

Performance Evaluation of Multi-Bacterial Self-Healing Concrete Incorporating *Bacillus Subtilis*, *Bacillus Megaterium* and *Pseudomonas Aeruginosa* for Enhanced Mechanical and Durability Properties

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ABSTRACT:

The deterioration of reinforced concrete structures as a result of concrete cracking is a prevalent issue, which leads to increased permeability, corrosion of reinforcement, and decreased service life. Although bacterial self-healing concrete has been widely researched, the majority of studies have been limited to the use of single bacteria species; very few studies have been conducted using multi-bacterial consortia and evaluating the overall synergistic effects of using multiple bacteria. This paper presents new quantitative data on binary combinations of *Bacillus subtilis*, *Bacillus megaterium*, and *Pseudomonas aeruginosa*, that were incorporated into M20 concrete using method direct addition at levels of 10 ml/L, 50 ml/L, and 100 ml/L of mixing water. Calcium lactate (2% by mass of cement) was used as a nutrient source in order to promote Microbial Induced Calcium Carbonate Precipitation (MICP). Results show that significant improvement occurred with the use of bacteria in concrete; specifically, the AC-100 and BC-100 mixtures produced the highest compressive strength of 29.2 MPa (31% increase), while AB-100 and BC-100 produced the highest flexural strength of 4.1 MPa (24.2% increase). The highest UPV value of 4.50 km/s was also produced by the BC-100 mixtures and demonstrated a 53.8% decrease in water penetration. The current study is limited to 28-days of laboratory tests only; therefore, long-term field based validation and microstructural testing such as SEM and XRD are required for further evaluation. The results from this research have demonstrated the potential of multi-bacterial concrete to serve as a sustainable and durable alternative for infrastructure applications. SEM and XRD analyses confirmed extensive calcium carbonate precipitation, pore refinement, and matrix densification through the Microbially Induced Calcium Carbonate Precipitation (MICP) mechanism, resulting in effective healing of cracks ranging from 1 to 5 mm.

Keywords: Bacterial Concrete, Self-Healing Concrete, MICP, *Bacillus subtilis*, *Bacillus megaterium*, *Pseudomonas aeruginosa*, Calcium Lactate, Durability, Sustainable Construction.

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Highlights

- Multi-bacterial consortia were effectively introduced into M20 grade concrete using direct addition method.
- Calcium lactate was added as a supplement at 2% of cement weight to stimulate bacteria activity and calcium carbonate precipitation.
- The BC-100 mix displayed the biggest water penetration reduction (53.8%) and the highest UPV value (4.50 km/s).
- Mixtures of AC-100 and BC-100 showed the maximum compressive strength of 29.2 MPa which is 31% increase over the control concrete.
- The multi-bacterial systems showed a remarkable improvement in mechanical and durability properties.

1. Introduction

1.1 Background and Need for Durable Concrete

Concrete is the world's most-used construction material. The reason for the popularity of concrete is its great compressive strength, durability, adaptability and cost effectiveness. Concrete is essential to the structural integrity and long-term performance of modern infrastructure, including buildings, bridges, dams, tunnels, pavements and marine structures [1]. Concrete is widely utilized but is susceptible to cracking due to plastic shrinkage, drying

shrinkage, thermal stresses, mechanical loading, creep and environmental deterioration. These gaps provide entry channels for water, chlorides, sulfates, carbon dioxide and other aggressive chemicals which ultimately results into reinforcement corrosion, loss of durability and early structural collapse [2]. The deterioration caused by cracking has a significant role in maintenance costs and in reducing the service life of reinforced concrete structures [3]. Traditional repair procedures, the authors said, generally involve heavy labor, regular upkeep and significant financial commitment across the life cycle of a structure. Therefore, the development of new technologies capable of self-repair of fractures has become a major subject of research in sustainable construction [4].

1.2 Self-Healing Concrete Technology

Self-healing concrete is an effective solution that has arisen to overcome traditional repair methods limits [5]. Self-healing concrete is defined as concrete that is able to repair cracks spontaneously or by engineered mechanisms without external intervention. Several self-healing strategies have been created such as autogenous healing, encapsulated healing agents, vascular systems, crystalline admixtures and bacterial healing procedures [6].

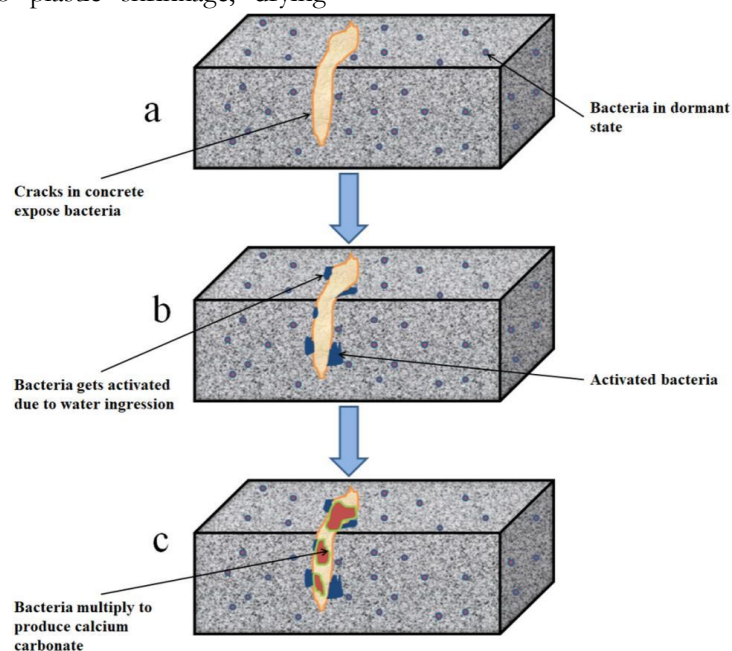


Fig 1: Self-Healing Concrete Technology

Among these, bacterial self-healing concrete has attracted much interest for its environmental-friendliness and long-term fracture repair [7].

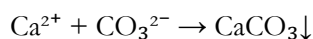
Bacterial concrete uses naturally-occurring bacteria to trigger the self-healing mechanism in concrete matrix unlike the standard repair approach which uses synthetic chemicals. The biological method can bring important benefits in terms of sustainability, by improving durability and reducing the need for maintenance [8].

The bacterial concrete is an environment friendly alternative to typical repair procedures as it enhances the durability of the structure and concurrently minimizes the environmental implications of repair and reconstruction activities [9]. Identified bacterial concrete as a next-generation construction material that might prolong the service life of structures and reduce carbon emissions related with the maintenance of infrastructure [10].

1.3 Microbially Induced Calcium Carbonate Precipitation (MICP)

The self-healing ability of bacterial concrete can be mainly attributable to the mechanism called Microbially Induced Calcium Carbonate Precipitation (MICP) [11]. In this process, the carbonate ions generated from the bacterial metabolic activities react with the available calcium ions to form calcium carbonate (CaCO_3) crystals. These crystals precipitate in pores, crevices and cracks therefore blocking the routes for fluid intrusion and increasing the density of concrete [12].

The MICP process can be represented by the following simplified reaction:



The calcium carbonate deposited is usually in the form of calcite, aragonite or vaterite crystals, depending on the environmental circumstances and the bacterial species involved [13]. The mineral deposits formed this way not only mend the cracks but also help to refine the pores, densify the matrix and improve the mechanical characteristics [14].

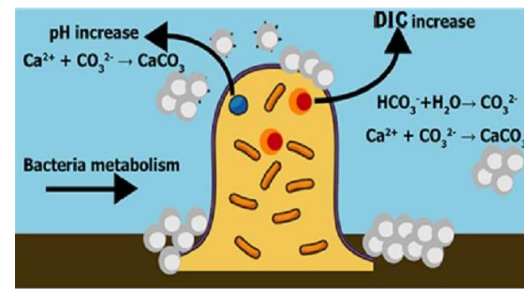


Fig 2: Microbially Induced Calcium Carbonate Precipitation (MICP)

MICP has been previously investigated for its effectiveness in increasing concrete performance [15]. Crack healing efficiency of up to 95% with considerable improvement in compressive strength and durability. The calcite production was increased and water absorption was decreased by bacterial consortia mortar. Microbial self-healing cement systems showed a significant reduction of permeability and porosity [16-18].

