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Original Article

Microbially Induced Calcite Precipitation (MICP)-Based Bio-Mediated Self-Repairing Concrete Incorporating Bacillus subtilis: Mechanical Performance and Chemical Durability Enhancement

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Abstract *- The use of concrete as the primary building material for infrastructure is widespread. The structural components of concrete tend to crack due to a variety of factors. These cracks allow harmful substances such as water, chlorides, sulfates, and carbon dioxide to enter the concrete and cause it to deteriorate. The maintenance and repair of cracked concrete increases the cost and ecological impact of constructing and operating these infrastructures. One way to manage this problem is to create Self-Repairing Concrete using biological materials. Several types of bio-based self-healing agents have been developed, and many have been verified in concrete. One such approach is called Microbially Induced Calcite Precipitation (MICP). This technique uses certain bacteria, such as Bacillus subtilis, that can survive within the concrete in a dormant state. Once the concrete is placed and water is introduced, these dormant bacteria become active. The bacteria secreted calcium carbonate fills the fissures in the concrete. The primary goal of this research was to use Bacillus subtilis to create bacterial concrete that heals itself. M25-grade concrete as per Indian Standards was used to prepare three types of concretes. The control concretes contained 0 cells/mL (M0). Bacterial concrete contained 10^5 cells/mL (B1) and 10^6 cells/mL (B2). The properties that were tested included workability, strength of compressive, split-tensile, and flexure, along with durability in alkaline-acidic environments. The results obtained indicate that the use of bacteria significantly improves the mechanical properties of concrete and durability. All of the bacterial concretes exhibited increased tensile, flexural and compressive strength. The B2 mix (10^6 cells/mL) exhibited the highest 28-day compressive strength. The durability tests indicated that bacterial concrete resists chemical deterioration better than conventional concrete. This study confirms that bacterial Self-Repairing Concrete is a sustainable substitute to usual concrete repair. The ability of bacterial concretes to autonomously seal microcracks and increase the durability of the concrete indicates that such concretes have great potential to be utilized in constructing long-lasting and low-maintenance infrastructures.*

Keywords *- Bacillus subtilis, Bio-mineralization, Bacterial concrete durability and strength enhancement.*

1. Introduction

1.1. General Overview

Being one of the most popular materials of construction, concrete benefits from high compressive strength, durability, and economic feasibility. It is a material that is used to construct almost every form of infrastructure. The major limitation of conventional concrete is the crack formation that is caused by the quasi-brittle nature of the material. Cracks can form at the various performing life stages of concrete. The causes of crack formation can include plastic shrinkage, thermal stresses, drying shrinkage, loading, and environmental exposure. These cracks provide pathways for aggressive substances such as water, chlorides, sulphates, alkalis, and carbon dioxide to enter the concrete structure.

The ingress of these substances causes deterioration processes such as reinforcement corrosion, carbonation, attack of sulphate, and silica-alkali-reaction, all of which shorten the service life of the concrete structure.

Commonly used are epoxy injection, surface coatings, patch repair, and jacketing to mitigate the effect of cracking. These methods are all temporary and involve costly maintenance procedures. Hence, there is a growing interest in Self-Repairing Concrete technologies.

1.2. Background and Motivation

The inspiration behind self-healing materials comes from the way in which biological systems are able to repair damaged biological tissues. This concept has been translated into the construction of self-healing cementitious materials.

In most cases, microcracks that are smaller than 0.2 mm in size can significantly increase the permeability of the concrete. The effects of cracking due to rebar corrosion are that nearly 50% of the concrete structure's deterioration; furthermore, 30-40% of the cost of owning and operating these structures is related to inspection and maintenance.

The above challenges have given rise to the need for innovative materials and technologies to provide long-term solutions to these problems. In this affection, one solution is to use of biologically induced self-healing through the use of microorganisms that are intelligent to precipitate minerals. This process is known as (MICP).

Among the various species of bacteria, Bacillus subtilis has been found to be the most appropriate to employ in concrete. The organism is well known for its ability to form endospores that allow it to survive the elevated alkalinity of the concrete. When water infiltrates the cracks in the concrete, the spores become metabolically active and form calcium carbonate that fills the cracks.

1.3. Need for Sustainable Construction Materials

The construction of infrastructures has a significant impact on the ecology. The production of cement, for instance, emits around 8% of the world's CO_2. The energy used in the repair and maintenance of construction materials further compounds the environmental problem.

Concrete that self-healing property bids a sustainable substitute to traditional construction materials. Self-healing materials will minimize the repair work required from construction firms and significantly reduce the use of construction materials.

Many of the United Nations sustainable development goals that the construction industry must pursue align with self-healing materials. These include goals related to infrastructure, climate change, and sustainable cities and communities. Self-healing materials can also provide increased durability for structures in extreme environments.

1.4. Concept of Bio-Mediated Self-Repairing Concrete

Concrete that has self-healing property is the name Self-Repairing Concrete, given to the ability that certain cement-based materials have to repair cracks that form in the construction material. This process is divided into two main types: autogenous and biological self-healing.

1.4.1. Autogenous Healing

Autogenous healing is the natural process that occurs within conventional concrete. The unreacted cement particles continue to hydrate and produce calcium carbonate, which assistances to closure the cracks. This reaction only works effectively for cracks less than 0.2 mm in width and over time.

1.4.2. Biological Self-Healing

Biological self-healing processes involve incorporating biological material, such as living bacteria and nutrients, into the concrete. The presence of water and cracks allows these dormant bacteria to become active and form calcite crystals within the cracks.

1.5. Mechanism of (MICP)

The process of biological self-healing involves the activity of microorganisms, referred to as (MICP). When water seeps into the cracks of the concrete, the bacterial spores become active and initiate the breakdown of organic nutrients.

