Excel International Journal of Technology, Engineering and Management
2026; Volume -13, Issue-2_Page_142-152

Excel International Journal of
Technology, Engineering & Management
Journal homepage: exceljournals.org.in
ISSN ONLINE: https://portal.issn.org/resource/ISSN/2349-8455
ISSN PRINT: https://portal.issn.org/resource/ISSN/2348-0378

SYNERGISTIC EFFECT OF METAKAOLIN AND BACILLUS SUBTILIS ON STRENGTH AND DURABILITY OF CONCRETE

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Published online: May 2026
DOI Link: https://doi.org/10.64971/j.cph.eijtem.v13.i2.17.2026

ABSTRACT:
In order to increase the durability of M25 grade concrete, this study examines the effectiveness of bacterial concrete, specifically using Bacillus subtilis and metakaolin as a partial cement alternative. Seven concrete mixes (M0-M6) with varying amounts of Bacillus and metakaolin were developed, along with a control mix. At 7 and 28 days, mechanical characteristics such flexural strength, split tensile strength, compressive strength and durability tests were evaluated. The best mix demonstrated a 28-day compressive strength of 39.2 MPa, split tensile strength of 3.4 MPa, and flexural strength of 5.7 MPa, indicating that bacterial concrete performed better than traditional concrete. Significantly, this mixture reduced water absorption by 30.8%, sorptivity by 25%, acid resistance by 40%, and chloride permeability by 43.75%. These improvements are ascribed to microbial-induced calcite precipitation, which helps close microcracks, and the pozzolanic activity of metakaolin, which refines the concrete's pore structure. All things considered, this study is in favor of using bacterial concrete as a long-lasting and sustainable substitute for traditional concrete, especially in challenging environmental circumstances.

KEYWORDS:
Bacterial Concrete, Bacillus subtilis, Metakaolin, MICP, Mechanical Properties.

1. Introduction:

The most popular building material is concrete, which is renowned for its strength and durability. Industrial byproducts have been added as a result of research to increase its properties [1-2]. Its low ductility and vulnerability to cracking, which shortens its lifespan due to water and hazardous ion infiltration, are its disadvantages. Conventional repair techniques are expensive and time-consuming. In order to improve durability and provide a long-term solution to current problems, "Bacterial Concrete" or "Self-Healing Concrete" has been created, which uses microorganisms to spontaneously fill cracks [3-4].

By using bacteria that promote calcite precipitation through a process known as Microbiologically Induced Calcite Precipitate (MICP), self-healing concrete, provides a crack treatment method [5-6]. Water activates unreacted cement, which expands and fills the gaps, allowing minor fractures, usually between

0.05 and 0.1 mm, to self-seal after being exposed to wet and dry cycles [7]. Because these bacteria can go latent for more than 200 years and speed up the mending of concrete structures, researchers are investigating the use of acid-producing microorganisms to improve the repair process for broader fractures [8-9].

