Investigating the repair of cracks through bacterial self-healing for sustainable concrete in aggressive sulfate attack environments

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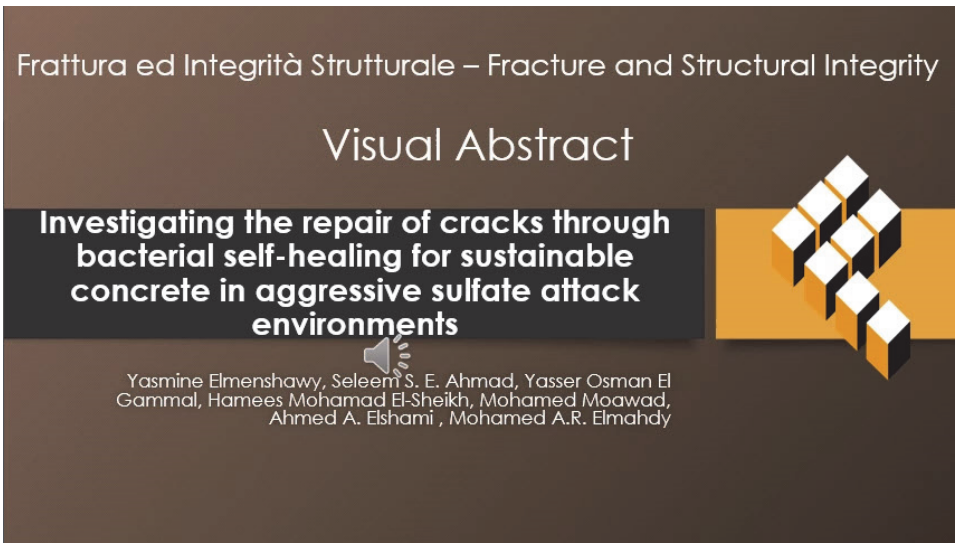

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INTRODUCTION

Bacterial self-healing efficiency in a higher sulfate attack environment is a significant issue as it forms expansive products that force the concrete capillaries open. The general scenario in concrete's early failure and degradation concerns is sulfate attack and sodium carbonate-induced corrosion of the reinforcing structure. Civil engineers need

special attention to develop concrete solutions to these adversities. In the self-healing concrete field, a few robust methods are available; however, the unique principle of bacterial mineral precipitation as an autonomous reaction in concrete involves special attention to the other chemical and physical processes. In addition, the biological options for concrete self-healing methods are not prominent enough because of their complexity, the requirement of additional nutrients or other precursors, the formation of undesirable by-products, the long-term adaptation of the microorganisms, a balance between desirable and undesirable reactions, and, very important, the biological activity.

The three processes of concrete technologies, as mentioned by Amirreza Talaiekhozan et al [1] contain (1) natural (2) chemical, and (3) biological processes. Autogenous self-healing in concrete is achieved through the hydration reaction of cementitious products or polymeric substances within the matrix [2]. The chemical healing process involves artificially promoting fracture healing by injecting chemicals into fractures, using techniques like glue-filled vessel networks, hollow pipettes, and encapsulated glue. Gollapudi et al.[3] introduced biological self-healing concrete, a sustainable method using specific bacteria strains to precipitate specific compounds from viruses, fungi, and bacteria.

Recent research has shown severe worry regarding the degradation of concrete caused by sulfate-bearing environments [4]. Sulfate ions infiltrate the cementitious matrix when cement mortars and concrete come into contact with sulfate-loaded surroundings during service life [5]. Chemical reactions with hydrated cement products occur when the elevated concentration of sulfate ions from the surface is transported into the bulk of the concrete [6].

As indicated in scholarly reports, the principal factor contributing to the deterioration of the concrete matrix due to sulfate exposure is a two-stage distress mechanism. This chemical process unfolds in two sequential stages. Initially, sulfate ions react with portlandite ($Ca(OH)_2$) to form gypsum $CaSO_4 \cdot 2H_2O$. Subsequently, the generated gypsum interacts with tricalcium aluminate (C_3A). Gypsum forms ettringite precipitates within the concrete's pore structure. In the second stage, the concrete undergoes swelling, cracking, and spalling due to the elevated crystallization pressure associated with the expansion of ettringite [7].

Physical sulfate attack (PSA) is the term used to describe this type of sulfate-induced degradation in which sulfate salt crystallization leaves concrete susceptible to harm. In this scenario, capillary rise and sulfate salt evaporation occur when the concrete surface touches a sulfate-bearing fluid. [8]. Mahmoud ZIADA et al [9] recommend using a bacterial crack treatment solution for structures subject to sulfate attack. Bacteria can survive in concrete during curing and after cracking, but their viability is influenced by various factors such as temperature, pH, and the specific bacterial strain used. Research indicates that certain Bacillus species can remain viable in the cement matrix, with some retaining functionality for up to 180 days under optimal conditions. [10]. The following sections detail the survival duration during curing and post-cracking. Survival During Curing, Optimal Conditions: Certain Bacillus species showed the highest viability when encapsulated, with effective healing observed within 14 to 28 days for cracks of approximately 0.13 mm. Survival After Cracking. Longevity: Bacteria can survive and remain active in concrete for extended periods, with some studies indicating effective self-healing capabilities even after 180 days [10]. Healing Efficacy: Cracks up to 0.4 mm can be effectively healed, with a success rate of 89.4% under optimal conditions. A material that can repair itself to its initial state is known as self-healing material. More than 20 years ago, the idea of self-healing concrete (SHC) that develops naturally over time has been recognized. It can be seen in several historic buildings that have survived for a long time despite receiving little maintenance [11].

Prior research has presented opposing perspectives on the effects of sulfate on concrete with low levels of bacteria. The behavior of native bacteria when exposed to sulfate has not been thoroughly explored, which could result in the death of bacteria. To fill this void, the study examines concrete's compressive and indirect tensile strengths. It also looks at how the loading ratio affects cracked specimens, compares crack formation before and after exposure to sulfate, and evaluates the rate of crack repair. Additionally, the research uses microstructure analysis through the Scanning Electron Microscope (SEM), the Energy-Dispersive Spectroscope (EDS), and X-Ray Diffraction (XRD) to validate the findings.

EXPERIMENTAL WORK

Test program

The current experimental work is an extension of the experimental program from Ref [12], which explored the effects of temperature variation on bacteria-infused concrete. This study focuses on examining the impact of exposing bacteria-infused concrete to sulfate. In this research, two different types of Bacillus bacteria, Bacillus Sphaericus (BS) and Bacillus Megaterium (BM), were employed to treat freshwater (FW) and sulfate (Sul) in concrete. The bacteria were added in varying concentrations (0%, 0.25%, 1%, 2.50%, and 5.00% by weight of the cement). Fig. 1 demonstrates the test program, which included 18 concrete mixes to examine these factors. Each mix produced three 100×100×100 mm cubic specimens, which were then tested for compressive strength after curing in freshwater or sulfate for 7, 28, 56, and 120 days,

in accordance with the guidelines of British Standards No. 12390-3:2019, . To calculate indirect tensile strength, three 100 x 200 mm cylindrical specimens were loaded to failure at the ages of 28, 56, and 120 days, following British Standards No. 1881-117:1983, To study the healing effects, three cubes and three cylinders were loaded with around 35% of the maximum load at 56 days old and were reloaded to failure at 120 days. Additionally, at 120 days, cubic specimens containing 2.5% and 5% of bacteria at 56 days old were tested using approximately 65% of the final load.

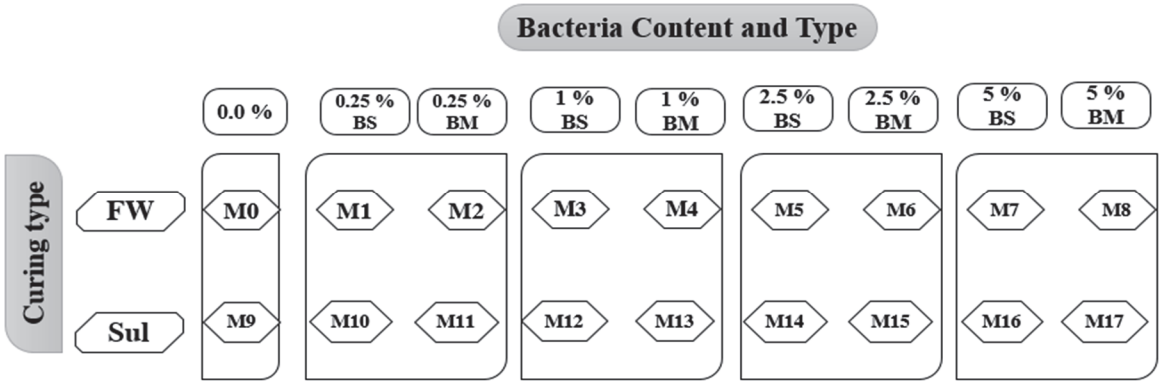


Figure 1: Flow chart for test program, where FW: fresh water; Sul: sulfate; M: Concrete mix; BS: Bacillus Sphaericus Bacteria and BM: Bacillus Megaterium Bacteria

