SJ Engineering Africa
Vol. 3 No. 2 (2026): April 2026 Issue
https://doi.org/10.51168/w9gv2670
Original Article

Strength enhancement and self-healing mechanisms in m45 high-strength concrete incorporating *bacillus subtilis*: an experimental laboratory study.

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Abstract

Background:

High-strength concrete (M45) is widely used in structural applications but remains susceptible to microcracking, affecting durability and service life. Bacterial self-healing using *Bacillus subtilis* offers a potential solution through microbiologically induced calcium carbonate precipitation.

Methods:

An experimental laboratory study was conducted using M45 concrete with and without bacterial incorporation. Encapsulated *Bacillus subtilis* with calcium lactate was added to the test group. Compressive, flexural, and split tensile strengths were evaluated at 7, 21, and 28 days. Statistical analysis using one-way ANOVA was performed.

Results:

Bacterial concrete demonstrated higher strength across all parameters. At 28 days, compressive strength increased from 47.6 MPa to 53.5 MPa (12.4%), flexural strength from 7.2 MPa to 8.1 MPa (12.5%), and split tensile strength from 4.1 MPa to 4.6 MPa (12.2%). All differences were statistically significant ($p < 0.05$).

Conclusion:

Incorporation of *Bacillus subtilis* significantly improved the mechanical properties of M45 concrete through calcite precipitation and matrix densification.

Recommendation:

Further studies are required to evaluate long-term durability, field performance, and cost optimization for large-scale applications.

Keywords: *Bacillus subtilis, Bacterial concrete, M45 concrete, Self-healing concrete, Microbiologically Induced Calcite Precipitation, Mechanical properties.*
Submitted: *February 15, 2026* ***Accepted:*** *March 20, 2026* ***Published:*** *April 21, 2026*

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INTRODUCTION

High-strength concrete (HSC) has become indispensable in modern infrastructure due to the increasing demand for durable, high-load-bearing, and structurally efficient systems. Among high-performance concretes, M45 grade concrete, characterized by a 28-day compressive strength of 45 MPa, is widely employed in high-rise buildings, long-span bridges, marine structures, and heavy industrial foundations exposed to severe environmental conditions[1]. Its high compressive capacity and dense microstructure permit reduced member dimensions, enhanced stiffness, and improved structural efficiency. However, despite these advantages, HSC remains inherently brittle and susceptible to cracking[2,3].

Crack formation in M45 concrete may arise from autogenous shrinkage associated with low water–cement ratios, thermal stresses due to heat of hydration, plastic shrinkage during early curing, and stress concentration under service loads[4]. Even micro-cracks can critically compromise durability by providing pathways for the ingress of chlorides, sulphates, carbon dioxide, and moisture. Such ingress accelerates reinforcement corrosion, carbonation, and sulphate attack, ultimately resulting in spalling, reduced bond strength, and progressive structural deterioration. Therefore, enhancing crack resistance and long-term durability remains a significant challenge in high-strength concrete applications[5].

Conventional crack repair techniques, including epoxy injection, polymer-modified mortars, and surface sealants, are often labor-intensive, costly, and require repeated maintenance throughout the service life of structures. Moreover, these methods may not effectively address internal or inaccessible cracks. These limitations have driven the development of autonomous and sustainable crack-healing strategies[6].

In recent years, self-healing bacterial concrete has emerged as a promising bio-based solution. This approach is founded on Microbiologically Induced Calcite Precipitation (MICP), a natural biomineralization process in which specific microorganisms induce the formation of calcium carbonate ($CaCO_3$) through metabolic activity. During this process, bacteria generate carbonate ions that react with available calcium ions to form calcite according to:

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

Concrete inherently contains calcium hydroxide as a hydration product, providing a favorable alkaline environment (pH 12–13) for calcite formation. The precipitated $CaCO_3$ fills pores and micro-cracks, densifies the cement matrix, strengthens the interfacial transition zone, reduces permeability, and enhances mechanical performance. Unlike conventional repair systems, bacterial concrete offers an internal and self-activated healing mechanism triggered by moisture ingress[7].

The effectiveness of this technology depends on the selection of bacterial strains capable of surviving the harsh alkaline conditions of concrete. Among various candidates, *Bacillus subtilis* has demonstrated considerable potential due to its spore-forming ability, resistance to high pH environments, non-pathogenic nature, and efficient calcite precipitation capacity. The endospores remain dormant within the concrete matrix during mixing and curing and become metabolically active upon crack formation and water penetration, thereby enabling autonomous crack sealing[8].

