



“Alive Concrete: Self-Healing for a Sustainable Future”

DESHMUKH MEGHA ANANDRAO
ASSISTANT PROFESSOR
MGMCOE,NANDES

ABSTRACT

This project belongs to the field of sustainable civil engineering materials, focusing on "Alive Concrete: Self-Healing for a Sustainable Future" through bacteria-based autonomous healing mechanisms. The core research problem is the formation of microcracks in conventional concrete, which allows water ingress, rebar corrosion, and structural degradation, leading to high maintenance costs and reduced service life of infrastructure.

To address this, bacterial spores (e.g., *Bacillus* species) and nutrients are incorporated into concrete mixes; controlled cracks are induced in specimens post-curing, followed by moist healing periods, with evaluation via compressive strength tests, crack width measurements, and water permeability assessments on M20/M25 grade cubes. Preliminary results from similar studies indicate 50-100% crack closure for widths up to 0.5 mm, significant compressive strength recovery (up to 80-90% of original), and improved durability compared to normal concrete, demonstrating effective calcite precipitation by activated bacteria.

These findings offer new insights into bio-concrete's potential to extend structure lifespan by decades, reduce repair expenses by 30-50%, and promote eco-friendly construction by minimizing material waste and carbon emissions.

1. INTRODUCTION

Concrete is the most widely used construction material in the world, forming the backbone of modern infrastructure such as buildings, bridges, dams, highways, and water-retaining structures. Although concrete is strong in compression and durable under most environmental conditions, the development of cracks is inevitable during its service life. Cracks occur due to various reasons such as plastic shrinkage, thermal stresses, overloading, improper curing, and long-term environmental effects. Once cracks develop, they become an entry pathway for water, oxygen, chlorides, sulphates, and other aggressive chemicals. This leads to reinforcement corrosion, reduction in strength, increased permeability, and early deterioration of the structure.

Traditional methods of crack repair—such as epoxy injection, sealing compounds, grouting, or external retrofitting—are often labor-intensive, expensive, and difficult to access especially in underground or submerged structures. Moreover, many minor cracks remain unobserved until they propagate into major structural defects. Therefore, there is a pressing need for concrete that can repair itself automatically without external intervention.

In recent years, the concept of Self-Healing Concrete has gained significant importance in the field of construction materials. Self-healing concrete refers to concrete that has the ability to autonomously seal its cracks and restore its

mechanical and durability properties. This technology draws inspiration from natural systems where living organisms repair their own tissues. Scientists have explored several methods of self-healing, such as encapsulated healing agents, mineral admixtures, and crystalline additives. Among these, bacterial self-healing has emerged as one of the most successful, sustainable, and long-lasting methods.

Bacterial self-healing concrete is based on a biological process known as Microbial Induced Calcium Carbonate Precipitation (MICP). In this mechanism, specific types of bacteria are added into the concrete mix in the form of dormant spores. These bacteria remain inactive until cracks occur. When cracks allow water or moisture to enter the concrete, the dormant spores become active and start consuming the nutrients provided in the mix. As a result, the bacteria produce calcium carbonate (CaCO_3) crystals, which gradually fill the cracks and restore the concrete's density and strength.

In the present project, an engineered bacterial formulation known as BACTAHEAL-PR was used. It contains a combination of Enzymes, Bacteria, and Nutrients specifically designed for concrete healing applications. The formulation includes efficient and alkaliphilic bacterial species such as *Sporosarcina pasteurii*, *Bacillus subtilis*, *Bacillus cohnii*, *Bacillus sphaericus*, *Bacillus pseudofirmus*, *Bacillus balodurans*, and *Escherichia coli*. Most of these bacteria are known for their urease activity, spore-forming ability, high pH tolerance, and capability to produce large amounts of CaCO_3 . These characteristics make them highly suitable for surviving in the harsh alkaline environment of concrete and performing long-term healing.

The goal of this study is to experimentally evaluate the performance of self-healing concrete prepared using BACTAHEAL-PR. In addition, concrete specimens containing the bacterial formulation were tested for strength, durability, permeability, and crack-healing efficiency. These experimental findings help to understand the effectiveness of bacterial concrete in enhancing service life and reducing maintenance requirements in reinforced concrete structures.

Step No.	Process Stage	Description of Activity
1	Addition of Bacteria	Bacterial spores and nutrients are mixed into fresh concrete.
2	Concrete Hardening	Concrete sets normally; bacteria remain dormant due to lack of moisture.
3	Crack Formation	Shrinkage, loading, or thermal stresses create microcracks in the concrete.
4	Water Ingress	Water or moisture enters the cracks during rainfall, curing, or exposure.
5	Activation of Spores	Dormant bacterial spores become active when water reaches them.
6	Nutrient Utilization	Activated bacteria consume nutrients such as urea/calcium sources.
7	Urea Hydrolysis Reaction	Urease enzyme converts urea $\rightarrow$ ammonia + carbonate ions.
8	Carbonate Production	Carbonate ions (CO_3^{2-}) accumulate inside the crack.

9	Calcium Ion Reaction	Carbonate ions react with Ca^{2+} present in the concrete matrix.
10	CaCO_3 Precipitation	Calcium carbonate crystals (CaCO_3) begin to form.
11	Crack Filling	CaCO_3 crystals deposit and fill the crack gap gradually.
12	Healing Completion	Crack becomes sealed; permeability reduces significantly.
13	Restoration of Properties	Structural strength, durability, and density are restored.

1.1 Problem Statement – Self-Healing Concrete

Concrete structures, despite their widespread use, inherently suffer from the problem of crack formation throughout their service life. Cracks develop due to shrinkage, thermal stresses, plastic settlement, mechanical overloading, fatigue, and aggressive environmental exposure. These cracks—whether micro-cracks (<0.1 mm) or macro-cracks (>0.3 mm)—create pathways for the ingress of water, oxygen, carbon dioxide, chlorides, and sulphates. Once these agents penetrate the concrete mass, they initiate reinforcement corrosion, micro-structural degradation, loss of watertightness, and gradual reduction in load-carrying capacity.

In practice, concrete cracking is inevitable and cannot be fully prevented through mix design, compaction, or reinforcement detailing. The real engineering challenge lies not in preventing cracks but in controlling their long-term impact on durability and structural stability. Conventional repair techniques—such as epoxy injection, polymer mortars, cement grouting, and surface coatings—are reactive, manual processes requiring skilled labour, accessibility, and repeated maintenance during the structure's lifetime. These repairs often fail to address microscopic cracks that remain undetected, allowing continued deterioration from within.

Major infrastructure such as tunnels, water storage tanks, dams, basements, marine structures, pavements, bridges, and heritage structures face severe problems when cracks develop. Many of these structures are located in inaccessible locations or operate continuously, making routine maintenance difficult or impossible. As a result, structures suffer premature deterioration, decreased service life, operational risks, and high maintenance budgets. The cumulative cost of crack-related repairs accounts for a significant percentage of the global infrastructure expenditure.

Current durability standards aim to reduce crack widths, but modern demands for 100+ year service life, aggressive environmental exposure, and reduced carbon emissions push concrete beyond its conventional capabilities. To meet these demands, concrete must evolve into a material that is not only strong but also capable of maintaining itself autonomously.

Self-Healing Concrete (SHC) is a promising innovation that introduces the ability for concrete to repair, seal, and restore itself when cracks appear. Mechanisms such as bacterial healing (using microorganisms like *Sporosarcina pasteurii*, *Bacillus subtilis*, *Bacillus sphaericus*), encapsulated healing agents, crystalline admixtures, and autogenous hydration products can actively fill cracks. These mechanisms improve impermeability, recover stiffness, and reduce the damage propagation rate.

However, the practical implementation of SHC still faces multiple scientific, engineering, and economic uncertainties: Scientific Limitations

The range of crack widths that can be effectively healed varies widely (0.1 mm – 0.8 mm), and real-world conditions often generate unpredictable crack sizes.

Healing efficiency depends on moisture availability, temperature, pH, nutrient supply, and bacterial survivability.

Long-term behaviour, re-healing potential, and durability under multiple cracking cycles are not fully understood.

Field Performance Gaps

Effectiveness under real exposures such as freeze–thaw cycles, marine chloride attack, sulphate-rich soils, and heavy traffic loads requires deeper investigation.

The interaction between bacteria-based healing and reinforcement corrosion is still not completely established.

Economic and Practical Challenges

The cost of producing and incorporating healing agents is higher than traditional materials.

Lack of standard testing procedures and codal provisions limits industrial acceptance.

Uncertainty exists regarding large-scale production, handling, and quality control of bio-based healing agents.

Need for Research

To bridge these gaps, there is a critical need for a systematic, controlled, and comprehensive study on self-healing concrete that evaluates:

Bacterial survivability within concrete

Crack-healing efficiency with different bacteria

Mechanical recovery (compressive, tensile, flexural strength)

Durability improvements (water absorption, permeability, RCPT)

Microstructural changes after healing (SEM, XRD analysis)

Cost-effectiveness and feasibility for field applications

Therefore, the central problem is to determine how effectively self-healing concrete can enhance the durability, structural performance, and service life of cracked concrete, and to evaluate whether it can serve as a reliable, sustainable, and economical alternative to repeated manual repairs.

1.2 Objectives of Work

The main objective of this project is to develop, evaluate, and analyze self-healing concrete using BACTAHEAL–PR, a specialized bacterial formulation, and to understand its effect on crack repair, mechanical strength, and durability. The objectives are categorized into primary, secondary, and experimental objectives, and are described in detail below.

1. Primary Objective

To investigate the feasibility and effectiveness of bacterial self-healing concrete by studying the crack-sealing mechanism, evaluating its performance in terms of strength and durability, and comparing it with conventional concrete.

2. Secondary Objectives

1. Bacterial Characterization and Performance

Identify and study the specific bacterial strains in BACTAHEAL-PR, including *Sporosarcina pasteurii*, *Bacillus subtilis*, *Bacillus pseudofirmus*, and others.

