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Research Article

Enhancing sustainability in construction: Exploring the self-healing mechanisms of bio-concrete

Subitha Thirupathi*[,1,a], Sasikumar Palanisamy[2,b], Venkateshan Asokan[3,c]

[1]*Dept. of Civil Eng.g, Avinashilingam Institute for Home Science and Higher Education for Women, Avinashilingam University, Tamilnadu, India*
[2]*Dept.of Civil Engineering, Kumaraguru College of Technology, Anna University, Tamilnadu, India*
[3]*Dept. of Civil Eng., Sri Muthukumaran Institute of Technology, Anna University, Tamilnadu, India*

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Abstract

This study evaluates the self-healing efficiency of bio-concrete incorporating *Bacillus megaterium* (067) and *Bacillus licheniformis* (598), combined with Steel Fibre Reinforced Concrete (SFRC), for sustainable construction. Concrete specimens were prepared with bacterial concentrations of 10^3, 10^5, and 10^7 cells/ml, alongside steel fibre additions of 1%, 1.5%, and 2% by weight. Artificial cracks were introduced, and the healing performance was assessed through visual and microstructural analyses. Mechanical properties, including compressive, split tensile, and flexural strengths, were systematically tested. Results showed significant enhancements, with compressive strength improving by up to 18% and crack widths reducing by over 60% due to microbiologically induced calcite precipitation (MICP). Scanning Electron Microscopy (SEM) confirmed calcite deposition within cracks, validating bacterial involvement in self-healing. This research highlights the potential of integrating bacteria into SFRC to improve mechanical durability and self-healing, offering an innovative path toward eco-friendly and resilient construction materials.

1.Introduction

Concrete is a fundamental component in Civil Engineering due to its high compressive strength, ready availability, and adaptability to various environmental conditions. However, it is inherently weak in tensile strength, which limits its use without reinforcement [1]. Under high loads, freezing and thawing cycles, and environmental fluctuations, concrete inevitably experiences cracking, compromising structural elements' durability and lifespan [2]. Key mechanical properties of concrete include compressive strength (CS), split tensile strength (STS), flexural strength (FS), and impact resistance (IR) [3]. Despite advances in reducing greenhouse gas emissions in construction, concrete remains susceptible to cracking due to shrinkage, salt crystallization, and chemical expansion [4,5]. These cracks permit the infiltration of aggressive agents, significantly reducing concrete's water resistance and durability [6]. At the same time, chloride ions in coastal regions can accelerate steel reinforcement corrosion, further weakening structures [7,8]. self-healing concrete bacteria can perform better in corrosive environmental conditions and seawater salinity, urease producing bacteria used and the mechanism of healing process. Research has shown that Bacillus subtilis bacteria can effectively heal microcracks in concrete exposed to corrosive environments, thereby improving its durability and mechanical properties [9]. Additionally, studies on bio-concrete in marine applications have demonstrated its enhanced self-healing ability in submerged and tidal conditions, particularly under sustained loading. Furthermore, the use of

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*Corresponding author: subitha_civil@avinuty.ac.in
[a]orcid.org/0000-0002-1646-1237; [b]orcid.org/0000-0002-9674-5319; [c]orcid.org/0009-0006-8570-0023;
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bio-carriers immobilized with marine bacteria has been found to positively impact the self-healing efficiency and mechanical properties of cementitious materials, further enhancing the performance of bio-concrete in challenging environments. [10].

Concrete has a natural healing ability for micro-cracks with widths below 200 µm, aiding in its resilience [11]. Studies have shown that bacterial incorporation in concrete enhances compressive strength over time, with $CaCO_3$ precipitating to fill pores and improve concrete microstructure [12]. Fibre reinforcement, particularly polypropylene fibre, has significantly reduced crack widths by bridging gaps between fibres [13]. Research has explored enhancing fibre-reinforced concrete (FRC) durability through integrating bacteria, with Bacillus subtilis being shown to improve crack healing and nutrient sources aiding the process [14, 15]. Fibre-reinforced cement Concrete (FRCC) has also demonstrated increased energy absorption capacity and tensile strength due to the post-cracking behaviour introduced by fibre additions [16-17]. Including *Bacillus* bacteria in concrete has been associated with a 23% improvement in compressive strength and the efficient healing of micro-cracks [18,19]. Studies on polypropylene fibre have further highlighted improvements in concrete's physical and chemical properties when fibres are incorporated [20, 21]. In addition to the improvements observed with polypropylene fibers, studies on self-healing concrete incorporating natural fibers have also demonstrated notable enhancements in both physical and chemical properties. These natural fibers provide to the overall strength and durability of structures while promoting self-healing mechanisms [10,22]. The integration of such fibers in bio-concrete systems further enhances crack repair and the longevity of structures, aligning with the growing trend of sustainable materials in construction [17]. Research on these innovative approaches underscores the potential for combining natural fibers with biologically active systems to create concrete that is both environmentally friendly and capable of self-repair in response to damage [23,24].