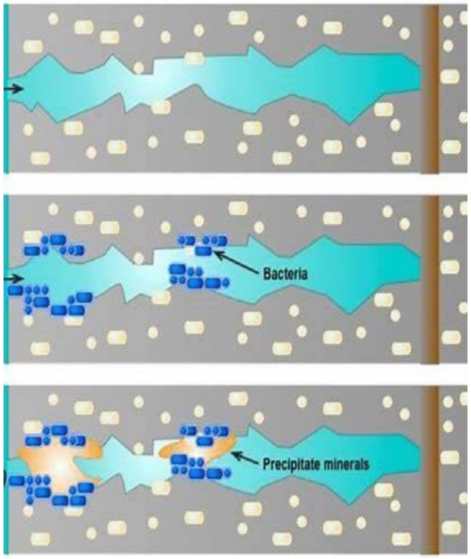

Figure 1. Bacterial concrete process

Despite being a common building material, concrete has decreased service life due to durability problems such corrosion and cracking. Conventional fixes are expensive and might not stop more deterioration. Bacterial concrete, which uses Bacillus subtilis for Microbially Induced Calcite Precipitation (MICP) to seal microcracks and increase resistance, is one possible remedy [10]. Furthermore, the microstructure of concrete is enhanced by metakaolin as an additional cementitious element; however, nothing is known about the combined impact on durability. In addition to durability tests including water absorption and permeability, this study evaluates bacterial concrete with metakaolin, concentrating on compressive, tensile, and flexural strengths [11-13]. Finding the best combinations to enhance performance under challenging situations is the goal. Reduced upkeep, longer lifespan, and improved chemical resistance are benefits of bacterial concrete. But there are drawbacks as well, such as higher building costs, difficulties with bacterial viability, and a lack of uniform testing protocols [14].

According to Chahal et al. (2012b), adding Sporosarcina pasteurii to fly ash concrete increased its strength and durability, resulting in a 22% increase in compressive strength and a fourfold decrease in water absorption because of a self-healing effect [15]. Additionally, this bacterium reduced the permeability and porosity of water. In addition to flourishing in alkaline concrete conditions, Chahal & Siddique (2013) found that S. pasteurii filled cracks by producing calcite, increasing compressive strength while reducing porosity and permeability [16]. Reddy et al. (2010) investigated Bacillus subtilis, which showed endurance in comparison to regular concrete and similarly increased compressive strength in cement mortar cubes [17]. According to Pei et al. (2013), after 28 days of curing, bacterial cell walls from B. subtilis caused a 15% improvement in compressive strength and a decrease in porosity [18]. In order to improve performance, Srivastava et al. (2012) investigated metakaolin and silica fume and suggested the ideal replacement levels in concrete [19]. In order to achieve fracture sealing and decreased permeability, Chintalapudi et al. (2016) used B. subtilis in self-healing concrete, highlighting the affordability of bio-based cement composites [20]. According to Ghosh et al. (2005), B. subtilis may efficiently precipitate calcite, resulting in a 15–25% increase in compressive strength [21]. While Wiktor and Jonkers (2011) verified that self-healing concrete could successfully close cracks up to 0.5 mm, Achal and Mukherjee (2015) discovered that the presence of bacteria enhanced hydration bonding and decreased water absorption [22-23]. According to Seifan et al. (2020), healing efficiency was strongly impacted by bacterial concentration [24]. Sabir et al. (2001) highlighted the importance of metakaolin in improving concrete strength, sulfate resistance, and efflorescence control; they suggested an 8–15% replacement rate for best outcomes [25]. According to Justice et al. (2005), improved interfacial transition zones (ITZ) in concrete with 10% metakaolin resulted in superior tensile and flexural strength [26]. According to Wang et al. (2014), metakaolin provides a denser matrix, improves durability, and decreases water absorption, all of which encourage bacterial calcite precipitation [27]. Overall, a number of studies show that bacterial activity can significantly improve the mechanical properties of concrete, and that metakaolin can further enhance these advantages through ITZ reinforcement.

The ideal bacterial cell concentration for concrete is examined in this study, with an emphasis on how various concentrations affect the material's mechanical and durability qualities. The goal of the research is to choose appropriate bacteria, like Bacillus subtilis, for bacterial concrete applications through literature review and lab testing. In order to evaluate the combined effects on strength, M25 grade mixes containing mineral admixtures such as metakaolin will be produced for both conventional and bacterial concrete. By combining different bacterial concentrations and mineral admixtures, the experiment seeks to identify the optimal ratios of bacterial concrete mixes for structural applications.

2. Materials and methods

2.1 Materials

Ordinary Portland cement (OPC) of 53 grade was sourced from the local market and consistently used throughout the investigation. A sample conforming to IS 12269:2013 was analyzed according to methodologies outlined in IS 4031: 1988 and IS 4032:1985.

The fine aggregate used in the study is locally derived river sand that has been cleaned and evaluated for specific gravity, fineness modulus, and bulk modulus in accordance with IS: 2386-1963 criteria. Crushed granite stones up to 20 mm in size are utilized as coarse aggregates and are sourced from local quarries. Clean water is required for mixing and curing of materials for bacterial concrete. Detailed results regarding the material properties are provided in Table 1.

