Original Article

Analysis of the Encapsulation of Bacillus Subtilis in Iron Oxide Nanoparticles for the Repair of Cracks and Improvement of Mechanical Properties of Self-Repairing Concrete Subjected to Saline Environments

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Abstract - Concrete faces durability challenges caused by moisture, temperature changes, and chemical attack, which generate cracking, reduce structural integrity, and increase maintenance needs. Cement production also produces considerable environmental impacts, reinforcing the need for sustainable repair strategies. This study evaluated the encapsulation of Bacillus subtilis in iron oxide nanoparticles to improve crack healing and mechanical performance of concrete under saline exposure. Nanoparticles were synthesized by chemical coprecipitation, and viable Bacillus subtilis colonies were isolated and encapsulated to ensure protection in the alkaline matrix. Encapsulated bacteria were incorporated into concrete at concentrations of 0, 2.8×10^9, 2.8×10^{12}, 2.8×10^{16}, 2.8×10^{18}, and 2.8×10^{20} cells/mL. Slump, compressive, tensile, and flexural strengths, as well as sulfate resistance, were evaluated using three replicates per dosage. The 2.8×10^{16} cells/mL dosage achieved the best performance, improving mechanical strength and sulfate resistance compared with the control mix. Crack-healing capacity increased by 18% in induced fissures and was effective in real cracks, confirming activation of the biological repair mechanism. A cost increase of S/70 demonstrated economic feasibility. The findings show that encapsulating Bacillus subtilis in iron oxide nanoparticles enhances durability and offers a sustainable solution for concrete in aggressive environments.

Keywords - Iron oxide nanoparticles, Bacillus subtilis, Self-healing concrete, Concrete exposed to salts.

1. Introduction

There's an expected continual growth in the global concrete market, expected to value USD 972.04 billion, with 4.7% growth annually in the following years, solidifying its position in the industry as the most used structural material in the upcoming years [1, 2]. This necessitates the need to take significant confortable the market growth of the construction sector in Peru, as evidenced by the 17.9% construction sector growth in 2025, as well as cement construction that relies on clinker binders, civil engineering construction, exemplified by the clinker cement production of 876,000 metric tonnes in March [3, 4]. This increasing growth in both sectors highlights the following two most significant challenges: The persistent need for construction materials that have lower environmental impacts without sacrificing their mechanical performance and durability. The high carbon output of cement production still accounts for 7% of global carbon [5, 6]. The durability deficit continues to be an issue, specifically in reinforced concrete

that is subjected to harsh service environments, and has driven the need for materials and technologies that reduce environmental impact without compromising mechanical performance and durability [7]. The second is the durability deficit of reinforced concrete exposed to aggressive service conditions.

Concrete structures operating under high temperature variations, sustained moisture, chloride ingress, and chemically active environments experience accelerated microcracking and enhanced transport processes within the cementitious matrix. These mechanisms promote stiffness degradation, increased permeability, and corrosion risk, leading to premature interventions that dominate life cycle costs and compromise structural reliability [8, 9]. In mature markets, the economic magnitude of this problem is reflected in direct repair expenditures, with annual spending close to USD 21 billion in the United States alone [10], together with

sustained growth of crack repair product markets [11]. Despite this investment, cracking remains a controlling factor for serviceability and durability rather than a resolved maintenance issue.

Industrially used repair products, such as cements, mortars, epoxies, polyurethanes, and acrylics, tend to be reactive and dependent on substrates for adhesion. The performance of all of these products is limited by their bond sensitivity, loss of adhesion caused by microcracking, poor thermal and mechanical compatibility, and degradation due to wet and saline environment exposure. This is especially the case for cement-based products. All of these factors reduce the ability to fully restore monolithic structural performance and, in turn, the service life of structural members. Because of this, these repair products are simply used to mitigate deterioration, instead of addressing the actual problem [12-14].

Microbial-induced carbonate precipitation has been studied as a way to build self-healing cementitious materials, capable of autonomously sealing microcracks in order to avoid costly and manual repairs [15]. The main challenge in the application of this technology is the 'inactivity' of the bacteria due to the cementitious matrix that lacks nutrients, is alkaline, and has a high chloride content. This is especially true in the case of cement-based products that are designed to be water impermeable (i.e., due to the addition of water-repelling chlorides). Many studies demonstrate crack closure, but few demonstrate the ability of the bacteria to survive the closure [16-18]. Even less is the ability for the bacteria to survive the closure and then the matrix to be subjected to saline water and then remain in a stagnant condition for a period of time. In addition, there is rarely a strong connection between the repair of a crack and the recovery of performance standards that are necessary for structural performance.

Proposed carriers improving retention of bacteria and stabilization of their microenvironment within the cement matrix include iron oxide nanoparticles functionalized with APTES. This increases the durability of bacteria and self-healing properties by strengthening calcite precipitation in cracks, compared to unprotected systems [19-21]. However, the most recent studies still do not provide integrated experimental data that distill the effect of nanoparticle-encapsulated Bacillus subtilis on crack closure and mechanical performance, particularly in saline conditions. These conditions pose the greatest healing demand and tend to be the least suitable for traditional repair materials.

Within this framework, the present research evaluates the encapsulation of Bacillus subtilis in APTES-coated iron oxide nanoparticles and quantifies its effectiveness for crack repair and mechanical performance improvement in self-healing concrete subjected to saline environments. By directly linking crack closure behaviour with mechanical response under aggressive exposure, the study provides a durability-driven basis for assessing this bio-nanotechnological approach against the limitations of current repair practices. It contributes quantitative evidence for its application in structural concrete systems.

2. Literature Review

There has been some study into the self-healing concrete technology, which can expand the longevity of building infrastructures and therefore save money. One solid body of research has been the use of microorganisms, which can foster the formation of calcium carbonate ($CaCO_3$) as a means of achieving a natural healing mechanism to close cracks. Based on the reviewed literature, there appear to be three principal approaches: bacterial encapsulation, calcium lactate addition, and microencapsulation.

Bacterial encapsulation has been the most frequently researched methodology, which aims to gain protection of the microorganisms from the concrete's high alkaline state and utilize the microorganisms when cracks are present. A UK-based civil engineering researcher studied self-healing concrete, which included spores of Bacillus pseudofirmus DMS 8715. They applied the concrete to test panels and found that there was very little change in average crack width over a six-month period under varying load conditions, and the cracks actually appeared to seal [22].

Also, in China, the School of Materials Engineering conducted experiments with spore-forming bacteria and identified calcium carbonate precipitation as a method for environmentally friendly concrete repair. Their experimental design spanned multiple compositions, including cement, fly ash, slag powder, water reducer, aggregates, and microbial agents, with varying dosages of 0, 13, and 23.5 kg/m³. Results demonstrated that concrete without the microbial agent reached approximately 50 MPa in compressive strength after 28 days, and that samples with 13 kg/m³ exceeded this value. In a real construction project, a 0.5 mm crack was observed to precipitate a white substance believed to be from bacterial activity. After sixty days, the crack had sealed, demonstrating the efficiency of biological self-healing [23].

The Laboratory of Molecular Environmental Microbiology in Korea examined microorganisms and their ability to precipitate calcium carbonate ($CaCO3$) in concrete. They highlighted that when bacteria are integrated into concrete, they can autogenously repair their own cracks, and thus, reduce repair and maintenance costs, while making it more durable by preventing water absorption and porosity. In addition, this method promotes positive environmental impacts by reducing the amount of cement needed. It is also noteworthy that the method can be used with ordinary concrete materials, and it does not reduce the concrete's compressive strength. However, it's not perfect; with low costs and low microbial survivability, it is not perfect. Even with these issues, the system is considered to be highly

innovative, particularly when trying to obtain proof of concept for an industrial-scale system [24, 25].

In China, during further studies at the School of Civil Engineering and Transportation, the different types of mechanical performances of concrete modified with Bacillus subtilis, sisal fiber, PVA, and expanded perlite were analyzed, with concrete being the focus of the study. The researcher was interested in the collective effect of each of the mentioned additives and the mechanical strength properties. The study concluded that the highest compressive strength was achieved when PVA and bacteria with a dosage of 2.0 optical density were used. Bacillus subtilis even further overshadowed the roles of sisal fiber and expanded perlite, thus playing a major role in the enhancement of concrete strength. Various studies have been conducted, and the results should indicate the possible role of bacteria in bioconstruction, which promotes the development of a better internal matrix that is capable of withstanding higher mechanical stresses [26].

As for the most pertinent strategies, one more common technique was the simple mixing of bacteria and a nutrient source, calcium lactate, which aids the process of calcium carbonate precipitation. Accordingly, in India, from the Institute of Technology, studies were carried out on the compressive strength of self-healing concrete, a developing technology with importance for prolonging the service life of a structure. The research used Bacillus subtilis at concentrations of 10^4, 10^5, and 10^6 cells/ml and calcium lactate at 0.985, 1.97, and 3.94 kg/m³ to determine the influence of these parameters on the compressive and flexural strength of the concrete. The findings indicate that the optimum values of mechanical properties were attained at 1.97 kg/m³ of calcium lactate and 10^5 cells/ml concentration of bacteria, which were greater than 40 MPa in compression and 4 MPa in flexural strength, after 56 days of curing, thus certifying the beneficial impact of this combination [27].

Moreover, the Civil Engineering Department of India studied the use of Bacillus Subtils spore and calcium lactate as a nutritious source, bearing in mind that concrete, one of the most common materials in construction, will always develop cracks. To improve mechanical performance, basalt fibre was incorporated. It was found that bacterial concrete increased its compressive strength as compared to conventional concrete and showed 20%, 24%, and 27% compressive strengths at water-cement ratios of 0.45, 0.4, and 0.35, respectively. This was accomplished with a mix design of 359 kg/m³ cement, 703.13 kg/m³ fine aggregate, 1222.33 kg/m³ coarse aggregate, 161.54 kg/m³ water, 10 CFU/ml bacterial concentration, calcium lactate 1.795 kg/m³, basalt fibre 4.05 kg/m³, and a chemical admixture of 1.79 kg/m³ [28, 29].

Also, in Nashik, self-healing concrete research was done in the Department of Civil Engineering in order to solve the frequent problem of the cracking of structures. Tests were done on the compressive strength and crack repair while using the bacterium Bacillus subtilis. It was found that at 4.5×10^{12} cells, the concrete reached a compressive strength of 32.2 MPa at 28 days. For the self-healing process, cubic specimens were made and cured with a whitish solution from a 1:2 mixture of the bacteria to water. A reduction in crack width was observed over time, confirming the system's ability to repair cracks [22].

