Research Article

Journal of Environmental Nanotechnology

# Effect of Microbial Calcite Precipitation on the Performance of Concrete Incorporating Different Bacterial Strains

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Received: 05.08.2025 Accepted: 23.09.2025 Published: 30.09.2025

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## ABSTRACT

Concrete is an unavoidable material of modern construction, but it remains susceptible to cracking, which reduces the strength and durability of the material over time. The need for sustainable alternatives is underlined by traditional repair methods that are often temporary and costly. The integration of bacteria in cementitious systems represents a bio-based approach, in which microbial-induced calcite precipitation (MICP) not only increases strength but also includes self-healing ability. In this study, three strains of bacteria, *Bacillus subtilis* (BS), *Sporosarcina pasteurii* (SP), and *Bacillus sphaericus* (BSp), were directly added to concrete to investigate the effect of these strains on the mechanical and durability properties of the concrete. The findings demonstrated that all bacterial concretes outperformed the control group. At 28 days, the compressive strength was maximum for BSp-1 at 39.62 MPa, followed by BS-2 at 38.36 MPa and SP-2 at 38.20 MPa. Microstructural study confirmed that the presence of calcite filling of pores and cracks leads to denser and more durable concrete. Among all three variants, the blended mix BSp-1 exhibited superior strength, microstructure, and durability. This study concluded that the addition of bacteria enhanced the strength, permeability, durability, and microstructural properties of the concrete.

**Keywords:** Bacterial concrete; Microbially Induced Calcite Precipitation (MICP); *Bacillus subtilis*; *Sporosarcina pasteurii*; *Bacillus sphaericus*; Self-healing properties.

## 1. INTRODUCTION

Concrete is the backbone of modern infrastructure and is widely used to construct buildings, bridges, highways, and dams. Its annual consumption worldwide has exceeded 10 billion tonnes and is the second most consumed substance following water (Hache *et al.* 2020; Danish *et al.* 2019; Soomro *et al.* 2023). The reasons for its dominance are obvious: flexible, somewhat cheap, and processed from raw materials, which are available in countless amounts in nature. However, for all these advantages, concrete has one very identified weakness - it is brittle and highly prone to cracking (Yoo *et al.* 2019; Zhang *et al.* 2021; Golewski, 2023). Cracks develop from drying shrinkage, thermal strains, overloading, and environmental strains. Such cracks, when initially small, affect the impermeability of the material where they exist because they form a conduit for aggressive agents, like water, chlorides, sulphates, and carbon dioxide (Gardner *et al.* 2018; Safiuddin *et al.* 2018; Yoo *et al.* 2019). Once they enter the matrix, they will act to corrode the reinforcement and decrease the durability of the structure significantly.

In order to reduce the issue of cracking, engineers have come up with a variety of traditional methods of repair over the years. They are epoxy injections, polymer coating, and surface sealant, which offer only temporary solutions by filling or sealing cracks (Issa, 2009; Min *et al.* 2025). These techniques, however, seldom become successful in the long run. Their bonding with the existing matrix is often ineffective; they are incompatible under changing environmental conditions and demand recurring maintenance interventions during the service life of a structure. These practices are more expensive, waste more energy and materials, and do not support sustainability in construction.

Researchers have responded by exploring the idea of self-healing concrete, which is a material that can automatically heal cracks without any outside help. It gets inspired by biological systems, whereby healing is natural. One of the most promising strategies that has been investigated is bacterial concrete (Wang *et al.* 2014; Belie *et al.* 2018; A. and Das, 2025; Sukumaran *et al.* 2025). The technique originated in the early 2000s and is based on microbially induced calcium carbonate precipitation (MICP) (Jonkers, 2007; Chaurasia *et al.* 2019; Nguyen *et al.* 2019; Ryparová *et al.* 2021; Nasser *et al.* 2022; B *et al.* 2025).

Bacterial strain selection is a key factor in achieving the success of bacterial concrete. Not every microorganism is capable of living in the extremely alkaline (pH > 12) concrete environment (Nodehi *et al.* 2022). Spores-forming, ureolytic, alkali-resistant strains are the most frequently studied strains capable of surviving the severe climate of cement hydration. Three of these are especially successful: *Sporosarcina pasteurii* (SP), *Bacillus subtilis* (BS), and *Bacillus sphaericus* (BSp) (Nguyen *et al.* 2019; Solarte *et al.* 2024; Reddy and Revathi, 2019; Yamasamit *et al.* 2023; Seo *et al.* 2022).

Autogenous healing is the method by which concrete heals itself. Autogenous healing is a process in which unhydrated cement particles interact with external water to seal cracks (Amoorezaei and Ghanbari, 2025). Bacteria remain alive in the cementitious setting and, on activation, generate carbonate ions that interact with calcium ions to form $CaCO_3$. The precipitated $CaCO_3$ seals the cracks and pores, restores water-tightness, and increases the strength and durability (Chaurasia *et al.* 2019; Nguyen *et al.* 2019; Nasser *et al.* 2022; Sunantha *et al.* 2025). The self-healing caused by bacteria reduces maintenance needs and extends the service life of concrete structures considerably (Khushnood *et al.* 2020).

Bacterial crack healing is an effective process and quite simple in its mechanism. As an example, urease enzymes are synthesized by *Sporosarcina pasteurii* in the presence of urea and hydrolyze urea into carbonate ions and ammonium ions (Henze and Randall, 2018; Brito and Kurda, 2021). The carbonate ions will then combine with calcium ions in the pore solution to create $CaCO_3$ crystals, which will gradually build up in the cracks and pores. In the same way, *Bacillus subtilis* uses calcium lactate to form the carbonate ions, which result in the same calcite precipitation (Sonali *et al.* 2020). A similar ureolytic activity occurs in *Bacillus sphaericus*. Not only does this biogenic precipitation seal cracks, it also enhances the microstructure by decreasing porosity and permeability, thus increasing long-term durability (Sandbhor *et al.* 2025). Several studies have established that bacteria-induced concrete has better strength, durability, and enhanced microstructural features as compared to conventional concrete (Khaliq and Ehsan, 2016; Gupta *et al.* 2017; Reddy and Kavyateja, 2019; Amoorezaei and Ghanbari, 2025; Jonkers, 2011).

The current study aims to fill the gap by experimentally testing the performance of the bacterial concrete with three strains: *Bacillus subtilis* (BS), *Sporosarcina pasteurii* (SP), and *Bacillus sphaericus* (BSp). The study is focused on the evaluation of mechanical properties, durability characteristics, and microstructural behavior. The findings provide clear and reproducible ideas for selection of strains and mix design, allowing for more durable and practical implementation of MICP concrete.

