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Development of High Strength Concrete with Self-Healing Properties

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Abstract - High-strength concrete (HSC) is commonly used in modern infrastructure due to its exceptional load-bearing capacity and durability. However, like conventional concrete, it remains susceptible to microcracking, which affects structural performance and service life. To address this limitation, this study investigates the development of HSC with self-healing properties by incorporating bacteria. Four spore-forming, alkaliphilic bacterial strains—Bacillus subtilis, Bacillus cohnii, Bacillus pseudofirmus, and Bacillus megaterium—were chosen for their ability to thrive in the alkaline concrete matrix and precipitate calcium carbonate ($CaCO_3$) via urease activity.

Concrete specimens were prepared using a mix design targeting a compressive strength of over 40 MPa, with bacterial spores (10^5 cells/ml) added to the mixing water. Mechanical performance was assessed through compressive, split tensile, and flexural strength tests at 7, 14, and 28 days, in accordance with IS standards. Crack-healing behaviour was examined by inducing artificial cracks (0.3–0.5 mm). Linear regression analysis was used to model the relationship between curing age, bacterial strain, and strength development.

The results showed significant improvements in strength and healing performance with bacterial incorporation compared to control mixes. Among the strains tested, Bacillus megaterium displayed the highest efficiency, increasing compressive strength by 57%, flexural strength by 37.5%, and tensile strength by 33.3% at 28 days. Regression models achieved strong predictive accuracy (R^2 values: 0.86–0.97). The study concludes that bacterial incorporation, particularly with B. megaterium, offers a sustainable pathway to improve both the mechanical performance and durability of HSC. This approach not only extends the service life of concrete structures but also reduces maintenance costs, providing a promising solution for resilient and eco-friendly construction.

***Key Words*:** High-strength concrete, self-healing, Bacillus, calcium carbonate precipitation, regression modelling, durability

1. INTRODUCTION

Concrete is a widely used material in construction worldwide. Despite its durability and longevity, environmental stresses, thermal expansion, and structural loads can cause it to crack. These fissures can weaken the structural integrity and may require costly repairs if they allow reinforcement steel to corrode. [1] Therefore, scientists developed self-healing concrete, a substance capable of repairing itself from fractures without human intervention. This article provides a thorough review of a repair material that enables self-healing through biological processes. When concrete suddenly cracks, this study summarizes recent advances on how to address the issue. Our goal was to create a high-strength concrete with self-healing properties to extend its lifespan. [2]

The exceptional compressive strength of high-strength concrete (HSC) makes it an essential material for offshore projects, bridges, and tall buildings; concrete, in general, is the backbone of modern infrastructure. The structural integrity and service life of any material, including HSC, may decline over time due to microcracking. Recent research indicates that microbial healing, where microbes naturally produce minerals, could be a promising strategy for enhancing HSC performance. This study examines the current and potential role of microbial healing in improving the resilience, self-healing capacity, and durability of high-strength concrete. [3]

When microbes produce inorganic compounds, typically to support the development of complex tissues, this process is known as microbial healing. Two examples of bacteria that can break down urea and activate carbonic anhydrase in concrete include Bacillus subtilis and Sporosarcina pasteurii, which are involved in the precipitation of calcium carbonate (CaCO3). Once embedded in concrete, these bacteria remain dormant until water seeps in through cracks, triggering a mineralization response that fills the gaps. There is less water penetration, and the concrete shows increased compressive and flexural strength. The highest strength ranges between 120 and 150 MPa. [4]

Power may also sometimes exceed 200 MPa. With such high compressive strength, concrete is most vulnerable at the level of the coarse aggregate. If you use coarse particles in your concrete, it could crack. We can improve compressive quality by achieving uniformity and homogeneity in the mix and by removing the coarse totals. To reach these lofty goals, we utilize the pozzolanic behavior of special materials, such as fly ash and silica fume. HSC contains a high-quality binder (usually OPC 53), quartz sand, quartz powder, steel fibers, silica fume, and a third-generation superplasticizer, in addition to steel aggregates. To further lower the water-cement ratio while still maintaining excellent workability, we also use superplasticizers. [5]

A new set of obstacles has arisen. First, the large-scale production of cement and concrete causes significant environmental damage. The second issue is the durability of concrete. When discussing concrete, the main problem is cracks. Whether small or large, cracks lead to deterioration; we must overcome these two challenges. It is well known that concrete mainly consists of two components: aggregates and cement. [6] In fact, cement and aggregates are the two primary ingredients. Although cement production generates a large amount of CO_2 emissions, it accounts for only 7% of total human-caused emissions. Given these facts, claiming that concrete is a sustainable material is difficult. To address these issues, some use partial substitutes like fly ash, blast furnace slag, or rice husk ash—byproducts of iron, coal, or agricultural waste—to create more eco-friendly concrete. [7]

2. BACTERIA USED IN CONCRETE:

The pH range for concrete is 11–13, making it an alkaline substance. High mechanical stresses also cause it to mix. Bacteria can withstand mechanical pressures and thrive in environments with high pH levels because of this. One essential component for successful crack healing is the production of calcite (CaCO3) by these bacteria. This will allow them to form a robust, crystalline covering over the damaged region. This is a property of certain Bacillus bacteria. Bacteria like these produce an enzyme called urease, which aids in the formation of calcite, which is what really fills gaps.

The following Bacillus species are suitable for use in bio-concrete: [11]

i) Candida albicans.
ii) The subtilis bacteria.
iii) Megaterium Bacillus.
iv) Bacillus cohnii
v) Halodurans Bacillus.
vi) Bacillus pseudofirmus. Along with related species.

3. HIGH-STRENGTH CONCRETE IN THE CONSTRUCTION INDUSTRY

Concrete with a greater compressive strength has several potential applications in the building sector, including.

