Research on Engineering Structures & Materials
www.jresm.org
MIM Research Group

Research Article

Mechanical and microstructural evaluation of bacterial and two-component bacterial concretes

C. M. Meera [*,a], Subha Vishnudas [b]

Faculty of Civil Engineering, Cochin University of Science and Technology, Cochin-22, Kerala, India

Article Info

Article History:

Received 04 Nov 2025

Accepted 18 Dec 2025

Keywords:

Bacillus subtilis;
Bacterial concrete;
Calcium lactate;
Kruskal-Wallis test;
Regression modelling;
Statistical analysis

Abstract

The serviceability and durability of concrete structures are often reduced by crack formation, which permits ingress of water and aggressive agents. This study presents an intrinsic self-healing approach using a two-component bacterial concrete system that combines *Bacillus subtilis* with a calcium source to promote in-situ biomineralization within the concrete matrix. The performance of this system was compared with bacterial concrete containing only micro-organisms and with normal concrete of grades M20, M25, and M30. Experimental evaluation included compressive, split tensile, flexural strength, modulus of elasticity, water absorption, and porosity tests. Scanning Electron Microscopy (SEM) confirmed calcite precipitation and microstructural densification in bacterial and two-component bacterial concretes. Statistical analysis using the Kruskal–Wallis test verified that the observed differences among the concrete types were highly significant ($p < 0.001$). Regression modelling further quantified the influence of concrete grade, age, and bacterial composition on strength parameters, achieving high predictive accuracy ($R^2 = 0.65–0.96$). The results demonstrate that the two-component bacterial system enhances both strength and durability, offering a sustainable and effective self-healing solution for extending the service life of concrete structures.

1. Introduction

The longevity of concrete structures hinges significantly upon the critical parameter of concrete durability, a determinant of their overall serviceability [1]. Cracking of concrete due to plastic shrinkage, settlement, delayed curing, etc. is a problem that affects durability. Tiny particles in fluids entering the cracks contribute to the widening and propagation of the cracks. This is primarily caused by the abrasion effects of these particles on the exposed crack surface. As a result, it becomes crucial to address and halt the initial progression of cracks.

Self-healing bacterial concrete is an intrinsic solution to this cracking problem [2,3]. This mechanism involves the use of microorganisms that can secrete or precipitate calcite ($CaCO_3$), a natural cementing agent, during their metabolic activities and aid in sealing cracks [4,5]. The process results in reduced porosity, permeability, and water absorption capacity, proportionally increasing concrete's durability and strength properties [6,7]. Chemical and physical treatments available for healing have shortcomings of non-reversible action, limited long-term performance, and high cost of construction. The biological approach is pollution free and natural and hence highly desirable [8,9,10].

Cracks up to a width of 0.2 mm, can be healed by autogenous healing in concrete. This mechanism entails the interaction between atmospheric carbon dioxide and calcium hydroxide generated during cement hydration, producing calcium carbonate ($CaCO_3$) that fills and seals cracks (Eq. 1) [11]. Since the material available for this process is limited, autogenous healing is also limited.

*Corresponding author: meeramilton@yahoo.co.in
[a]orcid.org/0000-0003-3117-0670; [b]orcid.org/0000-0003-3905-7058
DOI: http://dx.doi.org/10.17515/resm2025-1319me1104rs

In bacterial concrete, the bio-mineralization of calcium carbonate is materialized in three different means namely (i) ureolytic pathway, (ii) denitrification pathway and (iii) metabolic conversion of the organic compound pathway. In the ureolytic pathway, initially, urea reacts with water molecules to form ammonia and carbamic acid, which again reacts with water to form ammonia and carbonic acid. The products after initial ureolysis then equilibrate in the presence of water to form bicarbonate, ammonium ions, and hydroxyl ions. This process elevates the pH of the system and leads to the release of nitrogen oxides into the atmosphere. [12]. The denitrification pathway involves the production of calcium carbonate through the reduction of nitrate. This process includes the sequential reduction of nitrate to nitrite, nitric oxide, nitrous oxide, and nitrogen gas. Additionally, oxidizing organic compounds result in formation of carbon dioxide, water, and nitrogen gas, which ultimately leads to the formation of calcium carbonate [12].

In the metabolic conversion of the organic compound pathway, the active metabolic conversion of calcium nutrients by the bacteria in the concrete occurs (Eq. 1) [11,13]. This system is a two-component self-healing system with bacteria as the first component and an organic compound as the second component. The organic compound undergo metabolic conversion. The byproduct CO_2 reacts again with $Ca(OH)_2$, leading to the production of more calcite (Eq. 2). [12]

$$Ca(C_3H_5O_3)_2 + 6O_2 \rightarrow CaCO_3 + 5CO_2 + 5H_2O \tag{1}$$

$$5CO_2 + 5Ca(OH)_2 \rightarrow 5CaCO_3 + 5H_2O \tag{2}$$

In all these pathways, the electronegative charge of bacteria and its large ratio of surface area to volume attract calcium cations (Ca^{2+}) present in the cementitious matrix and aid in the precipitation of calcium carbonate [5]. The spores of bacteria of bacillus species adopted for this type of study are known to remain viable for up to 200 years and can withstand extreme mechanical and chemical stresses. The spores can remain dormant in the concrete matrix and be revived to their active state in contact with water [14,15]. The microbial bio-mineralization is found effective in arresting cracks up to a width of 0.3mm [16,17]. Although several recent studies emphasize that encapsulation or protective carriers (e.g., silica gel, lightweight aggregates, hydrogels) improve long-term bacterial survivability and controlled release within concrete, direct addition of spores has also been demonstrated as a viable approach for intrinsic self-healing. Reviews show that most bio concrete systems employ encapsulation to shield spores from the high pH, dehydration, and mechanical stresses during mixing and curing. [18,19] Studies using immobilizing agents such as iron-oxide nanoparticles have demonstrated that Bacillus subtilis spores remain viable and metabolically active until crack formation, successfully inducing $CaCO_3$ precipitation upon activation. [20] In the present study, unencapsulated spores were adopted to evaluate their inherent survivability and bio-mineralization potential. While encapsulation may further enhance long-term healing efficiency, direct addition allows assessment of the intrinsic viability of spores and their ability to precipitate calcite upon crack ingress and water activation.

A background investigation unveiled the potential for advancing a bio-based strategy aimed at self-healing concrete, harnessing bacterial agents to yield robust and environmentally sustainable construction materials. The realization of such a system necessitates a methodical progression marked by exhaustible experimentation to attain optimal outcomes. The present study centres on a meticulous appraisal of the mechanical characteristics and microstructural attributes of normal concrete, bacterial concrete and two-component bacterial concrete, spanning different concrete grades. Laboratory studies are conducted to explore the inherent properties of these concrete materials, thus laying the groundwork for the eventual realization of a self-healing concrete paradigm. In addition to experimental testing, statistical and predictive modelling approaches are employed to strengthen the analytical framework of the study. Non-parametric statistical testing using the Kruskal–Wallis method is conducted to verify the significance of variations among normal, bacterial, and two-component bacterial concretes. Furthermore, multiple linear regression models with interaction terms are developed to identify the key influencing parameters and to predict mechanical responses with high accuracy. These complementary analyses ensure that the observed improvements are not only experimentally validated but also statistically and computationally substantiated.

2. Materials and Methods

The study focuses on having a comprehensive idea of the behavior of bacteria in three different grades of concrete M20, M25, and M30. Normal concrete (NC), bacterial concrete (BC), and two-component bacterial concrete (TBC) specimens are prepared for various tests. Bacterial concrete is prepared by introducing bacterial agent at required concentration in a suspension state in concrete mixing water. Calcium lactate is added as a calcium source along with bacteria while preparing two-component bacterial concrete. Water curing is carried out until the specified date for testing.

2.1. Materials

Ordinary Portland Cement of 53 Grade is utilised for the experimental works, which adheres to the specifications outlined in IS:12269-2013 [21]. The cement tested as per IS:4031-1988 [22], has a specific gravity of 3.16. Well-graded crushed sand, passing through a 4.75 mm sieve is used as fine aggregates. The sand is tested as per IS:2386 (Part III) -1963 [23] and exhibits a specific gravity of 2.74, with water absorption 11% and fineness modulus 2.62. Fine aggregates were used in dry condition, and the required absorption water was added to bring them to saturated surface dry state prior to mixing, ensuring an accurate effective water–cement ratio. The coarse aggregate of 20 mm and downsize are tested as per IS:2386-1963 [23], and the specific gravity and water absorption are found to be 2.79 and 0.81% respectively. Locally available potable water, conforming to IS: 456-2000 [24], is used. To enhance the workability of concrete, Conplast SP 430, a chloride free sulphonated naphthalene formaldehyde based super plasticizing admixture, is incorporated. The admixture has a specific gravity of 1.18 at 25°C and is added to the concrete at a dosage of 8, 9, and 10 ml/kg weight of cement for M20, M25, and M30 grades of concrete, respectively.

