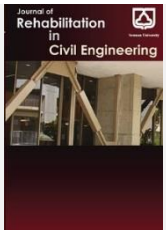

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Developing Bio-Based Self-Healing Concrete: An Eco-Friendly Strategy for Sustainability of Concrete

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ABSTRACT

There is a growing global demand for concrete, making it crucial to develop sustainable concrete solutions that address environmental concerns. Currently, approximately 7% of total human-caused CO2 emissions in the atmosphere come from cement production material. Techniques that incorporate bacteria to increase the lifespan of concrete elements will not only enhance their longevity but also contribute to more sustainable concrete materials for future generations. In this research, four different proportions of bacteria (0.5%, 1.5%, 3.5%, and 5%) and nutrients (0.10%, 0.30%, 0.5%, and 0.75%) were used relative to the weight of the cement. The objective of this research is to assess the capacity of bacteria to function as self-repairing agents in concrete elements, specifically their ability to fix cracks by producing calcium carbonate (CaCO3) in terms of spores that form within the concrete. The spores of the bacteria mixed directly into the concrete mixture remained viable for an extended period. The gradual reduction of pore diameter during the concrete setting likely limited the longevity and reproduction of spores, as the pore widths were reduced to 1µm or less. However, concrete mixed with bacteria exhibited a significantly higher production of minerals that fill cracks compared to normal concrete specimens. At various time intervals, the samples were tested for their compressive strength and microstructural characteristics using EDX, SEM, and XRD. The most significant improvement occurred at 28 days, with proportions of 1.5% bacteria and 0.3% calcium lactate relative to the weight of the cement, resulting in a 27.1% increase in compressive strength and the healing of cracks measuring between 1 and 3 mm. This suggests that the use of bacterial spores as a self-sealing agent holds enormous potential.

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1. Introduction

Concrete is one of the most crucial construction materials worldwide due to its universal availability and strong performance. The use of concrete is constantly increasing to meet the demands of a burgeoning population and the consequent booming infrastructure, with housing, transportation, highways, and industrial plants being the primary applications. Since the beginning of the Industrial Revolution, concrete has been the widely used construction material due to its unique properties, such as high compressive strength, durability in aggressive environmental conditions, and the ability to be easily cast into the required shape. However, it also has limitations, including its poor tensile strength and susceptibility to various types of deterioration. One of the primary limitations of concrete is that it may develop microcracks for several reasons, including drying shrinkage, heavy loads, temperature fluctuations, and the occurrence of earthquakes. Microcracks are typically fine and not readily apparent at first but can increase in size over time, effectively resulting in the loss of impermeable properties of the concrete, which renders it more vulnerable to penetration by harmful agents. These harmful external agents, such as chloride, sulfate, and carbonation resulting from increasing atmospheric CO2 levels, pose significant threats to structural durability [1]. Such assaults from the environment promote deterioration, reducing the serviceability of the concrete, which makes its resistance to penetration of great importance [2, 3]. Traditional surface treatments, including epoxies, silanes, and pore blockers, have been studied to enhance the durability of concrete. Yet, these approaches are widely debated for their limited long-term effectiveness, high maintenance requirements, cost, accessibility, and environmental implications [4]. Consequently, the use of self-healing, particularly bacterial-based self-healing concrete (BSSHC), holds promise for enhancing the durability and longevity of infrastructure.

Therefore, the priority is to develop new and efficient repair strategies that avoid the limitations associated with traditional approaches. Bacterial concrete, also known as self-healing concrete, is an innovative material designed to enhance durability by incorporating bacteria that autonomously repair cracks over time [5, 6]. This creative idea involves the use of bacteria, typically from the Bacillus species, which produce calcium carbonate when activated by water seeping into cracks. This natural process helps to fill the cracks, reducing the need for external repairs and preventing further deterioration [7]. One of the key benefits is its ability to autonomously heal cracks, which prevents the infiltration of harmful substances, such as water, chemicals, and gases, that could weaken the concrete [8]. This, in turn, helps to protect the internal steel reinforcement, reducing the risk of corrosion and extending the structure’s service life. Bacterial concrete is also more resistant to water permeability, making it ideal for structures exposed to moisture or requiring water retention, such as dams or bridges [9]. By minimizing crack propagation and reducing maintenance needs, this type of concrete promotes sustainability by decreasing the consumption of materials and energy for repairs [10].

Overall, the use of bacterial additives significantly enhances the durability of the concrete structure elements, offering a cost-effective solution for construction. According to [11, 12], concrete elements do not appear to be a sustainable material from an environmental standpoint. However, concrete is susceptible to cracking, which undermines its structural integrity and longevity. Durability difficulties can lead to considerable economic consequences, as evidenced by the enormous expenses associated with the repair and maintenance of concrete structure elements [11]. In 2002, America estimated that the yearly direct expenditure for repairing and maintaining concrete highways and bridges due to reinforcing corrosion was $ 4 billion [13, 14]. Cracking on the surface of the concrete's layer diminishes its durability, facilitating the infiltration of water and deleterious chemicals, which consequences in concrete matrix degradation and corrosion of the underlying steel reinforcement and increases repair and maintenance costs [14]. As of 2023, the aggregate expense of repairing and maintaining concrete highways and bridges constitutes a substantial portion of U.S. infrastructure expenditures. In 2023, governmental entities at the federal, state, and local

levels allocated roughly $249 billion for highway infrastructure [15], while the estimated backlog for essential highway and bridge repairs exceeds $1.1 trillion [16].

Concrete formulations are now more commonly incorporating various industrial by-products, such as fly ash, blast furnace slag, and silica fume, as substitutes for clinker (cement) to reduce production costs [17]. This method not only reduces costs but also enhances sustainability by conserving substantial quantities of clinker [18]. In developed countries such as the Netherlands, more than 65% of the clinker in cement is replaced with blast furnace slag [19]. Despite the longstanding recognition and implementation of the need for more sustainable cement-based materials, the advancement of sustainable repair and maintenance techniques for concrete buildings remains in its early stages of development. Generally, manual examination and remediation address durability concerns, such as crack development, by infusing epoxy, cement, and other synthetic fillers [21, 22, 23, 24, 25]. However, this issue still cannot be solved by attempting to minimize any adverse environmental effects from structural maintenance using Portland cement and other synthetic fillers. Numerous researchers are currently investigating a potential and sustainable repair approach involving mineral-producing bacteria. According to recent research, the use of microorganisms in ecological engineering is gaining attention. Several environmental applications have effectively utilized bacteria, including the extraction of compounds from wastewater [26], bio-remediation of polluted soil [27], and the reduction of greenhouse emissions from landfills [28, 29]. The use of this mineral-forming bacteria in various construction-related applications is increasing in the area of study. These bacteria have demonstrated efficacy in consolidating sand and restoring limestone monuments [30, 31, 32, 33, 34].

Recent research has investigated the usefulness of these bacteria in concrete, specifically for filling pores and cracks. This microbial method has the potential to transform concrete restoration by offering a sustainable, self-healing solution [32]. Researchers are investigating the potential of bacteria to enhance the longevity and resilience of concrete structures by naturally filling voids and cracks, thereby reducing the reliance on conventional repair techniques [36, 37, 34, 38]. This biological approach signifies a transition towards more sustainable and efficient practices in construction and maintenance. According to this research, bacteria belonging to the genus Bacillus were used to generate calcium carbonate-based minerals biologically. The formation of calcium carbonate by these bacteria occurs through an enzymatic process, in which urea (a chemical waste product resulting from the body's breakdown of protein) is decomposed into ammonia and carbon dioxide, leading to the production of carbonate ions that react with calcium ions to form solid calcium carbonate [36].

Even if, self-healing concrete, particularly bacteria-based self-healing concrete (BSSHC), represents a promising approach to enhancing the durability and service life of infrastructure, environmental consequences of the chemical reactions associated with these technologies must be examined carefully. For example, the release of ammonium ions during the microbial self-healing process is a significant concern. In BBSHC, bacteria such as Bacillus subtills degrade urea to form calcium carbonate, which seals cracks in concrete. But this reaction also produces ammonium. If they seep into groundwater or nearby ecosystems, ammonium ions can cause eutrophication and nitrogen loading in the environment. Another problem is that ammonium inside the concrete matrix might be oxidized to nitric acid, which lowers the local pH level and consequently increases the potential risk of steel reinforcement corrosion [37].