Materials

The cement used in the concrete mixes was standard Portland cement (OPC) obtained from a local company in Egypt, El Askry, with a specific gravity of 3.14, and it met the requirements of British Standards No 197-1 / 2011 and Egyptian Standard Specification No. 4756-1 / 2007. The fine aggregate used in this work was quartz-filled sand with an apparent specific gravity of 2.5, volume density of 1.73 t/m^3, and fineness modulus of 2.65, as measured in the lab. The coarse aggregate was crushed dolomite from Suez City's Ataka Mountain, with a 10 mm maximum aggregate size, apparent specific weight of 2.5, and a volume density of 1.36 t/m^3 as measured in the lab. The 10% silica fume (SF) SiO_2, which met the standard of ASTM C-1240-20, and type G superplasticizer called SikaViscoCrete-3425, which meets the standard of ASTM-C-494-20 categories G and F for superplasticizer, were added to cement content. Calcium lactate ($C_6H_{10}CaO_6$) from Oxford Lab Fine Chem LLP and magnesium sulfate ($MgSO_4$) were used as a chemical compound. Two ureolytic bacteria strains, Bacillus Megaterium (BM) and Bacillus Sphaericus (BS), were utilized for the research. These bacteria strains were acquired from the Microbiological Resources Centre (MIRCEN) in the microbiology lab at the Faculty of Agriculture, University of Ain Shams in Egypt. These bacteria were chosen for the study due to their safety and qualities, such as mineralization in the presence of a calcium supply, spore production, and ability to thrive in the water without a mobilizing agent, making them suitable candidates for use in the self-healing process for concrete.

Preparing a Bacterial Cell Suspension

The method for creating the bacterial cell suspension was carried out following the instructions provided by Elmahdy et al. [13]. The initial bacterial strains were kept refrigerated after being acquired from MIRCEN. Furthermore, the solution included sodium chloride, yeast extract, and beef extract at concentrations of 5 g/l, 2 g/l, and 5 g/l, respectively. The pH of the culture medium was brought to 7.20±0.20 using a pH meter to prepare it for autoclaving sterilization. The bacterial inoculation procedure was carried out to ensure sterility in a model AURA HZ 48 laminar flow cabinet. After being removed from the fridge, bacterial suspensions were kept in flask tubes. The medium's turbidity suggested the presence of microorganisms. The bacterial cells were taken out of the bio-media samples and diluted with distilled-water after being examined beneath a light microscope. Solid media were used to record bacterial colonies, diluted using culture media in order to reach a concentration of two-billion CFU/ml. The Seed and Tissue Pathology laboratory of the Faculty of Agriculture at Zagazig University in Egypt was the site of all microbiological procedures. The important steps for creating the bacterial cell suspension used in this study, was provided by Elmenshawy et al [12].

Mixing and Specimen Preparation

The quantities from the used materials required to produce 1 m^3 of concrete were determined and given in Tab. 1. The absolute volume method was used to check the total volume. For all combinations, the SF/C ratio was 10%, the W/C ratio was 0.4, the superplasticizer ratio was 0.50%, and the micro-nutrient ratio was 0.5% from cement content. The coarse

aggregate (dolomite) to fine aggregate (sand) ratio was maintained at 2:1 by weight. The Typical process used to develop the mix was applied to create the control samples (M0); however, bacteria were not included in the mix.

Mix ID	Bacteria type	Bacteria /cement (%)	Weight (kilogram per cubic meter)							Curing
			Cement	Bacteria	Water	Nutrient	Sand	Dolomite	Silica fume	
M0	-	0	450	0	171	0	592.88	1185.77	45	FW
M1	BS	0.25		1.125	169.87	2.25	590.97	1181.19		
M2	BM	0.25		1.125	169.87		590.97	1181.19		
M3	BS	1		4.5	166.5		587.95	1175.9		
M4	BM	1		4.5	166.5		587.95	1175.9		
M5	BS	2.5		11.25	159.75		582.84	1165.68		
M6	BM	2.5		11.25	159.75		582.84	1165.68		
M7	BS	5		22.5	148.5		571.88	1143.77		
M8	BM	5		22.5	148.5		571.88	1143.77		
M9	-	0		0	171	0	592.88	1185.77		Sulfate
M10	BS	0.25		1.125	169.87	2.25	590.97	1181.19		
M11	BM	0.25		1.125	169.87		590.97	1181.19		
M12	BS	1		4.5	166.5		587.95	1175.9		
M13	BM	1		4.5	166.5		587.95	1175.9		
M14	BS	2.5		11.25	159.75		582.84	1165.68		
M15	BM	2.5		11.25	159.75		582.84	1165.68		
M16	BS	5		22.5	148.5		571.88	1143.77		
M17	BM	5		22.5	148.5		571.88	1143.77		

Table 1: The Concrete mix ingredients.

There is a precise mixing procedure for each type of concrete mixture that needs to be followed. Cement, sand, and aggregate are the dry components mixed in a horizontal mechanical mixer pan. After adding the necessary quantity of Calcium lactate and silica fume, the mixture is mixed for two minutes at a low speed. After slowly adding the water and superplasticizer and mixing for about five minutes, the mixture should be equally dispersed. In relation to bacterial blends, mixing water is introduced first, followed by the simultaneous addition of BM or BS bacteria. Three layers of newly blended concrete are poured into molds, and a mechanical vibrator is used to compact the material for 30 seconds, as described in the ASTM C-192/C192M guidelines. The samples are taken out of the molds and soaked in faucet water for 7, 28, 56, and 120 days to get ready for test-taking. The mixing process of the samples is illustrated in Fig. 2.

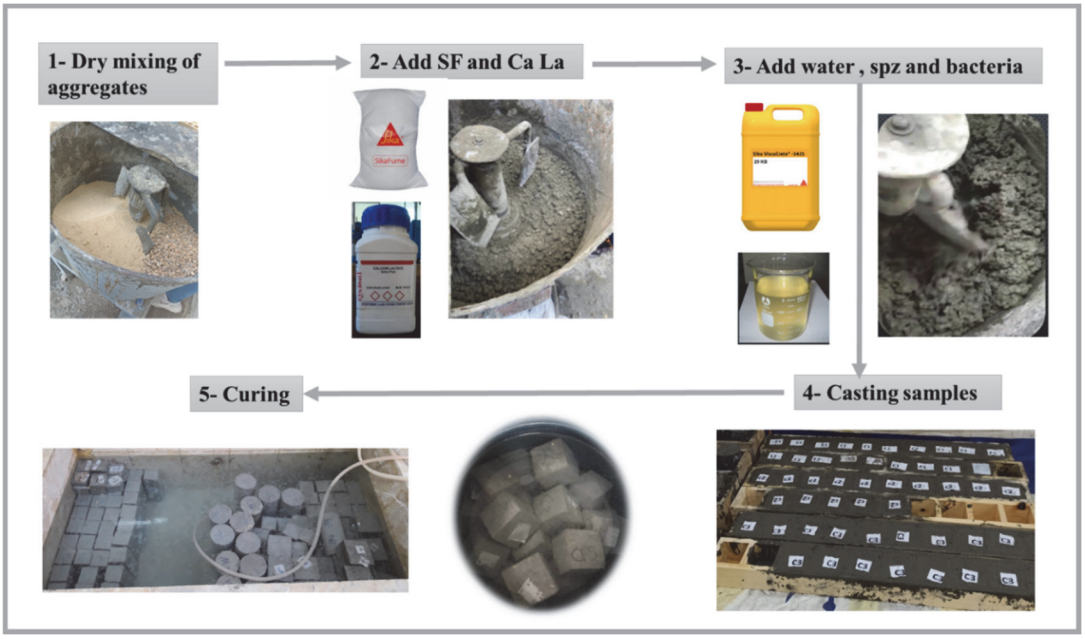


Figure 2: The sample mixing steps [13].

The creation of cracks

Three 100 x 100 x 100 mm cube samples were tested under load and observed for microcracks after 56 days. To ensure the completion of pozzolanic reactions, the specimens were carefully chosen and showed improved properties due to self-healing. Visual inspection confirmed the existence of cracks both before and after loading. Three 100×200 mm cylindrical specimens were also subjected to loads until failure. Certain specimens prone to cracking were tested at 35% of the maximum load. Cube specimens with 2.5% and 5% bacterial content at 56 days were loaded at 65% of the max load and tested at 120 days. Various mechanical, physical, and microstructure analysis tests were carried out.

The compressive strength was evaluated at intervals of 7, 28, 56, and 120 days after the initial casting, per the guidelines outlined in the BS EN 12390-3:2019. For each type of concrete, three specimen cubes measuring 100 x 100 x 100 mm were subjected to testing at different ages, and the average strength for each mix was calculated based on the results of these three specimens. Concrete cylinders with 100 x 200 mm dimensions were also subjected to the Brazilian test to assess indirect tension. Tests were performed using a universal testing machine at 28-, 56-, and 120 days following casting by the standards outlined in BS EN 1881-117:1983. Similar to the specimen cubes, the average strength for each concrete mix was computed based on the results from three specimen cylinders.