As for Taiwan's Department of Civil Engineering, Taiwan's Department of Civil Engineering looked into other methods of crack repair, since materials such as epoxies and acrylic resins are environmentally unsustainable. In this case, Bacillus pasteurii was used as a self-healing agent coupled with a urea (1 mol) and calcium acetate (0.5 mol) nutrient mixture. The mixture was used quó both cylindrical specimens and concrete beams. The greater calcium and urea soil was positive and resulted in the repair of cracks of 0.1 and 0.2 mm. This reinforced the need for positive results with adequate biological self-healing processes [30].

In a similar manner, the Department of Civil Engineering in India studied the use of Bacillus subtilis in enhancing the mechanical properties and minimizing the cracks of concrete. The tests showed that the specimens treated with bacterial solution attained 4 N/mm² and 0.16 N/mm² greater compressive and tensile strengths, respectively, than those treated with water only. Moreover, it was observed that the self-healing of concrete occurred much faster. This led the researchers to conclude that the use of this bacterium was appropriate for improving the mechanical properties of concrete and for structural repair applications as well [31].

Another relevant method is microencapsulation, which consists of embedding bacteria within microcapsules, or micrometric systems that disintegrate upon casuistic rupture, releasing microorganisms and nutrients at a controllable location. Likewise, it can preserve bacterial viability for long periods of time and initiate the repair process when it is needed. Thus, in Thailand, the Department of Civil Engineering studied microencapsulated bacteria for repairing shrinkage-induced cracks in concrete, having spores at 0, 0.5, and 1 percent proportions of the weight of the cement. Results were promising, as 1% addition sealed the cracks in 3 days. However, a decrease in compressive strength was observed after 28 days, which was attributed to the incorporation of nutrient(s) for bacterial activation [32].

2.1. Critical Synthesis Identified Gaps and Novelty of the Study

The reviewed literature confirms that self-healing concrete based on bacterial calcium carbonate precipitation shows significant potential for autonomous crack sealing and for improving selected mechanical and durability indicators [22-32]. However, most studies have focused on crack closure observations under controlled laboratory conditions or on

isolated gains in compressive strength, without systematically integrating mechanical recovery with the long-term stability of the bacterial system in aggressive environments [23, 27, 22, 31].

Recurrent limitations are also identified in bacterial incorporation methods. Approaches based on direct encapsulation, lightweight aggregates, hydrogels, or microcapsules have shown temporary improvements in bacterial viability, but exhibit accelerated degradation, loss of activity in highly alkaline matrices, and limited performance under saline exposure or prolonged wetting cycles [24, 25, 32]. Similarly, systems relying on external nutrient sources such as calcium lactate or urea have demonstrated short-term efficiency, but are constrained by rapid nutrient depletion and a progressive reduction in self-healing capacity [27, 28, 30].

A critical gap in the state of the art is the lack of studies that simultaneously evaluate bacterial viability, crack sealing performance, and mechanical response of concrete under saline conditions, despite this scenario representing one of the most demanding environments for durability in coastal and aggressive exposure infrastructures [22, 32]. Moreover, most investigations do not establish a clear link between the self-healing mechanism and mechanically relevant parameters, limiting the transferability of results to real engineering applications.

Within this context, the novelty of the present study lies in the integrated evaluation of Bacillus subtilis encapsulated in APTES functionalised iron oxide nanoparticles, analysing its combined effect on crack self-healing and mechanical performance of concrete under saline exposure. In contrast to previous investigations [22-32], this work directly links bacterial system stability with functional recovery of the material, providing experimental evidence oriented toward durability and structural applicability of bio-nanotechnological solutions in concrete.

3. Materials and Methods

3.1. Concrete

Concrete is a versatile construction material. It is made of liquid cement, water, and aggregates, and gains strength and durability during the curing process, solidifying into a rock-like state. Because of this flexibility, the material can be used in a range of pavements and structures. However, if used improperly or if there is insufficient attention to detail, it can weaken a structure's performance. Concrete's cracks are a big setback in the process. They can arise during the initial setting or later on during the hardened state. These can be caused by thermal changes, plastic shrinkage, or plastic settlement. Figure 1 shows a range of cracks that can be created in the setting process. Example (a) is a surface crack that is the result of drying shrinkage and thermal changes. Example (b) is a plastic shrinkage crack, which occurs when concrete that is in a plastic state starts to lose water. (c) shows a plastic settlement crack, which is created when freshly poured concrete settles around the reinforcements. Finally, (d) shows a structural crack, linked to the quality of a structure's design, load capacity, and the shifts in the structural elements.
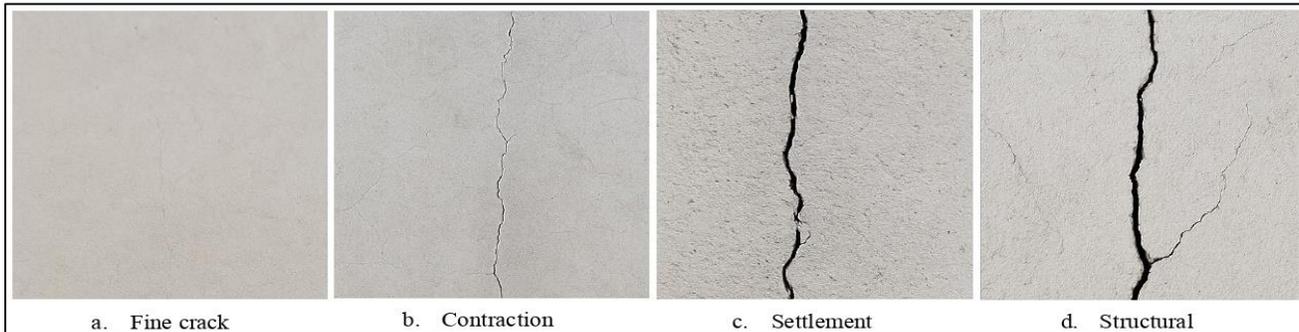

Fig. 1 Common types of cracks in concrete: (a) fine shrinkage cracks, (b) plastic shrinkage, (c) plastic settlement, and (d) structural cracks

3.2. Nanoparticles

Nanoparticles are particles measuring between 1 and 100 nanometres with unique properties due to their small size, as their large number of atoms on the surface gives them special characteristics such as high reactivity or conductivity [36]. They can form naturally through biological or geological processes, such as biomineralisation or rock erosion, or artificially synthesised using methods such as grinding, evaporation (top-down approach), or molecular self-organisation and chemical reduction (bottom-up approach) [37]. Figure 2 shows the top-down and bottom-up approaches used in the synthesis of nanoparticles. The top-down approach starts with a macroscopic or bulk material, which is reduced to the nanometric scale using physical techniques such as mechanical grinding, lithography, or ion etching. This method allows control over particle size through external processes, although it can generate a wide distribution of sizes and surface defects. In contrast, the bottom-up approach is based on the self-organisation of individual atoms or molecules, which, through chemical or physicochemical processes such as chemical reduction, controlled precipitation, or molecular self-assembly, form nanoparticles with high uniformity and precise control of their morphology. In this research, the

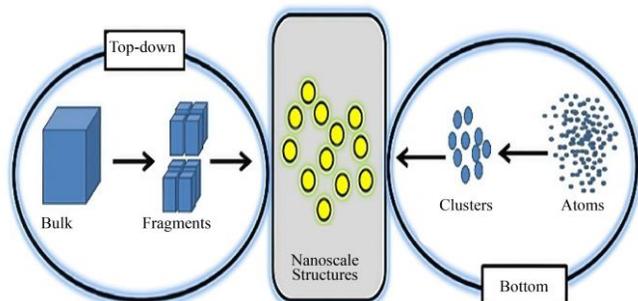bottom-up approach was used, specifically through the chemical coprecipitation method.

For the bottom-up synthesis of Iron Oxide Nanoparticles (IONPs), two solutions were prepared: solution A, containing 10.81 g of ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), and solution B, containing 5. 56 g of ferrous sulphate heptahydrate ($FeSO_4 \cdot 7H_2O$), both dissolved in distilled water. Solution B was added dropwise to solution A under vigorous stirring at 300 revolutions per minute. The mixture was then heated to 80°C until it turned black, indicating the formation of nanoparticles. Next, sodium hydroxide (NaOH) at 0.8 mol was added to induce precipitation, and stirring was continued at 300 rpm to ensure the dissolution of the NaOH. The nanoparticles were filtered, washed three times with ethanol and distilled water, and dried in an oven at 80 °C overnight. The procedure described is schematically represented in Figure 3.
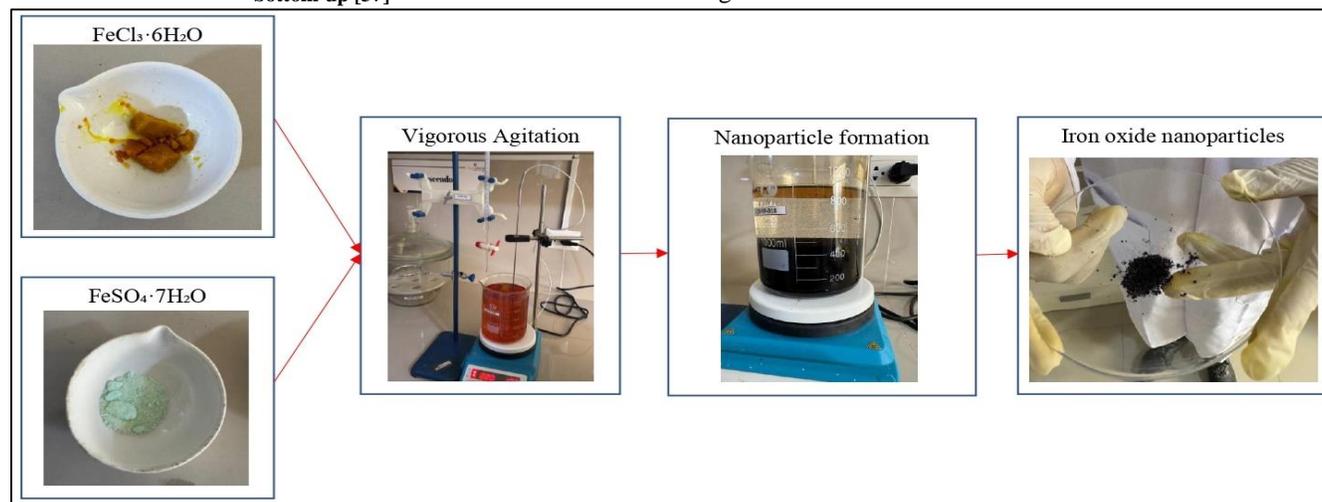