The environmental hazards associated with ammonium discharge worsen due to the lack of standardized international rules regarding self-healing concrete. Some other appropriate thresholds can be put into context in the meantime. For example, the introduction of fly ash in concrete is controlled by EN 450-1 and EN 197-1 in Europe, which, although not directly setting limits on ammonium content, recommends limiting ammonia to below 100 ppm due to health and odor issues [38]. In Germany, a lower recommendation of 50 ppm of ammonia in FA for use in cementation materials [39]. Occupational exposure to ammonia is also regulated in the United States by the Occupational Safety and Health Administration

(OSHA), which establishes a short-term exposure limit of 35 ppm over 15 minutes [40]. The UK Health and Safety Executive has restricted the 8-hour time-weighted average to 18 mg/m³. Although these standards are aimed at indoor air exposure rather than emissions from concrete operations, the consequences of unregulated ammonia generation are clear [41].

High ammonium content can cause eutrophication and poison aquatic life in aquatic environments. The European Commission's Water Framework Directive (Directive 2000/60/EC) establishes a framework for managing water quality, including the establishment of environmental quality standards for chemical substances, such as ammonium, in surface water bodies [42]. The US Environmental Protection Agency also sets criteria for nutrient pollution, with a focus on controlling nitrogen compounds (e.g., ammonium) to prevent nutrient over-enrichment and potential ecological effects in water bodies [43]. These criteria highlight the environmental implications of ammonium buildup and the need to regulate it under various conditions.

Apart from health risks, ammonium can affect the chemical and physical properties of concrete. The authors of this study have previously investigated the high ammonium content in additives (e.g., fly ash) and its potential to cause the release of ammonia during mixing and curing, which may impact both the environmental quality and the concrete quality. For example, concrete with fly ash containing more than 400 ppm ammonia may not meet the requirements for adding compressive strength or modifying the setting time [38].

Additionally, most self-healing systems are combined with supplementary cementitious materials (SCMs), such as ground granulated blast-furnace slag (GGBFS). The study, presented at the ASCP2023 - 7th Concrete Pavements Conference in Wollongong, Australia, observed the effect of high-volume GGBFS concrete on carbonation. Higher GGBFS content may result in a deeper carbonation depth, which can be a threat to the service life of concrete, especially in rigid road pavements. This impact should be weighed against the positive effects of SCM in lowering carbon footprints and improving sustainability [44]. In general, while success in combating climate change will be advanced if self-healing concrete can realize its full potential, environmental and related performance concerns will need to be addressed through appropriate material selection, meeting relevant thresholds, and ongoing research and development. To prevent the progression of these undesired and, in some cases, beneficial reactions and avoid as far as possible being harmed by their deleterious effects so that long-lasting health monitoring is not unacceptable, it is of the utmost importance to understand the impact of compound-forming reactions in general and that of ammonium bearing ones specifically.

Due to the equilibrium constant's pK-value being around 9.2, this reaction leads to a rise in pH from neutral to alkaline conditions. Acid-base chemistry uses the negative logarithm of the acid or base dissociation constant to indicate strength. Higher pKa means a weaker acid and more substantial base, while lower pKa means a stronger acid and lower base.

$$pK = -\log_{10} K \quad (1)$$

Where: K is the equilibrium constant of a reaction.
pK is the negative base of -10 logarithm of that equilibrium constant.

Under these alkaline conditions, carbonate and bicarbonate ions are produced, which then combine with calcium ions in the environment to precipitate and form calcium carbonate minerals [45]. However, a notable disadvantage of this reaction mechanism is that, alongside the production of carbonate ions, two ammonium ions are generated for every carbonate ion produced. The simultaneous generation of ammonium ions could potentially lead to excessive nitrogen accumulation in the environment, contributing to environmental nitrogen pollution if not properly managed [45]. Despite this, the process offers promise in areas such as construction and environmental engineering as researchers explore ways to mitigate ecological impact while harnessing the benefits of biological mineralization [49, 50, 51].

Many researchers have investigated bacteria that are present on the cracked concrete surface, typically from the external part of the concrete, often as part of surface treatments or repair methods [49]. This technique shows promise, although it has significant limitations. The urea-based system could potentially lead to environmental issues [50]. Manual inspection and the external application of bacteria to fissured regions are labor-intensive and necessitate repetition throughout the service lifetime of structures, rendering the technique expensive. The principal objective of this research was to investigate alternative bacterial routes that generate minerals autonomously, without external intervention, and to evaluate the viability of integrating bacteria directly into the concrete matrix during the casting procedure. Once integrated into the concrete, these bacteria will remain viable for extended periods and may generate significant amounts of minerals when required to seal or fill newly developed cracks. This method incorporates bacteria into concrete, enabling an autonomous self-healing process that minimizes the necessity for ongoing monitoring and repairs, hence decreasing long-term maintenance expenses and enhancing the sustainability of concrete structures [45].

Incorporating bacteria into the concrete would function as an internal self-healing mechanism, autonomously diminishing the permeability of the concrete matrix upon the emergence of fractures [54, 55]. This intrinsic healing mechanism would eliminate the need for manual examination and maintenance while concurrently enhancing the overall durability of the building. Furthermore, integrating these bacteria into the concrete formulation would provide substantial cost savings and ecological advantages by reducing the need for regular maintenance and the use of environmentally harmful repair substances [53]. The primary goal of this bacteria-incorporated concrete study effort was to identify bacteria capable not only of enduring the extreme conditions of the concrete matrix for extended periods but also of operating efficiently as self-healing agents. The bacteria remain alive despite the elevated pH, nutritional scarcity, and mechanical stressors characteristic of concrete [54]. Moreover, they must maintain the capacity to autonomously generate the requisite minerals to seal newly formed fissures, ensuring the long-term sustainability and durability of the concrete without continuous human oversight. Thus, the identification and examination of such microorganisms were essential for the advancement of an efficient self-healing concrete system.

While bio-based self-healing concrete is gaining popularity as a means to extend the lifespan of concrete, many questions remain unanswered. There is a significant lack of information regarding the bacteria's ability to survive in the concrete matrix over the long term. Although the surface application of bacteria in concrete has been studied, the internally incorporated viability and efficiency of bacteria in self-healing concrete have been underexplored. A better understanding of their viability, efficiency, crack-control behavior, and interaction with the cement matrix remains mandatory to realize autonomous, self-replicable bio-concrete for practical applications.

2. Methods

2.1. Research design

This research employs a systematic experimental methodology to investigate the impact of bacteria on the durability, strength, viability, porosity, and self-healing properties of concrete. The process begins with the cultivation of bacteria, particularly Bacillus species, which are recognized for their biomineralization capabilities. We prepare concrete specimens with and without bacteria, adhering to standard mixing, casting, and curing procedures to ensure consistency and accuracy. These function as experimental and control groups for comparative analysis. Core samples are periodically extracted and tested using microbial viability assays to evaluate bacterial viability within the concrete over time. The pore structure and strength of old samples are evaluated using mercury intrusion porosimetry (MIP), scanning electron microscopy (SEM), and strength tests to determine their resistance. The analyses facilitate the assessment of the impact

of bacterial inclusion on microstructure and mechanical performance over time. We evaluated the capacity of bacteria to induce biomineralization by identifying calcium carbonate deposits via X-ray diffraction (XRD) and energy-dispersive X-ray spectroscopy (EDX). Finally, we create artificial cracks in specific samples and subsequently subject them to wet-dry cycles. We assess the self-healing process through visual inspection, crack width measurements, and additional SEM analysis to confirm the presence of healing products. Figure 1 Conceptual framework depicted in diagrammatic form.