Table 1. Material properties

S.No	Property	Result
1	Specific gravity of cement	3.15
2	Fineness	5%
3	Initial setting time	36 minutes
4	Final setting time	290 minutes
5	Consistency	35 %
6	Specific gravity of FA	2.64
7	Specific gravity of CA	2.7
8	Fineness modulus of FA	4.8
9	Fineness modulus of CA	3.45

Metakaolin fine particles which improve compressive and flexural strength as cement replacement. It also enhances durability by reducing permeability and protecting against acid, sulfate, and chloride intrusion. Additionally, through microbial induced calcite precipitation (MICP), metakaolin aids in calcium carbonate precipitation, promoting self-healing in bacterial concrete. An optimal cement replacement level of 8–15%, particularly 10%, balances strength and workability. Physical properties of metakaolin are detailed in Table 2.

Table 2 Physical properties of metakaolin

Property	Value
Color	Off-white to light grey
Particle shape	Very fine, angular
Average particle size	2 µm
Specific gravity	2.5
Specific surface area	15,000 m^2/kg

On nutrient agar slants, the pure culture of Bacillus subtilis jc3 is kept as irregular, dry, white colonies. When necessary, a single colony is added to a 200 ml nutrient broth in a conical flask and shaken at 125 rpm while being incubated at 37°C. Yeast extract, NaCl, and peptone make up the medium. After two to three days, cultures are refrigerated and subcultured every ninety days to avoid infection. By transforming organic nutrients into insoluble calcite crystals, the bacteria are used in concrete to provide a self-healing mechanism that plugs fissures. The bacteria can repair larger cracks up to 0.5mm while preserving the integrity of the concrete. They are effective for cracks up to 0.2mm.

Figure 2. Bacteria

Coarse aggregate, fine aggregate, and cementitious material are piled in an electrically powered mixer during the mixing process. Bacteria (Bacillus subtilis) are added with water after dry mixing produces a consistent hue. According to IS: 10510 1983, workability tests are carried out using the compaction factor testing apparatus as soon as concrete is mixed.

2.2 Methodology

Before being mixed in a dry state on a non-absorbent platform, cement, fine aggregate, coarse aggregate, and supplementary cementitious ingredient metakaolin are carefully weighed in accordance with the mix percentage. Saturated surface dry (SSD) conditions were maintained for each aggregate. The concrete mix was created with a compressive strength of at least 25 MPa at 28 days, per IS: 10262 (2009). The cement to water ratio was established at 0.45. To measure compressive strength, a 150 x 150 x 150 mm concrete sample was cast. To find the split tensile strength and flexural strength, respectively, concrete beams of 700 x 150 x 150 mm and cylindrical specimens with a diameter of 150 mm and a height of 300 mm are cast. Every specimen is cured in water for 28 days.

Concrete tests included a number of evaluations: 100 mm cubes were oven-dried and submerged in water to measure water absorption. Sorptivity used cylindrical specimens that were sealed for unidirectional flow and had a diameter of 100 mm and a thickness of 50 mm. Acid resistance measured weight loss and strength decline using 100 mm cubes submerged in 5% sulfuric acid. In accordance with ASTM C1202 recommendations, cylindrical specimens were also employed in the RCPT. In order to control water flow, 150 mm cubes were sealed on all but one face. To guarantee consistent testing results, all specimens were cured in water for 28 days after demolding.