Fig. 2 Outline of nanoparticle synthesis methodologies: top-down and bottom-up [37]

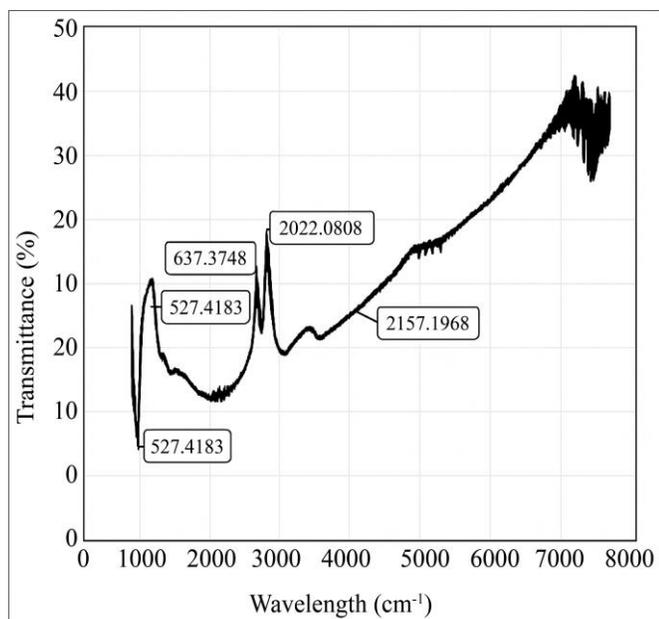**Fig. 3 Synthesis process of Fe₃O₄ nanoparticles by coprecipitation of ferrous and ferric salts with NaOH**

Fig. 4 FTIR spectrum of the iron oxide nanoparticles, showing characteristic absorption bands at 530 cm⁻¹ and 628 cm⁻¹ [37]

Figure 4 shows the spectra obtained by Fourier Transform Infrared Spectroscopy (FTIR) of the synthesised Fe₃O₄ nanoparticles, where absorption bands at 530 cm⁻¹ and 628 cm⁻¹ are identified, corresponding to the vibrations of the iron-oxygen (Fe–O) bond, confirming the formation of the characteristic structure of iron oxide.

A band at 2157 cm⁻¹ is also observed, attributed to compounds with carbon bonds, originating from the reagents and the aqueous medium used during synthesis. These results confirm that the nanoparticles have the appropriate composition for application in research.

3.2.1. Bacillus Subtilis

Bacillus subtilis is a gram-positive rod-shaped bacterium, commonly found in soil, non-pathogenic, and widely studied for its ability to form resistant spores, synthesise bioactive compounds, and degrade organic matter, making it useful as a model in microbiology and in industrial applications such as enzyme production, antibiotics, bioremediation, and controlled release of microorganisms [38, 39].

A nutrient agar and a Petri dish were acquired and then sterilized using an autoclave at 121 °C for 15 minutes. After both the nutrient agar and the Petri dish were cooled and brought into the lab, 5 grams of peptone were measured and mixed with 5 grams of NaCl and 28 grams of nutrient agar into a clean 1-liter glass bottle with 1 liter of distilled water. The bottle was heated to 100 °C to ensure that all the agar was dissolved, then the medium was cooled again before being poured into the plates. The newly created agar was allowed to solidify for 30 minutes at room temperature. The plates were placed into a laminar flow cabinet and subjected to UV light for 10 minutes to ensure that no environmental contaminants would interfere with the results. Using a sterile inoculating loop, a small amount of the bacterial sample was streaked onto the plates. The plates were then placed in an incubator at 37 °C for 24 hours to allow the bacterial colonies to grow. After incubation, bacterial growth was observed, ensuring that the Bacillus subtilis colonies were isolated, pure, and free of contamination, ready for further use, as shown in Figure 5.
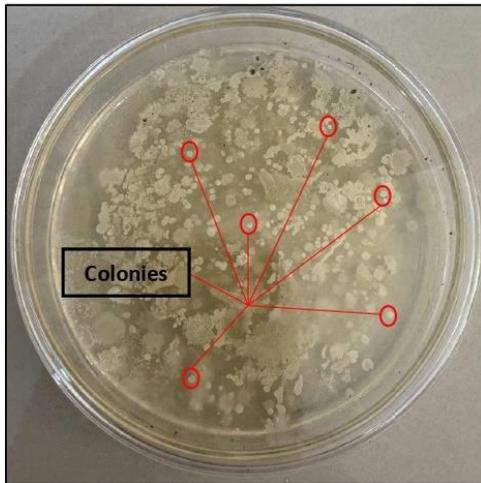

Fig. 5 Pure colonies of Bacillus subtilis (Own elaboration)

Once the Bacillus subtilis bacteria had been obtained, spores were produced using a process that began with the selection of a pure colony, previously isolated and free from contamination, using a sterile loop. This colony was transferred to a sterile conical tube containing a nutrient broth prepared with 0.5% peptone, 0.5% glucose, and 0.05% yeast extract, dissolved in distilled water.

The medium was sterilised in an autoclave at 121°C for 15 minutes. The liquid culture was then incubated at 37°C with agitation at 120 rpm for 24 hours to promote bacterial growth. After this time, to induce spore formation, the culture was placed in a water bath at 80°C for 10 minutes, which allowed the active cells to be eliminated and only the spores (Figure 6), which are more resistant, to be preserved. Finally, the mixture was centrifuged at 3000 rpm for 15 minutes, and the sediment containing the pure spores was collected and stored at 4 °C for later use or immediate encapsulation.
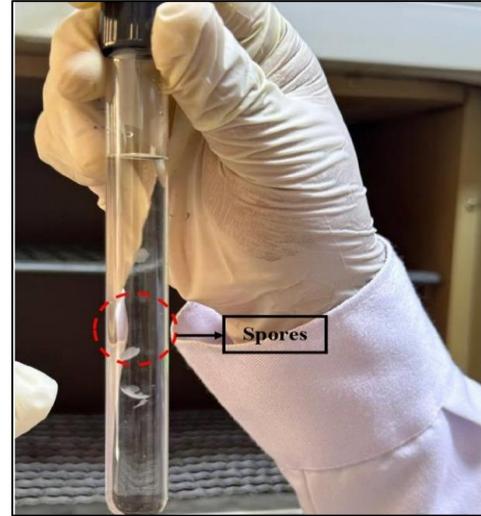

Fig. 6 Bacillus subtilis spores after heat treatment (80 °C, 10 min)

The bacterial concentration was estimated using equation (1):

$$Concentration \left(\frac{cells}{ml}\right) = \frac{Total\ number\ of\ cells}{Suspension\ volume\ (ml)} \qquad (1)$$

Once the Bacillus subtilis spores were obtained, they were encapsulated using iron oxide nanoparticles (IONP) at a concentration of 250 µg/ml [37]. Previously, these nanoparticles were dispersed in distilled water by sonication for 15 minutes at 35°C, in order to break up any agglomerates and form a stable suspension, also generating cavities that would facilitate binding with the spores. This IONP suspension was then mixed with the corresponding concentrations according to the dosages indicated in Table 1 and stirred for 2 minutes, thus achieving effective encapsulation and obtaining a homogeneous suspension of spores immobilised in the nanoparticles (Figure 7).

Fig. 7 Encapsulation of B. subtilis spores in Fe₃O₄-APTES nanoparticles by stirring and sonication.

3.3. Measurement Indicators

A concrete with a characteristic strength of f'c = 210 kg/cm² was designed by the laboratory C3 Ingeniería Especializada, in strict accordance with the ACI 211.1-91 guidelines for concrete mix proportioning. This strength level was selected because it represents a standard design value frequently used in structural concrete in Peru, ensuring practical applicability of the results to real construction scenarios.

To this base mixture, Bacillus subtilis encapsulated in iron oxide nanoparticles was incorporated at concentrations of 0, 2.8×10^9, 2.8×10^{12}, 2.8×10^{16}, 2.8×10^{18}, and 2.8×10^{20} cells/ml. The amounts used have been defended based on previous research and the establishment of the thresholds of bacterial tolerance in cementitious media. More accurately, Mahmood et al. [37] mentioned that encapsulation concentrations on the moderate side triggered the optimal improvements in crack repair efficiency and compressive strength.

Nevertheless, he mentioned that too little or too much encapsulated bacterial activity can repair the cracks and thus bacterial activity undershooting and bacterial overabundant. Therefore, this research aimed at establishing the optimal viable concentration of the Peruvian cement-bacteria system by starting at the lowest viable concentration of bacteria (10^9 cells/ml) and subsequently increasing to the upper threshold of 10^{20} cells/ml in order to examine the effects of saturation.

This approach allows for broader international comparisons and assessments of bacterial performance, survival, and self-healing at various levels of concentration, which is critical for the practical applicability of self-healing concrete in the future.

3.3.1. Materials Used

In Table 1, we can see the mixture design that seeks to analyze the effects of varying levels of encapsulated bacteria in concrete in the cell concentration range of 0 to 8.4×10^{21} cells/ml. The design employed 30 ml of distilled water per m³ [40, 41], which yielded a range of bacterial concentrations from 0 to 2.8×10^{20} cells/ml. Additionally, the design incorporated nanoparticles at a constant concentration of 7.5 mg/m³ [37]. As for the base materials, the quantities of cement (409 kg/m³), sand (941 kg/m³), and crushed stone (779 kg/m³) were kept constant in order to avoid variations that could affect the properties of the concrete. Likewise, a corrected water volume of 198 l/m³ was used for all mixtures, carefully adjusted to maintain a controlled water-cement ratio in each dosage. It is important to note that the 30 ml/m³ of bacterial solution was not added in addition to the 198 l/m³ of water, as it was incorporated by partially replacing the mixing water, keeping the total volume of liquid constant in order to ensure that the treated concrete had the same basic workability as the control concrete [41].

Table 1. Bacterial concentrations used in concrete mixtures

Concentration (cells/ml)	Cells (cells/m³)
0	0
2.8×10^9	8.4×10^{10}
2.8×10^{12}	8.4×10^{13}
2.8×10^{16}	8.4×10^{17}
2.8×10^{18}	8.4×10^{19}
2.8×10^{20}	8.4×10^{21}

Regarding the variation in the amount of water, the increase from the initial theoretical value of 185 litres to the final corrected value of 198 litres was necessary due to the actual moisture content of the aggregates used during the research, specifically in the fine aggregate (sand) and coarse aggregate (stone). Formula (2) was used to calculate this correction:

$$Corrected\ water\ (l) = \frac{Actual\ humidity - Absorptio}{100} x\ Dry\ aggregate\ mass \qquad (2)$$

When applying this formula to sand, which had an actual moisture content of 1.02%, absorption of 1.4% and dry mass of 941 kg, a correction of -3. 57 litres; while for the coarse aggregate, with actual moisture content of 0.48%, absorption of 1.66% and dry mass of 779 kg, the calculated correction was -9.23 litres. When the absolute values of both corrections were added together, a total of 12.8 additional litres was obtained, which was added to the initial theoretical volume of water. For this reason, the final corrected water volume for the mixture was 198 litres, a value adopted to ensure the workability and adequate consistency of the concrete.