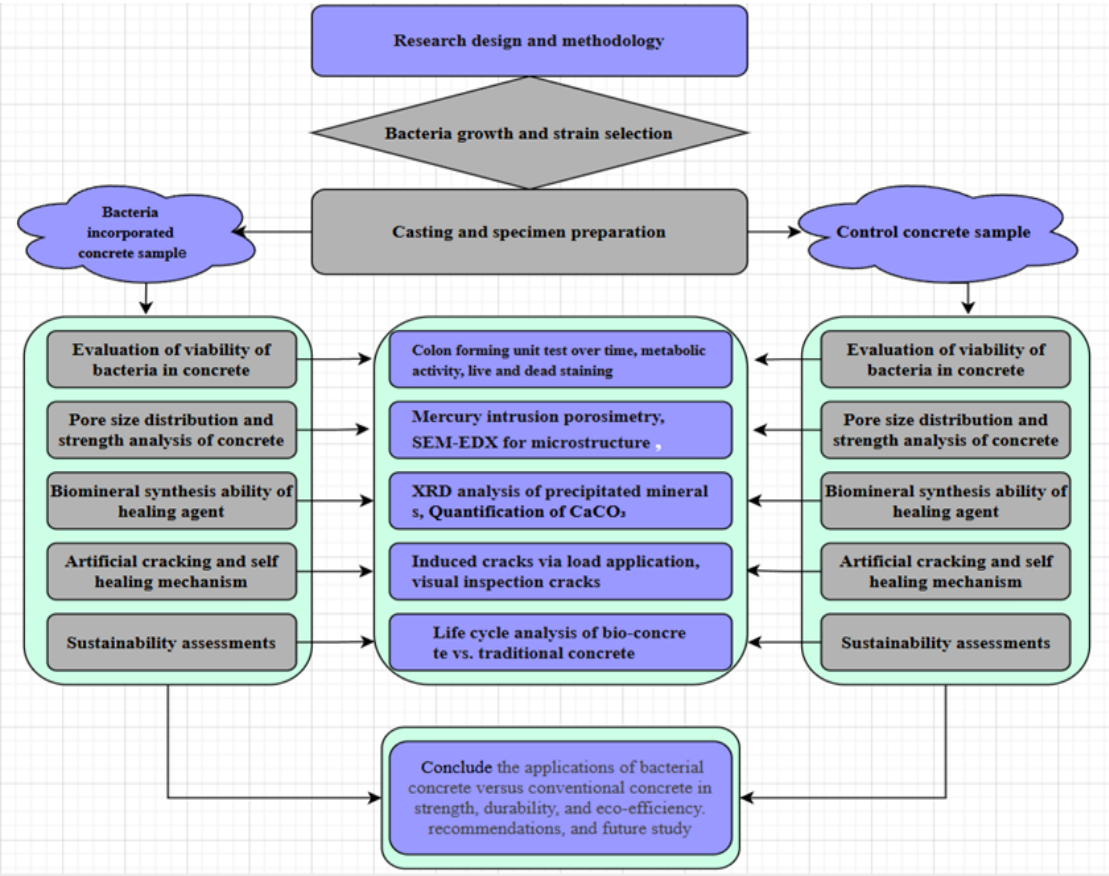

Fig 1. Overall research design flowchart.

2.2. Cultivation and growth of bacteria

The fresh concrete's matrix is highly alkaline, primarily due to the formation of calcium hydroxide (portlandite). In ordinary Portland cement (OPC), Portlandite is the most prevalent hydration product, following calcium-silicate-hydrate [45]. The increased alkalinity causes the capillary water in fresh concrete to exhibit pH levels that are typically between 10 and 12. Therefore, any spore of bacteria incorporated into the concrete matrix must not only resist the mechanical demands of the mixing process but also survive the highly alkaline environment for an extended duration. The elevated pH levels pose a considerable obstacle to bacterial life, as numerous microbes cannot flourish in such extreme conditions. Consequently, it is essential to choose bacteria that exhibit resilience to mechanical forces during mixing and can endure prolonged exposure to elevated alkalinity.

These specialist bacteria must preserve their viability within this adverse environment to fulfill their intended role, producing the essential minerals to seal cracks in the concrete elements. This requirement emphasizes the need to select resilient bacterial strains for self-healing concrete systems. The most promising candidates for integration into the concrete matrix seem to be alkaliphilic, spore-forming microorganisms. These bacteria thrive in the highly alkaline environment of concrete and can endure harsh conditions for extended periods. Since oxygen can permeate the matrix capillaries, rendering the concrete matrix, the chosen bacteria must also exhibit oxygen tolerance. Aerobic, alkaliphilic, and spore-forming

bacteria belonging to the Bacillus species are a promising source for potential self-repairing agents in concrete elements.

To assess the viability and efficacy of Bacillus subtilis in enhancing self-healing within the concrete matrix, we selected it for additional testing. This bacteria's spore-forming capability is particularly beneficial, allowing it to remain dormant under adverse conditions and reactivate when conditions for mineral production improve. Our aim is to create a resilient, self-healing system by selecting bacteria that can withstand the alkaline and oxygenated conditions of concrete while effectively sealing cracks over time. We obtained Bacillus subtilis bacteria, initially extracted from alkaline soil samples, from DVS BioLife, an industrial microorganism supplier located in Mandal, Hyderabad, Telangana, India [63]. The bacteria were grown in a liquid medium according to the supplier's specified instructions. The recommended medium consisted of 0.52 g of $NaHCO_3$, 3.4 g of meat extract, 0.63 g of Na_2CO_3, and 4.5 g of peptone per liter of distilled water, with a final pH of 9.8. However, the peptone/meat extract medium showed minimal sporulation (spore generation). The bacteria were grown on an alkaline mineral medium enriched with manganese to enhance sporulation. The medium was prepared using Milli-Q ultra-pure water, with the following components per liter: 0.232 g of $CaCl_2$, 0.3 g of KCl, 0.24 g of $MgCl_2 \cdot 6H_2O$, 0.18 g of NH_4Cl, 0.03 g of KH_2PO_4, 0.023 g of $MnSO_4 \cdot 2H_2O$, 2 ml of trace element solution SL12B, 0.3 g of yeast extract, 3.9 g of trisodium citric acid, 5.2 g of $NaHCO_2$, and 4.3 g of Na_2CO_3. This combination of ingredients ensured the media provided essential nutrients and trace elements needed for optimal bacterial growth. This modified media effectively improved the sporulation of Bacillus subtilis, rendering it more suitable for prospective use in self-sealing concrete.

To enhance bacterial growth, we cultured the bacteria aerobically in the flasks placed on a shaker table set to 150 rpm. As shown in Figure 2, bacterial growth and sporulation were systematically monitored and measured through colony counter and microscopic analysis. Upon reaching an aging phase characterized by a substantial quantity of spores, the cultures were treated to extract the bacterial spores and vegetative cells. The cultures were subjected to repeated centrifugation, followed by re-suspension of the cell pellets in sterilized tap water, thereby eliminating all dissolved culture components and residues. Following washing, the bacterial suspension was re-examined using a colony counter and microscopically to accurately determine the spore count. The produced suspensions were subsequently stored at 6°C in a refrigerator to maintain their integrity until utilized as an additive in cement paste. This process ensures the inclusion of only essential bacterial components in the cement mixture while eliminating extraneous residues, thereby enhancing the bacteria's efficacy in promoting the self-healing characteristics of the concrete. Then, bacterial growth and sporulation were systematically monitored in the laboratory, measured using a colony counter, and analyzed microscopically.

In Figures 2a-2h, the steps involved in cultivating the bacteria are illustrated. In the cultivation process, Fig. 2a shows Bacillus subtilis in powder form, which is used to initiate bacterial growth, while Fig. 2b represents calcium lactate, a key nutrient for bacterial growth. We prepare the media Fig. 2c using beakers, a stirrer for mixing, a pH meter for pH adjustment, and an autoclave for sterilization. After preparation, we transfer the media into flasks and place it on a shaker table running at 150 rpm Fig. 2d to ensure uniform mixing and aeration. The autoclave in Fig. 2e is used to sterilize the media and equipment, thereby eliminating potential sources of contamination. Inoculation Fig. 2f is performed in a clean area with an inoculating loop, Petri dishes, and a laminar flow hood to introduce Bacillus subtilis into the prepared media. Dry materials and tools undergo further sterilization in an oven (Fig. 2g) to ensure aseptic conditions are maintained. Finally, we monitored and evaluated bacterial growth through observation, using a colony counter Fig. 2h to quantify colony-forming units.

Fig. 2. Cultivation of bacteria and their growth.

