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An Experimental Study on the Strength Development of Microbial Concrete Containing Bacillus Subtilis

F Akter[1*], M S Islam[2], M H Apu[3], M Likuzzaman[3], M M Sarker[3], M M Mazumder[3]

[1]Department of Civil Engineering, Faculty, Stamford University Bangladesh,1217, Dhaka, Bangladesh
[2]Department of Civil Engineering, Faculty, East West University, 1212, Dhaka, Bangladesh
[3]Department of Civil Engineering, Stamford University Bangladesh, 1217, Dhaka, Bangladesh

Abstract: Bacterial or self-healing concrete employs specific bacterial strains to improve various performance characteristics, including mechanical strength, durability, and overall service behavior. These bacteria are spore-forming gram-positive microorganisms capable of consuming calcium lactate available in the concrete matrix. These microorganisms are capable of producing calcium carbonate (calcite), which naturally seals cracks and voids over time, thereby enhancing the concrete's mechanical properties. The present study focuses on how varying amounts of Bacillus subtilis affect the development of compressive strength in microbial concrete. For this purpose, a total of 36 cylindrical concrete specimens (100 mm × 200 mm) were prepared using a cement: sand: coarse aggregate ratio of 1:1.5:3 and a constant water-cement ratio of 0.45. The bacterial solution was prepared at a concentration of approximately 107 CFU/mL and added to the mix in quantities of 30 mL, 45 mL, and 60 mL per liter of mixing water. Compressive strength tests were carried out after 7, 14, and 28 days of curing. Additionally, load-displacement behavior and failure modes were analyzed. The findings confirmed that adding bacteria considerably enhanced the 28-day compressive strength of the concrete. Compared to the control concrete mix without bacteria, the strength increased by 47%, 63%, and 48% for specimens containing 30 mL, 45 mL, and 60 mL per liter of mixing water, respectively. In addition, the incorporation of bacteria improved the modulus of elasticity of the concrete. Among the three concentrations, 45 mL per liter of mixing water was found to be the optimal dosage for maximizing strength development.

Keywords: *Microbial Concrete; Bacillus Subtilis; Compressive Strength; Self- Healing Concrete; Failure Characteristics*

Introduction: Concrete is one of the most widely utilized construction materials due to its high compressive capacity, affordability, and ease of production. Despite these advantages, it is inherently brittle and susceptible to cracking under different loading and environmental conditions. Cracks may form because of factors such as freeze–thaw cycles, autogenous shrinkage, and applied mechanical or tensile stresses. Although microcracks alone may not immediately compromise the structural integrity of concrete, they can promote the ingress of water, chemicals, and other harmful agents such as chlorides and sulfates, eventually resulting in deterioration of the cement matrix and corrosion of embedded steel reinforcement [1]. The development of cracks ultimately reduces the material's mechanical strength and long-term durability, posing a threat to structural safety.

Researchers across the world have proposed numerous approaches to minimize the inherent limitations of conventional cement concrete [2]. Traditional surface repair techniques typically rely on organic polymer-based materials such as epoxy, silicone, acrylic, and polyurethane. Although these materials can fill microcracks, they often contain harmful chemicals and present several limitations that reduce their long-term effectiveness. Over the past few decades, the concept of self-healing concrete has gained considerable attention as a sustainable alternative capable of autonomously repairing microcracks in the structure [3, 4].

Among the different self-healing strategies, microbially induced calcium carbonate precipitation (MICP) has demonstrated exceptional promise. This process utilizes bacterial metabolic activity to precipitate calcium carbonate within cracks to seal them effectively. Spore-forming and gram-positive bacteria such as Bacillus Subtilis, Sporosarcina Ureae and Bacillus Pasteurii are commonly used in this regard. Because they have high alkali resistance and ability to survive within the harsh concrete environment. When moisture penetrates through cracks, dormant bacterial spores in the concrete become active and metabolize calcium-based nutrient such as calcium lactate. Thus, primary biomineralization process is initiated. During this process, the urease enzyme breaks down urea to produce ammonia and carbonate ions. The carbonate subsequently reacts with calcium ions to form calcium carbonate ($CaCO_3$) crystals, which deposit either directly at the crack location or within nearby regions of the concrete [5]. The secondary carbonation reaction also occurs naturally in all cement. The combined reactions are as follows:

For effective sustainable forest preservation and management initiatives, a data-driven, spatiotemporal assessment of the alterations in Sal Forest cover is essential [24], [25]. Some studies have used different models to estimate carbon sequestration.

