement hydration, leading to the formation of more calcium-silicate-hydrate gel than in the CN sample. C–S–H bond accounts for most of the physical, chemical and mechanical properties of cement mortars and concretes. In the CN sample, the partial filling could be attributed to the ongoing hydration of the cement grains, the precipitation of concrete fragments and potential formation of calcium carbonates.

Figure 2(a) highlighting the cement hydration products calcium silicate hydrate C–S–H and ettringite, Etr.without bacterial addition. Figure 2(b) shows the mortar with the addition of *Bacillus subtilis* spores by immersion. Figure 2(c) shows the mortar with phosphate buffer replacing water, calcium hydroxide, CH, C–S–H, and an intensive Etr are formed. Figure 2(d) shows the addition of *B. subtilis* in the mixing water, in which bioprecipitated $CaCO_3$ is highlighted.

Jonkers et al. [17] studied the crystallization of *Bacillus pseudofirmus*. These workers identified from SEM images that nucleation of calcite precipitation takes place at the bacteria cell walls. Thus availability/presence of bacteria cell wall is a necessary condition for calcite precipitation. The $CaCO_3$ precipitation is a function of ionic strength and pH in the medium [38].

Both Pei et al. [39] and Sookie et al. [40] suggested that bacteria cell wall served as nucleation sites for calcite precipitation in biochemical reactions. *Bacillus* bacteria precipitate calcite which is highly insoluble around their cell wall which acts as a nucleation site, increasing the impermeability of the mortar/concrete in the bacterial environment. This impervious layer results to an increase in compressive strength due to the lesser expansion within the cement matrix pores and eventually the overall durability performance of the cement specimen improves.

The bacteria cell wall acts like a nucleation site by providing a growth-inducing environment where the Calcium silicate hydrate (C–S–H) nucleation and growth appears to be due to the interactions

between a filler surface and calcium ions in the pore solution.

2.2. Mechanism of microbially induced calcite precipitation

The healing agent can be introduced in concrete/mortar matrix through a vascular network technique/immobilized or can be introduced directly during concrete/mortar preparation. The vascular technique has been said to be almost impractical on large scale by most researchers for some three main shortcomings. Firstly, due to the inability of the healing agent to maintain constant viscosity throughout its lifespan in the concrete/mortar lifespan [24]. Secondly, it would be difficult to distribute the vessels homogenously throughout the concrete/mortar matrix [15,41]. Thirdly, the incorporated vessels in the mortar/concrete matrix may lead to structural delamination.

MICP reactions can be summarized as given in Equations (1)–(18):

(a) Ureolysis

Some bacteria achieve MICP by the production of a urease enzyme. This enzyme uses urea as the main source of nitrogen, catalyzing the hydrolysis of urea into ammonium which creates a microgradient of concentration of carbonate and increases the pH at the site of cell attachment. This is achieved through a multiple of reactions leading to calcium carbonate precitation as follows:

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

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

$$2NH_3 + 2H_2O \rightleftharpoons 2NH_4^+ + 2OH^- \quad (3)$$

$$H_2CO_3 \rightleftharpoons HCO_3^- + H^+ \quad (4)$$

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

$$Ca^{2+} + \text{Bacterial cell} \rightarrow \text{Bacterial Cell} - Ca^{2+} \quad (6)$$

$$\text{Cell} - Ca^{2+} + CO_3^{2-} \rightarrow \text{Cell} - CaCO_3 \quad (7)$$

(b) Metabolic conversion of organic compound/heterotrophic bacteria

MICP can be due to increase in pH and production of carbonate by heterotrophic bacteria during aerobic oxidation of specific feed sources under alkaline conditions as shown in Equation I or II below:

(I) Use of lactate.

$$CaC_6H_{10}O_6 + 6O_2 \rightarrow CaCO_3 + 5CO_2 + 5H_2O \quad (8)$$

$$5CO_2 + 5Ca(OH)_2 \rightarrow 5\,CaCO_3 + 5H_2O \quad (9)$$

(II) Use of acetate.