The X-ray diffraction (XRD) method was utilized to investigate 75 different attributes of cementitious materials after 120 days, covering both hydrated and anhydrous cement phases. The samples underwent precise grinding in a tubular aerosol suspension chamber before being carefully positioned on a glass fiber filter. The study was carried out using an X-ray model X'-Pert ProPhillips MPD PW 3050/60 diffractometer at the National Research Centre, and a JEOL JSM-651OLV electronic microscope with a magnification capacity of 300,000x was used at Mansoura University Faculty of Agriculture in Egypt. Various magnifications, including 35X, 140X, 1000X, and 5000X, were selected for examining the samples. After a 120-day compressive strength test, concrete samples were collected from the deepest core of the broken specimens. These samples were dried at 70°C, affixed to holders using carbon adhesive, and then coated with gold using a sputter coating evaporator to enhance the imaging of the microstructure surface. The composition of the specimens was analyzed using the Oxford X-Max 20 energy-dispersive X-ray spectroscopy (EDS), providing a comprehensive understanding of the sample's composition. This method permitted a thorough analysis of the sample composition.

The study observed the regrowth and development of cracks using a stereomicroscope equipped with the OLYMPUS SZ 61 camera system. This equipment was situated in the Seed and Tissue Pathology laboratory at Mansoura University (Faculty of Agriculture). The research focused on artificial cracks in samples of different sizes, each with varying crack widths at 1, 7, and 120 days. Fig. 3 shows the experimental setup for visually measuring the cracks.

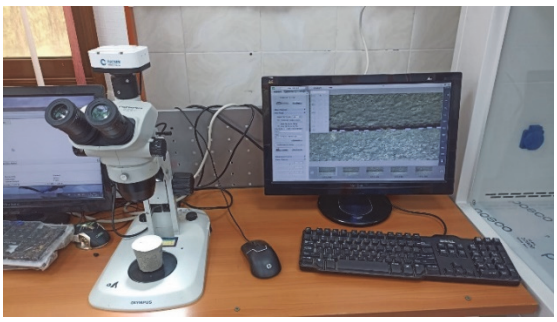


Figure 3: Experimental apparatus for visual measurements of cracks.

RESULTS AND DISCUSSION

Behavior of Compressive Strength - Specimens without pre-cracking

Figs. 4, A, B, C, and D display the results of compressive strength (Fcu) for specimens after curing in freshwater and sulfate for 7, 28, 56, and 120 days respectively. The results examine how bacteria type, quantity, and curing in sulfate impact concrete. The use of bacteria has improved the compressive strength of concrete by increasing the formation of calcite, which fills the pores in the binder matrix and increases compressive strength [14]. The M5 mix, which was cured in fresh water with 2.5% BM bacteria, had superior results in terms of augmenting compressive strength as compared to the M0 control mix. As can be seen in Fig. 4 (D), mix M5's compressive strength rose by 43.34% after 120 days. The compressive strength of BM bacteria increased by 37.94% when 5% of the bacteria were added to freshwater. According to these findings, utilizing 2.5% of both kinds of bacteria works better in freshwater than 5% because there isn't enough nutrient content to

sustain the larger degree of bacterial multiplication. The behavior of the mixtures treated with sulfate is similar to those treated with water at fresh water. The ideal percentage for each kind of bacteria was 2.5%, resulting in a 23.0% increase in compressive strength for BS (mix M14) and a 48.0% increase for BM (mix M15).

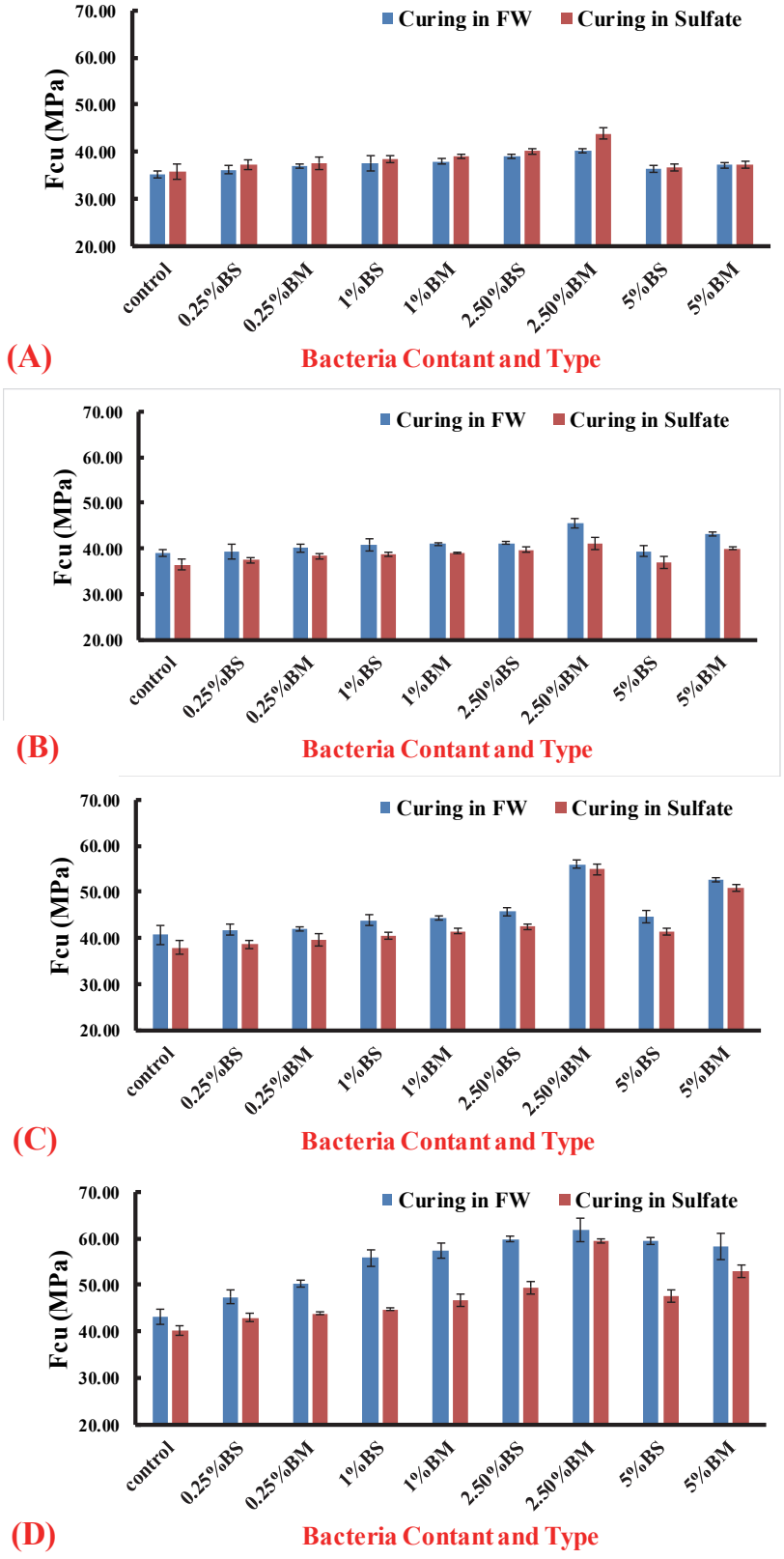


Figure 4: The impact of the curing type on concrete compressive strength for specimens without pre-cracking:(A) at the age of 7 days, (B) at the age of 28 days, (C) at the age of 56 days, and (D) at the age of 120 days.

The compressive strength of concrete under various curing conditions varied significantly depending on the types of bacteria (BM and BS). In general, the BM type outperformed the BS type in terms of significant performance improvement. This is explained by the fact that Bacillus Megaterium can generate more $CaCO_3$ to fill the pores and significantly boost compressive strength. The increased compressive strength shown in BM mixes over other mixes results from the greater $CaCO_3$ concentration.[15]. Calcium acetate is considered a more appropriate calcium source for reinforced concrete materials when MICP technology is used to improve the mechanical properties of microbial concrete.

The results in Fig. 4 clearly show how bacteria in concrete are affected by a sulfate attack. Concrete specimens containing bacteria and control samples were exposed to sulfate for 56 days. Durability parameters, such as compressive strength change, were assessed during this time. In the initial stages of observation, both control and bacterial specimens exhibited a marginal enhancement in compressive strength attributed to the sustained infiltration of sulfate ions into the cementitious matrix. This augmentation in compressive strength during the early phase of sulfate exposure can be attributed to the formation of expansive compounds like gypsum $CaSO_4 \cdot 2H_2O$ and ettringite, which serve to occupy pores and voids, thereby augmenting microstructural density [9]. A gradual loss in strength was observed at 120 days of immersion. The compressive strength of the control mix had decreased by 7.2% compared with the control specimen cured in FW. Because the enhanced sulfate penetration led to a higher accumulation of expanding products in the specimens' pores, this could account for the strength loss measured after seven days of immersion. On the other hand, various bacterial specimens exhibited excellent overall performance when exposed to sulfate. In the cementitious matrix, sulfate ion penetration was significantly decreased due to the biogenic precipitation of $CaCO_3$ crystals. The minimal infiltration of sulfate ions further reduces the production of expansive reaction products that cause concrete deterioration. Although there were some variations in compressive strength, the bacteria specimens did not show a significant decline in strength. Other researchers reported similar findings [16]. The presence of bacteria improved compressive strength by 6.9%,9.05%,11.3%,16.02%,23.0%,48.0%,18.4%, and 31.8%, respectively, for mixes M10, M11, M12, M13, M14, M15, M16, and M17 at the age of 120 days.