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Corresponding author details: Farjana Akter
E-mail address: farjanaakter@stamforduniversity.edu.bd
Tel: +8801757291985

These models include the Artificial Intelligence (AI) for Ecosystem Services (ARIES) model [26], [27], the Social Values for Ecosystem Services (SoLVES) model [28], and the Integrated Valuation of Ecosystem Services and Tradeoffs (InVEST)

A. Primary biomineralization:

$CO(NH_2)_2 + 2H_2O \rightarrow 2NH_4^+ + CO_3^{2-}$

$Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3\downarrow$

B. Secondary carbonation:

$CaO + H_2O \rightarrow Ca(OH)_2$

$Ca(OH)_2 + CO_2 \rightarrow CaCO_3\downarrow + H_2O$

The precipitated $CaCO_3$ (calcite) acts as a natural binding and sealing agent in the cracked concrete. It contributes to reduce the permeability and improve both compressive strength and durability of the concrete matrix.

Research investigations conducted in recent years have proven that microbial mineral precipitation, which is produced by the metabolic activities of beneficial bacteria, can effectively improve the overall behavior of concrete [6–9]. Some earlier studies demonstrated that cracks are healed successfully by using the Bacillus species in self-healing concrete [5,10]. Later, few researches enhanced knowledge that incorporating bacterial spores increased compressive strength by 15–25% and reduced water absorption by approximately 20%–35% compared to the control mix [11]. More recently, several studies demonstrated that appropriate volume of bacterial concentration, temperature and MICP lead to denser microstructures in the cracks [12–15]. Thus, it can improved compressive strength even up to 70%, greater resistance to chloride by approximately 20%–40% and enhanced long-term strength retention in concrete [16]. Furthermore, recent literature emphasizes that in-depth knowledge regarding the microbial concrete behavior requires the evaluation of durability, microstructure, and statistical correlations in addition to strength. Some studies have revealed that bacterial inclusion significantly decreases water absorption and chloride penetration by 30%–50% [17,18]. On the other hand, SEM and XRD analyses confirmed that formation of dense calcite crystals (2–10 μm in size) bridging the crack surfaces [19,20]. Some advanced research also integrates machine learning approaches to predict compressive strength (achieving prediction accuracies above 90%) and durability based on bacterial dosage (10^3–10^8 CFU/mL) and curing conditions (moisture, temperature [16,21]. Despite these advances, comprehensive experimental investigations remain limited till date. In particular, there is insufficient quantitative analysis of the relationship between bacterial dosage, curing age, and mechanical performance for Bacillus subtilis-based concrete systems for maximum performance [12].

This research explores how different quantities of *Bacillus subtilis* influence the mechanical response of concrete, particularly its compressive capacity and load–displacement performance. The bacterial cultures used in the work were grown under controlled laboratory conditions before being introduced into the concrete at three volume levels—30 mL, 45 mL, and 60 mL per liter of mixing water. After incorporating the microbial suspension into the fresh mix, cylindrical specimens were fabricated and subjected to curing for 3, 7, and 28 days. This study aims to identify the optimal bacterial dosage that maximizes the compressive strength in concrete.

Materials

Bacteria: *For* the experimental program, *Bacillus subtilis* was chosen as the microbial agent. This bacterium, which falls under the gram-positive category of the Bacillus genus, is known for its ability to form robust endospores. These spores enable the organism to persist for long periods in unfavorable environments where nutrients are scarce. B. subtilis is commonly found in surface soil layers and is also recognized as a harmless organism that can coexist within the human body. The bacterial strain was collected from the Department of Microbiology, Stamford University Bangladesh. The composition of the growth medium prepared for *Bacillus subtilis* in this investigation is presented in Table 1.

Table 1. Medium for the cultivation.

Bacteria Name	Materials of Culture Medium	Amount of Materials	Temperature & Conditions
Bacillus subtilis	Peptone	5 gm/L	-
	Beef Extract	3 gm/L	-
	Sodium Chloride (NaCl)	5 gm/L	-
	Distilled Water	1 L	-
	Sterilization of Nutrient Broth	-	121°C, 15 psi, 15 minutes (autoclave)
	Incubation of broth culture	-	37°C for 7 days
	Shaker incubation	-	37°C, 150–200 rpm, 24 hours

Cement: A locally manufactured Portland Composite Cement (PCC) was selected for the experimental work and sourced directly from the nearby market. The cement appeared as a consistent grey powder with a slight greenish tint and showed no signs of lump formation or physical defects. Its mechanical and physical characteristics are presented in Table 2. All examinations of the cement properties were performed following the relevant ASTM guidelines.