Table 3: Concrete mix proportion one kg per cubic meter

S.No	Cement	Fine aggregates	Coarse aggregate	Water
1	400	650	1200	180

Table 4: Bacterial concrete sample details

S.No	Sample	Description	Cement Replacement	Bacteria (Cell Conc.)
1	M0	Control (Conventional M25)	0% Metakaolin	No bacteria
2	M1	M25 + Metakaolin	10% Metakaolin	10^6 cells/mL
3	M2	M25 + *B. subtilis*	0% Metakaolin	10^6 cells/mL
4	M3	M25 + Metakaolin + *B. subtilis*	10% Metakaolin	10^6 cells/mL
5	M4	M25 + Metakaolin	10% Metakaolin	10^7 cells/mL
6	M5	M25 + *B. subtilis*	0% Metakaolin	10^7 cells/mL
7	M6	M25 + Metakaolin + *B. subtilis*	10% Metakaolin	10^7 cells/mL

3. Results and discussion

3.1Workability

The slump cone test uses a cone with particular measurements to assess the consistency and workability of fresh concrete. The test calculates the slump, which is the vertical difference between the concrete's initial height and its slumped state following compaction. The control mix (M0) had the maximum slump value of 85 mm among the different concrete mixes tested, suggesting better workability. Because of the fineness of metakaolin and the water absorption by bacterial cells, mixes containing both metakaolin and bacteria gradually lost their workability. Notably, all combinations showed acceptable workability for casting and compaction, but somewhat decreased workability was observed at higher bacterial concentrations (10^7 cells/mL).

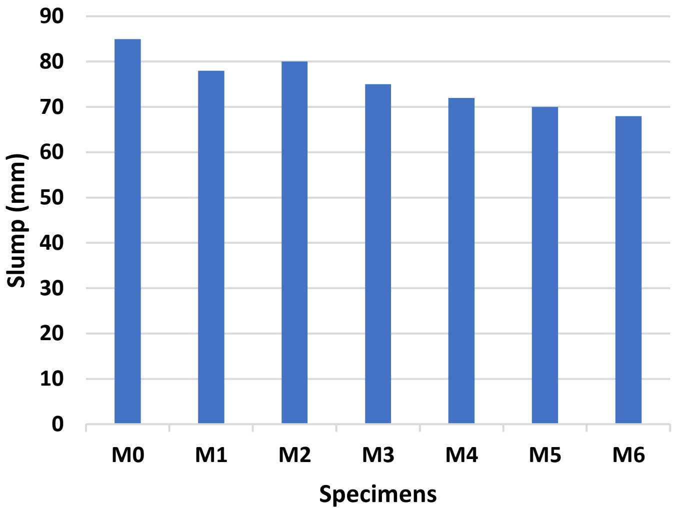

Figure 3. Slump test values

3.2 Compressive strength

For the control mix M0 (M25 grade), compressive strengths of 21.5 MPa at 7 days and 33.3 MPa at 28 days were noted. With Mix M1 (10% metakaolin) reaching 37 MPa and Mix M2 (Bacillus subtilis at 1×10^2 cells/mL) reaching 36 MPa, all adjusted combinations exceeded these values. Because of the synergistic effects of metakaolin and bacterial calcite, mixed M3 had the maximum strength (39.2 MPa). The best formulation combines 10% metakaolin with a bacterial concentration of 1×10^2 cells/mL, as seen by mix M6's peak strength of 39.6 MPa, whereas mixes M4 and M5 attained 35.3 MPa and 38.7 MPa, respectively.