Significant advancements in the growth of healing bacterial concrete and fibre-reinforced concrete, the simultaneous incorporation of *Bacillus megaterium* (BM) and *Bacillus licheniformis* (BL) within a Steel Fibre Reinforced Concrete (SFRC) matrix represents a novel approach in the field of sustainable construction. The existing literature primarily focuses on the use of a single microbial species or conventional polypropylene fibres, which limits the crack healing efficiency and mechanical performance of bio-concrete. This study distinguishes itself by optimizing bacterial concentrations (10^3, 10^5, and 10^7 cells/ml) alongside varying steel fibre content (1%, 1.5%, and 2%) to evaluate their synergistic impact on mechanical properties and self-healing capabilities. Furthermore, the integration of microbiologically induced calcite precipitation (MICP) with SFRC has not been extensively explored. By leveraging this unique combination, this research addresses the dual challenge of enhancing the mechanical durability and self-healing efficiency of concrete. The findings are expected to contribute to the growth of resilient and biodegradable construction materials, offering a significant step toward reducing the environmental effect of the construction production while improving structural performance and service life.

2.Materials and Methods

2.1 Cement

The Ordinary Portland Cement (OPC) of 53 grade is confirmed per the Indian code [6]. The sp. gravity of cement was 3.18. The mechanical properties of cement are illustrated in Table 1.

Table 1. Mechanical and Chemical properties of cement

Mechanical Properties	Values
Initial setting time	32 min
Final setting time	472 min
Fineness	2.77%
Consistency	32%
Sp. Gravity	3.14
Chemical Properties	%
SiO_2	22

CaO	64
Al_2O_3	5.45
Fe_2O_3	3.25
MgO	3.16
Na_2O	0.78
Loss on ignition	1.32

2.2 Fine Aggregate

River sand is the most suitable material for fine sand. The sp. Gravity of 2.69 and fineness modulus of 3.47% were used as fine aggregate. According to IS code [7], an aggregate retained on an IS 4.75mm sieve is called fine sand or aggregate as shown in (Fig. 1).

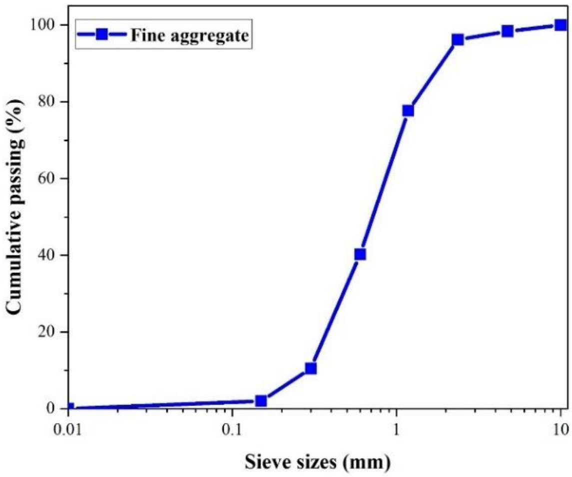


Fig. 1. Granularity diagram for fine aggregate analysis

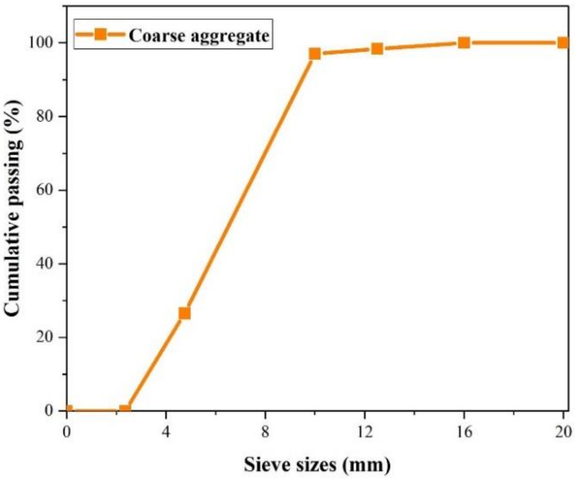


Fig. 2. Granularity diagram for coarse aggregate analysis

2.3 Coarse Aggregate

The coarse aggregate comprises granular and rough material from rocks and broken stones. In tests according to IS code [7,8], specific gravity was found to be 2.3 and water absorption at 0.68%. Granularity Diagram for Coarse Aggregate Analysis as shown in (Fig. 2).

2.4 Fly Ash

Table 2 illustrates the characteristics of fly ash, fitness, and specific gravity of 2.88 and 2.09

Table 2. Mechanical and Chemical properties of fly ash

Chemical properties	Values of fly ash %
SiO_2	46.42
Fe_2O_3	8.3
Al_2O_3	13.7
MgO	6.72
SO_3	4.45
CaO	0.16
Mechanical properties	Values for fly ash
Specific gravity	2.09
Fineness (m^2/kg)	288
Bulk density (kg/m3)	1100-1200

2.5 Silica Fume

Silica fume was purchased from the fibre regions of Chennai. The sp. gravity of silica fume was 2.63, and the properties of silica fume are illustrated in Table 3.