Statistical analysis

A fully randomized design was implemented to analyze the experimental data in Fig. 4, ensuring that each condition was equally represented without bias. Each mix underwent three replications during the curing process to bolster the reliability of the results. The measurements obtained were reported as mean values accompanied by their standard deviation (SD), clearly depicting variability within the data set.

To determine any significant differences between the mean values of the various experimental groups, a one-way analysis of variance (ANOVA) was performed. This statistical method allowed for the simultaneous assessment of potential differences among multiple groups. Following the ANOVA, Duncan's multiple range test was applied to identify specific group differences, with a significance level set at $p < 0.05$. This comprehensive approach enabled a thorough examination of the effects of different formulations on the outcomes measured.

Specimens with pre-cracking

The pozzolanic reaction was finished 56 days after the concrete was cast, and the main mechanism became continuous hydration because of the high percentage of unhydrated cement particles in the early stages.

The ratio of compressive strength recovery is higher in specimens with a higher bacteria content. For instance, bacteria BM at a 2.50% concentration shows a noticeable trend, and when 5.0% BM is used, the reloaded fractured specimens' compressive strength is 104.31% compared to 85.89% in unloaded specimens. This is due to an increase in calcium carbonate. The ratio of compressive strength recovery is higher in specimens with a higher bacteria content. This is due to an increase in calcium carbonate. Compared to the 35% preload, the 65% preload produced better outcomes. In Mix 8, the compressive strength recovery ratio for the 65% preload was 104.31%, while the recovery ratio for compressive strength was 107.5%. Compared to the 35% preload, the 65% preload produced better outcomes. In Mix 8, the compressive strength recovery ratio for the 65% preload was 104.31%, while the recovery ratio for compressive strength was 107.5% [17].

When cured with sulfate and preloaded by 35%, the reloaded fractured samples' compressive strengths in comparison to the unloaded specimens were 76.82%, 79.69%, 81.55%, 82.23%, 82.89%, 83.78%, 84.37%, 90.59%, and 94.34% for mixes M9, M10, M11, M12, M13, M14, M15, M16, and M17, as shown in Fig 5 (B). Comparing mix M8, preloaded by 35% and utilizing 5% bacteria BM at RT, with mix M17, cured with sulfate, it was shown that the reloaded cracked samples had compressive strengths of 104.31% and 94.34%, respectively, in comparison to the unloaded samples.

A study referenced as [17] found that a 65% preload produced better results than a 35% preload. In Mix 8 at room temperature, a 35% preload resulted in a compressive strength recovery ratio of 104.31%, while a 65% preload gave a recovery ratio of 107.5%. When mixed with sulfate and preloaded at 65%, the compressed strengths of reloaded cracked

samples for mixes M9, M14, M15, M16, and M17 were 46.64%, 90.54%, 91.34%, 92.33%, and 95.19% compared to unloaded samples. In Mix 32 with sulfate curing, a 35% preload led to a compressive strength recovery ratio of 94.34%, while a 65% preload resulted in a recovery ratio of 95.19%.

After an examination of the data, it was found that reload specimens with a 35% preload using mix M8 with 5.0% of BM and mix M7 with 5.0% of BS displayed compressive strengths of 104.31% for mix M7 and 102.24% for mix M8, as illustrated in Fig 5. In contrast to the specimens that were not reloaded, the compressive strengths of the reload specimens were 104.31% for mix M7 and 102.24% for mix M8 when comparing a 35% preload with mix M8 using 5.0% of BM and mix M7 using 5.0% of BS. This points to a significant difference in the compressive strengths between the reloaded and non-reloaded cracked specimens.

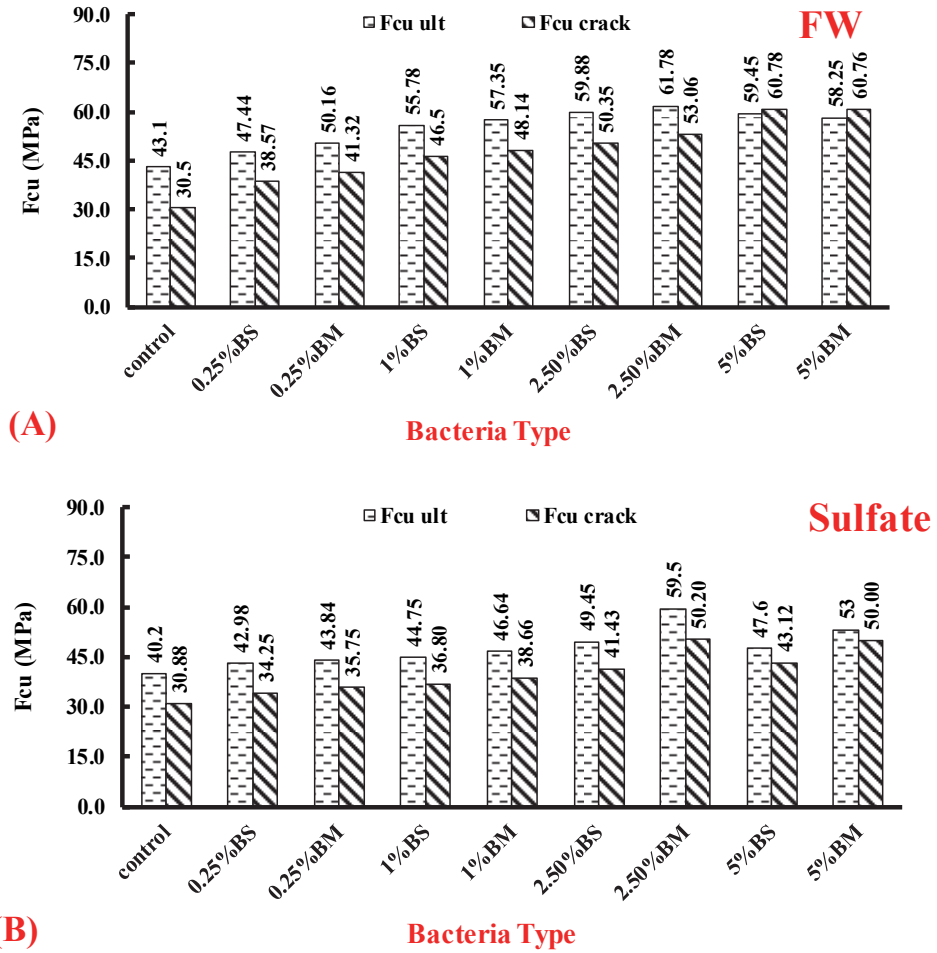


Figure 5: The impact on the compressive strength of strained, cracked specimens by 35% and specimens without prior cracks, due to the kind of bacteria (BM and BS) and bacterial content: When (A) in normal water and (B) cured in sulfate solution.

Based on prior studies, [18], the Bacillus Megaterium (BM), a type of bacteria from the Bacillus family, can increase the compressive strength of concrete by reducing voids within it. This is achieved through the growth of bacterial colonies and the subsequent precipitation of calcite, which fills the voids in the concrete, leading to a higher density and compressive strength. When subjected to a 65% preload, mix M7 with 5.0% of BM exhibited a compressive strength in cracked samples that was 104.31% of the strength of samples without a load, while mix M8 with 5.0% of BS showed a strength equal to 102.24% of the unloaded samples.

When comparing mix M17, which uses 5.0% of BM, with mix M31, which uses 5.0% of BS, in the case of preloading by 65% and curing in sulfate Fig. 6 (B), it was discovered that while the compressive strength of the reloaded cracked samples in mix M17 is equal to 95.19% of the unloaded samples, it is equivalent to 92.33% in mix 31.