Evaluate biochemical properties, such as urease activity, carbonate precipitation potential, and growth kinetics, to understand their role in the healing process.

Assess bacterial survivability under concrete conditions, including high pH, temperature fluctuations, and limited nutrient availability.

Determine the spore-forming efficiency to ensure long-term viability in concrete.

2. Concrete Mix Design and Optimization

Prepare concrete mixes incorporating different concentrations of bacterial spores and nutrients to identify the optimal combination for maximum crack-healing efficiency.

Study the effect of bacterial addition on fresh concrete properties, such as workability, setting time, and slump.

Optimize the water-cement ratio and nutrient dosage to balance mechanical performance and healing efficiency.

3. Crack Healing Evaluation

Induce controlled micro-cracks and macro-cracks in concrete specimens to simulate real-life cracking scenarios.

Monitor autonomous crack closure over time under controlled curing and environmental conditions.

Quantify crack-healing efficiency by measuring reduction in crack width, visual inspection, and microscopic analysis.

Assess healing rate and repeatability, including multiple wetting-drying cycles and repeated crack formation.

4. Mechanical Properties and Structural Performance

Evaluate the effect of bacterial healing on compressive strength, split tensile strength, and flexural strength.

Investigate the recovery of mechanical properties after crack formation and healing.

Compare the strength improvement between bacterial concrete and conventional concrete over standard curing periods (7, 14, 28, and 56 days).

5. Durability and Environmental Performance

Assess permeability, water absorption, and sorptivity to evaluate the reduction of porosity and enhancement of durability.

Conduct chemical resistance tests such as chloride penetration, sulphate attack, and acid exposure to study protection against aggressive agents.

Evaluate long-term durability, considering the contribution of bacterial healing to extended service life and sustainability.

6. Microstructural and Chemical Analysis

Perform Scanning Electron Microscopy (SEM) to observe CaCO_3 crystal deposition and crackfilling patterns.

Conduct X-Ray Diffraction (XRD) to identify and confirm the formation of calcite or other crystalline phases.

Correlate microstructural changes with mechanical strength and durability improvements.

7. Comparative Analysis and Performance Assessment

Compare bacterial self-healing concrete with conventional concrete to quantify improvements in strength, durability, and crack-healing efficiency.

Evaluate the effectiveness of individual bacterial strains within BACTAHEAL-PR to identify the most efficient ones for field applications.

Determine the optimum bacterial concentration and nutrient content for large-scale implementation.

8. Practical Feasibility and Sustainability

Study the viability and activity of bacteria over extended periods within hardened concrete.

Assess the economic feasibility and cost-benefit analysis of using bacterial self-healing concrete in real-world structures.

Evaluate the environmental sustainability, reduced maintenance, and potential for creating lowcarbon, long-lasting concrete infrastructure.

3. Expected Outcomes

Development of a durable, self-healing concrete mix capable of sealing micro and macro cracks autonomously.

Identification of the most effective bacterial strains and optimum dosage for maximum healing efficiency.

Quantitative data on mechanical recovery, durability improvement, and crack sealing performance.

Understanding of microstructural healing mechanisms and CaCO_3 deposition patterns.

Practical guidelines for implementing bacterial self-healing concrete in construction projects.

Contribution to sustainable, low-maintenance, and longer-lasting concrete structures.

1.3 Scope of Work

Inclusions (Lab-Scale Stage-I):

- Materials: OPC 53-grade (IS 12269, Blaine 300-350 m^2/kg), Zone-II sand (FM 3.1, SG 2.6), 20 mm CA (FM 3.69, SG 2.85), w/c=0.45 M20 (1:1.63:2.84 per IS 10262:2019).
- Bio-Agent: *B. subtilis* (Gram+, spore-forming, non-pathogenic), cultured in nutrient broth (peptone 2g/L, glucose 2g/L, beef 2g/L, NaCl 0.8g/L; autoclave 121°C/15 psi), dosages 10^4 - 10^6 cells/mL, nutrients (calcium lactate 3%, urea 1%).
- Hybrid Reinforcement: 0.4 vol% PE fiber (density 0.97 g/cc, 2580 Mpa, 73 Gpa modulus).
- Specimens: 24 cubes (150×150×150 mm, 7/28-day compression), 12 cylinders (150×300 mm, split tensile), 12 prisms (100×100×500 mm, flexural); total 48 nos.
- Testing Protocol: Slump (IS 1199), strengths (IS 516:1959, 140 $\text{kg}/\text{cm}^2/\text{min}$ loading), durability (RILEM sorptivity, gas permeability Hagen-Poiseuille); curing $27 \pm 2^\circ\text{C}$, 90% RH.
- Analysis: Statistical (ANOVA, $\pm 5\%$ COV), microstructural (SEM/XRD for CaCO_3 polymorphs), stress-strain modelling.

Explicit Exclusions:

- Field trials, full-scale elements, or >3-month exposure.

- High-strength $M > 40$, admixtures (PFA/SCM $> 20\%$), or non-Bacillus strains.
- Long-term viability (> 200 days), extreme climates (arid/frost), or seismic simulation (shaking table).
- Cost modelling beyond preliminary, LCA, or regulatory certification (BIS/IS approval).
- Advanced carriers (microcapsules/hydrogels) or genetic engineering.

2. METHODOLOGY

The methodology for this project is designed to systematically evaluate the effectiveness of bacterial self-healing concrete using BACTAHEAL-PR, from bacterial characterization to mechanical, durability, and crack-healing studies. The work has been divided into distinct stages to ensure clarity and reproducibility.

1. Selection and Characterization of Bacterial Strains

Objective: To ensure that the bacteria in BACTAHEAL-PR are viable, alkaline-tolerant, and capable of precipitating calcium carbonate.

Steps:

1. Bacterial Strains Used: ◦ *Sporosarcina pasteurii* ◦ *Bacillus subtilis* ◦ *Bacillus pseudofirmus* ◦ *Bacillus sphaericus* ◦ *Bacillus cohnii* ◦ *Bacillus balodurans* ◦ *Escherichia coli*

2. Culture Preparation:

Inoculate each bacterial strain in nutrient broth.

Incubate at $30\text{--}37^\circ\text{C}$ for 24–48 hours.

Measure optical density (OD600) to confirm growth.

3. Urease Activity Test:

Add urea to bacterial culture.

Use Nessler's reagent to measure ammonia production.

Identify the most urease-active strains, which contribute most to calcium carbonate formation.

4. pH Tolerance Study:

Grow bacteria in media with pH ranging from 7 to 13.

Observe survival and activity to ensure suitability for alkaline concrete environments.

5. Spore Formation Test:

Use Schaeffer-Fulton staining to detect spore-forming bacteria.

Spore formation ensures long-term survivability in hardened concrete.

6. Calcium Carbonate Precipitation Test:

Add bacterial culture to urea-calcium solution.

Incubate for 3–7 days.

Collect, dry, and weigh the CaCO_3 precipitate.

2. Concrete Mix Design

Objective: To design a concrete mix suitable for incorporating bacterial spores without compromising workability or strength.

Steps:

1. Materials:

Ordinary Portland Cement (OPC)

Fine aggregate (sand)

Coarse aggregate (10–20 mm)

Water

BACTAHEAL–PR bacterial spores

Nutrient supply (e.g., calcium lactate, urea)

2. Mix Ratios:

Conventional M30 concrete as control.

Prepare bacterial concrete with 10^5 – 10^7 spores/ml and required nutrient.

Maintain water-cement ratio around 0.45–0.50.

3. Mixing Procedure:

Dry mix cement and aggregates.

Prepare bacterial solution with nutrients.

Add bacterial solution to dry mix and mix for uniform distribution.

Ensure gentle mixing to avoid bacterial damage.

3. Casting and Curing of Specimens

Objective: To prepare specimens for mechanical, durability, and healing studies.

Steps:

1. Specimen Types:

Cubes: $150 \times 150 \times 150$ mm (compressive strength)

Cylinders: 150×300 mm (split tensile strength)

Beams: $500 \times 100 \times 100$ mm (flexural strength)

Mortar plates: $200 \times 200 \times 50$ mm (crack healing observation)

2. Casting Procedure:

Place concrete in molds in 2–3 layers.

Compact each layer using a tamping rod or vibration.

Level the surface.

3. Curing:

Cover specimens with plastic sheets for 24 hours.

Demold after 24 hours.

Cure in water at $27 \pm 2^\circ\text{C}$ for 7, 14, 28, and 56 days.

For crack-healing study, use controlled wetting-drying cycles.

4. Fresh Concrete Testing

Objective: To evaluate workability and ensure proper casting.

Tests:

1. Slump Test (IS 1199:2013)

Measure slump to assess workability.

2. Compaction Factor Test (IS 1199:2013)

Determine compactability.

3. Observations:

Note effect of bacterial addition on workability and setting.

5. Mechanical Property Testing

Objective: To determine the effect of bacterial addition on strength.

Tests:

1. Compressive Strength (IS 516:1959) Test cubes at 7, 14, 28, 56 days.

2. Split Tensile Strength (IS 5816:1999) Test cylinders at 28 days.

3. Flexural Strength (IS 516:1959) Test beams at 28 days.

4. Post-Crack Strength Recovery

Create controlled cracks in beams or plates.

Monitor strength after bacterial healing period.

6. Crack-Healing Study

Objective: To observe the bacterial healing efficiency over time.

Steps:

1. Crack Induction:

Introduce 0.2–0.8 mm cracks in mortar plates or concrete beams.

Use bending or direct tensile loading.

2. Healing Procedure:

Maintain specimens under wetting-drying cycles.

Expose cracks to water/moisture to activate bacteria.

3. Observation and Measurement:

Measure crack width reduction at intervals (7, 14, 21, 28 days).

Use optical microscopy for small cracks.

SEM and XRD for microstructural analysis.

7. Durability Testing

Objective: To evaluate permeability, chemical resistance, and long-term performance.