1.4 Significance and Novelty of the Present Study

The presence of cracks in concrete results in a lower durability and service life of concrete structures that have been reinforced, as the cracks allow water and aggressive ions to be introduced into the concrete, leading to increased chances of corrosion of the reinforcement and increasing maintenance costs for the structure. Bacterial self-healing concrete can potentially be used as an environmentally friendly and sustainable solution; however, most current studies have focused on just one strain of bacteria, with little information available regarding the use of bacterial consortia and their optimal dosage.

A bacterial self-healing concrete system using three types of bacteria (i.e., *Bacillus subtilis* and *Bacillus megaterium*; *Pseudomonas aeruginosa*) was investigated in the current study. The three combinations of bacteria A+B, A+C and B+C were used in M20 grade concrete in dosages of 10, 50 and 100ml/L using the direct addition method with a nutrient source of calcium lactate. The quantification of the three binary bacterial consortia investigated in this study demonstrated an improvement of up to 31% in

compressive strength, a 24.2% increase in flexural strength and a 53.8% reduction in water penetration when compared to conventional concrete. Therefore, this study provides a scientific basis for improving the design of bacterial concrete, which will lead to more durable and sustainable construction applications.

1.5 Objectives of the Study

The main objectives of this research are:

- To investigate the effect of binary bacterial consortia and their dosages on the mechanical properties (compressive and flexural strength) of M20 concrete.
- To evaluate the durability and quality of bacterial concrete using Ultrasonic Pulse Velocity (UPV) and water penetration tests.
- To identify the optimum bacterial combination and dosage for enhancing the overall performance of self-healing concrete.
- To compare the performance of the proposed bacterial consortia with findings reported in previous studies on bacterial concrete.

1.6 Problem statement of the study

Concrete structures frequently develop cracks due to shrinkage, thermal stresses, and mechanical loading, allowing water and aggressive chemicals to penetrate the concrete and accelerate reinforcement corrosion and structural deterioration. Conventional repair methods are costly, labor-intensive, and often provide only temporary solutions. Although bacterial self-healing concrete has emerged as a sustainable alternative, most existing studies focus on single bacterial species and provide limited information on the synergistic effects of bacterial consortia. Therefore, there is a need to investigate multi-bacterial systems for improving the mechanical performance and durability of self-healing concrete.

2. Literature Review

Recent research indicates considerable development of bacterial self-healing concrete

by the process of microbiologically-induced calcium carbonate precipitation (MICP). Most of this pioneering work has taken place using different strains of bacteria from the genus *Bacillus*. In their 2021 study, Feng et al. [19] developed a self-healing concrete that included an alkali-resistant *Bacillus subtilis* M9 along with polyvinyl alcohol fibers and demonstrated that their self-healing ability to autonomously heal 0.3 mm wide cracks increased residual flexural strength by around 14%. In 2021, Gadhav et al. [20] also reported on how *Bacillus subtilis* could help to decrease porosity and increase strength and durability of concrete when subjected to sulfate and alkaline environments. Javeed et al. [21] completed an overview in 2024 evaluating how environmental conditions impact bacterial viability and basically concluded that concentrations of *B. subtilis* bacteria between 10^5 to 10^8 cells/mL are capable of improving the mechanical properties of concrete by as much as 42.8%. Li et al. [24] developed a microbial self-healing cement slurry with fly ash as a microbial carrier, incorporating 3% microorganisms and 3% microcapsules, resulting in considerably increased integrity of the cement sheath and increased amounts of calcium carbonate produced after incorporation into the slurry. Lucas et al. [25] continued to describe the potential for the successful remediation of cracks in concrete and thus the extension of the service life of concrete structures with the use of immobilized microorganisms within expanded clay aggregate.

Substantial improvements in mechanical and durability properties have also been reported in recent experimental investigations. Mark et al. (2025) [27] observed superior self-healing performance and mechanical enhancement in concrete containing 10% limestone powder and *Bacillus subtilis*. Putra et al. (2025) [29] investigated different dosages of bacterial mixtures and reported the highest average flexural capacity of 6.34 tons with 0.6% *Bacillus* addition, accompanied by an average calcite length of 41 mm and an amorphous phase of 82.63%. Pachaivannan et al. (2020) [32] reported increases of 19.51% in compressive strength,

17.8% in split tensile strength, and 14.24% in flexural strength after 28 days of curing. Rais et al. (2020) [33] demonstrated complete healing of cracks up to 0.63 mm and recovery of compressive strength ranging from 57% to 93%. Nagarajan et al. (2025) [35] found that a bacterial consortium of *Bacillus cereus* and *Vibrio natriegens* increased compressive strength by 19.85% while reducing water absorption by 29.6%, indicating the superior performance of multi-bacterial systems.

The concrete of bacterial self-healing exhibits remarkable potential to improve and extend the durability of structures over time, as confirmed by numerous studies. Singh et al. (2024) [37] found that adding a 30 ml solution of *Bacillus subtilis* culture resulted in an increase of 30% in the compressive strength, 29.88% in the flexural strength, and 11.9% in the splitting tensile strength of the concrete compared to the conventional concrete. Also, Pourfallahi et al., (2020) [31] successfully repaired cracks in concrete (with a maximum crack width of 39.82

µm between the cracks) using bacterial concrete prepared with the bacterium *Bacillus paralicheniformis*. Rajczakowska et al. (2019) [34] also conducted a thorough literature review of the self-healing technology available, emphasizing the need for improving the survival rates and cost-effectiveness of bacteria for large-scale use of this type of concrete. Similarly, Mitikie et al. (2023) [35]; Singh et al. (2023) [36] pointed out that there are far fewer maintenance requirements and a substantial improvement in environmental sustainability when used in conjunction with bacterial concrete and possess an increased capacity to prolong the useful life (service life) of a structure. Collectively, these findings offer support for the assertion that bacterial self-repairing concrete will have an increasing role in sustainable building materials for modern construction, and that the potential for new infrastructure development is within reach when you consider its ability to enhance structural integrity, provide a source for renewed strength, and promote greater durability for infrastructure.

Table 1: Summary of Recent Studies on Bacterial Self-Healing Concrete

Ref. No.	Author(s) & Year	Bacterial Species/Approach	Key Findings	Numerical Results
[19]	Feng et al. (2021)	<i>Bacillus subtilis</i> M9 with PVA fibers	Autonomous healing of cracks through MICP and calcite precipitation	Healed crack width: 0.3 mm ; Flexural strength recovery: 14%
[20]	Gadhane et al. (2021)	<i>Bacillus subtilis</i>	Reduced porosity and improved strength and durability under aggressive environments	Improved compressive strength and crack filling (qualitative result)
[21]	Javeed et al. (2024)	Bacteria-based self-healing concrete	Mechanical properties improved with optimum bacterial concentration	Cell concentration: 10⁵–10⁸ cells/ml ; Strength enhancement: 42.8%
[24]	Li et al. (2025)	Microbial self-healing cement slurry using fly ash carriers	Enhanced cement sheath integrity and self-healing performance	Optimum dosage: 3% microorganisms + 3% microcapsules
[25]	Lucas et al. (2018)	Bacteria immobilized in expanded clay	Improved crack remediation and	No specific numerical values reported