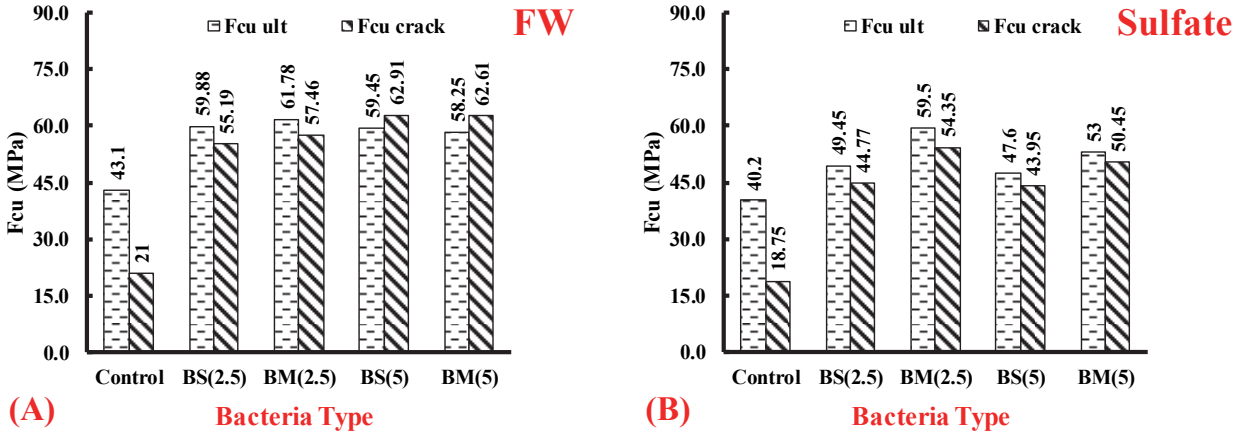
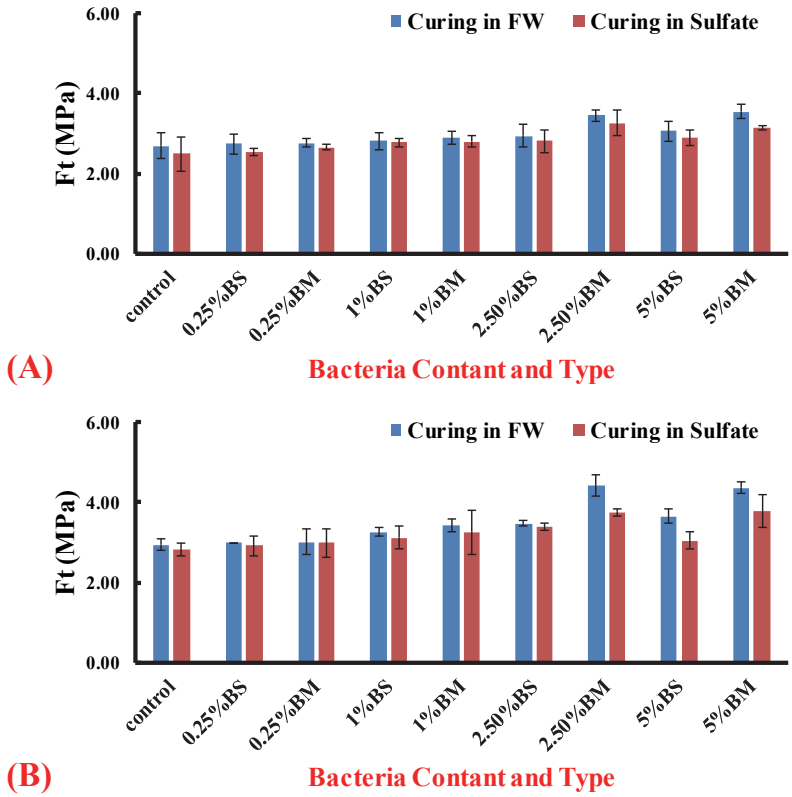


Figure 6: The impact of bacteria content and type (BM and BS) on the compressive strength of specimens with pre-cracking and specimens loaded to 65% after cracks have been reloaded: (A) at Fresh Water (FW), and (B) when curing in a sulfate environment.

Behavior of Indirect Tensile Strength - Specimens without pre-cracking

Figs. 7, A, B, and C show the indirect tensile strength (Ft) results for uncracked specimens at 28, 56, and 120 days. It examines how the concrete is affected by bacteria content, bacteria type, and different temperatures. Using 2.50% bacteria BM in Mix M5 led to the highest tensile strength results compared to control mix M0, with percentages of 27.88%, 50%, and 53.77% at 28, 56, and 120 days, respectively. This is due to the EPS layer created by the bacterial strain. Furthermore, the behavior of the mixtures in sulfate treatment was similar to that of water treatment. The optimal 2.5% percentage for both types of bacteria resulted in a 45.63% increase in compressive strength for BS (mix M14) and a 49.32% improvement for BM (mix M15).



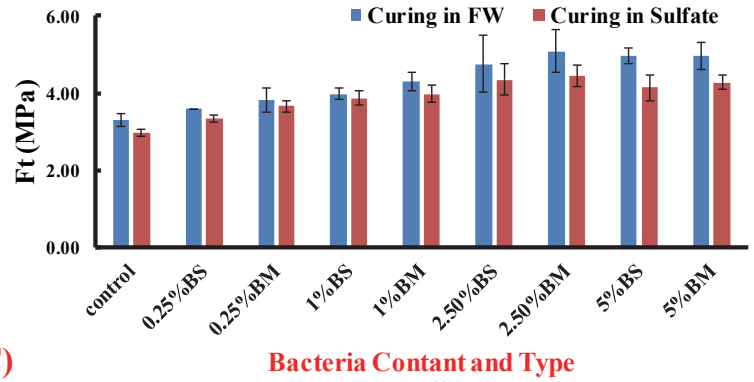


Figure 7: The impact of the curing type on concrete indirect tensile strength for specimens without pre-cracking:(A) at the age of 7 days, (B) at the age of 28 days, (C) at the age of 56 days, and (D) at the age of 120 days.

Substantial variations in the indirect tensile strength are observed when switching from BM to BS bacteria. A comparison of the two bacteria types reveals that BM generally outperforms BS regarding indirect tensile strength, as depicted in Fig. 8. The presence of calcium carbonate due to biochemical processes enhances the ability of the cement-sand matrix to resist loads [19]. Mixing 2.5% of BS bacteria increases M5's indirect tensile strength by 43.8%. Similarly, M6 experiences a 53.77% improvement in indirect tensile strength compared to using BM bacteria in the mixture.

This experiment is designed to investigate the impact of sulfate attack on bacteria in concrete. For 56 days, concrete samples containing bacteria and control samples were exposed to sulfate. Various durability measures, including changes in compressive strength, were evaluated during this time. The results are shown in Fig. 8. In the early stages of the experiment, both the control and bacterial samples experienced a slight increase in compressive strength due to the consistent infiltration of sulfate ions into the cementitious matrix. This boost in compressive strength is attributed to the formation of expansive compounds such as gypsum $CaSO_4 \cdot 2H_2O$ and ettringite, which help fill pores and voids, thereby increasing microstructural density [9]. A gradual decrease in strength was observed after 120 days of exposure. The compressive strength of the control mix had decreased by 11.07% compared to the control sample cured in FW. This strength loss could be due to the higher accumulation of expansion products in the pores of the samples as a result of enhanced sulfate penetration after seven days of exposure. In contrast, when exposed to sulfate, the bacterial samples showed remarkably good overall performance. The penetration of sulfate ions into the cementitious matrix was significantly reduced due to the biogenic precipitation of $CaCO_3$ crystals. The minimal infiltration of sulfate ions also reduced the production of harmful reaction products that can cause concrete deterioration. Although there were some variations in compressive strength, the bacteria samples did not show a significant decline in strength. The presence of bacteria resulted in improved indirect tensile strength by 12.08%, 22.80%, 29.87%, 33.55%, 45.64%, 49.33%, 38.93%, and 43.62%, respectively, for mixes M10, M11, M12, M13, M14, M15, M16, and M17 at the age of 120 days.

Specimens with pre-cracking

Once 56 days had passed since the pouring date, the pozzolanic reaction had fully completed, and constant hydration became the primary mechanism in the concrete because of the high proportion of cement particles that aren't hydrated. However, as concrete was exposed to environmental elements for extended periods, calcium carbonate became the primary mechanism. A maximum load of 35% was applied to each specimen. Fig. 8 shows the comparison between freshly cracked specimens and those without pre-cracking at 120 days. The recovery ratio of indirect tensile strength between reloaded broken samples and unloaded samples of the same mix increased due to the presence of bacteria. For example, when using bacteria BM at 2.50% in fresh water, the indirect tensile strength of the reloaded cracked samples compared to the unloaded samples was 96.27%, and at 5.0% BM, it was 99.4%. This is because of the creation of the Exopolysaccharide (EPS) Layer by the bacterial strain.[19].

Fig. 9 (a) illustrates the comparison between mix M6 with 2.5% of BM and mix M5 with 2.5% of BS. For mix M4, the compressive strength of reloaded cracked samples was measured at 96.27% contrasted with the unloaded specimens, while the indirect tensile strength of mix M7 was found to be 84.24%. The effect on the restoration of compressive strength is also discussed, with previous studies.[18]. highlighting the role of Bacillus Megaterium bacteria in forming calcium carbonate and enhancing material strength. In the case of curing in sulfate, as seen in Fig. 9 (B), the compressive strengths of reloaded cracked samples ranged from 76.17% to 92.99% for mixes M9 to M17. A comparison between mixed M8 at RT and mixed

M32 curing in sulfate revealed compressive strengths of 99.40% and 92.99% for mixes M8 and M17, respectively, using 5% bacteria BM.