Table 1. Mix proportions of concrete

Concrete Designation	Cement (kg/m^3)	Fine aggregate (kg/m^3)	Coarse aggregate (kg/m^3)	Water (kg/m^3)	Admixture (l/ m^3)	Bacteria concentration (cells/ml)	Calcium (kg/m^3)
M20NC	350	734	1272	157.5	2.45	-	-
M20BC	350	734	1272	157.5	2.45	10^5	-
M20TBC	350	734	1272	157.5	2.45	10^5	1.75
M25NC	366	720	1270	157.3	2.92	-	-
M25BC	366	720	1270	157.3	2.92	10^5	-
M30TBC	366	720	1270	157.3	2.92	10^5	1.83
M30NC	384	706	1268	157.4	3.45	-	-
M30BC	384	706	1268	157.4	3.45	10^5	-
M30TBC	384	706	1268	157.4	3.45	10^5	1.92

2.2. Bacterial Culture and Calcium Source

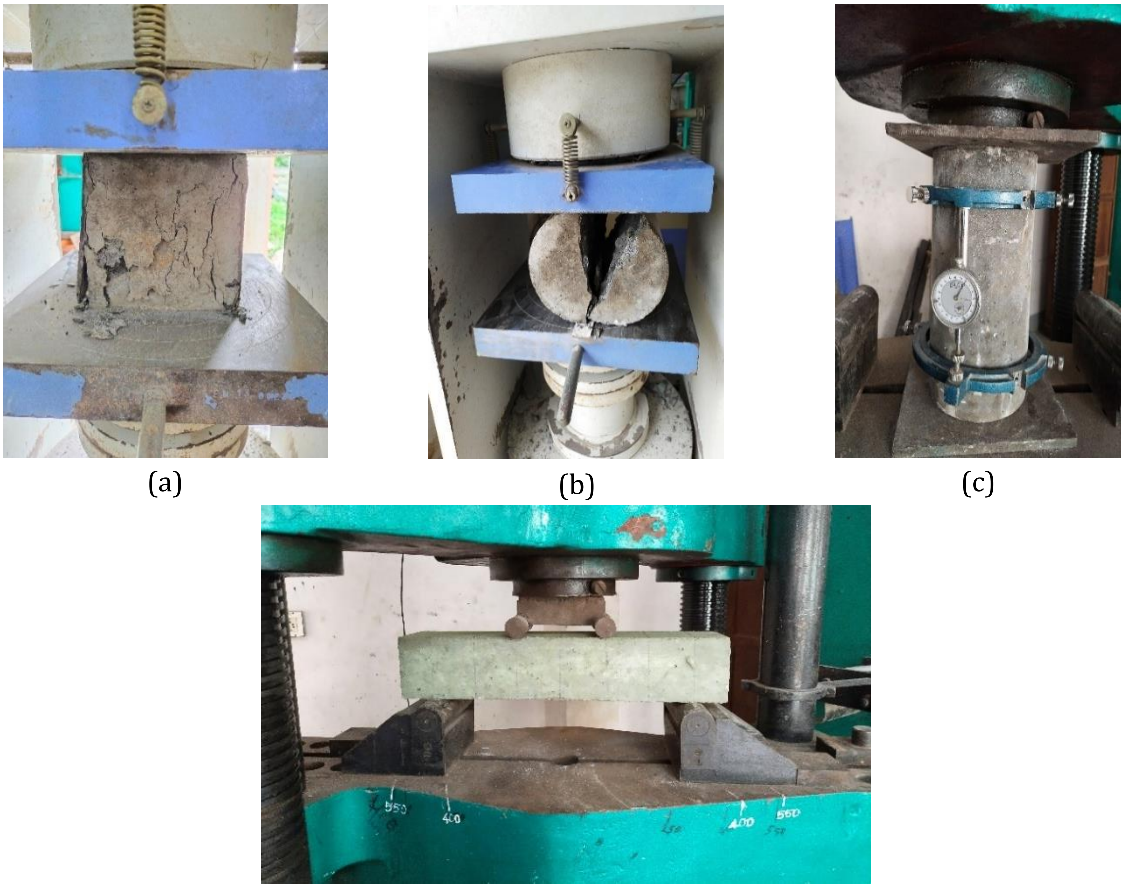
The microorganism, used in the study, is an aerobic, alkaliphilic, and spore-forming bacterial strain, *Bacillus subtilis JC3*, cultured at Kerala Agricultural University, Kerala, India. The bacterial culture is isolated from soil, grown in a nutrient medium for a concentration of 10^8 cells/ml, and stored at a temperature of less than 4°C. The nutrient medium used for growing the culture consisted of peptone (4.5 g/L), sodium chloride (3.5 g/L) and yeast extract (3.5 g/L) dissolved in 1000 mL of distilled water, as communicated by the supplier. For the preparation of bacterial concrete, as a first step, the obtained bacterial culture in the nutrient medium is normalized to the required concentration (10^3, 10^4, 10^5, 10^6, and 10^7 cells/ml) by serial dilution in the mixing water [25]. The water is then added to the dry ingredients. In a two-component bacterial concrete system, the calcium source added as a nutriment for bacteria is calcium lactate ($CaC_6H_{10}O_6$). The concrete mixing water dispersed with bacteria at an optimum concentration of 10^5 cells/ml as derived from compressive strength studies conducted on bacterial concrete and two-component bacterial concrete is adopted for further studies. The calcium lactate is added to the mixing water and dispersed with bacteria inoculum at a dosage of 0.5% by weight of cement.

2.3. Mix Proportion and Casting

Mix design is carried out for M20, M25, and M30 grades of concrete according to IS:10262-2019 [26]. The mix proportions of the materials for 1m^3 of concrete are shown in Table 1. The proportions of dry and wet ingredients are the same for both normal and bacterial concrete for the same grades. For each combination of concrete type (NC, BC, TBC), grade (M20, M25, M30), and test age (7, 28, 56, and 90 days), three replicate specimens (n = 3) were cast and tested for all mechanical tests (compressive strength, split tensile strength, flexural strength, and modulus of elasticity). Similarly, three specimens (n = 3) were used for each durability test (water absorption and porosity).

2.4. Testing Procedures

The specimens were prepared as per IS:516-1959 [27] for the concrete mix with and without the addition of microorganisms. Cube specimens measuring 150 mm × 150 mm × 150 mm were cast for the determination of compressive strength. The cylinder specimens of size 100mm diameter x 200mm height were used for testing split tensile strength and beam specimens of size 500mm x 100mm x 100mm were used for testing flexural strength Concrete is mixed in a concrete mixing machine for 2 minutes, cast and compacted on a tabletop vibrator for a minute, and is allowed to remain in iron moulds for the first 24 h at ambient temperature (27±2^{o}C). Specimens were water cured for 7, 28, 56, or 90 days depending upon the type of tests to be conducted.



(a) (b) (c)

(d)

Fig. 1. Experimental test setups and failure patterns (a) Compressive strength test showing crushing failure mode; (b) Split tensile strength test showing specimen failure; (c) Modulus of elasticity test set up; (d) Flexural strength test under two-point loading

Various tests are carried out to evaluate the performance of bacterial concrete as a building material. Strength assessment of the concrete specimens is done by conducting compressive strength test, split tensile strength test, and flexural strength test. A test for evaluating the modulus of elasticity is also conducted. A water absorption test is carried out to find the percentage of water absorption and porosity of concrete specimens. Microstructure evaluation of the concrete specimens is conducted by analysing Scanning Electron Microscopy (SEM) analysis. Statistical evaluation is performed to interpret the experimental results and determine the significance of differences among the various concrete types and grades.

Representative images of the experimental setups and typical failure patterns for the mechanical tests are provided in Figure. 1 to illustrate the testing conditions adopted in this study. Figure 1(a) shows a representative crushing pattern of a cube specimen subjected to the compressive strength test. Figure 1(b) depicts the test setup and typical splitting failure pattern of a specimen subjected to split tensile strength test. The compress meter arrangement used for elastic modulus determination is shown in Figure 1(c). The flexural test configuration under two-point loading is presented in Figure 1(d).

3. Results and Discussion

3.1. Compressive Strength

Compressive strength test is conducted for the normal, bacterial, and two-component bacterial concrete specimens of grades M20, M25, and M30 at ages 7, 28, 56, and 90 days as per Indian standard IS:516-1959 [27].

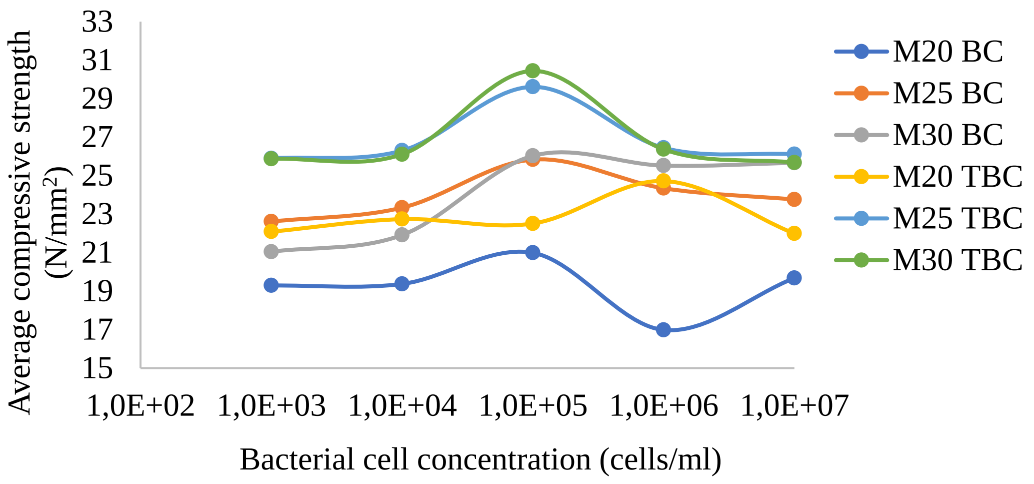


Fig. 2. Compressive strength of bacterial and two-component bacterial concrete of different grades, for varying bacterial cell concentrations for 7 days of curing in N/mm^2