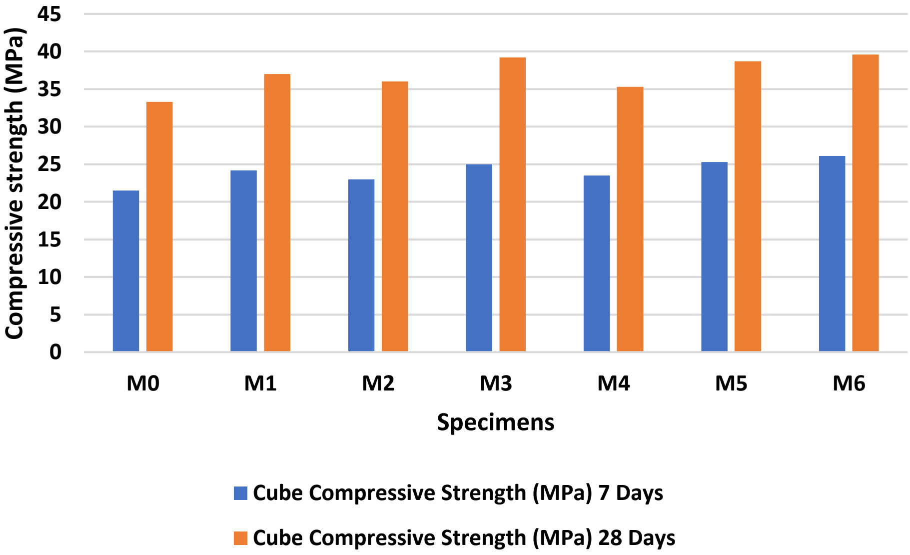

Figure 4. Compressive strength results

3.3 Strength tests

Because it is challenging to apply uniaxial tensile stresses, this study uses the split tensile strength test to assess the tensile strength of concrete. In compliance with IS 5816: 1999, cylindrical specimens measuring 150 mm in diameter and 300 mm in height were cast, cured for 7, 14, and 28 days, and then tested using a Compression Testing Machine (CTM). The test is positioning the specimen horizontally between bearing plates, increasing the load progressively until it fails, and recording the failure load.

The split tensile strength of the control mix M0 was 2.8 MPa after 28 days. Because of better densification and hydration in the interfacial transition zone (ITZ), modified mixes performed better than M0, with M1 reaching 3.1 MPa and M2 at 3.0 MPa. Higher pozzolanic concentration improves tensile resistance, as seen by M5's greatest strength of 3.3 MPa. Superior strengths of 3.4 MPa and 3.6 MPa were attained by M3 and M6, which used metakaolin and bacterial modifications to cause calcite precipitation.

Additionally, a two-point loading method is used to evaluate bending strength on prism samples that are placed above rollers to minimize torsion. Stresses are delivered gradually until breakage occurs. The analysis of failure modes and crack patterns for every specimen is noted in the study's conclusion.

The control mix M0 showed a flexural strength of 4.8 MPa after 28 days. Because of improved matrix continuity, modified mixes including metakaolin (M1, M4, M5) attained strengths between 4.9 MPa and 5.6 MPa. Microcrack healing was responsible for M2's strength of 4.9 MPa. Because of the combined impacts of metakaolin and bacterial activity on crack-bridging and stress distribution, mixes M3 and M6 showed the highest strengths, at 5.7 MPa and 5.9 MPa, respectively. M6 showed optimal suitability for high-bending strength specifications.

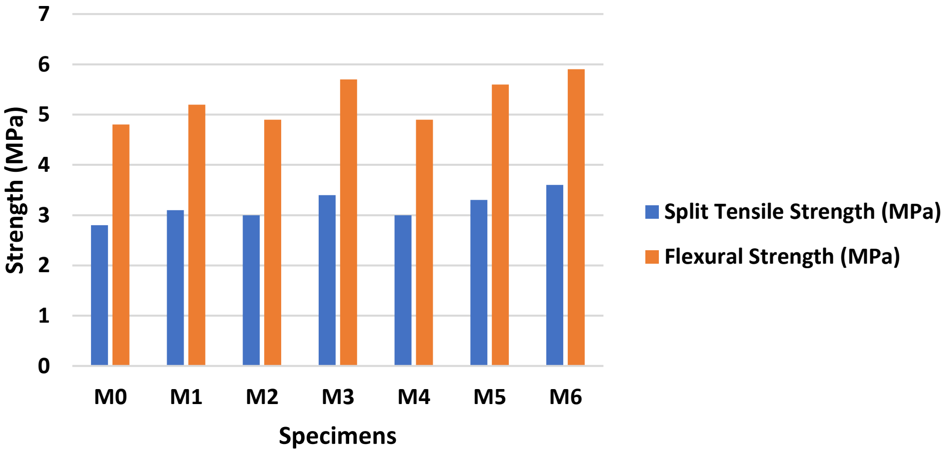

Figure 5 Split tensile strength results

3.4 Water Absorption & Sorptivity tests

By oven-drying specimens, submerging them in water, and monitoring weight changes, a water absorption test assessed the porosity and permeability of concrete. Higher percentages indicate greater porosity, while lower numbers indicate denser, more durable concrete. This evaluation, which is in line with Indian Standard IS 3085, emphasizes the potential advantages of bacterial calcite and metakaolin additions for improving the longevity and performance of concrete constructions.