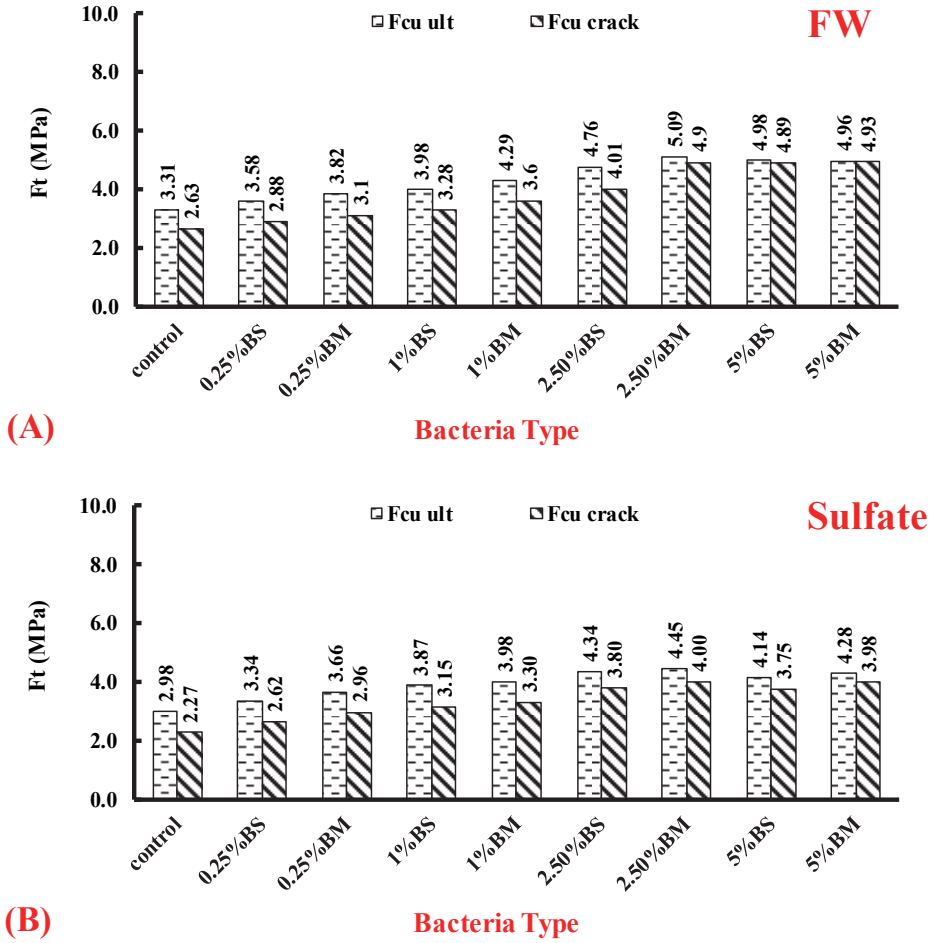


Figure 8: The effect of the type (BM and BS) and quantity of bacteria on the indirect tensile strength of concrete was examined for pre-cracked specimens and those reloaded with a 35% load. These effects were observed (A) during FW and (B) while cured in sulfate.

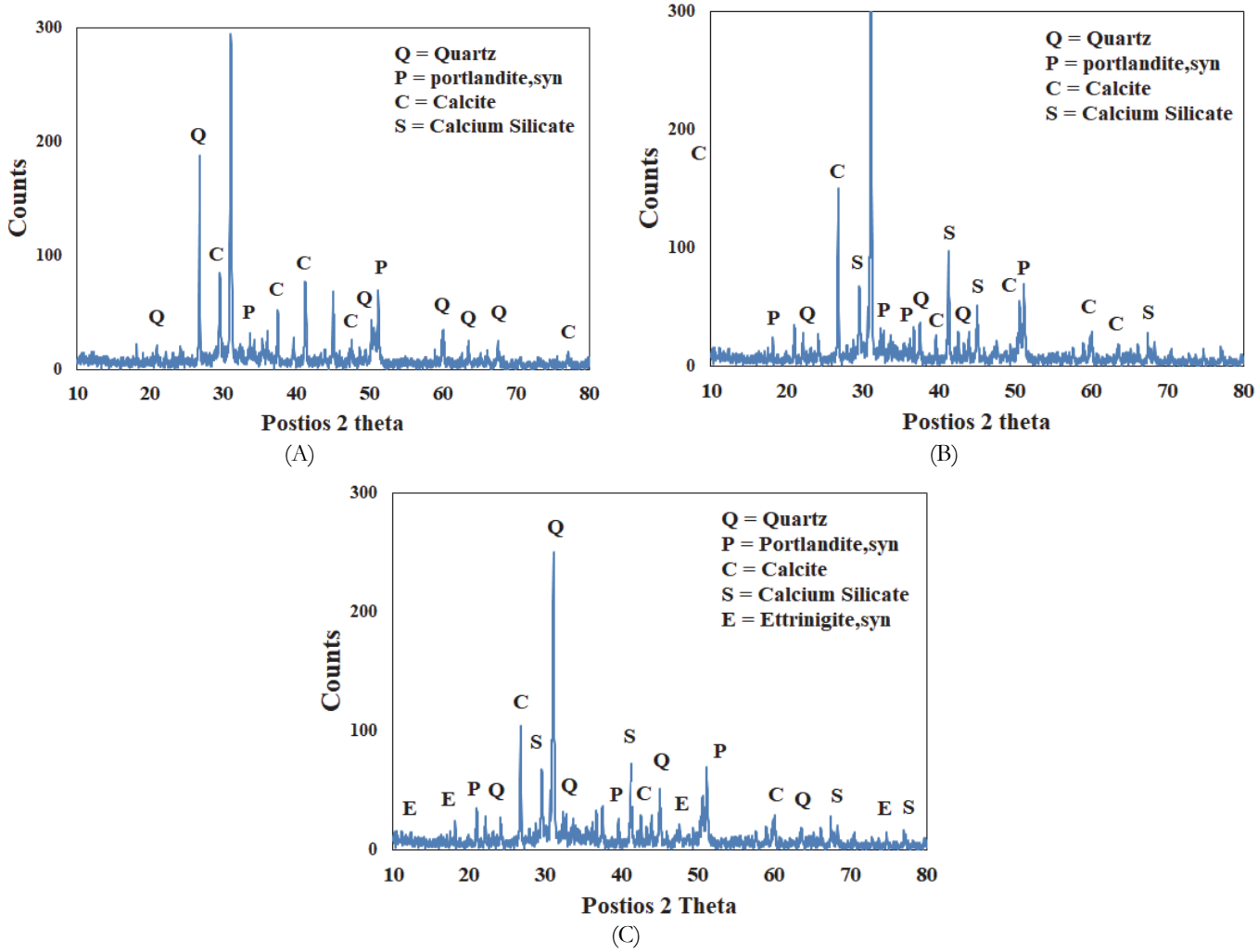
X-Ray Diffraction Analysis

This research used XRD analysis to detect different crystalline phases in concrete samples. The diffraction patterns for bacterial and control samples in various mixtures were compared after 120 days. Fig. 9 displays the outcomes for mixtures M0, M8, M10, M11, M13, and M32. It was observed that the XRD spectra of the bacterial samples displayed additional peaks not found in the control samples, suggesting a higher abundance of calcium in bacterial concrete. For the control mix M0 in Fig. 9 (A), most of the peaks were from silica and quartz because of sand grains, totaling nine. Other studies have reported similar results [20].

Types of Calcites Precipitated, Calcite Formation: The studies indicate that calcite is the predominant form of CaCO3 precipitated during microbial-induced carbonate precipitation MICP. Microbial Influence: Specific bacteria, such as certain Bacillus species, have been shown to effectively induce calcite precipitation, with varying efficiencies based on environmental conditions like pH and temperature [21].

Distinction Between Calcite Sources, Bacterial Impact: The calcite produced by bacteria is often purer and more crystalline than that formed through natural carbonization processes. For instance, Bacillus cereus demonstrated a high capacity for calcite precipitation, achieving significant crack healing in concrete., Natural Carbonization: In contrast, natural carbonization may yield less uniform calcite structures, potentially affecting the overall integrity of the concrete repair [12]. This indicates the bacteria may produce a new silicate phase within the concrete. The existence of crystalline C-S-H in differing intensities was also noted, possibly contributing to the increase in compressive strength. The XRD results confirmed the enhancement in compressive and indirect strength. Additionally, the analysis disclosed the existence of multiple quartz peaks, possibly due to the buildup of sand grains next to cementitious materials that can repair themselves.

The XRD analysis showed that $CaCO_3$ was formed in the empty spaces of the samples. The way $CaCO_3$ was created in concrete with bacteria differed from that in the control concrete. In bacterial concrete, the process of $CaCO_3$ creation was due to calcium hydroxide carbonation, an important product of cement hydration. When comparing the samples treated with water to those treated with sulfates, it was observed that the sulfate-treated samples had many peaks associated with ettringite, while the number of calcium peaks was reduced in these samples. Specifically, when comparing sample M8 to sample M17, it was found that M8 had nine calcium peaks, M17 had seven calcium peaks, and four ettringite peaks.



.Figure 9: XRD results for (A) M0, (B) M8, and (C) M17.

Scanning Electron Microscope (SEM).