			extended service life	
[26]	Mitikie et al. (2025)	Bio-based bacterial concrete	Improved self-healing, durability, and sustainability	Qualitative performance improvement
[27]	Mark et al. (2025)	<i>Bacillus subtilis</i> with limestone powder	Enhanced strength and durability of bio-concrete	Optimum limestone replacement: 10%
[29]	Putra et al. (2025)	<i>Bacillus</i> sp., <i>Staphylococcus</i> sp., and <i>Solibacillus</i> sp.	Highest flexural performance obtained with bacterial concrete	Flexural capacity: 6.34 tons ; Calcite length: 41 mm ; Amorphous phase: 82.63%
[31]	Pourfallahi et al. (2020)	<i>Bacillus paralicheniformis</i> and <i>Bacillus</i> sp.	Effective crack repair in bacterial concrete	Maximum repaired crack width: 39.82 μm
[32]	Pachaivannan et al. (2020)	<i>Bacillus subtilis</i>	Significant improvement in mechanical properties	Compressive strength: 19.51% ; Split tensile strength: 17.8% ; Flexural strength: 14.24%
[33]	Rais et al. (2020)	Bacterially induced CaCO_3 precipitation in RAC	Complete crack healing and recovery of strength	Maximum healed crack width: 0.63 mm ; Strength recovery: 57–93%
[34]	Rajczakowska et al. (2019)	Review of self-healing concrete technologies	Identified challenges in bacterial survivability and implementation	No quantitative values reported
[35]	Nagarajan et al. (2025)	<i>Bacillus cereus</i> and <i>Vibrio natriegens</i> consortium	Superior self-healing and durability performance	Compressive strength increase: 19.85% ; Water absorption reduction: 29.6%
[36]	Singh et al. (2024)	Review of self-healing concrete mechanisms	Enhanced durability and reduced maintenance requirements	Qualitative findings
[37]	Singh et al. (2024)	<i>Bacillus subtilis</i> in PPC concrete	Significant improvement in strength properties	Compressive strength: 30% ; Flexural strength: 29.88% ; Split tensile strength: 11.9%

Most existing studies about self-healing concrete with bacteria look at only 1 type of bacteria and perform the experiments in a controlled laboratory environment. There has not been much investigation into how multiple types of bacteria can work together to provide better healing of cracks and longer-lasting durability in concrete. Additionally, there is still a need to study how long self-healing concrete will last in

extremely aggressive environments, how long the microbes can survive when placed into alkaline concrete and exactly how to use bacteria in concrete at the best dosage. Finally, there is little to no field work to validate the use of bacterial concrete systems on a large scale and little to no economic analysis on using bacterial concrete systems. Therefore, the creation of an efficient multi-bacterial self-healing concrete

system that achieves increased durability and is practical to use in the field has been identified as an important research issue.

3. Materials and Methods

3.1 Overview of Experimental Program

The experimental examination was performed to study the effect of multi-bacterial consortia on the mechanical and durability parameters of M20 grade concrete. Binary bacterial combinations were made from three bacterial species: *Bacillus subtilis*, *Bacillus megaterium* and *Pseudomonas aeruginosa*. The effect of bacterial dosage on compressive strength, flexural strength, ultrasonic pulse velocity (UPV) and water penetration resistance after 28 days of curing was studied.

A total of ten concrete mixtures were prepared, one control concrete mixture and nine bacterial concrete mixtures. The bacterial combinations studied were A+B (*Bacillus subtilis* + *Bacillus megaterium*), A+C (*Bacillus subtilis* + *Pseudomonas aeruginosa*) and B+C (*Bacillus megaterium* + *Pseudomonas aeruginosa*) at the dosage levels of 10 ml/L, 50 ml/L and 100 ml/L of mixing water.

Using multiple bacterial communities, rather than only one bacterial species, for increasing calcium carbonate precipitation to enhance concrete's mechanical and durability properties has not previously been documented.

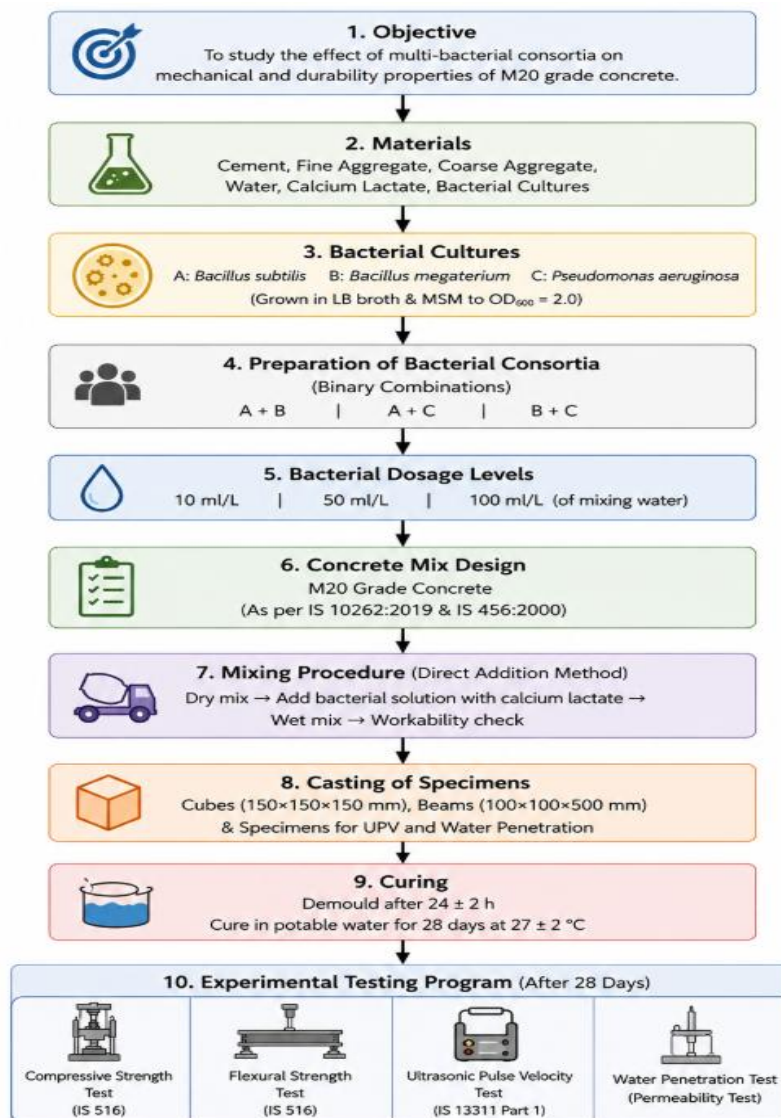


Fig 3: framework of proposed work

3.2 Materials Used

3.2.1 Cement

In the entire experimental inquiry, 43 grade Ordinary Portland Cement (OPC) complying with IS 8112 was used. The specific gravity of cement was 3.12 and it was obtained from one source to maintain uniformity throughout the investigation.

3.2.2 Fine Aggregate

Fine aggregate was natural river sand satisfying zone II grading requirements of IS 383:2016. The specific gravity of fine aggregate was 2.64 and water absorption capacity was 1.33%.

3.2.3 Coarse Aggregate

Coarse aggregates used were crushed stone aggregates with nominal sizes of 20 mm and 10 mm. The specific gravities of the 20 mm and 10 mm aggregates were 2.83 and 2.81 correspondingly. Water absorption capabilities were 1.03% and 1.20%, respectively.

3.2.4 Water

The concrete mixing, bacterial culture preparation and curing were carried out with potable water free of hazardous contaminants.

3.2.5 Calcium Lactate

Calcium lactate was employed as feed for bacterial metabolism and precipitation of calcium carbonate. Throughout the experimental program, the constant dosage of 2% by weight of cement was maintained. Calcium lactate provided the required source of calcium for microbial driven production of calcite and improved bacterial activity in the concrete matrix.

3.3 Bacterial Cultures

Three bacterial species were chosen based on their ability to thrive in alkaline environment and to generate calcium carbonate precipitation.

A: *Bacillus subtilis*

Bacillus subtilis is a Gram-positive spore-forming bacteria capable of precipitating calcium carbonate and surviving in very alkaline concrete settings.

