Engineering Research Journal
Journal homepage: https://erj.journals.ekb.eg/

Enhancing Concrete Performance through Microbial Intervention: A Comprehensive Experimental Study

Osama Ahmed Ibrahim[1,*], Ahmed I Hassanin Mohamed[1, 3], Wael Ibrahim[2], Raghda Osama Abd-Al Ftah[4], Shimaa R. hamed[5], Sherif Fakhry M. Abd-Elnaby[6]

[1] Construction Engineering Department, Faculty of Engineering, Egyptian Russian University Badr City, Cairo, Egypt.
[2] Civil Engineering Department, Faculty of Engineering, Helwan University, Al- Mattaria Branch. Cairo, Egypt
[3] Civil and Environmental Engineering Department, University of Missouri, United States.
[4] Housing and Building National Research Center, Cairo, Egypt.
[5] Microbial biotechnology department- biotechnology research institute- national research Centre.
[6] Construction Engineering Department, Faculty of Engineering, Egyptian Russian University/President of the Egyptian Russian University, Badr City, Cairo-Suez Road, 11829, Cairo, Egypt.

*Corresponding Author E-mail: osama-ahmed@eru.edu.eg

Abstract. The study investigates the impact of various microorganisms on concrete properties, focusing on enhancing compressive and tensile strength through microbial activity. A total of 48 bacterial and fungal isolates were sourced from soil samples in Egypt's El-Sharkia governorate, cultured, and identified using morphological and biochemical techniques. Concrete specimens were prepared in two phases: the first involved adding six microbial samples at 3% of the cement content, while the second phase tested selected microorganisms at 5%, 10%, and 15% concentrations. The research aims to explore microbial-induced calcium carbonate precipitation (MICP) as an innovative method for enhancing concrete performance. Concrete mixes were prepared using the British "DOE" method with varying proportions of microorganisms, particularly *Bacillus subtilis* and *Aspergillus fumigatus*, to assess their effects on mechanical properties. Compressive strength was evaluated over 7, 28, and 56 days. *Bacillus subtilis*-treated samples showed a 13.9% increase in compressive strength (29.83 MPa) compared to the control (26.23 MPa), while *Aspergillus fumigatus*-treated samples exhibited a 14.3% increase (29.96 MPa). Tensile strength tests revealed improvements of 12.8% and 21.1% for *Bacillus subtilis* and *Aspergillus fumigatus* samples, respectively, compared to the control. Scanning electron microscopy (SEM) analysis confirmed that *Bacillus subtilis* significantly enhanced calcium carbonate precipitation, improving pore-filling and crack-healing properties. These findings highlight the potential of MICP in advancing concrete technology, offering a promising approach to improving concrete's durability and structural integrity.

Keywords: Bio Concrete, MICP, $CaCO_3$ precipitation, Bio Cement, Sustainability.

DOI: 10.21608/erj.2024.311680.1087
Received 11 August 2024; Received in revised form 31 August 2024; Accepted 03 September 2024
Available online 01 December 2024

1 Introduction

Reinforced concrete, making up 80% of global infrastructure, is resilient yet prone to degradation from severe weather, causing costly maintenance. Researchers are exploring innovative strategies to enhance its mechanical properties and longevity to mitigate these issues [1].

In recent years, there has been a burgeoning interest in understanding the intricate role of microbial communities within the concrete, establishing a dynamic field of inquiry bridging the microbiology and construction sectors [2].

The study underscores the profound influence of microbial populations on concrete characteristics, thriving in its pores and fissures. Microbes engage in biogeochemical reactions, altering the material's composition and behavior [3]. Remarkably, these bacteria persist and adapt in conditions often considered hostile. Their metabolic activity actively transforms concrete, influencing properties like $CaCO_3$ precipitation and pore structure [4].

One of the principal effects of microbial activity in concrete is its significant impact on compressive strength and splitting tensile strength, two essential mechanical parameters that regulate the material's performance under load. Certain microbiological species, including *Bacillus* bacteria [5] and *Aspergillus Niger* fungi [6], have been shown in studies to have a remarkable ability to initiate Biomineralization processes within the concrete matrix. These activities cause the deposition of calcium carbonate crystals, which operate as a natural filler material, efficiently bridging micro cracks and gaps within the concrete structure.

Microbial activity induces the development of calcium carbonate crystals, which is an important method for increasing compressive strength. By filling in vacancies and micro-cracks, these crystals efficiently reinforce the concrete matrix, reducing crack propagation and increasing its ability to sustain compressive stresses [7]. In addition, another study clarified microbial activity in the concrete matrix improves splitting tensile strength, which is required to resist tensile forces perpendicular to the applied load [8].

The calcium carbonate crystals formed during Biomineralization operate as bridges across micro cracks and discontinuities in the concrete, effectively inhibiting crack propagation under tensile stress conditions [9]. This mechanism results in greater crack resistance and tensile strength, which improves the overall longevity and performance of the concrete construction [3].

Microbial Induced Carbonate Precipitation (MICP) has received a lot of attention in recent years as a method for mending civil engineering infrastructures because of its ecologically favorable and low-carbon properties, which are consistent with current development trends [10].

MICP can be achieved using a variety of mechanisms, including aerobic photosynthesis, methane oxidation, the sulfur cycle, and the nitrogen cycle, among others. Among these approaches, urea breakdown is commonly utilized due to the widespread distribution and ease of cultivation of urease-producing bacteria. This approach also provides easy control over the mineralization process, resulting in excellent mineralization efficiency [11].

Bacterial and fungal species capable of creating spores can survive hostile environments, with some spores remaining viable for up to 200 years. Biomineralization in microbial concrete creates fewer toxic by-products than traditional repair methods, making it an environmentally friendly option [12]. Increasing the cementation solution concentration boosts calcium carbonate precipitation, resulting in higher sand density, reduced permeability, and increased unconfined compressive strength (UCS), with a positive link between $CaCO_3$ content and UCS [13].

Microbial self-healing concrete uses microorganisms to biologically seal cracks, offering long-term crack reduction and repair superior to chemical sealants. Research shows microbial concrete has better mechanical properties, such as higher compressive, split tensile, and flexural strength, and greater resilience to strain and crack development compared to regular concrete. However, further research is needed to address ongoing challenges [14].

This study examines self-healing concrete's mechanical properties using metaheuristic approaches and response surface methodology (RSM), focusing on sustainable construction with bio-concrete technology based on bacterial concentration. Data analysis and sophisticated algorithms create cost-effective correlations between bacteria usage and concrete performance. Metaheuristic strategies like gray wolf optimization (GWO), multiverse optimization (MVO), and particle swarm optimization (PSO) surpass RSM in prediction accuracy, with GWO excelling at forecasting concrete slump, PSO at flexural strength, and MVO at compressive strength [15].

This study explores bio-concrete production using microbially-induced calcium carbonate precipitation (MICP) as a carbon-neutral alternative, aiming to enhance compressive strength and depth. Incorporating urease-active calcium carbonate powder (UACP) and an automated injection system, we achieved a compressive strength of 52.5 MPa and a cementation depth of 140 mm. These results highlight bio-concrete's potential for prefabricated load-bearing components, offering a sustainable replacement for conventional concrete [16].

The combination of autonomous and autogenous approaches in self-healing concrete employing *B. subtilis* bacteria, fly ash mineral admixtures, and polyvinyl alcohol (PVA) fibers. Mechanical and transport qualities for concrete restoration are evaluated in an experimental program using six concrete mixtures. The results show considerable increases in compressive and flexural strength, as well as decreases in sorptivity, especially when bacteria and PVA fibers are combined. Despite issues with bacterial survival, this hybrid technique holds promise for long-term concrete repair and maintenance [17].

This study examines the effect of three bacterial species, *Sporosarcina pasteurii*, *Bacillus megaterium*, and *Bacillus subtilis*, on the self-healing characteristics of fly ash concrete, with 20% cement replacement by fly ash. Tests for ultrasonic pulse velocity, impermeability, water absorption, and compressive strength show that fly ash and bacteria improve compressive strength and reduce water absorption, with Bacillus megaterium at a concentration of 105 being particularly effective [18]. Additionally, the impact of acid mine drainage (AMD) on concrete in the Iberian Pyrite Belt reveals significant deterioration in tensile and compressive strength, increased porosity and permeability, and a reduced modulus of elasticity, suggesting the need to revise international criteria correlating these strengths for AMD-exposed concrete [19].

Bacterial concrete (BC), which uses Microbiologically Induced Calcite Precipitation (MICP), is a promising option for crack closure, aided by urease enzyme-mediated calcium carbonate deposition. This study examines the impact of *Bacillus Megaterium* (BM) and *Bacillus Subtilis* (BS) bacteria at different concentrations (10^4, 10^5, and 10^6 cells/ml) on concrete strength parameters. The results indicate that ideal concentrations of 10^4 cells/ml for BM and 10^5 cells/ml for BS can improve concrete performance and strength [20].