Fine and Course Aggregates: In concrete, fine aggregates occupy the voids within the paste and help create a compact internal structure. For this study, Sylhet sand of a relatively coarse grade was selected, with particles small enough to pass through a 4.75 mm sieve. The characteristics of this sand are summarized in Table 3, and all evaluations were conducted in accordance with

ASTM specifications. Coarse aggregates, which make up the majority of the concrete volume, contribute significantly to the material's overall strength and abrasion resistance. Locally sourced stone chips with a maximum size of 20 mm were utilized for this purpose. Their physical properties are also presented in Table 3. All associated laboratory tests followed the relevant ASTM procedures [26–28].

Table 2. Physical and Mechanical Properties of Cement.

Material	Test Name	Results	Test Name	Results
Cement	Normal Consistency (ASTM C187-16) [22]	27.0 (%)	Mortar Compressive Strength: (ASTM C109) [24]	3 days: 2650 (psi) 7 days: 3240 (psi) 28 days: 4350 (psi)
	Initial setting Time Final Setting Time (ASTM C191-13) [23]	124.0 (min) 239 (min)		
	Specific Gravity (ASTM C 188-15) [25]	3.04		

Table 3. Properties of Fine and Coarse Aggregates.

Sl No.	Test Name	Fine Aggregate	Coarse Aggregate
1.	Specific Gravity (Fine: ASTM C128, Course: ASTM C127) [26,27]	2.60	2.90
2.	Absorption Capacity (%) [26,27]	0.81	2.14
3.	Fineness Modulus (ASTM C136) [28]	2.85	7.15

Water: Clean tap water, verified to be suitable for construction use, served as both the mixing medium and the curing agent for all specimens. The water met the necessary quality criteria for concrete preparation. For consistency across all batches, the proportion of water to cement was fixed at 0.45 throughout the study.

Methods

Cultivation of Bacteria: Bacillus subtilis was propagated using a liquid growth medium prepared from standard nutrient-broth ingredients. Peptone [Fig. 1], beef extract, and sodium chloride were combined with distilled water to formulate the medium, as outlined in Table 1. The prepared mixture was then sterilized in an autoclave at 121 °C and 15 psi for a 15-minute cycle. Once sterilization was completed and the medium cooled, the bacterial strain was introduced into the broth under oxygen-limited conditions to begin cultivation. Air content in the sealed vials was replaced entirely with CO_2 using a syringe needle system. The inoculated vials were then incubated at 37°C for 7 days to ensure sufficient bacterial growth. Staining and microscopic observation were performed to verify the morphology and viability of the bacteria. Calcium lactate served as the nutrient source for the bacteria in this investigation. Its presence supports the formation of calcium carbonate, which is the key mineral responsible for the self-healing action in microbial concrete. The container holding the calcium lactate used in the study is presented in Fig. 2.

Preparation of Bacterial Solution: The initial step in preparing the bacterial growth medium involved introducing 12.5 g of Nutrient Broth (media) into a 500 mL conical flask filled with distilled water to promote accelerated bacterial proliferation. The flask was then sealed using a dense cotton plug, subsequently made airtight with paper and a rubber band. Sterilization was achieved by heating the solution in a cooker for approximately 10 to 20 minutes. Post-sterilization, the medium was confirmed to be contaminant-free and displayed a clear orange color before the introduction of the inoculum. Following this, the flasks were opened, and precisely 1 mL of the precultured bacterium was aseptically added to the sterilized broth. The flask was then incubated overnight in a shaker at a constant speed ranging from 150 to 200 revolutions per minute (rpm). After 24 hours of incubation, the bacterial suspension developed into a cloudy, whitish-yellow solution, as shown in Fig. 3. For the preparation of sub-colonies, a pure culture was separated from the initial suspension and transferred onto a nutrient agar plate, where it was maintained [Fig. 4]. A nutrient agar plate essentially serves as a Petri dish containing a solidified growth medium that supports the cultivation and proliferation of microorganisms [Fig. 5]. As needed, an individual colony from this preserved culture could be inoculated into 100 mL of fresh nutrient broth contained within a conical flask, with optimal growth parameters maintained at a temperature of 37°C. The comprehensive procedure for combining the prepared bacterial solution with Calcium Lactate is illustrated in Fig. 6.