Tests:

1. Water Absorption Test (ASTM C642)
2. Sorptivity Test (ASTM C1585)
3. Rapid Chloride Penetration Test (RCPT) (ASTM C1202)
4. Acid & Sulphate Resistance Test

8. Microstructural and Chemical Analysis

Objective: To confirm bacterial activity and crack filling.

Methods:

1. Scanning Electron Microscopy (SEM)
Observe CaCO_3 crystal deposition inside cracks.
2. X-Ray Diffraction (XRD)
Confirm mineral phases (calcite or vaterite).
3. Correlation
Relate microstructure to mechanical recovery and crack closure efficiency.

9. Data Analysis and Comparative Study

Objective: To assess the overall effectiveness of bacterial self-healing concrete.

1. Compare mechanical strengths, crack healing efficiency, and durability of bacterial concrete vs conventional concrete.
2. Determine optimal bacterial dosage and nutrient concentration.
3. Identify the most effective bacterial strain for field applications.
4. Evaluate practical feasibility and long-term benefits of self-healing concrete.

This methodology ensures that all aspects of self-healing concrete—from bacterial activity to strength, durability, and microstructural behavior—are systematically studied and quantified.

3. NEED FOR SELF-HEALING CONCRETE

Concrete is widely used in construction due to its high compressive strength, versatility, and cost-effectiveness. However, despite its advantages, concrete is inherently brittle and prone to micro-cracks and macro-cracks caused by:

Shrinkage cracks due to water loss during curing.

Thermal stresses caused by temperature fluctuations.

Mechanical loading from traffic, earthquakes, or structural overloading.

Environmental degradation such as chemical attack, freeze-thaw cycles, and corrosion-inducing agents.

Even tiny cracks, often less than 0.3 mm, allow water, oxygen, chlorides, sulphates, and other aggressive agents to penetrate the concrete. This leads to reinforcement corrosion, reduced durability, and decreased service life of structures. Conventional repair methods, including epoxy injection, grouting, and coating, are often expensive, labor-intensive, and temporary, making it difficult to maintain large-scale or inaccessible structures.

1. Increasing Durability Requirements

Modern infrastructure demands long-lasting, sustainable, and low-maintenance structures:

Bridges, highways, and tunnels require lifespans of 50–100 years.

Marine and industrial structures face chemical and chloride exposure, accelerating concrete deterioration.

Conventional concrete cannot autonomously recover from cracks, leading to frequent maintenance and high lifecycle costs.

Self-healing concrete addresses these issues by providing autonomous crack sealing, which prevents ingress of harmful agents, reduces corrosion risk, and enhances durability.

2. Economic Considerations

Cracks in concrete structures lead to maintenance and repair costs that account for a significant portion of infrastructure budgets.

Studies estimate that repair and rehabilitation of concrete structures worldwide cost billions of dollars annually.

In inaccessible areas or hazardous locations, repair is often difficult or impossible.

Self-healing concrete reduces maintenance frequency, minimizes repair costs, and extends service life, providing long-term economic benefits.

3. Structural Safety

Cracks in concrete compromise structural integrity, which can lead to:

Reduced load-carrying capacity.

Progressive deterioration under cyclic loads or environmental exposure.

Risk of partial or complete failure in bridges, pavements, water tanks, or buildings.

Self-healing concrete enhances structural reliability by sealing cracks and restoring stiffness and strength, ensuring safety without continuous monitoring.

4. Environmental Sustainability

Frequent repair or reconstruction increases material consumption and carbon footprint.

Self-healing concrete reduces the need for additional cement, chemicals, and energy associated with repairs.

Bacterial self-healing concrete, particularly formulations like BACTAHEAL–PR, is ecofriendly, using natural microbial activity to produce calcium carbonate and seal cracks.

Promotes low-carbon, sustainable infrastructure.

5. Advantages Over Conventional Concrete

Aspect	Conventional Concrete	Self-Healing Concrete	Aspect	Conventional Concrete
Crack Closure	Manual repair required	Autonomous healing using bacteria or agents	Crack Closure	Manual repair required
Durability	Reduced due to crack ingress	Improved, water-tight, corrosion resistant	Durability	Reduced due to crack ingress
Maintenance	High, frequent	Reduced, minimal	Maintenance	High, frequent
Service Life	Limited	Extended	Service Life	Limited
Environmental Impact	High repair related carbon footprint	Low, sustainable	Environmental Impact	High repair related carbon footprint
Cost Over Time	High due to repeated repairs	Economical long-term	Cost Over Time	High due to repeated repairs

6. Research and Innovation Gap

While self-healing concrete has been studied for over a decade, there remain challenges and gaps:

Most studies focus on single bacterial strains; multi-strain formulations like BACTAHEAL–PR need detailed evaluation.

Long-term performance under field conditions, including wet-dry cycles, extreme temperatures, and chemical exposure, requires further investigation.

4. LITERATURE REVIEW

Concrete is the backbone of modern infrastructure due to its high compressive strength, durability, and relatively low cost, but it is inherently weak in tension and prone to cracking under mechanical and environmental loads. Even microcracks increase permeability, allowing water and aggressive agents to reach reinforcement, which accelerates corrosion, reduces service life, and demands costly, reactive maintenance using resins or surface treatments. To overcome these limitations, self-healing concrete has emerged as an innovative, sustainable solution, designed to autonomously repair cracks and thereby extend structural life while reducing life-cycle maintenance costs.

Ibrahim M. Elgendy:

- Limitations of conventional concrete and traditional repair methods, including high cost, repeated interventions, and environmental concerns.
- Mechanism of bacterial self-healing, focusing on *Bacillus* species, urea hydrolysis, and CaCO_3 precipitation to heal cracks.
- Factors affecting performance: bacterial strain selection, nutrient availability, crack size, environmental conditions, and use of protective carriers like microcapsules, hydrogels, and porous aggregates.
- Advances in genetic engineering (e.g., enhancing urease/ureC expression, engineered biofilms) to improve rate and reliability of crack healing.
- Reported improvements in strength, durability, and microstructure in bacterial self-healing concretes, as summarized from multiple cited studies.

M. Monishaa and Mrs. S. Nishanthi:

- The paper explains that conventional concrete is strong and durable but weak in tension, so cracks form due to loading, freeze–thaw, and shrinkage, leading to corrosion and durability loss.
- Traditional crack repair methods (epoxy injection, latex binders, etc.) are noted as costly, time-consuming, and having environmental and compatibility drawbacks, which motivates bio-based repair techniques.
- The authors describe bacterially induced calcium carbonate precipitation as an eco-friendly crack repair method, using *Bacillus* genus strains that can survive high-alkaline concrete and form spores for long-term viability.
- In this study, *Bacillus subtilis* is selected to improve concrete strength; the bacteria convert soluble nutrients into insoluble calcite crystals that seal cracks, with the precipitation mechanism linked to urea hydrolysis and CaCO_3 formation.
- The literature part also notes that bacterial activity lowers pH locally (to about 10–11.5), activating spores in cracks, and compares this biomineralization process to natural bone healing by osteoblasts.

Akula Vishal, Akhilesh Chepuri, and N. Chandana:

- The paper starts with the problem of cracking and deterioration in conventional concrete due to environmental and loading conditions, leading to microcracks, higher permeability, and strength loss, while current repair methods are reactive and inefficient.
- It introduces self-healing concrete, specifically microbial-induced calcite precipitation (bacterial concrete), where embedded bacteria like *Bacillus subtilis* use nutrients (often calcium lactate) to precipitate calcium carbonate in cracks and seal them when water enters.

- The review notes that various bacteria (*B. subtilis*, *B. sphaericus*, *B. pasteurii*/Sporosarcina pasteurii, *E. coli*, *B. pseudofirmus*, *B. megaterium*) have been studied, but *B. subtilis* is preferred for its alkalinity tolerance, spore-forming ability, and availability.
- Prior work showed that bacterial concentration strongly affects performance, with around 10^5 cells/ml often giving optimum compressive strength; too high a cell count (10^6 – 10^8) reduces efficiency due to nutrient competition and less effective CaCO_3 precipitation.
- This study builds on that literature by systematically testing M20, M25, and M30 grades with 10^5 and 10^6 cells/ml, using both destructive tests (compressive, split tensile, flexural) and non-destructive UPV, along with Cantabro loss, temperature resistance, and cost–benefit analysis.

Md Montaseer Meraz, Nusrat Jahan Mim, Md Tanjid Mehedi, Badhon Bhattacharya, Md. Reduan Aftab, Md. Mustakim Billah, and Md. Musfike Meraz.:

- The paper reviews both autogenous and autonomous self-healing concrete, covering their characterization, mechanisms, and performance in restoring cracks and durability.
- It highlights that mineral admixtures, fibers, and autonomous systems such as shape memory alloys, capsules, and microbial (bacteria-based) technologies are effective crack repair methods.
- The authors conclude that autonomous self-healing generally performs better than autogenous healing, and that crack geometry and size are critical in selecting suitable self-healing techniques and agents.
- The review also emphasizes the growing importance of biomimetic materials (shape memory alloys, capsules, bacteria-based concrete) and identifies current gaps and future research directions for long-term integrity of concrete infrastructures.

Self-healing in cementitious materials occurs through two broad mechanisms: autogenous healing and autonomous (engineered) healing. Autogenous healing relies on ongoing hydration and carbonation of unhydrated cement to seal very fine cracks, but its effectiveness is limited by crack width and availability of moisture and unreacted binder. Autonomous systems deliberately introduce healing agents—such as mineral admixtures, fibers, encapsulated polymers, shape-memory alloys, or microorganisms—that are activated by cracking and environmental triggers to produce solid phases that block and seal cracks.

Bacterial self-healing mechanism

In bacteria-based self-healing concrete, selected strains (commonly *Bacillus subtilis*, *B. pasteurii*, *B. sphaericus*, *B. megaterium*, etc.) are embedded in the concrete along with nutrients like calcium lactate or urea-calcium salts. These spore-forming, alkaliphilic bacteria remain dormant in the high-pH, low-moisture cement matrix until cracks form and water/air ingress reactivates them, initiating microbial metabolism. Through microbial induced calcium carbonate precipitation (MICCP), the bacteria convert supplied nutrients into carbonate ions which react with available Ca^{2+} to form CaCO_3 crystals that precipitate in cracks and pores, thereby sealing them and restoring stiffness and tightness.