The development of self-healing concrete, particularly bacterial concrete using *B. Megaterium*, offers a long-lasting and efficient method to cure concrete cracks, demonstrating superior self-healing and compressive strength compared to other methods and using *Bacillus Sphaericus* [21;22]. This study shows bacterial concrete as a sustainable alternative to traditional concrete, outperforming synthetic

polymer treatments in healing and sealing fractures [23]. Microbial carbonate precipitation improves concrete behavior, and microorganisms are promising in bio-cement production, as noted by Ibrahim et al. (2023) [24]. With rising environmental concerns from cement production, bio-cement, created through microbial-induced calcium carbonate precipitation (MICP), offers an eco-friendly alternative for various applications, addressing climate change and improving concrete crack healing mechanisms [25;26].

This review examines the limited research on AAS's self-healing properties, comparing various approaches and their impacts where Alkali-activated slag (AAS) offers environmental benefits and enhanced durability compared to ordinary Portland cement (OPC) but has challenges like higher shrinkage and cracking [27].

This study explores the incorporation of *Bacillus subtilis* in concrete, revealing significant improvements in compressive and tensile strengths, with the most effective results observed at a bacterial concentration of 10^8 cells/ml, particularly at early ages [28].

This study explores the use of *Bacillus subtilis* to heal AAS cracks through calcium carbonate precipitation. Incorporating calcium oxide (CaO) has been suggested to enhance AAS properties and support bacterial activity, though its effects have been inconsistent. The research finds that adding CaO at 7% of the binder significantly influences the self-healing efficiency and engineering properties of bio-AAS, recommending further correlation between free calcium ions and microbial activity [29].

This study focuses on the autogenic self-healing technique, examining four mixes with varying percentages of polyvinyl alcohol (PVA) fiber and 20% fly ash as a cement replacement. The compressive, flexural, and tensile strengths were tested after inducing cracks and curing for 28 days. The results revealed significant recovery in compressive strength, particularly in mixes with 1.5% and 2% PVA, highlighting the potential of this technique for crack recovery [30].

2 Experimental program

The methodology used in this research will be explained in figure 1 and then presented in detail in the following parts.

2.1 Materials

A wide range of microorganisms, as well as aggregates (particularly Dolomite), regular portland cement, and tap water were carefully chosen as samples for the study.

Collection and isolation of bacteria and fungi.

The current study focused on 48 isolates from soil samples collected in Egypt's El-Sharkia governorate. Soil samples were taken from root-free patches using Johnson's method [31]. The isolates were acquired by a normal serial dilution procedure. Bacterial isolates were grown on medium (g/l) containing glucose (20.0), $CaCO_3$ (1.0), NH_4NO_3 (0.8), K_2HPO_4 (0.6), KH_2PO_4 (0.05), $MgSO_4$.7H2O (0.05), $MnSO_4.4H_2O$ (0.1), and yeast extract (0.1). Plates were then incubated at 37ºC for 24 hours [32;33].The dilution-plate approach was used to identify soil fungus. Fungal isolates were cultured in a Czapek-Dox medium and incubated at 28ºC for 7 days [33]. Additionally, the current study used Czapek's agar to identify fungal isolates. Rose-Bengal and chloramphenicol were added to the medium to prevent bacterial growth [34]. Media were autoclaved at 121ºC for 15 minutes.

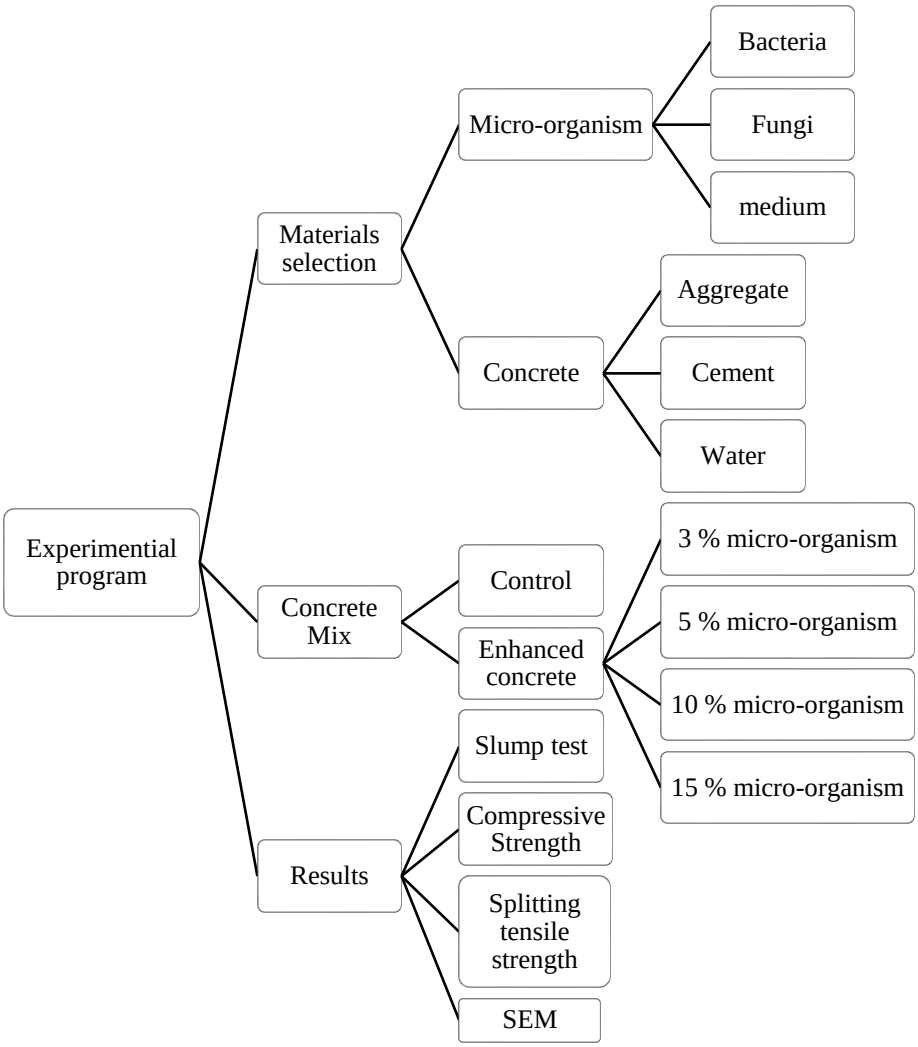


Fig.1. Overview of the experimental program conducted in this study.

Production media of bacteria and fungi.

Each bacterial strain was grown on a specific medium (peptone 4.0 g/l, yeast extract 2.0 g/l, and sucrose 20.0 g/l) [35] and inoculated with 2 ml of 24-hour-old cultures. The cultures were cultured at 37°C under static conditions for three days, while the fungal isolate was grown on a synthetic medium (malt, yeast, glucose, and peptone medium; MYGP) [37]6]. The experimental cultures were grown in 250 ml Erlenmeyer flasks, each containing 50 ml of synthetic media, and inoculated with 2 ml of 7-10 day-old cultures. For 7 days, fungal cultures were incubated at 28 °C under static conditions. Bacterial and fungal isolate cultures were combined and cultured under the same conditions.

Microbial identification

The higher producer fungi were identified using morphological characteristics and microscopic features examined by an optical light microscope (10×90) Olympus CH40 according to the following references: Ainsworth [38] as a dictionary of fungi, and Klich and Pitt [38] for *Aspergillus* species. Bacterial cells were stained with Gram stain using the procedure given by Shaffer and Goldin [39]. After staining, the morphology of bacterial cells, including shape and staining features, was evaluated using an optical light microscope (10×90, Olympus CH40).

Based on biochemical tests The pure isolated strain was identified to the genus level using Sneath's procedures [40] given in Bergey's Manual of Systematic Bacteriology as shown in Table 1 presents a detailed description of the microorganism samples utilized in our research study.

Table 1. Description of microorganism samples.

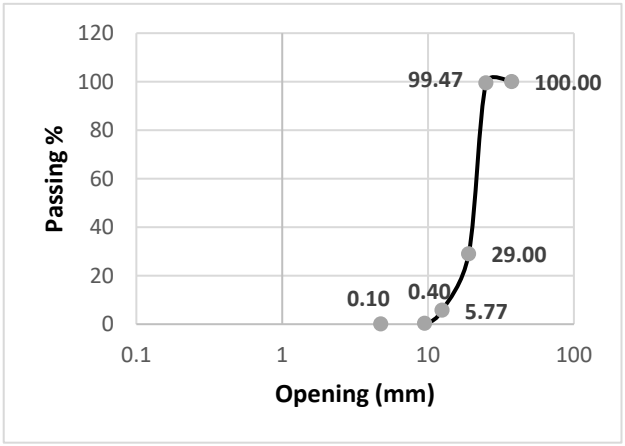
#	Micro-organism	Scientific name	Description
1	B9	*Bacillus subtilis*	Pure liquid sample from bacteria
2	F9	*Aspergillus fumigatus*	Pure fungal liquid sample
3	B1 +9	*Aspergillus fumigatus + Bacillus azotoformans*	Mixed liquid sample form fungi and bacteria
4	B1	*Bacillus azotoformans*	Pure liquid sample from bacteria
5	14	*Penicillium sp.*	Pure fungal liquid sample
6	3+14	*Penicillium sp. + Bacillus sp.*	Mixed liquid samples from fungi and bacteria

Coarse and fine aggregate.