**Table 1. Mix details of various bacterial strains with calcium lactate**

| Mix ID | Bacterial Strain | Concentration (cells/mL) | Calcium Lactate (% of cement) |
|---|---|---|---|
| BS-1 | *Bacillus subtilis* | $10^5$ | 0.5 |
| BS-2 | *Bacillus subtilis* | $10^6$ | 1.0 |
| SP-1 | *Sporosarcina pasteurii* | $10^5$ | 0.5 |
| SP-2 | *Sporosarcina pasteurii* | $10^6$ | 1.0 |
| BSp-1 | *Bacillus sphaericus* | $10^5$ | 0.5 |
| BSp-2 | *Bacillus sphaericus* | $10^6$ | 1.0 |

## 2. MATERIALS AND METHOD

### 2.1 Materials Utilised

#### 2.1.1 Cement

The study utilised the Ordinary Portland Cement (OPC) of 53 grade, which adheres to IS 12269:2013. The cement had a specific gravity of 3.15, a standard consistency of 31%, and setting times of 64 (initial) and 285 (final) minutes.

#### 2.1.2 Aggregates

Locally produced river sand conforming to Zone II according to IS 383:2016 was employed as fine aggregate, along with crushed stone aggregate of 20 mm size. The sand demonstrated a specific gravity of 2.63, a bulk density of 1525 kg/m³, and a fineness modulus of 2.8, whereas crushed granite coarse aggregate had a specific gravity of 2.8, and a bulk density of 1650 kg/m³.

#### 2.1.3 Water

The mixing and curing were carried out using potable tap water purified from organic impurities meeting IS 456:2000.

#### 2.1.4 Bacterial Strains

Three bacterial strains, *Bacillus subtilis* (BS), *Sporosarcina pasteurii* (SP), and *Bacillus sphaericus* (BSp), were obtained from Bioedge Solutions, Bengaluru, India. They were cultured in nutrient broth for 24–48 hours at 30–35°C, subsequently harvested through centrifugation, and diluted with sterile distilled water to create suspensions of $10^5$ and $10^6$ cells/mL.

#### 2.1.5 Nutrient Source

A nutrient source was included in the bacterial mixes to encourage precipitation of calcium carbonate by

microbes. Calcium lactate was chosen as the primary nutrition in this research due to its dual function of supplying calcium ions and serving as a carbon source. The bacteria employ lactate as an energy substrate, generating carbonate ions that subsequently react with calcium ions to produce solid $CaCO_3$. Calcium lactate was added in the concentrations of 0.5-1.0 percent of cement weight dissolved directly in the bacterial suspension to achieve uniform distribution of the additive throughout the fresh mix. It was chosen as an alternative to other salts, such as calcium chloride, calcium acetate, because it is biocompatible, less prone to corrosion, and it effectively promotes mineralization of bacteria.

## 2.2 Mix Proportions

Concrete mix was developed to M30 grade according to IS 10262:2019. The control mix (CM) was initially prepared without the inclusion of bacteria. The water-to-binder ratio was chosen at 0.45, and the mixture was formulated in the ratio of 1:1.6:3.0 (cement: sand: coarse aggregate). The bacterial suspensions were used to replace a part of the mixing water in the bacterial mixtures. The mix details adopted in the study are provided in Table 1. Two concentrations of each bacterial strain ($10^5$and$10^6$ cells/mL) were added to make six bacterial mixes besides the control. This variation was selected to analyze the impact of strain type and cell concentration on the performance of concrete.

## 2.3 Methodology

### 2.3.1 Formulation of Bacterial Suspension

The isolates of bacteria were cultured in nutrient broth until they attained the exponential growth phase, which is optimal for metabolic activity. After that, the cells were separated from the medium by centrifuging the cultures at 5000 rpm for 10 minutes. The cell density was adjusted to $10^5$ and $10^6$ cells/mL using a spectrophotometer to ensure accuracy after the harvested cells were re-suspended in sterile distilled water. These suspensions were used immediately in concrete mixing to ensure bacterial viability. Fig.1 depicts the process of carbonate formation facilitated by bacterial activity.



**Fig. 1: Carbonate precipitation by bacterial suspensions**

### 2.3.2 Mixing Process

The mixing of concrete was done in a laboratory pan mixer. Cement and aggregates were initially dry blended to form a homogeneous mixture. Water or bacterial suspension (according to the mix) was then added slowly, and the mixture was mixed. In the case of bacterial mixes, the suspension substituted the respective water amount entirely. The calcium lactate was dissolved in the bacterial solution, and the mixture was then mixed. The mixture was stirred until the mixture became homogeneous.

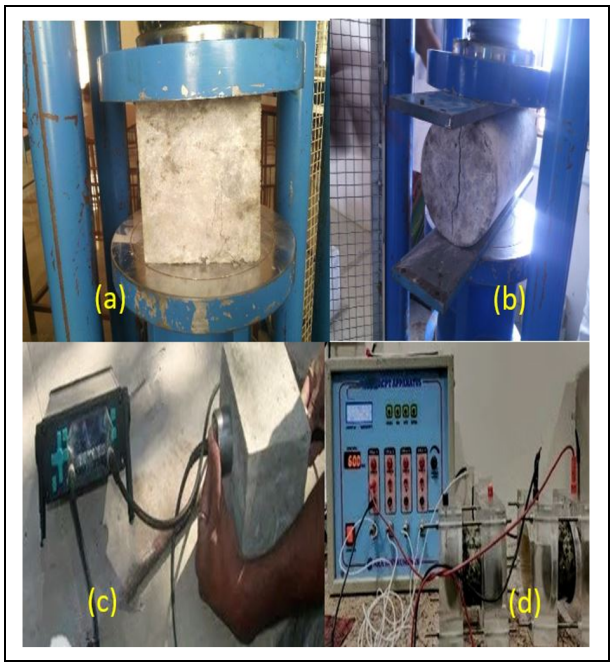



**Fig. 2: Laboratory testing methods for concrete specimens: (a) CS, (b) STS, (c) UPV and (d) RCPT**

### 2.3.3 Casting and Compaction

Standard cube moulds sized 15 cm, cylindrical moulds sized 15 × 30 cm, and prism moulds sized 10 × 10 × 50 cm were employed for casting. The air was eliminated by pouring the concrete in layers and consolidating it with a table vibrator. The surface was completed promptly after the casting procedure.

### 2.3.4 Curing

The test specimens were separated from their casts and placed in water curing at ambient temperature (27 ± 2 °C) after 24 hours. This mode of curing was chosen in order to simulate the conditions of a field, which is critical for the hydration of cement as well as for the activity of bacteria.

### 2.3.5 Testing Program

The test was carried out on the specimens post-curing to assess the impact of bacterial addition on the

strength and durability of the concrete. The compressive strength (CS) was evaluated using cubes in compliance with IS 516 (Part 1: 2021). The split tensile strength (STS) was measured in cylinders, while the modulus of rupture (MOR) was evaluated in prisms using two-point loading to determine the concrete's resistance to bending.