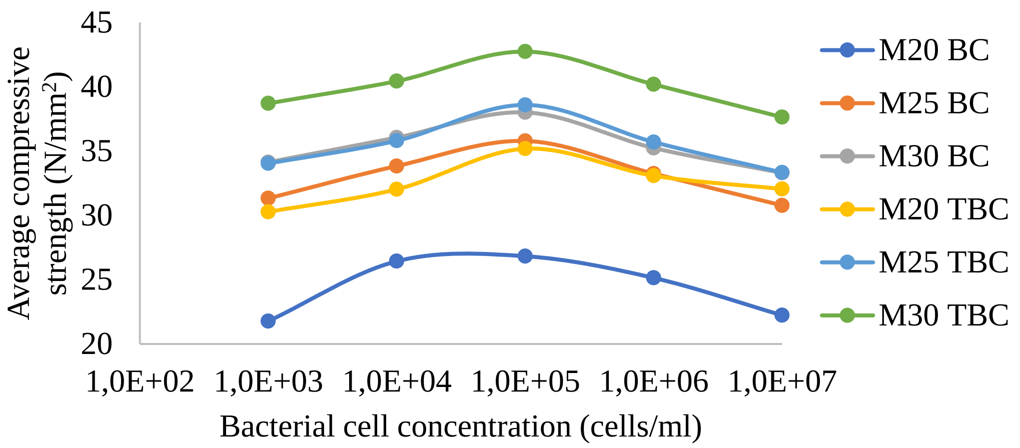


Fig. 3. Compressive strength of bacterial and two-component bacterial concrete of different grades for varying bacterial cell concentrations for 28 days of curing in N/mm^2

To determine the optimal cell concentration of bacteria, compressive strength tests are conducted on bacterial concrete specimens with varying bacterial concentrations of 10^3, 10^4, 10^5, 10^6, and 10^7

cells/ml. Figures 2 and 3 exhibit the variations in the compressive strength for different grades of bacterial concrete at 7 and 28 days, respectively, using different bacterial cell concentrations in the mixing water. The results demonstrate that at 7 and 28 days, the strength increases up to an optimum concentration of 10^5 cells/ml, after which it decreases for all three grades of bacterial concrete and two-component bacterial concrete. Consequently, the concentration of 10^5 cells/ml is chosen as the optimum level for further studies on bacterial and two-component bacterial concrete. Contemporary investigations have yielded analogous findings concerning the bacterial cell concentration [28]. The reduction in strength at higher bacterial concentrations is attributed to nutrient limitations within the cementitious matrix, which lead to incomplete or inefficient calcite precipitation when excess bacterial cells compete for limited resources. Prior studies report that overly dense bacterial populations may form micro-agglomerates that increase porosity or interfere with normal hydration, thereby reducing strength [11,15,17]. Consequently, concentrations above 10^5 cells/ml result in diminished biomineralization efficiency and lower compressive strength.

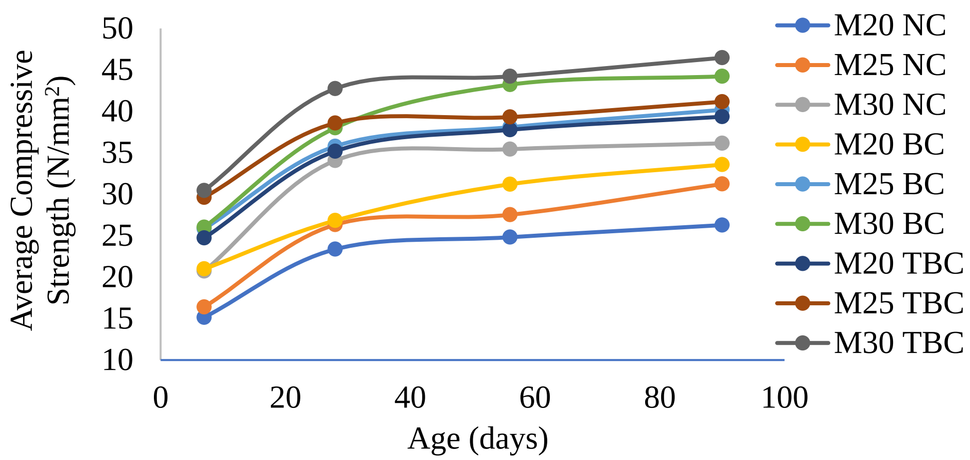


Fig. 4. Compressive strength of normal, bacterial, and two-component bacterial concrete of grades M20, M25, and M30 for the age of concrete in N/mm^2

Figure 4 represents the variation of compressive strength among different concrete specimens considered at ages 7, 28, 56, and 90 days. In all the cases, the average compressive strength exhibits a similar pattern of a steep curve up to 28 days and thereafter a flattened curve. This behaviour aligns with the general anticipation of normal concrete, which typically exhibits rapid strength improvement up to 28 days and a more gradual increase beyond that period. When bacteria are introduced into M20, M25, and M30 grades of concrete, the 28 days strength shows significant improvements of 14.8% (26.83 N/mm^2), 35.9% (35.79 N/mm^2), and 11.5% (38.01 N/mm^2), respectively, compared to normal concrete of the corresponding grades (23.36 N/mm^2, 26.33 N/mm^2, and 34.07 N/mm^2). Furthermore, the addition of calcium lactate in M20, M25, and M30 grades of two-component bacterial concrete with optimum bacterial concentration (10^5 cells/ml) exhibits improvements of 50.5% (35.19 N/mm^2), 46.5% (38.59 N/mm^2), and 25.4% (42.74 N/mm^2) in 28-day compressive strength, respectively, when compared to normal concrete.

3.2. Split Tensile Strength

The split tensile strength test is carried out on all the specimens at 28 days, in accordance with Indian standard IS:5816-1999 [29]. The test results are shown in Figure 5. Analysis of the results reveals discernible enhancements in the split tensile strength of bacterial concrete, indicating percentage improvements of 11.6% (3.39 N/mm^2), 8.2% (3.43 N/mm^2) and 4.3% (3.51 N/mm^2) in comparison to normal concrete (3.04 N/mm^2, 3.17 N/mm^2, and 3.36 N/mm^2), for M20, M25, and M30 grades, respectively. Notably, the two-component bacterial concrete shows superior increments of 16.3% (3.53 N/mm^2), 19.5% (3.79 N/mm^2), and 24.1% (4.17 N/mm^2), for M20, M25, and M30 grades, respectively, when compared with normal concrete. Furthermore, in relation to bacterial concrete, the two-component bacterial concrete displays augmentation rates of 4.2%, 10.4%, and 18.9% for M20, M25, and M30 grades, respectively. These improvements may be due to the incorporation of calcium lactate as well as the consequential pore-sealing mechanisms inherent to the two-component bacterial concrete. Ongoing scholarly investigations demonstrate concordant findings regarding the improvement of split tensile strength of bacteria incorporated concrete [8,28].

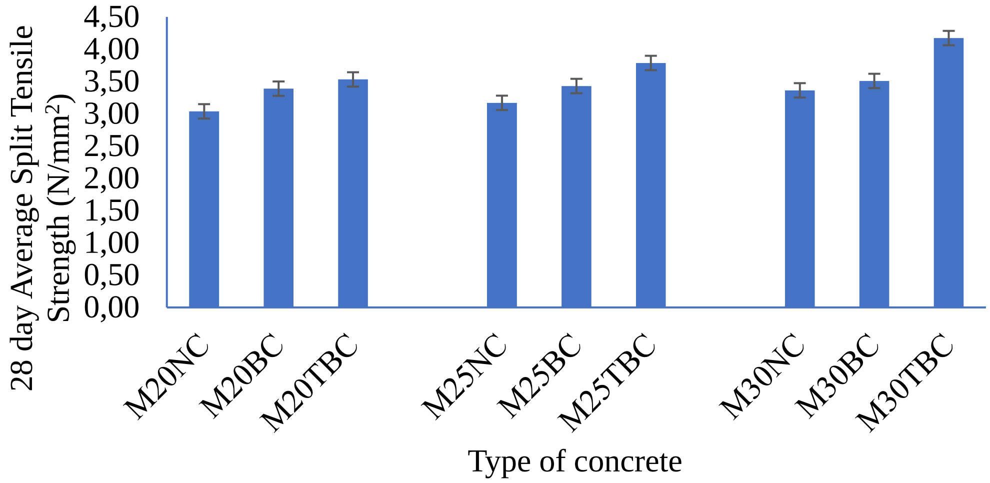


Fig. 5. Split tensile strength of normal, bacterial, and two-component bacterial concrete of grades M20, M25, and M30

3.3. Flexural Strength

The flexural strength of concrete specimens is determined as per Indian standard IS:516-1959 [27] for all specimens at 28 days. The results are graphically represented in Figure 6. The findings divulge that the flexural strength of bacterial concrete exhibits appreciable enhancement of 17.2% (5.67 N/mm^2), 15.4% (7.50 N/mm^2), and 14.2% (8.87 N/mm^2) in contrast to the corresponding normal concrete values (4.83, 6.50, and 7.77 N/mm^2) for M20, M25, and M30 grades, respectively. Recent scholarly research reveals that a commensurate increment in flexural strength of bacterial concrete is attained [8,28].