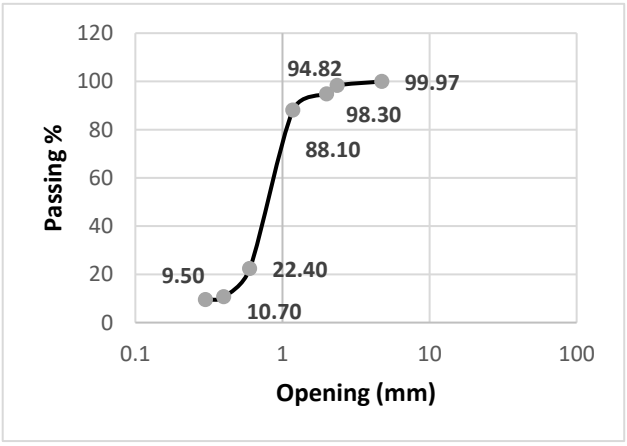
The natural sand was used as fine aggregates and crashed dolomites with a maximum size of 12.5 mm as coarse aggregate, as shown in Table 2 the properties of aggregate that used.

Table 2. Properties of aggregates.

Test	Specific weight	Volumetric weight	Fineness modulus	Materials finer than No 200 sieve
Result	2.57 gm / cm^3	1.42 gm / cm^3	2.54	1.78



A: Coarse aggregate



A: Fine aggregate

Fig.**2**. The gradation curve for the result of aggregate sieve analysis for A: coarse aggregate and B: fine aggregate the Egyptian code of concrete (ECP 203) [41].

Cement

Ordinary portland cement (42.5) N was utilized in this study with chemical and physical properties as shown in table 3 and table 4.

Table 3. Chemical analysis of cement.

Component	SiO_2	Al_2O_3	Fe_2O_3	CaO	MgO	SO_3	Na_2O	K_2O	Cl	C_3A	L.O.I
Percentage (%)	21	5.3	3.51	63.29	1.02	2.12	0.4	0.12	0.01	8.11	2.56

* Loss on Ignition (L.O.I)

Table 4. Physical analysis of cement.

Properties	Water percentage for standard consistency	Specific Surface area	Soundness	Initial Setting Time	Final Setting Time
Results	25.24 %	345 m^2 / kg	1 mm	135 min.	180 min.

Water

The mixing water used was tap water in the mixing of concrete and curing.

2.2 Specimen preparation.

The experimental program consisted of two phases: firstly, investigating the impact of 6 different microorganism samples on concrete with a 3% addition of cement content as shown in table 1; secondly, selecting two types of microorganisms based on phase one results and adding them at rates of 5%, 10%, and 15% of the cement content. Metal cube molds (150 x 150 x 150 mm) were used for concrete casting, followed by immersion in tap water at approximately 20°C until testing. compressive strength tests were conducted at 7, 28, and 56 days using a compression-testing machine with a capacity of 1500 KN and a loading rate of 2 KN/sec, as per ASTM 2016C standards [42] as shown in figure 3.

Fig. 3. Compressive strength test.

To evaluate the splitting tensile properties, cylindrical molds with dimensions of (150 mm in diameter and 30 in height) were used to measure the tensile strength of concrete indirectly according to (ASTM C496)[43] at ages of 7, 28, and 56 days as shown in figure 4.

Fig. 4. Splitting tensile strength test.

The slump test was performed per standard (ESS) No. 1658/2008 to evaluate the workability of fresh concrete as shown in figure 5.

Scanning electron microscopy (SEM) was used to determine the contents of concrete after microorganisms were added to it, as well as to study the surface of the sample, by enlarging a specified sample region with a concentrated high-energy electron beam.

3 Concrete mix design.

Our experimental program utilizes the British "DOE" methodology for concrete mix design, ensuring a meticulous balance of ingredients. Table 5 outlines the mixing proportions for both control mixes and subsequent phases. We aim to assess the influence of different microorganisms on concrete properties while maintaining overall mixing consistency. This approach allows for informed decisions on microorganism species and percentages, optimizing concrete performance. The goal is to make informed judgments about microorganism samples and percentages (by weight).

Table 5. Concrete mix design.

Mix Number	Component / m^3					
	Gravel (kg)	Sand (kg)	Cement (kg)	Water (kg)	W/C	Microorganism (kg)
M1	1240	623	350	140	0.4	10.5 (3% of the cement)
M2	1280	643	350	180	0.4	17.5 (5% of the cement)
M3	1280	643	350	180	0.4	35 (10% of the cement)
M4	1280	643	350	180	0.4	52.5 (15% of the cement)

4 Results and discussion

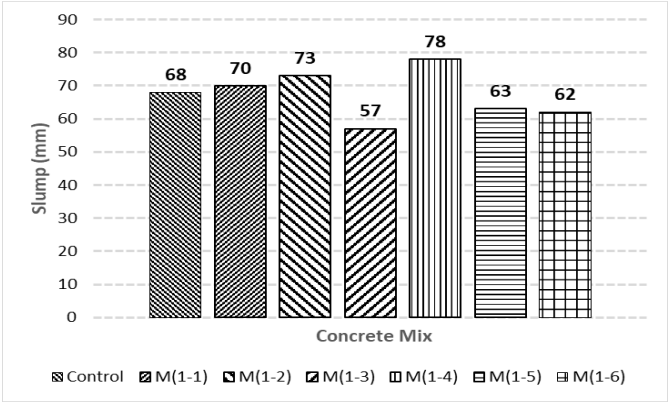
4.1 Fresh concrete

Slump test

The slump test is a critical measure for assessing the workability of fresh concrete, ensuring it meets specified standards. According to Egyptian code specifications [41], the ideal slump range is between 50 mm to 100 mm, indicating optimal workability. In the study, the obtained result is illustrated in Figures 5 A, and B, which fall well within this range, indicating favorable workability characteristics.

This suggests that fresh concrete possesses the desired consistency and flow properties necessary for effective placement and construction processes. This adherence to the specified limits is indicative of quality control measures being met, contributing to the overall integrity of the concrete structure.

A: The slump test for the sample is 70 mm.



B: The slump test results for samples with adding 3% microorganism from cement (by weight).

Fig. 5. The slump test results.

4.2 Hardened concrete

Compressive strength test for 6 samples with adding 3%

The compressive strength test results for 6 samples with adding 3% as shown in table 6, detail the performance of six samples compared to the control mixture. Figures 6, 7, and 8 graphically illustrate the compressive strength evolution over 7, 28, and 56 days, respectively, for these samples with different microorganisms. These visuals highlight the changes in compressive strength over time for each sample.

Table 6. Results of compressive strength tests on concrete cube for 6 samples with adding 3%.

Sample	Micro-organism	Addition percentage	Result (MPa)		
			7 days	28 days	56 days
M(1-1)	B9	3 %	12.45	20	23.47
M(1-2)	F9	3 %	10.4	19.12	24.89
M(1-3)	B1 +9	3 %	10.09	16.98	21.29
M(1-4)	B1	3 %	10.8	17.56	22.85
M(1-5)	14	3 %	10.27	16.94	21.07
M(1-6)	3+14	3 %	11.29	22.14	29.83
Control	-	0 %	10.18	16.54	21.07

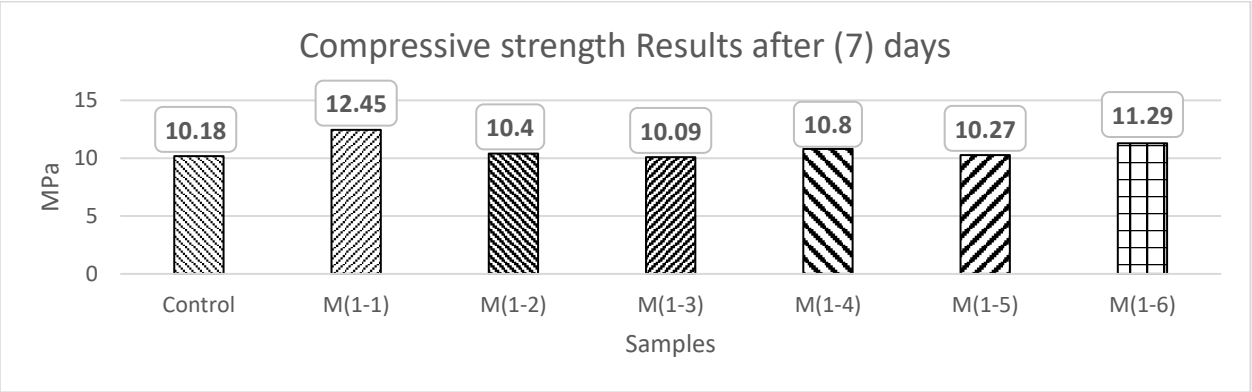


Fig. 6. Display the results of the compressive strength test after 7 days.

The 7-day compressive strength tests reveal a positive influence of microbial activity on concrete. Sample M(1-6) exhibited 11.29 MPa higher strength than the control, suggesting specific microbes contributed to early strength enhancement. Similarly, sample M(1-1) showed the highest strength at 12.45 MPa, indicating a significant microbial impact. These results highlight the potential of targeted microbial incorporation to improve early-stage concrete strength, valuable for construction applications as shown in Figure 6.