Table 3. Mechanical and Chemical properties of fly ash

Physical properties	Results
Physical level	Micronized powder
Odor	Odorless
Appearance	White powder form
Colour	White
The pH of 5% solution	6.9
Specific gravity	2.63
Moisture content	0.058%
Chemical properties	%
SiO_2	99.18
Al_2O_3	0.042
Fe_2O_3	0.038
TiO_2	0.001
MgO	0.000
K_2O	0.001
Na_2O	0.0022
Heavy metal Lead and Arsenic	Nil

2.6 Steel Fibre

SFRC is a composite material consisting of a concrete mix and steel fibres used as a reinforcing agent. In the concrete mix, the fibres are evenly distributed. The fibre characteristics and quantities are represented in Table 4.

Table 4. Steel fibre properties

Fibre properties	Quantity
Shape	(Deformed) Hooked end
Length	60 mm
Diameter	0.75 mm
Aspect ratio	80 (l/d)
Tensile strength	1100 N/mm^2
Unit weight	7850 kg/m^3
Elastic modulus, E	205 MPa

2.7 Chemical Admixture

Superplasticizer SP-43 was utilized in accordance with the specifications outlined in the IS codebook for concrete mix designs. This chemical admixture is designed to improve the workability of concrete without compromising its strength. It allows for the reduction of water content while maintaining or enhancing the flowability of the mix. The specific gravity of SP-43 is 1.08, indicating its density relative to water. This characteristic is important for accurately determining dosage rates and ensuring the proper balance between the admixture and other components in the mix. The correct usage of SP-43 contributes to achieving high-performance concrete with improved durability.

2.8 Concrete

This study used two natural mineral admixtures as a by-product; M40 grade concrete was used and set with a 0.3 water-cement ratio according to the Indian code [23]. Fly ash (FA) and Silica fume (SF) were used in varying proportions as a (partial replacement) for cement, such as 15% FA and 15% SF. The cultured bacteria were added to the concrete at various concentration levels, such as 10^3,10^5,10^7 cells/ml.

2.9 Bacteria Cultivation Process

Two bacterial species were obtained from a fermented mixture, and construction water samples were collected from a local construction site in Coimbatore, Tamil Nadu. A 1 ml or 1 g portion of each sample was introduced into an enriched nutrient medium and incubated at 37°C for 2 to 3 days. After sufficient bacterial growth, serial dilutions were carried out, and the cultures were transferred onto Urea agar with a pH of 9.4. The composition of the Urea agar included Urea (20 g/l), Sodium bicarbonate (2.12 g/l), NH_4Cl (10 g/l), Calcium chloride hydrate (25 g/l), and NB-Nutrient Broth (3.0 g/l). The plates were incubated for 2 to 3 days at 37°C. Following incubation, bacterial colonies showing crystal-like precipitation were identified and moved to Urea broth. Endospore staining was performed to confirm the presence of spores. The isolates were further tested for calcium carbonate precipitation by adding 2% urea and calcium chloride to the nutrient broth to maintain the ideal conditions. A 30 ml portion of this broth was inoculated with 0.6 ml of the isolated culture and incubated for 7 days at 30°C with shaking at 130 rpm. This process was repeated three times to ensure the isolates formed precipitates. The resulting calcium carbonate was filtered through Whatman filter paper, dried at 60°C for 3 hours, and weighed. Five distinct colonies were isolated using the spread plate method and sub cultured on nutrient agar slants. Nutrient agar is commonly used for growing a variety of bacteria and fungi, both in liquid and solid forms. The liquid form is referred to as Nutrient Broth, while the solidified version is called Nutrient Agar. When cooled below 45°C, the agar solidifies, providing a firm surface for bacterial growth and development. [26].