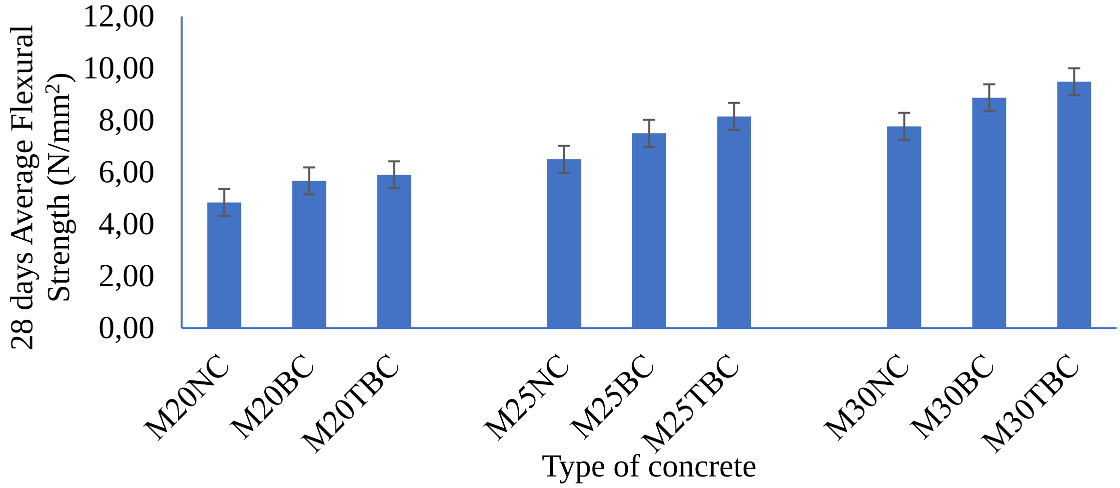


Fig. 6. Flexural strength of normal, bacterial, and two-component bacterial concrete of grades M20, M25, and M30

Furthermore, the application of two-component bacterial concrete yielded a more pronounced improvement of 22.1% (5.90 N/mm^2), 25.4% (8.15 N/mm^2), and 22.1% (9.48 N/mm^2) for M20, M25, and M30 grades, respectively, when compared with normal concrete. The improvement in flexural strength of the two-component bacterial concrete is due to the presence of calcium source within the concrete matrix, thus conferring incremental enhancements of 4.1%, 8.7%, and 7% for the M20, M25, and M30 grades, respectively.

3.4. Modulus of Elasticity

The modulus of elasticity is determined for all the specimens at 28 days, as per Indian standard IS:516-1959 [27]. The corresponding results are illustrated in Figure 7. The findings underscore that the bacterial concrete demonstrates remarkable increments in modulus of elasticity of 26% (29.72x10^3 N/mm^2), 52.1% (38.11x10^3 N/mm^2), and 47.4% (46.69x10^3 N/mm^2) when compared to normal concrete (23.58x10^3, 25.05x10^3, and 31.68x10^3 N/mm^2) across M20, M25, and M30 grades, respectively. Related studies also indicate enhancements in elasticity of concrete upon impregnation with bacteria [30]. Moreover, the two-component concrete specimens reveal a substantial enhancement in modulus of elasticity, exhibiting increments of 64.3% (38.74x10^3 N/mm^2), 62.4% (40.68x10^3 N/mm^2), and 53% (48.48x10^3 N/mm^2) in relation to normal concrete for the respective M20, M25, and M30 grades. The incorporation of a calcium source in two-component bacterial concrete, engenders a further augmentation of 30.3%, 6.7%, and 3.8% in the modulus of elasticity of bacterial concrete for the M20, M25, and M30 grades, respectively. This variation in improvement across concrete grades can be attributed to differences in the initial microstructural density. Lower-grade concrete such as M20 contains higher inherent porosity, providing more available void space for bacterial calcite precipitation and resulting in a greater increase in stiffness. In contrast, higher-grade mixes like M30 already possess a denser and more compact matrix with fewer voids, limiting the extent of additional microstructural densification achievable through biomineralization. Consequently, the relative improvement in modulus of elasticity decreases as the concrete grade increases.

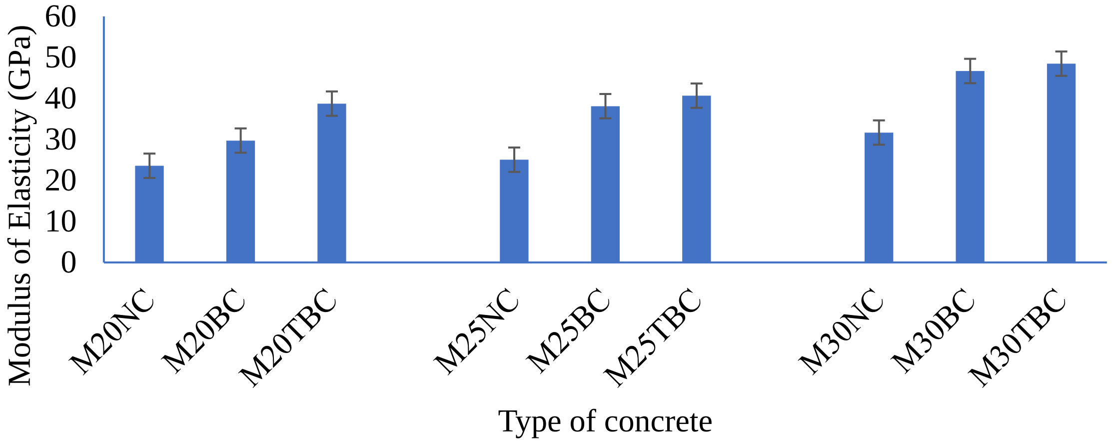


Fig. 7. Modulus of elasticity of normal, bacterial, and two-component bacterial concrete of grades M20, M25, and M30

3.5. Water Absorption Test

Water absorption test is carried out for all specimens at ages 28, 56, and 90 days, as per ASTM C 642-13 [31] and the corresponding results are illustrated in Figure 8. Notably, the water absorption values exhibit a significant reduction in the case of the two-component bacterial concrete, recording values of 4.97%, 4.11%, and 3.90% for M20, M25, and M30 grades, respectively, at 28 days. These findings denote substantial improvements of 42.5%, 43.4%, and 34.7%, respectively, in comparison to the water absorption rates observed in normal concrete (8.65%, 7.26%, and 5.97% for M20, M25, and M30 grades, respectively, at 28 days). Similarly, the percentage water absorption of bacterial concrete measures 5.15%, 4.89%, and 4.52% for M20, M25, and M30 grades, respectively, at 28 days, signifying a reduction of 40.4%, 32.6%, and 24.3%, respectively, in contrast to normal concrete. Furthermore, when compared directly with bacterial concrete (BC), the two-component system exhibited additional reductions of 3.5%, 16.0%, and 13.7% for the M20, M25, and M30 grades, respectively. These values confirm the beneficial role of the added calcium source in enhancing biomineralization efficiency and reducing pore connectivity more effectively than BC alone. This trend of two-component bacterial concrete displaying the lowest water absorption, followed by bacterial concrete (which exhibits lower absorption than normal concrete but higher than two-component bacterial concrete), persisted at both the 56 and 90-day intervals.

The water absorption curve, irrespective of the specimen type, demonstrated a consistent decreasing trend with increasing age. In normal concrete, this decline could be attributed to the phenomenon of autogenous healing, which contributes to pore filling. In the case of bacterial concrete and two-component bacterial concrete, the diminishing trend of the curve is postulated to result from a combination of autogenous healing and bacterial bio-mineralization, contributing to pore filling. In bacterial concrete, microorganisms effectively harness available calcium ions within the concrete matrix to facilitate the precipitation of calcite. On the contrary, in two-component bacterial concrete, microorganisms utilise the metabolic conversion of the applied calcium source to precipitate a more substantial quantity of calcite. Consequently, it is inferred that the two-component concrete experiences a more pronounced reduction in pores, thereby leading to a more extensive decrease in water absorption when compared to bacterial concrete.

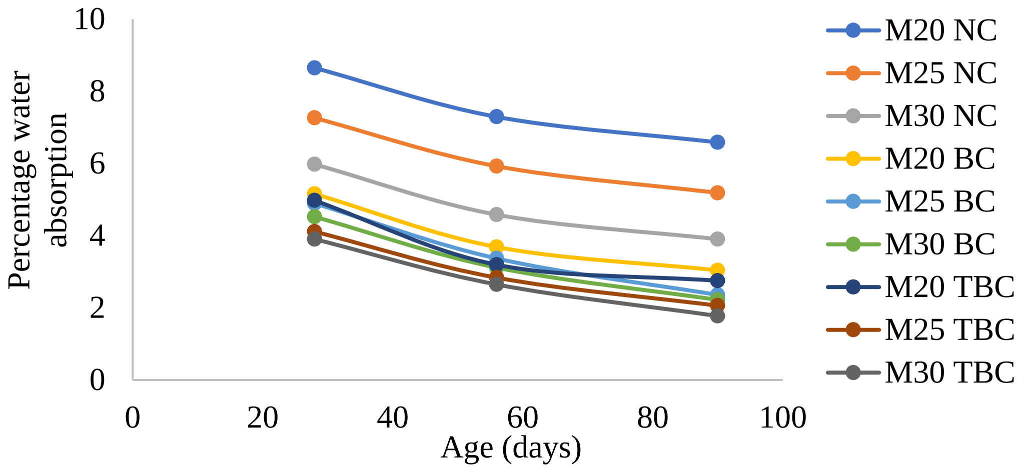


Fig. 8. Water absorption percentage of normal, bacterial, and two-component bacterial concrete of grades M20, M25, and M30 at 28, 56, and 90 days of age

3.6. Porosity

To comprehend the water transport dynamics within the pore structure of concrete, the porosity of the concrete specimens is assessed. Porosity is quantified using Eq (3):

$$n = {V_v}/{\rho_w V} = {W_{sat} - W_{dry}}/{\rho_w V} = {W_w}/{\rho_w V} \tag{3}$$

where n = porosity, V_v = volume of voids in the specimen, V = total volume of the specimen, ρ_w = unit mass of water, W_{sat} = weight of saturated specimens, W_{dry} = weight of oven dried specimens at 105°C and W_w = weight of absorbed water in the concrete specimen [28].

