Use of *Bacillus Species* Bacteria in Protecting the Concrete Structures from Sulphate Attack - A Review

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**Abstract:** Deleterious ions in the environment such as sulfates may degrade the concrete structures. The interaction of cement hydration products with these destructive agents contributes to severe durability threat of the concrete structures. External sulfate attack is well-known for causing permanent changes in concrete. Microbially induced calcium carbonate (MICP) precipitation has been considered as a unique technique in enhancing the durability properties of concrete. This review paper discusses the possibility of bio-deposition from MICP process as a barrier in microbial treated concrete against the penetration of sulfate ions in a sulfate-rich environment. The effect associated with chemical and physical sulfate attack is discussed in line with the mechanical properties of cement such as compressive strength whereas microscopic evaluation is based on scanning electron microscopy studies. The shortcomings associated with sulfate ions in cement-based materials and the positive effects of incorporating *bacillus species* bacteria in sulfate rich areas is discussed. This review found that, MICP can significantly reduce the ingress of sulfate ions in cement-based materials, which results in improving the mechanical properties of the cement mortar/concrete.

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**Keywords:** Sulfate attack; MICP; Bacteria; Concrete; Degradation

**Graphical Abstract:**

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1. Introduction

The benefits associated with concrete such as accessibility of raw materials, minimal preparation cost, high compressive strength and durability has made it to be the most affordable and widely used construction material. Large volumes of concrete are used annually across the world for construction of buildings, roads, sewer systems and bridges. While concrete is the most preferred building material, it is susceptible to the aggressive materials such as sulfates, carbon dioxide, and chlorides. The ingress of these agents into the concrete matrix causes serious durability threat on the concrete structures [1, 2].

Most of the concrete structures are suffering from durability threats due to the presence of micro and macro cracks that may develop during the service life of the concrete structures. These cracks form suitable pathways for the ingress of harmful substances into the concrete matrix [3, 4]. The presence of aggressive agents in the concrete matrix triggers early failure of
the cement based structures. Amongst the discussed deleterious ions are sulfates. Sulfates are known to cause deleterious effects on cement based structures and several methods have been adopted by many researchers to reduce the sulfate attack on concrete. Concrete structures erected in sulfate rich-environment suffer great extent of damage as a result of sulfate attack. Presence of sulfate ions in concrete structures trigger an increase in porosity, expansion, and cracking. These affects the mechanical properties and hence resulting to reduced durability of the cement based materials [5,6]. The placed concrete intermingles with the sulfates from the ground water resulting to dilapidation of the inherent concrete properties. This phenomenon is referred to external sulfate attack [7]. External sulfate attack caused by the ingress of sulfate ions from the environment into the concrete has been discussed in several studies [8-10]. Georges et al., 2016 [11] reported that concrete structures placed in sulfate rich areas suffers from sulfate attack. This is due to the ingress of sulfate ions into the cement matrix [12, 13]. The mechanism of degradation of cement based materials due to the sulfate attack is now comparatively well-known [14, 15]. Sulfate ions upon ingress into the concrete matrix can modify the chemical equilibrium between the solid phase and the interstitial phase in the cementitious matrix [16, 17]. The chemical reaction between the hydration products of the cement and the sulfates from the environment occurs as a result of diffusion of these ions to the concrete surface [19, 20]. Calcium hydroxide (CH) may react with sulfates forming gypsum. The formed gypsum further reacts with tricalcium aluminate from clinker to form ettringite. Ettringite is an expansive needle like crystalline substance and the expansion of concrete/mortar is associated with its formation [21, 22]. The crystallized ettringite causes expansive forces within the concrete matrix, leading to cracking and spalling [23]. The equation below shows the formation of ettringite.

$$3\text{CSH}_2 + \text{C}_3\text{A} + 26\text{H} \rightarrow \text{C}_6\text{A}_3\text{S}_3\text{H}_{22}$$ (1)

Among the most consistent theories derived from most of the experiments with respect to sulfate attack is the crystal growth theory. This theory defines the crystallization pressure produced by supersaturation and confinement. The driving force for the crystallization according to crystal growth theory is the super saturation of the pore solution with respect to ettringite [24 - 26]. Microstructural tests have shown that the reaction of sulfates and monosulfates in pockets does not cause any expansion. However, when the pockets are depleted the finely intermixed monosulfates in the C-S-H reacts to form expansive ettringite [27]. Experimental results have shown that sulfate attack leads to not only expansion of cement based materials but also causes softening and decohesion [28, 29].