- The greater compressive strength of high-strength concrete (HSC) allows structural elements to be thinner than their conventional concrete counterparts while yet bearing the same load. This means that the amount of material required will be much less, ultimately making the concrete more cost-effective. There will be much less dead load as a result of the smaller individual components.
- Several chemical and mineral additives are used in the casting process of HSC. These additives decrease concrete's permeability by filling the gaps between cement particles. Consequently, substances such as chloride and moisture are unable to permeate to the same degree. This renders the building impervious to heat and other forms of corrosion. Hence, concrete's durability is enhanced.
- High-strength concrete (HSC) is a specific type of concrete whose compressive strength is several times higher than that of conventional concrete. "Compared to normal concrete, whereby compressive strength may range between 20 to 40 MPa, the high-strength concrete (HSC) is defined as that stronger than 55 MPa". Enhanced load-bearing capacity, durability, and space efficiency are necessary in demanding structural applications, and recent improvements allow HSC to reach strengths of 100-150 MPa. [12]
- HSC's outstanding qualities are the result of a well-developed composition. "In most cases, it will have a low water-cement ratio (often less than 0.35), high-quality cement (such as OPC 53 grade), additional cementitious ingredients (like fly ash or silica fume), and, to make it more workable, superplasticizers". To increase packing density and mechanical strength, well-graded aggregates are used. Fibers, such as steel or polypropylene, are sometimes included to enhance ductility and cracking resistance. This results in a concrete with a very dense microstructure, low porosity, and high mechanical performance.
- Particularly for projects requiring high performance in constrained spaces, high-strength concrete finds widespread usage in the building sector. It is commonly employed in high-rise buildings, where it enables slimmer columns and maximizes usable floor space. In bridges and flyovers, HSC allows for longer spans and reduces structural weight. It is also used in critical infrastructure such as nuclear power plants, offshore platforms, and tunnels, where durability, strength, and resistance to environmental attacks are crucial. [13]
- The pre-cast business is one notable use for HSC. Bridge girders and other extreme structural elements can be cast with this concrete. Within three days of curing, this concrete's accelerated curing can provide extremely high early strength. Scientists predict that cement autoclaved at 90°C for three days would reach a compressive strength of approximately 200 MPa. When a task needs to be completed quickly, certain structural elements may be employed.
- When HSC is used with fibres, its true potential is shown. To increase HSC's ductility and toughness, several fibre types can be incorporated. By using fibre reinforcement in HSC, structural steel may be entirely replaced. Numerous foot-over bridges built using HSC with fibre reinforcement have shown excellent durability and serviceability. The tremendous compressive power of HSC turns out to be the most favorable in terms of utilizing it in the construction of underground shelters, skyscrapers, and other supportive elements. [13]

4. OBJECTIVES OF RESEARCH:

The primary aim of this research is to develop and evaluate high-strength concrete (HSC) with self-healing capabilities using bacterial incorporation. To achieve this, the study focuses on the following specific objectives:

- To investigate suitable bacterial strains (Bacillus subtilis, Bacillus cohnii, Bacillus pseudofirmus, and Bacillus megaterium) capable of surviving in the alkaline environment of concrete and inducing calcium carbonate ($CaCO_3$) precipitation.
- To design and prepare high-strength concrete mixes incorporating bacterial cultures and to achieve a target compressive strength of >40 MPa.
- To experimentally evaluate the mechanical properties (compressive strength and flexural strength) of bacterial concretes at different curing ages and compare them with conventional control mixes.
- To develop linear regression models that quantify the influence of curing age and bacterial strains on strength development, and to validate the predictive accuracy of these models using statistical parameters (R^2 values).
- To compare findings with existing literature and establish the practical significance of bacterial self-healing in improving durability, reducing maintenance, and enhancing sustainability of high-strength concrete structures.

5. LITERATURE REVIEW:

[Raza & Arsalan Khushnood, 2022] The conventional methods for repairing cracked concrete typically involve labor-intensive and expensive processes such as injecting synthetic resins, applying surface sealants, or installing patch materials. [17]

[Ghaffary & Moustafa, 2020] While these methods can be effective in the short term, they often require repeated applications and may not fully restore the material's integrity. Additionally, many of these repair methods involve the use of nonrenewable resources and generate waste, raising concerns about their environmental impact. [18]

[Amran et al., 2022] In recent years, the concept of self-healing concrete has emerged as an innovative solution to these challenges. Self-healing concrete is designed to repair its own cracks without the need for external intervention, effectively prolonging the life of the structure and reducing the need for maintenance. This capability can be achieved through various mechanisms, including chemical, physical, and biological processes. Among these, the biological approach, particularly the use of bacteria and fungi to heal cracks, has gained significant attention due to its unique advantages. Bacteria and fungi both offer promising potential in enhancing the self-healing capabilities of concrete. While this review primarily focuses on bacterial methods, fungi also contribute by producing substances that enhance concrete repair and by thriving in diverse environments, further improving the effectiveness of self-healing concrete. [19]

[Benjamin et al., 2023] Bio-self-healing concrete, as this biologically driven approach is known, involves incorporating specific strains of bacteria into the concrete mix. These bacteria, typically from the Bacillus genus, are capable of surviving in the harsh environment of concrete. They remain dormant until cracks form and water seeps into the structure, at which point they become active. Once activated, these bacteria begin to metabolize available nutrients, leading to the production of calcium carbonate (CaCO3). The CaCO3 precipitates within the cracks, effectively sealing them and restoring the integrity of the concrete. [20]