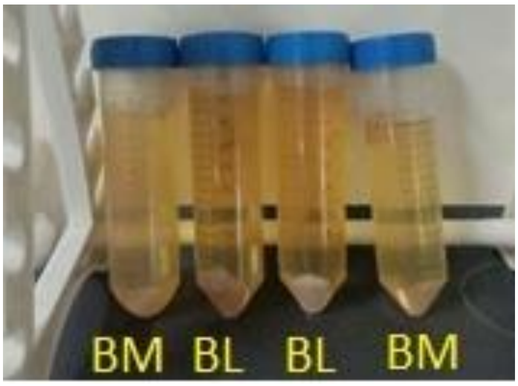


Fig. 3. Cultured *Bacillus megaterium* and *Bacillus licheniformis*

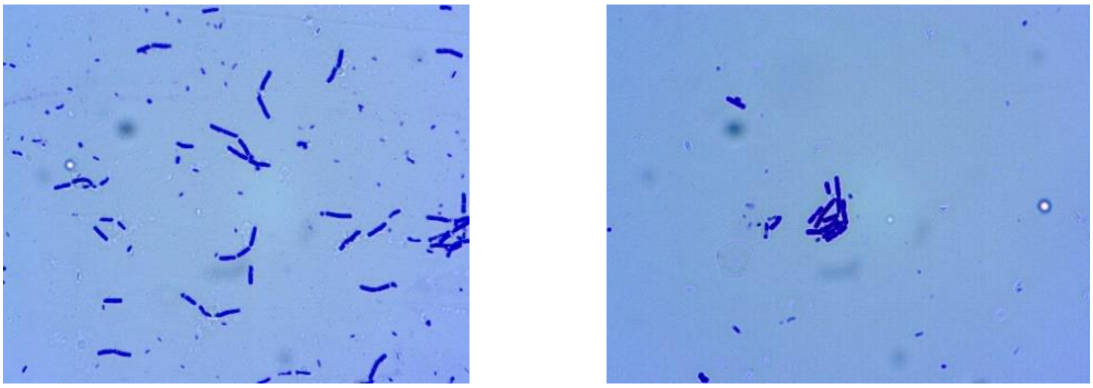


Fig. 4. Microscopic images of *Bacillus megaterium* and *Bacillus licheniformis*

3. Experimental Investigations

3.1 Proportioning of Mixes

In the trial mix design, a conventional trial mix was developed (without fibre and bacteria culture) on a trial mix to achieve a 28-day cube of 40 N/mm^2. The materials are available in the local market. The mix proportion has been determined to reach the target design strength of 48N/mm^2 without using fibre and bacteria. By weight of cement (C), admixtures (A), fine aggregate (FA), coarse aggregate (CA), fibre, and bacteria were used in 1%,1.5%, and 2%. We could observe and evaluate the mechanical behavior of bio concrete with fibre and bacteria more effectively. The mechanical behavior of bio concrete and mixes in Table 5 were used. As for the conventional mixture, 40 N/mm^2 (without fibre and bacteria) mixing procedures were carried out as per codes [24,25].

Table 5. Bio concrete mixtures for 1m^3 volume of concrete

Materials	Quantities			
	M 1	M 2	M 3	M 4
Cement (kg)	350	350	350	350
Fly ash(kg)	65	65	65	65
Silica fume(kg)	65	65	65	65
Water	160	160	160	160
Fine aggregate (kg)	720	720	720	720
Coarse aggregate (kg)	1230	1230	1230	1230
Superplasticizer	4.2	4.2	4.2	4.2
W/c	0.36	0.36	0.36	0.36
Bacterial Sample	-	-	*B. megaterium*	*B. licheniformis*

3.2 Proportions of Test Specimens

Bio concrete is characterized by its Compressive Strength (CS), Split Tensile Strength (STS), and Flexural Strength (FS), which is prepared at various ages, like 7 days, 14 days, and 28 days. CS and STS were tested on a compression testing machine (CTM), and FS was tested on the universal testing machine (UTM) to evaluate the strength properties [27]. The specimen was cast like a 150 mm X 150 mm X 150 mm cube, a 150 mm diameter X 300 mm length cylinder, and a 500 X 100 X 100mm prism. The design target strength is achieved at 28 days of 48 N/mm^2 without fibre and bacteria. Summarizing the number of specimens and tests as shown in Table 6.

Table 6. Summarizing the number of specimens and tests

Mix Design	Specimen Shape	Specimen Size	Number of Specimens	Purpose
M1	Cube	150x150x150	3	Compressive Strength(CS)
M1	Cylinder	150x300	3	Split Tensile Strength(STS)
M1	Prism	100x100x500	3	Flexural Strength(FS)
M2	Cube	150x150x150	3	Compressive Strength(CS)
M2	Cylinder	150x300	3	Split Tensile Strength(STS)
M2	Prism	100x100x500	3	Flexural Strength(FS)
M3	Cube	150x150x150	3	Compressive Strength(CS)
M3	Cylinder	150x300	3	Split Tensile Strength(STS)
M3	Prism	100x100x500	3	Flexural Strength(FS)
M4	Cube	150x150x150	3	Compressive Strength(CS)
M4	Cylinder	150x300	3	Split Tensile Strength(STS)
M4	Prism	100x100x500	3	Flexural Strength(FS)

4. Results and Discussion

4.1 Compressive Strength

The results of this study indicate clear improvement in compressive strength with the incorporation of both steel fibers and bacterial additives in bio-concrete. Specifically, the compressive strength increased from 48.15 N/mm^2 (without fiber) to 52.38 N/mm^2 (with fiber), exceeding the target design strength of 48 N/mm^2. A further increase in compressive strength was observed when the fiber content was increased from 0% to 1.5%, with the 28-day strength reaching 52.90 N/mm^2. This demonstrates that steel fibers significantly contribute to improving the concrete's structural integrity. The observed 9.86% increase in compressive strength is consistent with prior research, which indicates the positive effects of steel fiber reinforcement on concrete performance as shown in (Fig. 5). The addition of bacteria, particularly *Bacillus megaterium* and *Bacillus licheniformis*, further enhanced the compressive strength from 50.94 N/mm^2 to 51.45 N/mm^2 illustrated in table 7. This suggests that the bacteria, likely due to their role in self-healing mechanisms, contributed to the improved durability and strength of the concrete. The optimum concentration of 10^5 cells/ml for the bacteria provided the best enhancement in compressive strength, which aligns with other studies that have highlighted the importance of bacterial concentration in bio-concrete.