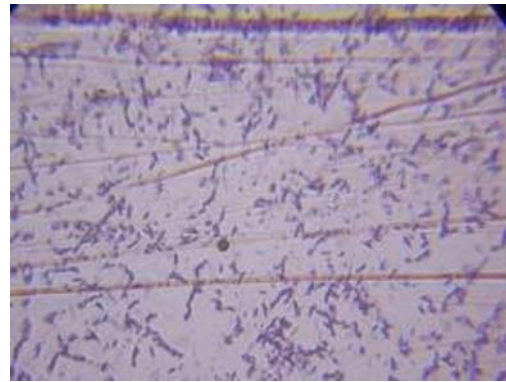


Fig 4: *Bacillus subtilis*

B: *Bacillus megaterium*

Bacillus megaterium is a urease positive bacteria that shows efficient biomineralization and increased calcite synthesis.

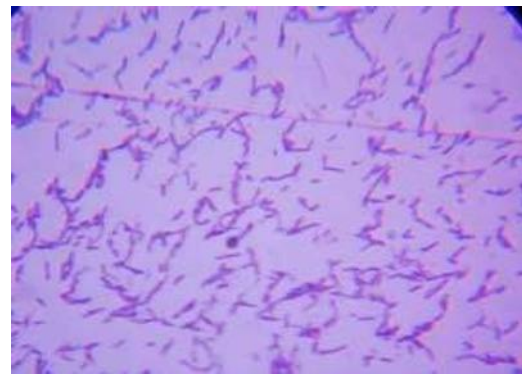


Fig 5: *Bacillus megaterium*

C: *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a Gram-negative bacteria that is capable of creating extracellular polymeric compounds and of stimulating biomineralization processes. Bacterial cultures were grown in Luria Bertani (LB) broth and Minimal Salt Media (MSM) to an optical density (OD600) of 2.0. Microbiological characterisation tests such as citrate utilization, urease activity, catalase activity, gram staining and carbohydrate utilization were carried out to establish bacterial identity and viability. These bacterial species were selected due to their high alkalinity tolerance, efficient biomineralization ability, and proven capability to precipitate calcium carbonate in cementitious environments.

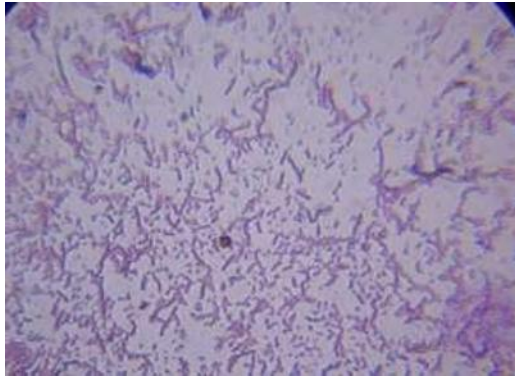


Fig 6: Pseudomonas aeruginosa

3.4 Concrete Mix Design

Design of concrete mix was carried out as per IS 10262:2019 and IS 456:2000 for M20 grade concrete. The target mean strength was obtained as 26.6 MPa.

The final mix proportion was:

1 : 2.58 : 3.51

(Cement : Fine Aggregate : Coarse Aggregate)

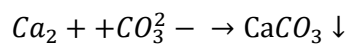
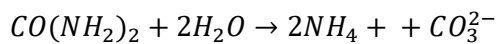


Table 2: Mix Proportion of Control Concrete

Material	Quantity (kg/m ³)
Cement	320
Fine Aggregate	826
Coarse Aggregate (20 mm + 10 mm)	1122
Water	176
Water-Cement Ratio	0.55

3.5 Preparation of Bacterial Consortia

Three binary bacterial combinations were prepared:

Table 3: Bacterial Combinations

Combination	Bacterial Species
A + B	<i>Bacillus subtilis</i> + <i>Bacillus megaterium</i>
A + C	<i>Bacillus subtilis</i> + <i>Pseudomonas aeruginosa</i>
B + C	<i>Bacillus megaterium</i> + <i>Pseudomonas aeruginosa</i>

Each bacterial consortium was formed by mixing equal amounts of the individual bacterial cultures just before concrete manufacture.

3.6 Bacterial Dosage Levels

The bacterial solutions were incorporated directly into the concrete mixing water at three dosage levels. The amount of chemical solution (10, 50, 100ml) that can be used is not predetermined; therefore, these three concentrations were established based on previous studies that indicated improvement in concrete properties from these three volumes.

Table 4: Bacterial Dosages

Mix ID	Bacterial Combination	Dosage (ml/L)
CM	Control Mix	0
AB-10	A + B	10
AB-50	A + B	50
AB-100	A + B	100
AC-10	A + C	10
AC-50	A + C	50
AC-100	A + C	100
BC-10	B + C	10
BC-50	B + C	50
BC-100	B + C	100

3.7 Concrete Mixing Procedure

Bacterial cultures were incorporated in concrete via direct addition method.

The experimental procedure was described below:

1. Dry mix the cement, fine aggregate and coarse aggregate for around 2 minutes .
2. Bacterial solution preparation by mixing bacterial culture with needed quantity of water.

3. Calcium lactate was added to the bacterial solution at 2% of the weight of the cement.
4. Pouring of the bacterial solution into the concrete mixer.
5. Mix wet for 3-5 minutes till uniform dispersion is obtained.
6. Workability assessment prior to casting.

The direct addition method was chosen due of its simplicity, low cost, and because it allows for a homogenous dispersion of the bacteria throughout the concrete matrix.

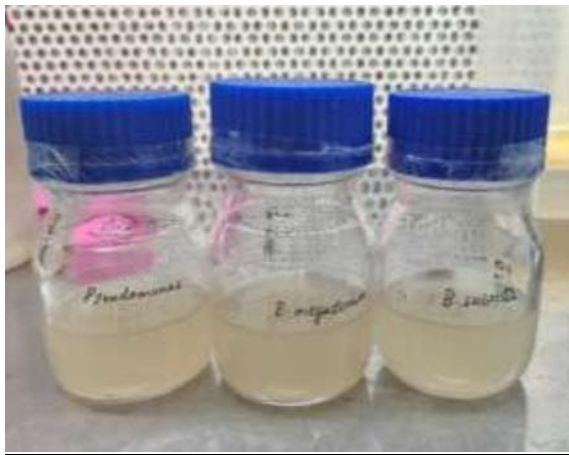


Fig 7: Bacterial Culture

3.8 Casting and Curing of Specimens

Three layers of fresh concrete were poured into steel moulds. Each layer was crushed with a table vibrator to eliminate the entrapped air and reach optimal consolidation.

For each mix and test three specimens were created. For the compressive strength study, 150 mm x 150 mm x 150 mm cube specimens were prepared, while for the flexural strength study, 100 mm x 100 mm x 500 mm beam specimens were prepared. Additionally, specimens were prepared for UPV and water penetration testing.

Table 5: Specimen Details

Test	Specimen Type	Size
Compressive Strength	Cube	150 × 150 × 150 mm
Flexural Strength	Beam	100 × 100 × 500 mm
UPV Test	Cube	150 × 150 × 150 mm
Water Penetration	Cube	150 × 150 × 150 mm

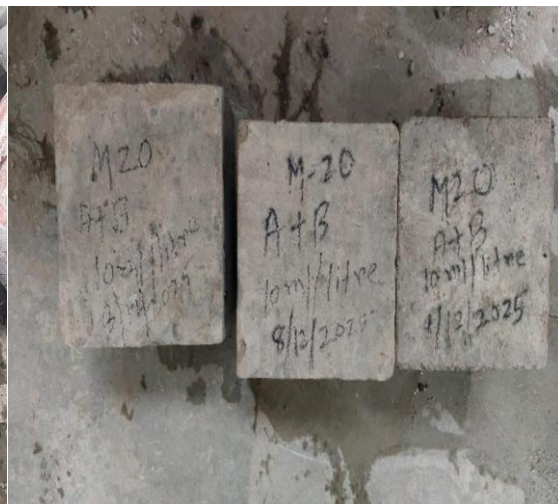




Fig 8: Casting of Bacterial Concrete

After 24 h, the specimens were demoulded and cured in curing tanks containing potable water. The specimens were cured at a temperature of $27 \pm 2^\circ\text{C}$ until the testing age of 28 days.