An examination of the area plot, as illustrated in Figure 9, reveals a consistent decrease in porosity as the concrete grade escalates. Notably, the introduction of bacterial agents contributes to a significant reduction in porosity within bacterial concrete, resulting in decreases of 36.5% (12.93), 31% (12.22), and 25.5% (11.18) in comparison to the respective porosity values observed in normal concrete (20.35, 17.70, and 15) for M20, M25, and M30 grades, respectively. This reduction in porosity is even more pronounced in the case of two-component concrete, displaying a reduction of 39% (12.40), 41.5% (10.36), and 33.5% (9.98) for M20, M25, and M30 grades, respectively. Furthermore, when compared directly to bacterial concrete, the two-component system achieved additional porosity reductions of 4.0%, 15.2%, and 10.8% for M20, M25, and M30 grades, respectively, confirming the enhanced pore refinement provided by the added calcium source. It is postulated that the formation of calcite crystals, facilitated by bacterial bio-mineralization, underpins the observed reduction in porosity within bacterial concrete. It is further inferred that the enhanced biomineralization resulting from the addition of an accessible calcium source leads to a greater reduction in porosity in the two-component bacterial concrete.

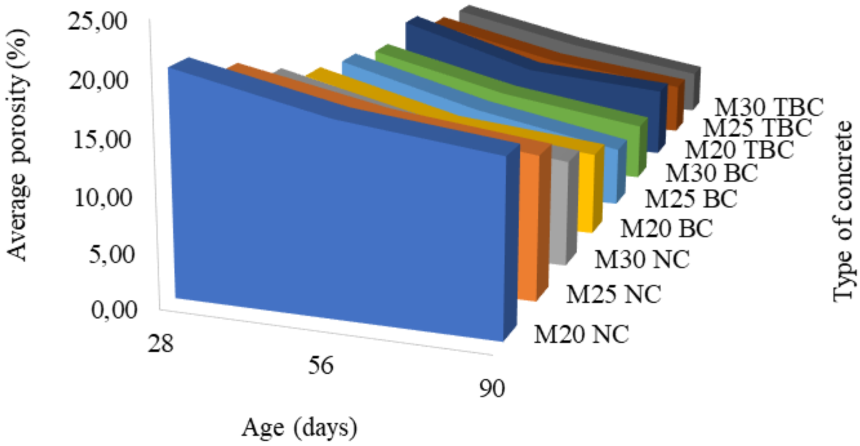
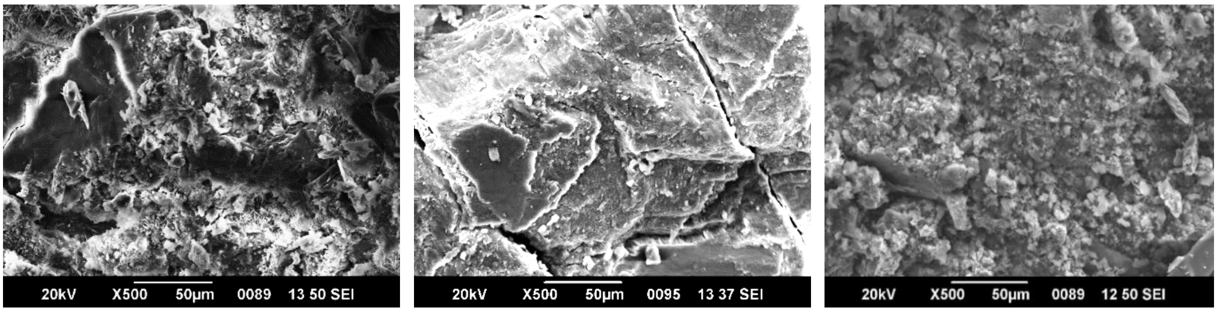


Fig. 9. Average porosity percentage of normal, bacterial, and two-component bacterial concrete of grades M20, M25, and M30 at 28, 56, and 90 days of age

4. Microstructure Evaluation

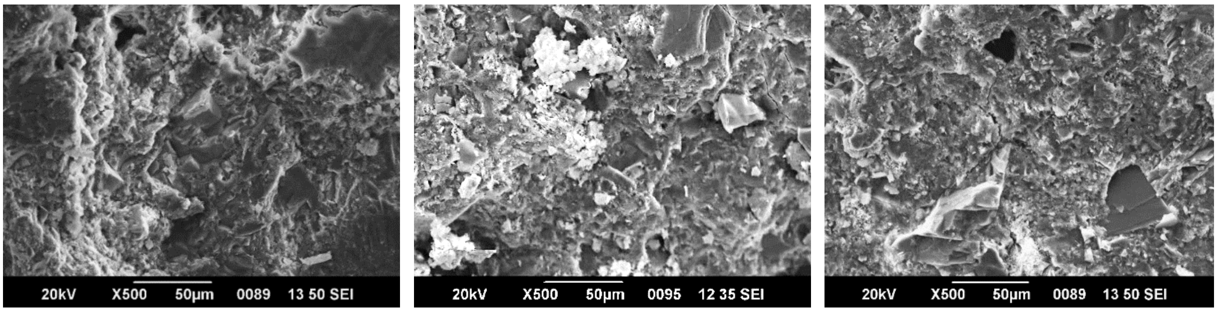
Scanning Electron Microscopy (SEM) imaging of concrete specimens is taken at a magnification of 5000x using a JSM-6390 scanning electron microscope. SEM images showing normal, bacterial, and two-component bacterial concrete specimens of grades M20, M25, and M30 are presented in Figures 10 to 12. These images offer valuable insights into the microstructure characteristics of the concrete specimens. A detailed examination of SEM images of normal concrete specimens reveals the presence of distributed pores within the concrete matrix, along with the formation of occasional calcium carbonate (calcite) crystals (Figure 10). These crystals could be the product of autogenous healing in normal concrete.

In Figure 11, SEM images of bacterial concrete specimens exhibit reduced voids and presence of dense calcite precipitation in the form of calcite crystals when compared to normal concrete. These crystals are assumed to be a result of the bacterial bio-mineralization taking place in the concrete matrix. Furthermore, SEM images of two-component bacterial concrete (Figure 12) illustrate an even lesser occurrence of voids and a denser precipitation of calcite crystals expected to be attributed to the metabolic conversion of added calcium lactate. Distinct bacterial spores or rod-shaped cells are not clearly visible in the SEM images, which is expected because bacterial morphology may not remain visually detectable, as cells often become encapsulated within hydration products or coated by calcite in the high-alkaline cementitious environment [11,15]. However, the dense rhombohedral calcite deposits and crystal agglomerations observed in the BC and TBC specimens (Figures 11 and 12) are consistent with microbially induced calcite precipitation reported in Bacillus-based studies [11,17]. These features indicate that bacterial cells or their remnants likely acted as nucleation sites during early calcite formation, even though they are not visually distinguishable in the hardened concrete. Collectively, these SEM images vividly exemplify the successful incorporation of bacteria and a calcium source within the concrete matrix, highlighting their bio-mineralization ability and their positive influence on the microstructure of concrete. The presence of calcite crystals and pore-filling effect indicates the potential of two-component bacterial concrete to enhance its mechanical properties and self-healing capacity [6,7].