Supplementary cementitious materials (SCMs) such as fly ash, natural Pozzolana and slag have been incorporated in cement to mitigate the sulfate attack [30 - 34]. These materials however, do not effectively protect the physical sulfate attack [28, 35 and 23]. Other materials such as epoxy based fillers or silane-based water repellant have been used widely to repair concrete cracks associated with physical sulfate attack. Their short term efficiency and negative environmental impact has been an issue for the repair industry [20]. These polymeric materials are expensive and can only be applied from outside where the cracks are visible. The precipitation of calcium carbonate by calcifying bacteria through a process referred to bio deposition has been suggested as a remarkable method for improving the durability of cement based materials. This method facilitates self-healing of concrete cracks reducing the risks of harmful materials from ingestion into the concrete [36]. Chigozirim et al., [37] defined microbially induced calcium carbonate precipitation (MICP) as the ability of microbes to produce calcium carbonate extracellularly through a metabolic activity. Several researchers have shown the potential of using calcifying bacteria via biominerization to improve the durability of cement-based materials [38-40].

2. Biomineralisation

Mineralisation used in civil engineering often refers to production of minerals, primarily carbonate products. Chigozirim et al., [37] defined biomineralisation as the process by which living organisms produce minerals through metabolic activities from their interaction with the environment. In biomineralisation, living organisms produces inorganic mineral phases with a biopolymer [41]. The involved microorganisms secrete one or more metabolic products that react with ions or compounds in the environment resulting in the subsequent deposition of mineral particles as metabolic products [42]. These metabolic activities may result in selective cementation by producing moderately insoluble organic and inorganic compounds. The formed compounds which can serve as cementitious materials are referred to bio cement. Biocement comprising of an alkalophilic microbe, substrate solution and calcium ion solution has attracted much attention as a "green" material. It relies on microbially induced calcium carbonate precipitation (MICP) [43]. Many researchers have shown that MICP can improve the concrete strength and durability of cement-based materials [44-46]. The process involves enzymatic reactions. Urease produced by the bacteria hydrolyses urea, and calcium is utilized as energy source to form biocement [47].
In MICP process, microorganisms play a key role in maintaining an alkaline environment via their innumerable physiological activities. These activities ensure an increase in pH and dissolved inorganic carbon [48]. Rong et al., [49] and Ariyanti et al., [48] reported that biocementation can be effective in binding sand grains for making bio sands of adequate compressive strength as shown in Figure 1. Ghosh et al., [50] reported compressive strength increasing up to 25% when pure mortar was mixed with shewanella species. The increase in compressive strength was attributed to biocementation.

Ureolytic activity involves consumption of urea to form ammonia and carbamate. The formed carbamate spontaneously hydrolyses to form an additional ammonia and carbonic acid [51]. The formed products trigger formation of bicarbonate, increasing the pH that ultimately shifts the bicarbonate equilibrium resulting to formation of carbonate ions. As a result of high pH in the cell, there is need for a high extracellular calcium ion concentration and a low extracellular proton concentration to facilitate the secretion of carbonate ions. High pH favors the formation of carbonates from bicarbonates [52, 47]). Equations 2-8 reveals the mechanism of ureolytic activity.

$$\text{CO(NH}_2\text{H}_2 + \text{H}_2\text{O} \rightarrow \text{NH}_2\text{COOH} + \text{NH}_3 \quad (2)}$$

$$\text{NH}_2\text{COOH} + \text{H}_2\text{O} \rightarrow \text{NH}_3 + \text{H}_2\text{CO}_3 \quad (3)$$

$$\text{H}_2\text{CO}_3 \leftrightarrow \text{HCO}_3^- + \text{H}^+ \quad (4)$$

$$2\text{NH}_3 + 2\text{H}_2\text{O} \leftrightarrow 2\text{NH}_4^+ + 2\text{OH}^- \quad (5)$$

$$\text{HCO}_3^- + \text{H}^+ + 2\text{NH}_4^+ + 2\text{OH}^- \leftrightarrow \text{CO}_3^{2-} + 2\text{NH}_4^+ + 2\text{H}_2\text{O} \quad (6)$$

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

$$\text{Cell} - \text{Ca}^{2+} + \text{CO}_3^{2-} \rightarrow \text{Cell} - \text{CaCO}_3 \quad (8)$$

Carbonic anhydrase enzyme assists the interconversion of carbon dioxide and bicarbonate and promotes the precipitation of calcium carbonate [53]. Carbonic anhydrase plays a vital role in concentration of CO$_2$. Equations 9-10 show how the carbonic anhydrase in presence of bicarbonate as the source of the dissolved inorganic carbon catalyses bicarbonate into carbon dioxide [54].