3.3.2. Physical Properties
Slump

The slump test is used to measure the consistency or workability of fresh concrete, indicating how fluid or dry it is before hardening. To ensure the correct execution of the procedure, standard MTC E-705 [42] was used. According to this standard, the test was performed with an Abrams cone that was filled in three equal layers, each compacted with 25 blows of a metal rod; then, the concrete was levelled, and the cone was removed vertically in 5 to 10 seconds. Finally, the difference in height between the cone and the highest point of the settled concrete was measured, which represents the slump value in centimetres, as shown in Figure 8.
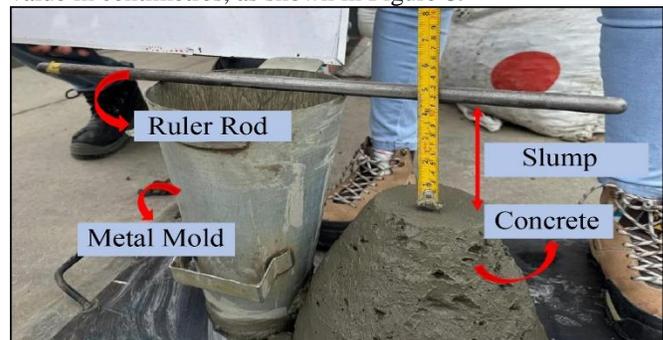

Fig. 8 Slump test of fresh concrete using an Abrams cone.

3.3.3. Mechanical Properties

Compressive strength determines the ability of concrete to withstand loads without fracturing. In this test, in accordance with standard MTC E 704 [42], cylindrical specimens measuring 15 cm in diameter and 30 cm in height were prepared and cured for 7, 14, and 28 days. They were then subjected to continuous axial loading in a hydraulic press until they reached their failure point. Additionally assessed was the indirect tensile strength. This assesses the concrete's ability to withstand split tensile forces. For this purpose, as per the standards of MTC E 708 [42], cylindrical test specimens of 15 x 30 cm, 7, 14, and 28 days were prepared. These were horizontally positioned in a testing machine, and a diametral load was applied to them until they failed. Flexural strength was also assessed, which is the ability of the concrete to withstand deflection caused by forces applied transverse to the length of the member. In compliance with MTC E 711 [42] standards, prisms of 15 x 15 x 60 cm were used, with a 28-day cure, and these were deflected by a load applied at mid span until failure.

3.3.4. Resistance to Sulfates

The sulphate resistance test was carried out using cylindrical concrete test tubes measuring 15 cm in diameter and 30 cm in height (6in× 12in), in accordance with Peruvian Technical Standard E.060 [43] for severe sulphate exposure. The sodium sulphate (Na_2SO_4) solution was prepared by dissolving 10 grams of Na_2SO_4 in one litre of drinking water and left to stand for 24 hours. The test samples were completely immersed in this solution and exposed for 7, 14, 28, and 56 days, following the procedures established in ASTM C1012 Standard Test Method for Length Change of Hydraulic-Cement Mortars Exposed to a Sulfate Solution. At the end of each immersion period, the test samples were removed and subjected to an axial compression test in a universal testing machine, applying load until failure, as shown in Figure 9.

Fig. 9 Cylindrical concrete test tube after compression testing

3.3.5. Self-Repairing Concrete

The self-repairing concrete test was performed on disc-shaped specimens (4″ diameter × 2″ height). After standard curing for 28 days, a controlled load equivalent to 60% of their maximum compressive strength (determined according to ASTM C39/C39M) was applied to induce cracks. Once the cracks were generated, the specimens were transferred to a humid curing environment for an additional 28 days, allowing the activation of bacteria present in the concrete, which promoted the formation of calcium carbonate ($CaCO_3$) as a repair agent. Over the 56-day monitoring period, crack evolution showed a progressive closure accompanied by $CaCO_3$ precipitation within the fissures, confirming the activation of the biological self-healing mechanism. This test was fundamental for evaluating the behaviour of concrete with bacterial self-healing capacity.
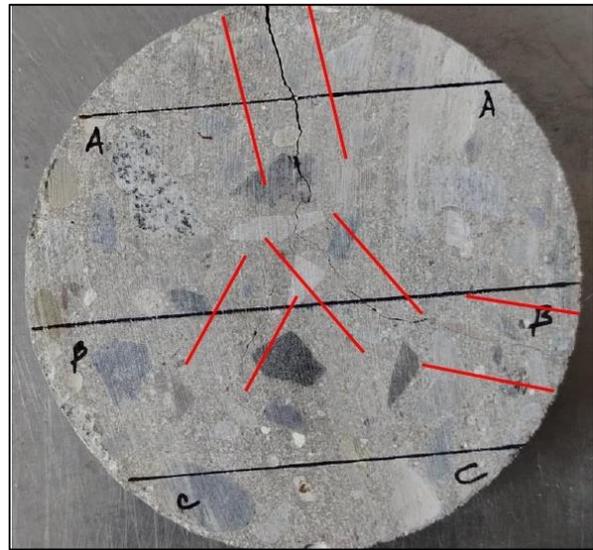

Fig. 10 Initial cracking induced in the conventional concrete disc before the commencement of the 28-day biological curing period.

3.3.6. Application in Real Cracks with Optimal Dosage

The previously established optimal dosage, which utilises concrete discs measuring 4 inches in diameter and 2 inches in height, was used to carry out the application in real cracks. For the repair operation, a bacterial paste was made by mixing encapsulated bacteria and distilled water in a 1:20 ratio, using 1 mL of bacterial culture of concentration M3 ($2.8×10^{16}$ cells/mL) and 7.5 mg/m³ of nanoparticles, together with 20 mL of water and stirring until a thick grey paste was formed.

The cracks in the specimens were then located, and only cracks with a thickness of 1 to 3 mm were selected. The bacterial paste was meticulously applied to the cracks, making sure that it was completely penetrated in depth. The samples were kept in a controlled environment, and during this time, the samples were monitored. The samples were monitored repeatedly, and to demonstrate the self-repair action caused by the bacteria, measurements were taken, and the cracks were closed (Figure 11).

Fig. 11 Application of a paste containing encapsulated B. subtilis (2.8×10^16 cells/ml) on a real crack

4. Results
4.1. Slump

The results from the slump test for the various mixtures of fresh concrete and encapsulated Bacillus subtilis at different concentrations as shown in Figure 12 (0, 2.8×10^9, 2.8×10^{12}, 2.8×10^{16}, 2.8×10^{18} and 2.8×10^{20} cells/ml), and the slump values of 4 1/4, 4 1/2, 4 3/4, 5, 5 3/4, 6 inches. As the concentration of the bacteria increases, so does the fluidity of the concrete, which can be explained by the enclosed bacteria suspension medium containing distilled water. The additional water from the suspension increases the effective water of the mixture but does not change the water/cement ratio. Consistency and workability are therefore improved. The enhanced workability of the concrete allows for easy placement and is therefore suitable for slabs, pavements, strip footings, and elements with moderate reinforcement. Concrete is needed that has medium to high fluidity, which allows for the free movement of placement with no risk of segregation.
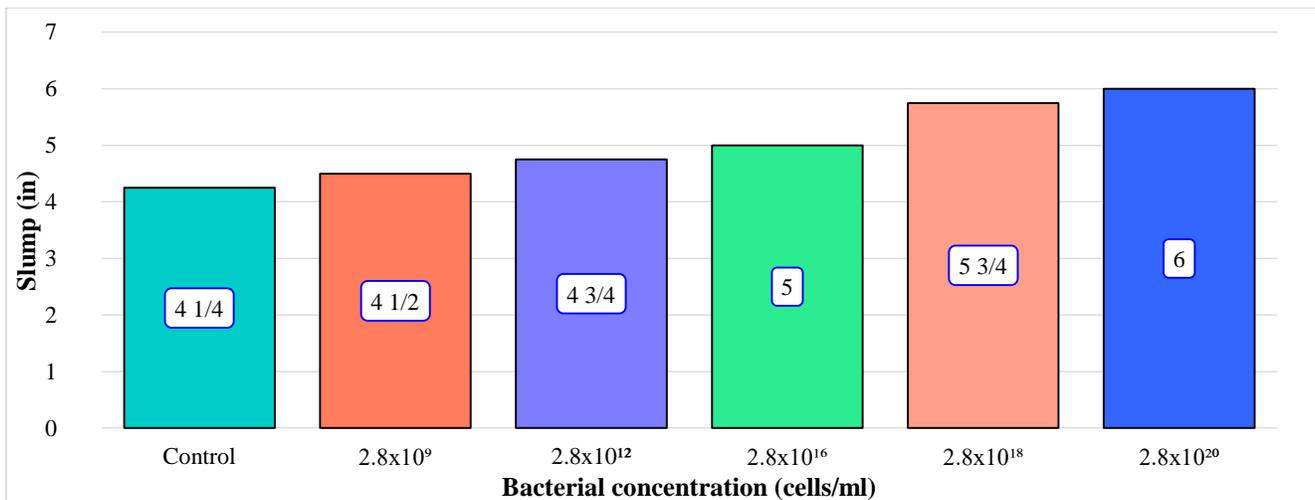

Fig. 12 Variation in the settlement of fresh concrete with different concentrations of encapsulated Bacillus subtilis

4.2. Compressive Strength

The different concrete mixture compressive strength results are shown in Figure 13 for the 7, 14, and 28-day assessments. The control mixture, which did not have bacterial addition, achieved strengths of 141.80 kg/cm² at 7 days, 194.31 kg/cm² at 14 days, and 213.17 kg/cm² at 28 days. The mixture with added encapsulated Bacillus subtilis progressively increased strength. Mixture M1 (2.8×10^9 cells/ml) achieved strengths of 148.95, 201.84, and 216.54 kg/cm² at 7, 14, and 28 days, respectively. Mixture M2 (2.8×10^{12} cells/ml) achieved values of 155.89, 207.65, and 221.97 kg/cm², while mixture M3 (2.8×10^{16} cells/ml) achieved the highest strengths of 163.31, 216.01, and 238.76 kg/cm² over the same periods. In mixtures M4 (2.8×10^{18} cells/ml) and M5 (2.8×10^{20} cells/ml), although M4 and M5 values exceeded the control, a slight decrease and a lesser value relative to M3 was observed with values of 160.83 and 157.39 kg/cm² at 7

days; 213.23 and 210.09 kg/cm² at 14 days; and 235.11 and 231.05 kg/cm² at 28 days, respectively.