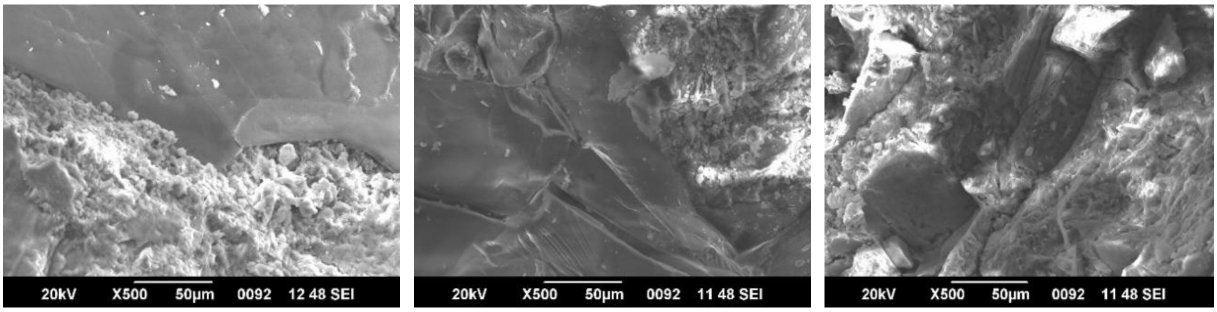
(a) (b) (c)

Fig. 10. SEM Image of (a) M20NC (b) M25NC (c) M30NC specimens



(a) (b) (c)

Fig. 11. SEM Image of (a) M20BC (b) M25BC (c) M30BC specimens



(a) (b) (c)

Fig. 12. SEM Image of (a) M20TBC (b) M25TBC (c) M30TBC specimens

5. Statistical Analysis

To ensure the reliability and robustness of the experimental observations, statistical evaluation is performed to verify whether the observed variations among normal, bacterial, and two-component bacterial concretes are statistically significant. The analysis focuses on identifying the influence of bacterial incorporation and calcium supplementation on key performance parameters through non-parametric testing. Statistical analysis is conducted to compare various test results, considering compressive strength as a strength parameter, modulus of elasticity, and percentage water absorption as a permeability parameter. Due to the non-normal distribution of the population, the Kruskal-Wallis test (KW test), which does not assume a normal distribution of residuals, is adopted for the analysis [32].

Table 2. Statistical test results testing 'all at a time'

Test	BC	NC	TBC	BC	NC	TBC	KW Test
Compressive strength test	36.79±5.63	29.46±4.67	40.53±3.43	***	***	***	***
Modulus of Elasticity	38.17±7.35	26.77±3.74	42.63±4.47	***	***	***	***
Water absorption test	3.59±1.04	6.15±1.50	3.14±0.99	***	***	***	***

*Significance codes: 0 <= '***' < 0.001 < '**' < 0.01 < '*' < 0.05*

A comprehensive statistical assessment is first performed to determine whether significant variations exist among the groups (NC, BC, and TBC) when all variables are analyzed collectively. The results in Table 2 demonstrate that all variables collectively show significant differences among the groups (NC, BC, and TBC). The obtained p-values (< 0.001) indicate very strong evidence that the differences among the groups remain highly significant in the KW Test. Next, the KW test is performed separately for each variable to evaluate whether there are significant differences among the groups for each variable individually. The results in Table 3, considering each variable individually, also indicate significance in the KW test. The obtained p-values (<0.01) strongly indicate significant differences for each variable among the groups.

Based on the test results, it can be concluded that the addition of bacteria in bacteria concrete and the inclusion of a calcium source in two-component bacterial concrete positively influence compressive strength, modulus of elasticity, and water absorption. Additionally, the age and grade of concrete also exert a significant influence on the concrete parameters [33,34]. The application of the Kruskal–Wallis test establishes that the mechanical and permeability characteristics of the concrete types differ significantly ($p < 0.01$). This statistical confirmation substantiates the experimental trends observed in compressive strength, modulus of elasticity, and water absorption, thereby validating the role of bacterial biomineralization in enhancing overall concrete performance.

Table 3. Statistical test results testing 'with all variables'

Variable	Levels	Test	NC	BC	TBC	KW Test
Age	28 days	Compressive strength test	30.01±5.32	36.74±7.56	40.19±4.17	**
	56 days		29.09±5.34	37.07±5.75	40.94±4.19	***
	90 days		29.29±3.67	36.56±3.55	40.46±1.75	***
	28 days	Modulus of Elasticity	23.58±0.20	29.72±0.34	38.73±0.02	***
	56 days		25.05±0.25	38.11±0.10	40.68±0.28	***
	90 days		31.68±0.10	46.69±0.39	48.48±0.32	***
	28 days	Water absorption test	6.14±2.04	3.54±1.26	3.23±1.33	**
	56 days		6.15±1.74	3.60±1.19	3.03±1.02	***
	90 days		6.16±0.40	3.64±0.70	3.16±0.57	***
Concrete Mix	M20	Compressive strength test	24.82±1.31	30.54±3.00	37.44±2.20	***
	M25		28.36±2.26	38.02±2.02	39.68±1.27	***
	M30		35.21±1.06	41.82±3.97	44.47±1.67	***
	M20	Modulus of Elasticity	23.58±0.20	29.72±0.34	38.73±0.02	***
	M25		25.05±0.25	38.11±0.10	40.68±0.28	***
	M30		31.68±0.10	46.69±0.39	48.48±0.32	***
	M20	Water absorption test	7.51±1.12	3.96±0.97	3.64±1.02	***
	M25		6.12±1.01	3.54±1.12	3.00±0.91	***
	M30		4.82±0.99	3.29±1.01	2.77±0.93	***

6. Regression Analysis

While the Kruskal–Wallis test provides evidence of statistically significant group-wise differences, regression modelling is employed to further quantify the influence of key variables and their interactions on concrete performance [35.36]. Multiple linear regression (MLR) models are developed to predict strength parameters and to identify the most influential factors affecting bacterial and two-component bacterial concretes. In addition, the regression analysis facilitates quantitative evaluation of model accuracy and predictive capability using standard error metrics and goodness-of-fit indicators. For compressive strength, bacterial concentration was treated as a quantitative variable due to the evaluation of multiple cell concentrations (10^3–10^7 cells/ml), whereas for split tensile and flexural strength, bacterial presence at the optimum dosage (10^5 cells/ml) was treated as a component variable.

6.1. Compressive Strength

A linear regression model was developed to predict compressive strength. The model performance results indicate that the regression model achieves a validation RMSE of 6.46 MPa, a coefficient of determination (R^2) of 0.7625, and a mean absolute error (MAE) of 4.87 MPa, with a corresponding mean squared error (MSE) of 41.7 $(MPa)^2$.

Table 4. Regression summary for compressive strength

	Estimate	SE	tStat	pValue
Intercept	-0.02819	2.712514	-0.01039	0.991734
Grade of concrete	0.745328	0.098789	7.544626	6.68E-11
Age in days	0.204805	0.015967	12.82692	5.27E-21
Bacterial concentration	0.81645	0.2027	4.027865	0.000128
Calcium component	8.720814	1.704952	5.114991	2.15E-06

The regression coefficients provided in Table 4 represent a multiple linear regression model predicting compressive strength based on grade of concrete, age in days, bacterial concentration, and calcium component. Grade of concrete, age in days, bacterial concentration, and calcium component are all statistically significant predictors ($p < 0.05$), while the intercept is not statistically significant. Higher concrete grades and longer curing ages are associated with increased compressive strength. The statistically significant coefficients for bacterial concentration and calcium component indicate the contribution of microbial activity and calcium availability to strength enhancement. Overall, the model shows that grade of concrete and curing age are the most influential parameters, with bacterial concentration and calcium component providing additional positive contributions to compressive strength.

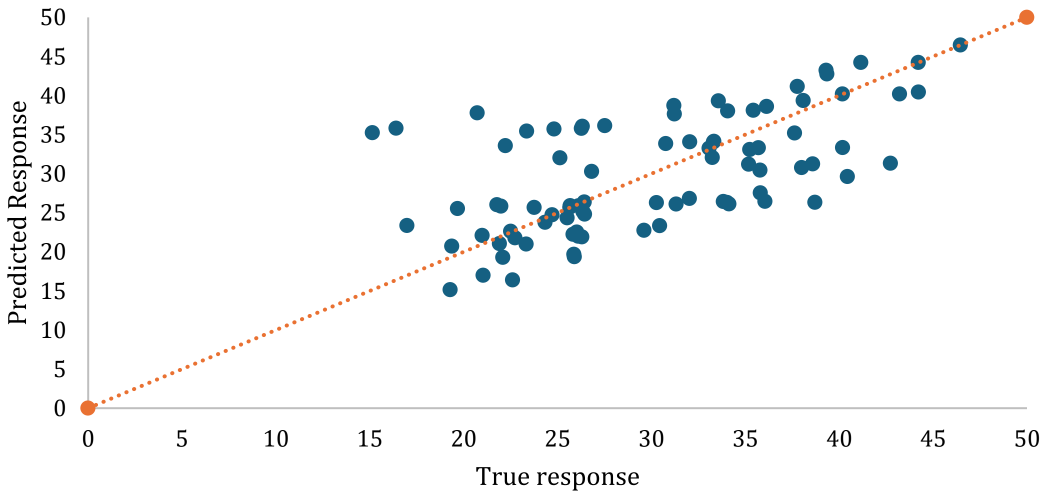


Fig. 13. Predicted versus true compressive strength

The scatter plot compares predicted versus true compressive strength responses from the regression model (Figure 13). Most points cluster around the 45-degree reference line, reflecting good agreement between the model predictions and experimentally measured compressive strength values. Some deviations from the line are observed; however, the spread remains moderate and consistent with the reported R^2 value, indicating reliable predictive performance across the investigated strength range.

6.2. Split Tensile Strength

The linear regression model demonstrates satisfactory predictive performance, with a validation RMSE of 0.253 MPa and a validation mean squared error (MSE) of 0.0639 $(MPa)^2$, indicating relatively small prediction errors. The R-squared value of 0.6537 shows that the model explains approximately 65% of the variance in split tensile strength. The mean absolute error (MAE) of

0.179 MPa further indicates that the model predictions remain close to the experimentally measured values.

Table 5. Regression summary for split tensile strength

	Estimate	SE	tStat	pValue
Intercept	2.28560195	0.291666	7.836378	6.10308E-08
Grade of concrete	0.036172053	0.011226	3.222106	0.003773805
Bacterial Component	0.050515059	0.022452	2.24987	0.034325214
Calcium component	0.775969175	0.224524	3.456059	0.002145743

The regression output presented in Table 5 shows that grade of concrete ($p = 0.0038$), bacterial component ($p = 0.034$), and calcium component ($p = 0.0021$) are statistically significant predictors of split tensile strength, while the intercept is also statistically significant. The positive regression coefficients indicate that increases in grade of concrete, bacterial component, and calcium component contribute positively to split tensile strength. These results highlight the combined influence of material grade, microbial activity, and calcium availability on tensile performance.