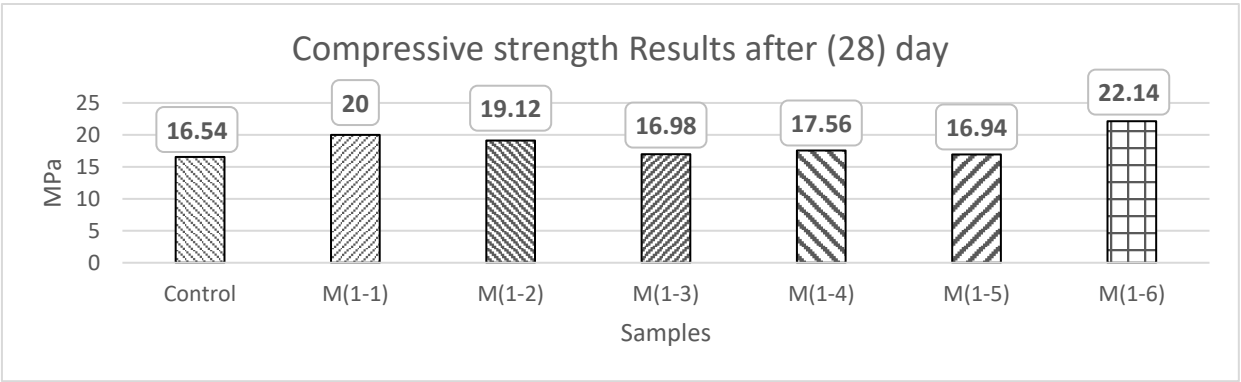


Fig. 7. Display the results of the compressive strength test after 28 days.

The data from the 28-day compressive strength tests further confirm the positive impact of microbial activity on the concrete samples. Figure 7 indicates that sample M(1-6) shows a significant increase in strength over the control, registering at 22.14 MPa. This suggests that the microbial treatment in M(1-6) continues to contribute to strength gain beyond the initial curing period.

Similarly, sample M(1-1) maintains a higher compressive strength compared to the control, demonstrating the lasting effect of its specific microbial content. The consistent performance of these samples from 7 to 28 days could imply that microbial activity contributes not just to the early strength of concrete but also to its development over time.

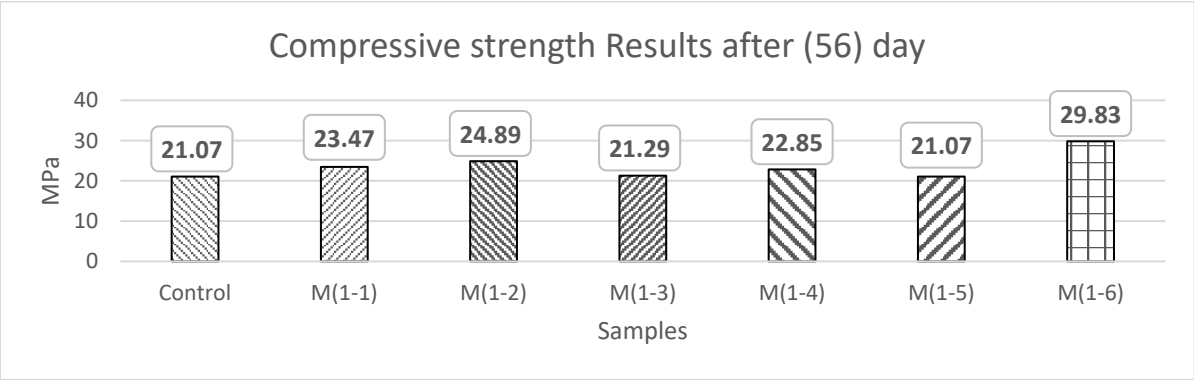


Fig. 8. Display the results of the compressive strength test after 56 days.

The 56-day compressive strength results further illustrate the beneficial impact of microbial treatment in concrete samples. As shown in Figure 8, sample M(1-6) exhibits a significant increase in strength, with a value of 29.83 MPa, which is substantially higher than the control sample's 21.07 MPa. This indicates that the microbial activity in M(1-6) affects the compressive strength over an extended period.

The trend observed in sample M(1-1), with a strength of 23.47 MPa, suggests a persistent positive effect of its microbial content on the concrete's strength development. The consistent performance of these samples from the early stages to 56 days post-casting implies that the microbes may play a role in the long-term strength gain and curing processes of the concrete.

These results support the potential of microbial addition as a strategy to increase the compressive strength of concrete, offering benefits for structures requiring higher strength thresholds. The continued increase in strength also suggests that microbial activity may be involved in processes that contribute to concrete curing and densification.

Concrete with adding different percentage of micro-organism.

Two different samples of microorganisms were carefully introduced using addition ratios of 5%, 10%, and 15%. To speed up the treatment process, prepared samples were immersed in a treatment basin filled with tap water. These studies sought to determine the effect of microbial intervention on the structural integrity of the specimens. This extensive explanation will provide insights into the observed effects and trends caused by the addition of various microorganisms at varying ratios to conventional cubic, cylindrical specimens and sample for SEM test.

Compressive strength test

Table 7 presents the compressive strength test results of concrete cubes augmented with microorganisms *Bacillus subtilis* and *Aspergillus fumigatus* over 56 days. The data exhibit an overall trend where samples containing these microorganisms demonstrate an increase in compressive strength over time when compared to the control.

Table 7. Results of compressive strength tests on concrete with adding different percentage of micro-organism.

Sample	Micro-organism	Addition percentage	Result (MPa)		
			7 days	28 days	56 days
Control	-	0 %	14.18	24.36	26.23
M(2-1)	B9	5 %	17.83	25.47	29.83
M(3-1)	B9	10 %	16.8	24.98	28.23
M(4-1)	B9	15 %	16	24.49	27.74
M(2-2)	F9	5 %	14.8	26	29.96
M(3-2)	F9	10 %	14.45	25.25	28.58
M(4-2)	F9	15 %	14.32	24.63	27.47

The outcomes of concrete with adding different percentage of micro-organism of compressive strength assessments are visualized in Figure 9, displaying the results after 7 days. Figure 10 illustrates the findings following 28 days, while Figure 11 portrays the results at the 56-day. These figures provide a graphical representation of how the compressive strength evolves for the two different samples of microorganisms.

The compressive strength results after 7 days, as shown in Figure 9, indicate that the incorporation of microorganisms into the concrete mix has yielded varying effects on the samples' early strength development. The control sample, without microbial treatment, shows a compressive strength of 14.18 MPa, which serves as a benchmark for comparison.

Sample M(2-1), with the highest compressive strength of 17.83 MPa, demonstrates a significant improvement over the control. This suggests that the microorganisms present in this sample may have a robust effect on the early strength gain of the concrete, possibly through mechanisms such as Biomineralization.

On the other hand, samples M(3-1), M(4-1), M(2-2), M(3-2), and M(4-2) show a compressive strength range from 14.32 MPa to 16.8 MPa, all exceeding the control sample to various extents. These outcomes indicate that the different microorganisms used in these samples have a positive impact on the concrete's initial compressive strength, albeit to varying degrees.

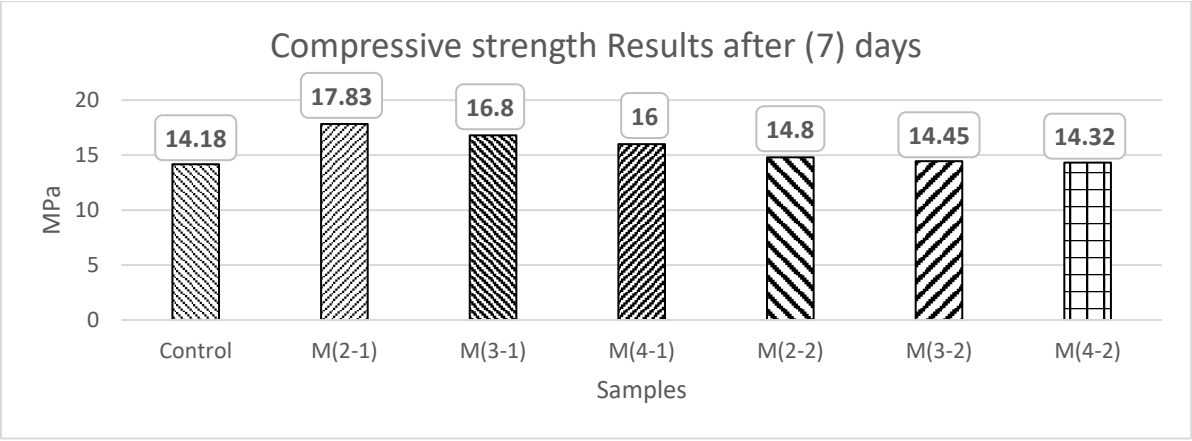


Fig. 9. Display the results of the compressive strength test after 7 days.

The compressive strength results after 28 days, as displayed in Figure 10, indicate a consistent pattern of strength development among the concrete samples treated with microorganisms. All treated

samples show compressive strengths that are competitive with or exceed that of the control, which measures at 24.36 MPa.