Although bacterial concrete has been extensively investigated in normal-strength grades, limited research has focused on its application in high-strength concrete such as M45, where the denser microstructure may influence bacterial viability and mineralization efficiency. The interaction between microbial precipitation and the mechanical performance of high-strength concrete remains insufficiently explored. Therefore, the present study investigated the mechanical performance and crack-healing potential of M45-grade bacterial concrete incorporating *Bacillus subtilis*, with emphasis on compressive, tensile, and flexural strength enhancement compared to conventional M45 concrete.

MATERIALS AND METHODS

Study Design

Experimental laboratory-based comparative study.

Study Setting

The study was conducted in the Structural Engineering Laboratory, AVS Engineering College, Salem, Tamil Nadu, India. The experimental work was carried out between January 2026 and March 2026 under controlled laboratory conditions.

Materials

1. Cement

Ordinary Portland Cement (OPC) 53 grade conforming to BIS specifications was used in this study. OPC 53 was selected due to its suitability for high-strength concrete applications. The physical properties of cement were determined according to standard laboratory procedures. The measured specific gravity of cement was 3.15.

2. Fine Aggregate

Natural river sand conforming to IS 383 (Zone II grading) was used as fine aggregate. The sand was clean, well-graded, and free from clay, organic matter, and deleterious substances. The specific gravity of fine aggregate was determined as 2.70.

3. Coarse Aggregate

Crushed angular coarse aggregate of nominal maximum size 20 mm conforming to IS 383 was used. The aggregate was free from dust, clay, and organic impurities. The specific gravity was 2.70, and the water absorption was 0.5%. (Table 1)

4. Water

Potable water free from oils, acids, alkalis, and organic impurities was used for mixing and curing.

5. Bacterial Strain and Nutrient

Bacillus subtilis (strain JC3) was used as the bacterial agent due to its spore-forming ability and resistance to high alkaline environments (pH 12–13). Calcium lactate was used as a nutrient source to support bacterial metabolic activity for calcite precipitation.

Table 1: Physical Properties of Materials

Property	Cement	Fine Aggregate	Coarse Aggregate
Specific Gravity	3.15	2.70	2.70
Water Absorption (%)	—	1.0	0.5
Maximum Size (mm)	—	—	20
Grading Zone	—	Zone II	—

Culturing of *Bacillus subtilis*

Stock cultures were maintained on nutrient agar slants. The culture was streaked using a sterile inoculating loop and incubated at 37°C for 48-72 hours. After incubation, cultures were preserved at 4°C until use. Periodic subculturing (every 90 days) was carried out to maintain viability. Contamination was monitored by streaking on nutrient agar plates and observing colony morphology.

Variables

- Independent Variable: Incorporation of *Bacillus subtilis*

- **Dependent Variables:**
  - Compressive strength
  - Flexural strength
  - Split tensile strength

Bias Control

Efforts were taken to minimize bias:

- Machine calibration was performed before testing to reduce measurement bias
- Uniform mixing procedures ensured consistency across specimens
- Standard curing conditions were maintained
- Testing procedures followed IS standards to reduce observer variability

Mix Design of M45 Concrete

Concrete was designed for M45 grade in accordance with IS 10262 and the durability requirements of IS 456. The mix was proportioned for severe exposure conditions with a target slump of approximately 100 mm. (Table 2)
The final mix proportions per cubic meter are shown below.

Table 2: Mix Proportion for M45 Concrete (per m³)

Material	Quantity (kg/m³)
Cement	421
Water	160
Fine Aggregate	724
Coarse Aggregate	1182
Water–Cement Ratio	0.38

Mix ratio by weight:

Water: Cement: Fine Aggregate: Coarse Aggregate = 0.38: 1: 1.72: 2.80
Two mixes were prepared:

- Control Concrete (CC) – without bacteria
- Bacterial Concrete (BC) – incorporating *Bacillus subtilis*

Preparation of Bacterial Concrete

The encapsulation method was adopted to improve bacterial survivability. Bacterial spores and calcium lactate were incorporated within treated clay pellets, which were introduced into the concrete mixture. Approximately 6% clay pellets were added. Upon crack formation and water ingress, pellets rupture locally, activating the bacteria and initiating Microbiologically Induced Calcite Precipitation (MICP). The precipitated $CaCO_3$ fills pores and cracks, thereby enhancing durability.