2.3. Test procedure and specimen preparation

We prepared concrete samples with and without added bacteria to investigate several factors. These include the longevity of bacteria within the concrete, changes in pore sizes over time, and the impact of bacteria on the concrete's strength and self-healing ability. This research utilized OPC 43-grade cement, conforming to ASTM C150 specifications. The cement exhibited key qualities, including a specific gravity of 3.15, a fineness of 363 m^2/kg, a standard consistency of 32%, an early setting period of 45 minutes, and a final setting time of 6 hours. The concrete mixtures were formulated to attain an ideal equilibrium between workability and strength, utilizing a water-to-cementitious ratio of 0.5 and a sand-to-cementitious mass ratio of 2. The proportions were meticulously selected to guarantee both the simplicity of mixing and the resilience of the concrete. The dimensions of the concrete specimens were customized to satisfy the precise criteria of several mechanical tests. For instance, in Figure 3, we created cubes measuring 15 cm x 15 cm x 15 cm for compressive strength evaluation, providing a uniform dimension that enables a precise comparison of strength across various concrete mixtures. Additionally, we used cylindrical specimens measuring 30 cm in height and 15 cm in diameter for artificial crack testing, following established norms and techniques. Figures A to C explain the protocol of concrete preparation. Figure A illustrates casting, where concrete is placed into molds. Figure B is all about demolding and setting the concrete out, and Figure C is about curing, which is the method of maintaining the concrete's hardened state. This systematic approach guaranteed a reliable and uniform assessment of the concrete's mechanical and self-healing characteristics. Spores in bacterial specimens ranged from 1 to 10 × 10^9 cm^3 in cement stone.

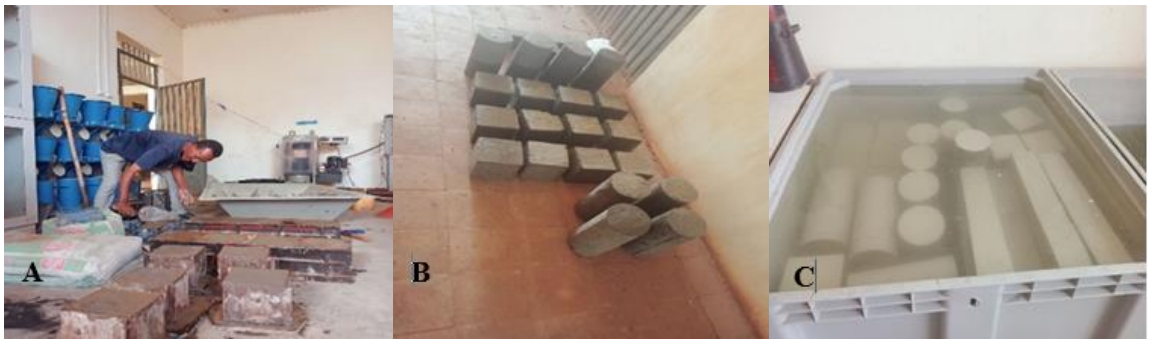

Fig.3. Preparation of specimens and curing.

2.4. Evaluation of bacteria viability in concrete

According to the ASTM C39/C39M specifications for M25 grade concrete, Table 1 presents the concrete mix design employed in this investigation for one-meter cubes of concrete specimens. The mix was used to make both regular and bacterial concrete. Normal concrete was used as a control (0%), and four different proportions of bacteria were added: 0.5%, 1.5%, 3.5%, and 5% of the cement weight. One gram of bacterial cells in powder form contains a concentration of 1 × 10^9. We have attempted to study and evaluate the survival rate of inserted bacterial spores (Bacillus subtilis spores at a concentration of 3.5×10^9 spores/cm³) in specimens of concrete-size cubes and cylinders arranged with a water-to-cement ratio of 0.5. For assessing bacterial viability over time, concrete samples were preserved for 7 and 28 days and analyzed using the most-probable-number (MPN) method, which relies on serial dilution. During this procedure, aged concrete specimens were crushed into a fine powder using a hammer and then suspended in a portion of the growth medium. The suspension was uniformly mixed via frequent agitation on a vortex mixer and ultrasonic treatment in a water bath. Subsequently, serial dilutions (eight replicates) were performed, comprising eleven successive tenfold dilutions into an alkaline growth medium within 96-well microtiter plates. Positive bacterial proliferation in the wells was readily discernible by the heightened turbidity of the medium, signifying bacterial activity. The MPN values, indicative of the estimated count of live bacteria, were calculated using a software program based on the methodology of [55]. This approach allowed for a precise and systematic evaluation of bacterial survival within the concrete matrix over an extended period. We can learn a great deal about how well the bacteria can survive the harsh conditions inside the concrete by observing their viability. These conditions include high alkalinity, low moisture, and limited access to nutrients. The results also help me understand the long-term benefits of using bacteria in self-healing concrete.

Table 1. Concrete mix proportions.

Quantity of material in (kg/m3)	
Cement (kg/m^3)	364.32
Sand (kg/m^3)	733.7
Aggregate (kg/m^3)	1,078
Water (kg/m^3)	182.16 kg (liters)
Bacteria in %	0, 0.5, 1.5, 3.5, and 5

2.5. Pore size distribution and strength analysis of aging concrete specimens

The research investigated the distribution of pore diameter sizes and the possible adverse effects of additive agents on the strength of concrete specimens cured for 7, 14, 28, 56, 90, 120, and 150 days. The concrete specimens, measuring 15 cm × 15 cm × 15 cm, were formulated with a water-to-cement ratio of 0.5, and their pore size distribution was evaluated using a mercury intrusion porosimeter (MIP), specifically the Micromeritics AutoPore IV Mercury Porosimeter. For the MIP testing, the aged specimens were initially fragmented into smaller pieces, measuring approximately 0.6 cm × 0.6 cm × 0.6 cm, using a chisel. The fragments were subsequently frozen in liquid nitrogen and subjected to cryo-vacuum evaporation for three weeks to ensure the elimination of pore water. Subsequently, the MIP experiments were conducted to examine the pore structure. The study also examined the development of compressive strength in cement stone specimens, comprising cubes with dimensions of 15 cm × 15 cm × 15 cm and cylindrical samples with dimensions of 30 cm × 15 cm for tensile strength assessments. The specimens were created with a w/c ratio of 0.5, including bacterial spores in the mixture. Bacterial spores are incorporated into the powder at four distinct proportions: 0.5%, 1.5%, 3.5%, and 5% of the cement weight, along with calcium lactate as a nutrient. Among these three distinct concentrations of bacteria, a 1.5% bacteria-to-cement weight ratio yields a 27.1% increase in compressive strength compared to standard concrete and the other bacteria concentrations. Then, the potential biomineral was determined using Scanning Electron Microscopy (SEM) with energy-dispersive X-ray Spectroscopy (EDS/EDX). The compressive strength of the specimens was

investigated using a compressive strength testing apparatus, facilitating a thorough examination of the impact of bacterial concentrations on strength development.

2.6. Concrete with incorporated healing agents and its biomineral synthesis ability

The specimens of the concrete measuring (15 x 15 x 15) cm^3 were prepared as control samples and with the incorporation of Bacillus subtilis spores at a different concentration of spores/cm^3, added at four different proportions: 0.5%, 1.5%, 3.5%, and 5% of the cement weight, along with 0.10%, 0.30%, 0.5%, and 0.75% of calcium-lactate respectively by the cement weight. The mixture was arranged with a water-to-cement ratio of 0.5 and permitted to cure for 7, 14, 28, 56, 90, 120, and 150 days. After the curing time, the specimens were fragmented into smaller segments. The pieces of the concrete were then cultured in tap-water at room temperature (27 ± 2°C) for one week to promote mineral formation on the fractured surfaces. The specimen pieces were washed with distilled water and examined using environmental scanning electron microscopy (ESEM) to investigate this mineralization process. No further treatment was necessary before the ESEM study, which facilitated the observation of mineral deposits on the fracture surfaces and provided insight into the concrete's self-healing capabilities, attributed to the presence of bacterial spores.

2.7. Artificial cracking and self-healing mechanism

We examined the self-healing capacity of concrete by casting cylindrical samples with different bacterial proportions relative to cement weight. The object was assessing the degree of crack sealing brought on by bacterially generated mineral precipitation and its effect on compressive strength. After curing, artificial cracks were introduced in all specimens to simulate real-world situations where concrete structures develop cracks over time. These cracks were allowable for the comparative investigation of bacterial self-healing effects across different bacterial concentrations, specifically 0% (control), 0.5%, 1.5%, 3.5%, and 5% of cement weight. Observations indicate that the highest bacterial concentration of 5% produced the highest amount of mineral precipitation, efficiently sealing more cracks than lower bacterial concentrations and standard concrete. The bacteria facilitated the development of calcium carbonate ($CaCO_3$), which was essential for sealing the fractures and enhancing the concrete's durability. The 5% bacterial sample's compressive strength was lower than the other samples' despite its enhanced capacity for self-healing. The inclusion of both 5% bacteria and 0.75% nutrients changed the cement's hydration process, resulting in prolonged setting periods, which is why the strength decreased. The mineral forms of all cylindrical samples are shown in Figure 10, which indicates a trend where more crystal formation in fractures is correlated with higher bacterial content. The process of self-healing mechanism is depicted in Figure 4. The dormant bacteria around the fissure are active when water enters into a new break in the concrete. They start to grow after activation and generate minerals as metabolic byproducts, most notably calcium carbonate. Over time, these minerals accumulate and form a protective barrier by sealing the cracks. This preserves the embedded steel reinforcement from damage by external substances like sulfates and chlorides, while also assisting in restoring the concrete's integrity.