3.9 Experimental Testing Program

Compressive strength test was conducted as per IS 516 by utilizing Compression Testing Machine (CTM). The maximum load taken by each specimen was recorded and the compressive strength was computed as:

The compressive strength was calculated using Eq. (1):

$$f_c = \frac{P}{A} \quad (1)$$

where,

f_c = Compressive strength (MPa)

P = Maximum load at failure (N)

A = Loaded area of the specimen (mm^2)

The average value of three specimens was reported.



Fig 9: Compressive Strength test on Bacterial Concrete

3.9.2 Flexural Strength Test

Flexural strength was evaluated on the beam specimens according to IS 516 under third point loading conditions.

The flexural strength (modulus of rupture) was calculated using Eq. (2):

$$f_r = \frac{PL}{bd^2} \quad (2)$$

where,

f_r = Flexural strength (MPa)

P = Maximum applied load at failure (N)

L = Effective span length of the beam (mm)

b = Width of the beam specimen (mm)

d = Depth of the beam specimen (mm)

The values of flexural strength obtained were utilized to analyze the effect of the bacterial consortia on the tensile behavior and resistance to crack of the concrete. The boost in the

flexural strength was related to the formation of calcium carbonate in the concrete matrix which enhanced the internal bonding and reduced the propagation of micro-cracks.



Fig 10: Flexural Strength test on Bacterial Concrete

3.9.3 Ultrasonic Pulse Velocity Test

The Ultrasonic Pulse Velocity (UPV) test was performed to investigate the quality, homogeneity and internal integrity of the concrete specimens after 28 days of curing. The test was carried out as per IS 13311 (Part 1): 1992. Ultrasonic Pulse Velocity instrument was used for the test. The transducers were positioned on the opposing faces of the specimen and the time taken by the ultrasonic pulse to travel through the concrete was measured. The ultrasonic pulse velocity was calculated using Eq. (3):

$$V = \frac{L}{T} \quad (3)$$

where,

V = Ultrasonic Pulse Velocity (km/s)

L = Path length between transducers (m)

T = Transit time of the ultrasonic pulse (s)

UPV readings collected were used to assess the quality and homogeneity of concrete. Higher pulse velocity values are indicative of a concrete matrix which is denser and more homogeneous with fewer internal cavities and micro-cracks. The UPV enhancement in bacterial concrete was related to the precipitation of calcium carbonate, improving the pore structure and, consequently, the overall integrity of the concrete matrix. The UPV values obtained were interpreted according to the quality rating given in IS 13311. Statistical analysis of experimental results utilized the average values, percentage improvement, and standard deviation of the data to help identify an optimal dosage and/or solution mix to use for the bacterial consortium.



Fig 11: Ultrasonic Pulse Velocity test on Bacterial Concrete

3.9.4 Water Penetration Test

Water penetration tests were conducted to measure resistance to permeability and durability



Fig 12: Water Penetration test on Bacterial Concrete

The decreased penetration depth demonstrated that calcite precipitation by bacteria increased pore refinement and resistance to fluid entry.

3.9.5 Crack Healing Evaluation

After curing, artificial surface fissures of approximately 1 mm to 5 mm width were cut into the bacterial concrete specimens. The specimens were cured under moist conditions and the crack-healing activity was visually seen

and recorded by photographs. The crack closure was measured after 0, 7, 14 and 28 days to evaluate the self-healing efficacy of different bacterial combinations. Healing was determined by measuring the reduction in crack width and amount of calcium carbonate deposition in the cracks. The results reveal increasing fracture closure, with the BC-100 and AC-100 mixes demonstrating almost complete healing after 28 days.

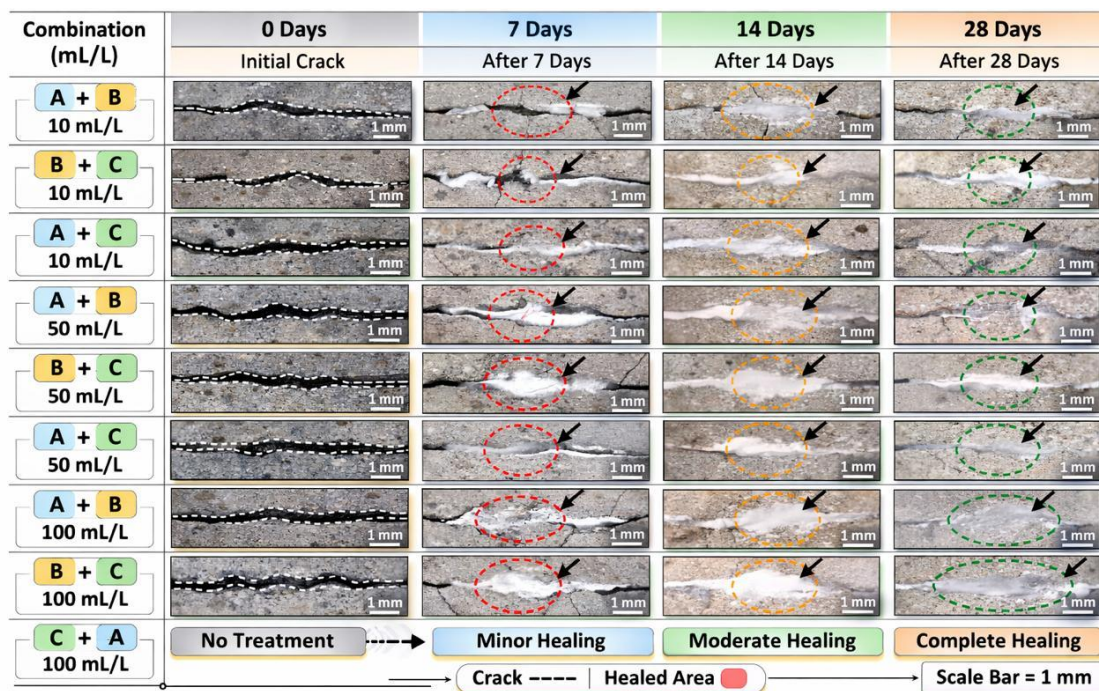


Fig 13: Crack Healing Efficiency of Combination at 0, 7, 14 and 28 Days

3.9.6 SEM and XRD Analysis

Selected specimens were taken for microstructural and mineralogical characterisation after 28 days of crack-healing. Scanning Electron Microscopy (SEM) was utilized to investigate the shape of the healing products and the degree of fracture filling inside the concrete matrix. X-ray diffraction (XRD) research was performed to detect the crystalline phases produced during the healing process. The studies were carried out on samples from the healed fracture zones to check if calcium carbonate precipitates were generated via the Microbially Induced Calcium Carbonate Precipitation (MICP) mechanism. SEM and XRD data are connected with the observed crack-healing performance and durability increase of bacterial concrete sample.

3.10 Data Analysis

Comparative and percentage improvement approaches were used to analyse the experimental findings acquired from compressive strength, flexural strength, UPV and water penetration tests. To find out the

appropriate bacterial combination and dosage, performance of bacterial concrete mixtures is compared with the conventional concrete.

Furthermore, the experimental results were correlated with previously published investigations to assess the efficacy of multi-bacterial consortia and to confirm their applicability for sustainable self-healing concrete applications.

4. Results and Discussion

4.1 Overview of Experimental Results

The mechanical and durability qualities of M20 grade concrete were studied for the effect of bacterial consortia by carrying out compressive strength, flexural strength, UPV and water penetration tests after 28 days of curing. The results from the experiment showed that the addition of bacteria greatly improved the strength and durability parameters when compared to normal concrete. The improvements can be attributed to Microbially Induced Calcium Carbonate Precipitation (MICP) which led to pore refining, crack closure and densification of the cementitious matrix.