The results indicate that the addition strength of mixtures with encapsulated Bacillus subtilis is due to the process of Microbially Induced Calcium Carbonate Precipitation (MICP). In this process, the activities of the bacteria that are metabolically active cause the precipitation of calcium carbonate in the pores and microcracks, which, in turn, densifies the internal matrix, decreases the porosity, and improves the load transfer capacity. The most efficient result was found in M3 (2.8×10^{16} cells/ml), where the precipitation of calcium carbonate occurred optimally and uniformly, which, in turn, bolstered the concrete structure. The lower value of performance in M4 and M5 is due to the oversaturation of the bacteria, which created localized clusters that restricted the uniform calcium carbonate deposition

within the matrix. The added value obtained from this particular study is in line with past recorded data.

While Bacillus subtilis and calcium lactate resulted in an increment in compressive strength in India between 20-27% [29, 22], in China, microbial agents resulted in compressive strength of over 50 MPa at 28 days [24]. Strengths of 32.2 MPa were also achieved in Nashik after 28 days [23]. Although the cited works did not address it, the unique aspect of our study is the usage of iron oxide nanoparticle

encapsulation, which guarantees both the bacterial viability and efficiency in saline conditions. This aspect of our study is what distinguishes it from the cited works.

The following sections present compressive strength values in kg/cm² because of the customary practice in Peru, where this is the unit of measure for concrete design and control, and this measure is approximately equivalent to MPa (1 MPa $\approx$ = 10.2 kg/cm²).
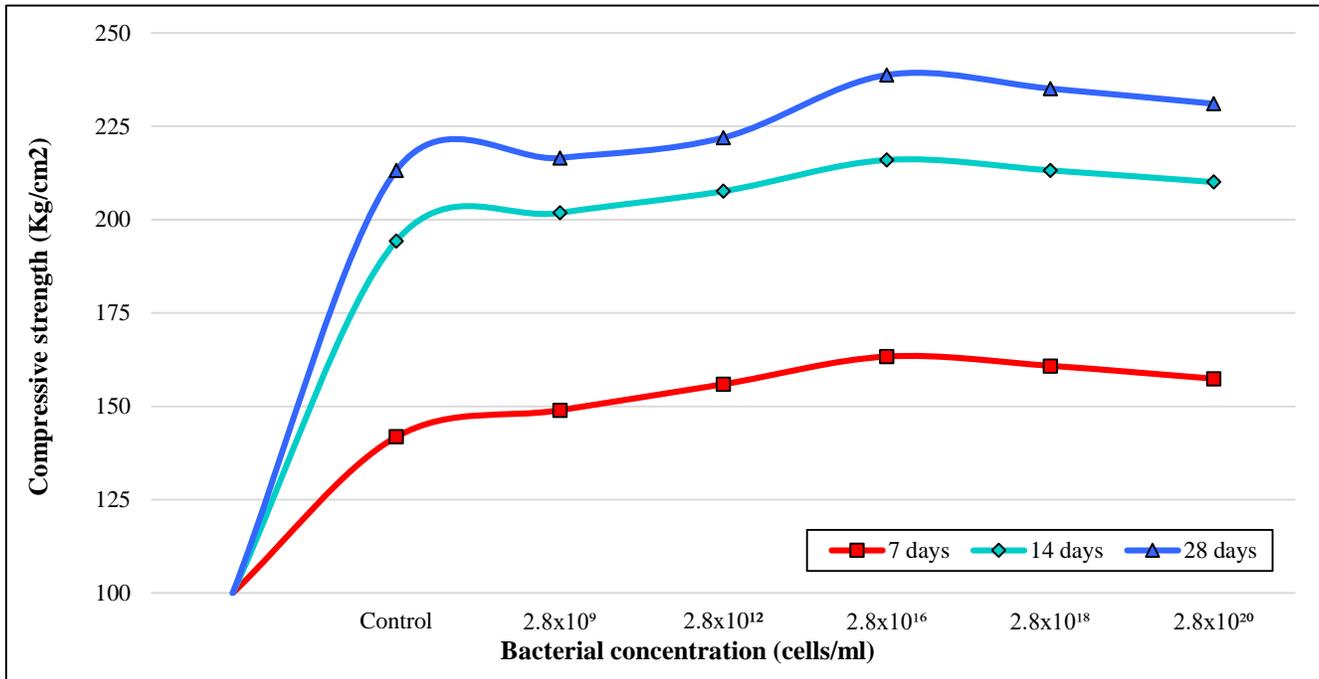

Fig. 13 Compressive strength of concrete mixtures with encapsulated Bacillus subtilis, evaluated at 7, 14, and 28 days

4.3. Tensile Strength

The results for tensile strength are illustrated in Figure 14 for each different concrete mixture after 7, 14, and 28 days. Control samples (without any encapsulated Bacillus subtilis) achieved scores of 13.56, 17.01, and 21.34 kg/cm², respectively, on days 7, 14, and 28. Mixtures with various proportions of encapsulated bacteria improved significantly, particularly mixture M1 (2.8×10^9 cells/ml) which achieved 14.34, 17.18, and 21.78 kg/cm²; mixture M2 (2.8×10^{12} cells/ml) which improved to 14.95, 17.89, and 22.06 kg/cm²; and mixture M3 (2.8×10^{16} cells/ml) with 15.08, 18.71, and 23.18 kg/cm², respectively, the largest improvements of the group. Mixtures M4 (2.8×10^{18} cells/ml) and M5 (2.8×10^{20} cells/ml) recorded respective scores of 14.93, 18.34, and 23.01, and 14.21, 18.01, and 22.95 kg/cm². These scores confirm the positive impact encapsulated Bacillus subtilis has on concrete tensile strength, particularly at the M3 dosage.

These improvements can be attributed to certain beneficial traits of the bacteria: (i) During the process of Microbially Induced Calcium Carbonate Precipitation

(MICP), which is a metabolic function of Bacillus subtilis, some of the pores and microcracks were filled with $CaCO_3$ crystals, thereby creating a denser and a more compact matrix; (ii) filling of the $CaCO_3$ crystals improved the bond at the interfacial transition zone (ITZ) that is the weakest zone and in most cases, the zone that is most affected by tensile loads, between the cement paste and the aggregates; and (iii) the encapsulation defended bacterial viability and ensured that there was continuous and uniform $CaCO_3$ deposition during the entire hydration process. These mechanisms all work together to enhance the load transfer and cracking bridging capacity, which directly led to a higher tensile strength, and in particular, mixture M3 had the highest tensile strength. This agrees with prior research conducted in India, which reported that there is a bacterial curing process that increases tensile strength by 0.16 N/mm² (6%) as compared to conventional curing [32]. On the other hand, the encapsulation in the study at hand reported numbers that were almost 9% which underlines the importance of the use of protecting bacteria with nanoparticles.
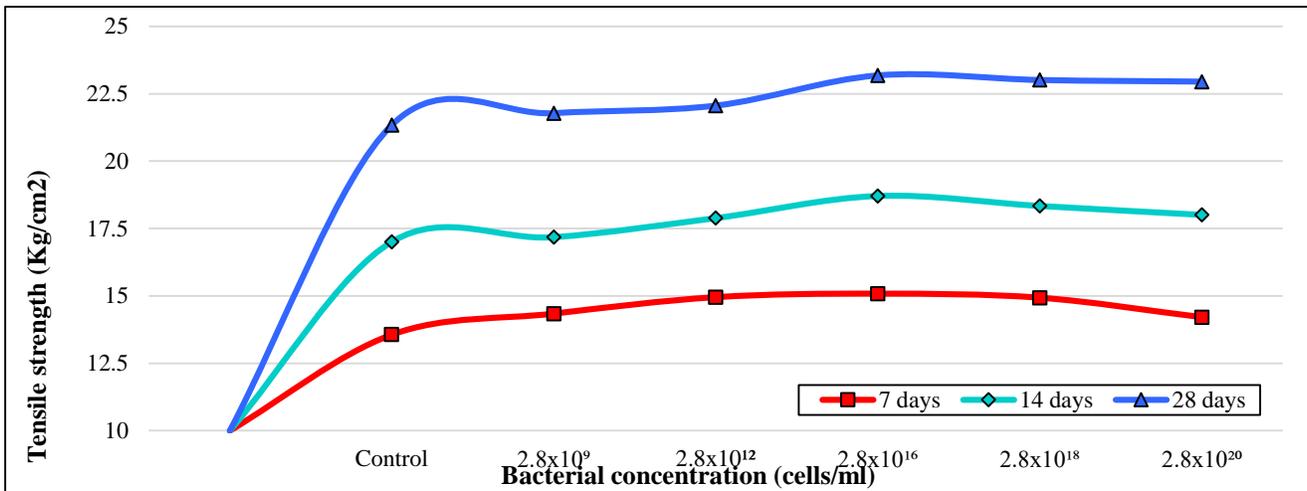
Fig. 14 Tensile strength of concrete mixtures with encapsulated Bacillus subtilis, evaluated at 7, 14, and 28 days

4.4. Flexural Strength

In Figure 15, we show results for the 28-day flexural strength for various concrete mixes containing encapsulated Bacillus subtilis. The control mixture without encapsulated bacteria showed a strength value of 15.64 kg/cm². The mixes with encapsulated bacteria showed considerable improvements, with M1 (2.8×10^9 cells/ml) being the most remarkable at 16.31 kg/cm², which is higher than the control. With the increase in bacterial concentration, the strength also increased: mixture M2 (2.8×10^{12} cells/ml) reached 17.03 kg/cm², and mixture M3 (2.8×10^{16} cells/ml) obtained the highest value with 17.65 kg/cm². In contrast, mixtures M4 (2.8×10^{18} cells/ml) and M5 (2.8×10^{20} cells/ml) showed slightly lower values, with 17.08 kg/cm² and 16.82 kg/cm², respectively. These results demonstrate the positive effect of encapsulated bacteria on the flexural strength of concrete, with mixture M3 being the most efficient. Unlike compressive and indirect tensile strength, which were monitored at 7, 14, and 28 days to evaluate strength development, flexural strength was assessed only at 28 days. This decision is based on the fact that flexural strength is more sensitive to early-age microstructural defects, which can cause high variability, and because the bacterial self-healing mechanism requires sufficient time to consolidate calcium carbonate precipitation. Therefore, the 28-day age, in accordance with ASTM C78/C78M, was selected as the most reliable reference to assess the real contribution of Bacillus subtilis encapsulation to flexural performance.