Notably, sample M(2-2) registers the highest compressive strength at 28 days, achieving 26 MPa. This reinforces the potential of using specific microorganisms to achieve greater strength in concrete, aligning with the results observed at the 7-day mark. The data suggests that microbial activity contributes positively not only in the initial setting phase but also throughout the curing process.

Samples M(2-1), M(3-1), and M(3-2) also demonstrate increased compressive strengths compared to the control, with sample M(3-1) showing a notable increase to 24.98 MPa. These variations in strength gains across different microbial treatments imply that the type and activity of the microorganisms may influence the extent of the compressive strength enhancement.

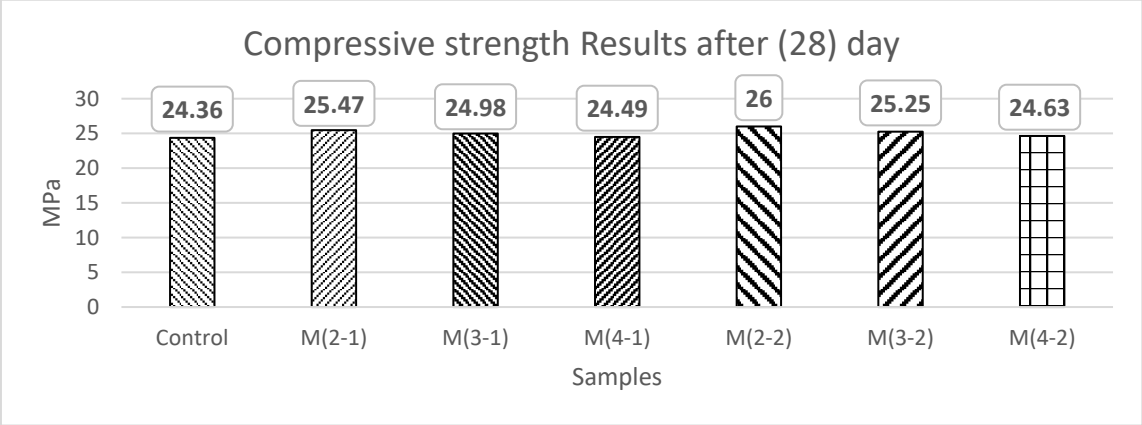


Fig. 10. Display the results of the compressive strength test after 28 days.

The 56-day compressive strength results presented in Figure 11 continue to support the observation that microorganisms can positively influence concrete strength. The control sample shows a strength of 26.23 MPa, which establishes the baseline for this evaluation period.

Sample M(2-2) exhibits the highest compressive strength at 29.96 MPa, suggesting that the specific microbial treatment applied in this sample has a sustained and beneficial impact on the concrete's strength development. Similarly, sample M(2-1) also shows a significantly higher strength of 29.83 MPa compared to the control.

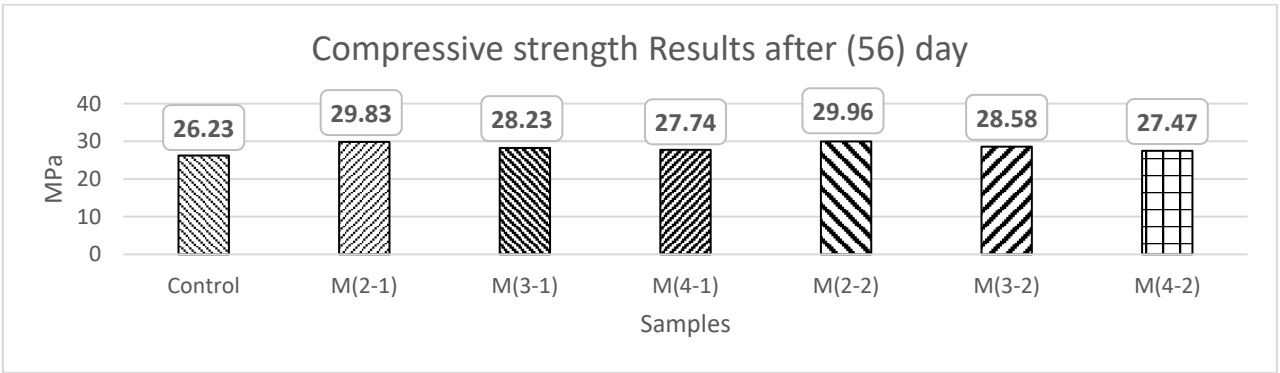


Fig. 11. Display the results of the compressive strength test after 56 days.

Particularly, samples M(2-1) with microorganism *Bacillus subtilis* and M(2-2) with microorganism *Aspergillus fumigatus* show a substantial increase in compressive strength after 56 days, achieving 29.83 MPa and 29.96 MPa respectively, compared to the control's 26.23 MPa. This trend suggests that

the presence of these microorganisms within the concrete matrix contributes to a continued increase in compressive strength beyond the typical 28-day observation period.

The observed pattern across the three testing intervals for both microorganisms indicates that the biological activity contributes not only to early strength gains but also continues to have a positive effect on the concrete's mechanical properties in the longer term. It suggests that microbial-induced processes, such as calcium carbonate precipitation, may continue beyond the initial stages of curing, potentially resulting in further pore refinement and micro-crack healing over time.

Splitting tensile strength test

Table 8 shows the results of the splitting tensile tests on cylindrical specimens at ages 7, 28, and 56 days respectively.

As shown in Table 8 reveals the progression of splitting tensile strength over time for concrete cylinders treated with microorganisms *Bacillus subtilis* and *Aspergillus fumigatus*.

Table 8. Results of splitting tensile strength on concrete with adding different percentage of micro-organism.

Sample	Microorganism	Addition percentage	Result (MPa)		
			7 days	28 days	56 days
Control	-	0 %	0.98	2	2.13
M(2-1)	B9	5 %	1.21	2.21	2.41
M(3-1)	B9	10 %	1.04	2.1	2.21
M(2-2)	F9	5 %	1.15	2.25	2.58
M(3-2)	F9	10 %	1.01	2.17	2.49

The control sample shows a modest increase in tensile strength from 0.98 MPa at 7 days to 2.13 MPa at 56 days, setting a baseline for the performance of the untreated concrete. In comparison, the samples treated with *Bacillus subtilis* and *Aspergillus fumigatus* microorganisms consistently outperformed the control in terms of tensile strength at each time interval.

Sample M(2-1), treated with microorganism *Bacillus subtilis*, demonstrates a marked improvement in early tensile strength at 7 days (1.21 MPa), which further increases to 2.41 MPa by 56 days. Similarly, sample M(2-2), with microorganism *Aspergillus fumigatus*, shows initial strength at 7 days (1.15 MPa) and the highest strength among all samples at 56 days (2.58 MPa). This suggests that both *Bacillus subtilis* and *Aspergillus fumigatus* contribute positively to the tensile capacity of the concrete.

The continued increase in tensile strength over time for samples containing *Bacillus subtilis* and *Aspergillus fumigatus* indicates that microbial activity may be enhancing the internal cohesion of the concrete matrix. Microbial-induced Biomineralization, specifically the precipitation of calcium carbonate, is known to fill pores and micro cracks, which could account for the observed increases in tensile strength.

The outcomes of the second phase splitting tensile strength assessments are visualized in Figure 12, displaying the results after 7 days. Figure 13 illustrates the findings following 28 days, while Figure 14 portrays the results at the 56-day. These figures provide a graphical representation of how the splitting tensile strength evolves for the two different samples of microorganisms.

Figure 12 shows the splitting tensile strength results after 7 days for concrete samples with and without microbial treatment. The control sample shows a tensile strength of 0.98 MPa, setting the baseline for early tensile strength development.

Sample M(2-2) demonstrates the highest tensile strength at this early stage, with 1.15 MPa, suggesting that the microorganism used in this sample significantly enhances the concrete's ability to resist tensile stresses. Sample M(2-1) also shows a marked improvement with a tensile strength of 1.21 MPa.

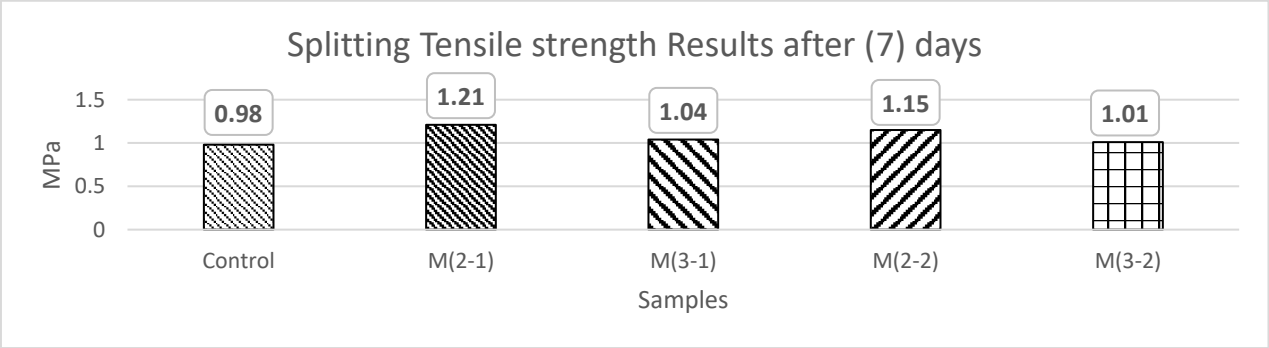


Fig. 12. Display the results of the splitting tensile strength test after 7 days.