To prepare the microbial admixture, the bacterial suspension was first adjusted so that the number of viable cells reached about 10^7 CFU per milliliter. Instead of adding a fixed amount, three separate doses of 30 mL, 45 mL, and 60 mL were measured for every liter of mixing water used in the concrete batch. This microbial solution was introduced directly during the mixing stage, allowing the cells to disperse uniformly throughout the fresh concrete.

Preservation and Handling of *Bacillus subtilis* Stock Cultures: To maintain the bacterial source used in this study, *Bacillus subtilis* was first grown on a solid nutrient medium prepared in slant form. A small amount of the organism was transferred onto the surface of the medium with a sterilized loop, after which the tubes were kept at 37 °C to allow the cells to multiply. Once a sufficient layer of growth had formed, the slants were placed in cold storage so the culture could remain viable for future experimental procedures.

Calcium Lactate as Nutrient: In this study, the self-healing concrete was prepared using *Bacillus subtilis* along with calcium lactate, which served as the nutrient source for bacterial activity. The Calcium Lactate is applied directly into the wet concrete mix during the preparation. This calcium lactate acts as the food source and supports the bacteria once they become active. When the concrete mass develops micro-cracks and is penetrated by water, the dormant bacteria consume the provided calcium lactate. This metabolic activity triggers a mechanism referred to as Microbially Induced Calcite Precipitation (MICP) [12]. In this process, the bacteria utilize oxygen along with the supplied nutrients, leading to the transformation of soluble calcium lactate into insoluble calcium carbonate ($CaCO_3$), commonly known as limestone. The resulting limestone solidifies within the crack network and sealing the defect effectively. Thereby, it aiding in the restoration of the material's durability and structural integrity. A crucial benefit of this technology is the long-term viability of the repair mechanism, as these bacteria are capable of remaining in a latent or dormant stage for 50 years or even longer [10]. However, in this study, calcium lactate was incorporated into the concrete mixtures at a dosage equal to 2% of the cement weight. A cultured suspension of *Bacillus subtilis*, prepared at a concentration of 1×10^7 CFU/mL, was added to the fresh concrete to evaluate its influence on the material's properties.

Concrete Mixing and Casting of Specimens: A mix proportion of 1:1.5:3 (cement : sand : coarse aggregate) by weight was adopted for preparing the concrete. The batchwise quantities of the individual materials used in the concrete mixture are provided in Table 4. Total 36 cylinders Concrete mixing was carried out using a drum-type mixing machine [Fig. 7]. Cylindrical specimens having a diameter of 4 inches and a height of 8 inches were cast in accordance with ASTM requirements [29]. Prior to mixing, both fine and coarse aggregates were maintained in a saturated surface-dry (SSD) condition. All materials were weighed accurately, and the entire mixing & Testing procedure followed the guidelines as per ASTM standards [29,30].

Fig. 1: *Peptone and Beef Extract.*

Fig. 2: Calcium Lactate.

Fig. 3: *Stock culture of Bacillus subtilis in the sterilized flask.*

Fig. 4: Addition of Bacterium Solution on nutrient agar plate.

Fig. 5: *Colony of Bacillus subtilis.*

Fig. 6: *Preparation of Bacterial Solution with Calcium Lactate.*

A slump test [31] was performed to assess the workability of the freshly prepared concrete mix, as shown in Fig. 8. The concrete was then placed into clean, oiled steel cylindrical molds in three successive layers, ensuring proper compaction of each layer (Fig. 9). The top surface was leveled and finished with a trowel. Immediately after casting, the specimens were covered with moist burlap and allowed to rest at room temperature to complete the initial setting. After 24 hours, the samples were removed from the molds and transferred to a water-curing tank, where they remained until their designated testing age.

Table 4. Mix Proportion of Concrete Materials (per batch).

Concrete Type	W/C Ratio	Water (ml)	Cement (kg)	Fine Aggregate (kg)	Coarse Aggregate (kg)	Bacteria Solution (ml)	Calcium Lactate (gm)	Mix Ratio (by wt)
Ref.	0.45	3460	7.68	11.5	23	-	-	1:1.5:3
BC-30	0.45	3256	7.68	11.5	23	104	155	1:1.5:3
BC-45	0.45	3304	7.68	11.5	23	156	155	1:1.5:3
BC-60	0.45	3252	7.68	11.5	23	208	155	1:1.5:3

Fig. 7: Addition of prepared bacterial solution.