The simplified biochemical reaction is:

$$Ca(c_3H_5O_3)_2 + 7O_2 \rightarrow CaCO_3 + 5CO_2 + 5H_2O$$

The produced carbon dioxide reacts with water to formulate carbonate ions:

$$CO_2 + H_2O \rightarrow H_2CO_3 \rightarrow HCO_3^- \rightarrow CO_3^{2-}$$

These carbonate ions syndicate by means of calcium ions present in the cement matrix:

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

The resulting calcium carbonate crystals fill cracks and pores, reducing permeability and restoring the integrity of the cementitious matrix.

1.6. Research Problem

Cracking is the prime cause of deterioration in concrete structures. Even the presence of hairline cracks of a width of 0.05 – 0.2 mm can significantly increase the permeability of concrete to aggressive substances that degrade the material. The repair techniques used to repair these cracks are generally costly. As such, there is a critical need to develop an autonomous concrete that can fix the cracks that form in it.

Bacillus subtilis is a microorganism that shows promise as a resource of providing self-healing properties to the concrete. However, the efficiency of this biological agent to repair cracks in concrete be subject to several aspects, such as bacterial concentration.

1.7. Scope of the Study

This examine aims to consider the practise of Bacillus subtilis to provide biological self-healing properties to M25 grade concrete.

Three different mixes of concrete will be prepared:

- M0 - control concrete
- B1 - bacterial concrete with 10^{5}cells/mL
- B2 - bacterial concrete with 10^{6}cells/mL

The strength of the concretes, namely split tensile, compression, and flexure, will be experimented as per Indian Standards. Additionally, acid and alkaline resistance tests will be performed. Finally, the healing of cracks will also be observed.

2. Literature Review

2.1. Research Gap

Recent literature confirms that MICP is now a well-established strategy for improving concrete performance, although reported gains vary widely across bacterial species, dosage, and delivery method. In a 2025 synthesis of macrostructural MICP studies, Odeyemi et al. [9] reported that compressive strength typically increases by 20–50%, flexural strength by 19–66%, and tensile strength by 30–63% across the published record, while water absorption and permeability are commonly reduced by 15–31% and 44–55%, respectively. These wide ranges indicate that treatment outcome is highly sensitive to strain selection, nutrient formulation, and curing regime, and that findings from one bacterial system cannot be assumed to transfer directly to another - a limitation that the present study addresses by isolating the effect of Bacillus subtilis concentration alone, under a single, standardised Indian mix design.

Several recent critical reviews indicate that most experimental MICP studies examine only a narrow set of properties in isolation. Wong et al. [10] observed that although the mechanisms of microbial calcite precipitation are increasingly well characterised at laboratory scale, comparatively few studies report compressive, tensile and flexural strength together with multi-parameter chemical durability (acid, alkaline and chloride-ion permeability) within the same experimental matrix, which limits the ability to draw an integrated picture of performance. Similarly, Nasser et al. [11], working with Bacillus pasteurii and Bacillus sphaericus, restricted their durability assessment mainly to general degradation indices without RCPT-based permeability classification, while Javeed et al. [12] noted that implementation technique (direct mixing, encapsulation, or surface application) is rarely varied alongside bacterial concentration within a single study, making it difficult to isolate the individual contribution of cell density. The present investigation is structured to close this gap: three concentrations (0, 10^5, 10^6 cells/mL) of Bacillus subtilis are evaluated across compressive, split-tensile and flexural strength as well as acid resistance, alkaline resistance and RCPT-based chloride permeability within one coherent mix design, enabling direct, internally consistent comparison across mechanical and durability domains.

Comparative benchmarking against the wider literature reveals both consistency and divergence. Alemu et al. [13], reviewing multiple bacterial concrete studies, reported average tensile and flexural strength improvements of approximately 24.3% and 17.2% respectively; the improvements recorded in the present study (16.1% tensile and 18.6% flexural at the higher bacterial concentration) fall broadly within this reported envelope, corroborating the underlying trend while indicating that the strengthening achieved here sits toward the moderate end of the range reported for Bacillus-based systems. Yamasamit et al. [14] and Vishal et al. [15], both working with Bacillus subtilis at cell concentrations comparable to those used here, likewise reported concentration-dependent improvements in strength and self-healing, but their work was confined to mortar or to a single concrete grade. The use of standard M25-grade concrete prepared and tested strictly to Indian Standards (IS 10262:2019, IS 456:2000, IS 516:2018, IS 5816:1999) in the present work extends this evidence base to a code-governed Indian construction context that remains comparatively under-represented in the international literature.

On the durability side, Rajasekar et al. [16] and Zheng et al. [17] both confirmed that microbially precipitated calcite reduces chloride-ion permeability, but their treatments were applied through surface curing or post-cast surface application rather than by incorporating the bacterial suspension directly as mixing water, as adopted in this study. Nain et al. [18] reported compressive and tensile strength gains from Bacillus incorporation but did not extend their assessment to chemical durability, leaving the acid- and alkaline-resistance behaviour of Bacillus subtilis-based concrete comparatively less documented than its mechanical behaviour. Hareru et al. [20] examined the microstructural basis of MICP bio-cement but did not correlate microstructural findings with a full RCPT-based permeability classification. The present study addresses this shortfall directly by pairing SEM, EDX, and XRD microstructural evidence with quantitative RCPT permeability classification (ASTM C1202) for both control and bacterial concretes, thereby linking observed microstructural densification to a standardised durability metric. It is also notable that the environmental life-cycle implications of bacterial concrete remain comparatively under-explored. Justo-Reinoso et al. [19], in one of the few life-cycle assessment (LCA) studies of bacteria-based self-healing concrete, found that the net environmental benefit depends strongly on bacterial cultivation method and application scale. The present study does not include a full LCA, and this is acknowledged as a limitation; nonetheless, the reductions achieved here in RCPT charge passed (58.9%) and in acid/alkaline strength loss (up to 54.5% and 48.3%, respectively) point to a strong potential for extending structural service life and reducing the frequency of repair interventions, which is itself a proxy for reduced life-cycle material and carbon expenditure. Overall, the literature confirms the mechanistic plausibility and broad effectiveness of Bacillus-mediated MICP, but reveals a persistent gap in studies that (i) isolate bacterial concentration as the sole experimental variable, (ii) evaluate mechanical strength and multi-mode chemical durability within a single, standardised mix design, and (iii) do so under Indian material and testing standards. This study is positioned to directly address that three-part gap.