Figure 13 provides the splitting tensile strength results of concrete samples after 28 days, showcasing the continued influence of microbial treatment. The control sample's tensile strength is measured at 2 MPa.

At this phase, all microbial-treated samples exceed the control in tensile strength, with sample M(2-2) achieving the highest value at 2.25 MPa. This demonstrates a consistent trend where microbial activity contributes to an increase in the tensile strength of concrete over time. Sample M(2-1) also shows significant strength gain with a value of 2.21 MPa.

The increase in tensile strength suggests that the biomineralization process, possibly facilitated by microbial action, is effective not only in the initial stages but also in the maturation of the concrete. The ongoing formation of calcium carbonate within the matrix may be responsible for the increased tensile strength, as it could help to bridge cracks and improve aggregate bonding.

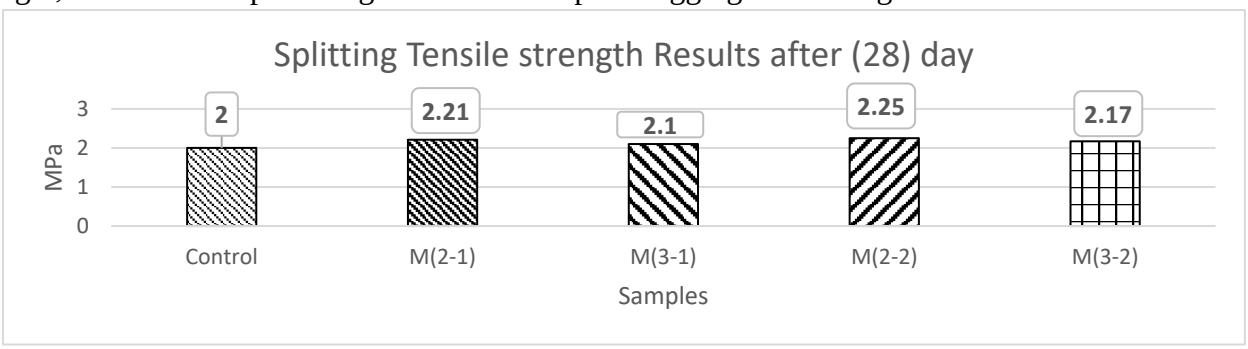


Fig. 13. Display the results of the Splitting tensile strength test after 28 days.

Figure 14 illustrates the splitting tensile strength results of concrete samples after 56 days, indicating the endurance of the microbial treatment effects. The control sample presents a tensile strength of 2.13 MPa.

The data reveals that samples treated with microorganisms generally maintain a superior tensile strength over the control throughout the extended curing period. Specifically, sample M(2-2) shows a

notable increase with a tensile strength of 2.58 MPa, the highest among the group, while sample M(2-1) also demonstrates a significant improvement at 2.41 MPa.

The continued increase in tensile strength suggests that microbial processes, such as calcium carbonate precipitation, contribute to ongoing benefits in concrete cohesion and crack healing beyond the early stages of setting.

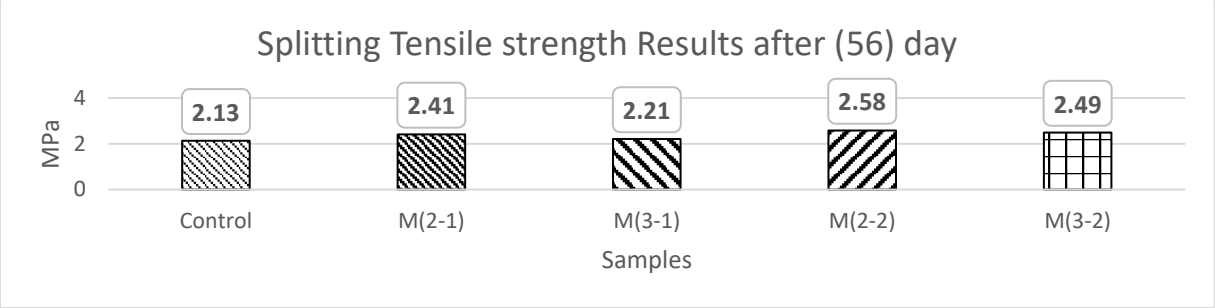
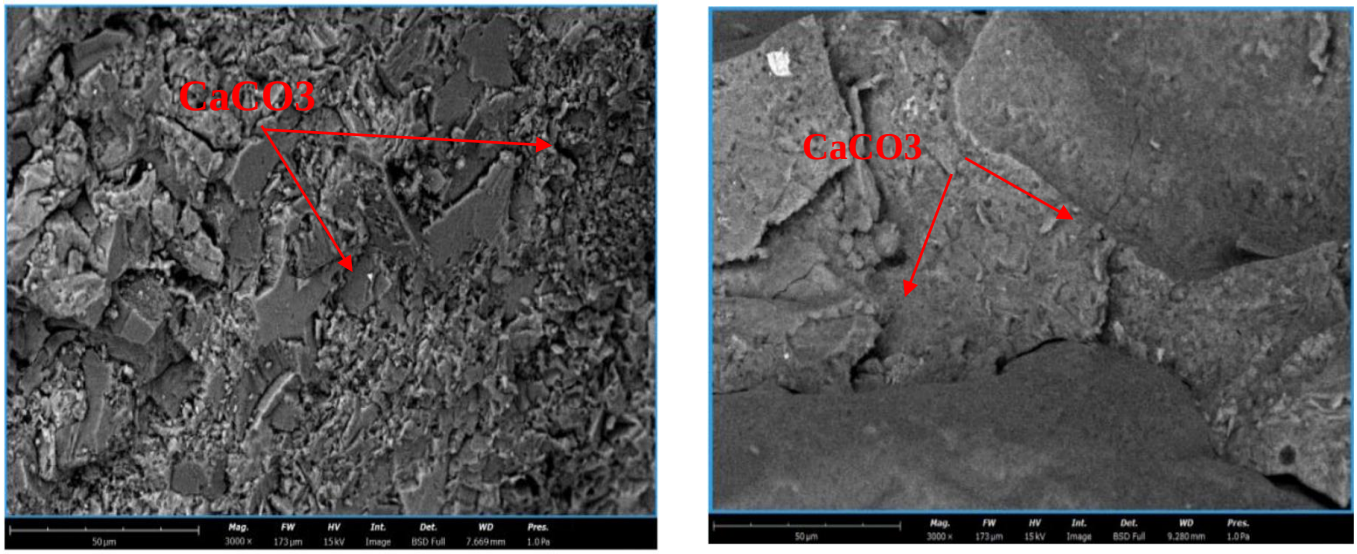


Fig. 14. Display the results of the splitting tensile strength test after 56 days.

Scanning electron microscopy (SEM).

The microstructure investigation utilized a JEOL-JSM 5600 LV microscope with 6587 EDS detectors at 15 KV accelerating voltage. Fragments from the compressive strength test, 1-2.5 cm long, were affixed to a testing plate with carbon tape and magnified from 500× to 1500× for SEM imaging. SEM images were analyzed with an energy - dispersive X-ray (EDX) detector to assess concrete matrix components. *Bacillus subtilis* and *Aspergillus fumigatus* showed superior outcomes for concrete mechanical qualities, prompting further SEM analysis to study their effects on the sample surface and microbial preparations.

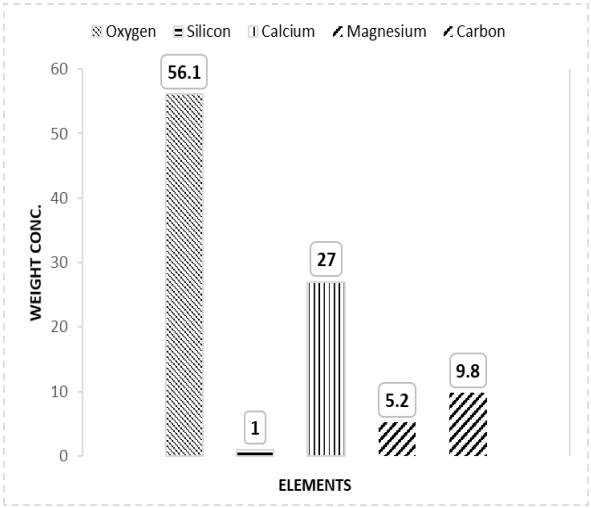
Analyzing the sample with added microorganisms.