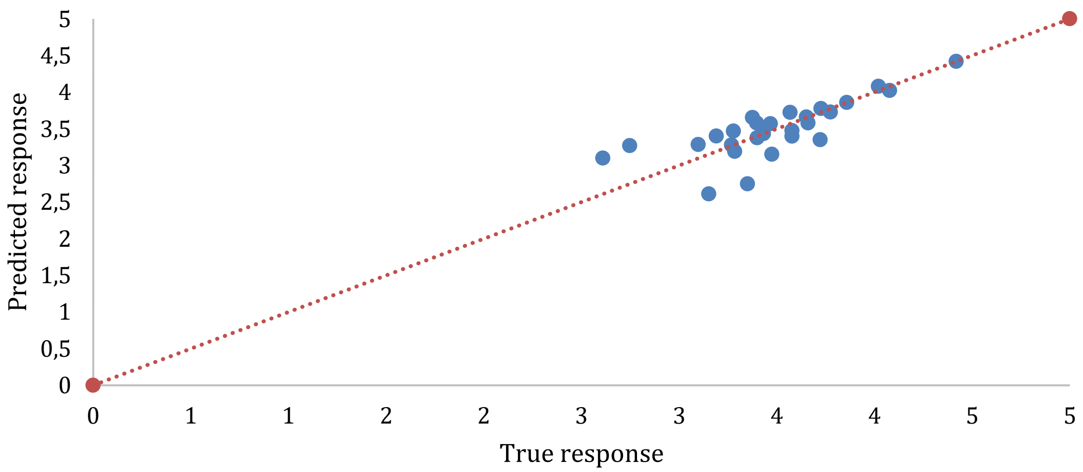


Fig. 14. Predicted versus true split tensile strength

The scatter plot (Figure 14) displays predicted versus true split tensile strength responses from the regression model. The majority of data points align reasonably well with the diagonal reference line, indicating good agreement between predicted and measured values. Some scatter is observed, which is consistent with the moderate R^2 value; however, the absence of pronounced outliers or systematic bias suggests that the model provides reliable predictions within the investigated data range.

6.3. Flexural Strength

The linear regression model as represented in Table 6 demonstrates excellent predictive performance, as reflected by a validation RMSE of 0.3067 MPa and a validation mean squared error (MSE) of 0.0941 $(MPa)^2$, meaning that prediction errors are small on average. An R-squared value of 0.96 indicates that the model explains 96% of the variance in flexural strength, providing very high accuracy and demonstrating strong agreement between predicted and observed values. Additionally, the mean absolute error (MAE) of 0.261 MPa confirms that the predictions remain consistently close to the actual values, highlighting the model's reliability.

The regression results show that grade of concrete ($p < 0.001$), bacterial component ($p < 0.001$), and calcium component ($p = 0.0013$) are all statistically significant predictors in the model, while the intercept is also statistically significant. The strong positive coefficient for grade of concrete and the bacterial component indicates that increases in these variables are associated with higher flexural strength values. The calcium component, with a smaller positive coefficient, also exerts a significant positive effect. The very low p-values and high t-statistics confirm the strength of these predictors, highlighting their importance in explaining variations in flexural strength (Table 6).

Table 6. Regression summary for flexural strength

	Estimate	SE	tStat	pValue
Intercept	-1.730555	0.354564	-4.8808	6.27E-05
Grade of concrete	0.323888	0.013647	23.73304	1.13E-17
Bacterial Component	9.78E-06	1.36E-06	7.164692	2.69E-07
Calcium component	1.00E+00	2.73E-01	3.663763	1.29E-03

The scatter plot (Figure 15) of predicted versus true flexural strength responses demonstrates a close alignment of data points along the 45-degree reference line, indicating that the model's predictions closely match the experimentally measured values across the investigated range. Minor deviations from the line are observed, with no evident systematic bias or major outliers, confirming that the model exhibits high predictive accuracy and reliability. Collectively, the regression analyses confirm that both grade of concrete and bacterial incorporation are statistically dominant predictors of mechanical strength. The high R^2 values (0.65–0.96) demonstrate that the developed models accurately represent the relationship between experimental parameters and performance outcomes. This quantitative modelling approach enhances the predictive understanding of biomineralization effects in bacterial concretes, complementing the statistical validation obtained through the Kruskal–Wallis test.

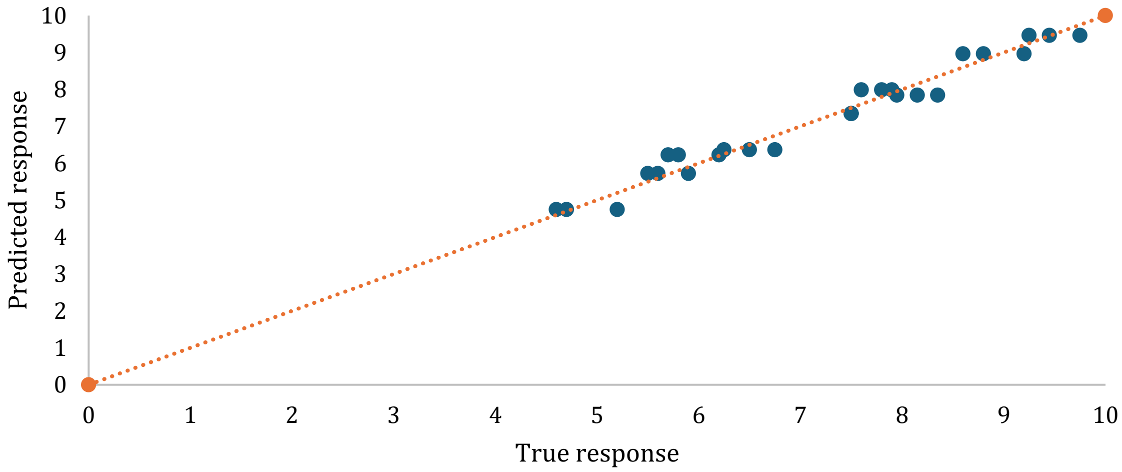


Fig. 15. Predicted versus true flexural strength

7. Conclusion

The study confirms that bacterial biomineralization serves as an effective intrinsic mechanism for improving the strength and durability of concrete. The major findings and implications are summarized as follows:

- Enhanced mechanical performance: The incorporation of Bacillus subtilis and a calcium source significantly improved compressive, split tensile, and flexural strengths—by up to 50%, 24%, and 25%, respectively, compared with normal concrete. The optimum bacterial concentration was identified as 10^5 cells/ml, consistent with prior studies [1,9].
- Improved durability and impermeability: The two-component bacterial concrete exhibited more than 40% reduction in water absorption and porosity, demonstrating a denser, less permeable matrix. The improvement is attributed to the bacterial conversion of calcium lactate into calcium carbonate, effectively sealing voids and microcracks [11,13].
- Microstructural confirmation: SEM analysis revealed dense calcite precipitation and reduced pore connectivity in bacterial and two-component bacterial concretes. The two-component

system showed the most extensive and uniform calcite deposition, validating the biomineralization-driven densification mechanism [10,11].

- Statistical validation: The Kruskal–Wallis test confirmed highly significant differences ($p < 0.001$) among concrete types, while regression analysis ($R^2 = 0.65–0.96$) identified grade of concrete and bacterial component as the key predictors of performance. These trends are consistent with established regression-based strength models [30,31].
- Practical and sustainability implications: The two-component bacterial system provides a robust, eco-efficient, and self-healing concrete with enhanced durability, reduced maintenance needs, and extended service life. This approach offers a viable pathway toward sustainable and resilient infrastructure development [2,4].

In conclusion, this study demonstrates that the integration of *Bacillus subtilis* and a calcium source through a two-component bacterial concrete system effectively enhances the mechanical strength, durability, and microstructural integrity of concrete. The synergistic bacterial biomineralization mechanism promotes in-situ calcium carbonate precipitation, leading to a denser and more impermeable matrix. The strong agreement between experimental, microstructural, and statistical analyses confirms the robustness of this bio-based approach. Overall, the proposed two-component bacterial concrete offers a sustainable, self-healing, and eco-efficient alternative to conventional concrete, with significant potential for application in long-lasting and low-maintenance infrastructure systems.

Although the study demonstrates clear improvements in strength and permeability characteristics, certain limitations must be acknowledged. The long-term viability of bacterial spores beyond 90 days was not evaluated, and the self-healing capacity was not directly quantified through controlled crack-width recovery tests. Environmental influences such as wet–dry cycles, temperature variations, carbonation, and exposure to aggressive chemicals were also not assessed. These factors could influence actual field performance, and future studies incorporating accelerated aging, in-situ viability tracking, and standardized crack-healing protocols are recommended.