$$\text{HCO}_3^- \rightarrow \text{H}_2\text{O} + \text{CO}_2 \quad (9)$$

$$\text{Ca}^{2+} + 2\text{HCO}_3^- \rightarrow \text{CaCO}_3 + \text{H}^+ + \text{HCO}_3^- \quad (10)$$

### 2.1 Biodeposition/ Bioremediation

MICP deposits carbonate crystals inside the surface pores of cement-based materials preventing the ingress of external deleterious materials into the concrete matrix [55]. Several researchers have explored MICP for protection and consolidation of ornamental limestone [56]. Biodeposition treatment has been applied in five different types of limestone to investigate the effect of pore structure on the protective performance of a MICP based surface treatment [57]. It was also found that biodeposition reduced water absorption and gas permeation on cementitious materials.

D. Muyck reported that decrease in capillary suction and decrease in gas permeability was a result of deposition of calcite layer on surface of the cement [45]. Biodeposition results in plugging of pores and formation of biofilms on the surface of cement based materials. MICP technique has been used to remove sulphate and clean crusts from marble monuments [58]. *Desulfovibrio desulfuricans* an anaerobic bacterium converted calcium sulphate into calcium carbonate as product of MICP as shown in Equation 11 [59].

$$6\text{CaSO}_4 + 4\text{H}_2\text{O} + 6 \text{CO}_2 \rightarrow 6\text{CaCO}_3 + 4\text{H}_2\text{S} + 2\text{S} + 11\text{O}_2 \quad (11)$$

Bioremediation in concrete materials has been carried out in two ways. Firstly the bacteria cells with proper nutrition, urea and calcium source are assimilated into the concrete matrix during casting. In this scenario, the microbes trigger the formation of biominerals when the cracks occur. The biominerals forms calcites that seal the cracks [60]. The second method involves...
application of the bacteria cell on the surface of the concrete cracks. From the previous studies carried out by [44] SEM scans showed calcite precipitation in healing areas of cement mortar surface where cracks of width of 3mm and depth of 18.8mm in mortars were healed by *Sporosarcina pasteurii*. Ramachandran *et al.*, [44] initiated the MICP based bioremediation of concrete materials. Sand mixed with *Sporosarcina pasteurii* cell were applied in cracks in mortars to improve the compressive strength by 61%. In a separate experiment, Ramachandran *et al.*, [44] employed polyurethane–encapsulated bacterial cells in order to protect the bacteria cells from the high pH of cement [61]. There was a significant increase in compressive strength of the remediated concrete of 12% at 7 days and only 3% increase at 28 days. The sealing of cracks by calcite precipitated by *B. sphaericus* resulted in reduction of water permeability and crack bridging was demonstrated by an increase in ultra-sonic pulse velocity [62]. Table 1 summarizes the bioremediation efficiencies in crack healing of cementitious materials as reported by several authors.

| Specimen and Microbe | Specimen Dimension (mm) | Crack Size (mm) | Bioremediation efficiency | Author |
|----------------------|-------------------------|-----------------|----------------------------|--------|
| Concrete prisms      | 160 × 160 × 70          | d = 20, w = 0.3 | Decrease in water permeability, crack bridging | De Belie *et al.*, 2009 [63] |
| *B. sphaericus*      |                         |                 |                            |        |
| Reinforced mortar    | 40 × 40 × 160           | Multiple cracks, w = 0.05-1.00 mm | Self-healing, oxygen diffusion barrier | Wiktor *et al.*, 2011[64] |
| *B. alkalinitrilicus* |                         |                 |                            |        |
| Cement mortar        | 50.8 × 50.8 × 50.8      | d = 25.4, w = 3.175 | Improvement in compressive strength (61%) | Ramachandran *et al.*, 2001[44] |
| *S. pasteurii*       |                         |                 |                            |        |
| Cement mortar        | 50.8 × 50.8 × 50.8      | d = 25.4, w = 3.18 | Improvement in compressive strength (12%) | Bang SS *et al.*, 2001[61] |
| *S. pasteurii* (encapsulated) |                   |                 |                            |        |
| Cement mortar        | 40 × 40 × 40            | -               | Protect crack against water ingress | Jonkers HM *et al.*, 2008[65] |
| *B. pseudofirmus B. cohnii* |                   |                 |                            |        |
| Concrete prisms      | 160 × 160 × 70          | d = 20, w = 0.3 | Decrease in water permeability, visual crack sealing, high pH protection of bacteria | Van Tittelboom *et al.*, 2010[62] |
| *B. sphaericus*      |                         |                 |                            |        |
| Reinforced prism     | 40 × 40 × 360           | d = 20, w = 0.35-0.50 | Higher strength regain and more pronounced decrease of water permeability | Wang JY *et al.*, 2012[60] |
| *B. sphaericus*      |                         |                 |                            |        |
| Reinforced prism     | 30 × 30 × 360           | Multiple cracks, w = 0.20-0.22 | Self-healing (48-80%), lower water permeability | Wang JY *et al.*, 2014[66] |

3. Effects of Sulphate Attack on Cement Based Materials

In this segment, we summarize the results obtained so far on the effects associated with sulphate attack on the cement-based materials when exposed to sulphate solutions.