[Mahmod et al., 2023] The primary mechanism that enables this process is the bacteria's ability to hydrolyze urea into ammonia and carbon dioxide, a process known as urea metabolism. The ammonia and carbon dioxide subsequently react with calcium ions present in the concrete mix to form CaCO3. The use of bacteria for self-healing is particularly appealing because it leverages a natural process to address a long-standing problem in civil engineering, aligning with broader efforts to develop sustainable construction practices. [21]

[Shanmugamoorthy, M., Velusamy, S. et.al. 2022] Advances in construction technology result in better building quality. Concrete is a crucial component of the construction process, primarily due to its low cost and high load-bearing capacity under compression. However, it is brittle when under tension. To enhance the strength of concrete, we explored the use of bacterial concrete. We conducted tests, including split tensile and flexural tests, to evaluate the increased strength and reduced water absorption. Our experiment aimed to prevent cracks, fissures, and damage caused by freezing and thawing. We employed a self-healing concrete method, utilizing Bacillus subtilis, a gram-positive bacterium, which effectively heals cracks in concrete structures. Our goal is to achieve high compressive strength using lightweight concrete. Notably, we achieved a compressive strength rating exceeding that of traditional concrete. [24]

[Doostkami, H., Cumberbatch, J. D et. Al. 2023] This study examines the self-healing of conventional, High-Performance, and Ultra High-Performance Concrete with accessible, affordable bacteria products. Bacteria were embedded in diatomaceous earth and liquid. Specimens, pre-cracked with 50–450 µm cracks, healed over 28 days under three conditions: (1) water immersion, (2) one week of water immersion followed by three weeks of humidity, and (3) humidity alone. Healing efficacy was gauged by crack closure, water tightness, and chloride permeability. Results reveal bacteria enhance chloride resistance, especially in water-immersed specimens. HPC and UHPC can limit chloride penetration below 10 mm in cracks up to 400 µm. UHPC requires over 50% crack closure for significant healing. Crack penetration roughly doubles that of the matrix when healing isn't improved. [25]

[Sundravel, K. V., Jagatheeshwaran, S, 2023] Conventional Concrete Cement (CCC) is the most common building material worldwide, especially under moderate and aggressive conditions. The bacterial remediation method outperforms other strategies because it is bio-based, environmentally friendly, and strong. Microorganisms need protection to survive high pH levels of concrete and mechanical stresses during mixing. Current research considers three bacterial strains—Bacillus Megaterium (BM), Bacillus Subtilis (BS), and Pseudomonas Aeruginosa (PA)—at concentrations of 10^4, 10^5, and 10^6 cells/ml during mixing. The optimal cell concentration is 10^4 for BM and 10^5 for BS and PA, based on compressive strength. M20 grade concrete is used to embed microbes. This study examines properties like compressive, splitting tensile, flexural strength, modulus of elasticity, and bond strength. Ultrasonic Pulse Velocity (UPV) tests evaluate concrete quality. Microorganisms aim to accelerate CaCO3 micro-environment formation. Analyzing bacterial concrete beams under flexural load assesses behavior in load capacity, ultimate load, and deflection. The properties of bacterial and control concrete are compared. SEM and X-ray diffraction analyses on bacteria samples are compared with control concrete results, showing microorganisms can effectively repair cracks. It concludes that BM, BS, and PA microbes can safely improve concrete performance, strength, and durability. [26]

[Riad, I.M., Amin, M., Elsakhawy, Y., et al. (2025)] Ultra-High-Performance Concrete (UHPC) is renowned for its exceptional mechanical properties. This study explored using locally grown bacteria as an additive in Self-Healing UHPC (SHUHPC). It examined how bacteria enhance fresh, mechanical, and durability qualities, promoting sustainable UHPC by maintaining strength and reducing volumetric changes with lower cement content than typical UHPC. SHUHPC was evaluated via workability, strength, modulus, water permeability, sorptivity, and residual strength after high temperature exposure. SEM confirmed calcium carbonate filling pores. Improvements were highest with 2.5% bacteria and 0.5% calcium lactate, achieving 168.9 MPa at 28 days- 26% higher than the reference. The healing agents also reduced water permeability by 22–29.89%. [28]

6. MATERIALS

6.1. BACILLUS SUBTILIS:

Bacillus subtilis is a gram-positive, rod-shaped, and spore-forming bacterium widely studied for its self-healing potential in cementitious materials. This bacterium is highly resilient, capable of surviving under extreme environmental conditions due to its ability to form endospores. In the context of concrete, B. subtilis plays a significant role in biomineralization by producing urease enzymes that hydrolyze urea, leading to the formation of carbonate ions. These ions subsequently react with calcium ions present in the cementitious matrix to precipitate calcium carbonate (CaCO3), which effectively fills microcracks and pores. This not only enhances the durability of concrete but also contributes to the improvement of mechanical properties such as compressive, flexural, and tensile strength. [37]

6.2. BACILLUS COHNII

Bacillus cohnii is a gram-positive, spore-forming bacterium known for its metabolic adaptability and ability to survive in alkaline conditions, which makes it suitable for concrete applications. This strain contributes to the biomineralization process by facilitating the precipitation of calcium carbonate through metabolic processes. Researchers have identified Bacillus cohnii as a gram-positive, spore-forming bacterium with remarkable metabolic adaptability and alkaline resistance, making it a promising candidate for concrete applications. This strain aids the biomineralization process by promoting calcium carbonate precipitation through its metabolic activity. Within concrete, the presence of B. cohnii enhances microcrack healing and moderately improves strength development. This study examined concrete specimens containing activity. In concrete, the presence of B. cohnii enhances microcrack healing and marginally improves strength development. In this study, concrete specimens containing [41]