Fig. 5. Compressive strength test

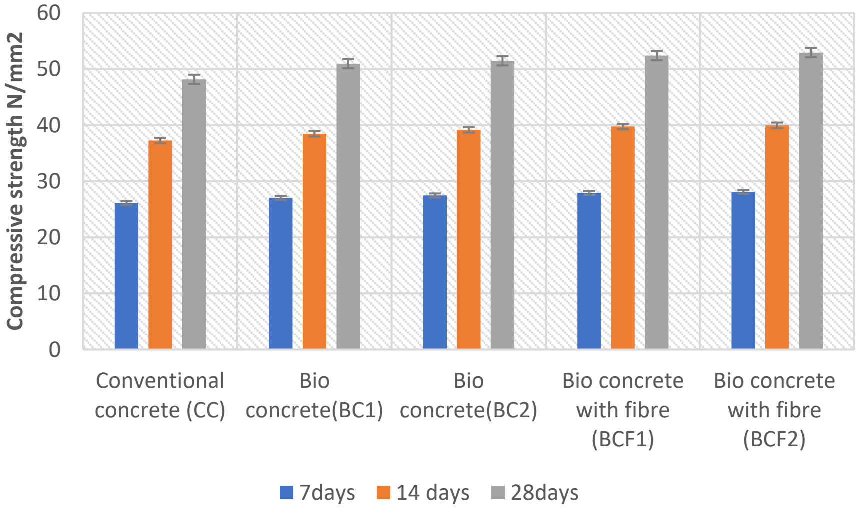


Fig. 6. Compressive strength at 7,14,28 days)

Table 7. Compressive strength result

Mix ID	% Of fibre	Average compressive strength test results in N/mm^2			% Increase over the conventional mix in 28 days
		7days	14 days	28 days	
Conventional concrete (CC)	_	26.10	37.25	48.15	-
Bio concrete (BC1) (*B. megaterium*) (10^5)	_	27.00	38.45	50.94	5.79
Bio concrete (BC2)	_	27.45	39.15	51.45	6.85

(*B. licheniformis*) (10^5)					
Bio concrete with fibre (BCF1) (10^5)	1.00	27.92	39.74	52.38	8.78
Bio concrete with fibre (BCF2) (10^5)	1.50	28.10	39.95	52.90	9.86

The optimal fiber percentage for maximum compressive strength was found to be 1.5%, reinforcing the importance of a balanced mix of fiber and bacterial additives to achieve high-strength bio-concrete [28]. The results support the hypothesis that a synergistic effect between steel fibers and bacteria can significantly improve the mechanical properties of concrete, suggesting a promising avenue for future development of self-healing, durable concrete as shown in (Fig. 6).

4.2 Split Tensile Strength (STS)

Table 8 shows different percentages of fibre and bacteria. STS of bio concrete after 7,14, and 28 days with an increase in fibre percentage from 0 to 1.5%, the 28 days split tensile strength increased from 4.85 to 6.00 N/mm^2.When the fibre percentage 1 - 1.5 % increased, the split tensile strength increased slightly from 5.9 to 6.00 N/mm^2. When the coupled effect of fibre and bacteria was added, it increased the strength from 10.10 to 23.71%. According to (Fig.7) a fibre percentage of 1.5% is the best value for the combined effect of fibre and bacteria addition for split tensile strength of 6.00 N/mm^2.The addition of bacteria and fibre increased the STS from 0 to 23.71% over the conventional concrete sample, and the optimum percentage of bacteria (10^5) cells/ml, with split tensile strength, increased from 5.68 to 6 N/mm^2 of bacteria, as shown in (Fig. 8).

Fig. 7. Split tensile strength

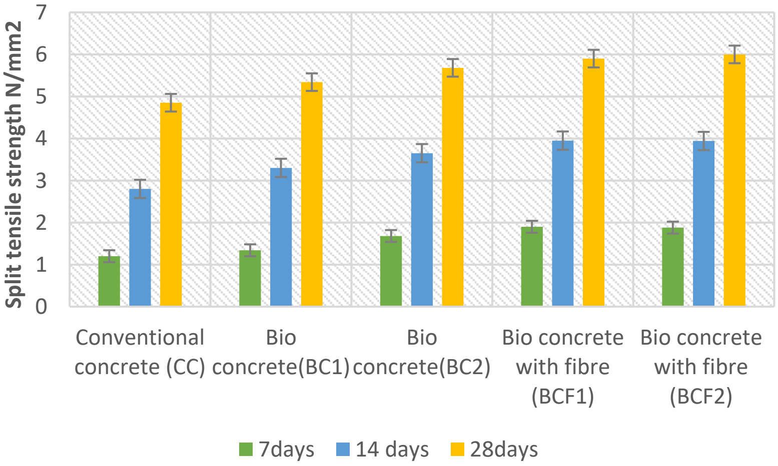


Fig. 8. Split tensile strength increased over the conventional concrete in percentage

Table 8. Split tensile strength result

Mix ID	% Of fibre	Average split tensile strength test results in N/mm^2			% Increase over the conventional mix on 28days
		7days	14 days	28days	
Conventional concrete (CC)	_	1.2	2.8	4.85	-
Bio concrete(BC1) (*B. megaterium*)(10^5)	-	1.34	3.3	5.34	10.10
Bio concrete(BC2) (*B. licheniformis*) (10^5)	_	1.68	3.65	5.68	17.11
Bio concrete with fibre (BCF1) (10^5)	1%	1.90	3.95	5.90	21.64
Bio concrete with fibre (BCF2) (10^5)	1.5%	1.88	3.94	6.00	23.71