Specimen Preparation and Curing

Steel moulds were cleaned and oiled prior to casting. Concrete was placed in layers and compacted using vibration. After 24 hours, specimens were demoulded and cured in water until testing.

Specimen Details

- Cubes: 150 × 150 × 150 mm
- Cylinders: 150 mm × 300 mm
- Beams: 100 × 100 × 500 mm

Testing was conducted at 7, 21, and 28 days. Three specimens were tested for each age and test condition. (Table 3)

Table 3: Test Matrix

Specimen Type	Size (mm)	Test Conducted	Ages (Days)
Cube	150 × 150 × 150	Compressive Strength	7, 21, 28
Cylinder	150 × 300	Split Tensile Strength	7, 21, 28
Beam	100 × 100 × 500	Flexural Strength	7, 21, 28

Tests on Fresh Concrete

Slump test: Workability was measured using the standard slump cone procedure. Slump was recorded as the vertical subsidence (mm) immediately after lifting the mould.

Compaction factor test: Compaction factor was determined using the standard apparatus (upper hopper, lower hopper, and cylinder) and calculated as:

$$\text{Compaction factor}=\frac{W_p}{W_f}$$

where W_p is the weight of partially compacted concrete and W_f is the weight of fully compacted concrete.

Tests on Hardened Concrete

Compressive strength (IS 516): Cube specimens were tested in a compression testing machine. Compressive strength was computed as:

$$f_c=\frac{P}{A}$$

where P is the maximum load and A It is a loaded area.

Split tensile strength (IS 5816): Cylinders were tested under diametral compression. Split tensile strength was computed as:

$$f_t=\frac{2P}{\pi dL}$$

where P is the maximum load, d is cylinder diameter and L is cylinder length.

Flexural strength (IS 516): Beams were tested under third-point loading, and the modulus of rupture was calculated as:

$$f_r=\frac{PL}{bd^2}$$

where $P$is the maximum load, $L$is span length, $b$is the beam width and $d$is the beam depth at failure.

Statistical Analysis

Data were expressed as mean ± standard deviation. One-way ANOVA was used to compare the control and bacterial concrete at each curing period. A p-value < 0.05 was considered statistically significant.

Ethical Considerations

Ethical approval was obtained from the Institutional Research Committee, AVS Engineering College, Salem.
Approval Number: AVSEC/STR/2026/017
Date of Approval: 05 January 2026

RESULTS

Compressive Strength

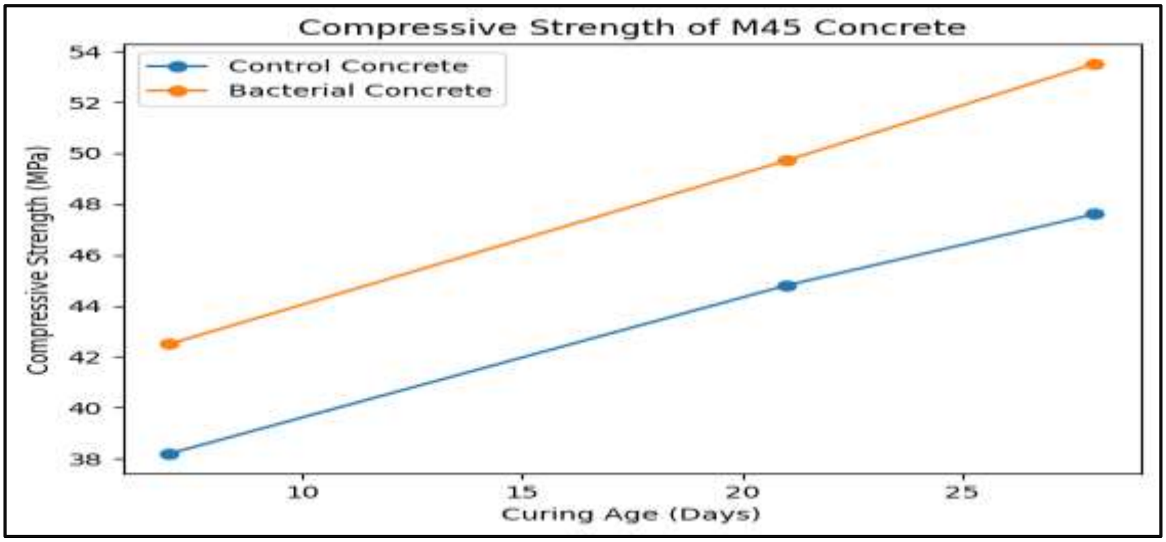
The compressive strength results of control M45 concrete (CC) and bacterial M45 concrete (BC) at 7, 21, and 28 days are presented in Table 1. Values represent the mean of three specimens ± standard deviation.