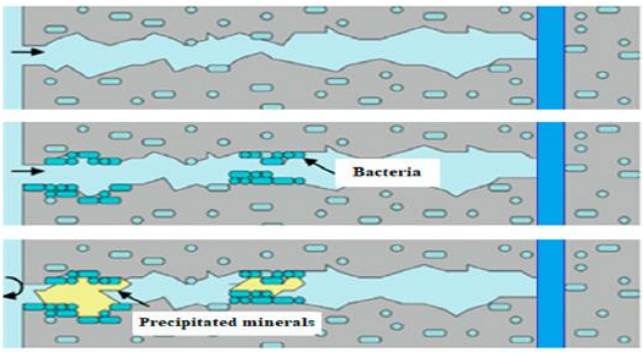

Fig. 4 The process of self-healing mechanism in self-healing concrete.

3. Results and discussion

3.1. Spore generation in bacteria

Adding calcium lactate to the growing media significantly accelerated the generation of bacterial spores. To produce the growth medium for bacterial cultures, we mixed 28 grams of calcium lactate powder with 1000 ml of purified water. The medium was fully dissolved by heating the solution to its boiling point. After complete dissolution, we autoclaved the solution at 15 pounds of pressure (121°C) for 15 minutes to eliminate any potential impurities. Following sterilization, the medium was cooled to a temperature range of 45°C to 50°C, confirming its readiness for use. The fluid was subsequently homogenized and dispensed into sterile petri dishes under aseptic conditions for additional research. A close examination of bacterial cultures growing in Figure 5A under a microscope revealed that spore development occurred within vegetative cells, indicating the formation of endospores. Following vegetative cell senescence, individual spores persisted in the culture. Using environmental scanning electron microscopy (ESEM) analysis, as shown in Figure 5B, made it easy to visualize these spores because their cell walls were robust. The measured spore sizes often varied from 0.6 to 1 μm, validating the effective development of endospores in the nutrient agar media.

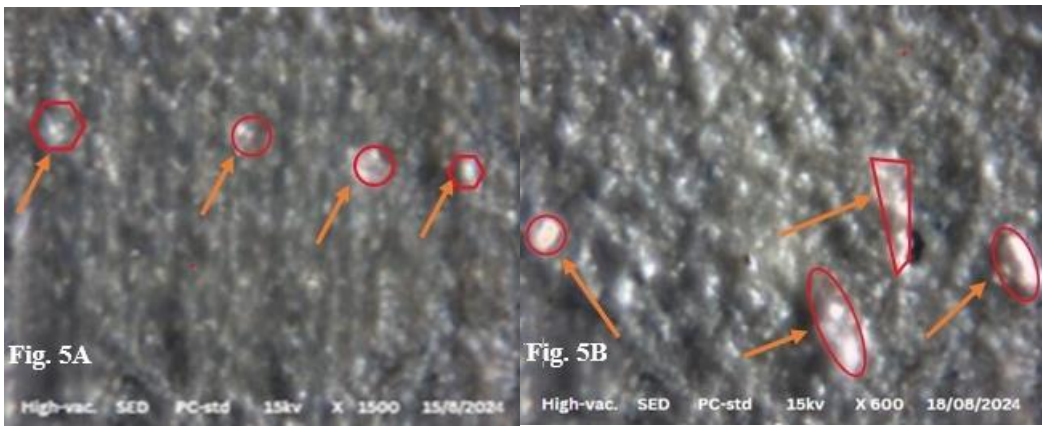

Fig 5. An optical photomicrograph 1500x shows Bacillus. S with clear internal spores Fig.5A, while an ESEM photomicrograph 600x shows spores that are separate and up to 1 μm in diameter Fig.5B.

3.2. Spore viability within incorporated cement stone

In aged concrete specimens, the MPN approach enabled the assessment of feasible bacterial cells, vegetative cells, and bacterial spores. The procedure involves extracting the bacteria from the concrete matrix and dispersing them into a single-cell solution. This necessitated the crushing and pulverization of the concrete using a strong mechanical force, followed by ultrasonic treatment. We evaluated the survivability of spores within the cement stone of the 3.5% bacterial concrete concentration among the four tested bacterial concentrations. We expected a relatively low output of bacterial cells due to the rigorous nature of this technique. For specimens cured for 7 days, approximately 1% of the initially inserted spores lost their viability, and around 2.5 × 10^7 of the 3.5 × 10^9 spores per cm^3 of concrete were recovered, as shown in Figure 6. Despite its modest recovery rate, we consider it acceptable, given the inherent difficulties in the extraction procedure. The survivability of spores in cement stone is contingent upon the bacterial species, cement composition, and environmental conditions. Encapsulation in carriers such as silica gel improves resilience to elevated pH and thermal hydration. Pore size affects viability; smaller pores restrict space, whereas larger pores enhance survival but compromise strength. Spores can remain alive for extended periods, activating upon the creation of cracks to initiate $CaCO_3$ precipitation, thereby facilitating self-repair. Viability can be evaluated by plate counting, fluorescence assays, scanning electron microscopy (SEM), and strength assessments.

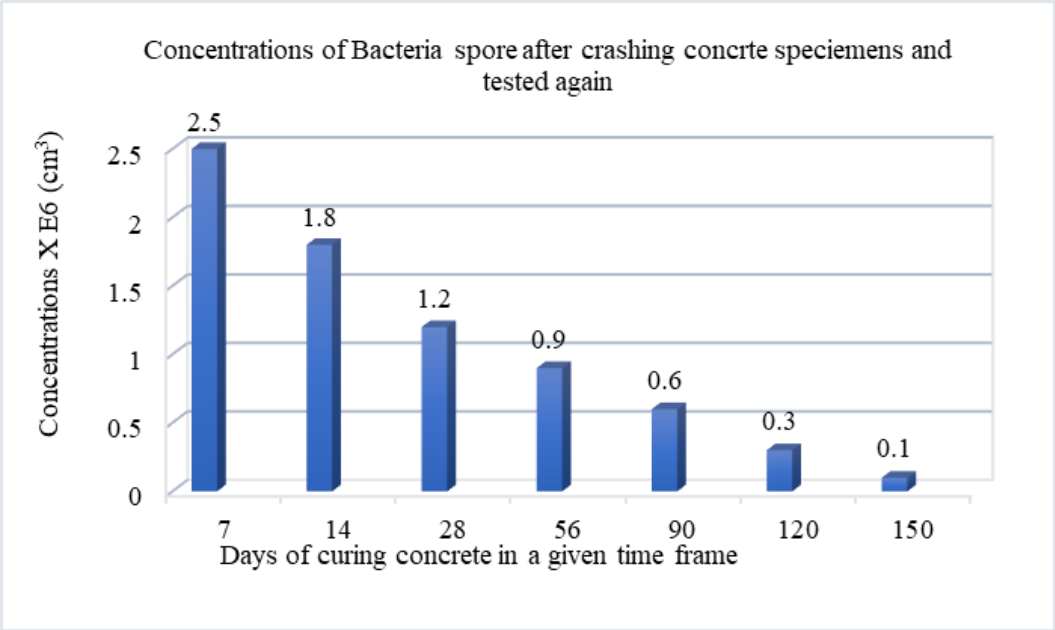

Fig. 6. MPN estimation was used to assess 3.5% of Bacillus subtilis spores viability in aged concrete specimens.

As the amounts of viable cells reduced significantly with the increasing age of the concrete specimens, it can be summarized that the feasibility of spores incorporated into the concrete diminished over time. The number of viable cells detected in concrete specimens cured for 120 and 150 days fell below the detection limit of the MPN technique ($<0.5 \times 10^3$ cells/cm³), suggesting that the majority of spores mixed directly into the concrete paste had lost viability and dormant within five months, because of pore size of the concrete. Simultaneously, control specimens, prepared without the addition of bacteria, also do not yield any feasible bacterial cells. These results confirm that the nonsterile tap water used during concrete preparation contained less than 0.5×10^3 cement-stone-resistant bacteria, if any, further underscoring the lack of external bacterial contamination in the concrete mix.