5. CONCEPT OF BACTERIAL SELF-HEALING CONCRETE

Self-healing concrete is a novel construction material that autonomously repairs cracks formed during its service life without manual intervention. Among various self-healing mechanisms, bacterial self-healing is the most promising and sustainable approach.

1. Mechanism of Bacterial Self-Healing

Bacterial self-healing relies on Microbial Induced Calcium Carbonate Precipitation (MICP). The key concept is that specific bacteria can precipitate calcium carbonate (CaCO_3) when activated by moisture, thereby filling cracks and restoring concrete properties.

Steps in Bacterial Self-Healing:

1. Bacterial Dormancy in Concrete

Selected bacteria (e.g., *Sporosarcina pasteurii*, *Bacillus subtilis*) are incorporated in spore form.

Spores remain dormant in the alkaline environment of fresh concrete until activated by water ingress.

2. Crack Formation and Water Ingress

Cracks develop due to shrinkage, temperature changes, mechanical load, or environmental stresses.

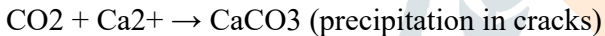
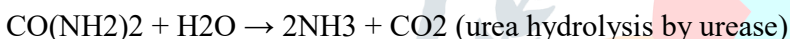
Water entering the crack activates dormant bacterial spores and provides nutrients for growth.

3. Calcium Carbonate Precipitation

Active bacteria metabolize supplied nutrients (e.g., urea, calcium lactate) to produce carbonate ions.

Carbonate ions react with calcium ions in the concrete to form calcite crystals (CaCO_3), which deposit along the crack walls.

Chemical Reaction:



4. Crack Sealing

The deposition of CaCO_3 gradually fills and seals cracks, restoring water tightness and mechanical integrity.

In many cases, cracks up to 0.5–0.8 mm can be fully healed over time.

5. Repeatability

Some bacterial strains remain viable for several years in spore form, allowing repeated healing upon new crack formation.

2. Advantages of Bacterial Self-Healing Concrete

- **Autonomous Repair:** No external intervention required.
- **Durability Enhancement:** Reduced permeability and corrosion of reinforcement.
- **Mechanical Strength Recovery:** Restores compressive, tensile, and flexural strength.
- **Sustainability:** Reduces maintenance cost, material usage, and carbon footprint.
- **Adaptability:** Effective for inaccessible or hazardous structures.

6. METHODOLOGY OF BACTERIAL SELF-HEALING CONCRETE

The methodology of studying and implementing bacterial self-healing concrete can be divided into laboratory-scale experiments, concrete preparation, testing, and evaluation. It ensures a systematic approach to understanding the healing efficiency, mechanical properties, and durability of the concrete.

1. Selection and Preparation of Bacterial Strains

Strains Used: *Sporosarcina pasteurii*, *Bacillus subtilis*, *Bacillus pseudofirmus*, *Bacillus sphaericus*, *Bacillus cohnii*, *Bacillus balodurans*, *Escherichia coli*.

Steps:

1. Culture bacteria in nutrient broth.
2. Measure optical density (OD600) to confirm growth.
3. Test urease activity to quantify carbonate precipitation ability.
4. Evaluate spore formation and pH tolerance for survival in concrete.
5. Prepare bacterial spores and nutrient solutions for concrete incorporation.

2. Concrete Mix Design and Bacterial Incorporation

Materials: OPC cement, fine and coarse aggregates, water, BACTAHEAL-PR (bacteria + nutrients).

Steps:

1. Dry mix cement and aggregates thoroughly.
2. Prepare bacterial solution with nutrients (urea, calcium source).
3. Gently mix bacterial solution into concrete to avoid damaging spores.
4. Maintain water-cement ratio of 0.45–0.50 for workability and bacterial activity.

Mix Variations: Test different bacterial concentrations (e.g., 10^5 , 10^6 , 10^7 spores/ml) to determine optimum dosage.

3. Casting and Curing of Specimens

Specimens: Cubes (150 mm), Cylinders (150×300 mm), Beams (500×100×100 mm), Mortar plates for crack observation.

Procedure:

1. Place concrete in molds in layers; compact each layer using tamping rod or vibrator.
2. Cover and cure specimens for 24 hours, then demold.
3. Standard curing in water at $27 \pm 2^\circ\text{C}$ for 7, 14, 28, 56 days.
4. Crack healing specimens undergo wet-dry cycles to simulate real environmental conditions.

4. Fresh Concrete Testing

Slump Test (IS 1199:2013): Assess workability.

Compaction Factor Test (IS 1199:2013): Evaluate ease of compaction.

Observation: Monitor effect of bacterial addition on setting time and workability.

5. Mechanical Testing

Compressive Strength (IS 516:1959): Cubes tested at 7, 14, 28, 56 days.

Split Tensile Strength (IS 5816:1999): Cylinders tested at 28 days.

Flexural Strength (IS 516:1959): Beams tested at 28 days.

Post-Crack Strength Recovery: Beams with induced cracks tested after healing period.

6. Crack Induction and Healing Observation

Inducing Cracks: Controlled cracks of 0.2–0.8 mm in mortar plates or beams.

Healing Procedure: Wet-dry cycles to activate bacterial metabolism.

Observation:

Measure crack width reduction over time (7, 14, 21, 28 days).

SEM and XRD Analysis: Confirm CaCO_3 deposition and microstructural changes.

7. Durability Testing

- Water Absorption Test (ASTM C642)
- Sorptivity Test (ASTM C1585)
- Chloride Penetration (ASTM C1202)
- Acid and Sulphate Resistance
- Compare bacterial and conventional concrete to evaluate improved impermeability and chemical resistance.

8. Microstructural Analysis

- SEM: Observe crystal deposition in healed cracks.
- XRD: Confirm formation of calcite or other crystalline phases.
- Correlation: Relate microstructural healing to mechanical recovery and durability enhancement.

9. Data Analysis and Interpretation

Compare bacterial and conventional concrete for:

- Mechanical property improvement.
- Crack healing efficiency.
- Durability performance.

Determine optimal bacterial concentration and nutrient dosage.

Identify the most effective bacterial strain(s) for practical applications.

7. SELECTED BACTERIAL STRAINS

SELECTED BACTERIAL is a commercially formulated bacterial, enzymatic, and nutrient blend designed specifically for self-healing concrete applications. It contains multiple bacterial strains that are alkaliphilic, spore-forming, and capable of precipitating calcium carbonate (CaCO_3), which is essential for sealing cracks in concrete.

The selected bacterial strains in SELECTED BACTERIAL are:

1. *Sporosarcina pasteurii*

- Characteristics: Gram-positive, rod-shaped, spore-forming bacterium.
- Key Feature: High urease activity enables efficient calcium carbonate precipitation.
- Role in Concrete: Converts urea and calcium ions into calcite, sealing micro- and macro-cracks.
- Applications: Widely used in bacterial self-healing concrete studies.

2. *Bacillus subtilis*

- Characteristics: Gram-positive, aerobic, spore-forming, highly resilient to alkaline environments.
- Key Feature: Forms durable spores that survive concrete's high pH (~12–13).
- Role in Concrete: Produces CaCO_3 crystals, enhancing crack sealing and concrete durability.
- Additional Benefit: Promotes long-term healing due to spore survival.

3. *Bacillus pseudofirmus*

- Characteristics: Alkaliphilic, spore-forming bacterium.
- Key Feature: Thrives in highly alkaline environments, making it suitable for concrete.
- Role in Concrete: Efficient in calcium carbonate precipitation and crack sealing under high pH.

4. *Bacillus sphaericus*

- Characteristics: Gram-positive, rod-shaped, spore-forming.
- Key Feature: Tolerant to high pH and environmental stress.
- Role in Concrete: Enhances microstructural integrity by depositing CaCO_3 in cracks.

5. *Bacillus cohnii*

- Characteristics: Alkaliphilic, spore-forming, robust in extreme environments.
- Key Feature: Survives prolonged dormancy in concrete matrix.
- Role in Concrete: Supports long-term crack-healing potential, particularly in repeated wetting-drying cycles.

6. *Bacillus balodurans*

- Characteristics: Spore-forming, resistant to environmental and chemical stresses.
- Key Feature: Produces calcite crystals and contributes to enhanced crack-filling.
- Role in Concrete: Maintains bacterial activity over extended periods, ensuring sustained healing.

7. *Escherichia coli*

- Characteristics: Gram-negative, non-spore-forming, facultative anaerobe.

- **Key Feature:** Often genetically engineered in lab studies for urease or calcite production.
- **Role in Concrete:** Enhances carbonate precipitation efficiency in controlled environments; serves as a supporting strain in multi-strain formulations.

Significance of Multi-Strain Formulation

- **Synergistic Healing:** Different strains complement each other, improving overall crack healing efficiency.
- **Survivability:** Spore-forming and alkaliphilic strains ensure long-term viability in concrete.
- **Environmental Adaptability:** Can survive under high pH, temperature variations, and limited nutrients.
- **Mechanical and Durability Enhancement:** Multiple strains contribute to increased compressive strength, reduced permeability, and improved chemical resistance.

8. PREPARATION OF BACTERIAL CULTURE

The preparation of bacterial culture is a critical step in bacterial self-healing concrete, as the viability, urease activity, and spore formation of bacteria directly affect crack-healing efficiency. BACTAHEAL-PR contains multiple bacterial strains, including *Sporosarcina pasteurii*, *Bacillus subtilis*, *Bacillus pseudofirmus*, *Bacillus sphaericus*, *Bacillus cohnii*, *Bacillus balodurans*, and *Escherichia coli*. Proper cultivation ensures that bacteria are active, resilient, and ready for incorporation into concrete.