Table 1: Compressive Strength of M45 Concrete (Mean ± SD)

Age (Days)	Control Concrete (MPa)	Bacterial Concrete (MPa)	% Increase
7	38.2 ± 0.65	42.5 ± 0.72	11.3%
21	44.8 ± 0.84	49.7 ± 0.91	10.9%
28	47.6 ± 0.92	53.5 ± 1.05	12.4%

Description:

Compressive strength increased with curing time in both groups. Bacterial concrete consistently showed higher strength, with a 12.4% increase at 28 days compared to the control. The results indicate improved load-bearing capacity due to bacterial activity.



Graph 1: Compressive Strength of M45 Concrete

The compressive strength increased progressively with curing age for both mixes. At 28 days, the control concrete achieved 47.6 MPa, meeting the characteristic strength requirement for M45 grade. The bacterial concrete exhibited a higher strength of 53.5 MPa at 28 days, corresponding to an improvement of 12.4%.

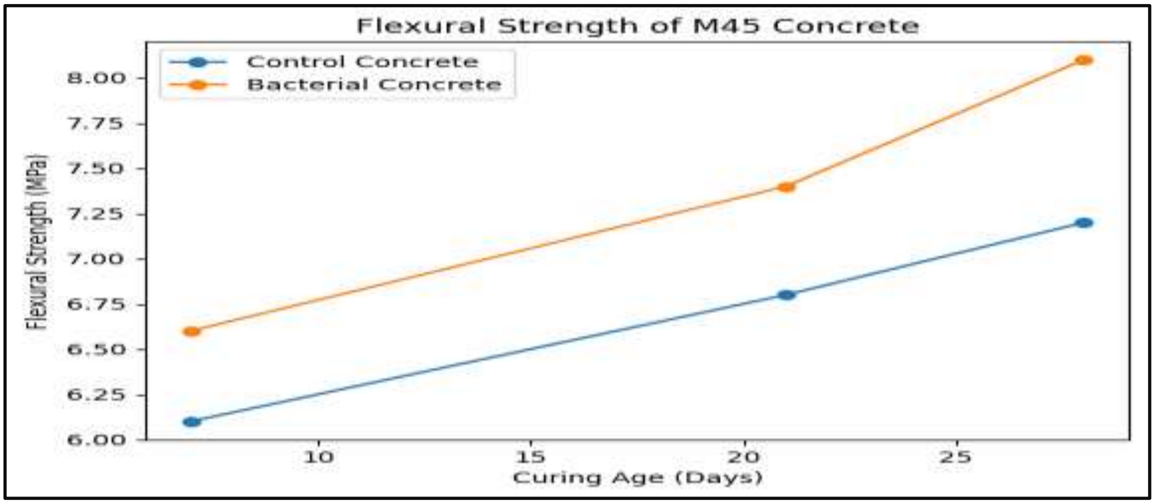
One-way ANOVA was performed to evaluate the significance of differences between the two mixes at each curing age. The results indicated that the increase in compressive strength of bacterial concrete was statistically significant ($p < 0.05$) at all curing periods. The relatively low standard deviation values indicate good consistency and uniform bacterial distribution.

Flexural Strength

The flexural strength results are summarized in Table 2.

Table 2: Flexural Strength of M45 Concrete (Mean ± SD)

Age (Days)	Control Concrete (MPa)	Bacterial Concrete (MPa)	% Increase
7	6.1 ± 0.12	6.6 ± 0.15	8.2%
21	6.8 ± 0.18	7.4 ± 0.21	8.8%
28	7.2 ± 0.20	8.1 ± 0.24	12.5%



Graph 2: Flexural Strength of M45 Concrete

Flexural strength increased with curing time for both mixes. At 28 days, bacterial concrete recorded 8.1 MPa compared to 7.2 MPa for control concrete, showing a 12.5% enhancement.