3.3. Strength and pore size distribution in samples

Evaluations were conducted on the strength development and pore size distribution of aging concrete specimens with and without integrated agents. Mercury intrusions porosimeter (MIP) analysis revealed two main pore diameter size classes inside the concrete matrix: 0.01–0.1 µm and 0.1–1 µm. We noted a significant alteration, with younger specimens (cured for 7 and 14 days) displaying a greater relative abundance of bigger pores, while older specimens (cured for 120 and 150 days) showed a tendency towards smaller pores. As the concrete specimens matured, the reduction of larger pores, generally measuring 0.5–1. µm (the size range for bacterial spores) became apparent. Compressive strength tests have demonstrated the diverse effects of various healing agents on concrete strength over time. When a large number of bacterial spores were added, the strength decreased by less than 10 to 15% in samples that were cured for different periods, as shown in Figure 7. Among these three distinct concentrations of bacteria, a 1.5% bacteria-to-cement weight ratio yields a 27.1% increase in compressive strength compared to normal concrete and the other bacteria concentrations. Numerous chemical substances, including calcium acetate, yeast extract, and peptone, significantly reduced strength, with peptone being particularly detrimental. The incorporation of calcium lactate either preserved strength in specimens cured for 3 and 7 days or marginally enhanced it in those cured for 28 days.

A quantitative relationship between pore size and compressive strength has been documented in the literature. According to the finding of [56], pore size reductions greatly enhance compressive strength. He found that compressive strength increased as pore volume decreased in this region. Pores smaller than 1

µm had a minimal impact on strength, whereas those between 0.01 and 1 µm exhibited a weaker effect. Pores greater than 1 µm drastically damage structural integrity. In our research, we suggest that lowering pore diameters to below 1 µm can increase compressive strength by 15–27%, depending on the degree of densification. This result highlights the importance of pore structure refinement for concrete performance, particularly in systems with bacterial self-healing, where calcium carbonate densification and precipitation limit pore size dispersion.

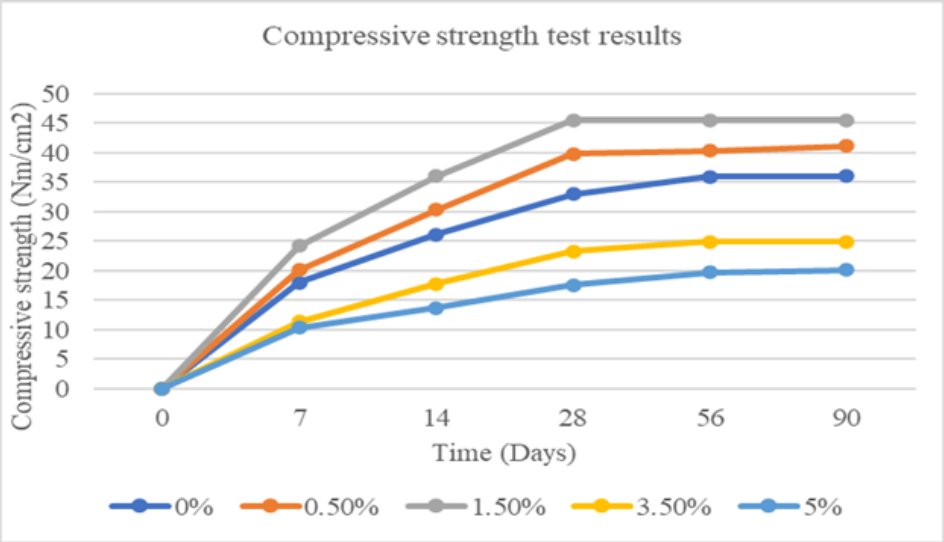

Fig.7. Compressive strength development in aged concrete specimens, both control and bacteria-incorporated.

3.4. Healing agent and its biomineral producing capacity

Concrete specimens without bacteria, cured for 7 and 28 days, exhibited the emergence of tiny particles, often measuring between 1 and 4 µm, on their crack surfaces, as shown in Figures 8A and 8B. The presence of substantial fibrous debris was a prominent feature on the fracture surfaces of the younger, 7-day-old specimens. This fibrous substance was not that match present on the fracture surfaces of older specimens cured for 28 and above days. The crack surfaces of concrete specimens containing the healing agent (bacteria and calcium lactate) appeared distinct. In specimens cured for seven days, substantial mineral-like particles, ranging in size from 18 to 78 µm, were observed on the fracture surfaces.

However, these larger particles were absent from the surfaces of specimens cured for 28 days, as shown in Figures 8C and 8D. Notably, the fracture surfaces of the older specimens, which had been treated for 28 days, were significantly different from those of the control group. This suggests that the healing process, particularly the formation of larger mineral particles, is more pronounced in the initial stages of curing. Self-healing concrete utilizes spore-forming bacteria, such as Bacillus species, and a supplement like calcium lactate. When water-filled cracks surface, then dormant bacteria become active, triggering metabolic processes that lead to biomineralization, primarily the precipitation of calcium carbonate. Cracks are filled, and structural integrity is restored. Bacterial viability, nutritional availability, and environmental factors affect biomineral production. Hydrogels and lightweight aggregates prolong and improve bacterial performance. Mineral deposition and healing effectiveness are evaluated using scanning electron microscopy (SEM), X-ray diffraction (XRD), and strength tests.

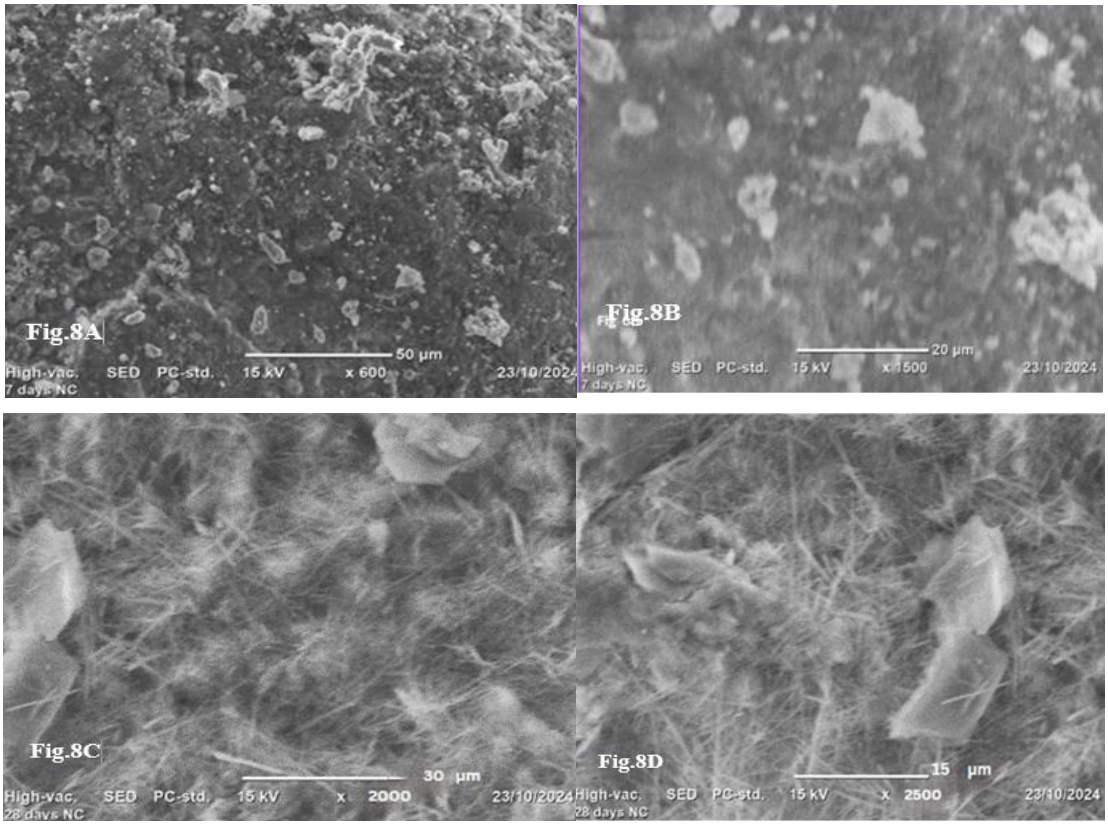

Fig. 8 Control concrete specimens that did not contain any incorporated healing agent showed cracks after 7 days of curing, as observed at 600× magnification (panel A) and 1500× magnification (panel B). Additionally, concrete specimens that did not contain any incorporated healing agent exhibited cracks after 28 days of curing, as observed at 2000× magnification (panel C) and 2500× (panel D).