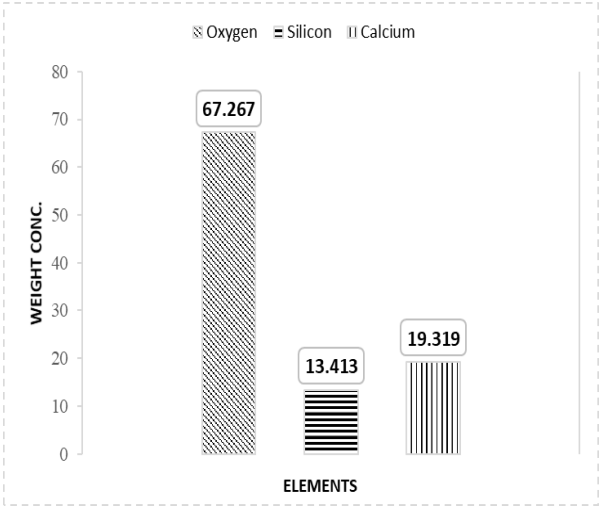
A: Bacillus subtilis ***B: Aspergillus fumigatus***

Fig. 15. Show SEM test results for the sample with microorganisms.

The figure (16-A) shows that the sample contains C (Carbon), O (Oxygen), Mg (Magnesium), Si (Silicon) and Ca (Calcium), and the figure (16-B) show that the sample contain O (Oxygen), Si (Silicon) and Ca (Calcium)



A: Weight conc.

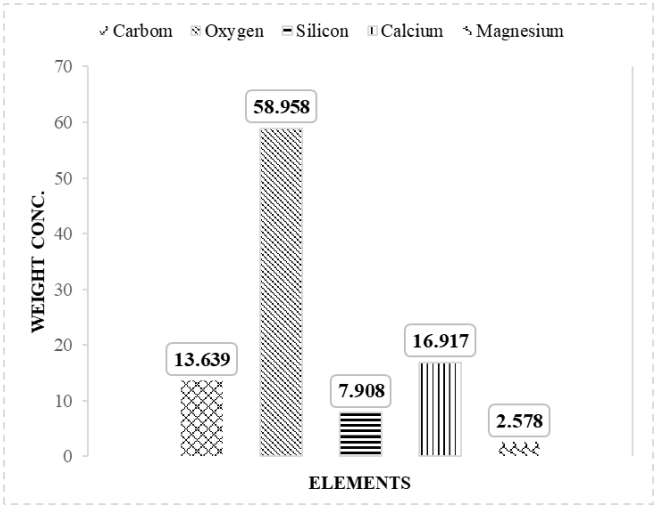


B: Weight conc.

Fig. 16. Show the results of SEM test for the sample with microorganisms; A: *Bacillus subtilis* and B: *Aspergillus fumigatus.*

Analyzing the control sample.

A: Control sample



b: Weight conc.

Fig. 17. Show the results of the SEM test for the control sample.

Figure 17 show that the sample contains C (Carbon), O (Oxygen), Si (Silicon), Mg (Magnesium), and Ca (Calcium).

The experimental results show a significant variation in the performance of various microorganisms used in bioconcrete in terms of calcium precipitation. In comparison to the control group, the presence of *bacillus subtilis* bacteria resulted in a significant increase in calcium content, and weight concentration (50.1% increase). In contrast, the use of *Aspergillus fumigatus* resulted in a rather small improvement, with calcium concentrations of 14.2% in terms of weight concentration.

The findings underscore the vital role of microorganisms in bioconcrete repair, particularly in facilitating pore filling via calcium carbonate precipitation. This method displays the efficacy of bioconcrete in self-healing and suggests the potential for enhancing regeneration qualities through selective microorganism choice.

5 Conclusion

This research Enhancing Concrete Performance through Microbial Intervention through A Comprehensive Experimental Study deals with the use of different microorganisms to improve the mechanical properties of concrete. The study demonstrates that microorganisms can significantly enhance the performance of concrete through MICP, contributing to the filling of pores and healing cracks in concrete structures.

There is a significant relationship between the mechanical properties of the concrete and the added microbial bacteria. The experimental results clearly demonstrate that the inclusion of specific microbial strains, such as *Bacillus subtilis* and *Aspergillus fumigatus*, positively influences both the compressive and tensile strengths of the concrete over time.

Compressive Strength: The study shows that concrete samples treated with *Bacillus subtilis* and *Aspergillus fumigatus* exhibited a substantial increase in compressive strength compared to the control samples. This increase was particularly pronounced after 56 days, with *Bacillus subtilis*-treated samples achieving up to 29.83 MPa and *Aspergillus fumigatus*-treated samples reaching 29.96 MPa, compared to the control's 26.23 MPa.

The increase in compressive strength is attributed to microbial-induced calcium carbonate precipitation, which enhances the concrete's density and fills micro-cracks, leading to improved mechanical properties.

Splitting tensile Strength: The splitting tensile strength tests also reflect the beneficial effects of microbial addition. Samples treated with *Bacillus subtilis* and *Aspergillus fumigatus* consistently outperformed the control, with significant improvements observed at both early (7 days) and later (56 days) stages. For example, the tensile strength of *Aspergillus fumigatus*-treated samples reached 2.58 MPa at 56 days, compared to the control's 2.13 MPa.

This improvement in tensile strength suggests that microbial activity contributes to internal cohesion within the concrete matrix, likely through mechanisms such as biomineralization and micro-crack healing.

Microstructural Analysis: SEM analysis further supports these findings by revealing increased calcium content in the microbial-treated samples, particularly those with *Bacillus subtilis*, which showed a 50.1% increase in calcium concentration compared to the control. This suggests that the bacteria facilitate the formation of calcium carbonate, which is critical for enhancing the concrete's mechanical properties.

The addition of microbial bacteria such as *Bacillus subtilis* and *Aspergillus fumigatus* is directly linked to the observed improvements in both compressive and tensile strengths of the concrete. These enhancements are largely due to the microbes' ability to precipitate calcium carbonate, leading to a denser and more resilient concrete structure.

Recommendations for Practice: As we look to the future of construction, the results of this study suggest an exciting and practical application: integrating microbial treatments into the concrete mix. *Bacillus subtilis* and *Aspergillus fumigatus* have proven their worth as concrete enhancers, offering a sustainable and innovative approach to creating stronger, longer-lasting structures. These

microorganisms could be the key to developing self-healing concrete, reducing the need for repairs and extending the lifespan of buildings and infrastructure. The message is clear: embracing this microbial technology can lead to concrete that not only meets but also exceeds modern performance standards. Imagine a world where concrete repairs itself, adapts to environmental stresses, and remains robust for decades—this is no longer a distant possibility but a tangible reality, ready to be explored and implemented. As we move forward, further research and field trials will be essential to refine these techniques and scale them for widespread use, ushering in a new age of intelligent, bio-enhanced construction and create a new construction material using microbial technology

6 Future Recommendations

- **Microbial Mix Mastery:** Explore the ideal mix of *Bacillus subtilis* and *Aspergillus fumigatus*, fine-tuning their concentrations and application methods to boost concrete strength. Discovering the best strains and curing conditions could unlock even greater benefits.
- **Durability:** Assess how microbial treatments hold up over the long haul. Test concrete in real-world conditions like extreme weather and chemical exposure to ensure it remains strong and durable.
- **From Lab to Site:** Move beyond lab experiments by conducting large-scale field trials.
- New Microbial mixes: Investigate other microorganisms that might enhance concrete properties.
- **Nanomaterials:** Explore the optimal use of nanomaterials to enhance concrete without driving up costs.

References

1. Sumit Joshi, Shweta Goyal, Abhijit Mukherjee, M Sudhakara Reddy, Microbial healing of cracks in concrete: a review, *Journal of Industrial Microbiology and Biotechnology*, Volume 44, Issue 11, 1 November 2017, Pages 1511–1525, https://doi.org/10.1007/s10295-017-1978-0
2. Ding, Y. A Bibliography Study of Self-Healing Concrete. Preprints 2024, 2024011134. https://doi.org/10.20944/preprints202401.1134.v1
3. Jiang, L., Li, P., Wang, W., Zhang, Y., & Li, Z. (2023). A self-healing method for concrete cracks based on microbial-induced carbonate precipitation: bacteria, immobilization, characterization, and application. *Journal of Sustainable Cement-Based Materials*, *13*(2), 222–242. https://doi.org/10.1080/21650373.2023.2263447
4. An, B.; Wang, Y.; Huang, Y.; Wang, X.; Liu, Y.; Xun, D.; Church, G. M.; Dai, Z.; Yi, X.; Tang, T. C.; Zhong, C. Engineered living materials for sustainability. *Chem. Rev.* 2023, 123, 2349– 2419, DOI: 10.1021/acs.chemrev.2c00512
5. Hamza, O., Esaker, M., Abogdera, A. and Elliott, D., 2024. Bio-protection of cementitious materials below ground: The significance of natural soil environments. *Developments in the Built Environment*, 17, p.100331. https://doi.org/10.1016/j.dibe.2024.100331