4.3 Flexural Strength (FS)

Flexural strength after 7, 14, and 28 days with different percentages of fibre and bacteria is shown in (Fig. 9) and Table 9. Increase the fibre percentage from 0 to 1.5 % in 28 days flexural strength from 8.24 N/mm^2 to 10.80 N/mm^2. With the increase in fibre percentage from 1 to 1.5%, the flexural strength increased slightly from 10.18 to 10.80 N/mm^2. The fibre percentage of 1.5% is the optimum value of fibre addition for a flexural strength of 10.80 N/mm^2.The coupled effect of fibre and the addition of bacteria for flexural strength from 0 to 31.06% over the conventional sample fibre is 1.5%, and bacterial 10^5 cells/ml optimum concentration levels as shown in (Fig.10)

Fig. 9. Flexural strength test

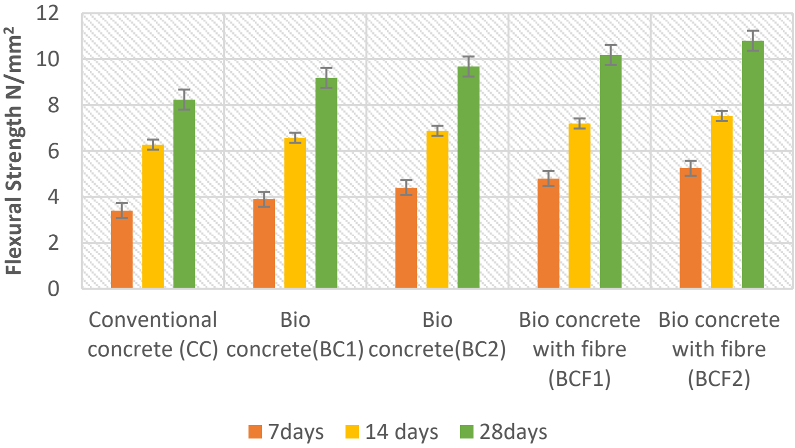


Fig. 10. Flexural strength test results at different ages (7,14, and 28 days)

Table 9. Flexural strength result

Mix ID	% Of fibre	Average split tensile strength test results in N/mm^2			% Increase over the conventional mix on 28days
		7days	14 days	28days	
Conventional concrete (CC)	_	3.4	6.28	8.24	-
Bio concrete(BC1) (*B. megaterium*)(10^5)	_	3.9	6.58	9.18	11.40
Bio concrete(BC2) (*B. licheniformis*) (10^5)	_	4.4	6.88	9.68	17.47
Bio concrete with fibre (BCF1) (10^5)	1%	4.8	7.20	10.18	23.54
Bio concrete with fibre (BCF2) (10^5)	1.5%	5.25	7.52	10.80	31.06

4.4 Healing Process

The self-healing process in concrete begins soon after cracks form, and this study indicates that within the first 7 days, the cracks start to self-repair. The primary mechanism for self-healing in this context is the precipitation of calcium carbonate ($CaCO_3$). When cracks appear on the concrete surface, certain conditions (such as moisture and nutrients) trigger the bacteria within the concrete to become active, leading to the precipitation of calcium carbonate.