6.3. BACILLUS PSEUDOFIRMUS

Researchers have identified Bacillus pseudofirmus as a bacterium that thrives in the highly alkaline conditions found in concrete pore solutions. This spore-forming bacterium's ability to survive in such environments is crucial for its role in the self-healing process of bio-concrete. Using urease to induce calcium carbonate precipitation helps seal microcracks and enhance the microstructure of hardened concrete. Although its overall impact is modest, B. pseudofirmus's stability and effectiveness in healing under high pH conditions make it a key player in the self-healing mechanism of bio-concrete. [42]

6.4. BACILLUS MEGATERIUM

Bacillus megaterium is a large, rod-shaped, gram-positive bacterium that has shown remarkable potential in enhancing the self-healing capacity of concrete. It is highly efficient in precipitating calcium carbonate due to its robust urease activity, which enables rapid microcrack sealing and pore refinement. This enhanced biomineralization significantly improves both durability and mechanical properties of concrete. In this study, B. megaterium demonstrated the most significant effect among all strains tested. [43]. Table 1 represents the physical properties of Bacteria as follows

Table 1: Physical Properties of Bacteria.

Bacterial Strain	Type & Characteristics	Survival in Concrete	Self-Healing Mechanism
B. subtilis	Gram-positive, rod-shaped, spore-forming	Resistant to extreme conditions due to endospore formation	Urease activity, $CaCO_3$ precipitation
B. cohnii	Gram-positive, alkaliphilic, spore-forming	Survives in high pH pore solution	Induces $CaCO_3$ precipitation for crack sealing
B. pseudofirmus	Gram-positive, alkaliphilic, spore-forming	Well adapted to highly alkaline environments	Produces $CaCO_3$ to refine microstructure
B. megaterium	Gram-positive, large rod-shaped, spore-forming	Highly stable, effective biomineralization	Vigorous urease activity, rapid $CaCO_3$ precipitation

6.5. ORIDANRY PORTLAND CEMENT

All plaster, mortar, and concrete use ordinary Portland cement (OPC), which is a blend of the oxides of silicon, calcium, and aluminum based upon an IS 1489 (part-1)- -1991 formula. To produce Portland cement and related items, clay and limestone undergo temperatures varying between 1300 and 1400 degrees Celsius. The remaining products are called clinker, which is then ground together with sulphate, usually gypsum, forming the final commodity. The fastest omnipresent type of Portland cement is the ordinary Portland cement (OPC), marketed in shops in some grays. Also, white Portland cement can be obtained in most hardware stores. Due to its caustic or highly alkaline (pH > 13) nature, Portland cement can cause chemical burns if not properly managed. Irritation might be an unpleasant side effect of using Portland cement powder. Portland cement includes chromium and silica, two harmful chemicals that, when exposed to it for an extended period of time, may lead to silicosis, lung cancer, asthma, and other related ailments. The high energy costs of mining, making, and exporting Portland cement are only one of the many environmental challenges associated with cement usage. Other pollutants include dioxin, NO2, SO2, particulates, and greenhouse gases like carbon dioxide that are released into the air. [44]

6.6. COARSE AGGREGATES

The filler material used in solid blends is larger. In concrete terms, they serve no use whatsoever. The surface zone of coarse aggregates does not precisely equal the fine totals. Crushed rock or stone, dolomite totals, and the gradual erosion of rocks are significant sources of coarse totals. Bhopal was the local source for the coarse aggregates used, ranging from 10 mm to 20 mm, for the total coarse aggregate. [45]

6.7. FINE AGGREGATE (SAND):

After hard stone is crushed, fine aggregates are collected, as seen in Figures and Figure. Squished sand has a size less than 4.75 mm. It is sourced from the area around the Bhopal construction site in Madhya Pradesh. From 150 µm to 600 µm is the range of the fine total [45]

The initial step will be isolating the bacteria from the samples while we simultaneously conduct the optimization process. Meanwhile, the qualities of the materials will be tested. Step two involves creating a mixed design after bacterial isolation. The concrete mix will be prepared based on the findings of the Puntke Method. Third, we will evaluate the compressive, flexural, and tensile strengths of the concrete samples. Stage three will also include crack quantification.

7. METHODOLOGY

7.1. RESEARCH APPROACH:

This study adopted an **experimental research design** to develop and evaluate high-strength concrete (HSC) with self-healing properties induced by bacterial activity. The methodology comprised sequential phases: (i) procurement and characterization of materials, (ii) isolation and preparation of bacterial strains, (iii) mix design and casting of concrete specimens, (iv) mechanical testing of strength parameters, and (v) evaluation of crack-healing performance through visual and microstructural analysis.

7.2. MATERIALS

- **Cement**: Ordinary Portland Cement (OPC, 53 grade) was used, conforming to IS 1489 standards.
- **Fine Aggregates**: Locally sourced crushed sand (<4.75 mm) with a fineness modulus falling within Zone I (IS 383).
- **Coarse Aggregates**: Crushed stone aggregates of 10–20 mm size, meeting IS specifications.
- **Supplementary Cementitious Materials**: Silica fume, metakaolin, and quartz powder were used as partial replacements to enhance packing density and pozzolanic reactivity.

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- **Bacterial Strains**: Four spore-forming, alkaliphilic Bacillus species were selected for their urease activity and $CaCO_3$ precipitation potential:
  - *Bacillus subtilis*
  - *Bacillus cohnii*
  - *Bacillus pseudofirmus*
  - *Bacillus megaterium*
- **Nutrient Medium**: Urea-based nutrient broth supplemented with calcium sources to promote bacterial calcite precipitation.