$$CH_3COO^{1-} + 2O_2 \rightarrow 2CO_2 + OH^{1-} + H_2O \quad (10)$$

$$CO_2 + OH^{1-} \rightarrow HCO_3^{1-} \quad (11)$$

$$HCO_3^{1-} + OH^{1-} \rightarrow CO_3^{2-} + H_2O \quad (12)$$

(c) Denitrification

Bacterial denitrification process causes a localized pH rise due to the production of hydroxyl ions which initiates MICP without pH buffering [42]. The carbon (IV) oxide gas produced in the presence of rising pH produces the carbonate ion which on being in contact with calcium ion results in MICP as shown by the following equations:

(I) Dissimilatory nitrate reduction.

$$\text{Organic compound} + a\,NO_3^- + b\,H^+ \rightarrow c\,CO_2 + d\,H_2O + e\,N_2 \quad (13)$$

$$CO_2 + 2OH^- \rightarrow CO_3^{2-} + H_2O \quad (13)$$

$$Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3 \quad (14)$$

(II) Dissimilatory sulphate reduction.

$$CaSO_4 + 2(CH_2O) \rightarrow CaS + 2CO_2 + 2H_2O \quad (15)$$

$$CaS + 2H_2O \rightarrow Ca(OH)_2 + H_2S \quad (16)$$

$$2CO_2 + 2H_2O \rightarrow H_2CO_3 \quad (17)$$

$$Ca(OH)_2 + H_2CO_3 \rightarrow CaCO_3 + 2H_2O \quad (18)$$

The dissimilatory sulphate reduction may not be a suitable concrete/mortar remediation process as it uses up gypsum as shown in Equation (15) above. It may also produce acidic products hence deleterious to concrete/mortar. The sulphides formed reacts with available metal ions forming insoluble sulphides which clogs the pores in the cement matrix binding the particles together hence beneficial. However, the compaction created by the formation of the sulphides is unstable as they can chemically or biologically be oxidized to sulphuric acid or sulphates under aerobic conditions hence detrimental to concrete/mortar [43].

Table 1 gives the details of the mechanism of precipitation of various bacteria, source of calcium nutrients and mode of healing agent embedment into mortar/concrete matrix.

Ureolysis is the most studied pathway for MICP. This could be attributed to the fact that urea hydrolyzing microorganisms are abundant in nature, making the urea hydrolysis process common to soils worldwide [44]. Urease producing microorganisms are known to induce MICP in the presence of urea and calcium ion [45]. Ureolytic bacteria are also able to produce urease in presence of urea causing the production of the carbonate ion necessary for MICP process. These bacteria are also able to withstand relatively high pH levels compared to the pH of mortar/concrete matrix without suppressing their microbial growth/processes. Most of the other abundant bacteria that are nonureolytic suppress urease in the prescence of ammonia and other nitrogen containing compounds [44].

2.3. *Atmospheric carbon (IV) oxide gas concrete/mortar carbonation*

The process of carbonation of concrete occurs at carbon (IV) oxide gas, CO_2, concentrations of as low as 0.03% by volume of air in the rural air upto 0.3% in large cities. It has been found that the rate of carbonation of concrete increase with increase in the concentrations of CO_2. Atmospheric carbonation process most readily involves $Ca(OH)_2$, [46,47]. On $Ca(OH)_2$ getting depleted for instance by secondary reaction with pozzolanic silica, the carbonation of calcium silicate hydrate (CSH) as well tricalcium silicate (C_3S) and dicalcium silicate (C_2S) is also possible [48,49].

$$Ca(OH)_2 + CO_2 \rightarrow CaCO_3 + H_2O \quad (19)$$

Hydration products (calcium silicate hydrate or CSH gel) and even the residual unhydrated compounds, tricalcium silicate (C_3S) and dicalcium silicate (C_2S) present in all concretes, also react with CO_2 according to the following reactions [47,48]:

$$CSH + 3CO_2 \rightarrow (3CaCO_3.2SiO_2 + 3H_2O) \quad (20)$$

$$C_3S + 3CO_2 \rightarrow H_2O.SiO_2.H_2O + 3CaCO_3 \quad (21)$$

$$C_2S + 2CO_2 \rightarrow H_2O.SiO_2.H_2O + 2CaCO_3 \quad (22)$$

Generally, reactions (19)–(22) may ionically be summarized as:

$$Ca^{2+} + 2OH^- + CO_2 \rightarrow CaCO_3 + H_2O \quad (23)$$

These atmospheric carbonation processes are irreversible. By reacting with OH^-, they lower the hydrated cement alkalinity/pore solution pH. This destroys the

structural stability of concrete/mortar reducing its service life [10].