3.1 Chemical Sulphate Attack

3.1.1. Compressive Strength

According to Joshi *et al*. findings [67], the performance of cement both mortar prisms and concrete cubes were subjected to sulphate environment using the outlined exposure regimes (as shown in Table 2). Concentrations of the nutrient broth medium, urea and calcium were 1.3% w/v, 2% w/v and 25mM w/v, respectively.

It was found that, the compressive strength of test cements treated and cured with bacteria solutions indicated as BAT and BST increased as compared to the blank. The treated test cements exhibited 35% increase in compressive strength while the test cement cured through spraying with bacteria solution gave an increase in compressive strength up to 16% as compared to the control test cements. The authors attributed the compressive strength increase to biocementation. Mittermayr *et al.*, [68] showed that the increase in compressive strength could be as a result of densification of cementitious matrix at microstructural level. Najjar *et al.*, [69] in their work reported that ingress of sulfate ions into the concrete matrix triggers the formation of expansive products that fills the pores and voids leading to the densification of microstructure...
Table 2. Exposure of test cements (concrete cubes and mortar prisms) in both blank and bacterial solutions

| Specimens      | Material Used                              | Mechanism of curing                                      |
|----------------|--------------------------------------------|----------------------------------------------------------|
| Control Sample | Cement:Sand:Course Aggregate, Water/Cement =0.5 | Water curing for 28 days                                 |
| Concrete Cubes | Bacterial admixed treated (BAT)            | Submersion in NB media, urea CaCl\(_2\) and bacterial culture for 28 days |
|                | Cement:Sand:Course Aggregate Bacterial Culture/Cement =0.5 |                                             |
|                | Bacterial spray treated (BST)              | Bacterial spray on specimens twice a day till 28 days     |
|                | Cement:Sand:Course aggregate, Water/ cement =0.5 |                                             |
| Control Sample | Cement:Sand, =0.47                        | Water curing for 28days                                 |
| Mortar Prisms  | Bacterial Admixed Mortar (BAM)             | Submersion in NB media, Urea CaCl\(_2\) and bacterial culture for 28days |
|                | Cement: Sand Bacterial culture/ cement=0.47 |                                             |
| Bacterial Spray mortar (BSM) | Cement:Sand =0.47 | Water/Cement Bacterial Spray on specimens twice a day till 28 days |

during the initial exposure of concrete to sulfate rich areas. Summit et al., [67] observed severe strength loss in control test cement after 12 months of exposure to sulphate solutions. The compressive strength of the control test cement (blank) decreased by 30% as compared to the initial strength before exposure while the bacteria treated test cement showed no significant drop in strength. This decrease in strength was attributed to increased penetration of sulfate salts initiating higher buildup of expansive products in the pores of the control test cements. Formation of ettringite crystals reduces the quantity of CH and C\(_3\)A of the cementitious matrix and the salt crystallization pressure within the pores of the cement mortar [70, 71]. Several other authors have also shown the increase in compressive strength as a result of incorporating bacteria [72-75]. While the studies are based on simulated laboratory experiments, it would be necessary if beneficial bacteria were incorporated into a sulfate rich environment where biodegrading bacteria are present such as sewage set up. This would help establish whether it would be suitable to introduce the remedial bacteria during the mixing as mix water or use the remedial bacteria solution in curing the concrete.

3.1.2 Visual Appearance/ Observations

The studies conducted by [67] clearly showed distinct difference in control specimens versus bacterial treated specimens when subjected into sulfate solutions. According to the authors there was clear sign of degradation of the control specimen after 12 months of exposure to sulfate solution. The bacterial treated specimen did not show any sign of degradation. Figures 2, 3 and 4 show the appearance of control specimens, BAT and BST respectively when exposed to sulfate solutions. These results are according to Sumit et al., findings [67].