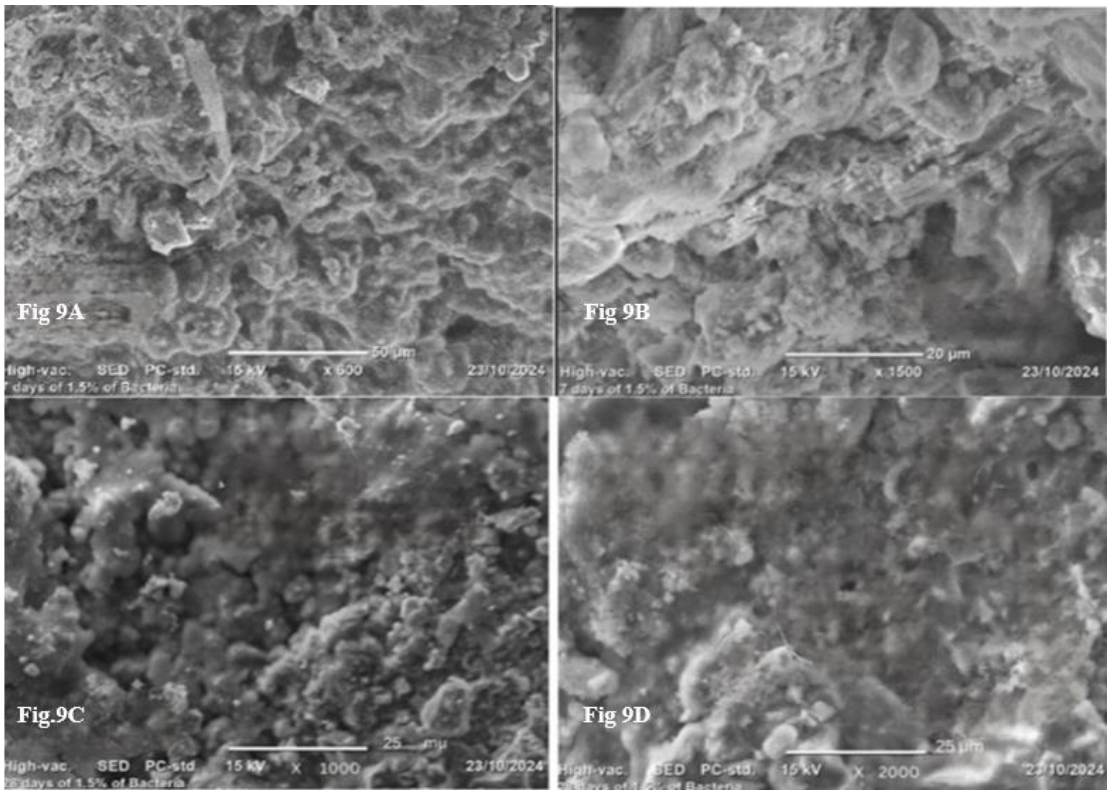

Fig.9 Shows concrete specimens with a healing agent were cracked after 7 days of curing (panels A: 600× and B: 1500× magnification) and 28 days of curing (panels C: 1000× and D: 2000× magnification). In specimens A and B, relatively large mineral precipitates, sized 18–78 µm, are visible. In contrast, those in specimens C and D are observed to be 5 -8 µm in size.

As shown in Figure 9, concrete specimens with a healing agent were cracked after 7 days of curing (panels A: 600× and B: 1500× magnification) and 28 days of curing (panels C: 1000× and D: 2000× magnification). In specimens (A and B), relatively large mineral precipitates, sized 18–78 µm, are visible, and 5-8 µm in size are observed in specimens (C and D).

3.5. Artificial cracking and healing mechanism

We examined cylindrical concrete specimens with varying bacterial concentrations (0%, 0.5%, 1.5%, 3.5%, and 5% of the cement weight) to evaluate their self-healing capabilities through artificial cracks, as shown in Figure 10. The sample with 5% bacteria showed the most mineral buildup, successfully closing 1- to 3-millimeter cracks, more than the others, because it produced more calcium carbonate ($CaCO_3$). This sample demonstrated the lowest compressive strength. The lower strength is due to a longer setting time caused by the mix of 5% bacteria and 0.75% nutrients, which interfered with the cement's hardening process.

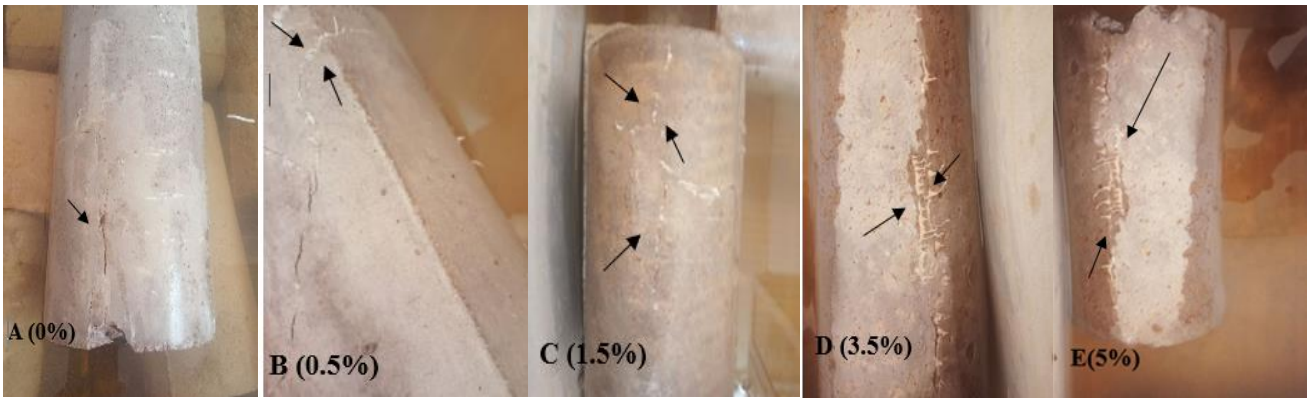

Fig. 10 Artificial cracking healing mechanism.

4. Discussions

4.1. Overall, the discussion of results

The primary goal of this research is to determine if bacteria embedded within the concrete matrix can act as self-healing agents, facilitating the automatic repair of newly formed cracks. A major concern regarding crack formation is the significant rise in permeability, which increases the risk of harm to both the concrete elements and the embedded reinforcement by facilitating the liking of water and corrosive substances. Bacterial-induced mineral precipitation may plug these fissures, thus decreasing permeability. Since bacteria act as catalysts, it is essential to incorporate a mineral precursor chemical into the concrete matrix, allowing the repair process to occur independently. We likely limit the quantity of this mineral precursor because excessive amounts may negatively impact other critical aspects of concrete, such as setting time and overall strength.

Thus, employing reduced quantities of the precursor compound is expected to effectively repair microcracks smaller than 1 to 3 mm in diameter, but it may prove inadequate for addressing larger macrocracks. This study demonstrated that an agent consisting of bacteria and calcium lactate promotes the growth of large mineral-like particles on the cracked surfaces of young concrete samples cured for 7 and 14 days. We found significant amounts of these precipitates, ranging in size from 18 to 78 µm. While further research is needed to confirm the effectiveness of this method in reducing material permeability, the initial findings are promising. Analysis by the ESEM indicates that the generated minerals are abundant and structurally resilient (Fig. 9A and 9 B). These crystals or minerals are likely calcium carbonate, produced through the bacterial metabolic breakdown of calcium lactate and calcium elements within the concrete. This metabolic response underpins the self-repairing mechanism and demonstrates the potential for enhancing the longevity of concrete buildings through the following reactions. The synthesis of calcium

carbonate ($CaCO_3$) within concrete involves a sequence of interactions between calcium ions (Ca^{2+}) and carbonate ions (CO_3^{2-}), often augmented by bacterial activity. Bacillus species can metabolize urea ($CO(NH_2)$ via an enzymatic mechanism known as urea hydrolysis. The urease enzyme facilitates the decomposition of urea into ammonia (NH_3) and carbon dioxide (CO_2). Then, ammonia (NH_3) interacts with water to form ammonium ions (NH_4^+) and hydroxide ions (OH^-):

$$CO(NH_2)2 + H_2O \rightarrow 2\ NH_3 + CO_2 \quad (2)$$

$$NH_3 + H_2O \rightarrow NH_4^+ + OH^- \quad (3)$$

During urea hydrolysis, the carbon dioxide (CO_2) produced reacts with water to form carbonic acid (H_2CO_3). This carbonic acid subsequently dissociates into bicarbonate ions (HCO_3^-) and carbonate ions (CO_3^{2-}). In the end, the free calcium ions (Ca^{2+}) in the concrete, which originate from cement or additional calcium sources such as calcium lactate, react with carbonate ions (CO_3^{2-}) to form calcium carbonate ($CaCO_3$), a solid mineral that is formed.