2.2. Novelty and Contributions

The core intention of this research is to determine the influence of bacterial concentration on the mechanical and durability properties of bacterial Self-Repairing Concrete according to Indian Standards. This examine aims to further add to existing literature and practice by investigating the properties of bacterial Self-Repairing Concrete integrating Bacillus subtilis.

More specifically, this research will compare the effects of two discrete concentrations of bacteria (10^5 and 10^6cells/mL) in the M25 grade concrete prepared as per Indian Standards. In addition, there will be tests to govern the durability of concrete in acidic and alkaline environments. Furthermore, the calcite precipitation formed by the bacteria will be measured. The outcomes of this examine will lead to the enhancement of sustainability to self-healing concrete.

2.3. Objectives

The main objective of this examine is to determine if bacterial Self-Repairing Concrete using Bacillus subtilis is feasible.

- Develop bacterial concrete mixes by means of Bacillus subtilis in M25 concrete.
- Evaluate the effect of bacterial concentration on the strength of concrete.
- Assess the durability of bacterial concrete in acidic and alkaline environments.
- Investigate the restoring capacity of bacterial concrete.
- Recognise the optimum bacterial attentiveness to enhance the concrete strength and durability.

2.4. Research Hypothesis

The main hypothesis of this research is that the use of Bacillus subtilis will lead to (MICP) in the concrete that will enhance the mechanical and durability properties of the concrete.

Specifically, the following hypotheses are made:

- The precipitation of $CaCO_3$ by bacteria enhances the mechanical properties of concrete.
- The higher the concentration of bacteria, the higher the degree of biomineralization, until a point of saturation.
- The usage of bacterial concrete enhances the chemical durability of concrete.
- Concrete containing approximately cells/mL will outperform concrete with lower concentrations of these bacteria.

2.5. Methodology Adopted

This study involves the selection and characterization of materials, preparing bacterial culture, and designing the concrete mix as per IS 10262:2019. Three mixes, including the control mix (M0) and two bacterial mixes (B1, B2), were cast and tested for self-healing. Experimental Methodology Flowchart for Preparation and Evaluation of Bacterial Concrete is shown in Figure 1.

Fig. 1 Experimental methodology flowchart for preparation and evaluation of bacterial concrete

3. Characterization of Materials

3.1. Introduction

The quality of the conventional materials used in the formulation of bacterial Self-Repairing Concrete and their compatibility with the biological agent have to be ascertained before the preparation of the concrete mix. The different materials utilised in this study are OPC 53 grade cement, manufactured sand, crushed granite coarse aggregate, potable water, and Bacillus subtilis. The specifications of these materials are tested as per the relevant IS std, such as IS 12269:2013, IS 383:2016, IS 4031:1988, and IS 456:2000.

3.1.1. Cement

OPC 53 grade as per IS 12269:2013 used, whose details are revealed in Table 1.

Table 1. Physical characteristics of cement

Property	Observed Value
Specific Gravity	3.14
Standard Consistency	31%
Initial Setting Time	35 min
Final Setting Time	590 min
Fineness (90 µm sieve residue)	4%

The outcomes of the tests on the cement show that the cement met the required standards and was suitable for the study.

3.1.2. Fine Aggregate

Table 2 shows the parameters of M-sand used, which falls in Zone II as per IS 383:2016.

Table 2. Engineering properties of Fine Aggregate

Property	Observed Value
Specific Gravity	2.63
Fineness Modulus	2.42
Water Absorption	1.25%
Bulk Density	1630 kg/m³

3.1.3. Coarse Aggregate

Table 3 illustrates that crushed granite of 20 mm size was utilized as the CA.

Table 3. Engineering properties of CA

Property	Observed Value
Specific Gravity	2.71
Water Absorption	0.45%
Fineness Modulus	7.12
Aggregate Impact Value	19%
Aggregate Crushing Value	22%

3.1.4. Water

Potable water was the ingredient in the formation of bacterial concrete. As per IS 456:2000, the water used was free from harmful substances.

The water quality test results revealed the following:

- pH = 7.3
- Total Dissolved Solids (TDS) = 75 mg/L

3.1.5. Bacterial Culture - Bacillus Subtilis

The biological component used in this study is the bacterial culture Bacillus subtilis. This is an aerobic, spore-forming, and alkaliphilic microorganism that can survive in alkaline environments.

This bacterial strain is selected due to its proven ability to induce MICP. The bacterial growth medium comprised of the mentioned contents: peptone (5 g/L), sodium chloride (5 g/L), beef extract (3 g/L), and calcium lactate (10 g/L)

The bacterial culture has been incubated at 37°C for 3600min. After incubation, the bacterial solution was diluted to prepare two concentrations of bacterial suspension:

- B1: 10^5 cells/mL
- B2: 10^6 cells/mL

This bacterial suspension will be used as a substitute for mixing water in bacterial concrete.

MICP is the process that gives bacterial concrete its ability to heal itself. The chemical reaction is as follows:

$$Ca(C_3H_5O_3)_2 + 7O_2 \rightarrow CaCO_3 + 5CO_2 + 5H_2O \; CO_2 + H_2O \rightarrow H_2CO_3 \rightarrow HCO_3^- \rightarrow CO_3^{2-} \; Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3 \downarrow$$

The ensuing calcium carbonate precipitation rebuilds the cracks in the concrete, illustrated in Table 4.