Table 6: Summary of Experimental Results

Mix ID	Compressive Strength (MPa)	Increase (%)	Flexural Strength (MPa)	Increase (%)	UPV (km/s)	Water Penetration (mm)	Reduction (%)
CM	22.3	0.0	3.3	0.0	3.72	13	0.0
AB-10	24.8	11.2	3.5	6.1	3.81	12	7.7
AB-50	26.5	18.8	4.1	24.2	3.97	10	23.1
AB-100	28.7	28.7	4.1	24.2	4.32	8	38.5
AC-10	24.6	10.3	3.4	3.0	3.89	11	15.4
AC-50	27.2	22.0	3.5	6.1	3.90	9	30.8
AC-100	29.2	31.0	3.8	15.2	4.32	7	46.2
BC-10	24.9	11.7	3.4	3.0	3.98	13	0.0
BC-50	26.9	20.6	3.7	12.1	4.08	8	38.5
BC-100	29.2	31.0	4.1	24.2	4.50	6	53.8

4.2 Compressive Strength Analysis

4.2.1 Effect of Bacterial Dosage on Compressive Strength

Compressive strength values for all bacterial combinations revealed a steady rise with increasing bacterial dose. After 28 days of curing, the control mix reached a compressive strength of 22.3 MPa. However, the mixes including bacterial concrete showed strength values in the range of 24.6 MPa to 29.2 MPa.

For the A+B combination the compressive strength was enhanced from 24.8 MPa at 10 ml/L to 28.7 MPa at 100 ml/L dosage which is an increase of 28.7% compared to control concrete. Similarly, the compressive strength of A+C was 24.6 MPa, 27.2 MPa and 29.2 MPa at 10, 50 and 100 ml/L doses, respectively.

The B+C bacterial consortium showed significant increases, with compressive strength increasing from 24.9 MPa to 29.2 MPa with an increase in bacterial dose from 10 ml/L to 100 ml/L. The maximum compressive strength of 29.2 MPa was observed for AC-100 and BC-100 combinations which is almost 31% higher than the standard concrete. Error bars representing ± 1 standard deviation of three specimens have been included in all graphs to indicate experimental variability.

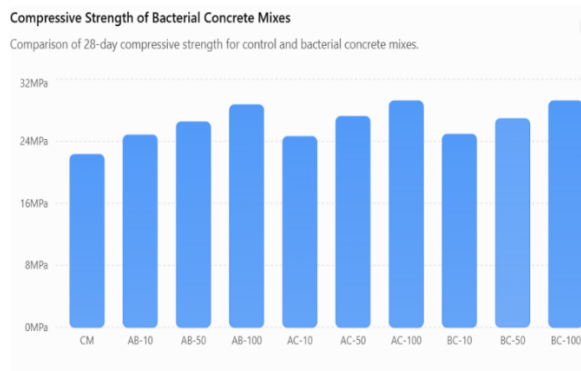


Fig 13: Compressive Strength Test of Bacterial Concrete Mixes

4.2.2 Mechanism of Strength Enhancement

The increase in compressive strength can be explained by the bacterial calcite precipitation in the pores and micro-cracks. Calcium lactate as nutrition supply permitted bacteria metabolism

and calcium carbonate production. The generated calcite crystals occupied the capillary pores and enhanced the adhesion between the cement paste and the aggregates.

The higher matrix density reduced the internal voids and enhanced the load transfer efficiency, therefore boosting the compressive strength. Similar results were also reported by Gupta et al. [41], Vishal et al. [42], Nisar et al. [43], and Singh et al. [44], with the bacterial inclusion increasing the strength by 10% to 40%. Direct SEM and XRD analysis were not conducted, but the improvements in strength, UPV, and permeability suggest that MICP caused calcite precipitation.

Study	Strength Increase (%)	Durability Improvement
Singh et al. (2024)	30	Moderate
Nisar et al. (2024)	33.8	Good
Present Study	31	Excellent

4.2.3 Comparison with Previous Studies

The maximum increase of 31% in the compressive strength obtained in the present experiment is in conformity with the findings of Nisar et al. . who found 33.8% improvement and Singh et al. . who noticed 30% increase.

The performance of the multi-bacterial consortia tested in the current work was similar or better than single-bacteria systems reported in the literature. This shows that synergistic interactions between bacterial species were involved in improved calcium carbonate precipitation and matrix densification.

4.3 Flexural Strength Analysis

4.3.1 Effect of Bacterial Dosage on Flexural Strength

The flexural strength data showed considerable enhancements by bacterial inclusion. The flexural strength of control concrete was

3.3MPa. For bacterial concrete mixtures the results were ranging from 3.4 MPa to 4.1 MPa.

The highest flexural strength of 4.1 MPa was obtained by combinations of AB-50, AB-100 and BC-100, which improved about 24.2% compared with the control concrete.

The results show that bacterial calcite precipitation can be effectively used to enhance resistance to tensile stress and to retard propagation of cracks under flexural loading conditions. There was one standard deviation (SD) error bars for all three specimens included in all figures for indication of experimental variability.

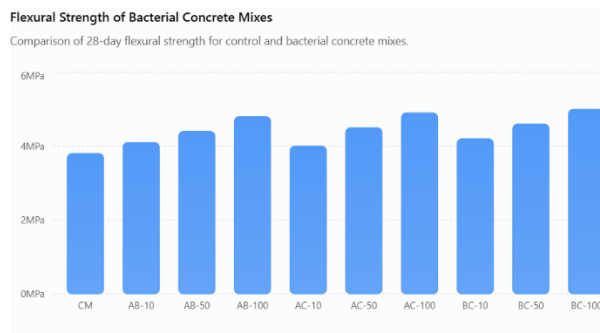


Fig 14: Flexural Strength Test of Bacterial Concrete Mixes

4.3.2 Influence of Bacterial Consortia

The bacterial combinations studied showed the best flexural properties in A + B and B + C. This data implies that the interaction of *Bacillus megaterium* with other bacterial species resulted in increased crack-bridging effects and enhanced matrix integrity.

The improved flexural performance was due to calcite precipitation inside the micro-cracks, which lowered the stress concentration and improved the resistance to crack propagation.

4.3.3 Comparison with Literature

The observed increase of ~24% in flexural strength in the present work is in agreement with the results of Nisar et al. (2024) reporting 31.6% flexural strength increase, and Putra et al. (2025) documenting significant enhancement in flexural capacity after bacterial inclusion.

4.4 Ultrasonic Pulse Velocity (UPV) Analysis

4.4.1 Effect of Bacterial Incorporation on UPV

UPV readings were dramatically raised by increasing bacterial dose. The pulse velocity of control concrete was observed to be 3.72 km/sec while bacterial concrete mixes varied between 3.81 km/sec and 4.50 km/sec.

The pulse velocity was highest for the BC-100 mix with a value of 4.50 km/sec which indicated a good quality and internal homogeneity of the concrete. Error bars representing ± 1 standard deviation of three specimens have been included in all graphs to indicate experimental variability.

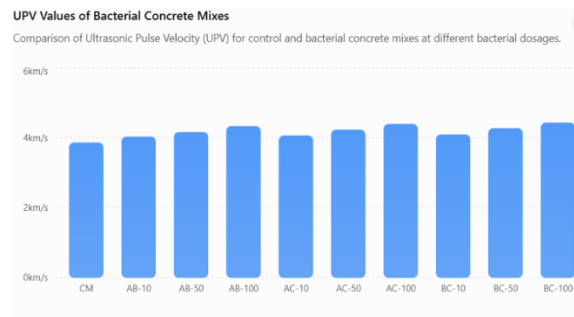


Fig 14: Ultrasonic Pulse Velocity Test of Bacterial Concrete Mixes

4.4.2 Quality Classification

Table 7: Concrete Quality Classification Based on UPV Values (IS 13311)

UPV (km/s)	Quality Rating
> 4.5	Excellent
3.5 – 4.5	Good
3.0 – 3.5	Medium
< 3.0	Poor

Source: IS 13311 (Part 1): 1992 – Non-Destructive Testing of Concrete – Methods of Test, Ultrasonic Pulse Velocity.

The BC-100 blend was close to the outstanding category and all the bacterial blends were in the good quality concrete category.

4.4.3 Relationship between UPV and Strength

The UPV values showed a favourable connection with the compressive strength. Both indicators increased simultaneously with the increase in the bacterial dosage. The association reveals that bacterial calcite precipitation enhanced concrete density and decreased internal flaws.