A non-destructive Ultrasonic Pulse Velocity (UPV) test was conducted to identify defects within the samples and to determine the overall quality of the samples without destruction, in accordance with IS 13311 (Part 1). To ensure the required durability, Saturated water absorption (SWA) along with rapid chloride permeability tests (RCPT) were conducted. The SWA test provided data on the pore structure and permeability. The RCPT conducted in accordance with ASTM C1202 indicated the resistance of the concrete against the diffusion of chloride ions. Furthermore, the microstructure was examined using Scanning Electron Microscopy (SEM) to analyze the surface alterations induced by bacteria. Fig.2 depicts the laboratory tests carried out on concrete specimen.

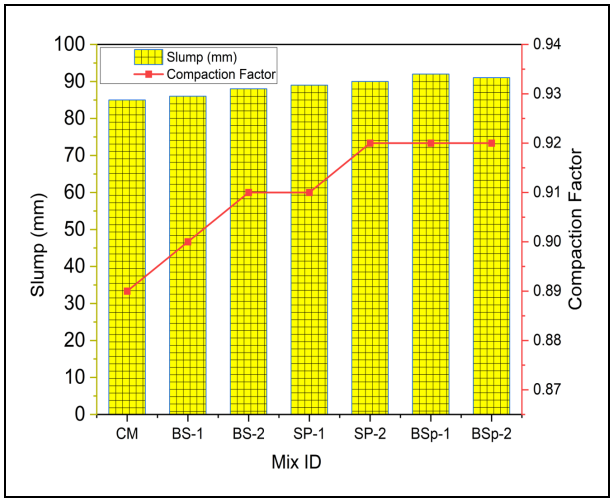



**Fig.3: Results of workability assessment**

## 3. EXPERIMENTAL OUTCOMES AND DISCUSSION

### 3.1 Workability of Fresh Concrete

The test data indicated that the slump values of the control matrix were 85 mm, whereas the bacterial mixes indicated 92 mm slump. The compaction factor was between 0.89 and 0.92, as shown in Fig.3. The compaction factor was 0.90-0.91, and the slump was 86-88 mm with the inclusion of *Bacillus subtilis* (BS), indicating that the higher the bacteria concentration ($10^6$ cells/ml), the better it performed (Kandaswamy *et al.* 2024). The values of *Sporosarcina pasteurii* (SP) were higher (slump of 89 mm to 90 mm and compaction factor of 0.91 to 0.92), which could be attributed to its high urease activity that facilitated greater precipitation of calcium carbonate (Khoshtinat, 2023). The optimal performance was achieved using *Bacillus sphaericus* (BSp), with a slump of 91 to 92 mm and compaction factor settling at 0.92, despite a lower level of concentration. This implies that *B. sphaericus* is very effective in enhancing mix workability, as well as mix density.

### 3.2 Mechanical Properties

Mechanical testing is crucial in assessing the overall performance and strength of concrete. The findings demonstrate the impact of bacterial inclusion on the material's load-bearing capability and structural integrity across several circumstances. The mechanical performance is listed in Table 2.

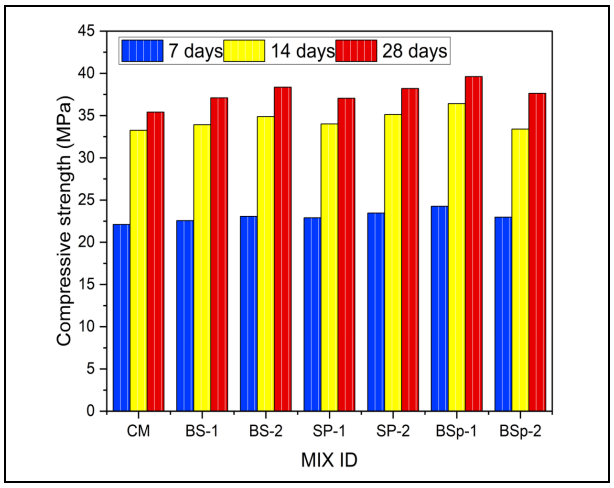



**Fig. 4: CS of control and bacterial concrete samples**

#### 3.2.1 Compressive strength (CS)

The CS performance clearly shows the positive impact of bacterial inclusion in concrete. Bacterial mixes were stronger than the control at all ages, but the strength increased more significantly at 28 days. The control mix attained 35.40 MPa at 28 days, while bacterial mixes varied from 37.05 to 39.62 MPa, indicating strength enhancements of around 5–12%, as depicted in Fig. 4. *Bacillus sphaericus* at a higher concentration (BSp-1) was the strongest, with 39.62 MPa. However, *Sporosarcina pasteurii* (SP-2) and *Bacillus subtilis* (BS-2) also improved significantly by around 8 percent. The enhancement in strength can be attributed to the microbiological precipitation of $CaCO_3$, which fills the gaps and microcracks, reduces porosity, and hence raises the density of the cement matrix (Danish *et al.* 2020). This contributes to a highly developed pore structure and interfacial transition zones (ITZ) between the paste and aggregate.

The same increases in the strength of the concrete by bio-mineralization by ureolytic bacteria have been reported earlier. The more significant influence of

bacterial activity on later ages, and especially the increased strength gains at 28 days. These findings validate earlier research and confirm the concept that the incorporation of bacteria, specifically *Bacillus sphaericus*, could be an efficacious approach to enhancing the CS of concrete (Pachaivannan et al. 2020; Khaudiyal *et al.* 2022).

### 3.2.2 Split Tensile Strength (STS)

The outcomes of STS indicated that at 7 and 28 days, all bacterial combinations increased the tensile strength more than the control mix. The STS of CM was 2.35 MPa at 7 days, with higher values being 2.47 to 2.73 MPa between bacteria mixes, which is an improvement of approximately 5-16%. The CM attained 3.61 MPa by 28 days, compared with the bacterial mixes of 3.80 to 4.20 MPa as illustrated in Fig. 5. The *Bacillus sphaericus* mix (BSp-1) recorded the highest strength, approximately 16 % higher than the control.

The biogenic calcite closes fine cracks and increases the strength of the ITZ between the paste and aggregates, thus decreasing stress concentrations that normally cause tensile failure. The tensile behavior of bacterial concrete has also shown similar enhancement, with researchers reporting that bacterial activity increases crack resistance and slows crack propagation. This supports the fact that bio-concrete can be used to considerably increase tensile strength to help combat one of the fundamental drawbacks of conventional concrete (Mondal *et al.* 2018; Achal *et al.* 2011).