3.1.3 Change in Mass

Ingress of sulfate ions into the cement mortar/ concrete causes increase in mass by about 0.8% [67]. Maes et al., [76] reported that the increase in mass of concrete / mortar exposed to sulphates could be attributed to the formation of expansive products. The authors argued that the sulphate ions reacts with the hydration products leading to compacted microstructure. According to [77] a high porous concrete gains more mass due to high rate of sulphate ions ingress. Najjar et al., [78] reported that higher pore volume contributes to the transportation of sulfate ions hence filling of concrete pores with expansive products.
Each concrete cube measured 100 mm x 100 mm x 100 mm.

Figure 2. Visual exposure of control specimen (100x100x100mm) after sulfate exposure at the age of (A) 30 days, (B) 90 days, (C) 180 days, (D) 270 days and (E) 365 days.

Each concrete cube is 100 mm x 100 mm x 100 mm

Figure 3. Visual Appearance of BAT specimen (100x100x100mm) after sulfate exposure at the age of (A) 30 days, (B) 90 days, (C) 180 days, (D) 270 days and (E) 365 days.

Each concrete cube is 100 mm x 100 mm x 100 mm

Figure 4. Visual Appearance of BST specimen (100x100x100mm) after sulfate exposure at the age of (A) 30 days, (B) 90 days, (C) 180 days, (D) 270 days and (E) 365 days.
3.2 Physical Sulfate Attack

Cement based materials are prone to physical sulfate attack. This attack to the concrete/ cement mortar is characterized by formation of salt efflorescence on the surface of the concrete/ mortar. Nehdi et al., [77] reported that deterioration mechanism in physical sulfate attack is due to stress development from the salt crystallization pressure in the pore structure. Sulfate ions penetrates into the concrete pores through capillary suction [79]. Sulfate salts crystallizes on the concrete surface since at the upper dry surface, the rate of evaporation exceeds the rate of capillary rise. Scherer G.W. reported that salt solution uptake into the pores by capillary pressure is dependent on the pore distribution of porous body as well as its wetting behavior [80].

According to Sumit et al., [67] thick deposition of salt efflorescence was observed on the upper surface of the control specimens. The authors further noted that there was a crack developed on the control specimens after 90 days of exposure. The bacterial treated specimens showed excellent resistant to sulfate attack. There was no visible damage such as surface scaling or crack formation observed in the bacteria treated samples (BSM and BAM) as indicated in Table 2. The authors attributed the resistance to calcite precipitation. The figures below show the influence of physical sulfate attack as reported by Joshi et al. (Figures 5, 6 and 7) [67].

![Figure 5](image_url)

Figure 5. Typical salt efflorescence development in control prism after exposure at the age of (a) 30 days, (b) 90 days (c) 180 days (d) 270 days (e) 365 days.

![Figure 6](image_url)

Figure 6. BSM prism after exposure sulfate at the age of (a) 30 days (b) 90 days (c) 180 days (d) 270 days (e) 365 days.
Each mortar prism is 285 mm x 25 mm x 25 mm

3.3 Microscopic Evaluation
Summit et al. [67] reported increased ettringite formation at the age of 90 days in control specimens cured in sodium sulfate solution, whereas the bacterial cured specimens exhibited increased calcium carbonate formations. The growth of calcite might be attributed to MICP. In other studies, [81-84] SEM results showed that the concrete mixed with bacteria had improved the microstructure which was attributed to calcite precipitation by the bacteria. According to Iheanyichukwu and Vijay reports [37, 85], the incorporation of bacteria into the concrete improved the overall microstructure of the concrete. The calcite precipitation filled the pores of the concrete reducing any ingress of external materials such as sulphates. Figure 8 depicts an example of SEM images for concrete with and without bacteria solution [37]. As seen in Figure 8, concrete with bacteria showed improved morphology as opposed to plain concrete. The authors attributed this behavior to calcite precipitation by the bacteria.

Figure 7. BAM Prism after sulfate exposure at the age of (a) 30 days (b) 90 days (c) 180 days, (e) 365 days

Figure 8. SEM images showing (a) Normal concrete, (b) Bacterial concrete, (c) 5% RHA concrete, and (d) Bacterial concrete with 5% RHA [85].
4. Conclusion

This review work has established that cement-based materials exposed to sulfate rich-environment are prone to chemical and physical sulfate attack. Durability of such structures is reduced significantly. Further, it was shown that MICP improves the mechanical properties of cement-based materials. As such, the ingress of sulfate ions in microbial treated concrete/ mortar is reduced significantly resulting to increased compressive strength. However, it was found that there is a need for conducting more research about the use of remedial bacteria in environment with deleterious bacteria. This includes environment with sulfate bacteria such as sewage cases. Many structures have failed especially in Kenya in such environments where remedial measures have not been taken early in advance. Such cases may involve preparation of concrete with remedial bacteria.

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