3.1.6. Overview of the Material Attributes

Table 4. Summary of material properties

Material	Specific Gravity	Water Absorption (%)
Cement	3.14	—
Sand (Fine Aggregate)	2.63	1.25
Coarse Aggregate	2.71	0.45
Bacillus subtilis (culture)	—	—
Water	—	—

3.2. Significance of Material Characterization

The characterization of the materials used determines the basis on which the mix design and the various tests will be carried out. The compatibility of the materials and the biological agent is particularly important for the survival of the biological agent. Through proper characterization, the desired properties of fresh, mechanical, and durability can be maintained.

4. Experimental Investigation on Bacterial Self-Repairing Concrete

4.1. Introduction

This chapter describes the experiments conducted on bacterial Self-Repairing Concrete using Bacillus subtilis. Three mixes of bacterial concrete were prepared. The properties of control concrete (M0), bacterial concrete at the dosage of cells/mL (B1), and bacterial concrete at the dosage of cells/mL (B2) were evaluated.

The experiments include the evaluation of fresh concrete, mechanical properties, and durability. All the tests are performed as per the relevant Indian Standards.

4.2. Mix Design

The concrete designed in this experiment is M25 grade as per IS 10262:2019 and IS 456:2000. The bacterial solution is used as a replacement for mixing water while maintaining the w/c.

The target mean strength is determined by:

$$f_{cm} = f_{ck} + 1.65S$$

The w/c was fixed at 0.45.

Table 5. Design stipulations

Parameter	Value
Grade of Concrete	M25
Type of Cement	OPC 53 Grade
Maximum Size of Aggregate	20 mm
Specific Gravity of Cement	3.14
Specific Gravity of Fine Aggregate	2.63
Specific Gravity of Coarse Aggregate	2.71
Water–Cement Ratio	0.45
Workability (Slump Range)	75–100 mm
Exposure Condition	Severe

Table 6. Mix proportions

Ingredient	Ratio	Quantity (kg/m³)
Cement	1.00	383.16
FA	1.50	674.69
CA	2.68	1142.35
Water	0.45	172.42
Bacillus subtilis solution	—	Replaces water in B1 and B2

Table 7. Identification of mixes

Mix ID	Description	Bacterial Concentration
M0	Control concrete	-
B1	Bacterial concrete	10^5 cells/mL
B2	Bacterial concrete	10^6 cells/mL

4.3. Specimen Preparation, Casting, and Curing

The concrete was mixed in a tilting drum mixer. The dry components were first mixed, and then the water or bacterial suspension was added gradually. The specimens were compacted and left undisturbed for 24 h. After that, they were demoulded and cured in potable water maintained at for seven, fourteen, and 28 days, as shown in Figure 2.

Fig. 2 Preparation and casting of concrete cube specimens for compressive strength testing

4.4. Workability Assessment

The workability was determined as per IS 1199:1959 specified in Table 8.

Table 8. Slump test results

Mix ID	Slump (mm)	Workability	Remarks
M0	82	Medium	Satisfactory
B1	78	Medium	Slightly stiffer
B2	75	Medium	Lower flow due to higher cell density

The slump values of all mixes fell within the target range of 75-100 mm. As shown in Figure 3, a slight reduction in slump is seen with increasing bacterial Concentration.

Fig. 3 Slump test of fresh concrete

4.5. Compressive Strength

The compressive strength determined as per IS 516:2018 are given in Table 9.

Table 9. Specimen strength (Compression)

Mix ID	7-Day (MPa)	14-Day (MPa)	28-Day (MPa)	% Increase over Control (28-day)
M0	22.4	29.6	36.5	-
B1	24.2	32.8	40.2	+10.1%
B2	37.5	41.5	49.5	+35.6%

The results implied bacterial mixes had higher compressive strength than the control mix. The increased strength of these mixes was due to the precipitation of $CaCO_3$ by the microbes shown in Figure 4.

Fig. 4 CTM used for determining the compressive strength of specimens

4.6. Split Tensile Strength

Split tensile strength was determined on cylinder specimens as per IS 5816:1999, results shown in Table 10, and experimental set up shown in shown in Figure 5

Table 10. Specimen strength (Split Tensile)

Mix ID	28-Day Split Tensile Strength (MPa)
M0	3.1
B1	3.4
B2	3.6

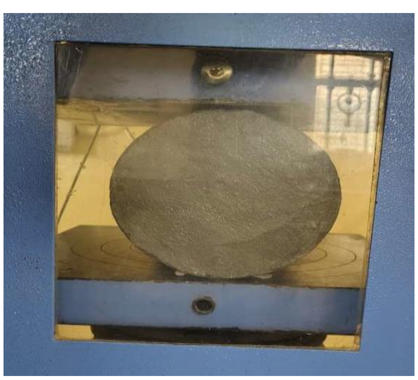

Fig. 5 Split tensile strength test of concrete cylinder

4.7. Flexural Strength

This was measured on beam specimens under two-point loading as per IS 516:2018 calculated in Table 11

Table 11. Specimen strength (Flexural)

Mix ID	28-Day Flexural Strength (MPa)
M0	4.3
B1	4.7
B2	5.1

The rise in flexural strength indicates that the material is more resistant to cracking due to flexure. The B2 mix showed the highest flexural performance, as shown in Figure 6.

Fig. 6 Flexural strength test of beam specimen

4.8. Durability Evaluation

The durability of the specimens was tested through immersion in acid and alkaline solutions.

4.8.1. Acid Resistance

Standard size cubes have been cured and deep in 5% H_2SO_4 for 28 days, shown in Figure 7, whose values revealed in Table 12.

Fig. 7 Acid resistance test

Table 12. Acid resistance results

Mix ID	% Weight Loss	% Strength Loss
M0	4.8	11.2
B1	3.1	6.4
B2	2.7	5.1

The bacterial mixes showed lower weight and strength loss than the control mix, indicating better resistance to acid attack due to calcite-based pore blocking shown in Figure 8.

Fig. 8 Alkaline resistance test

4.8.2. Alkaline Resistance

Similarly, cubes were deep in 5% NaOH for 28 days, whose outcomes are shown in Table 13.