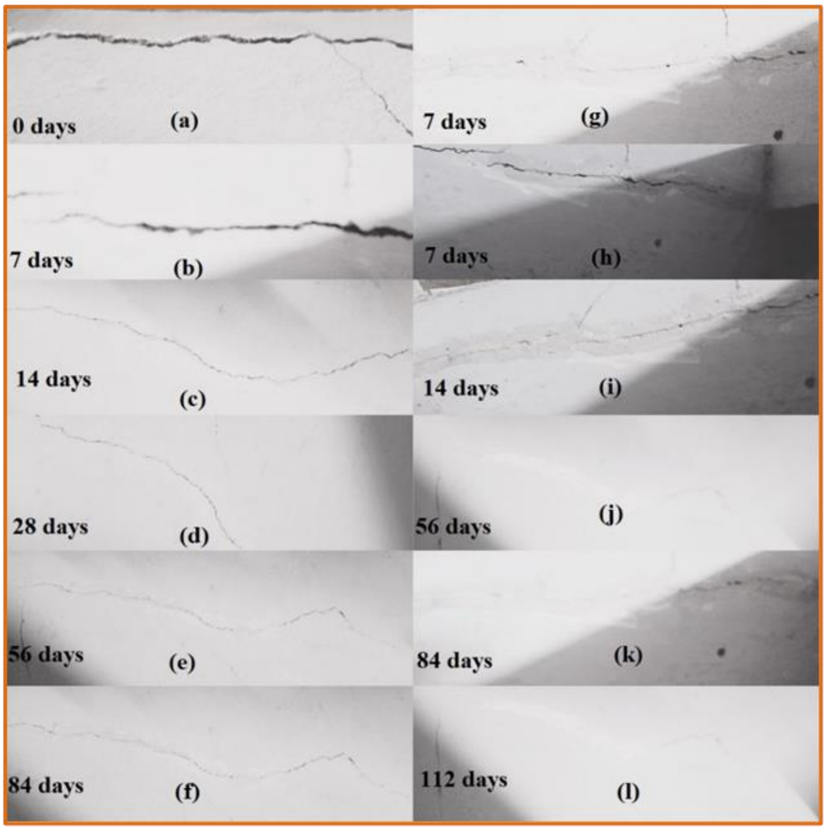
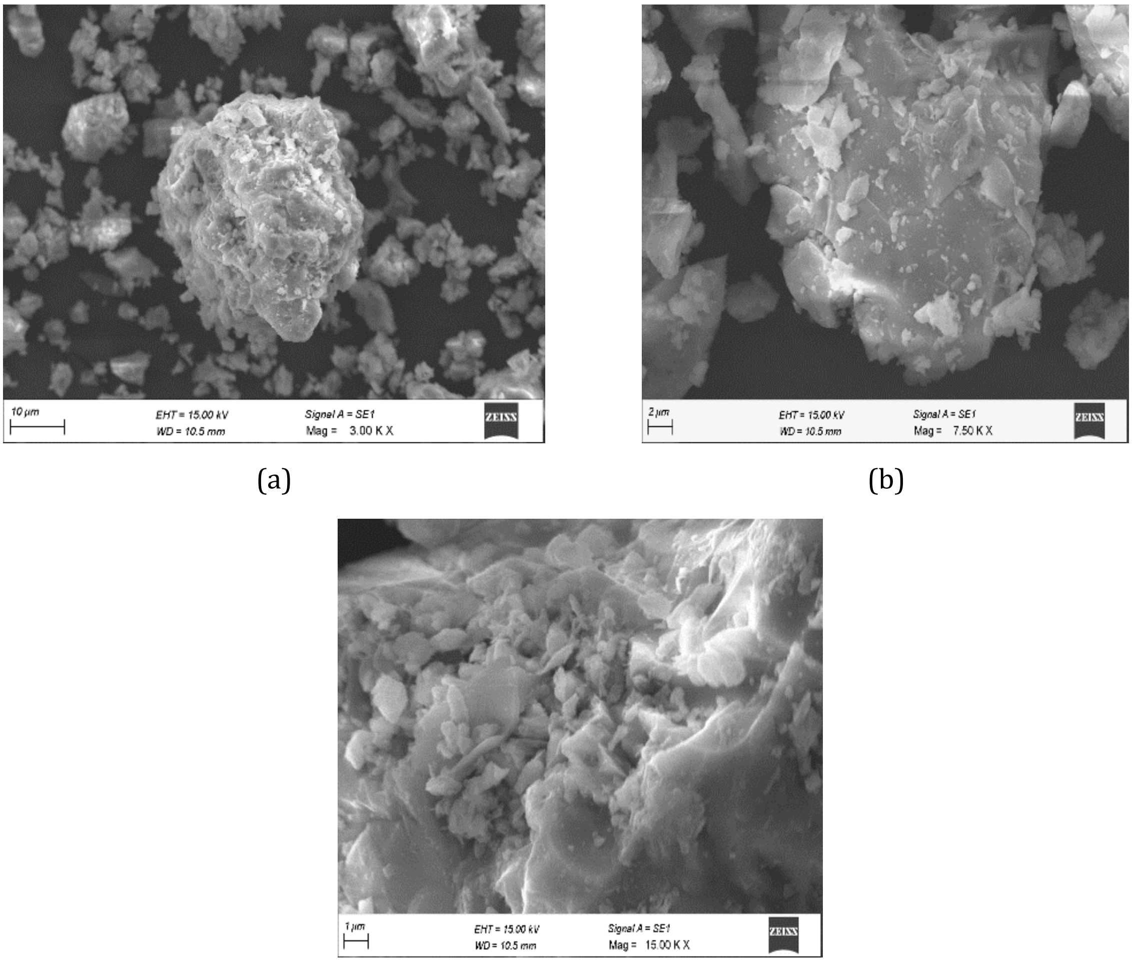


Fig. 11. Healing Process

This precipitation fills the cracks, effectively "healing" the concrete. In this case, after 112 days, the cracks in the non-bacterial concrete specimens were fully healed due to the complete deposition of calcium carbonate, as illustrated in (Fig. 11i). In the bacterial concrete specimens, however, the healing process is more gradual. After 28 days, the cracks in these specimens were only partially healed, as shown in (Fig. 11d). Over time, calcite precipitates (a form of calcium carbonate) continued to form within the cracks. By the 84-day mark, calcite was visible within the cracks of the bio concrete specimens, and by the end of 84 days, these bacterial concrete specimens had achieved complete crack healing, as seen in (Fig. 11k). This process demonstrates the effectiveness of using bacterial-based self-healing in concrete, with bacteria promoting the formation of calcium carbonate that gradually seals the cracks, enhancing the structural integrity of the concrete over time.