SEM and EDS tests were utilized to analyze various bacterial and control concrete samples cured in freshwater or sulfate to study the microstructure of self-healing materials. SEM micrographs were captured using magnifications (35X, 140X, 1000X, and 5000X) at the 120-day casting age. As seen in Figs. 10 and 11, the results indicated that control concrete samples contained more pores and voids than concrete samples contaminated with bacteria. Furthermore, concrete with bacteria exhibited denser hydration products and improved performance, suggesting a strengthening effect. Fig. 11 shows the presence of bacteria spores in concrete gaps, categorized as active spores that produce calcium and inactive void-filling vesicles. The SEM revealed increased calcium carbonate and calcite crystals as the concrete aged due to bacterial activity. These findings demonstrated the effects of different mixes on the presence of ettringite magnesium deposits and calcite crystals [22]. Bacterial Precipitation: Incorporating bacteria such as Bacillus Sphaericus (BS) and Bacillus Megaterium (BM) can enhance the self-healing properties of concrete by precipitating calcium carbonate ($CaCO_3$), which can fill cracks and reduce permeability, thus limiting the ingress of harmful agents that may promote thaumasite formation. The risk of thaumasite formation is not considered in this specific study because thaumasite typically forms in environments with low temperatures (around 5 °C) [23].

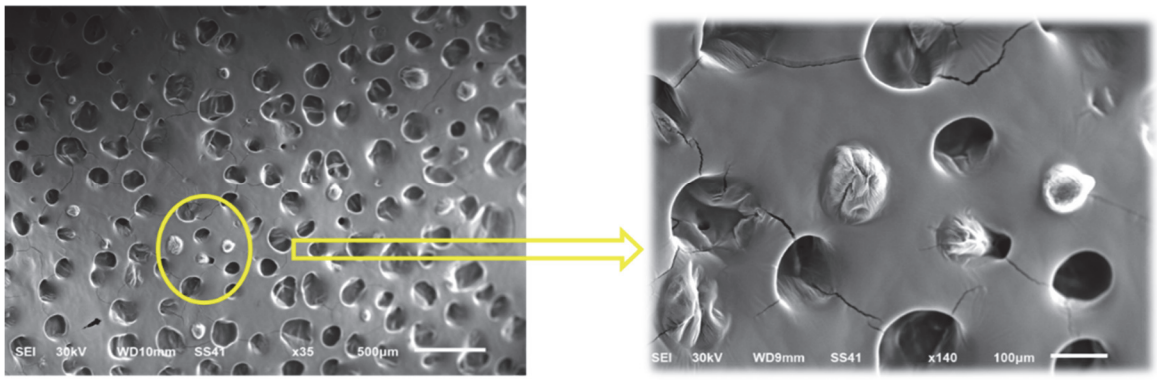
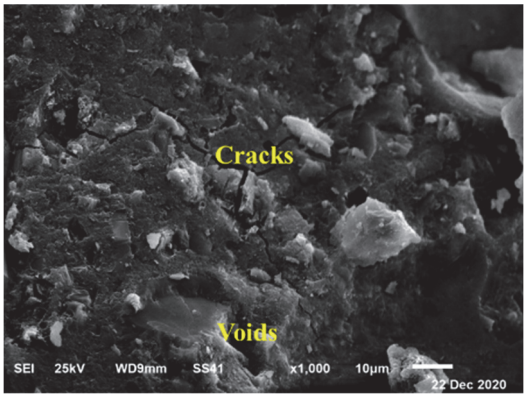
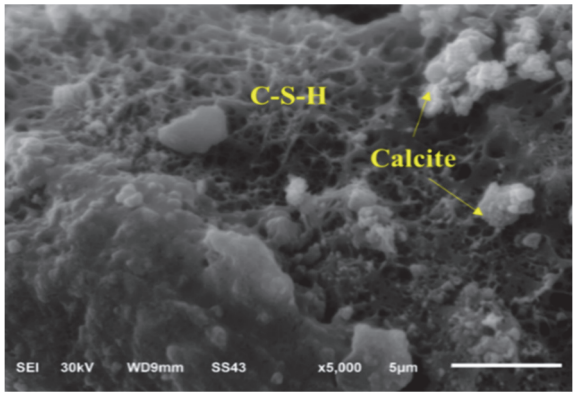


Figure 10: SEM images show filling pores for M8-containing BM bacteria.



(A)



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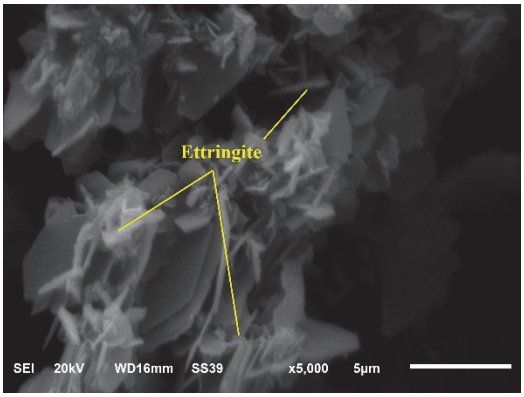
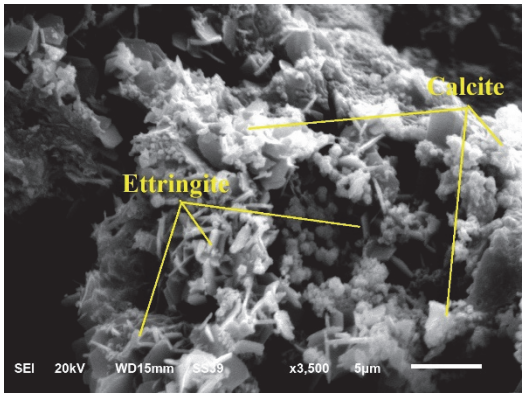
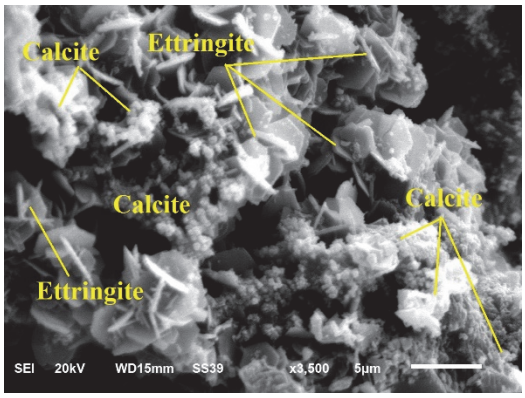


(C)



(D)



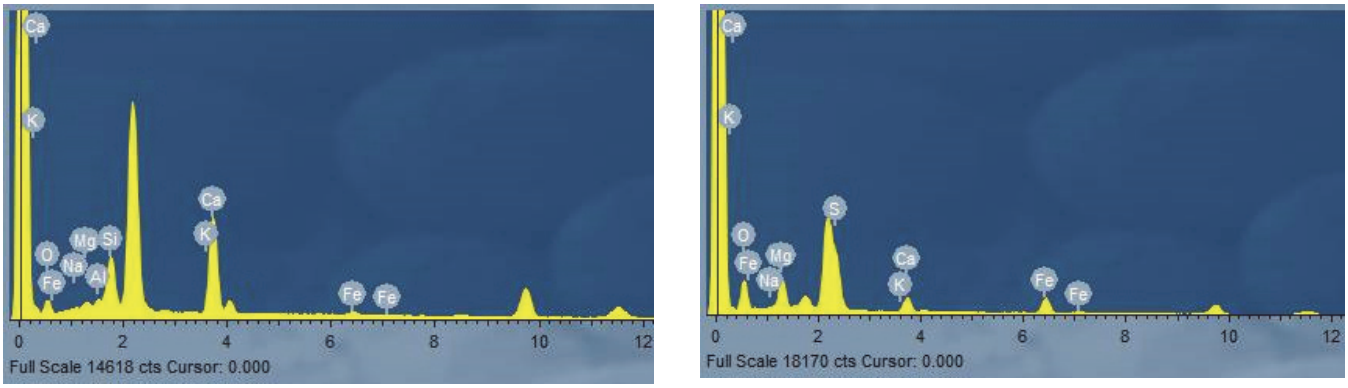
(E)

Figure 11: SEM images for mixes (A) M0, (B) M8, (C) M9, (D) M16, and (E) M17 at 120 days.

Energy-Dispersive X-Ray Spectroscopy (Eds)

The analysis of calcite growth was validated using energy-dispersive X-ray spectroscopy (EDS), which involved examining the peaks that represent the sample's chemical makeup. The elemental components of concrete mixtures were found to include wollastonite (Ca), silicon dioxide (Si), calcium (Ca), oxygen (O), and aluminum oxide (Al). EDS analysis also confirmed that the precipitate is calcium carbonate, comprised of Ca, C, and O atoms. Fig. 12 illustrates the presence of calcium peaks in all samples, with the peaks increasing in proportion to the concentration constant of bacteria and intensifying when spores are added.

Notably, bacterial concrete specimens exhibited a significant increase in the quantity of CaO compared to control specimens, suggesting that these results are attributed to the growth of microorganisms[24]. EDS testing indicated that the calcium percentages were 23.5% for the 5% BM bacterial mixture and at sulfate, while the corresponding mixture at FW had a value of 22.2%. Additionally, the magnesium percentages in EDS testing were found to be 18.03% for the 5% BM bacterial mix and at sulfate, while the corresponding mixture at FW had a value of 2.0%. These findings provide detailed insight into the composition and growth dynamics of the concrete samples.