Table 13. Alkaline resistance results

Mix ID	% Weight Loss	% Strength Loss
M0	3.6	8.7
B1	2.5	5.2
B2	2.2	4.5

The bacterial concrete also showed improved alkaline resistance, with B2 performing best.

Mass and strength loss were determined using:

$$\%W_L = \frac{W_i - W_f}{W_i} \times 100 \quad \%S_L = \frac{f_i - f_f}{f_i} \times 100 \quad 4.9$$

Performance Interpretation

The overall performance enhancement may be expressed as:

$$HE(\%) = \frac{X_b - X_0}{X_0} \times 100$$

The results obtained from the experiments are confirmatory of the beneficial effects that B. subtilis has on the strength and durability of the concrete. Among all the concrete mixes that were prepared and tested, the one with the highest number of B. subtilis cells (10 6 cells/mL) had the best performance.

5. Results and Discussion

5.1. Opening Discourse

What follows is the presentation and comparison of the mechanical strength and lasting endurance of bacterial concrete made with Bacillus subtilis in contrast to conventional concrete. The diverse kinds of strength and endurance of the concrete will be reviewed.

5.2. Compressive Strength

Strength in compression is one of the primary indicators of the load that concrete can carry. The results of the compressive strength test are presented in Figure 9.

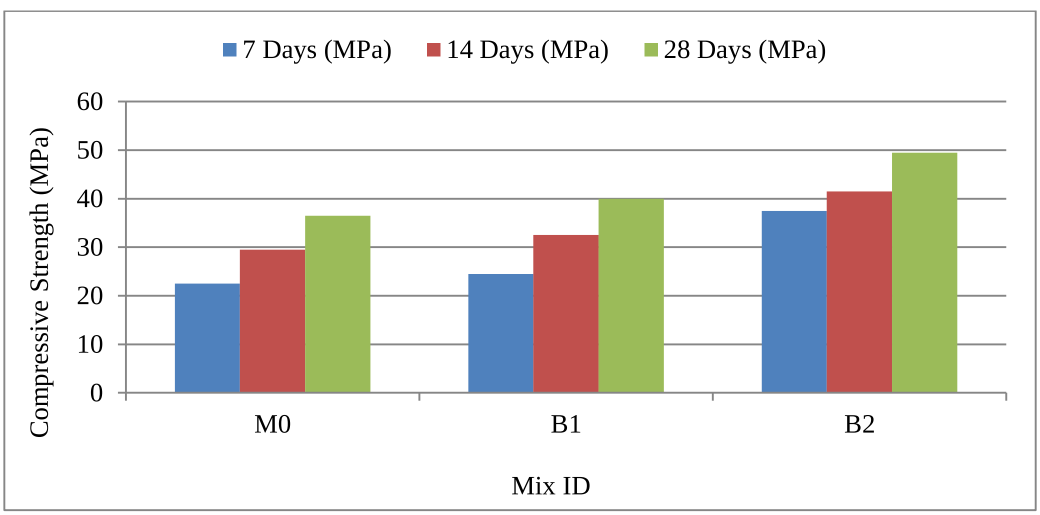

Fig. 9 Compressive strength development of concrete mixes

Both bacterial mixes revealed better strength than the control concrete. At 28 days, the strength improved from 36.5 MPa (M0) to 40.2 MPa (B1) and 49.5 MPa (B2).

The enhance in strength is attributed to the formation of MICP. The B2 mix, with a strength of 10^6 cells/mL, outperformed the other two mixes.

5.3. Split Tensile Strength

This indicates the resistance of concrete to crack initiation and propagation. The 28-day results are given in Figure 10.

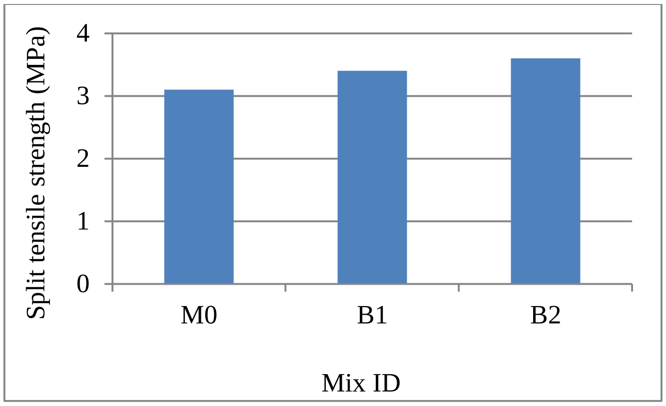

Fig. 10 Split tensile strength of mixes

Associated to the control mix, the tensile strength increased by approximately 9.7% for B1 and 16.1% for B2. This increased strength suggests that the bacterial calcite is contributing to the properties of the cement matrix.

5.4. Flexural Strength

The strength in flexural is an important property to consider for components such as slabs, pavements, and beams. The 28-day results are shown in Figure 11.

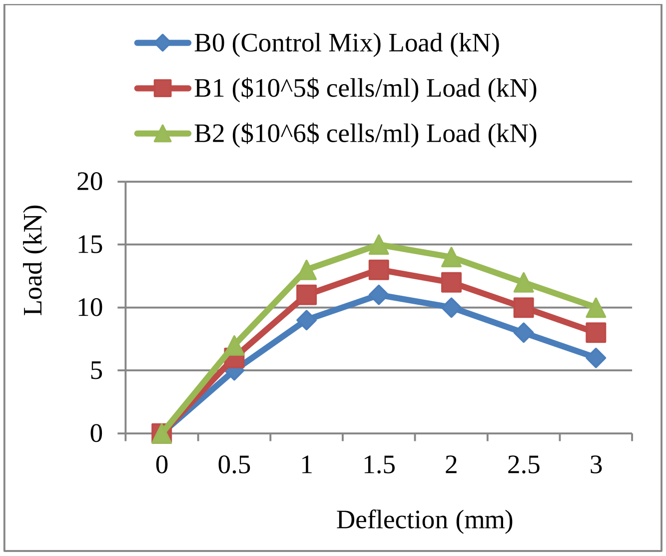

Fig. 11 Load-deflection response of concrete mixes

The flexural strength of B1 and B2 was increased by about 9.3% and 18.6% as compared to the control mix. The improvement of the flexural strength suggests that bacterial mineralization is effective in increasing the crack resistance of the materials. The presence of white deposits under the loading zone also indicates the precipitation of $CaCO_3$ and the healing of cracks.