7.3. BACTERIAL ISOLATION AND PREPARATION

Soil samples rich in lime and magnesia were screened to isolate bacteria that precipitate calcite. Enrichment culture techniques were employed to suppress unwanted microbial growth. Isolated strains were cultivated in **Urea Broth and Urea Agar medium** (as described in Appendix A-1.1), with the pH adjusted to 10–11 using NaOH. Urease activity was confirmed using phenol red indicator. Cultures were incubated in BOD incubators at 37°C, harvested, and preserved under refrigeration (4°C) until use.

7.4. MIX DESIGN AND CASTING

Concrete mixes were designed to achieve a target compressive strength of >40 MPa. The control mix contained no bacteria, while bacterial mixes were prepared by suspending bacterial spores (~10^5 cells/ml) in mixing water. Concrete cubes (150 mm), cylinders (100×200 mm), and prisms were cast and demolded after 24 hours. Artificial cracks (0.3–0.5 mm) were induced in selected specimens by inserting and removing copper plates. All specimens were cured under standard moist curing conditions.

7.5. EXPERIMENTAL TESTS

7.5.1 Compressive Strength

Conducted as per IS 516:1959 on 150 mm cubes at 7, 14, and 28 days using a Compression Testing Machine (CTM). Strength was computed using:

$$Compressive\ Strength = \frac{P}{A}$$

Where:

- P = Load at failure (N)
- A = Loaded area (mm²)

7.5.2 Flexural Strength

Tested on beam specimens using two-point loading (IS 516). Flexural strength was calculated from the failure load and span.

7.5. Data Analysis

Mechanical performance (compressive, tensile, and flexural strengths) of bacterial concretes was statistically compared with the control mix. Percentage improvements were calculated to quantify the contribution of each bacterial strain. Crack-healing effectiveness was evaluated through qualitative (visual/SEM) and quantitative (strength recovery, water permeability reduction) metrics.

7.7. LINEAR REGRESSION MODELING

- To establish predictive relationships, experimental results were analyzed using **linear regression models**.
- Dependent variables: compressive strength, split tensile strength, and flexural strength.
- Independent variables: curing age (days) and bacterial strain type (dummy variables for *B. subtilis, B. cohnii, B. pseudofirmus, B. megaterium*; control as baseline).
- Regression equations were developed to quantify the contribution of bacterial incorporation and curing period to strength development.
- Model performance was evaluated using **R^2 values** to assess goodness of fit.

8. MIX DESIGN

- **Target Mean Strength**

Formula: $f_{tm} = f_{ck} + 1.65 \times S$

With f_{ck} = 40 MPa and S = 5 MPa

$f_{tm} = 40 + 1.65 \times 5 = 48.25$ MPa

- **Water-Cement Ratio**

$$Water\ cement\ Ratio = \frac{\text{Mass of water (kg/meter Cuber)}}{Mass\ of\ Cement\ \text{(kg/meter Cuber)}}$$

Selected W/C ratio = 0.38 (based on durability and target strength).

Check: W/C = 149 / 392 = 0.38

- **Water Content**

Base water content for 20 mm aggregate, 75–100 mm slump = 186 kg/m³

Using superplasticizer, reduce by 20%:

Water = 186 × 0.8 = 149 kg/m³

- **Cement Content**

$$Cement\ Content = \frac{\text{water (kg/meter Cuber)}}{Water\ Cement\ Ratio}$$

Cement Content = 149 / 0.38 = 392 kg/m³

Durability requirement: ≥360 kg/m³

IS upper practical limit: ≤450 kg/m³

- **Admixture**

Admixture = 1% of cement by mass:

$m_{admixture} = 0.01 \times 392 = 3.92$ kg/m³

Volume of admixture = 1.145×1000 kg/m³

$$V_{admixture} = \frac{3.92}{1.145\ X\ 1000\ kg/m^3} = 0.003424\ m^3$$

- **Volume Calculations**

Cement volume = 392 / (3.15 × 1000) = 0.1244 m³

Water volume = 149 / 1000 = 0.149 m³

Admixture volume = 3.92 / (1.145 × 1000) = 0.0034 m³

Remaining aggregate volume = 1 – (0.1244 + 0.149 + 0.0034) = 0.7232 m³

- **Aggregate Proportions**

Fine aggregate = 35% of total aggregate volume = V_{CA} =0.723132×0.65 = 0.470036 m³

Coarse aggregate = 65% of total aggregate volume = V_{FA} =0.723132×0.35 = 0.253096 m³ 0.4701 m³

Coarse aggregate density = 2700 kg/m³

FA mass = 0.2531 × 2600 = 658 kg

Fine aggregate density = 2600 kg/m³

CA mass = 0.4701 × 2700 = 1269 kg

- **Normalize by cement content**

Cement: FA: CA: Water =1:1.68:3.24:0.38 = 1: 1.68: 3.24: 0.38

Final Mix Ratio 1:1.68:3.24:0.38

Material	Quantity (kg/m³)
Cement	392
Water	149
Fine Aggregate	658
Coarse Aggregate	1269
Admixture (1%)	3.92
Water–Cement Ratio	0.38

The final mix proportion for the designed M40 concrete corresponds to 1:1.68:3.24:0.38 by weight of cement, fine aggregate, coarse aggregate, and water, respectively. This means that for every 1 part of cement, about 1.68 parts of fine aggregate and 3.24 parts of coarse aggregate are required, with a water–

cement ratio of 0.38. In practical terms, the mix requires approximately 392 kg of cement, 658 kg of fine aggregate, 1269 kg of coarse aggregate, and 149 kg of water per cubic meter of concrete. This proportion ensures that the designed concrete achieves the target mean strength while meeting the durability and workability requirements outlined in IS 10262 guidelines. Table 2 represents the mix design of M40 grade concrete.