The performance enhancements observed in this study indicate strong potential for deploying two-component bacterial concrete in practical infrastructure such as canal linings, retaining walls, pavements, water-retaining structures, and marine components where resistance to moisture ingress and reduced maintenance are critical. The denser matrix and self-healing ability can substantially reduce repair cycles, offering estimated lifecycle cost savings of 20–40% as supported by similar biomineralization-based systems. The process of microbially induced calcite precipitation requires minimal energy input and avoids carbon-intensive repair materials, thereby improving sustainability metrics and lowering the environmental footprint of infrastructure. Thus, the proposed system aligns with modern requirements for durable, low-carbon, and long-service-life construction materials.

Acknowledgement

The authors acknowledge that this study was supported by the TEQIP Phase III Seed Money Project for Research Projects, a Government of India initiative assisted by the World Bank, through the School of Engineering, Cochin University of Science and Technology (CUSAT). The authors also express their sincere gratitude to DST-SAIF, Cochin, Kerala, India, for providing access to SEM facilities at subsidized rates, which greatly contributed to the successful completion of this research work.

References

[1] Ozmen HB, Inel M. Effect of concrete strength and detailing properties on seismic damage for RC structures. Res Eng Des. 2024;1(1):1-11. https://doi.org/10.17515/rede2024-005en1124rs

[2] Van Tittelboom K, De Belie N, De Muynck W, Verstraete W. Use of bacteria to repair cracks in concrete. Cem Concr Res. 2010;40:157-66. https://doi.org/10.1016/j.cemconres.2009.08.025

[3] Van Breugel K. Self-healing material concepts as solution for aging infrastructure. In: 37th Conference on Our World in Concrete and Structures. 2012. p. 1051-7.

[4] Chithra S, Senthil Kumar SRR, Chinnaraju K, Alfin Ashmita F. A comparative study on the compressive strength prediction models for high performance concrete containing nano silica and copper slag using

regression analysis and artificial neural networks. Constr Build Mater. 2016;114:528-35. https://doi.org/10.1016/j.conbuildmat.2016.03.214
[5] Uddin MN, Tafsirojjaman T, Shanmugasundaram N, Praveenkumar S, Li LZ. Smart self-healing bacterial concrete for sustainable goal. Innov Infrastruct Solut. 2023;8(1):46. https://doi.org/10.1007/s41062-022-01020-6
[6] Vashisht R, Shukla A. Potential application of bacteria to improve the self-healing and strength of concrete. J Build Pathol Rehabil. 2020;5(1):10. https://doi.org/10.1007/s41024-020-0073-5
[7] Khaliq W, Ehsan MB. Crack healing in concrete using various bio influenced self-healing techniques. Constr Build Mater. 2016;102:349-57. https://doi.org/10.1016/j.conbuildmat.2015.11.006
[8] Prasad CV, Lakshmi TV. Experimental investigation on bacterial concrete strength with Bacillus subtilis and crushed stone dust aggregate based on ultrasonic pulse velocity. Mater Today Proc. 2020;27:1111-7. https://doi.org/10.1016/j.matpr.2020.01.478
[9] Bhaskar S, Hossain KMA, Lachemi M, Wolfaardt G, Kroukamp MO. Effect of self-healing on strength and durability of zeolite-immobilized bacterial cementitious mortar composites. Cem Concr Compos. 2017;82:23-33. https://doi.org/10.1016/j.cemconcomp.2017.05.013
[10] Ozmen HB, Tanrıverdib B. Concrete paradox: Economic importance, environmental impacts, and the sustainability of concrete material. Research & Design 2025;2(2):1-19. https://doi.org/10.17515/rede2025-008en0801rs
[11] Jonkers HM, Thijssen A, Muyzer G, Copuroglu O, Schlangen E. Application of bacteria as self-healing agent for the development of sustainable concrete. Ecol Eng. 2010;36(2):230-5. https://doi.org/10.1016/j.ecoleng.2008.12.036
[12] Sidiq A, Gravina R, Giustozzi F. Is concrete healing really efficient? A review. Constr Build Mater. 2019;205:257-73. https://doi.org/10.1016/j.conbuildmat.2019.02.002
[13] Tziviloglou E, Wiktor V, Jonkers HM, Schlangen E. Bacteria-based self-healing concrete to increase liquid tightness of cracks. Constr Build Mater. 2016;122:118-25. https://doi.org/10.1016/j.conbuildmat.2016.06.080
[14] Algaifi HA, Bakar SA, Sam AR, Ismail M, Abidin AR, Shahir S, Altowayti WA. Insight into the role of microbial calcium carbonate and the factors involved in self-healing concrete. Constr Build Mater. 2020;254:119258. https://doi.org/10.1016/j.conbuildmat.2020.119258
[15] De Muynck W, Cox K, De Belie N, Verstraete W. Bacterial carbonate precipitation as an alternative surface treatment for concrete. Constr Build Mater. 2008;22(5):875-85. https://doi.org/10.1016/j.conbuildmat.2006.12.011
[16] Aytekin B, Mardani A, Yazıcı Ş. State-of-art review of bacteria-based self-healing concrete: biomineralization process, crack healing, and mechanical properties. Constr Build Mater. 2023;378:131198. https://doi.org/10.1016/j.conbuildmat.2023.131198
[17] Ghosh S, Biswas M, Chattopadhyay BD, Mandal S. Microbial activity on the microstructure of bacteria modified mortar. Cem Concr Compos. 2009;31(2):93-8. https://doi.org/10.1016/j.cemconcomp.2009.01.001
[18] Wong PY, Mal J, Sandak A, Luo L, Jian J, Pradhan N. Advances in microbial self-healing concrete: A critical review of mechanisms, developments, and future directions. Science of The Total Environment. 2024 Oct 15;947:174553. https://doi.org/10.1016/j.scitotenv.2024.174553
[19] Javeed Y, Goh Y, Mo KH, Yap SP, Leo BF. Microbial self-healing in concrete: A comprehensive exploration of bacterial viability, implementation techniques, and mechanical properties. Journal of Materials Research and Technology. 2024 Mar 1;29:2376-95. https://doi.org/10.1016/j.jmrt.2024.01.261
[20] Mahmood F, Kashif Ur Rehman S, Jameel M, Riaz N, Javed MF, Salmi A, Awad YA. Self-healing bio-concrete using Bacillus subtilis encapsulated in iron oxide nanoparticles. Materials. 2022 Nov 3;15(21):7731. https://doi.org/10.3390/ma15217731
[21] IS 12269: 53 Grade Ordinary Portland Cement. Bureau of Indian Standards, New Delhi; 2013.
[22] IS 4031 (Part 1): Methods of physical tests for hydraulic cement - Determination of fineness by dry sieving. Bureau of Indian Standards, New Delhi; 1988.
[23] IS 2386 (Part III): Methods of test for aggregates for concrete - Part 3: Specific gravity, density, voids, absorption and bulking. Bureau of Indian Standards, New Delhi; 1963.
[24] IS 456: Plain and reinforced concrete - Code of practice (Fourth Revision). Bureau of Indian Standards, New Delhi; 2000.
[25] Amjad H, Khushnood RA, Memon SA. Biomimetic robust self-healing of Bacillus subtilis immobilized through sisal fiber for next-generation concrete infrastructure. Constr Build Mater. 2023;368:130299. https://doi.org/10.1016/j.conbuildmat.2023.130299
[26] IS 10262: Concrete Mix Proportioning - Guidelines (Second Revision). Bureau of Indian Standards, New Delhi; 2019.
[27] IS 516: Method of Tests for Strength of Concrete. Bureau of Indian Standards, New Delhi; 1959.

[28] Seshagiri Rao MV, Srinivasa Reddy V, Sasikala Ch. Performance of microbial concrete developed using Bacillus subtilis JC3. J Inst Eng (India) Ser A. 2017;98(4):501-10. https://doi.org/10.1007/s40030-017-0227-x
[29] IS 5816: Splitting Tensile Strength of Concrete - Method of Test. Bureau of Indian Standards, New Delhi; 1999.
[30] Nguyen TH, Ghorbel E, Fares H, Cousture A. Bacterial self-healing of concrete and durability assessment. Cem Concr Compos. 2019;104:103340. https://doi.org/10.1016/j.cemconcomp.2019.103340
[31] ASTM C 642-13. Standard Test Method for Density, Absorption and Voids in Hardened Concrete. ASTM International, West Conshohocken, Pennsylvania; 2009. p. 1-3.
[32] Kruskal WH, Wallis WA. Use of ranks in one-criterion variance analysis. J Am Stat Assoc. 1952;47:583-621. https://doi.org/10.1080/01621459.1952.10483441
[33] Kheder GF, Gabban AA, Abid SM. Mathematical model for the prediction of cement compressive strength at the ages of 7 and 28 days within 24 hours. Mater Struct. 2003;36(10):693-701. https://doi.org/10.1007/BF02479504
[34] Zain MFM, Abd SM. Multiple regression model for compressive strength prediction of high performance concrete. J Appl Sci. 2009;9:155-60. https://doi.org/10.3923/jas.2009.155.160
[35] Atici U. Prediction of the strength of mineral admixture concrete using multivariable regression analysis and an artificial neural network. Expert Syst Appl. 2011;38(8):9609-18. https://doi.org/10.1016/j.eswa.2011.01.156
[36] Keith A, Marill MD. Advanced statistics: linear regression, part II: multiple linear regression. Acad Emerg Med. 2004;11:94-102. https://doi.org/10.1197/j.aem.2003.09.006