**Table 2. Mechanical properties on various mixes**

| Mix Identification | CS (MPa) | | | STS (MPa) | | MOR (MPa) | | UPV (km/s) |
|---|---|---|---|---|---|---|---|---|
| | 7 d | 14 d | 28 d | 7 d | 28 d | 7 d | 28 d | 28 d |
| CM | 22.11 | 33.26 | 35.40 | 2.35 | 3.61 | 2.99 | 4.60 | 4.32 |
| BS-1 | 22.56 | 33.92 | 37.10 | 2.49 | 3.84 | 3.13 | 4.82 | 4.45 |
| BS-2 | 23.06 | 34.87 | 38.36 | 2.55 | 3.93 | 3.22 | 4.95 | 4.62 |
| SP-1 | 22.90 | 34.00 | 37.05 | 2.52 | 3.87 | 3.09 | 4.75 | 4.55 |
| SP-2 | 23.45 | 35.13 | 38.20 | 2.54 | 3.91 | 3.17 | 4.88 | 4.60 |
| BSp-1 | 24.26 | 36.41 | 39.62 | 2.73 | 4.20 | 3.30 | 5.07 | 4.85 |
| BSp-2 | 22.97 | 33.40 | 37.63 | 2.47 | 3.80 | 3.18 | 4.89 | 4.79 |

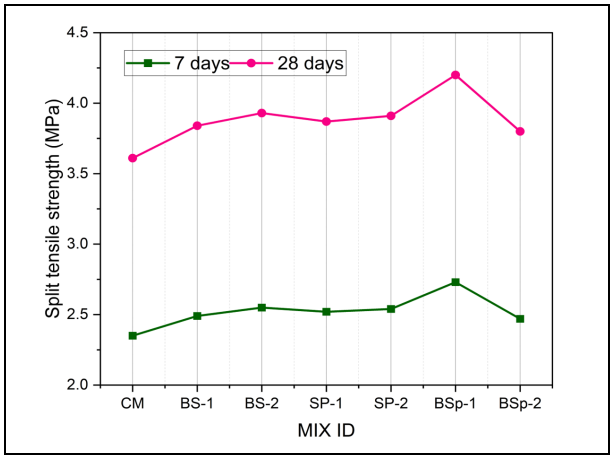



**Fig. 5: STS of control and bacterial concrete samples**

### 3.2.3 Modulus of Rupture (MOR)

The MOR results revealed that the integration of bacteria showed a definite positive impact on the flexural performance of concrete. At 7 days, the values were 2.99 MPa for CM and 3.30 MPa for the *Bacillus sphaericus* mix (BSp-1), which demonstrates an improvement of around 5 to 10 percent. The control exhibited a maximum value of 4.60 MPa at 28 days, while the bacterial mixtures reached peak values ranging from 4.75 to 5.07 MPa. BSp-1 had the maximum flexural strength at 5.07 MPa, representing a 10 percent enhancement relative to the control. The MOR is especially strongly influenced by the microstructural soundness of the cement matrix, and the existence of bacterial calcite will contribute to slowing down the initiation and development of the crack (Kurda *et al.* 2022; Andalib *et al.* 2016).

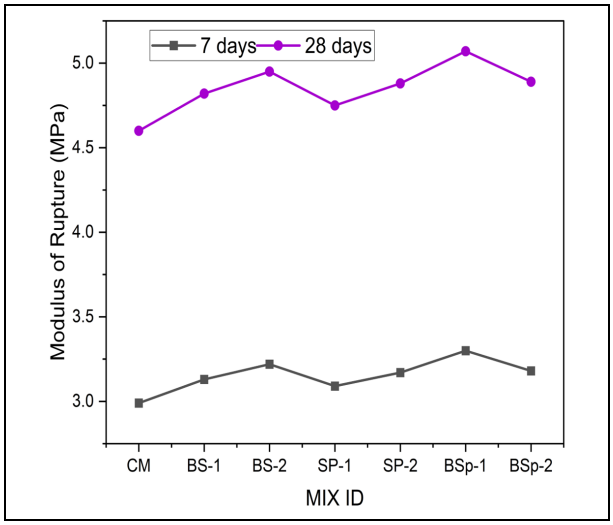



**Fig. 6: MOR values for control and bacterial concrete mixes**

### 3.2.4 Ultrasonic Pulse Velocity (UPV)

The UPV measurements at 28 days indicated an apparent development in the internal properties of

bacterial concrete over the control. The UPV of 4.32 km/s obtained for the control mix is considered good quality by IS 13311:1992. Comparatively, the bacterial concretes reported bigger values between 4.45 and 4.85 km/s, which imply that they possessed denser and homogenized microstructures. Fig. 7 shows that the maximum value was recorded in the BSp-1 blend of *Bacillus sphaericus* at 4.85 km/s, approximately 12 % higher than the control. There were also other significant improvements seen in other mixes like BS-2 4.62 km/s and SP-2 4.60 km/s. These increased velocities have been explained by the deposition of biogenic calcium carbonate that fills pores, microcracks, increases the continuity of the matrix, and minimizes the sound wave discontinuities (Bashaveni and Pannem, 2024). Altogether, the findings of the UPV support the mechanical strength results and prove that the combination of bacteria boosted the strength of concrete and its overall quality (Aldea *et al.* 2000).

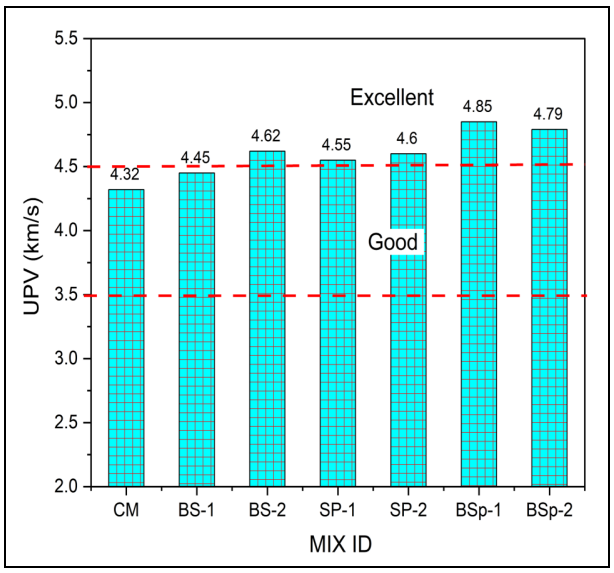



**Fig.7: Test outcomes on UPV test**

## 3.3 Durability Performance

The resilience of concrete to endure unfavorable conditions without compromising its strength is a significant attribute, illustrating its endurance. Saturated Water Absorption (SWA) and Rapid Chloride Permeability (RCPT) assessed the durability of bacterial concrete in this study. These tests determine the permeability of water or chloride ions through the concrete, directly influencing its long-term performance.