5.5. Durability Performance

Durability was evaluated through acid and alkaline resistance tests to simulate aggressive field conditions.

5.5.1. Acid Resistance

The bacterial concrete samples exhibited much lower levels of deterioration compared to the control concretes when exposed to acidic environments. The strength loss for B1 and B2 concretes was reduced by 42.9% and 54.5% compared to the M0 control, respectively. This result is attributed to the calcite filling of the pores and cracks in the concrete structures, which limits the penetration of the acid into the concrete matrix.

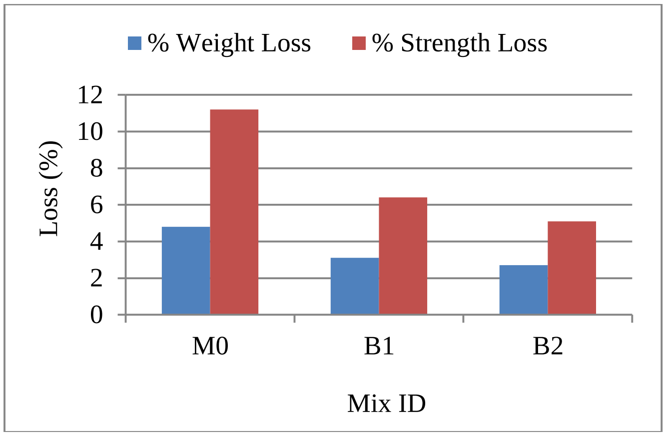

Fig. 12 Acid resistance performance of concrete mixes

5.5.2. Alkaline Resistance

The results also make clear that the bacterial concrete had superior resistance to chemical attack. This confirms that B. subtilis not only improves the strength of the concrete but also increases its durability.

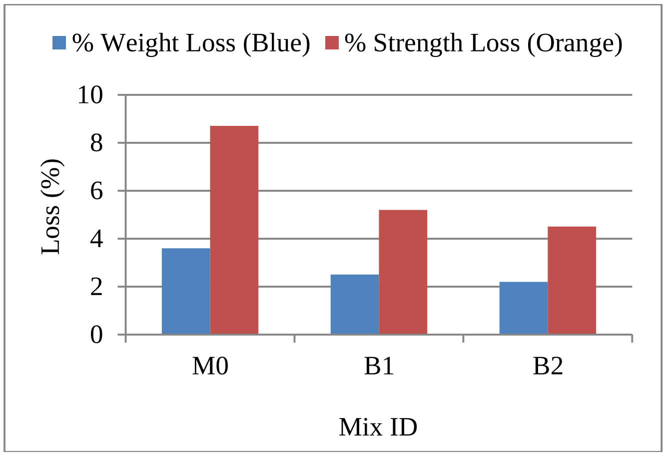

Fig. 13 Alkaline resistance performance of concrete mixes

5.6. Microstructural Observations

5.6.1. SEM Microstructural Analysis

SEM analysis was employed to investigate the microstructural characteristics of bacterial concrete and to confirm the occurrence of MICP. The micrograph of the control specimen (Figure 14(a)) reveals the typical heterogeneous and porous nature of the cement matrix. In contrast, bacterial-treated specimens show some microstructural modifications. The micrograph of the sample collected from the surface region of the remediated crack (Figure 14(b)) reveals the presence of calcite precipitation deposited onto and between the sand particles, indicating that these calcite crystals act as natural cementing agents.

Additionally, the micrograph of the sample collected from the interior region of the crack (Figure 14 (c)) shows the presence of well-developed calcite crystals that are filling the microcracks and voids within the structure. The micrograph of the deepest interior region of the crack (Figure 14 (d)) reveals that the calcite deposits are visible within the rod-shaped cavities that correspond to the bacterial cells.

These observations confirm that the metabolic activity of the bacteria promoted the precipitation of calcite crystals that effectively sealed the microcracks within the cement matrix.

The formation of such mineral phases also reduces the penetrability of the concrete and improves the bonding between the hydration products. These microstructural observations help to explain the improved mechanical properties of microbial concrete as seen in the experimental results.

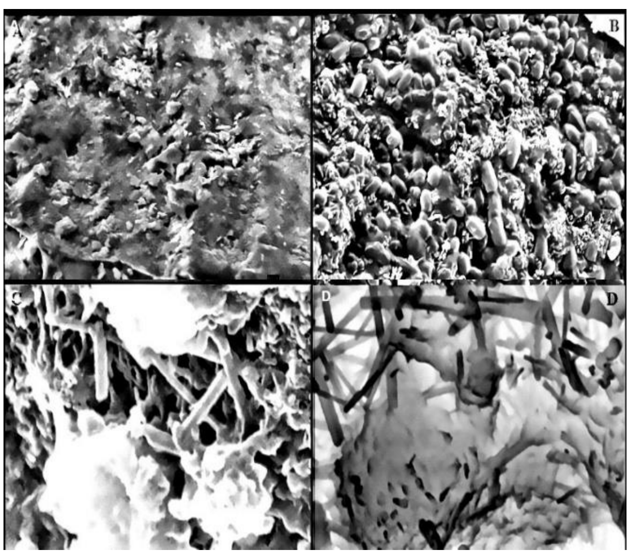

Fig. 14 SEM microstructural images illustrating microbial-induced calcite precipitation in bacterial concrete

5.6.2. EDX Spectrum of Microbial Sand Columns Treated with Bacillus Subtilis

The EDX spectrum indicates the presence of elements such as O, Si, Ca, and C, which indicates the formation of CaCO3 due to the metabolic activities of bacteria. Other elements, such as Al, Na, Cl, and Fe, are detected in smaller amounts and are from impurities in the concrete components.