Tests of water absorption show that modified concrete is more durable than the control mix (M0), which had a maximum water absorption of 5.2%. This was lowered to 4.5% with the addition of metakaolin (M1) and 4.7% with microbiological concrete (M2). Even greater results were obtained from mixed mixes, with M6 showing the lowest absorption at 3.6%, representing a 30.8% reduction, and M3 at 3.9%, representing a 25% reduction. In comparison to M3 (10^2 cells/mL), higher bacterial densities, as observed in M6 (10^2 cells/mL), further reduced absorption by 7.7%. Together, our findings support the development of a solid, impermeable concrete matrix that improves durability as water absorption drops from M0 to M6.

By measuring the water's capillary suction in concrete, sorptivity is a crucial metric for evaluating the longevity of structures exposed to moisture. In one experiment, concrete specimens were partially submerged, and water absorption was monitored over time. Pore connection is reflected in initial absorption rates; stronger durability and resistance to water penetration are shown by lower sorptivity values. The cumulative water absorption per unit area over time is used to calculate the sorptivity coefficient.

Capillary water absorption is significantly reduced in modified concrete mixtures; the control mix (M0) has a sorptivity value of 0.120 mm/min½. Sorptivity is reduced by 12.5% to 0.105 mm/min^3 with metakaolin (M1) and 8.3% to 0.110 mm/min½ with bacterial concrete (M2). Even larger reductions were seen in blended mixtures, with Mix M6 at 0.090 mm/min½ (25% reduction) and Mix M3 at 0.095 mm/min½ (20.8% reduction). The sorptivity of M6 is 5.3% lower than that of M3 due to increased bacterial activity, which improves pore blockage and shows better resistance to moisture and hostile ions.

Mix	**Water Absorption (%)**	**Sorptivity (mm/min½)**
M0	5.2	0.120
M1	4.5	0.105
M2	4.7	0.110
M3	3.9	0.095
M4	4.3	0.100
M5	4.1	0.098
M6	3.6	0.090

Table 5 Water Absorption & sorptivity values

3.5 Acid resistance test

Concrete cubes were immersed in a 5% sulfuric acid solution for 28 days in order to assess their acid resistance and replicate circumstances found in industrial and marine settings. To maintain a constant concentration, the submerged specimens were swirled in the acid after 28 days of cure. In order to evaluate weight loss and durability using residual compressive strength, the concrete was dried, weighed, and rinsed after exposure. The findings showed that specimens with higher strength and less weight loss had better acid resistance, underscoring the significance of assessing concrete's performance in corrosive environments to improve its durability.

Significant improvements in durability against chemical exposure, especially acid resistance, are revealed by the investigation on treated concrete. Poor acid resistance was demonstrated by the control mix (M0), which lost 6.5% of its weight. The addition of metakaolin (M1) further decreased weight loss to 5.2% (20% improvement), although bacterial concrete (M2) performed better with a 5.5% weight loss (15.4% improvement). The blended mixes performed better; Mix M3 had a weight reduction of 4.3% (33.8% improvement) and Mix M6 had the lowest weight loss of 3.9% (40% improvement). In terms of resistance, M6 outperformed M3 by 9.3%, demonstrating the advantages of increased calcite deposition and decreased calcium hydroxide concentration, both of which promote acid resistance.