The results obtained are consistent with previous studies, such as those by Reddy & Kavyateja [22], who reported flexural strengths exceeding 4 MPa when using Bacillus subtilis and calcium lactate, and by Nuaklong [30], who observed that microencapsulated bacteria achieved complete sealing of shrinkage cracks within three days. However, unlike those studies, the present research found that the encapsulated bacteria not only promoted effective crack closure but also enhanced flexural strength without compromising compressive strength, thereby demonstrating the balance achieved with the optimal M3 dosage.
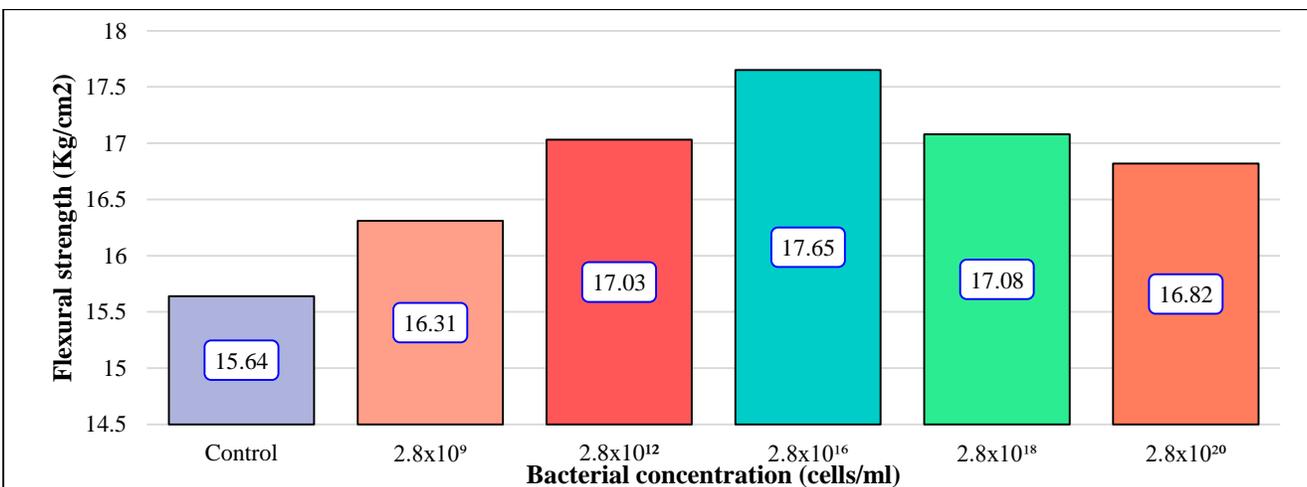

Fig. 15 Flexural strength of concrete mixtures with encapsulated Bacillus subtilis, evaluated at 28 days

4.5. Self-Repairing Concrete

Figure 16 presents the results concerning the comparison of the widths of the cracks in concrete samples with different concentrations of encapsulated *Bacillus subtilis*, assessing both before and after 28 days of curing. Initially, i.e., before the curing process starts, the crack width shows no trend in relation to the increase in encapsulated bacteria concentration. This is also clear from the very low value of the coefficient of determination $R^2 = 0.0159$, which shows a very weak correlation (linear). After 28 days of the curing process, more definitive trends in the reduction of crack widths become evident with a higher concentration of encapsulated bacteria, with $R^2 = 0.5134$, suggesting a moderate correlation in the effective dosage toward closure of the cracks. The mixtures

M1, M2, M3, M4, and M5 recorded a total reduction of the fissure width, while M1 alone showed a significant partial reduction from 2.30 mm to 0.20 mm. In contrast, the control sample (without the addition of encapsulated bacteria) maintained its crack without variation (0.10 mm). This improvement in the behaviour of the treated mixtures is attributed to the biological action of *Bacillus subtilis*, which favoured the precipitation of calcium carbonate ($CaCO_3$), contributing to the sealing of the cracks. However, the behaviour was not strictly linear at all concentrations, which could be due to saturation effects, interaction with the concrete matrix, or limitations in bacterial metabolic activity at high concentrations.
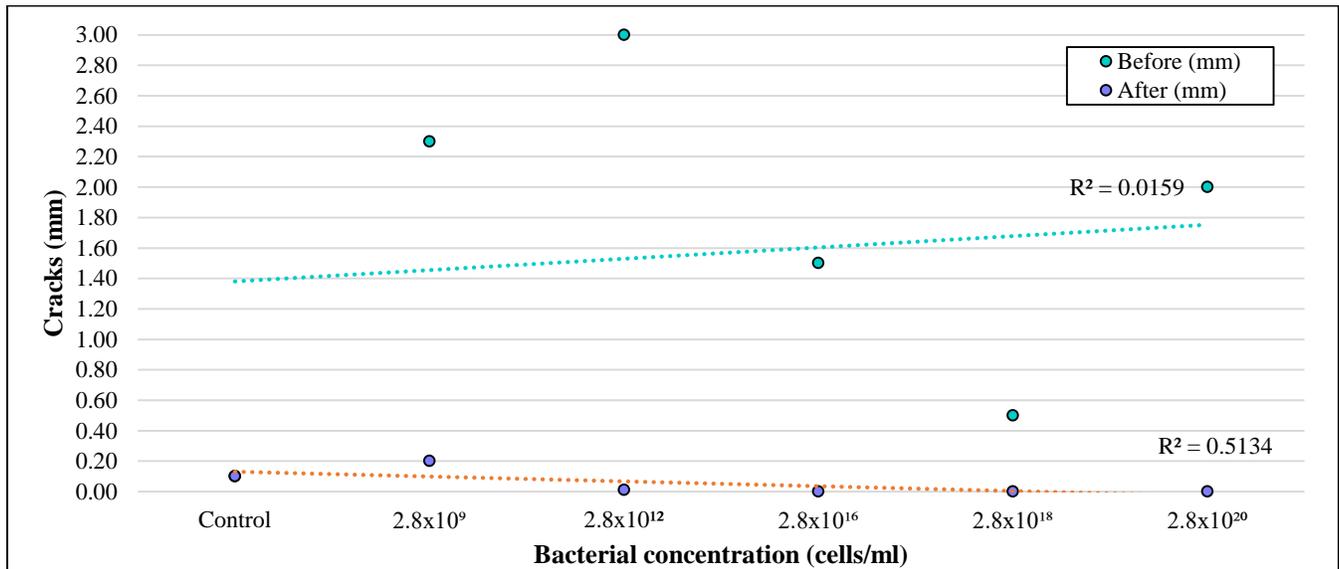

Fig. 16 Comparison of crack width before and after biological curing in concrete discs with different concentrations of encapsulated *Bacillus subtilis*.

In this study, crack width measurements were primarily taken along the B-B axis, which corresponds to the central region of the specimens. This selection was made because the central area more accurately represents the behaviour of the concrete matrix, minimising the influence of edge effects that are more evident along the A-A and C-C axes. Consequently, the results provide a more reliable assessment of self-healing effectiveness.

Figure 17 shows the cracks observed in concrete discs treated with different concentrations of encapsulated *Bacillus subtilis*, evaluated before and after 28 days. In item (a), corresponding to the control sample without bacterial addition, an initial crack of 0.10 mm is observed, which, as shown in item (b), remained unchanged after 28 days. In item (c), corresponding to the dosage of 2.8×10^9 cells/ml, the initial crack was 2.30 mm, and in item (d), after 28 days, it was reduced to 0.20 mm. The observation of a greyish coloration caused by calcium carbonate precipitation indicates that the biological curing process has begun. Sample e, with a

concentration of 2.8×10^{12} cells/ml, had an initial crack of 3.00 mm, which was considerably diminished to 0.01 mm after 28 days (item f), indicating almost complete crack repair. In item (g), which corresponds to 2.8×10^{16} cells/ml, an initial crack of 1.5 mm was observed, which completely vanished after 28 days (item h), demonstrating highly effective self-healing properties. Regarding the dosage of 2.8×10^{18} cells/ml, item (i) had an initial crack of 0.5 mm, which also completely sealed after 28 days (item j), showing clear calcium carbonate deposits. Lastly, the mixture with the highest concentration (2.8×10^{20} cells/ml), represented in item (k), the initial crack was 2.00 mm, and after 28 days (item l), the crack was tb 0.00 mm. However, despite the complete closure, the performance was not as favorable as in the intermediate dosages, suggesting, once again, the existence of an optimal concentration of encapsulated *Bacillus subtilis* to enhance the self-repair process.

The advancements noted can be credited to the microbial-induced calcium carbonate precipitation (MICP), resulting

from the microbial activity of Bacillus subtilis. This process led to: (i) the entrapment of microspores and microfractures with $CaCO_3$, which diminishes permeability; (ii) bolstering the Interfacial Transition Zone (ITZ) between the paste and aggregates, which is often the most fragile zone; and (iii) increasing stress distribution within the concrete microstructure through the gradual and progressive closure of cracks. The encasement in iron-oxide NPs assured sustained

bacterial activity in the high-alkaline matrix of concrete, which allowed for gradual release.