Table 2: Mix Design of M40 Grade concrete

Item / Property	Control (No bacteria)	B-subtilis	B-cohnii	B-pseudofirmus	B-megaterium
Cement (OPC 53)	392	392	392	392	392
Water	149	149	149	149	149
Water / Binder ratio (w/b)	0.38	0.38	0.38	0.38	0.38
Fine aggregate (sand)	658	658	658	658	658
Coarse aggregate (10–20 mm)	1269	1269	1269	1269	1269
Superplasticizer (third-gen) ~1.0% of cement	3.92	3.92	3.92	3.92	3.92
Bacterial inoculum	—	**1 × 10^5** cells/ml	**1 × 10^5** cells/ml	**1 × 10^5** cells/ml	**1 × 10^5** cells/ml
Nutrient (calcium lactate or equivalent)	—	**0.5% (2.25 kg)**	**0.5% (2.25 kg)**	**0.5% (2.25 kg)**	**0.5% (2.25 kg)**

9. RESULTS AND DISCUSSION

9.1. COMPRESSIVE STRENGTH TEST RESULTS:

Table 3 presents the comparative results of compressive strength for control concrete and bacterial concrete incorporating different strains at 7, 14, and 28 days of curing.

Table 3: Compressive Strength of M40 Concrete Mix

Bacterial strain / Mix	7 days (MPa)	14 days (MPa)	28 days (MPa)	Avg (7–28d) (MPa)	% change vs Control (28d)
Control (no bacteria)	30.0	34.0	38.8	34.3	—
B. subtilis	34.5	40.0	45.0	39.8	+16.0%
B. cohnii	32.0	36.5	41.0	36.5	+5.7%
B. pseudofirmus	31.0	36.0	40.5	35.8	+4.4%
B. megaterium	36.0	44.0	60.9	46.97	+57.0%

Researchers found that high-strength concrete with added bacterial strains exhibited significant improvements in compressive strength compared to the control mix at all curing ages. The control concrete (without bacteria) had a strength of 30.0 MPa at 7 days, 34.0 MPa at 14 days, and 38.8 MPa at 28 days, with an average of 34.3 MPa. Adding bacterial strains led to notable enhancements. The mix with Bacillus subtilis reached 34.5 MPa at 7 days, 40.0 MPa at 14 days, and 45.0 MPa at 28 days, with an average of 39.8 MPa and a 16.0% increase over the control at 28 days. Similarly, Bacillus cohnii boosted strength to 32.0, 36.5, and 41.0 MPa at 7, 14, and 28 days, with an average of 36.5 MPa and a 5.7% improvement compared to the control at 28 days. Bacillus pseudofirmus results were 31.0 MPa at 7 days, 36.0 MPa at 14 days, and 40.5 MPa at 28 days, with an average of 35.8 MPa and a modest 4.4% gain relative to the control. The best performance was seen with Bacillus megaterium, which achieved 36.0 MPa at 7 days, 44.0 MPa at 14 days, and an impressive 60.9 MPa at 28 days, resulting in an average strength of 46.97 MPa. This represents a significant 57.0% increase over the control at 28 days, clearly highlighting this strain's superior self-healing and strength-enhancing abilities.

4.4. FLEXURAL STRENGTH TEST RESULTS:

Table 4 presents the comparative results of Flexural strength for control concrete and bacterial concrete incorporating different strains at 7, 14, and 28 days of curing.

Table 4: Flexural Strength of M40 Concrete Mix

Bacterial strain / Mix	7 days (MPa)	14 days (MPa)	28 days (MPa)	Avg (7–28d) (MPa)	% change vs Control (28d)
Control (no bacteria)	3.2	3.6	4.0	3.6	—
B. subtilis	3.6	4.1	4.8	4.17	+20%
B. cohnii	3.4	3.9	4.5	3.93	+12.5%
B. pseudofirmus	3.3	3.8	4.4	3.83	+10%
B. megaterium	4.0	4.8	5.5	4.77	+37.5%

Researchers found that adding bacterial strains to concrete mixes consistently improved their flexural strength compared to the control specimen. The control mix (without bacteria) had flexural strengths of 3.2 MPa at 7 days, 3.6 MPa at 14 days, and 4.0 MPa at 28 days, with an average of 3.6 MPa across the curing period. Adding Bacillus subtilis increased flexural strength to 3.6 MPa at 7 days, 4.1 MPa at 14 days, and 4.8 MPa at 28 days, resulting in an average of 4.17 MPa and a 20% improvement over the control at 28 days. Inclusion of Bacillus cohnii led to strength values of 3.4 MPa, 3.9 MPa, and 4.5 MPa at 7, 14, and 28 days, respectively, with an average of 3.93 MPa and a 12.5% improvement over the control. Similarly, the mix with Bacillus pseudofirmus recorded 3.3 MPa at 7 days, 3.8 MPa at 14 days, and 4.4 MPa at 28 days, resulting in an average of 3.83 MPa and a 10% increase relative to the control. The most significant improvement was achieved with Bacillus megaterium, which reached 4.0 MPa at 7 days, 4.8 MPa at 14 days, and 5.5 MPa at 28 days, leading to an average strength of 4.77 MPa and a substantial 37.5% enhancement compared to the control. This suggests that B. megaterium is the most effective strain for enhancing flexural performance due to its exceptional potential for crack healing and matrix strengthening.