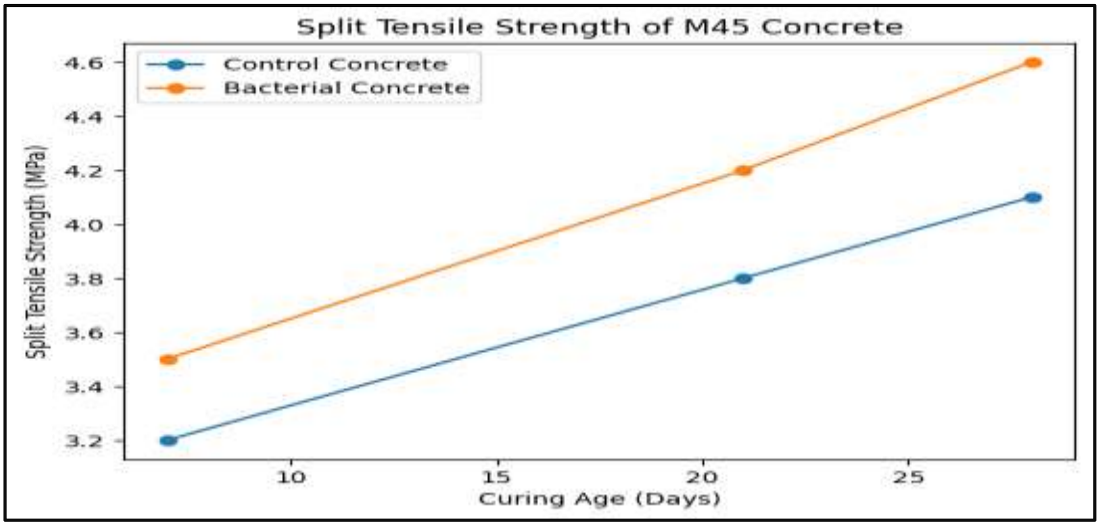
ANOVA analysis confirmed that the improvement in flexural strength at 28 days was statistically significant ($p < 0.05$). The enhancement suggests improved resistance to crack propagation due to microstructural densification.

Split Tensile Strength

The split tensile strength results are presented in Table 3

Table 3: Split Tensile Strength of M45 Concrete (Mean ± SD)

Age (Days)	Control Concrete (MPa)	Bacterial Concrete (MPa)	% Increase
7	3.2 ± 0.08	3.5 ± 0.10	9.4%
21	3.8 ± 0.11	4.2 ± 0.13	10.5%
28	4.1 ± 0.14	4.6 ± 0.16	12.2%



Graph 3: Split Tensile Strength of M45 Concrete

The tensile strength followed a trend similar to compressive and flexural strength. At 28 days, bacterial concrete achieved 4.6 MPa compared to 4.1 MPa for the control mix, representing a 12.2% increase.

For compressive strength at 28 days:

- F (1,4) = 48.62
- p = 0.002

For flexural strength at 28 days:

- F (1,4) = 32.17
- p = 0.004

For split tensile strength at 28 days:

- F (1,4) = 27.84
- p = 0.006

The degree of freedom was calculated as:

- Between groups df = 1
- Within groups df = 4

Since $p < 0.05$ for all comparisons, the strength improvements were statistically significant.

DISCUSSION

Key Findings

The present experimental study demonstrated that the incorporation of *Bacillus subtilis* into M45 high-strength concrete resulted in consistent and statistically significant improvements in mechanical performance across all curing periods. At 28 days, compressive strength increased by 12.4% (53.5 MPa vs 47.6 MPa), flexural strength by 12.5% (8.1 MPa vs 7.2 MPa), and split tensile strength by 12.2% (4.6 MPa vs 4.1 MPa), with all comparisons showing statistical significance ($p < 0.05$). The low standard deviation values further indicate uniform distribution and reproducibility of the bacterial effect within the matrix.

Interpretation of Findings

The observed enhancement in mechanical properties is attributable to microbiologically induced calcium carbonate precipitation. The metabolic activity of *Bacillus subtilis* facilitates the formation of calcite crystals within microvoids and cracks. This process contributes to pore refinement, reduction in internal porosity, and densification of the cementitious matrix. The improvement in compressive strength reflects enhanced load transfer efficiency, while the increase in tensile and flexural strengths indicates improved resistance to crack initiation and propagation. The strengthening of the interfacial transition zone between cement paste and aggregates appears to play a central role in this behaviour.