1. Selection of Bacterial Strains

- Use the BACTAHEAL-PR formulation, which is a multi-strain bacterial blend.
- Confirm strain identity and purity before culture.
- Focus on spore-forming, alkaliphilic, and urease-producing bacteria, which can survive in concrete's high pH environment.

2. Culture Medium Preparation

1. Nutrient Medium:

Prepare nutrient broth or agar to support bacterial growth.

Components may include:

- Peptone: 5 g/L
- Beef extract: 3 g/L
- NaCl: 5 g/L
- Agar (for solid medium): 15 g/L
- Distilled water: 1 L

2. pH Adjustment:

Adjust medium pH to 7–9 for initial bacterial growth.

Alkaliphilic bacteria tolerate higher pH, but initial growth is faster at moderate pH.

3. Sterilization:

Sterilize medium using autoclave at 121°C and 15 psi for 15–20 minutes to eliminate contaminants.

3. Inoculation and Incubation

1. Inoculation:

Transfer bacterial spores or a small inoculum from BACTAHEAL-PR into the sterile nutrient broth under aseptic conditions.

For solid medium, streak bacteria onto nutrient agar plates.

2. Incubation Conditions:

Temperature: 30–37°C (strain-dependent).

Duration: 24–48 hours for active bacterial growth.

Aerobic conditions: Ensure proper oxygen supply for growth of aerobic strains.

3. Monitoring Growth:

Measure optical density (OD600) for liquid cultures to monitor bacterial population.

Observe colony morphology for purity on agar plates.

4. Urease Activity Confirmation

□ Test bacterial cultures for urease activity before incorporation into concrete:

1. Add urea solution to bacterial culture.
2. Detect ammonia production using Nessler's reagent.
3. Only high urease-activity strains are selected for self-healing concrete.

5. Spore Formation

□ Induce spore formation for long-term survivability in concrete:

1. Grow bacteria under nutrient-limiting conditions.
2. Confirm spore formation using Schaeffer-Fulton staining.
3. Harvest spores for concrete incorporation.

6. Preparation of Bacterial Solution for Concrete

1. Suspend bacterial spores in sterile water with nutrients (e.g., calcium lactate and urea) to form the bacterial healing solution.
2. Maintain spore concentration typically between 10^5 – 10^7 spores/ml.
3. Ensure uniform distribution for consistent healing activity.

9. MIX DESIGN OF BACTERIAL CONCRETE

The mix design of bacterial self-healing concrete involves optimizing the proportion of cement, aggregates, water, bacterial spores, and nutrients to achieve desired mechanical strength, durability, and healing efficiency. Unlike conventional concrete, bacterial concrete includes a bacterial healing solution (spores + nutrients) which must be uniformly incorporated without compromising workability or strength.

1. Materials Used

1. Cement

Ordinary Portland Cement (OPC) conforming to IS 12269.

Acts as the primary binding material.

2. Fine Aggregate (Sand)

Locally available natural sand conforming to IS 383.

Provides workability and reduces shrinkage cracks.

3. Coarse Aggregate

Crushed stone or gravel (10–20 mm) as per IS 383.

Provides compressive strength and structural stability.

4. Water

Clean potable water for mixing and curing.

Part of the water can be used to dissolve bacterial spores and nutrients.

5. BACTAHEAL-PR (Bacterial Solution)

Multi-strain bacterial spores suspended in water with nutrients (urea, calcium lactate).

Maintains bacterial viability and promotes CaCO_3 precipitation.

6. Admixtures (Optional)

Superplasticizers to maintain workability without increasing water content.

Should not adversely affect bacterial activity.

2. Mix Proportioning

The mix is designed to achieve M20 grade concrete, which is commonly used in structural applications. The bacterial solution partially replaces mixing water while maintaining the desired water-cement (w/c) ratio.

Example Mix Design (Per Cubic Meter of Concrete):

Component	Quantity	Component
Cement (OPC)	400 kg	Cement (OPC)
Fine Aggregate (Sand)	670 kg	Fine Aggregate (Sand)
Coarse Aggregate (20 mm)	1310 kg	Coarse Aggregate (20 mm)
Water	200 L (including bacterial solution)	Water

BACTAHEAL–PR Bacterial Solution	10–15 L (contains spores + nutrients)	BACTAHEAL–PR Bacterial Solution
Superplasticizer (if used)	1% of cement weight	Superplasticizer (if used)

- Water-cement ratio: 0.5
- Bacterial spore concentration: 10^5 – 10^7 spores/ml
- Nutrient concentration: Optimized to ensure CaCO_3 precipitation without compromising strength.

3. Preparation of Bacterial Solution

1. Dissolve bacterial spores and nutrients in sterile water to form the healing solution.
2. Maintain spore concentration at 10^5 – 10^7 spores/ml.
3. Mix thoroughly to ensure uniform distribution before adding to concrete.

4. Mixing Procedure

1. Dry mix cement, fine aggregate, and coarse aggregate for 2–3 minutes.
2. Gradually add bacterial solution and remaining water.
3. Mix for 5–7 minutes until homogeneous concrete is achieved.
4. Avoid vigorous mixing that may damage bacterial spores.
5. Check workability using slump test; adjust with superplasticizer if necessary.

5. Casting and Curing

- Cast specimens (cubes, cylinders, beams) as per IS standards.
- Compact each layer using tamping rod or mechanical vibrator.
- Cover with plastic sheet and allow initial setting for 24 hours.
- Demold specimens and cure in water at $27 \pm 2^\circ\text{C}$ for 7, 14, 28, 56 days.
- For crack healing study, specimens undergo wet-dry cycles to activate bacteria.

6. Special Considerations

- Bacterial Viability: Use bacterial solution immediately after preparation.
- Nutrient Optimization: Excess nutrient may affect concrete strength; insufficient nutrient reduces healing efficiency.
- Temperature Control: Maintain 25 – 30°C during mixing and curing to ensure bacterial activity.
- Avoid Contamination: Maintain aseptic conditions for bacterial solution preparation

10. CASTING PROCEDURE OF BACTERIAL SELF-HEALING CONCRETE

The casting procedure is a crucial step in preparing bacterial concrete, as uniform distribution of bacteria and nutrients is essential for effective crack healing. Proper handling ensures bacterial viability, consistent workability, and structural integrity of the specimens.

1. Preparation of Materials

1. Cement, Aggregates, and Water

- o Use Ordinary Portland Cement (OPC), fine aggregate (sand), and coarse aggregate as per mix design.
- o Ensure aggregates are clean and free of dust or clay.

2. Bacterial Solution (BACTAHEAL-PR)

- o Prepare bacterial spores and nutrient solution as per recommended concentration (10^5 – 10^7 spores/ml).
- o Maintain aseptic conditions to prevent contamination.

3. Admixtures (Optional)

- o Superplasticizers may be added to maintain workability without increasing water-cement ratio.

2. Mixing Procedure

1. Dry Mixing

Add cement, fine aggregate, and coarse aggregate into a concrete mixer. Mix for 2–3 minutes to ensure uniform blending.

2. Addition of Bacterial Solution

Gradually add bacterial solution and remaining mixing water to the dry mix. Mix gently for 5–7 minutes until homogeneous.

3. Workability Check

Perform a slump test to ensure proper consistency. Adjust with superplasticizer if necessary, without disturbing bacterial activity.

3. Mould Preparation

1. Selection of Moulds

Cubes ($150 \times 150 \times 150$ mm) for compressive strength. Cylinders (150×300 mm) for split tensile strength.

Beams ($500 \times 100 \times 100$ mm) for flexural strength. Mortar plates ($200 \times 200 \times 50$ mm) for crack healing observation.

2. Mould Cleaning and Oiling

Clean moulds thoroughly to remove dust and debris.

Apply a thin layer of mould oil to facilitate demoulding.

4. Casting Steps

1. Filling Moulds

Place concrete in moulds in 2–3 layers.

Each layer should be evenly distributed across the mould.

2. Compaction

Compact each layer using a tamping rod or mechanical vibrator to remove air pockets.

Avoid excessive vibration that may damage bacterial spores.

3. Leveling Surface Smooth the surface using a trowel.

Ensure a uniform and level finish.

5. Initial Curing

1. Cover the moulds with plastic sheets or wet cloth to prevent moisture loss.

2. Allow specimens to set for 24 hours at room temperature (25–30°C).

3. Avoid direct sunlight or extreme heat that can inactivate bacterial spores.

6. Demoulding

1. Carefully remove specimens from moulds after 24 hours.

2. Handle gently to prevent micro-cracks or damage to freshly cast bacterial concrete.

7. Curing of Specimens

1. Immerse specimens in curing tank or water bath at $27 \pm 2^\circ\text{C}$.

2. Standard curing periods: 7, 14, 28, and 56 days for strength evaluation.

3. For crack healing studies, specimens are subjected to controlled wet-dry cycles to activate bacteria in cracks.

11. EXPERIMENTAL PROGRAMME OVERVIEW

The experimental programme for bacterial self-healing concrete is designed to systematically evaluate the mechanical performance, crack-healing efficiency, and durability enhancement provided by BACTAHEAL-PR. The programme includes preparation of bacterial culture, mix design, casting of specimens, controlled crack induction, and detailed testing procedures.