### 3.3.1 Saturated Water Absorption

The obtained water absorption results clearly indicate that incorporation of bacteria decreases the permeability of concrete, as depicted in Fig.8. This was observed to have the highest absorption of the control mix (CM) at 4.84% with much lower values ranging between 3.30% and 3.75%, which corresponded to a reduction of approximately 22-32%. The minimal was received in the *Bacillus sphaericus* mix (BSp-1) with 3.30%. This reduction can be attributed to the bacterial accumulation of calcium carbonate in pores and spaces, which enhances the microstructure and diminishes water infiltration (Phung *et al.* 2013).

This follows the earlier findings of Wang *et al.* (2012) and Achal *et al.* (2011a), who stated that bacterial activity decreases porosity and enhances impermeability of concrete. The reduction of water absorption leads to a direct proportional rise in the durability, which proves the positive effect of bacteria in altering the pore structure of the concrete matrix (Achal *et al.* 2011b; Wang *et al.* 2012).

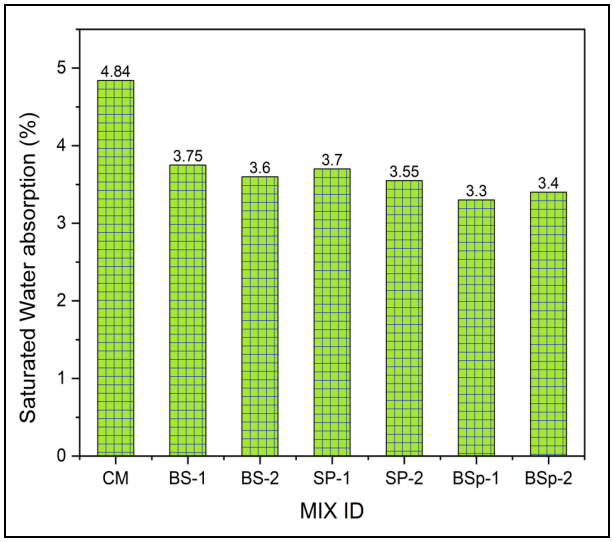



**Fig.8: Test results on Saturated water absorption**

### 3.3.2 Rapid Chloride Permeability (RCPT)

The RCPT results validated that bacterial activity markedly enhanced the durability of concrete by lowering chloride ion permeability. The RCPT of CM was 2980 C at 30 days, which corresponds to the moderate range (2000 to 4000 C) of chloride permeability according to ASTM C1202. Bacterial concretes had lower values between 2370 and 2700 C, which represents a reduction of around 10–20% compared to CM, as illustrated in Fig. 9. At 60 days, the control decreased to 2320 C, remaining within the moderate range, while bacterial mixtures declined to between 1920 and 2145 C. The highest value was with the *Bacillus sphaericus* mix (BSp-1), at 1920 C, which is quite near the low permeability range (1000 to 2000 C). This implies that as curing time is prolonged or optimum bacterial dosage is obtained, chloride permeability may be lowered further (Chahal *et al.* 2012; Nosouhian *et al.* 2016). This enhancement may be explained by the precipitation of calcium carbonate by microbes when $CaCO_3$ crystals occupy pores and micro-cracks, which in

effect prevents the chloride transportation pathways (Pachaivannan *et al.* 2020).

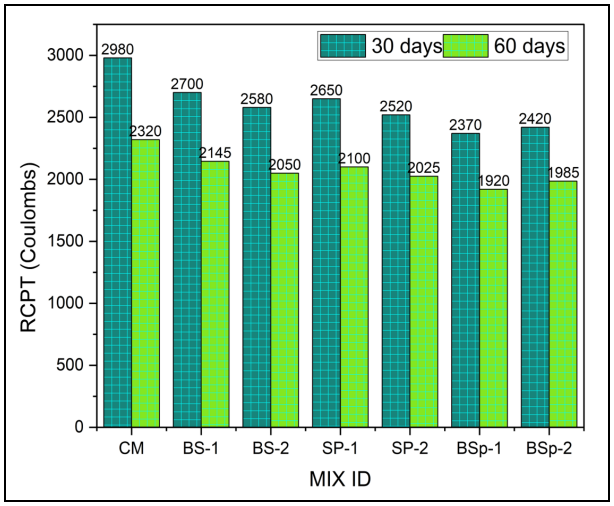



**Fig. 9: Comparison of RCPT values between control and bacterial concretes**

## 3.4 SEM Analysis

The optimal mixtures were chosen for SEM evaluation to examine the surface morphology and microstructural characteristics of the concrete. The micrographs provide detailed information regarding the hydration products, bonding integrity, and pore architecture of the mixture. The investigation demonstrates the association between microstructural properties and the mechanical and durability performance of concrete, hence validating the effectiveness of the selected optimal mixtures.

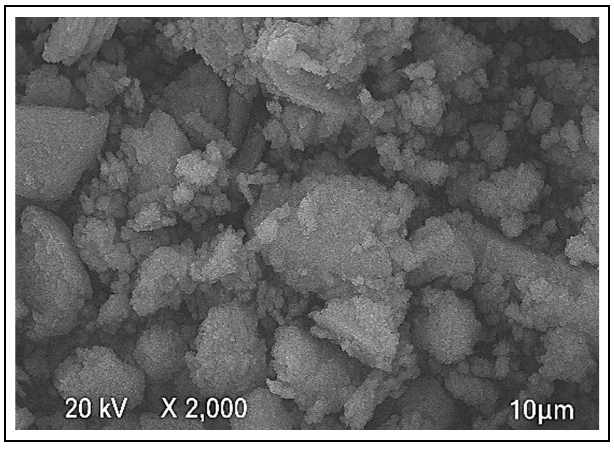



**Fig. 10: SEM micrograph of *Bacillus subtilis* (BS-2) concrete mix**

The SEM photograph of the treated *Bacillus subtilis* (BS-2) specimen depicts the thick deposition of crystalline substance in the cement matrix as in Fig.10. The precipitated $CaCO_3$ can be seen as crystallized structures that are tightly packed around the cement particles and that seem to fill the micro-voids and bridge the gaps (Luo and Qian, 2016; Althoey *et al.* 2023). This microstructural change indicates that the bacteria were active in the cementitious setting and stimulated mineralization, resulting in refinement of pores. This deposition leads to better densification, lower permeability, as well as better strength of the concrete. The observed morphology confirms the ability of *Bacillus subtilis* to initiate effective self-healing at a microscopic level, correlating with its potential to enhance and prolong the service life of concrete structures.