Alternatively, CO_2 may dissolve in pore solution forming carbonic acid which degrades cement matrix according to the following equations:

$$CO_2 + H_2O \rightarrow H_2CO_3 \quad (24)$$

$$H_2CO_3 + H_2O \rightarrow {HCO_3}^{1-} + {H_3O}^{1+} \quad (25)$$

$${HCO_3}^{1-} + H_2O \rightarrow {CO_3}^{2-} + {H_3O}^{1+} \quad (26)$$

$$H_2CO_3 + Ca(OH)_2 \rightarrow CaCO_3 + 2H_2O \quad (27)$$

$$H_2CO_3 + CaCO_3 \rightarrow Ca(HCO_3)_2 \quad (28)$$

Equation (28) shows that significant quantities of CO_2 results in conversion of insoluble beneficial $CaCO_3$ to the soluble $Ca(HCO_3)_2$ which is easily leached out thus decreasing the porosity of hydrated cement [48].

This could imply that, while carbonation from microbial organisms (MICP) is beneficial to cementious structures, the atmospheric carbonation is deleterious predominantly on mortar/concrete compressive strength, porosity and hardness. It could also imply that atmospheric carbonation affects pozzolanic Portland cements (PPC) more than ordinary Portland cements (OPC). This could be attributed to the late hydration in PPC which is interfered with when the reaction between atmospheric CO_2 and the residual unhydrated compounds occurs (Equations 20–22).

3. Optimum conditions for biocementation

3.1. pH tolerance

Concrete/mortar matrix has a pH range of between 11.5 and 13.5. These high pH values are a requirement in MICP, where the organic compounds are degraded into carbon (IV) oxide and water. Sahoo et al. [51] observed that due to the high pH of the mortar/concrete matrix, bacterial cells grow slowly at the initial curing period as they accustom themselves to the new high pH environment. If the pH media is beyond what a given bacteria can withstand, the bacteria either gets dead or turns into an endospore.

pH levels in the mortar/concrete matrix affect the bacterial growth/survival. [40], tested the effect of pH on the bacterial growth. In their studies they used both *Bacillus pasteurii* and *Bacillus sphaericus* and exposed them to nutrients with pH range of 4–12. They found out that *Bacillus pasteurii* had optimum growth in the pH range of 7.0–9.0 while *B. sphaericus* optimum growth was at the pH range of 8.0–9.0.

Wu et al. [50] cultured both *Bacillus cereus* and *B. sphaericus* in Luria Bertani broth at the pH range of 8–12.5 at both 37 °C and 50 °C for 24 h. They found out that *B. cereus* did not survive above pH 9.0 while *B. sphaericus* survived in the media in the pH range of 8.0–12.5 at both 37 °C and 50 °C and thus suitable for fresh mortar/concrete with pH of about 11.5–12.5 and the temperature of 37–50 °C. Sahoo et al. [51] on culturing *Bacillus megaterium*, found out that the maximum pH was 8.9 at both 37 °C and 50 °C after 24 h of curing till 120 h. Schwantes-Cezaro et al. [52] in their work observed that the optimum pH for *B. subtilis* in their phosphate-buffered nutrient media is 9.0.

Arunachalan et al. [53] in their work observed that the optimum pH for urease enzyme is in the range of 7.5–8.0. They further found that the urease activity increased gradually from pH 6.0 to 8.0. However, the pH of reactant medium was found to increase gradually during the urea hydrolysis due to the production of ammonia. The other product during ureolysis and microbial respiration is carbon (IV) oxide which acts as a buffer to the pH rise.

The influence of pH on MICP is complex because it affects various processes

such as microbial activity, urease activity, and calcite solubility. The initial pH medium increased during the precipitation for all MICP bacterial species, thus changing the environment for optimum precipitation. Various species of MICP forming bacteria adapt to a given pH range inorder to optimize calcite precipitation with an alkaline media being favourable to the process.

3.2. Temperature tolerance

The microbial activity and growth are less sensitive to the temperature within the range of 20–30 °C. The rate of urea hydrolysis is insignificantly higher at 30 °C as compared with 20 °C. Increment in temperature after 30 °C does not promote the decomposition rate of urea [54]. Temperatures mainly vary with latitude, altitude, incident solar radiation, moisture content, conduction, thickness of the structure among other factors. In terms of urease enzyme, [54], on using *B. megaterium* found out that urease activity increased with increasing temperature from 10 °C and reached an optimum at 60 °C. Urease activity was inhibited by 100 °C. Dhamia et al. [54] findings were consistent with the findings of both [55] and [56].