(A) (B)

Figure 12: EDS spectra of mixes (A) M8 and(B) M17.

Surface Crack Healing Analysis

In Fig. 13, the crack healing process is visually depicted for control and microbial concrete samples with varying levels of BS and BM bacteria. These samples were subjected to curing for 7 and 120 days in freshwater and sulfate solutions. The presence of white precipitates indicates a reduction in crack width across all scenarios. Moving on to Fig. 14, the proportion of healing for mixes M0, M5, M6, M8, M14, M15, M16, and M17 was calculated using Eq1 from a previous study [25]. The findings reveal that the rate of crack healing is positively correlated with higher bacterial content of both BS and BM. For instance, in mix M6, the cracks exhibited healing percentages of 19.7%, 89.69%, and 94.42% after 28, 56, and 120 days, respectively. In comparison, mix M8 displayed healing percentages of 37.28%, 91.06%, and 95.36% over the same periods. Samples treated with sulfates showed more significant healing rates at earlier stages than those treated with water only, likely due to sulfate deposition in the cracks. For example, in mix M14, the healing percentages were 13.26%, 84.55%, and 90.1% after 28, 56, and 120 days respectively, while in mix M15, the percentages were 16.84%, 87.66%, and 91.66% for the same periods. Furthermore, the crack healing rate for 5% of bacteria was higher than 2.5%. For mix M16, the healing percentages were 24.93%, 88.13%, and 92.62% after 28, 56, and 120 days respectively, and for mix M17, the percentages were 39.82%, 90.15%, and 94.88% for the same periods.

$$Heahling\ Rate\% = \frac{initial\ \ crack\ width - final\ crack\ width}{initial\ crack\ width} * 100$$

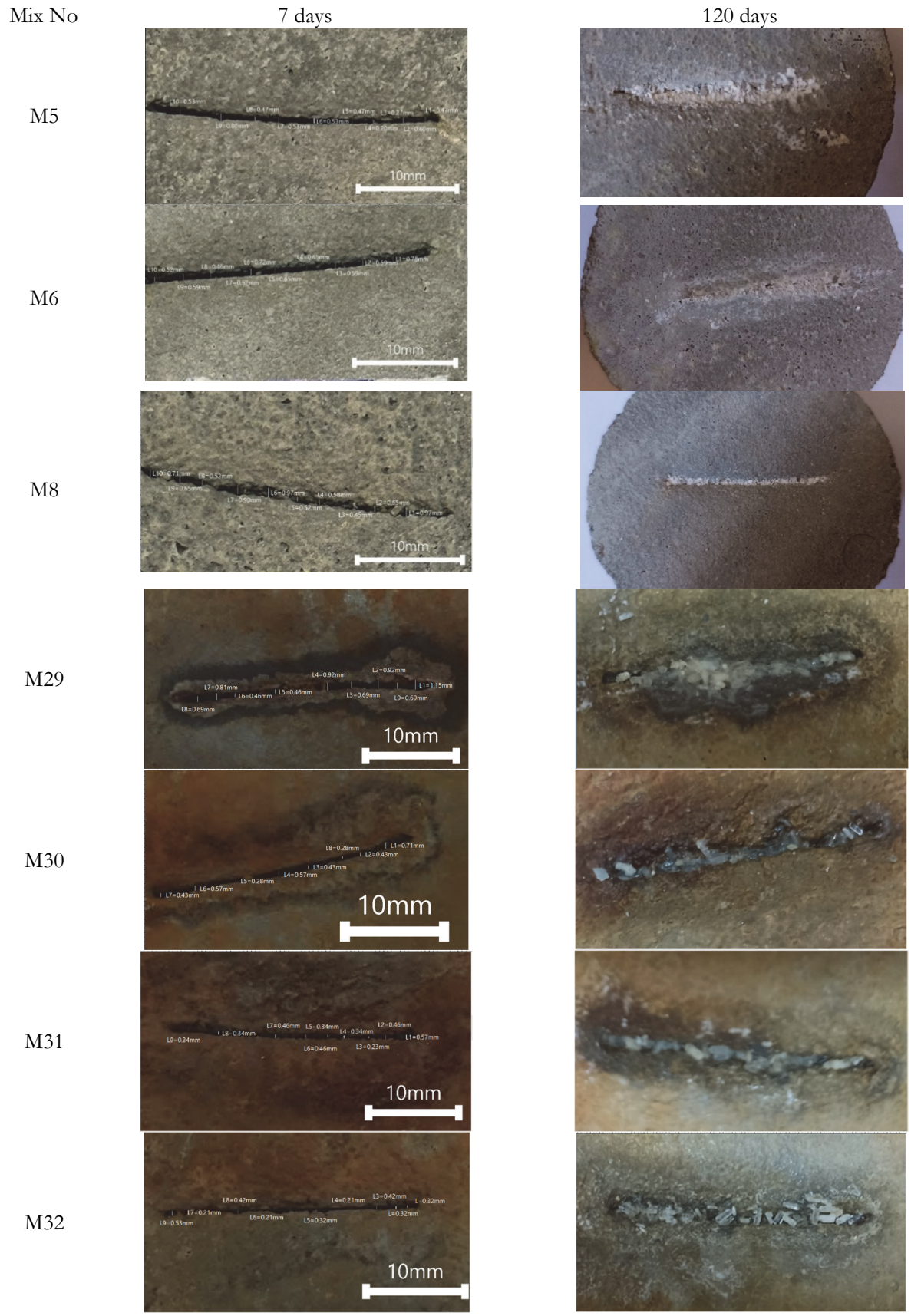


Figure 13: Development of both bacteria's crack healing at different mixtures.

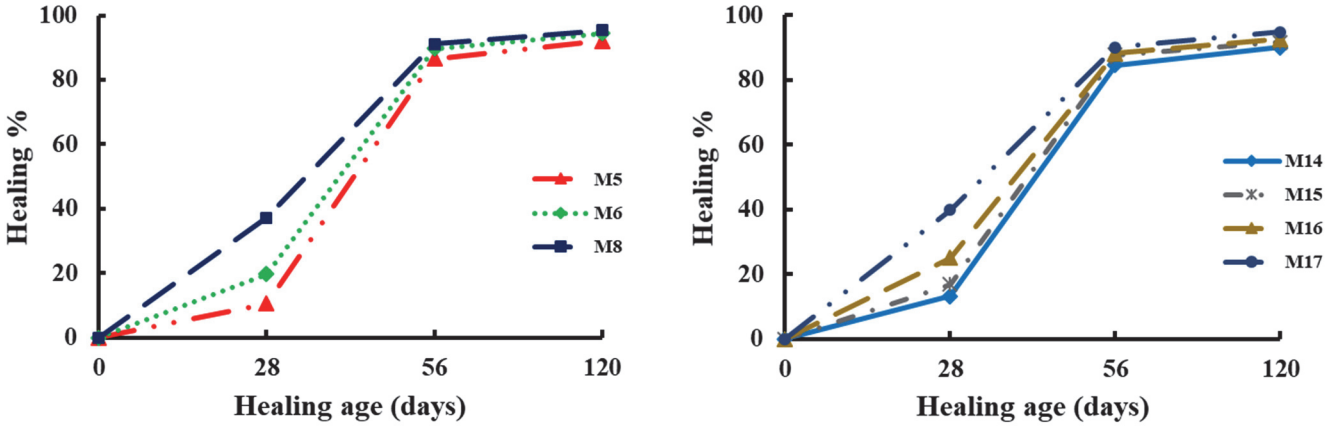


Figure 14: Crack healing rates of bacteria for (A): mixes M5, M6, M8, (B) mixes M14, M15, M16, M17.

CONCLUSIONS

The experimental results presented in this work reveal the following conclusions:

- Incorporating bacteria into concrete improves its mechanical properties and ability to heal cracks.
- In freshwater, the best percentage for both types of bacteria was determined to be 2.5%. As a result, the compressive strength of BS increased by 38.93%, and BM increased by 43.34%.
- Curing in sulfate reduces compressive strength. The control mix decreased by 7.2%, while the mix with 2.5% BM decreased by 3.82%.
- In curing in sulfate, the optimal ratio for both types of bacteria was 2.5%, resulting in a 22.84% improvement in compressive strength for BS and a 47.65% improvement for BM.
- The compressive strength, indirect tensile strength, and flexural strength results for reloading cracked bacterial concrete specimens at 120 days improved compared to the similar mix specimens without pre-cracking.
- The ratio of the compressive strength recovery of the reloaded cracked samples to the unloaded samples was 107.48% for mix M17, indicating that loading by 65% was superior to loading by 35%.
- Analysis of concrete specimens using SEM, EDS, and XRD revealed that the added bacteria could produce significant CaCO3, indicating possible effectiveness in fracture repair.
- The number of calcium peaks reached 8 in M8; however, the number of calcium peaks was 5 in M0.

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