6. Min, J., Son, Y., Jang, I., Yi, C. and Park, W., 2024. Managing two simultaneous issues in concrete repair: Healing micro cracks and controlling pathogens. *Construction and Building Materials*, 416, p.135125. https://doi.org/10.1016/j.conbuildmat.2024.135125.
7. Chen, H.J., Lo, Y.H., Tang, C.W. and Chang, H.W., 2024. Applying Microbial-Induced Calcium Carbonate Precipitation Technology to Improve the Bond Strength of Lightweight Aggregate Concrete after High-Temperature Damage. *Applied Sciences*, 14(4), p.1416. https://doi.org/10.3390/app14041416.
8. Javeed, Y., Goh, Y., Mo, K.H., Yap, S.P. and Leo, B.F., 2024. Microbial self-healing in concrete: A comprehensive exploration of bacterial viability, implementation techniques, and mechanical properties. *Journal of Materials Research and Technology*, 29, pp.2376-2395. https://doi.org/10.1016/j.jmrt.2024.01.261.
9. Wang, K., Zheng, M., Yan, S., Gao, Z., Hu, Y., Peng, L., Zhang, Y. and Wang, Z., 2024. Study on the influence mechanism of calcium carbonate particles on mechanical properties of microcrack cement. *Construction and Building Materials, 411*, p.134563. https://doi.org/10.1016/j.conbuildmat.2023.134563
10. Viado, M.J., 2024. Encapsulating immobilized ureolytic bacteria yields self-healing concrete apropos sustainable transportation materials: A review. In E3S Web of Conferences (Vol. 488, p. 03019). EDP Sciences. https://doi.org/10.1051/e3sconf/202448803019
11. Zhang, Y.S., Liu, Y., Sun, X.D., Zeng, W., Xing, H.P., Lin, J.Z., Kang, S.B. and Yu, L., 2024. Application of microbially induced calcium carbonate precipitation (MICP) technique in concrete crack repair: A review. Construction and Building Materials, 411, p.134313. https://doi.org/10.1016/j.conbuildmat.2023.134313
12. Van Wylick, A., Rahier, H., De Laet, L. and Peeters, E., 2024. Conditions for CaCO3 Biomineralization by Trichoderma Reesei with the Perspective of Developing Fungi-Mediated Self-Healing Concrete. Global Challenges, 8(1), p.2300160. https://doi.org/10.1002/gch2.202300160
13. Duo, L., Kan-liang, T., Zhang, H., Yu-yao, W., Kang-yi, N. & S., Z. 2018. Experimental investigation of solidifying desert aeolian sand using microbially induced calcite precipitation. Construction and Building Materials.251-262. https://doi.org/10.1016/j.conbuildmat.2018.03.255
14. Udhaya, S., Devi, V.V., Philips, J. and Lija, R.L., 2023. Experimental study on bio-concrete for sustainable construction. Materials Today: Proceedings. https://doi.org/10.1016/j.matpr.2023.03.676
15. Onyelowe, K.C., Adam, A.F., Ulloa, N., Garcia, C., Andrade Valle, A.I., Zúñiga Rodríguez, M.G., Zarate Villacres, A.N., Shakeri, J., Anyaogu, L., Alimoradijazi, M. and Ganasen, N., 2024. Modeling the influence of bacteria concentration on the mechanical properties of self-healing concrete (SHC) for sustainable bio-concrete structures. Scientific Reports, 14(1), p.8414. https://doi.org/10.1038/s41598-024-58666-8
16. Smirnova, M., Nething, C., Stolz, A. et al. High strength bio-concrete for the production of building components. npj Mater. Sustain. 1, 4 (2023). https://doi.org/10.1038/s44296-023-00004-6
17. Hassanin, A., El-Nemr, A., Shaaban, H.F. et al. Coupling Behavior of Autogenous and Autonomous Self-Healing Techniques for Durable Concrete. Int J Civ Eng 22, 925–948 (2024). https://doi.org/10.1007/s40999-023-00931-4

18. Sadeghpour, M. and Baradaran, M., 2023. Effect of bacteria on the self-healing ability of fly ash concrete. Construction and Building Materials, 364, p.129956. https://doi.org/10.1016/j.conbuildmat.2022.129956
19. Davila, J.M., Sarmiento, A.M., Grande, J.A. and Luís, A.T., 2023. Different evolution of tensile and compressive strength in concrete affected by acid mine drainage. Construction and Building Materials, 397, p.132351. https://doi.org/10.1016/j.conbuildmat.2023.132351
20. Singh, O.P., Kulhar, K.S. and Upadhyai, R.P., 2023. The comparison of the experimental investigations of strength characteristics of conventional and bacterial concrete. Materials Today: Proceedings. https://doi.org/10.1016/j.matpr.2023.06.035
21. Rajawat, S.P.S., Rajput, B.S., Sharma, M. and Jain, G., 2023. Exploring the potential of bacterial concrete: An analysis of self-healing capabilities and compressive strength. Materials Today: Proceedings. https://doi.org/10.1016/j.matpr.2023.07.358
22. Helal, Z., Salim, H., Ahmed, S., Hesham, E.E., Ibrahim, A. and Elmahdy, M., 2024. Sustainable Bacteria-Based Self-Healing Steel Fiber Reinforced Concrete. Case Studies in Construction Materials, p.e03389. https://doi.org/10.1016/j.cscm.2024.e03389
23. Nisar, M. and Singh, B., 2024. An Experimental Study on Strength and Self-Healing Characteristics of Bacterial Concrete. International Journal of Innovative Research in Engineering & Management, 11(1), pp.10-14. https://acspublisher.com/journals/index.php/ijirem/article/view/13200
24. Ibrahim, O., Abbas, A., Hassanin, A., Ibrahim, W., Abd-Elnaby, S. (2023). 'A Literature Review of Bio-cement: Microorganisms, Production, Properties, and Potential Applications.', ERU Research Journal, 2(4), pp. 554-574. doi: 10.21608/erurj.2023.321807
25. Devgon, I., Sachan, R.S.K., Devgon, J. and Karnwal, A., 2024, February. Bio-cement: A sustainable approach in the construction sector. In AIP Conference Proceedings (Vol. 2986, No. 1). AIP Publishing. https://doi.org/10.1063/5.0194118
26. Nur, M.M.A. and Dewi, R.N., 2024. Opportunities and challenges of microalgae in biocement production and self-repair mechanisms. Biocatalysis and Agricultural Biotechnology, p.103048. https://doi.org/10.1016/j.bcab.2024.103048
27. State-of-the-Art Report: The Self-Healing Capability of Alkali-Activated Slag (AAS) Concrete. In Materials (Vol. 16, Issue 12, p. 4394). MDPI AG. https://doi.org/10.3390/ma16124394.
28. Efficiency of Bacteria-Based Self-Healing Mechanism in Concrete. In Materials Science Forum (Vol. 1089, pp. 135–143). Trans Tech Publications, Ltd. https://doi.org/10.4028/p-tc6w54.
29. The Efficiency of Calcium Oxide on Microbial Self-Healing Activity in Alkali-Activated Slag (AAS). In Applied Sciences (Vol. 14, Issue 12, p. 5299). MDPI AG. https://doi.org/10.3390/app14125299.
30. Spotlight on mechanical properties of autogenic self-healing of concrete. In: 2nd International Summit on Civil, Structural, and Environmental Engineering, 18-20 March, Florence, Italy. (In Press) https://repository.uwl.ac.uk/id/eprint/11216
31. Johnson LF, EA Curl, JM Bono, HA Fribourg.Methods for Studying Soil MicrofloraPlant Disease Relationships. Minneapolis Publishing Co., USA, 1959, 178.
32. Kim S; S Ahu; W Seo; G Kwan; Y Park,J. Microb.Biotechnol.,1998, 8, 178-181.
33. Robinson M; J Riov; A Sharon,Appl. Environ. Microbiol., 1998, 64, 5030-5032.
34. Martin JP,Soil Sci., 1950, 69, 215-233.
35. Jiang ZD; PR Jensen; W Fenical,Biog. Med. Chem. Lett., 1999, 9(14), 2003-2006.

36. Masoud W; CH Kaltoft,Inter. J. Food Microbiol.,2006, 106, 229-234.
37. Ainsworth GC.Ainsworth and Bisby's Dictionary of the Fungi. Commonwealth Mycological Institute, Kew, Surrey, England, 1971.
38. Klich, M.A.; Pitt, J.I. (1992). A laboratory guide to the common Asperigillus species and their teleomorphs. Commonwealth scientific and industrial research organization, division of food processing, North Ryde, Australia.
39. Shaffer JG, MMS Goldin.Microbiologic Methods. In: Clinical Diagnosis by Laboratory Methods (Eds. I Davidsohn,BB Wells), W.B. Saunders Company. Inc., Philadelphia, London, 1963, 718-719.
40. Sneath HA.Endospore-Forming Gram-Positive Rods and Cocci. In: Bergey's Manual of Systematic Bacteriology (Eds.PHA Sneath, NS Mair, ME Sharpe, JG Holt), Lippincott Williams and Wilkins Company, Baltimore, Beverly presses. Inc., USA, 1986, 1005-1141.
41. Egyptian code of concrete (ECP 203).
42. ASTM C109, 2016c. Standard Test Method for Compressive Strength of Hydraulic Cement Mortars (Using 2-in. or [50-mm] Cube Specimens).ASTM International, West Conshohocken, USA.
43. ASTM C496 / C496M-17, Standard Test Method for Splitting Tensile Strength of Cylindrical Concrete Specimens, ASTM International, West Conshohocken, PA, 2017.