5. Microstructural Analysis

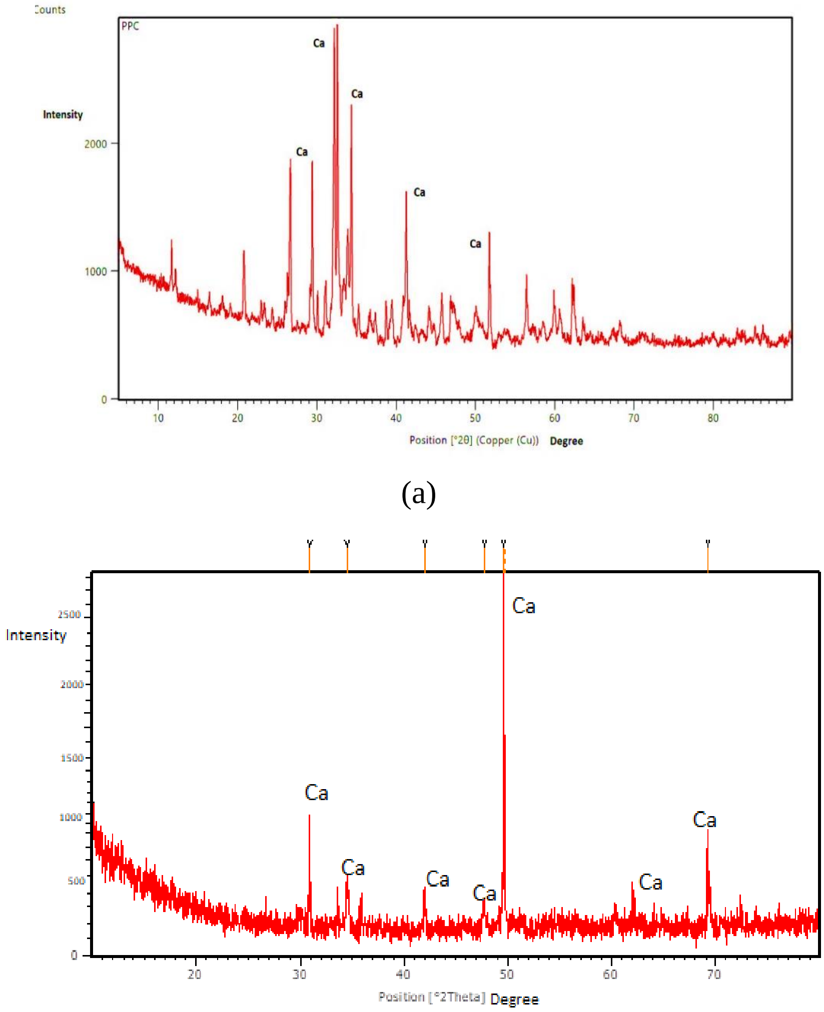
Calcite precipitation in concrete specimens was observed through the activity of *Bacillus megaterium*, *Bacillus licheniformis*, and a mixture of IS 1 and IS 2. The growth of calcite crystals, interspersed with bacterial cells, was detected in the specimens, as shown in Fig. 12b, with larger crystals visible in Fig. 12c. Bacteria were found in close contact with the calcite crystals in samples 2b and 2c. The cultures of IS 1 and IS 2 significantly enhanced the strength of the structures compared to conventional bio-concrete, as shown in Fig. 13b, and traditional concrete, as seen in Fig. 12, where no crystals were visible. This absence of calcite crystals in the traditional concrete suggests that calcite-precipitating bacteria were not present in those samples [30,31]. The XRD analysis was performed at a step size of 2θ, starting at 10° and ending at 80°. The scanning was conducted in continuous mode with a fixed divergence slit at a temperature of 25°C, using Cu Kα radiation as the source. Calcite ($CaCO_3$) was detected throughout the scan range, as illustrated in Fig. 13.



(a) (b)

(c)

Fig. 12. SEM of (a), (b) concrete specimen with B.megaterium and B. licheniformis representing the calcite crystals, (c) concrete specimen with IS 1 and IS 2 bacterial mixture representing the presence of enlarged crystals, showing higher microbially induced calcite precipitation



(a)

(b)

Fig. 13. XRD Analysis (a) Conventional concrete (b) Isolated Microbial concrete

6. Conclusions

This study establishes a correlation between the integration of bacterial strains and steel fibres in cementitious composites and the enhancement of both structural integrity and autonomous healing capabilities. The bio-concrete mix achieved compressive strengths of 49.25 N/mm^2 without fibres and 52.38 N/mm^2 with steel fibres, surpassing the targeted design strength of 48 N/mm^2 and outperforming traditional concrete. The self-healing efficiency of the bio-concrete was fully realized within 84 days, with approximately 60-70% strength recovery observed by day 28. Microstructural analyses, including XRD and SEM, long-established the presence of calcite ($CaCO_3$) in healed cracks, validating the role of microbiologically induced calcite precipitation (MICP) in crack healing. These findings demonstrate that standardized strains of *Bacillus megaterium* and *Bacillus licheniformis* significantly enhance both the mechanical properties and durability of bio-concrete. Further optimization of production parameters holds the potential to amplify these benefits, paving the way for the development of highly durable, sustainable construction materials.

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