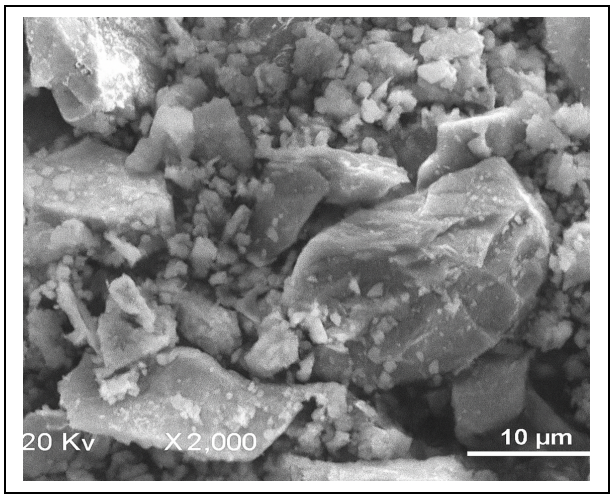



**Fig. 11: SEM micrograph of *Sporosarcina pasteurii* (SP-2) concrete mix**

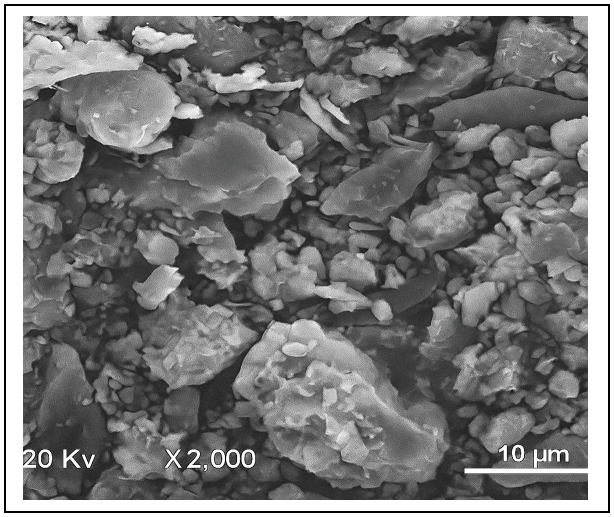



**Fig. 12: SEM micrograph of *Bacillus sphaericus* (BSp-1) concrete mix**

The SEM photograph of the *Sporadiscina pasteurii* (SP-2) treated sample indicates that there is a high level of crystalline deposition throughout the cement matrix, as shown in the Fig.11. The precipitated $CaCO_3$ is seen as fine particles which coat the surfaces of the hydration products in large numbers and get attached

to the larger cementitious fragments in a very strong way (Jena *et al.* 2020). The presence of such deposits, in comparison to untreated concrete, suggests that the micro-organisms present are highly active, and urease produced by *S. pasteurii* decomposes urea to produce carbonate ions, which react with calcium to form $CaCO_3$. This even distribution of the mineral precipitates not only fills the micro-cracks and voids, but also leads to a more compact microstructure.

The SEM micrograph of the treated specimen *Bacillus sphaericus* (BSp-1) demonstrates the presence of crystalline precipitates embedded into the cementitious matrix, as demonstrated in Fig. 12. The deposits of the $CaCO_3$ are relatively coarse and irregular in shape and are scattered across the hydration products as filling the holes and the surface cracks. In contrast to *Sporosarcina pasteurii*, the crystal is more localized in its mineralization, demonstrating relatively slow kinetics of precipitation. However, $CaCO_3$ deposition leads to refinement and densification of the matrix pores, which is likely to enhance resistance to permeability and increase durability. The results indicate that although *B. sphaericus* is an effective self-healing promoter, its functionality is not as consistent as *S. pasteurii* and yet has great structural advantages, especially in minimizing the number of micro-pores (Jena *et al.* 2020).

## 4. CONCLUSIONS

Three bacterial strains (*Bacillus subtilis*, *Sporosarcina pasteurii*, *Bacillus sphaericus*) were directly incorporated into concrete using the direct mixing method. The bacterial inclusion improved fresh workability, as reflected by higher slump and compaction factor values compared to the control. Bacterial concretes exhibited significant enhancement in mechanical strength due to microbially induced calcite precipitation, which blocked pores and microcracks, thereby increasing load-bearing capacity. Improved durability was observed in terms of reduced water absorption, lower chloride penetration, and diminished microcrack formation, with evidence of self-healing behavior. The SEM analysis confirmed $CaCO_3$ deposition in optimal bacterial mixes, validating bacterial activity in microstructural refinement.

## FUNDING

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

## CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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## REFERENCES

Achal, V., Mukherjee, A. and Reddy, M. S., Effect of calcifying bacteria on permeation properties of concrete structures, *J. Ind. Microbiol. Biotechnol.,* 38(9), 1229–1234(2011a). https://doi.org/10.1007/s10295-010-0901-8

Achal, V., Mukherjee, A. and Reddy, M. S., Microbial Concrete: Way to Enhance the Durability of Building Structures, *J. Mater. Civ. Eng.,* 23(6), 730–734(2011b). https://doi.org/10.1061/(ASCE)MT.1943-5533.0000159

Aldea, C. M., Song, W. J., Popovics, J. S. and Shah, S. P., Extent of Healing of Cracked Normal Strength Concrete, *J. Mater. Civ. Eng.*, 12(1), 92–96(2000). https://doi.org/10.1061/(ASCE)0899-1561(2000)12:1(92)

Althoey, F., Zaid, O., Arbili, M. M., Martínez-García, R., Alhamami, A., Shah, H. A. and Yosri, Ahmed, M., Physical, strength, durability and microstructural analysis of self-healing concrete: A systematic review, *Case Stud. Constr. Mater.,* 18, e01730(2023). https://doi.org/10.1016/j.cscm.2022.e01730

Amoorezaei, K. and Ghanbari-Ghazijahani, T., A comprehensive review: Self-healing methods and cementitious composites, *Struc.,* 72, 108038(2025). https://doi.org/10.1016/j.istruc.2024.108038

Andalib, R., Abd Majid, M. Z., Hussin, M. W., Ponraj, M., Keyvanfar, A., Mirza, J. and Lee, H. S., Optimum concentration of Bacillus megaterium for strengthening structural concrete, *Constr. Build. Mater.,* 118, 180–193(2016). https://doi.org/10.1016/j.conbuildmat.2016.04.142

ASTM C1202 – Standard test method for electrical indication of concrete's ability to resist chloride ion penetration, *ASTM International* (2012).