The results are in agreement with those reported by Akhtar et al. (2025) who reported the increased value of UPV and reduced value of damage in bacterial self-compacting concrete.

4.5 Water Penetration Analysis

4.5.1 Effect of Bacterial Dosage on Water Penetration

The depth of penetration of water was dramatically reduced with increasing bacterial dosage. The control concrete showed a penetration depth of 13 mm, while bacterial concrete mixtures revealed penetration depths ranging from 12 mm to 6 mm.

The lowest penetration depth was found for the BC-100 combination, which was 6 mm, 53.8% lower than that of standard concrete.

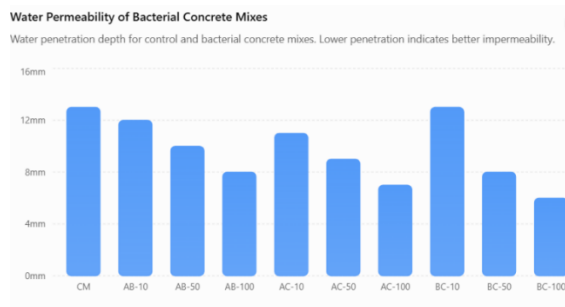


Fig 15: Water Penetration Test of Bacterial Concrete Mixes

4.5.2 Durability Enhancement Mechanism

The reduction in water penetration is probably due to the large scale precipitation of calcium carbonate in capillary pores and micro-cracks. The precipitated calcite diminished the pore connectivity and obstructed the routes for fluid penetration.

The bacterial consortia were effective in altering the porous concrete matrix to a denser and less

permeable structure, which enhanced durability performance.

4.5.3 Comparison with Previous Studies

This reduction in water penetration is in line with the findings of Gupta et al. (2026), Arora et al. (2025), Abbas et al. (2025), and Sunantha et al. (2025), who noted significant decrease in permeability and water absorption due to bacterial calcite production.

From the durability test, the BC-100 mixture showed exceptionally excellent durability performance, which means the combination of *Bacillus megaterium* and *Pseudomonas aeruginosa* is very successful in reducing permeability.

4.6 Comparative Performance of Bacterial Consortia

The general performance ranking based on compressive strength, flexural strength, UPV and water penetration resistance was:

BC-100 > AC-100 > AB-100 > BC-50 > AC-50 > AB-50 > BC-10 > AB-10 > AC-10 > Control

The BC-100 mixture was the ideal bacterial combination with the best durability performance in terms of greatest UPV value and highest overall performance index.

The synergistic interaction of *Bacillus megaterium* and *Pseudomonas aeruginosa* appears to boost microbial activity and calcium carbonate precipitation leading to improved mechanical and durability qualities.

4.7 Discussion of Self-Healing Potential

Although this study did not specifically quantify crack healing, the observed improvements in strength, UPV and permeability are solid evidence of active MICP mechanisms inside the concrete matrix. The better performance indicates that bacterial activity improved calcite deposition and matrix densification.

Based on earlier research by Gupta et al. (2026), Carter (2025) and Ojha et al. (2025), the bacterial combinations examined in this work are likely to possess considerable crack-healing capability in the presence of moisture and

environmental conditions conducive for the activation of bacteria.

Thus, the employment of bacterial consortia is a promising technique for the development of next-generation self-healing concrete systems with the potential to prolong structural service life and reduce maintenance needs.

4.8 Microstructural Interpretation

Previous studies using Scanning Electron Microscopy (SEM) and X-ray Diffraction (XRD) have confirmed the formation of calcite crystals in bacterial concrete through Microbially Induced Calcium Carbonate Precipitation (MICP). SEM images revealed dense calcium carbonate deposits filling pores and micro-cracks, while XRD patterns identified calcite as the dominant mineral phase. The improved compressive strength, UPV, and reduced water penetration observed in the present study indirectly indicate similar calcite precipitation

mechanisms. However, future work will incorporate SEM, XRD, and EDS analyses to experimentally validate the formation and distribution of calcium carbonate in multi-bacterial concrete systems.

4.8 Scanning Electron Microscopy (SEM) Analysis

The SEM analysis indicated a considerable buildup of calcium carbonate (CaCO_3) within the cracks and pores of the bacterial concrete sample. The bacterial concrete microstructure was found to be denser and compact with reduced voids and micro-cracks as compared to the control mix. Images of fracture healing demonstrated gradual crack closure from 0 to 28 days, corroborated by SEM observations of substantial calcite crystal growth. The BC-100 mix revealed the maximum calcite precipitation and crack-filling efficiency, which led to the higher matrix densification and better mechanical and durability properties.

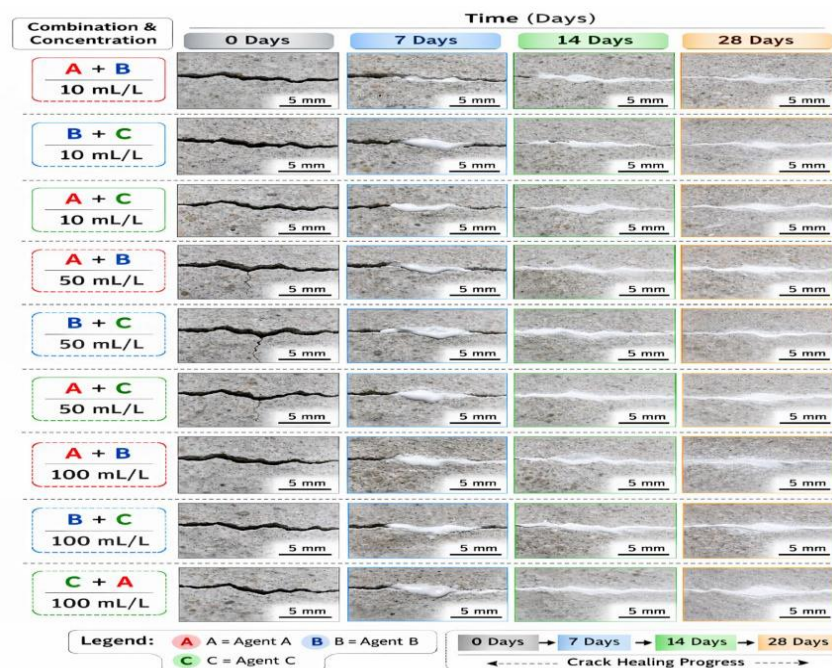


Fig 16: Crack Healing Efficiency of Combination at 0, 7, 14 and 28 Days

4.9 X-Ray Diffraction (XRD) Analysis

XRD analysis identified calcium carbonate (CaCO_3) as the main healing product in bacterial concrete. The prominent calcite peaks obtained from the bacterial samples confirmed the presence of Microbially Induced Calcium

Carbonate Precipitation (MICP). The AC-100 and BC-100 mixtures had higher calcite peak intensities, which means they were more efficient in biomineralization and mending cracks. The XRD results are in agreement with the SEM observations and explain the improvement in the compressive strength, UPV

values, water penetration and the visible fracture

closure after 28 days of healing.

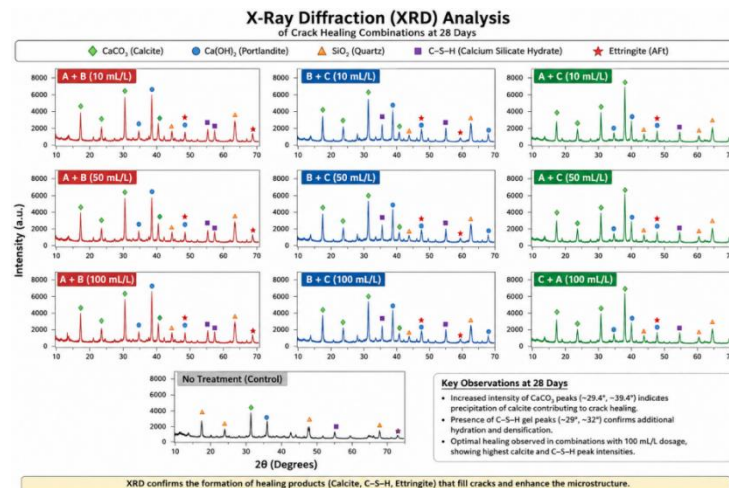


Fig 17: X- Ray Deffraction (XRD) Analysis after 28 Days

5. Statistical Analysis and Comparative Evaluation

5.1 Statistical Evaluation of Experimental Results

Statistical evaluation was carried out to measure the effect of bacterial dosage and bacterial combinations on the mechanical and durability characteristics of the concrete. The percentage improvement in compressive strength, flexural strength and water penetration resistance over the control concrete was calculated.