Fig. 8: Concrete after Mixing.

Fig. 9: Slump Test (25mm).

Results And Discussion: A Universal Testing Machine (UTM) with an automated data collection system till failure was used to test the compressive strength of every concrete specimen. The tests were performed on 36 nos concrete cylindrical specimens. Bacteria dosages of 0 ml (Reference), 30 ml, 45 ml, and 60 ml of Bacillus subtilis solution per liter of mixing water were added to the concrete specimens for each group of specimens. The concrete mix having 0ml bacteria contents represent control concrete sample which was used to compare the strength development of bacteria induced concrete specimens. Compressive strength tests were conducted after 3, 7, and 28 days of curing. For each mix group, the average value of three specimens was taken as the representative strength (Table 5).

Fig. 10 illustrates the compressive strength development of microbial concrete at various curing ages and bacterial concentrations of 30 ml, 45ml, 60ml. For comparison, strength values for control concrete and microbial concretes are shown side by side. From the graph, it is seen that the strength increases for each dose of bacteria at any curing age. However, the increase in strength is not proportional with respect to bacteria concentration. For bacteria content up to 45 ml, there is an increasing trend of strength enhancement, followed by a reduction trend from 45 ml to 60 ml of bacteria content. For example, at 28 days curing age, the strength enhancement is observed as 46.67%, 62.68%, and 48.05% for bacteria contents of 30 ml, 45 ml, and 60 ml, respectively. A maximum strength enhancement of around 63% is achieved at 45 ml bacteria concentration. The compressive strength enhancement up to 70% is reported for similar studies of Microbial concrete [16]. The observed decrease in compressive strength beyond the optimum bacterial concentration may be attributed to the excessive bacterial biomass and non-uniform $CaCO_3$ precipitation in the concrete. It may obstruct hydration and introduce micro-voids between paste and aggregates. This occurrence may likely to weaken the interfacial transition zones and adversely affect strength development of concrete. [10,11,18]

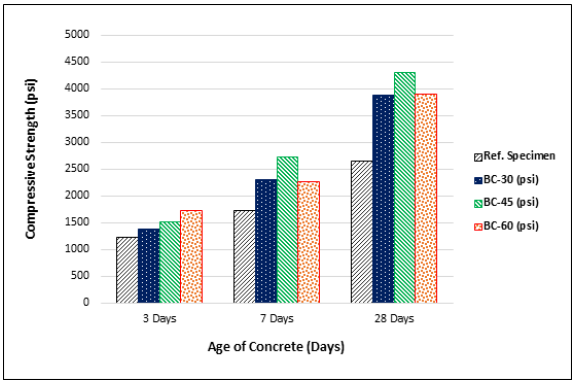
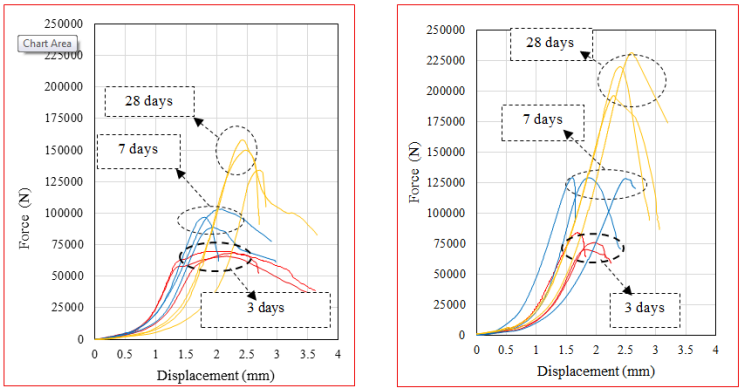
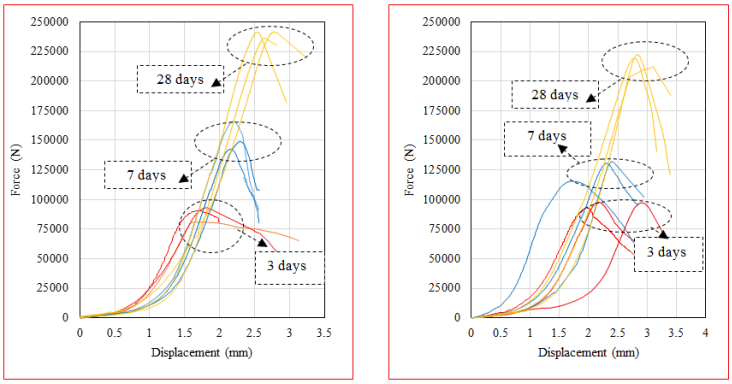


Fig. 10: Effects of concrete age on the Compressive strength of the concrete.