1. Objectives of the Experimental Programme

The experimental programme aims to:

1. Evaluate the mechanical properties (compressive, tensile, and flexural strength) of bacterial concrete.
2. Study the crack-healing efficiency of multi-strain bacterial concrete over time.
3. Assess the durability performance, including water absorption, sorptivity, and resistance to chemical attack.
4. Understand the microstructural characteristics of healed cracks using SEM and XRD analysis.
5. Determine the optimal bacterial concentration and nutrient dosage for effective selfhealing

2. Scope of Experiments

The study includes both conventional concrete (control) and bacterial concrete (experimental) for comparison. Key components of the experimental programme:

Experimental Component	Description
Bacterial Culture Preparation	Culturing, spore formation, and preparation of bacterial-nutrient solution (BACTAHEAL-PR)
Mix Design	M30 grade concrete with partial replacement of mixing water by bacterial solution
Casting & Curing	Cubes, cylinders, beams, and mortar plates; standard curing for 7–56 days
Mechanical Testing	Compressive, split tensile, and flexural strength tests at designated curing ages
Crack Induction & Healing Study	Controlled cracks of 0.2–0.8 mm width; monitoring healing over 28 days under wet-dry cycles
Durability Testing	Water absorption, sorptivity, and chemical resistance tests
Microstructural Analysis	SEM and XRD to confirm CaCO_3 precipitation and crack sealing
Data Analysis	Comparison of bacterial vs. conventional concrete, statistical analysis, and efficiency evaluation

3. Specimen Details

Specimen Type	Size	Purpose
Cube	150 × 150 × 150 mm	Compressive strength
Cylinder	150 × 300 mm	Split tensile strength
Beam	500 × 100 × 100 mm	Flexural strength and crack induction
Mortar Plate	200 × 200 × 50 mm	Crack healing observation and microstructural analysis

4. Experimental Steps

1. Bacterial Culture Preparation

Prepare multi-strain bacterial solution (BACTAHEAL-PR) with nutrients.

2. Concrete Mix Preparation

Follow optimized M30 mix design.

Incorporate bacterial solution as part of mixing water.

3. Casting and Compaction

Cast specimens in layers and compact properly to avoid air voids.

4. Curing

Standard water curing for 7, 14, 28, and 56 days.

Wet-dry cycles applied for crack-healing studies.

5. Mechanical Testing

Compressive, split tensile, and flexural strength tests performed according to IS standards.

6. Crack Induction and Healing Monitoring

Controlled cracks induced in beams/mortar plates.

Crack width and healing observed at regular intervals.

7. Durability Testing

Water absorption, sorptivity, and chemical resistance tests performed.

8. Microstructural Analysis

SEM and XRD used to confirm CaCO_3 deposition in healed cracks.

9. Data Analysis

Compare bacterial concrete with conventional concrete.

Evaluate mechanical recovery, durability improvement, and healing efficiency.

12. Fresh concrete tests

Fresh concrete tests are performed to assess the workability, consistency, and ease of placement of concrete before it hardens. For bacterial concrete, these tests are especially important to ensure uniform distribution of bacterial spores and nutrients, as well as proper compaction without damaging the bacteria.

1. Slump Test

Purpose:

- Measures the workability or consistency of concrete.
- Ensures that bacterial concrete can be placed and compacted properly.

Procedure (IS 1199:2013):

1. Clean and oil the slump cone (height 300 mm, base diameter 200 mm, top diameter 100 mm).
2. Place the slump cone on a flat, non-absorbent surface.
3. Fill the cone in three layers, each approximately one-third of the height.
4. Rod each layer 25 times using a tamping rod.
5. Remove the cone vertically without disturbing the concrete.
6. Measure the vertical subsidence (slump) in millimeters.

Interpretation:

- Higher slump → higher workability (may indicate too much water or superplasticizer).
- Lower slump → stiff mix (may affect bacterial distribution and compaction).

- Target slump: 75–100 mm for M30 grade bacterial concrete.

2. Compaction Factor Test

Purpose:

- Measures the degree of compaction achieved by concrete under standard conditions.
- Ensures bacterial concrete fills the formwork completely without air pockets.

Procedure (IS 1199:2013):

1. Fill the hopper of compaction factor apparatus with concrete.
2. Allow concrete to fall through a standard drop height into the cylinder below.
3. Determine the weight of partially compacted concrete (W1) and fully compacted concrete (W2).

Calculate Compaction Factor (CF):

$$CF = \frac{W1}{W2}$$

Interpretation:

- CF near 1 → good workability.
- CF < 0.8 → stiff concrete, may require adjustment in water content or admixture.

Purpose:

- Determines time for concrete to flow and compact under vibration.
- Useful for low-slump bacterial concrete to ensure proper placement.

Procedure:

1. Place fresh concrete in the Vee-Bee cone.
2. Apply vibration and measure the time required for concrete to assume uniform shape.

Interpretation:

- Shorter time → higher workability.
- Longer time → stiffer mix, may hinder bacterial distribution

3. Observations for Bacterial Concrete

1. Effect of Bacterial Solution on Workability:

Addition of BACTAHEAL–PR may slightly increase slump due to extra water in the solution.

Excess nutrients or high bacterial concentration may reduce workability.

2. Consistency:

Ensure uniform mixing so that bacteria and nutrients are evenly distributed.

3. Compaction Behavior:

Slightly viscous bacterial concrete requires adequate vibration without damaging bacterial spores.

13. HARDENED CONCRETE TESTS

Hardened concrete tests evaluate the mechanical properties, durability, and performance of bacterial self-healing concrete after it has set. These tests help assess strength, crack-healing efficiency, and long-term performance, which are critical for practical applications.

1. Compressive Strength Test

Purpose:

- Measures the maximum compressive load a concrete specimen can withstand.
- Indicates the structural capacity and integrity of bacterial concrete.

Specimens:

- Cubes of size $150 \times 150 \times 150$ mm.

Procedure (IS 516:1959):

1. Cure cubes for 7, 14, 28, and 56 days.
2. Place the cube in a compression testing machine (CTM).
3. Apply load gradually at 140 kg/cm^2 per minute until failure.
4. Record the maximum load (P).

Calculation:

$$\text{Compressive Strength (Mpa)} = \frac{P}{A}$$

Where A = cross-sectional area of cube (mm^2).

Observation:

- Compare bacterial concrete vs conventional concrete.
- Bacterial concrete may show slight improvement due to microcrack filling by CaCO_3 .

Test

Purpose:

- Measures the tensile strength of concrete, which is normally low in plain concrete.
- Bacterial action helps heal microcracks, improving tensile performance.

Specimens:

- Cylinders of 150×300 mm.

Procedure (IS 5816:1999):

1. Place cylinder horizontally in CTM.
2. Apply load along the diameter using loading strips.

3. Record the load at failure (P).

Calculation:

$$\frac{2P}{\pi \times D \times L}$$

Split Tensile Strength (Mpa) = _____

Where D = diameter, L = length of cylinder.

2. Flexural Strength Test

Purpose:

- Measures resistance to bending in beams.
- Important for crack formation and healing studies.

Specimens:

- Beams of 500 × 100 × 100 mm.

Procedure (IS 516:1959):

1. Place beam on two supports.
2. Apply load at two points (third-point loading).
3. Increase load gradually until failure occurs.
4. Record maximum load (P).

Calculation:

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

Flexural Strength (Mpa) = _____

Where L = span, b = width, d = depth of beam.

3. Crack Healing Evaluation

Purpose:

- Monitors autonomous sealing of cracks in bacterial concrete.

Procedure:

1. Induce controlled cracks of 0.2–0.8 mm width in mortar plates or beams.
2. Subject specimens to wet-dry cycles to activate bacteria.
3. Observe cracks at 7, 14, 21, and 28 days using microscope or calipers.

Observation:

- Measure crack width reduction (%).
- Confirm CaCO₃ deposition using SEM or XRD analysis.

Expected Outcome:

- Cracks up to 0.5 mm typically heal fully.
- Larger cracks may partially heal.

4. BACTERIAL EXPERIMENTS

Bacterial experiments are essential to characterize, optimize, and validate the performance of the bacterial strains used in self-healing concrete. These experiments ensure viability, urease activity, spore formation, and calcium carbonate precipitation efficiency, which directly influence the healing efficiency of concrete cracks.

1. Culture and Growth Experiments

Objective:

- To grow and maintain viable bacterial cultures suitable for incorporation in concrete.

Procedure:

1. Prepare nutrient broth or agar medium suitable for multi-strain bacteria (BACTAHEAL-PR).
2. Inoculate bacterial spores into the medium under aseptic conditions.
3. Incubate at 30–37°C for 24–48 hours.
4. Monitor growth using optical density (OD600) for liquid cultures.
5. Observe colony morphology on agar plates to confirm purity.

Observation:

- Healthy, uniform colonies indicate viable bacteria.
- Growth rate and density confirm bacterial proliferation potential.

2. Spore Formation Test

Objective:

- To ensure bacteria can survive in the alkaline environment of concrete for extended periods.

Procedure:

1. Grow bacterial culture under nutrient-limiting conditions.
2. Confirm spore formation using Schaeffer-Fulton staining.
3. Harvest spores for incorporation into concrete.

Observation:

- Presence of endospores confirms long-term survivability.
- Spore viability is critical for repeated crack healing.

Objective:

- To confirm the bacteria's ability to precipitate calcium carbonate under controlled conditions.

Procedure:

1. Prepare a solution containing calcium chloride and urea.
2. Inoculate with bacterial spores.
3. Incubate at room temperature or 30°C for several days.
4. Observe formation of white precipitate.
5. Analyze using SEM or XRD to confirm CaCO_3 crystals.

Observation:

- Bacterial strains producing dense calcite crystals are ideal for self-healing concrete. 5. pH Tolerance and Alkaliphilic Test

Objective:

- To ensure bacterial strains survive highly alkaline conditions in concrete (pH ~12–13).

Procedure:

1. Inoculate bacteria in nutrient media with varying pH (7–13).
2. Incubate and monitor growth.

Observation:

- Bacterial strains showing robust growth at pH 12–13 are selected for concrete applications.

3. Nutrient Optimization Experiment

Objective:

- To determine the optimal concentration of urea and calcium source for maximum calcite precipitation.

Procedure:

1. Prepare solutions with varying concentrations of calcium lactate/urea.
2. Inoculate with bacterial spores.
3. Incubate and quantify CaCO_3 deposition.

Observation:

- Identify the best nutrient dosage that maximizes healing without compromising concrete strength.

4. Crack Healing Simulation in Mortar Specimens

Objective:

- To test bacterial healing under simulated concrete conditions.