The presence of Ca–C–O phases confirms the precipitation of calcite, which leads to densification and increased durability of the concrete structure.

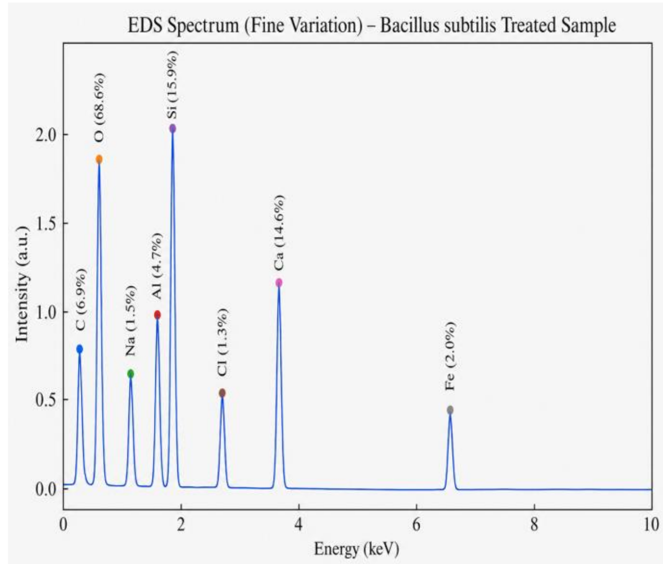

Fig. 15 EDX spectrum of bacterial concrete showing elemental composition

5.6.3. XRD Analysis

XRD analysis performed on the microbial-treated sand and concrete specimens revealed the formation of minerals that are consistent with calcite ($CaCO_3$) formation. The XRD spectrum of the treated samples shows strong peaks from quartz (SiO_2) present in the sand at around $2\theta \approx 26.6°$.

In addition, however, there are distinct diffraction peaks from calcite ($CaCO_3$) present at around $2\theta \approx 29.4°$, 39.4°, and 43.1°, indicating the formation of calcite by the microbial species. The formation of these calcite crystals in the matrix indicates that the treatment reduced the porosity of the sand and improved the bonding between the particles.

The presence of these crystalline carbonate minerals indicates that the microbes were able to mineralize the substrate and form an improved, stronger material.

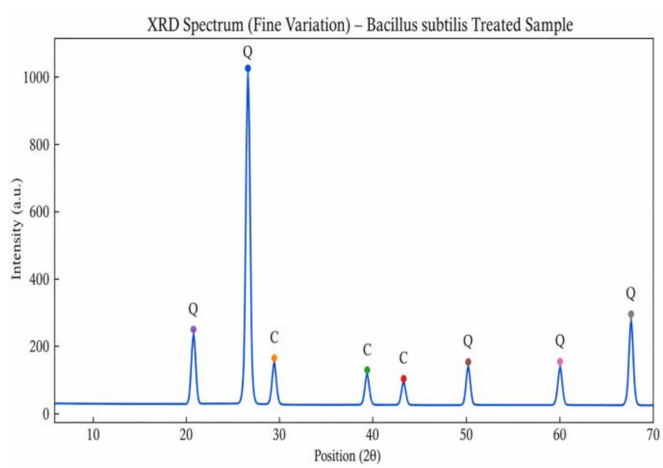

Fig. 16 XRD spectrum of microbial sand columns treated with bacillus subtilis

Fig. 17 Showing crack healing

5.7. RCPT Charge vs Time Interpretation

The charge passed during the 6-hour period is an indication of the permeability of the tested specimens. The control specimens accumulated a higher charge, reaching 3176 Coulombs after 6 hours, and exhibiting moderate permeability (ASTM C1202).

In contrast, the specimens treated with Bacillus subtilis reached only 1304 Coulombs, indicating low penetrability. The difference in the amount of charge passed after the first hour of the test indicates that the bacterial treatment results in improved resistance to penetration of the specimens.

This is due to the sleet of calcium carbonate within the pores and cracks of the concrete via MICP. The resulting densification of the concrete structure limits the movement of chloride ions and increases the durability and resistance of the concrete to chloride-induced corrosion.

Table 14. RCPT charge values and permeability assessment for control and bacillus subtilis modified concrete

Treatment	Charge Passed (Coulomb) – Trial 1	Trial 2	Trial 3	Average Charge Passed (Coulomb)	Permeability Class (ASTM C1202)
Control	2887	3248	3392	3175.67	Moderate
Bacillus subtilis	1328	1268	1317	1304.33	Low

Fig. 18 Experimental setup for RCPT and preparation of electrolyte solutions for bacterial concrete specimens

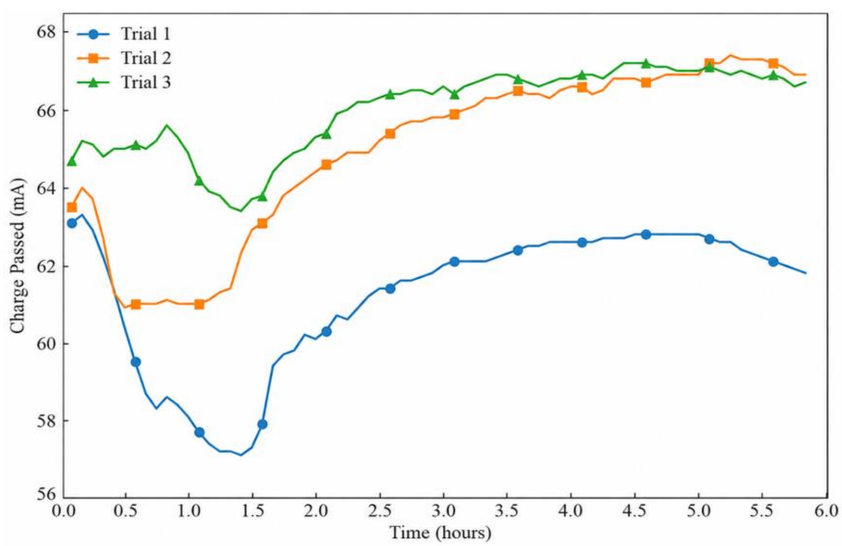

Fig. 19 Variation of charge passed (mA) with time during the RCPT test for Bacillus subtilis-based concrete specimens tested in triplicate