Bashaveni, B. and Pannem, R. M. R., Development of self-healing property in self-compacting concrete, *Case Stud. Constr. Mater.,* 20, e02942(2024). https://doi.org/10.1016/j.cscm.2024.e02942

Belie, D. N., Gruyaert, E., Al-Tabbaa, A., Antonaci, P., Baera, C., Bajare, D., Darquennes, A., Davies, R., Ferrara, L., Jefferson, T., Litina, C., Miljevic, B., Otlewska, A., Ranogajec, J., Roig-Flores, M., Paine, K., Lukowski, P., Serna, P., Tulliani, J., Vucetic, S., Wang, J. and Jonkers, H. M., A Review of Self-Healing Concrete for Damage Management of Structures, *Adv. Mater. Interf.*, 5(17), 1800074(2018). https://doi.org/10.1002/admi.201800074

Brito, D. J. and Kurda, R., The past and future of sustainable concrete: A critical review and new strategies on cement-based materials, *J. Clean. Prod.,* 281, 123558(2021). https://doi.org/10.1016/j.jclepro.2020.123558

Chahal, N., Siddique, R. and Rajor, A., Influence of bacteria on the compressive strength, water absorption and rapid chloride permeability of fly ash concrete, *Constr. Build. Mater.,* 28(1), 351–356(2012). https://doi.org/10.1016/j.conbuildmat.2011.07.042

Chaurasia, L., Bisht, V., Singh, L. P., and Gupta, S., A novel approach of biomineralization for improving micro and macro-properties of concrete, *Constr. Build. Mater.,* 195, 340–351(2019). https://doi.org/10.1016/j.conbuildmat.2018.11.031

Danish, A., Salim, M. U. and Ahmed, T., Trends and developments in green cement: A sustainable approach, *Sustain. Struc. and Mater.*, 2, 45–60(2019). https://doi.org/10.26392/SSM.2019.02.01.045

Danish, A., Mosaberpanah, M. A. and Salim, M. U., Past and present techniques of self-healing in cementitious materials: A critical review on efficiency of implemented treatments, *J. Mater. Res. Technol.,* 9(3), 6883–6899(2020). https://doi.org/10.1016/j.jmrt.2020.04.053

Gardner, D., Lark, R., Jefferson, T. and Davies, R., A survey on problems encountered in current concrete construction and the potential benefits of self-healing cementitious materials, *Case Stud. Constr. Mater.,* 8, 238–247(2018). https://doi.org/10.1016/j.cscm.2018.02.002

Golewski, G. L., The Phenomenon of Cracking in Cement Concretes and Reinforced Concrete Structures: The Mechanism of Cracks Formation, Causes of Their Initiation, Types and Places of Occurrence, and Methods of Detection—A Review, *Build.,* 13(3), 765(2023). https://doi.org/10.3390/buildings13030765

Gupta, S., Pang, S. D. and Kua, H. W., Autonomous healing in concrete by bio-based healing agents – A review, *Constr. Build. Mater.,* 146, 419–428(2017). https://doi.org/10.1016/j.conbuildmat.2017.04.111

Hache, E., Simoën, M., Seck, G. S., Bonnet, C., Jabberi, A. and Carcanague, S., The impact of future power generation on cement demand: An international and regional assessment based on climate scenarios, *Int. Econ.,* 163, 114–133(2020). https://doi.org/10.1016/j.inteco.2020.05.002

Henze, J. and Randall, D. G., Microbial induced calcium carbonate precipitation at elevated pH values (>11) using *Sporosarcina pasteurii*, *J. Environ. Chem. Eng.,* 6(4), 5008–5013(2018). https://doi.org/10.1016/j.jece.2018.07.046

IS 12269:2013 – Ordinary Portland cement, 53 grade — Specification. New Delhi: BIS (2013).

IS 383:2016 – Coarse and fine aggregate for concrete — Specification. New Delhi: BIS (2016).

IS 456:2000 – Plain and reinforced concrete — Code of practice. New Delhi: BIS (2000).

IS 10262:2019 – Concrete mix proportioning — Guidelines. New Delhi: BIS (2019).

IS 516 (Part 1):2021 – Method of tests for strength of concrete. New Delhi: BIS (2021).

IS 13311 (Part 1):1992 – Non-destructive testing of concrete — Ultrasonic pulse velocity method. New Delhi: BIS (1992).

Issa, C. A., Methods of crack repair in concrete structures, Failure, Distress and Repair of Concrete Structures, *Woodhead Publishing,* 169–193 (2009)., https://doi.org/10.1533/9781845697037.2.169

Jena, S., Basa, B. and Chandra, P. K., A Review on the Bacterial Concrete Properties, *IOP Conf. Ser. Mater. Sci. Eng.,* 970(1), 012004(2020). https://doi.org/10.1088/1757-899X/970/1/012004

Jena, S., Basa, B., Panda, K. C. and Sahoo, N. K., Impact of *Bacillus subtilis* bacterium on the properties of concrete, *Mater. Today Proc.* 32(4), 651–656(2020). https://doi.org/10.1016/j.matpr.2020.03.129

Jonkers, H. M., Self-Healing Concrete: A Biological Approach, Self-Healing Concrete: A Biological Approach*, Springer Dordrecht*, 195–204 (2007). https://doi.org/10.1007/978-1-4020-6250-6_9

Jonkers, H. M., Bacteria-based self-healing concrete, *Heron,* 56(1/2), 1–12(2011).

Kandaswamy, S., Shanmugam, B., Munuswamy, M. G., Thirumoorthy, P., Ashokkumar, L. and Sisubalan, S., A comprehensive study on microbial self-healing concrete for sustainable construction, *Matér. Rio. Jan.,* 29(2), e20240181(2024). https://doi.org/10.1590/1517-7076-rmat-2024-0181

Khaliq, W. and Ehsan, M. B., Crack healing in concrete using various bio influenced self-healing techniques, *Constr. Build. Mater.,* 102349–357(2016). https://doi.org/10.1016/j.conbuildmat.2015.11.006

Khaudiyal, S., Rawat, A., Das, S. K. and Garg, N., Bacterial concrete: A review on self-healing properties in the light of sustainability, *Mater. Today Proc.,* 60, 136–143(2022). https://doi.org/10.1016/j.matpr.2021.12.277

Khoshtinat, S., Advancements in Exploiting *Sporosarcina pasteurii* as Sustainable Construction Material: A Review, *Sustain.,* 15(18), 13869(2023). https://doi.org/10.3390/su151813869

Khushnood, R. A., Qureshi, Z. A., Shaheen, N. and Ali, S., Bio-mineralized self-healing recycled aggregate concrete for sustainable infrastructure, *Sci. Tot. Environ.,* 703, 135007(2020). https://doi.org/10.1016/j.scitotenv.2019.135007

Kurda, R., Salih, A., Shakor, P., Saleh, P., Alyousef, R., Ahmed, H. and Aslani, F., Mix design of concrete: Advanced particle packing model by developing and combining multiple frameworks, *Constr. Build. Mater.,* 320, 126218(2022). https://doi.org/10.1016/j.conbuildmat.2021.126218

Luo, M. and Qian, C. X., Performance of Two Bacteria-Based Additives Used for Self-Healing Concrete, *J. Mater. Civ. Eng.,* 28(12), 04016151(2016). https://doi.org/10.1061/(ASCE)MT.1943-5533.0001673

Mondal, S. and Ghosh, A., Microbial concrete as a sustainable option for infrastructural development in emerging economies, Urbanization Challenges in Emerging Economies: Resilience and Sustainability of Infrastructure*, ASCE Press,* 413–423(2018).