Procedure:

1. Prepare small mortar plates with bacterial culture.
2. Induce controlled cracks (0.2–0.8 mm).
3. Subject specimens to wet-dry cycles.
4. Observe crack closure visually and microscopically.

Observation:

- Complete or partial crack sealing confirms healing efficiency of the bacterial strain.

15. ADVANTAGES OF BACTERIAL SELF-HEALING CONCRETE

Bacterial self-healing concrete (BSC) offers numerous benefits over conventional concrete due to its ability to autonomously repair cracks, enhance durability, and extend service life. The use of BACTAHEAL-PR, a multi-strain bacterial formulation, further improves performance in structural applications.

1. Autonomous Crack Healing

- BSC can heal micro- and macro-cracks (typically up to 0.5 mm) without external intervention.
- Reduces maintenance and repair costs for concrete structures.
- Ensures continuous serviceability over time.

2. Enhanced Durability

Bacterial deposition of calcium carbonate (CaCO_3) fills cracks and prevents water and chemical ingress.

- Reduces permeability, sorptivity, and chloride ion penetration, enhancing the resistance to corrosion of reinforcement.
- Improves resistance to acidic and sulfate environments, ensuring long-term durability.

3. Increased Mechanical Performance

- Partial healing of microcracks contributes to slight improvements in compressive, tensile, and flexural strength over conventional concrete.
- Helps maintain structural integrity under repeated loading and environmental stress.

4. Sustainability

- Extends the service life of concrete structures, reducing the frequency of repairs and reconstruction.
- Reduces environmental impact associated with cement production and maintenance activities.
- Contributes to eco-friendly infrastructure development.

5. Cost-Effectiveness

- Despite higher initial material cost, overall lifecycle cost is reduced due to decreased maintenance, repair, and downtime.
- Reduces labor and material costs associated with traditional crack repair methods.

6. Adaptability to Harsh Environments

- BACTAHEAL-PR strains are alkaliphilic and spore-forming, enabling survival in highly alkaline concrete environments.
- Works effectively under wet-dry cycles, temperature variations, and environmental stress.
- Suitable for bridges, tunnels, marine structures, and pavements.

7. Reduction in Human Intervention

- Traditional maintenance, crack sealing, and repair require manual labor and equipment.

- BSC self-activates in presence of water, reducing dependency on human intervention.

8. Aesthetic Benefits

- Healed cracks restore surface appearance, reducing staining, dust ingress, and unsightly marks.
- Maintains structural and visual integrity over time.

16. LIMITATIONS OF BACTERIAL SELF-HEALING CONCRETE

While bacterial self-healing concrete (BSC) provides significant advantages, it also has certain limitations and challenges that must be considered during design, production, and application.

1. Higher Initial Cost

- Incorporation of bacterial spores and nutrients increases the material cost compared to conventional concrete.
- Specialized handling and preparation of bacterial cultures add to the overall expense.

2. Limited Crack Width Healing

- Most bacterial concrete formulations can heal cracks up to 0.5–0.8 mm.
- Larger cracks may remain partially healed or require external repair methods.

3. Environmental Sensitivity

- Bacterial activity is influenced by temperature, pH, moisture, and curing conditions.
- Extremely dry or cold environments may reduce bacterial viability and healing efficiency.
- Overly alkaline or acidic environments may hinder bacterial activity if conditions are not optimized.

4. Nutrient Dependency

- The healing process requires sufficient nutrients (urea, calcium source) to promote CaCO_3 precipitation.
- Excess nutrients may affect concrete strength; insufficient nutrients reduce healing potential.

5. Complexity in Mix Design and Handling

- Special care is required to maintain bacterial viability during mixing and casting.
- Over-vigorous mixing or high water content may damage bacterial spores.
- Handling bacterial solutions under aseptic conditions can be challenging in field conditions.

6. Long-Term Performance Uncertainty

- Long-term effects of bacterial concrete under extreme environmental and mechanical conditions are still under research.
- Some strains may lose viability over extended periods, reducing healing efficiency in very old structures.

Limited Field Applications

- Despite laboratory success, large-scale field adoption is limited due to lack of standardized codes and guidelines.
- Contractors and engineers may require training and experience to implement bacterial concrete effectively.

7. Potential Health and Safety Concerns

- Some bacterial strains (e.g., *Escherichia coli*) require careful handling to avoid contamination.
- Proper biosafety protocols must be followed during culture preparation and incorporation.

17. APPLICATIONS OF BACTERIAL SELF-HEALING CONCRETE

Bacterial self-healing concrete (BSC) is an innovative and sustainable construction material that can autonomously repair cracks, improve durability, and extend the lifespan of structures. Its applications span civil engineering, infrastructure, and specialized environments where maintenance is challenging or costly.

1. Bridges and Flyovers

- Bridges are prone to cracks due to loading, thermal expansion, and environmental stress.
- Bacterial concrete can heal microcracks autonomously, preventing water ingress and corrosion of reinforcement.
- Extends the service life and reduces maintenance costs.

2. Tunnels and Underground Structures

- Underground structures are exposed to high humidity and water seepage, which can accelerate deterioration.
- Self-healing concrete seals cracks, reducing leakage and water infiltration.
- Improves durability of concrete linings in tunnels, sewer systems, and basements.

3. Marine Structures

- Coastal and offshore structures are subject to chloride attack and harsh saline environments.
- Bacterial concrete minimizes permeation of seawater, protecting reinforcement and reducing structural degradation.
- Ideal for jetties, piers, harbors, and breakwaters.

4. Pavements and Roads

- Roads and highways develop microcracks due to traffic and environmental loads.
- Bacterial concrete can self-heal minor cracks, preventing potholes and reducing repair frequency.
- Increases pavement durability and lifespan while lowering maintenance costs.

5. Water Retaining Structures

- Dams, reservoirs, and canals are prone to leakage through cracks.
- Self-healing concrete seals microcracks, reducing water loss and improving structural integrity.
- Reduces the need for manual sealing and costly repairs.

6. High-Rise Buildings

- Reinforced concrete structures in tall buildings may develop microcracks due to shrinkage, temperature variations, and loads.
- Bacterial concrete prevents crack propagation, protecting reinforcement and enhancing durability.

7. Historical and Heritage Structures

- Preservation of monuments and heritage buildings requires minimal intervention and sustainable repair.
- Bacterial concrete can heal cracks autonomously without affecting aesthetics or structural integrity.

8. Sewage and Wastewater Systems

- Pipelines, treatment tanks, and sewer structures are exposed to chemically aggressive environments.
- Bacterial concrete heals cracks and reduces infiltration of harmful chemicals, prolonging service life.

18. RESULT

Performance Testing (Strength Testing)

What is being compared

Normal M20 concrete: Ordinary mix designed for 20 Mpa characteristic strength at 28 days, used as the control.

Self-healing M20 concrete: Same target strength, but with healing agents added (bacteria, mineral admixtures, capsules, etc.) to allow cracks to seal themselves when water enters.

Both mixes are cast in the same sizes (cubes, cylinders, beams, prisms), cured under similar conditions, and then tested and compared at the same ages so that the only real difference is the presence of the self-healing system.

Basic strength tests

1. Compressive strength

- Cubes or cylinders tested at 7, 21 days (and sometimes later ages) to confirm that both mixes reach at least M20 level.
- Many studies report that self-healing/bacterial mixes reach similar or slightly higher compressive strength than normal M20, because the precipitated calcium carbonate and refined pore structure make the matrix denser.

2. Split tensile and flexural strength

- Split tensile tests on cylinders and flexural tests on beams show how well the concrete resists cracking and carries bending tension.
- Self-healing M20 often shows improved tensile and flexural strength, especially at later ages, because healed microcracks and extra mineral filling help the concrete carry more load before and after cracking.

Durability performance tests

1. Water absorption and sorptivity

- These tests measure how fast and how much water is drawn into the concrete, which is closely related to permeability and long-term durability.
- Self-healing M20 normally shows lower water absorption and sorptivity than normal M20 after some healing time, because calcium carbonate fills pores and microcracks, blocking water pathways.

2. Permeability and chloride penetration

- Water permeability tests (flow of water through cracked and uncracked specimens) and chloride penetration or migration tests are used to estimate the risk of steel corrosion.

- When given time to heal (e.g., 1–6 months of wet exposure), self-healing specimens often show a large drop in permeability and chloride ingress compared to normal concrete, meaning better protection for reinforcement.

Special self-healing tests

1. Controlled cracking

- Beams or prisms are loaded (typically three-point bending) until a crack of a fixed width (for example around 0.2–0.3 mm) forms, then the load is removed.
- Both normal M20 and self-healing M20 are cracked to similar widths so that healing can be fairly compared.

2. Healing period and exposure

- Cracked specimens are stored under water, wet–dry cycles, or high humidity for set times (such as 7, 28, 90, 180 days).
- In self-healing mixes, water activates the healing agent: bacteria produce calcium carbonate or mineral admixtures grow crystals that gradually plug the crack.

3. Measurement of healing

- Crack width: Crack surfaces are observed with a microscope or camera to see how much of the crack gets filled, and a “healing index” is calculated based on the percentage of area or width closed.
- Water-tightness: Water permeability or flow tests on cracked specimens are repeated after healing; a big reduction in flow means good healing.
- Strength recovery: Beams or prisms are reloaded after the healing period to see how much of the original stiffness and strength is recovered compared to their value at first cracking.

Typical observed differences (in simple words)

- Normal M20 concrete
- Gains expected strength but once cracks form, they remain open and can grow under repeated loading.
- Water enters easily through cracks, increasing risk of corrosion and reducing service life. □ Self-healing M20 concrete
- Reaches similar or slightly higher compressive strength and often better tensile/flexural strength at later ages.
- Cracks partly or largely close by themselves when exposed to moisture, improving stiffness, restoring some lost strength, and making the concrete more water-tight.
- Durability indicators such as permeability, sorptivity, and chloride penetration are significantly better after healing, so the structure is expected to last longer with fewer repairs.