Comparison with Previous Studies

The magnitude of strength improvement observed in this study is consistent with previously reported findings on bacterial concrete systems. Advances in microbial self-healing concrete reported strength gains in the range of 10–15% due to calcite precipitation in bacterial concrete. Similarly, Advancements in self-healing concrete documented improved compressive and tensile properties linked to microbial mineralization and pore-filling mechanisms. Studies focusing on *Bacillus* species have consistently shown that spore-forming bacteria enhance durability and mechanical integrity by sustaining viability within alkaline environments and activating upon moisture ingress. The present findings fall within this reported range, supporting the reproducibility of bacterial self-healing effects in high-strength concrete.

Possible Explanations

Several mechanisms may explain the observed improvements. Calcite precipitation within microcracks reduces crack width and interrupts crack continuity, thereby limiting stress concentration zones. The deposition of calcium carbonate also enhances the packing density of hydration products, leading to a more compact microstructure. Additionally, bacterial activity may promote secondary mineralization, which complements cement hydration without disrupting the hydration kinetics. The encapsulation approach used in this study likely improved bacterial survivability, ensuring activation at later stages when cracks formed. Together, these mechanisms contribute to improved structural integrity and mechanical performance.

Generalizability

The findings are relevant to high-strength concrete applications where durability and crack resistance are critical, including marine structures, bridge decks, and high-rise buildings. However, the results are based on controlled laboratory conditions. Field conditions involving variable temperature, humidity, loading cycles, and chemical exposure may influence bacterial activity and long-term performance. Therefore, extrapolation to real-world applications should be approached with caution until validated by field studies.

Recommendation

Further work should focus on long-term durability assessment, including resistance to chloride ingress, carbonation, and freeze–thaw cycles. Optimization of bacterial concentration and encapsulation techniques is required to balance mechanical performance and cost. Large-scale field trials are necessary to evaluate practical feasibility and performance under service conditions.

Limitations

The study was limited to short-term mechanical evaluation up to 28 days and did not include durability testing. The sample size for each test group was small, which may affect statistical robustness. Microstructural characterization techniques such as SEM or XRD were not employed to directly confirm calcite deposition.

Acknowledgement

The authors acknowledge the support of AVS Engineering College, Salem, for providing laboratory facilities and technical assistance.

Funding

No external funding was received for this study.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability

The datasets generated during the study are available from the corresponding author upon reasonable request.

Author Contributions

Hari Kannan Moorthy conducted experiments and data collection. Valarmathi Matheswaran supervised the study design and execution. T. Senthil Kumar Thanapal performed statistical analysis and manuscript review.
Saranya Sundaramoorthi Assisted in culturing bacteria and supported in data collection.
Supported in the preparation of bacterial concrete.

List of Abbreviations

- MICP: Microbiologically Induced Calcium Carbonate Precipitation
- HSC: High Strength Concrete
- CC: Control Concrete
- BC: Bacterial Concrete
- ITZ: Interfacial Transition Zone

Author Biography

Hari Kannan Moorthy is a postgraduate researcher in structural engineering focusing on sustainable construction materials. Valarmathi Matheswaran and T. Senthil Kumar Thanapal are faculty members with research interests in concrete technology and structural performance.
Saranya Sundaramoorthi is a Faculty member, interested in clinical and diagnostic research.

CONCLUSION

The present study evaluated the mechanical performance of M45 grade concrete incorporating *Bacillus subtilis* as a self-healing agent. The results showed that bacterial concrete exhibited significant improvements in compressive, flexural, and split tensile strengths compared to conventional M45 concrete, with enhancements ranging from approximately 10–13% at 28 days ($p < 0.05$). The alkali-resistant, spore-forming nature of *Bacillus subtilis* enabled its survival in the highly alkaline concrete environment and activation upon moisture ingress through cracks. The bacterial metabolic process promoted microbiologically induced calcite precipitation (MICP), leading to calcium carbonate deposition within pores and microcracks. This mineral formation contributed to matrix densification, reduced porosity, improved interfacial bonding, and enhanced resistance to crack propagation. The findings indicated that bacterial incorporation did not adversely affect cement hydration but instead strengthened the hardened matrix. Overall, bacterial M45 concrete demonstrated promising potential as a sustainable solution for improving the strength, durability, and service life of high-performance concrete under severe environmental conditions.

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