$$CO_2 + H_2O \rightarrow H_2CO_3 \rightarrow HCO_3^- + H^+ \quad (4)$$

$$Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3 \quad (5)$$

The process of bacterial-mineral production utilizing calcium lactate presents an alternative to the urea-based mechanism examined in previous research. The urease system generates a significant amount of ammonia, which increases the likelihood of reinforcement corrosion [60] and the concrete matrix's susceptibility to degradation, particularly when bacteria convert it into nitric acid [57]. The calcium lactate pathway, on the other hand, avoids these issues. A significant discovery of this research is that the mineral formation by the integrated two-component repairing agent only appears limited to fresh concrete. We observed substantial precipitate development in specimens aged 7 days, but this phenomenon decreased in those cured for 28 days and above. The capacity of bacteria used as self-healing agents to produce spores, which demonstrate significant resistance to mechanical stress and can survive for extended periods — potentially up to 200 years in arid conditions —intimately links their efficacy [58]. This robustness highlights their potential for long-term application in self-repairing concrete systems, but efficacy in aged concrete remains a barrier. This study shows that the functionality of bacteria added to concrete decreases over time, most likely because the bacteria become less viable with time. The gradual reduction in matrix pore size appears to be connected to the decline in spore viability.

The mercury intrusion porosimeter (MIP) research indicated that the bigger pore size category of 0.1–0.9 μm in the younger specimens of 7 to 14 days cured concrete specimens significantly diminished after 28 days, yielding smaller pores (0.01–0.1 μm). The larger pores in younger concrete may accept bacterial spores, typically measuring 0.8–1 μm in diameter; however, the diminished pore sizes in older concrete presumably lead to the crushing of these spores. This crushing not only reduces the spores' survival but also restricts their ability to create minerals, thereby diminishing the concrete's self-healing capabilities. To preserve the viability and functionality of bacterial spores, a practical approach could involve encapsulating or immobilizing them within a protective cover matrix before incorporating them into the fresh concrete mixture. An advantageous strategy could involve encapsulating spores within sol-gel-like substances, such as inorganic oxide matrices. Previous studies have shown that encapsulating Bacillus subtills spores in silica sol-gels effectively preserves their ability to grow. A proposed technique involves incorporating air-entraining chemicals into the elements of a concrete- matrix to generate isolated micropores, thus providing a safe habitat for the survival and functionality of bacterial spores over time. This would alleviate the problem of spore degradation in aging concrete and potentially improve long-term mineral yield.

5. Conclusions

This research suggests that spore-forming bacteria have the potential to be utilized, particularly in combination with calcium lactate, to enhance the self-healing properties of concrete through biomineralization. The addition of calcium lactate to the bacterial growth medium significantly accelerated spore formation, with spore sizes ranging between 0.6 to 1 μm, as confirmed by microscopic and ESEM. After being incorporated into the concrete matrix, the spores appeared to show an initial level of viability except for the material cured up to 7 days, where 1% of the spores initially inserted were dead, and 2.5×10^7 of the 3.5×10^9 spores per cm^3 of concrete were recovered. However, the survival properties of the spores diminished markedly with time, with viability being reduced to below-detectable levels in samples cured for 120 to 150 days. This suggests that bacterial spores can survive in the hostile environment of a concrete matrix during the early stages of healing; however, their long-term ability to perform this process is decreasing, which may lead to compromised long-term self-healing performance.

The results showed that pore structure is a crucial factor for bacterial viability and cement strength. Mercury intrusion porosimetry revealed that with the increase in concrete age, the larger pores decreased by less than 1%. This improvement in pore structure was accompanied by an enhanced compressive strength, consistent with the literature, where densification of pore size has been proportionally related to mechanical performance. It is important to note that, even if the integration of bacteria, particularly at higher concentrations such as 5%, increased calcium carbonate, it reduced the overall strength (by less than 10 to 15 %) because of the delay of setting time and hydration, but due to the calcium carbonate precipitation could compensate for this drawback, especially at early curing times. Concrete with 1.5% bacteria by weight of cement exhibited superior strength and generated calcium carbonate for healing cracks measuring 1 to 3 mm, compared to other bacteria-infused mixtures and standard concrete.

At the early stages of healing, the effectiveness of the healing agent was more pronounced as mineral particles (ranging from 18 to 78 μm) formed in the cracked area of the bacterial specimens. This deposition was missing in older samples, and this evidence suggests that bacterial healing action is higher at early ages. Artificial cracking experiments also confirmed this, with the results showing that an increased bacteria concentration led to rapid crack closure through increased $CaCO_3$ production. However, this came at the cost of delaying setting and increasing porosity, which did not lead to enhanced compressive strength.

Overall, bacteria-based self-healing concrete has the potential to enhance durability by repairing early cracks; however, the sustainability of its performance is challenged due to the reduction in bacterial viability over time. The research emphasizes the optimization of bacterial concentration, curing time, and encapsulation strategies to balance the retention of strength and effective healing. We suggest that the researcher be directed to understand the long-term viability of spores better and to study the encapsulation method for the long-term functional service life of the self-healing system in concrete.

Based on the problems that have been found with self-healing concrete, such as its decreasing effectiveness over time, the lower feasibility of bacterial spores, and the lower number of bacterial spores in older concrete, we suggest the following solutions:

Encapsulation of Bacterial Spores: To prevent bacterial spores from being crushed and dying in old concrete, researchers should consider encapsulating them in protective materials, such as sol-gel matrices or silica-based compounds. This can provide physical protection and maintain bacterial viability for extended periods.

Use of air-entraining agents: Incorporating air-entraining agents into the fresh concrete mixture generates isolated micropores that allow bacterial spores to exist without exposure to compressive forces, thereby aiding in the preservation of their functionality as the concrete matures.

Optimization of mineral precursors: The concentration of calcium lactate or other mineral precursors must be carefully regulated to guarantee adequate nutrition availability for bacteria during the concrete's lifespan. This can improve long-term bacterial activity and self-healing capacity.

Development of slow-release nutrient systems: Integrating a slow-release nutrient system within the concrete matrix may prolong the viability and activity of bacterial spores, thereby sustaining self-healing capabilities for a prolonged duration.

Further investigation of Bacterial strains: Investigating alternative strains of Bacillus or other bacteria that exhibit greater resilience to aging or the variable conditions of concrete may assist in preserving viability and prolonging the material's self-healing capabilities.

List of Abbreviations

OPC……Ordinary Portland cement
$NaHCO_3$……. Sodium bicarbonate
Na_2CO_3……...Sodium carbonate
CaCl2……. Calcium Chloride
KCl ……Potassium chloride
$MgCl_2$ ……Magnesium Chloride
$NaHCO_2$……. Sodium bicarbonate
KH_2PO_4 ……. Potassium dihydrogen phosphate
ASTM……American Society for Testing and Materials
MPN……...Most-probable-number
MIP……. Mercury intrusion porosimeter
SEM……Scanning Electron Microscopy
EDX…….……. Energy Dispersive X-ray Spectroscopy
ICCR……. Indian council for cultural relations

Availability of data and materials

All data generated or analyzed during this study are included in this published article and are available on request from the corresponding author.

Conflicts of interest

The authors declare that they have no competing interests.

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Authors contribution statement

Habtamu Teshome: Conceptualization, methodology, designing; collecting data; conducting experments; wrting orginal draft; analyzing mechanical and microstructural features; interpreting the results and reviewing and edting.

Battula Saradhi: Conceptualization; methodology; supervision; analayzing mechanical and microstructural features and interprating the result; and reviewing and editingAcknowledgement.

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