Thus, the improvement is not only linked to dosage but also to the biological process and the protective role of encapsulation, which together explain the effectiveness of crack closure in the tested specimens.
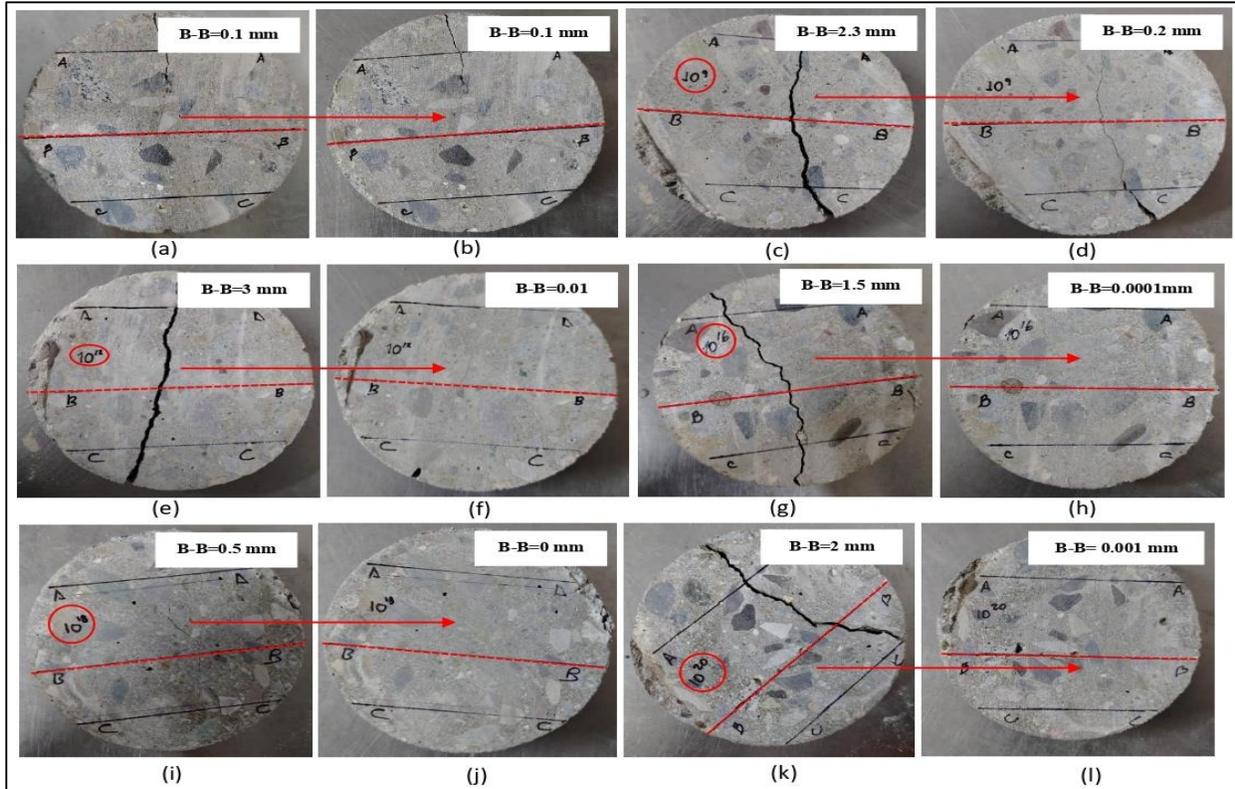

Fig. 17 Creation of cracks with dosage (o): (a) initial crack; (b) healed crack. Creation of cracks with dosage of 2.8×109: (c) initial crack; (d) healed crack. Cracks with dosage of 2.8×1012: (e) initial crack; (f) healed crack. Cracks with a dosage of 2.8×1016: (g) initial crack; (h) healed crack. Cracks with a dosage of 2.8×1018: (i) initial crack; (j) healed crack. Cracks with a dosage of 2.8×1020: (k) initial crack; (l) healed cracks

4.6. Resistance to Sulphates

Figure 18 shows the results of the compressive strength of concrete samples exposed to sulphate attack, evaluated at 7, 14, 28, and 56 days. It was observed that the addition of Bacillus subtilis significantly improved the behaviour of concrete in sulphate environments. Without the addition of bacteria, the control sample obtained the following compressive strengths: 146.32 kg/cm² at 7 days, 199.87 kg/cm² at 14 days, 219.14 kg/cm² at 28 days, and 235.45 kg/cm² at 56 days. In comparison, samples with bacteria 2.8×10^9 cells/ml obtained the following strengths: 152.43 kg/cm², 207.08 kg/cm², 222. 67 kg/cm², and 241.86 kg/cm², respectively; at 2.8×10^{12} cells/ml, 160.11 kg/cm², 213.27 kg/cm², 227.91 kg/cm², and 248.02 kg/cm²; and at 2.8×10^{16} cells/ml, the highest compressive strengths recorded were 169.29 kg/cm², 222.53 kg/cm², 244.10 kg/cm², and 256.73 kg/cm². At 2.8×10^{18} cells/ml, the compressive strengths were

167.44 kg/cm², 218.64 kg/cm², 240.35 kg/cm², and 254.61 kg/cm². With 2.8×10^{20} cells/ml, compressive strengths of 164.92 kg/cm², 216.53 kg/cm², 236.89 kg/cm², and 251.56 kg/cm² were obtained. This demonstrates that bacteria, in addition to assisting with internal self-compaction and the recovery of microcracks, also reinforced the concrete matrix in resisting sulphate deterioration.

Calcium carbonate precipitation that was microbially induced (MICP) helps to explain the increased resistance to sulphate, as it densifies the matrix and reduces the permeability by sealing pores and microcracks. Additionally, being encapsulated in iron oxide nanoparticles that were coated with APTES provided some important technical benefits. The nanoparticles have protective microenvironments that cause the preservation of bacterial viability, and this microenvironment allows for the bacterial

metabolism to be sustained under extreme and aggressive alkaline conditions. The APTES coating was also beneficial because it improved the cement matrix compatibility, reduced particle agglomeration, and promoted even dispersion of the bacteria and nucleation sites for $CaCO_3$.

Due to this, the microstructure was densified. This microstructure also increased the resistance to the percolation of SO_4^{2-}, which reduces expansive products that cause secondary cracking and loss of strength (ettringite and gypsum). Increased paste-aggregate adhesion, stress transfer, and durability were due to the precipitation of calcium carbonate in the Interfacial Zone of the matrix (ITZ) that added more bound water. The enhanced performance of the M3 mixtures (2.8×10^{16} cells/ml) was due to the well-balanced bacterial density, ionic abundance, and excellent dispersion, which was a result of Fe_3O_4–APTES encapsulation, while excessive amounts resulted in agglomeration and oversaturation, thus compromising the sealing uniformity and effective overall reduction.
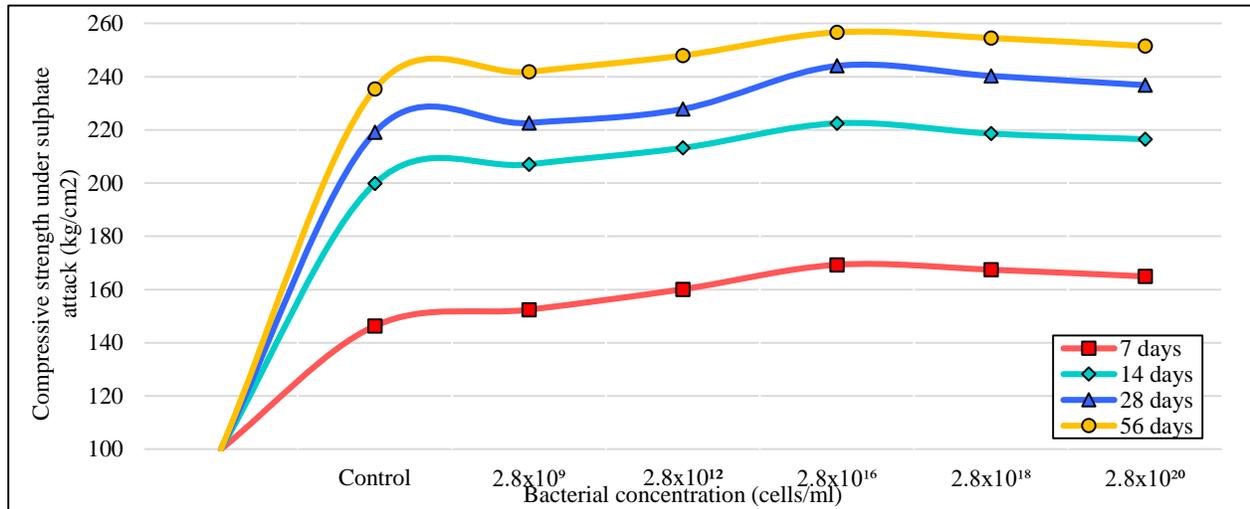

Fig. 18 Compressive strength evaluation of cylindrical concrete specimens treated with encapsulated Bacillus subtilis and exposed to sulphate attack

4.7. Application to Real Cracks

Figure 19 shows the crack widths measured before and after applying a bacterial paste made with encapsulated bacteria and distilled water in a 1:20 ratio, using the optimal dosage of 2.8×10^{16} cells/ml. This paste was applied superficially to concrete discs with actual cracks between 0 and 1.5 mm. The results show a slight reduction in crack width in all cases, from values such as 0.90, 0.50, 0.30, 0.70, 1.00, and 0.80 mm to 0.80, 0.45, 0.20, 0.65, 0.90, and 0.80 mm, respectively. However, this reduction was insignificant, which is reflected in the coefficients of determination obtained: $R^2 = 0.0824$ before treatment and $R^2 = 0.1329$ after treatment, indicating a weak relationship between the application and the decrease in crack width. This is because, unlike the internal self-repair process, where bacteria are incorporated into the concrete from the outset and act from within, in this case, the action was more superficial and external, generating a visible but limited closure in depth, probably due to the lower penetration of precipitated calcium carbonate into the crack.
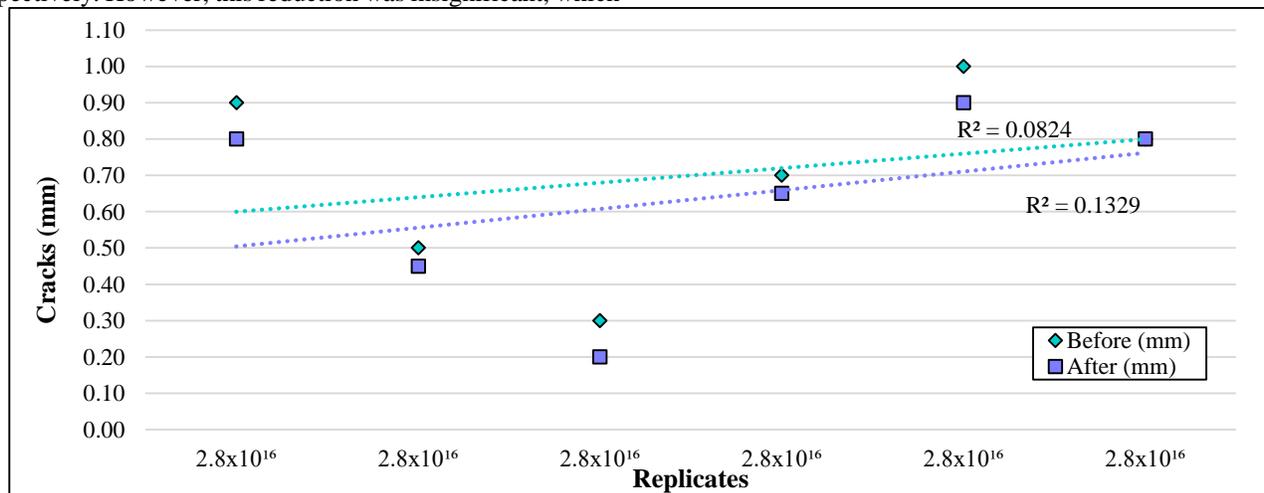

Fig. 19 Comparison of actual crack width before and after treatment with bacterial paste