The optimum temperature for bacterial activity is different with the optimum temperature for calcite precipitation. Urease was found to be active at temperatures between 10 °C and 60 °C, with the urease activity being at its peak at 60 °C, while calcite precipitation increased between 20 °C and 30 °C with calcite precipitation being at its peak at 30 °C. In the temperature range of 30–50 °C, there is a significant decrease in calcite precipitation, with the least precipitation observed at 50 °C. Perhaps there is no direct correlation between temperature and MICP deposits since calcite solubility decreases with an increase in temperature, thus affecting calcite precipitation.

3.3. Nutritional requirements

The source of calcium ion during biocementation dictates the type of crystal formed, the size, morphology and the degree of crystallization. Calcium chloride induces the formation of calcite (rhombohedral crystals), calcium acetate induces the formation of aragonite (lamellar/needle-like crystals) and calcium lactate induces the formation of the complex vaterite (hexagonal crystals) [57]. Numerous studies have reported the use of 3 g/l of nutrient broth, with different chemicals for different bacteria *species* as shown in Table 1 into the treatment solution to sustain the growth and viability of urease producing bacteria [24,58]. The supply of nutrients is to ensure the bacteria can sustain long enough to support MICP in order to achieve the desired structural performance.

Several studies have illustrated that these bacteria form thick membrane spores, which can survive without nutrients for up to 200 years [24]. The endospores are also postulated to be able to remain dormant and be able to withstand environmental changes, chemicals, ultraviolet radiations as well as mechanical stresses for hundreds of years [17,24].

The supply of enough, appropriate, and sufficient nutrients to bacteria ensures that they get the energy sources that can initiate their growth from the endospores and sustain them long enough. The concentration of nutrients and their salinity influences the MICP process. High salinity has inhibitory effect on microbial activity and MICP.

The urease enzyme, supplied directly into the mortar/concrete or produced by the bacteria, decompose urea through a chemical reaction known as hydrolysis of urea;

$$CO(NH_2)_2 + 2H_2O \rightleftharpoons 2\ NH_4^+ + CO_3^{2-} \quad (29)$$

The ammonium ions released from urea hydrolysis results in local pH rise and

commences the MICP process. The high pH at localized area increases the tendency for the bacteria cell wall to act as a nucleation site for calcite precipitation. Calcite precipitation is through the combination of carbonate ion (CO_3^{2-}) from the hydrolysis of urea and the calcium ion (Ca^{2+}) from calcium supplied from a soluble calcium salt or from the bacteria cell wall.

The calcite precipitated is responsible for the biocementation of mortar/concrete. Numerous and living bacteria support adequate calcite precipitation in mortar prisms, perhaps enhancing achievement of the desired properties.

4. Physio-mechanical properties of hydrated cements

4.1. Consistency and setting time

Several studies have found that the initial and final setting time for mortar/concrete with beneficial bacteria solution is always slightly higher than the one without bacterial solution. It has also been noted that the concentration of bacteria in the mix water does not have significant effect on both initial and final setting time of bacterial mortar/concrete. The addition of bacteria spore powder in concrete/mortar either retard or accelerate the setting time of concrete/mortar depending on the calcium source. Luo and Qjan [16] and Zhang et al. [36] in their studies reported that calcium lactate retards the setting time, while calcium formate and calcium nitrate accelerating the setting time of concrete/mortar.

Thiyagarajan et al. [59] in their work, determined the consistency and setting time of control and *B. pseudofirmus* bacterial concrete at cell concentration of between 10^6 and 10^8 cells/ml and obtained the results as shown in Table 1. Wu et al. [50] in their work used *B. sphaericus* to make bacterial solution with a cell concentration of 10^6 cells/ml to make mortar and also obtained results as shown in Table 2.

From the results, the consistency of the bacterial concrete is slightly lesser than for the control concrete. The researchers noted that the water requirement for bacterial concrete is lesser than for the control concrete. The initial and final setting time for bacterial concrete is slightly higher than the control concrete.