Fig. 11 presents the load–deformation curves for both reference and microbial indicating concrete specimens. From these graphs, it is observed that microbial concrete exhibits greater deformation at ultimate loads showing improved ductile behavior compared to the reference (control) concrete particularly for higher bacterial doses at 28 days curing ages. The calcite precipitation inside the concrete cracks is principally responsible for this improved ductile behavior, which enables higher energy absorption under compressive stress. [4,6,8,32].



(a) Reference specimens (b) BC-30



(c) BC-45 (d) BC-60

Fig. 11: Load Displacement curve of the concrete specimens (3 days, 7 days and 28 days).

During compression testing, the failure patterns of all specimens were carefully observed with the identification of the various cracks and their patterns. Fig. 12 presents the failure modes for both reference and microbial concrete specimens at 28 days of curing. It is seen that most of the failure patterns are shear-type. However, some microbial concrete specimens displayed a cone and split-type failure. It is due to improved internal bonding and crack-bridging effects induced by bacterial activity [6].

Ref *BC-30* *BC-45* *BC-60*

Fig. 12: Failure Pattern of Concrete Cylindrical Specimen at 28 days.

The modulus of elasticity (Ec) for all specimens was calculated using stress–strain data obtained during compressive testing [33]. The findings are summarized in Table 5.

Table 5 Elasticity Modulus of Microbial and Reference Concrete.

Variant	Age of Specimen	Avg. Compressive Strength, f_C (N/mm^2)	Enhancement in Δf_C (%)	Modulus of Elasticity, E_C (N/mm^2)	Enhancement in ΔE_C (%)
Reference Specimen	3 Days	8.68	-	13846	-
	7 Days	12.29	-	16478	-
	28 Days	18.76	-	20359	-
BC-30	3 Days	9.78	13	14701	6
	7 Days	16.42	34	19045	16
	28 Days	27.52	47	24656	21
BC-45	3 Days	10.80	24	15445	12
	7 Days	19.41	58	20708	26
	28 Days	30.52	63	25967	28
BC-60	3 Days	12.23	41	16434	19
	7 Days	16.04	31	18826	14
	28 Days	27.78	48	24772	22

From the Table 5, it is evident that all microbial concrete specimens exhibited higher modulus of elasticity values compared to the control concrete. This indicates better stiffness and load- resisting capacity in bacterial concrete. However, the enhancement of Ec values for microbial concrete follow the similar trend as that for compressive strength values. Furthermore, the maximum modulus of elasticity recorded was 25,967 N/mm² for the mix containing 45 mL of bacterial solution at 28 days. This represents an enhancement of approximately 28% compared with the control concrete at the same curing age. These results demonstrate that adding Bacillus subtilis improves both elasticity and compressive strength, especially at the optimum dosage of 45 ml per liter of mixing water.

Conclusions: Based on the limited number of variables studied including bacterial concentration, curing ages, and test properties the following conclusions have been derived from this experimental study:

1. For all curing periods and concentrations, adding bacteria (Bacillus subtilis) to the concrete mix significantly increased its compressive strength. The strength enhancement increased with the bacterial dosage up to 45 ml/1000ml, after which a slight decline was observed at 60 ml/1000ml.
2. Among the three bacterial concentrations, the maximum compressive strength increase of around 63% was recorded at 45 ml bacteria content as compared to the non-bacterial concrete after 28 days curing. For 30 ml and 60 ml dosages, strength gains were around 47% and 48%, respectively.
3. The incorporation of bacteria also resulted in an improvement in the modulus of elasticity, showing a trend consistent with the observed increase in compressive strength. The maximum enhancement of approximately 28% as was observed for the 45 ml/1000ml of bacterial concentration at 28 days.
4. Most of the failure patterns were of shear type, but some cone and split failures were also observed in bacterial concrete, indicating a possible improvement in ductile behaviour and internal bonding particularly for higher doses.
5. A bacterial dosage of 45 ml per Liter of mixing water is found as the optimum concentration for strength and overall performance of bacterial (microbial) concrete.

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Conflict of Interest: The author declares that no competing interests influenced the execution or outcomes of this research.

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