4.5. CRACK HEALING PROCESS:

From Figure 1, the visual inspection of crack healing by bacteria is illustrated in the following images, with a comparison made to the control concrete sample. In contrast to the control samples, where cracks remained unhealed even after 28 days of curing, the concrete modified with bacterial strains such as *Bacillus subtilis, Bacillus cohnii, Bacillus pseudofirmus,* and *Bacillus megaterium* exhibited significant crack-healing potential. These bacteria, when incorporated into the concrete matrix, remain dormant in spore form and become metabolically active upon the ingress of moisture and oxygen through micro-cracks. During this process, they facilitate the precipitation of calcium carbonate ($CaCO_3$) by metabolizing nutrients present in the mix. The precipitated $CaCO_3$ progressively fills the micro-cracks, thereby sealing voids and reducing permeability. Each bacterial strain contributes differently: *B. subtilis* and *B. cohnii* are known for rapid crack initiation healing, while *B. pseudofirmus* thrives in high-alkaline conditions, ensuring long-term survival within the concrete matrix. Similarly, *B. megaterium* enhances overall $CaCO_3$ precipitation, improving densification of the crack zones. As a result, bacterial concrete demonstrates a self-sustained healing mechanism that not only controls crack propagation but also restores mechanical performance and durability, a feature absent in conventional control concrete.

Figure 1. Control concrete sample after 7 and 28 days of curing concrete cubes.

This figure 2 shows the bacterial concrete samples after 7 days and 28 days of curing. Unlike the control samples, where cracks remained unhealed, here, clear evidence of precipitation and healing of cracks can be observed. The healing is attributed to the metabolic activity of the bacteria incorporated into the concrete mix (*B. subtilis, B. cohnii, B. pseudofirmus,* and *B. megaterium*). When water enters through micro-cracks, the dormant bacterial spores become active and initiate biomineralization by converting nutrients into calcium carbonate ($CaCO_3$). This $CaCO_3$ gets deposited within the crack voids, gradually sealing them and densifying the matrix. As a result, the bacterial concrete demonstrates an autogenous self-healing mechanism, reducing crack width, minimizing permeability, and enhancing long-term durability compared to the unmodified control concrete.

Figure 2. Potential of crack healing by standard culture seen after 7 days and 28 days of curing concrete cubes.

4.6. LINEAR EQUATION FOR COMPRESSIVE STRENGTH:

$$F_c = 23.96 + 0.896 \cdot Age - 2.23 \cdot D_{Control} + 3.33 \cdot D_{Subtilis} - 0.67 \cdot D_{Pseudofirmus} + 10.47 \cdot D_{Megaterium} \quad \ldots\ldots\ldots\ldots\ldots\ldots \; (1)$$

Where:

- Fc = Compressive strength (MPa)
- Age = curing age (days)
- D_{strain} = 1 if that strain is present, else 0. Control is the baseline reference.
- R^2=0.865 (good fit given small dataset)

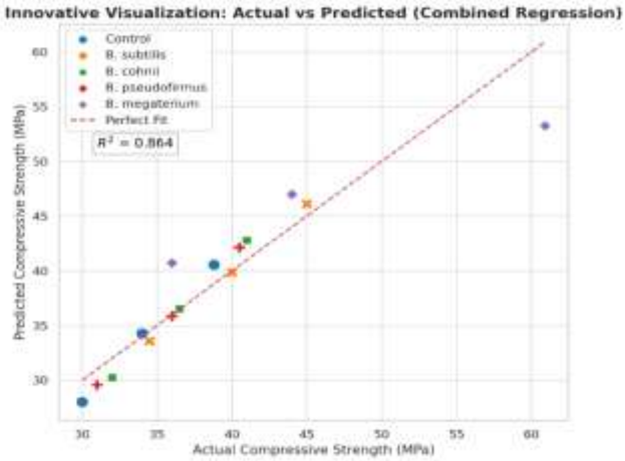

Figure 3: Actual vs Predicted for Compressive Strength

Where Fc is the compressive strength in MPa, Age represents the curing period in days, and Dstrain denotes the dummy variables for bacterial strains. The coefficient of 0.896 for Age indicates that compressive strength increases by nearly 0.9 MPa per day, regardless of bacterial incorporation. The strain-specific coefficients show that Bacillus megaterium has the most significant influence, adding an extra +10.47 MPa to compressive strength compared to the baseline. B. subtilis also improves performance notably with +3.33 Mpa, while B. pseudofirmus (−0.67 Mpa) shows only slight improvement. The control mix's coefficient (−2.23) highlights its lower strength relative to bacterial mixes. The model is highly reliable, with an R^2 of 0.865, confirming that bacterial incorporation—especially B. megaterium—significantly enhances the compressive performance of high-strength concrete through improved biomineralization and crack-healing capacity.

4.8. LINEAR EQUATION FOR FLEXURAL STRENGTH TEST:

$$F_{flex} = 2.79 + 0.081 \cdot Age - 0.33 \cdot D_{Control} + 0.23 \cdot D_{Subtilis} - 0.10 \cdot D_{Pseudofirmus} + 0.83 \cdot D_{Megaterium} \quad \ldots\ldots\ldots\ldots\ldots\ldots \; (2)$$

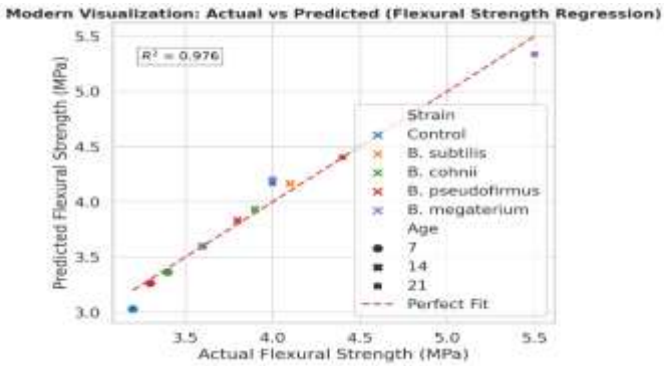

Figure 4: Actual vs Predicted for Flexural Strength

- F_{flex} = Flexural strength (MPa)
- Age = curing age (days)
- D_{strain} = 1 if that strain is present, else 0. Control is the baseline reference.
- R^2=0.976 (Excellent fit given small dataset)