Min, W., Lu, P., Liu, S. and Wang, H., A Review of Crack Sealing Technologies for Asphalt Pavement: Materials, Failure Mechanisms, and Detection Methods, *Coat.,* 15(7), 836(2025). https://doi.org/10.3390/coatings15070836

Nasser, A. A., Sorour, N. M., Saafan, M. A. and Abbas, R. N., Microbially-Induced-Calcite-Precipitation (MICP): A biotechnological approach to enhance the durability of concrete using Bacillus pasteurii and *Bacillus sphaericus*, *Heliyon,* 8(7), e09879(2022). https://doi.org/10.1016/j.heliyon.2022.e09879

Nguyen, T. H., Ghorbel, E., Fares, H. and Cousture, A., Bacterial self-healing of concrete and durability assessment, *Cem. Concr. Compos.,* 104, 103340(2019). https://doi.org/10.1016/j.cemconcomp.2019.103340

Nodehi, M., Ozbakkaloglu, T. and Gholampour, A., A systematic review of bacteria-based self-healing concrete: Biomineralization, mechanical, and durability properties, *J. Build. Eng.,* 49, 104038(2022). https://doi.org/10.1016/j.jobe.2022.104038

Nosouhian, F., Mostofinejad, D. and Hasheminejad, H., Concrete Durability Improvement in a Sulfate Environment Using Bacteria, *J. Mater. Civ. Eng.,* (1), 04015064(2016). https://doi.org/10.1061/(ASCE)MT.1943-5533.0001337

Pachaivannan, P., Hariharasudhan, C., Mohanasundram, M. and Anitha Bhavani, M., Experimental anaylsis of self healing properties of bacterial concrete, *Mater. Today Proc.,* 33, 3148–3154(2020). https://doi.org/10.1016/j.matpr.2020.03.782

Phung, Q. T., Maes, N., Schutter, G. D., Jacques, D. and Ye, G., Determination of water permeability of cementitious materials using a controlled constant flow method, *Constr. Build. Mater.,* 47, 1488–1496(2013). https://doi.org/10.1016/j.conbuildmat.2013.06.074

Ranjith, A. and Das, B. B., Self-healing bacterial concrete: A bibliometric overview and state-of-the-art review on fundamentals, techniques and future perspectives, *J. Build. Eng.,* 104, 112324(2025). https://doi.org/10.1016/j.jobe.2025.112324

Reddy, B. M. S. and Revathi, D., An experimental study on effect of *Bacillus sphaericus* bacteria in crack filling and strength enhancement of concrete, *Mater. Today Proc.,* 19, 803–809(2019). https://doi.org/10.1016/j.matpr.2019.08.135

Reddy, P. and Kavyateja, B., Experimental Study on Strength Parameters of Self Repairing Concrete, *Ann. Chim. - Sci. Matér.,* 43(5), 305–310(2019). https://doi.org/10.18280/acsm.430505

Ryparová, P., Prošek, Z., Schreiberová, H., Bílý, P. and Tesárek, P., The role of bacterially induced calcite precipitation in self-healing of cement paste, *J. Build. Eng.,* 39, 102299(2021). https://doi.org/10.1016/j.jobe.2021.102299

Safiuddin, Md., Kaish, A. B. M. A., Woon, C. O. and Raman, S. N., Early-Age Cracking in Concrete: Causes, Consequences, Remedial Measures, and Recommendations, *Appl. Sci.*, 8(10), 1730(2018). https://doi.org/10.3390/app8101730

Sandbhor, S., Apte, S., Puranik, S., Asati, M. and Rai, S., Technique for improving concrete characteristics with bacillus: Insights on strength and durability, *MethodsX*, 14, 103151(2025). https://doi.org/10.1016/j.mex.2024.103151

Seo, J., Kim, H., Kim, S., Park, S., Bae, J. H., Kim, N., Jang, D. and Lee, H. K., *Sporosarcina pasteurii*-induced hydration and shrinkage properties of Portland cement, *Constr. Build. Mater.,* 356, 129213(2022). https://doi.org/10.1016/j.conbuildmat.2022.129213

Solarte, A., Choque, B., Perez, Y. C. and Amaya, S. U., Structural performance of self-healing concrete by Bacillus bacteria with addition of rice husk ash, *Struc.,* 61, 106111(2024). https://doi.org/10.1016/j.istruc.2024.106111

Sonali, S. D. C., Ruben, N., Sri, R. C. M. and Venkatesh, C., Performance studies on rate of self healing in bio concrete, *Mater. Today Proc.,* 27, 158–162(2020). https://doi.org/10.1016/j.matpr.2019.09.151

Soomro, M., Tam, V. W. Y. and Jorge, E. A.C., Production of cement and its environmental impact, *Recycled Concrete, Woodhead Publishing,* 11–46 (2023). https://doi.org/10.1016/B978-0-323-85210-4.00010-2

Sukumaran, A., Johnpaul, V., Balasundaram, N. and Senthil, K. S., Bacterial concrete: the future of self-healing and sustainable infrastructure, *MethodsX,* 15, 103569(2025). https://doi.org/10.1016/j.mex.2025.103569

Sunantha, S., Nagarajan, P., Sudhakumar, J., Thomas, B. S., Sankaranarayanan, P., Pitchaikani, S. and Suchithra, T. V., A comprehensive screening on the effect of Bacillus cereus and Vibrio natriegens bacterial consortia in cement mortar, *Sci. Rep.,* 15(1), 20917(2025). https://doi.org/10.1038/s41598-025-94641-7

Wang, J. Y., De Belie, N. and Verstraete, W., Diatomaceous earth as a protective vehicle for bacteria applied for self-healing concrete, *J. Ind. Microbiol. Biotechnol.,* 39(4), 567–577(2012). https://doi.org/10.1007/s10295-011-1037-1

Wang, J. Y., Soens, H., Verstraete, W. and De Belie, N., Self-healing concrete by use of microencapsulated bacterial spores, *Cem. Concr. Res.,* 56139–152(2014). https://doi.org/10.1016/j.cemconres.2013.11.009

Yamasamit, N., Sangkeaw, P., Jitchaijaroen, W., Thongchom, C., Keawsawasvong, S. and Kamchoom, V., Effect of *Bacillus subtilis* on mechanical and self-healing properties in mortar with different crack widths and curing conditions, *Sci. Rep.,* 13(1), 7844(2023). https://doi.org/10.1038/s41598-023-34837-x

Yoo, D. Y., Kim, S., Kim, M. J., Kim, D. and Shin, H. O., Self-healing capability of asphalt concrete with carbon-based materials, *J. Mater. Res. Technol.,* 8(1), 827–839(2019). https://doi.org/10.1016/j.jmrt.2018.07.001

Zhang, S., Han, B., Xie, H., An, M. and Lyu, S., Brittleness of Concrete under Different Curing Conditions, *Mater.,* 14(24), 7865(2021). https://doi.org/10.3390/ma14247865