5.8. Integrated Strength–Durability Performance Analysis

The integrated radar diagram hereafter doth portrays the integrated performance of the strength and durability measures of both the control and the bacterial concretes. The type of concrete that is endowed with Bacillus subtilis bacteria shows a nobler excellence in terms of strength, as well as in terms of acid, alkali, and RCPT trial resistance.

The higher measures of these qualities that are present in bacterial concrete do arise from the presence of MICP. The calcite crystals that are created by the bacteria fill the pores within the concrete, increasing the strength and durability of the concrete structure.

5.9. Mechanistic Interpretation

The improved performance of the bioactivity in the presence of Bacillus subtilis is recognized to the biomineralization activity of these bacteria. In the presence of moisture and nutrients, the bacteria perform metabolism to yield carbonate ions that react with calcium ions to form calcite:

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

The overall microbial conversion of calcium lactate is:

$$Ca(C_3H_5O_3)_2 + 7O_2 \rightarrow CaCO_3 + 5CO_2 + 5H_2O$$

The precipitated CaCO3 acted as:

- a microfiller that reduces the pore connectivity,
- a crack-sealing product that restores the crack discontinuity, and
- a densifying agent that enhances the interfacial strength.

Therefore, bacterial concrete exhibited increased strength, decreased permeability, and improved resistance to acid and alkaline attack.

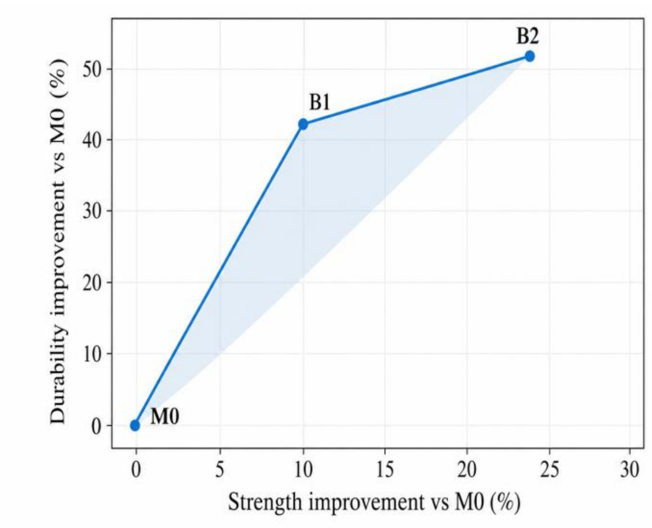

Fig. 21 Strength-durability performance envelope diagram

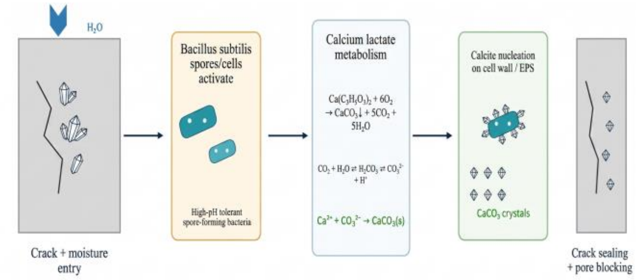

Fig. 22 Schematic illustration of MICP mechanism and its influence on the behavior of bacterial concrete

6. Conclusion

6.1. Concluding Remarks

- Compressive Strength: Increases by 10.1% (B1) and 35.6% (B2) compared to the control concretes – indicating that the biogenic precipitation of CaCO3 leads to densification of the matrix.
- Split Tensile Strength: Increases by 9.7% (B1) and 16.1% (B2), reflecting the improved cracking resistance of the concretes.
- Flexural Strength: Increases by 9.3% (B1) and 18.6% (B2), indicating the improved resistance of the concretes to bending.
- Workability: Decreases by 5-8% in slump; however, the slump of all concretes remains within the acceptable range of 75-100 mm.
- Acid Resistance: The reduction in strength loss is 42.9% in B1 and 54.5% in B2 compared to the control concretes when exposed to 5% H_2SO_4.
- Alkaline Resistance: The reduction in strength loss is 40.2% in B1 and 48.3% in B2 compared to the control concretes when exposed to 5% NaOH.
- RCPT Permeability: The charge passed is reduced by 58.9%, from 3175.7 Coulombs (moderate permeability) in control concretes to 1304.3 Coulombs (low permeability) in bacterial concretes.
- The bacterial concretes containing 10^6 cells/mL (B2) exhibit the best performance overall – achieving approximately 35% improvement in strength and nearly 50% improvement in durability relative to conventional concrete.
- The improvements in most of the properties are due to (MICP), which densifies the matrix of the concretes and reduces their permeability. This suggests the potential of bacterial concretes as a sustainable and self-healing construction material.

6.2. Engineering Significance

The findings of this research show that bacterial Self-Repairing Concrete has great potential as a material for constructing durable and low-maintenance structures and infrastructure. The ability of bacterial components to autonomously seal cracks in concrete enhances the durability of the construction.

These types of construction materials are ideal for structures exposed to aggressive environments, such as marine environments, sewers, pavements, underground constructions, and water-retaining constructions. Therefore, the incorporation of biological functionality into construction materials is a promising development.

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