Table 6 Acid resistance test results

Mix	Weight Loss (%)
M0	6.5
M1	5.2
M2	5.5
M3	4.3
M4	4.8
M5	4.5
M6	3.9

3.6 RCPT test

The Rapid Chloride Permeability Test (RCPT), which is essential for assessing corrosion hazards in structural reinforcement, evaluated concrete's permeability to chloride ions in compliance with ASTM C1202. Concrete examples measuring 200 mm in height and 100 mm in diameter were made, hardened for 28 days, and then cut into discs that were 50 mm thick. They were vacuum-saturated and submerged in de-aerated water after having their sides coated with epoxy to limit ion mobility. Saturated specimens were put in cells with 0.3N sodium hydroxide and 3% sodium chloride solutions during the test, and current was measured for six hours using a 60V DC voltage. Permeability was determined by the ensuing charge, which was measured in coulombs; higher durability was indicated by lower charges. The findings emphasized how bacterial calcite precipitation and metakaolin reduce pore connectivity.

According on RCPT data, changed concrete mixes' chloride ion permeability has significantly lowered. Charge passed was 3200 coulombs for the control mix (M0), 2600 coulombs (18.75%) for metakaolin (M1), and 2800 coulombs (12.5%) for bacterial concrete (M2). Even more benefits were shown in the mixed mixes; Mix M6 had the lowest charge of 1800 coulombs, which was 43.75% less than the control. Mix M3 had a reduction of 34.4%, with 2100 coulombs. Additionally, M6 exhibited 14.3% lower permeability than M3, highlighting how higher bacterial concentrations can improve resistance to corrosion caused by chloride by lowering ion transport and pore connections.

Table 7 RCPT test results

Mix	Charge Passed (Coulombs)	Permeability level
M0	3200	Moderate
M1	2600	Moderate
M2	2800	Moderate
M3	2100	Low
M4	2400	Moderate
M5	2300	Moderate
M6	1800	Low

3.7 Water permeability test

Concrete's ability to withstand water seepage is measured by a water permeability test, which is essential for the durability of underground and water-retaining constructions. After 28 days of curing, concrete samples (150 mm³) are covered on all but one side to permit unidirectional water flow. The specimens are examined for water penetration depth after being subjected to 5 kg/cm² pressure for 72 hours. A denser microstructure and improved resistance to water infiltration are indicated by a lower penetration depth.

The results show that because bacterial concrete has less water permeability, it is more durable. Water penetration was maximum in the control mix (M0) and decreased by 12–18% in the bacterial concrete (M2). Penetration was further reduced by 15–20% when metakaolin (M1) was added. Significant reductions of 30–45% were achieved by combined mixes (M3 and M6), with M6 exhibiting the lowest penetration depth, indicating its greater impermeability. The combination of bacterial calcite and metakaolin is responsible for this improved performance, which makes the concrete suited for maritime and water-retaining structures by improving pore structure, obstructing capillary channels, and repairing microcracks. M6's improved durability is confirmed by its low permeability classification.

Table 8 Water permeability test results

S. No	Mix ID	Depth of Penetration (mm)	Quality
1	M0	75	Moderate
2	M1	60	Good
3	M2	65	Moderate
4	M3	52	Good
5	M4	58	Good
6	M5	55	Good
7	M6	42	Excellent

4 Conclusions

When compared to conventional M25 concrete, the experimental investigation of bacterial concrete with metakaolin showed significant improvements in mechanical qualities. The highest mechanical strengths were obtained when 10% metakaolin and Bacillus subtilis bacteria were combined; the compressive strength was 39.6 MPa, the split tensile strength was 3.6 MPa, and the flexural strength was 5.9 MPa. Because of its mechanical capabilities and self-healing qualities, this synergy improves the concrete's performance and highlights the potential of bacterial concrete as a sustainable building material. Durability testing showed a 43.75% decrease in chloride permeability, an improvement in acid resistance, and a 30.8% reduction in water absorption. To further improve the durability, the study suggests future research in long-term performance, microstructural analysis, alternative bacterial strains, carrier material optimization, sustainability evaluations, and advanced materials integration.

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Venkatesh Kondamuru et.al, Synergistic Effect Of Metakaolin And Bacillus Subtilis On Strength And Durability Of Concrete, Excel International Journal of Technology, Engineering and Management, 2026; Volume -13, Issue-2_Page_142-152. DOI Link: https://doi.org/10.64971/j.cph.eijtem.v13.i2.17.2026

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