Figure 20 shows concrete discs treated superficially with the encapsulated bacterial paste, using the optimal dosage of Bacillus subtilis (2.8×10^{16} cells/ml). Although the application is external and does not act inside the concrete, partial sealing of the cracks is evident. For example, in item (a), there is effective coverage of the crack, while in item (b), a similar result can be seen. In item (c), the crack is still visible, with no apparent closure due to the action of the product, and in item (d), a superficial application without complete sealing can be seen. In item (e), in addition to not achieving structural closure, small gaps can be seen where the paste did not completely cover the crack. Finally, in item (f), the behaviour is comparable to that of the other samples, showing a slight superficial improvement, although without achieving deep internal repair.
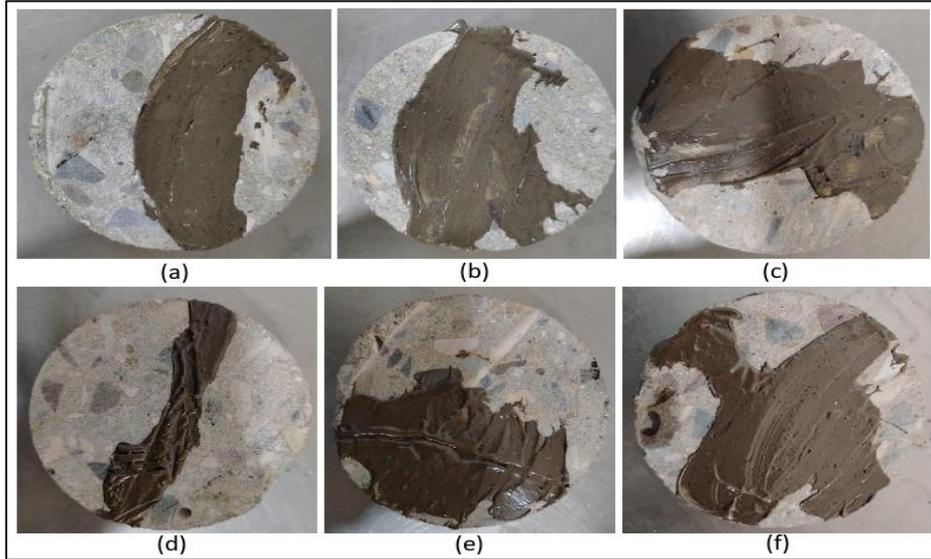

Fig. 20 Concrete discs superficially treated with paste containing encapsulated Bacillus subtilis (optimal dose of 2.8×10^{16} cells/ml)

4.8. Cost Analysis

Figure 21 shows the variation in total cost after adding different concentrations of encapsulated Bacillus subtilis (cells/ml) to the concrete. It can be seen that the treatment without bacterial addition had a cost of S/685.4, while concentrations of 2.8×10^9, 2.8×10^{12}, 2.8×10^{16}, 2.8×10^{18}, and 2.8×10^{20} cells/ml generated costs of S/ 743.4, S/ 749.4, S/ 755.4, S/ 761.3, and S/ 767.3, respectively. Although at first glance there may appear to be a slight variation in costs, these values are entirely justified given the highly favourable technical impact observed, especially at a concentration of 2.8×10^{16} cells/ml, which demonstrated an impressive ability to repair cracks in the cement matrix. This dosage enabled *Bacillus subtilis* activation within the concrete matrix, inducing calcium carbonate $CaCO_3$ precipitation and promoting biocementation that effectively sealed microcracks and enhanced structural integrity. The cost assessment accounted for bacterial inputs together with labor, constituent materials, cement, coarse and fine aggregates, water, and equipment, ensuring a robust technical and economic evaluation. The results indicate a technically viable and cost-efficient solution for crack treatment without introducing disproportionate additional costs.
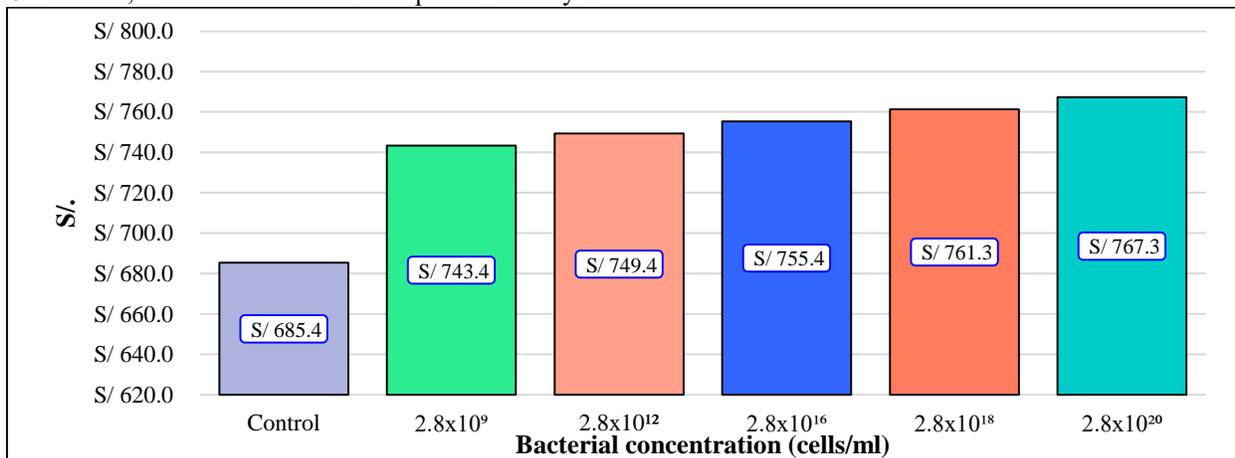

Fig. 21 Unit cost analysis of concrete with different concentrations of encapsulated Bacillus subtilis

5. Discussion

The properties of both fresh and hardened concrete with encapsulated Bacillus subtilis showed steady improvements. Mahmood et al. [37] reported up to 25.9% higher compressive strength. We achieved a maximum increase of 12% at 28 days with 2.8×10^{16} cells/ml. This variance may be due to methodology differences. While Mahmood et al. conducted studies with optimized nanoparticle–bacteria ratios and under controlled inducement of crack conditions, our study assessed several dosages within the framework of ACI 211.1, and designed the mix with a focus on delayed bacterial release and on bacterial activity longevity. Nonetheless, the compressive strength improvements (12%), tensile strength (9%), and flexural strength (13%) were consistent and verified the structural advantages of encapsulation and $CaCO_3$ precipitation, even though at a smaller scale compared to Uddin et al. [15] and Tomal [44].

This study shows an unprecedented example of near-complete crack closure, as fissures of 1.5 mm were reduced to levels undetectable by a standard crack comparator ruler (0.0001 mm). This not only shows reduction due to bacteria but also due to the encapsulated nano-particles, which, as evident by the mixing process and bacterial bioactivity, maintained bacterial viability and metabolic activity within the crack zone. When compared to Doostkami et al. [17, 45], who reported closure of cracks up to 370 μm, our results suggest encapsulation improves repair mechanisms to accommodate larger fissures.

The durability of the concrete matrix against chemical attack complements the 9% improvement of sulfate resistance at 56 days, hence bacterial action is not limited to crack filling. While Mahmood et al. [37] and Uddin et al. [15] emphasized the benefits of the early age, we have the findings of improvement during the medium-term under aggressive conditions. Nevertheless, this study did not cover performance evaluation with chlorides, carbonation, and freeze-thaw cycles, so additional studies are needed. If durability is to be extended, as evidenced by iron oxide nanoparticles and environmental impact lessened by the nanoparticles, the use of iron oxide nanoparticles in this study will have to be assessed to determine if the durability has extended the service life and lessened the maintenance. A life cycle assessment is needed to determine the extent of the impact of the produced nanoparticles.

The applicability of the findings in large-scale batching is less certain. While the mechanical resistance of the cores and protective sampling under lab mixing are certified, the certainty is lost under large-scale batching, where the mixing energy, the type and amount of raw materials, and the environmental conditions are variable. Provided that the encapsulation of iron oxide produced at a reasonable cost, it would be economically feasible to use the encapsulated iron oxide in standard batching.

6. Conclusion

The presence of Bacillus Subtilis at various concentrations improved the fresh and hardened state properties of the concrete. Mixtures containing B. Subtilis at 2.8 x 1020 cells/ml showed a workability improvement of 41% in the slump test (6" vs. 4.25" for the control), improving the ease of placement in complicated geometry elements. The mixture with 2.8 x 1016 cells/ml yielded the most consistent results in hardened concrete: in the compression test, 12% (238.76 vs. 213.17 kg/cm^2) improvement, 9% (23.18 vs. 21.34 kg/cm^2) in tensile strength, and flexural strength improved 13% (17.65 vs. 15.64 kg/cm^2). The self-healing mechanism added 99.99% crack self-healing in comparison to the control, with a reduction of 1.5mm to undetectable thresholds. The biocement also demonstrated 9% improvement in sulfate resistance at 56 days. The most economical option, 2.8 x 1016 cells/ml, showed a marginally increased cost of S/ 70 over the concrete and showed the most performance improvement among the mixtures tested.

The study is not without its shortcomings, despite these commendable findings. The 28-day evaluation period is far too short to make assessments about the long-term precipitation of $CaCO_3$ and the potential of bacterial activity to fizzle out. The curing conditions were very controlled, which do not mimic the majority of construction sites where variability in temperatures, humidity, and exposure to different chemicals may affect bacterial viability. Moreover, the scope of durability is mostly limited to sulfate resistance without taking into consideration several other important mechanisms, such as chloride penetration, carbonation, freeze-thaw cycles, shrinkage, and permeability. The biogenic repair process is not directly validated because there was no microbiological analysis done post-tests to confirm the survival and activity of the bacteria.

In future studies, it would be useful to include a wider dosage range with additional intermediate concentrations to more precisely delineate optimal thresholds and saturation boundaries. Evaluation timeframes longer than 90-365 days would be important to ascertain time-dependent durability performance. Furthermore, it would be best to undertake this technology validation in actual construction environments, or within a construction-analog environmental simulation (e.g., variable temperature, humidity, and contaminants) to assess all construction-durability relevant parameters. Most primary research is likely to focus on additional strains of calcifying bacteria (e.g., Sporosarcina pasteurii, Bacillus megaterium) and other, non-iron oxide nanoparticle encapsulation carriers, as well as other durability features. Also of critical importance is the need for life cycle assessments of the nanomaterials to determine whether the environmental costs of the materials are outweighed by the intended durable-use benefits. The broadest application of this technology would be in developing predictive models for crack healing to be used in the real world.

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