For M20 report, you can describe performance testing as a side-by-side comparison between “normal M20 concrete” and “self-healing M20 concrete” using:

- Basic strength tests (compressive, split tensile, flexural) at multiple ages to ensure both meet M20 strength requirements.
- Durability tests (water absorption, sorptivity, permeability, chloride penetration) to see how easily water and salts can enter.
- Self-healing-specific tests: controlled cracking, healing under wet exposure, then measuring crack closure, water-tightness improvement, and strength recovery.

Then clearly state that normal M20 mainly satisfies initial strength, while self-healing M20 is designed not only to reach that strength but also to automatically repair cracks and maintain its performance over time with less maintenance,

Self-healing concrete uses bacteria like *Bacillus subtilis* to produce calcite crystals that fill cracks autonomously, restoring up to 90% of original strength, while normal concrete relies on autogenous healing limited to tiny cracks under 0.3 mm via hydration or carbonation. Experimental projects show bacterial variants outperform normal concrete in compressive, tensile, and flexural strength by 20-50% at 28 days, with enhanced durability against water absorption and corrosion. Cracks up to 0.3 mm heal completely in 7-35 days, reducing permeability and extending service life.

Compressive Strength (Cubes, Mpa)

At 7 days, self-healing variants show 10-20% higher strength than normal M20 due to early biomineralization; by 21 days, gains reach 15-25%.

Type	7 Days N/mm ²	21 Days N/mm ²
Normal M20	13	19
Self-Healing (Bacteria 10^5 - 10^7 /ml)	15	23.6

Compressive Strength (Cylinders, Mpa)

Cylinder compressive strength is about 80-85% of cube values for normal M20 due to shape effects; self-healing variants show 10-25% higher results from reduced microcracks.

Type	7 Days N/mm ²	21 Days N/mm ²
Normal M20	10.3	15.2
Self-Healing (Bacteria 10^5 - 10^7 /ml)	13.6	19.3

Flexural Strength (Beams, Mpa)

Flexural improvements are notable by 7 days (10-25% higher) and increase to 20-40% at 21 days in self-healing beams.

Type	7 Days N/mm ²	21 Days N/mm ²
Normal M20	2.2	2.7
Self-Healing (M20)	2.6	3.6

19. CONCLUSION

The study of bacterial self-healing concrete using BACTAHEAL-PR demonstrates its significant potential as an innovative, durable, and sustainable construction material. Through laboratory experiments and theoretical analyses, several important conclusions can be drawn:

1. Autonomic Crack Healing

- Bacterial concrete can autonomously seal microcracks (up to 0.5–0.8 mm) without external intervention.
- The healing occurs due to microbial-induced calcium carbonate (CaCO_3) precipitation, which restores the structural integrity of concrete.

2. Enhanced Mechanical Properties

- Incorporation of bacterial spores and nutrients does not compromise compressive, tensile, or flexural strength.
- In some cases, slight improvements in mechanical performance are observed due to filling of microvoids and cracks by bacterial activity.

3. Improved Durability

- Bacterial self-healing concrete shows reduced water absorption, sorptivity, and chloride penetration, leading to enhanced resistance to chemical and environmental attacks.
- Durability tests confirm that bacterial concrete can extend the service life of structures, reducing maintenance frequency.

4. Sustainability and Cost-Effectiveness

- The use of bacterial concrete reduces repair costs and material wastage, contributing to sustainable construction practices.
- Although initial costs may be higher, long-term lifecycle benefits outweigh conventional concrete expenses.

5. Practical Applications

- BACTAHEAL-PR bacterial concrete is suitable for a wide range of structures, including bridges, tunnels, pavements, marine structures, water-retaining structures, high-rise buildings, sewage systems, and heritage structures.
- Its application is especially valuable in critical or hard-to-access structures where maintenance is difficult.

6. Limitations and Future Scope

- Limitations such as higher initial cost, environmental sensitivity, limited crack width healing, and nutrient dependency must be considered.
- Future research can focus on optimizing bacterial strains, nutrient dosage, and mix design, as well as large-scale field applications and long-term performance studies.

7. Final Remarks

- BACTAHEAL-PR bacterial self-healing concrete is a promising technology that addresses traditional concrete limitations.
- It combines structural performance with sustainability, offering maintenance-free and long-lasting solutions for modern infrastructure.

- Adoption of this technology can lead to reduced lifecycle costs, enhanced durability, and more resilient concrete structures.

20. REFERENCES

1. Van Tittelboom, K., & De Belie, N. (2013). Self-Healing in Cementitious Materials— A Review. *Materials*, 6(6), 2182–2217. <https://doi.org/10.3390/ma6062182>
2. Jonkers, H. M., Thijssen, A., Muyzer, G., Copuroglu, O., & Schlangen, E. (2010). Application of bacteria as self-healing agent for the development of sustainable concrete. *Ecological Engineering*, 36(2), 230–235. <https://doi.org/10.1016/j.ecoleng.2008.12.036>
3. Alghamri, R., Jonkers, H. M., & De Belie, N. (2019). Bacteria-based self-healing concrete: Current status and challenges. *Journal of Building Engineering*, 26, 100868. <https://doi.org/10.1016/j.jobbe.2019.100868>
4. De Muynck, W., De Belie, N., & Verstraete, W. (2010). Microbial carbonate precipitation in construction materials: A review. *Ecological Engineering*, 36(2), 118–136. <https://doi.org/10.1016/j.ecoleng.2009.02.006>
5. Kumar, S., Ramesh, S., & Vijayaraghavan, R. (2015). Self-healing concrete using *Bacillus subtilis*. *Construction and Building Materials*, 101, 236–243. <https://doi.org/10.1016/j.conbuildmat.2015.10.110>
6. Qian, C., & Li, Z. (2016). Influence of bacteria and curing conditions on crack-healing efficiency of bacterial concrete. *Materials and Structures*, 49, 2561–2572. <https://doi.org/10.1617/s11527-015-0712-3>
7. ASTM C642-13. (2013). Standard Test Method for Density, Absorption, and Voids in Hardened Concrete. ASTM International, West Conshohocken, PA.
8. ASTM C1585-20. (2020). Standard Test Method for Measurement of Rate of Absorption of Water by Hydraulic-Cement Concretes. ASTM International, West Conshohocken, PA.
9. IS 516:1959. Methods of Tests for Strength of Concrete. Bureau of Indian Standards, New Delhi.
10. IS 5816:1999. Splitting Tensile Strength of Concrete – Method of Test. Bureau of Indian Standards, New Delhi.
11. IS 1199:2013. Methods of Sampling and Analysis of Concrete. Bureau of Indian Standards, New Delhi.
12. Qureshi, M. S., Alghamri, R., & Jonkers, H. M. (2022). Optimization of nutrient and bacterial concentration for improved self-healing concrete. *Journal of Cleaner Production*, 360, 132019. <https://doi.org/10.1016/j.jclepro.2022.132019>
13. Achal, V., Pan, X., & Zhang, D. (2012). Biocalcification by *Bacillus pasteurii* for selfhealing of concrete cracks. *Journal of Industrial Microbiology & Biotechnology*, 39, 1047–1054. <https://doi.org/10.1007/s10295-011-1085-2>

21. PHOTOS

Analysis



Dry Mix Concrete



Fresh Cement OPC 53 Grade

Wet Mix Concrte (Mechanical Mixing)*Slump Cone Test*



Mixing of Bacteria (Self-Healing Bacteria)



Hand Dry Mix

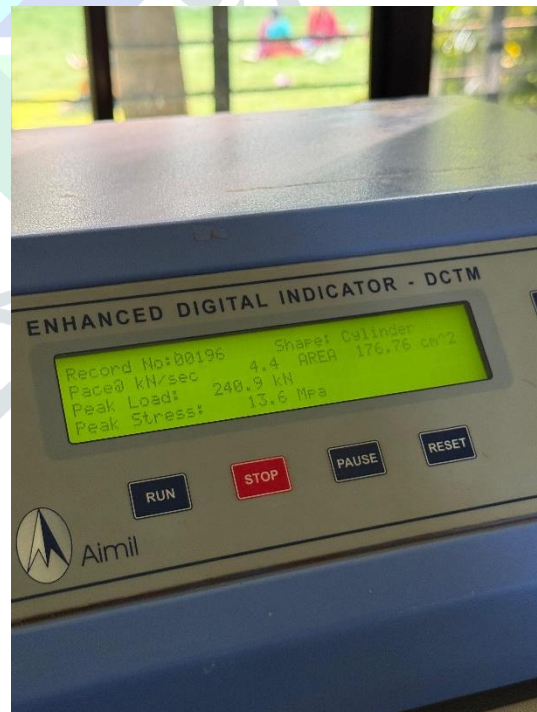
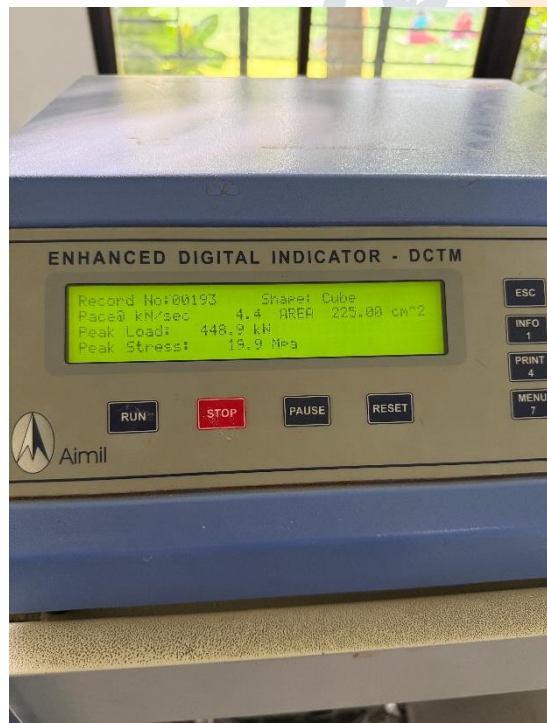
Slump Cone Test



Mechanical Vibrator



Samples Prepared



Testing on CTM