4.2. Compressive strength

Compressive strength is the capacity of a material or structure to withstand loads tending to reduce size. Several studies have documented compressive strength improvements on bacterial containing cementitious material of between 9% and 25% by 28th day of curing [38,60]. Different researchers have documented both positive and negative effects on compressive strength depending on bacterial strain, cell concentration or concrete age [38,61]. These nutrients affect compressive strength and cement hydration. Wang [23] found out that calcium nitrate as a bacterial nutrient accelerated cement hydration while yeast extract significantly delayed the hydration and resulted in a lower hydration

Table 2. Consistency and setting time.

	Fresh properties			
	Control concrete	Bacterial concrete	Bacteria used	Reference
Consistency	30.5%	29%		
Initial setting	215 min	230 min	*B. pseudofirmus*	Thiyagarajan et al. [59]
Final setting	420 min	450 min		
Consistency	34.5%	32%		
Initial setting	52 min	50 min	*B. sphaericus*	Sahoo et al. [51]
Final setting	360 min	367 min		

degree at the 7th day of curing and a reduced compressive strength at the 90th day of age.

Chahal et al. [62] used 10% fly ash-substituted cement with a 10^5 cells/ml *Sporosarcina pasteurii* bacteria solution and observed a 20% improvement in compressive strength. Nosouhian et al. [21] in their work used 15% silica fume-substituted cement with 2×10^9 cells/ml *Sporosarcina pasteurii* accompanied by *B. subtilis* and observed a 20% enhancement in compressive strength at the 28th day of curing. Achal et al. [19] used cement substituted with fly ash at varied concentrations of 10%, 20%, and 40% in mortar with a specific *B. subtilis* cell concentration and an observed compressive strength improvement of 19%, 14%, and 10%, respectively, at the 28th day of curing. The compressive strength improvement in bacterial concrete/mortar added to silica fume could be attributed to calcium carbonate precipitation in the pores of cement—sand matrix.

In contrast, there are contradictory findings in regard to the influence of bio-based healing agents on compressive strength. [23], on embedding encapsulated *B. sphaericus* in mortar, obtained decrease in compressive strength of between 15% and 34% on a cracked mortar between the 7th and 28th day of curing. Achal et al. [60] in their literature review reported a reduction in compressive strength on using *B. sphaericus* at a cell concentration of 5×10^8 cells/mm^3. Ersan et al. [63] in their study, using encapsulated *Sporosarcina pasteurii*, reported a decreased compressive strength on concrete in the 7th and 28th day of curing by 63% and 60%, respectively.

Generally, there is a significant increase in bacterial concrete/mortar compressive strength as compared to the conventional concrete/mortar. The type of biocementation bacteria used determines the extent of compressive strength enhancement. This could be attributed to the activity of different bacterial species such as ureolytic, halophilic, alkaliphilic, cyano-bacteria, heterotrophic, or denitrifying.

4.3. Water absorptivity

Permeability of cement concrete/mortar depends on pore network of cementitious material Permeability is thus an important property for ingress of substances that may cause degradation of concrete/mortar. Permeability is considered to be a fundamental property for portraying the durability of cement concrete/mortar. Chahal et al. [62] in their work observed a decrease in water permeability in their concrete containing *Sporosarcina pasteurii* bacteria and fly ash compared with concrete without the bacterial content. [60] cast cubes with the addition of *B. megaterium* and its nutrients.

Table 3. Water absorption of different bacteria after 28 days of curing.

Bacteria	Author	Water absorption after 28 days of curing
B. sphaericus	De Muynck et al. [11], Achal et al. [19]	45–50% less than controlled concrete sample
B. subtilis	Reddy et al. [2], Muhammad et al. [6], Pei et al. [39]	Nearly 50% less than controlled concrete sample
B. megaterium	Dhamia et al. [54]	46% less than controlled concrete sample
Sporosarcina pasteurii	Achal et al. [19], Pei et al. [39]	50–70% less than controlled concrete sample
B. pseudofirmus	De Muynck et al. [11], Maheshwaran et al. [58]	50% less than controlled concrete sample

They observed water absorption reduction of more than three times in the bacterial concrete than in the control one. Siddique et al. [64] added *Bacillus aeris* bacteria in their concrete and observed a reduction in water absorption. All these studies attributed a decrease in water permeability and porosity in the bacterial concrete to calcium carbonate precipitation within the cured concrete pores as shown in Figure 1.