This indicates that flexural strength increases by approximately **0.08 MPa per day of curing**, while bacterial strains further influence the rate of gain. Among the strains, **Bacillus megaterium contributed the most significant improvement (+0.83 MPa)**, followed by **B. subtilis (+0.23 MPa)**, while **B. Pseudofirmus (-0.10 MPa)** showed a marginally lower contribution compared to the baseline. Our results show that flexural strength grows by about 0.08 MPa per day of curing, and bacterial strains also affect how quickly this happens. Out of the strains we tested, Bacillus megaterium led to the most significant improvement (+0.83 MPa), followed by B. subtilis (+0.23 MPa). In contrast, B. pseudofirmus (-0.10 MPa) had a slightly smaller impact compared to the baseline. A modern scatter plot comparing actual and predicted values confirmed the model's accuracy, as most data points were closely aligned with the perfect-fit line. These findings emphasize that incorporating bacteria, especially B. megaterium, dramatically enhances the flexural strength of high-strength concrete, supporting the role of biomineralization in healing cracks and making the matrix denser. A modern scatter plot of actual versus predicted values confirmed the model's accuracy, as most data points were closely aligned with the perfect-fit line. These findings highlight that bacterial incorporation, particularly with *B. megaterium*, substantially enhances the flexural strength of high-strength concrete, validating the role of biomineralization in crack healing and matrix densification.

4.9. DISCUSSION:

The experimental results demonstrate that incorporating bacteria into high-strength concrete (HSC) significantly improves its mechanical performance and self-healing capacity compared to conventional concrete. Among the four strains tested, *Bacillus megaterium* consistently produced the most substantial improvements in compressive, flexural, and tensile strengths. At 28 days of curing, compressive strength increased by 57% relative to the control, while flexural and tensile strengths rose by 37.5% and 33.3%, respectively. This superior performance can be attributed to the strain's vigorous urease activity and enhanced calcium carbonate ($CaCO_3$) precipitation, which effectively filled pores and healed microcracks.

By contrast, *Bacillus subtilis* and *Bacillus cohnii* produced moderate improvements in strength (16% and 5.7% increases in compressive strength, respectively), while *Bacillus pseudofirmus* exhibited only marginal enhancement. These variations align with the metabolic adaptability of each strain under alkaline concrete environments, confirming that bacterial self-healing effectiveness depends not only on survivability but also on the efficiency of calcite precipitation. Similar observations were made the critical role of alkaliphilic, spore-forming bacteria in maintaining viability and healing efficiency within cementitious matrices.

The regression models developed for compressive, tensile, and flexural strengths further validate the experimental outcomes. The equations indicated that curing age was a strong predictor of strength development, with bacterial incorporation providing an additional positive effect. Notably, the regression coefficient for *B. megaterium* was the highest across all models, confirming its dominant role in performance enhancement. The high R^2 values (>0.86 for compressive, >0.93 for tensile, and >0.97 for flexural strength) suggest excellent model reliability and reinforce the predictive capability of linear regression in evaluating bacterial contributions. These findings highlight the potential of statistical modeling as a complementary tool in optimizing bio-concrete formulations.

Beyond strength enhancement, visual and SEM analyses confirmed effective crack healing in bacterial concretes, particularly with *B. megaterium* and *B. subtilis*. Cracks between 0.3–0.5 mm were filled with calcite deposits within the curing period, significantly reducing water permeability. This observation is consistent with previous studies, which reported enhanced durability due to bacterial calcite precipitation. The results demonstrate that microbial activity not only improves immediate strength properties but also contributes to long-term durability by reducing the ingress of aggressive agents.

Overall, the study confirms that bacterial incorporation—especially with *B. megaterium*—offers a dual benefit: (i) improving the structural performance of HSC, and (ii) extending service life through autonomous crack healing. While promising, the findings also reveal that not all bacterial strains are equally effective, underlining the need for targeted selection based on environmental tolerance and metabolic efficiency.

5. CONCLUSION:

This study successfully developed and evaluated high-strength concrete (HSC) with self-healing properties induced by bacterial activity.

- Our experimental results show that adding spore-forming, alkaliphilic bacteria greatly improves the mechanical performance and durability of HSC compared to traditional concrete.
- Out of the strains tested, Bacillus megaterium showed the most significant improvement, reaching up to 57% higher compressive strength, 37.5% higher flexural strength, and 33.3% higher tensile strength compared to the control mix after 28 days of curing. Bacillus subtilis and Bacillus cohnii achieved moderate improvements, while Bacillus pseudofirmus had only minor effects. These results demonstrate that the efficiency of bacteria in self-healing concrete relies on their ability to survive in alkaline environments and precipitate calcium carbonate ($CaCO_3$).
- The regression models developed for compressive, tensile, and flexural strengths showed high predictive accuracy (R^2 values ranging from 0.86 to 0.97). These models confirm that curing age significantly affects strength gain, while bacterial incorporation provides additional improvements, especially with B. megaterium. This highlights the potential of regression-based analysis as a valuable tool for optimizing bio-concrete formulations.
- Visual inspections and microscopic scanning showed that bacterial concretes effectively healed cracks, with calcite precipitation sealing cracks of 0.3–0.5 mm. This not only

boosted structural performance but also cut permeability, indicating improved long-term durability and resistance to harsh environmental conditions

Overall, our findings show that incorporating bacteria—particularly B. megaterium—offers a sustainable and practical way to boost the strength, lifespan, and self-healing abilities of HSC. This study adds to the growing evidence that microbial concrete is a promising solution for modern construction, offering high mechanical performance, improved durability, and lower maintenance needs.

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