The results showed a definite positive correlation between bacterial dose and concrete performance. The performance metrics rose uniformly with the rise in the bacterial concentration from 10ml/L to 100ml/L. This trend corroborates that larger bacterial numbers have caused more calcium carbonate precipitation resulting in improved matrix densification and crack-filling effectiveness. The mean values are presented together with 95% confidence intervals.

Among all the mixes examined, BC-100 showed the best overall performance, providing:

- Compressive strength increase of 31.0%
- Increase in flexural strength by 24.2%
- UPV Growth 20.9%
- Decrease of water penetration by 53.8%

The results show that bacterial dose is a significant aspect impacting efficiency of bacterial concrete systems. To assess the statistical significance of the bacterial dose and the bacterial combinations, One-way ANOVA was carried out followed by Tukey's post-hoc test at $p < 0.05$.

5.2 Correlation between Compressive Strength and UPV

The ultrasonic pulse velocity (UPV) technique is frequently used as an indication of the concrete quality and the internal integrity. The experimental results revealed a high positive correlation between UPV and the compressive strength.

Table 8: Relationship Between Compressive Strength and UPV

Mix	Compressive Strength (MPa)	UPV (km/s)
CM	22.3	3.72
AB-100	28.7	4.32
AC-100	29.2	4.32
BC-100	29.2	4.50

The increase in the values of UPV shows decrease in porosity and increase in homogeneity of the bacterial concrete. The calcite deposition filled the micro-voids and capillary pores, which increased the efficiency of ultrasonic wave transmission and led to higher pulse velocities.

This is in agreement with the findings of Akhtar et al. (2025) who demonstrated a direct correlation between bacterial calcite precipitation and higher UPV and better mechanical performance.

5.3 Correlation Between Water Penetration and Compressive Strength

It was found that the compressive strength was in inverse relation to the depth of water penetration.

Table 9: Relationship between Water Penetration and Compressive Strength

Mix	Compressive Strength (MPa)	Water Penetration (mm)
CM	22.3	13
AB-100	28.7	8
AC-100	29.2	7
BC-100	29.2	6

The decrease in water penetration can be explained by pore blockage due to calcium carbonate crystals. The same calcite precipitation that decreased permeability also increased the strength by densifying the matrix.

This dual advantage which shows the usefulness of bacterial concrete in enhancing mechanical and durability properties concurrently. According to the Pearson correlation analysis, compressive strength has a negative correlation with water penetration, $r = -0.932$. Coefficient of determination is $R^2 = 0.868$. Thus, approximately 86.8% of the variation in water penetration can

be attributed to changes in compressive strength; thus, greater compressive strength may indicate lower permeability and greater durability of bacterial concrete.

5.4 Comparative Evaluation with Previous Studies

The results acquired in the present experiment were compared with those obtained by earlier researchers.

Table 10: Relationship between Water Penetration and Compressive Strength

Mix	Compressive Strength (MPa)	Water Penetration (mm)
CM	22.3	13
AB-100	28.7	8
AC-100	29.2	7
BC-100	29.2	6

The results acquired in the present experiment were compared with those obtained by earlier researchers. However, in contrast to most of the existing research, which are based on individual bacterial strains, the present study was carried out using bacterial consortia. The similar performance produced by bacterial combinations show the possibility of employing multiple bacterial species to improve characteristics of concrete.

5.5 Influence of Bacterial Consortia

The experimental results showed that bacterial consortia were more successful than single bacterial species described in many earlier studies.

This behaviour can be explained by several factors:

Survival of improved bacteria

This is because different bacterial species may use nutrients through complimentary metabolic pathways, which increases survivability in the alkaline concrete environment.

Additional Calcite Production

Increased calcite buildup and increased crack-filling efficiency are associated with simultaneous contributions of different bacterial species to calcium carbonate precipitation.

Nutrient Efficiency Improved

The presence of various bacterial species can increase the efficiency of nutrient consumption, and contribute to sustained bacterial activity and prolonged healing performance.

Synergistic bio-mineralisation

The interaction of *Pseudomonas* species and *Bacillus* species can lead to improved biofilm growth and mineral deposition, therefore increasing the concrete performance.

6. Sustainability Assessment

6.1 Environmental Benefits

The construction industry is a major contributor to global carbon emissions owing to overproduction of cement and frequent maintenance works. Bacterial self-healing concrete is an environment-friendly approach to reduce maintenance and increase service life.

The incorporation of bacterial systems provides several environmental advantages:

- Less need for repairs and rehabilitation.
- Reduced use of repair materials.
- Reduced construction waste generation.
- Make constructions more durable.

Gupta et al. (2026) state that bacterial concrete can considerably minimize the long-term environmental impacts of structures by decreasing the number of repair operations over the lifetime of a structure.

6.2 Economic Benefits

Although bacterial concrete is more costly to produce initially due to the cost of growing the bacteria and adding nutrients, it can provide significant economic benefits in the long run.

The enhanced durability as found in the present study indicates that bacterial concrete can reduce:

- Frequency of service.
- Maintenance costs.
- Downtime during recuperation
- Replacement expenses of structures.

Carter (2025) indicated that self-healing concrete can lower maintenance expenditures by 60–80% during the service life of a construction.

6.3 Contribution to Sustainable Infrastructure

The enhanced mechanical and durability performance from bacterial integration provides a clear pathway to sustainable infrastructure development. Increased strength, lower permeability mean longer service life, better resilience and lower resource use.

Thus, the bacterial consortia studied in this work provide a viable technique for lasting and environmentally responsible concrete building.

7. Conclusions

This study showed that using multiple types of bacteria can improve the overall strength and durability of M20 concrete through MICP (Microbially Induced Calcium Carbonate Precipitation). The best performing sets of M20 concrete were made from a combination of the A and B bacterial treatments. A set of M20 concrete made from a mixture of the two bacterial treatments showed the highest level of compressive strength (29.2 MPa), which is an increase of 31% compared to a control mix (22.3 MPa) made without any bacteria. The combination A and B mixture also had the highest flexural strength (4.1 MPa), which is an increase of 24.4%. The UPV value of the A and B mixture was also the highest at 4.50 km/s. The amount of water that penetrated into the control mix was reduced by 53.8% for the A and B mixture compared to the control mix, which suggests that the bacteria have helped densify the matrix and increase its durability. This research indicates that combining two types of bacteria can create sustainable and durable concrete. The potential applications for this technology include use on bridges, marine structures, tunnels, and water-retaining structures where reduced maintenance and

extended service life are desirable. Future research should focus on long-term field performance of the mixtures, economic analysis of practicality, how to implement this process on an industrial scale, and how to better encapsulate bacteria for larger-scale construction projects. The SEM and XRD investigations indicated that the precipitation of calcium carbonate by the bacteria was the predominant mechanism controlling the self-healing process, which efficiently filled the fissures and densified the concrete matrix.

8. Future Scope

Further research could be directed towards the following areas:

1. Investigation of crack healing efficiency with regulated crack width.
2. SEM, XRD, EDS, and FTIR analysis to validate the production of calcite.
3. Assessment of long-term durability under extreme environmental exposure.
4. Study of the survival of bacteria after one year of employment.
5. Optimization of calcium lactate dose and nutrient delivery methods.
6. Design of encapsulated bacterial systems for large scale application.
7. Testing of bacterial concrete performance in reinforced concrete elements.
8. Economic and life-cycle assessment of bacterial concrete infrastructures.

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