Several researchers have investigated the effect of MICP on water permeability involving different bacteria after 28 days of curing and their results are summarized in Table 3 in the following page.

From Table 3, it is evident that bacterial concrete/mortar have a higher water permeability resistance than control concrete/mortar regardless of the species of *Bacillus* bacteria used. Amongst all the *Bacillus* species used the water porosity reduced by between 45% and 50%, except *Sporosarcina pasteurii* where the reduction was upto 70%. This implies that there is no significant difference in water porosity with the species of bacteria used.

4.4. Durability properties of hydrated cements

Despite the various concrete/mortar advantages, it has a high tendency to form cracks allowing aggressive substances to penetrate into the structure. Permeability or cracks are one of the main causes of concrete/mortar deterioration and a decrease in durability. Cracking in concrete structures affects the materials structural integrity and durability [65]. These cracks have a severe negative influence in aggressive environment with regard to chloride and sulphates penetration [66]. The presence of these cracks forms a path way for ingress of aggressive materials/ions such as sulphates and chlorides that decrease the service life and the durability of the concrete structures [17,67].

4.5. Chloride ingress

Majority of the researchers have used Rapid chloride permeability test to investigate chloride ion ingress. Chahal et al. [62] on using *Sporosarcina pasteurii* bacterial concrete observed 380 coulombs chloride penetration as compared with their control concrete which allowed 762 coulombs penetration. Nosouhian et al. [21] in their work observed the reduced chloride ion penetration in their concrete containing *Sporosarcina pasteurii* and *B. subtilis* bacteria and fly ash separately compared with concrete without the bacterial content. [64] on using *Bacillus aerius* bacterial cells in their work, noted reduced total charge passed through their bacterial concrete by 55.8%, 49.9%, and 48.4% compared to the control concrete at the curing age of 7, 14, and 56 days, respectively.

Maes and De Belie [66] focused on using encapsulated polyurethane with concrete of any mixture-induced cracks of 100 and 300 μm. They observed 83% and 67% decrease in chloride ingress on exposure of the cracks to encapsulated polyurethane followed by the chloride ingress. The same workers also observed that autonomous healing is able to seal crack widths of 100 and 300 μm for chloride penetration in 67% and 33% of the cases, respectively.

There is a significant reduction in chloride ion permeability in bacterial concrete/mortar for all *Bacillus species* considered. This could imply that the proper service life of concrete/mortar structures exposed to aggressive ions is well defined by the ability of the concrete/mortar to resist the penetration of chloride ions.

5. Conclusion

1. The traditional crack repair systems have multiple demerits such as

different thermal expansion coefficients compared to concrete/mortar, health and environmental hazards, requires manual inspection and repair, expensive and sometimes interferes with the aesthetic value of the structure.

2. The use of denitrifying cultures for concurrent self-healing and production of corrosion inhibiting nitrites are a promising new construction technology. The contradictory effect on compressive strength by MICP could be attributed to brittleness of precipitated $CaCO_3$.
3. This review has identified that *Bacillus species* bacteria can be used for enhancing concrete/mortar physico-mechanical properties when some necessary optimum conditions are provided.
4. The efficacy of calcium carbonate precipitation can be influenced by the type of bacteria, concentration of bacterial cells, pH, temperature and amount of calcium, and the nutrients added to the medium.
5. Compressive strength of mortar/concrete was found to increase with an increase in bacterial concentration up to 109 cells/ml. However, further increase of any *Bacillus* spp. bacteria concentration was found to reduce the compressive strength both at the 7 day and at 28 day of curing.
6. Comparative studies have exhibited that there are both merits and limitations on bacterial healing agents and strategy. Moreover, most studies have focused more on durability side than on the mechanical properties. Thus, there is need to further investigate to establish the more reliable and efficient self-healing strategy as well as examine the durability of bio-crack repair technique.
7. Further work is also necessary for better understanding of the self-healing efficiency and its variability so as to allow proper selection of the MICP bacteria and the optimum conditions that will allow production of an alternative and high-quality concrete/mortar. When all this is achieved and documented, then engineers, contractors and owners could be convinced of the suitability to practice application of specific *Bacillus* species either as an environmentally responsible course of action, for mortar/concrete surface crack healing, to improve the durability properties or to lower the ingress of ions into the cement matrix on large scale or in the field.

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