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Review Article

A Review of Biomineralization as Solution for Roads and Infrastructures Concrete Sustainability

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Abstract

Concrete cracks in roads and infrastructure are ubiquitous due to environmental factors, fatigue, and material degradation. Applying bacteria with self-healing capabilities in concrete matrices is proposed as a solution. These bacteria, activated by water and oxygen ingress, produce calcium carbonate through biomineralization. They are improving structural integrity while reducing the adverse effects of chemical and water infiltration. The quantity of Bacillus bacteria to be added to the concrete mixture is an integral part of the standardization of the self-healing mechanism. 10^5 - 10^8 cells/mL of spores experienced improvement in mechanical properties and self-healing efficiency. Various Bacillus strains, such as *Bacillus sphaericus*, *Bacillus subtilis*, and *Bacillus megaterium*, are typically utilized in self-healing. The by-product of biomineralization, calcium carbonate, is an autonomous crack and pore sealer, which can be evaluated via SEM, XRD, and XDS. The study highlights the testing methodologies used to examine calcite deposition. Also, it reiterates the importance of urease activity evaluation before bacterial propagation to confirm the occurrence of the biomineralization process. Moreover, the article reiterates the bacteria's history, origin, and pathogenicity, bridging the gap concerning bacteria propagation safety and the need for industry-accepted standards and certification procedures. The transition from laboratory experiments to large-scale implementation is advocated to demonstrate bacterial concrete's sustainability and economic feasibility for broader industry adoption. Finally, bacteria concrete is a ground-breaking approach that unites construction and biology for long-term sustainable transportation materials and construction.

Keywords: Transportation Materials; Self-Healing Concrete; Biomineralization; Infrastructure Sustainability.

1. Introduction

The demand for innovative materials that balance ecological responsibility and structural resilience has been essential throughout the nations' challenge with the environmental effects of growing transportation networks. In this framework, the incorporation of self-healing concrete is a noteworthy frontier that presents a revolutionary method for augmenting the sustainability of transportation materials [1, 2].

Concrete is one of the most critical, adaptable, diverse, heterogeneous, and widely utilized construction materials in the world [3-5]. However, this material is brittle and likely to crack due to the concrete's relatively low-tension strength, varying stresses, and atmospheric conditions that accelerate the deterioration of the structures [4]. The subsequent fissures make the concrete susceptible to detrimental effects by allowing hazardous material-containing liquid and gas to infiltrate the concrete matrix [6, 7]. Non-structural fractures have a tangential impact on the service life of the structure. In contrast, structural cracks significantly reduce the load-bearing strength. Indeed, cracks undermine

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durability through the deterioration of the concrete's integrity and the reinforcement bars oxidation [7]. Repairing the shattered section through concrete rebuilding and maintenance will be highly costly, labor-intensive, inconvenient, and could harm the environment [3, 4, 8]. Researchers have been creating a technique known as self-healing to minimize concrete structure degradation in response to cracking and developing structural damage [9].

Self-healing was first recognized in 1836. Since then, countless researchers have investigated novel approaches to functionalize it in concrete [10]. Self-healing concrete represents an innovative development in construction materials, aiming to address one of the primary challenges in structural longevity and their subsequent deterioration [11] by reducing porosity and permeability by introducing bacterial spores into concrete matrices, which decreases the penetration of chloride and the probability of the sulfate exposure of materials [4]. Recently, other accessible artificial self-healing materials have been evaluated, such as shape-memory materials, fly ash, slag, silica particles, chemical encapsulation, and hollow fibers, which can restore mechanical performance and transport qualities even after significant damage. It is recognized that this process is an autogenous self-healing mechanism; structures can be made more reliable and safer, which can have a significant positive impact on the environment without requiring any human interference [11]. However, the autogenous mechanism is only effective for early cracks, where materials can mend gradually in the presence of water. Further, crack sealing has only been observed during the initial recovery and is insufficient for the long-term healing of cracks [12].

A different method is called "microbial-based self-healing," an autonomous mechanism that promotes concrete structure repair without needing an external sealing agent by employing the inherent attributes of bacteria [11]. Shashank et al. [13] examined the direct application of Bacillus Subtilis and Bacillus Sphaericus through a three-point bending test in concrete that contained silica fumes and calcium lactate. Research findings indicate that Bacillus subtilis improves their durability, fracture behavior, and deflection in concrete samples. Further, the study found that it would be feasible to use bacteria in concrete to modify reinforced concrete's fracture and deflection parameters [13]. However, research must delve further into the biomineralization process to properly evaluate the crack-sealing efficacy, employing advanced laboratory equipment such as Scanning Electron Microscopy (SEM) further to confirm the establishment of calcite [6]. The authors recommend that encapsulation strategies be considered in the self-healing process to obtain more significant healing and compressive strength results than direct bacteria application in concrete mixtures [14].

Furthermore, Khushnood et al. [6] experimented with recycled concrete aggregate (RCA) to carry Bacillus Subtilis. At 28 days of healing, infilled fissures with calcite precipitation indicate bio-mineralization caused by microbial metabolic activity. In comparison to the direct addition of bacteria to the concrete mix, which resulted in a 0.6mm healed crack and a 69% recovery index [6], there was a considerable reduction in crack width of up to 0.7mm and a 76% strength recovery index [6]. In another study, Rajawat et al. [14] ingested Bacillus megaterium spores in a variety of carriers, including lightweight aggregate (LWA) and Exfoliated Graphite Nano Platelet (xGnP). After 14 and 28 days, the fractures included significant calcium carbonate crystals ($CaCO_3$), a critical healing component that assists in reducing crack width in bacteria-infused specimens. However, urease activity and calcite precipitation must be determined before encapsulating bacteria spores to guarantee calcite formation through biomineralization [15]. Furthermore, Sohail et al. [16] encapsulated the Bacillus cereus strain in sodium alginate beads. The chosen strain could fill cracks with widths ranging from 0.162 mm to 0.67 mm, whereas the Sporosarcina pasteurii strain could fill cracks with widths ranging from 0.200 mm to 4.7 mm. The X-ray diffraction (XRD) spectra and SEM images showed calcium carbonates in the fractures. However, the Bacillus Cereus strain may be hazardous to humans [17]. Future research must verify the pathogenicity of the strain to be used in upscale manufacturing [11] to address one of the challenges related to self-healing: construction professionals are cautious and unaware of bioprocesses [18].

Bacillus bacteria have been misconstrued as hazardous to human health [18]. In this study, the pathogenicity and substantial advantages of bacteria from the Bacillus family are reaffirmed to bridge the gap that suggests that human psychology is the primary barrier to their adoption of self-healing concrete. Furthermore, this study evaluated recent research on self-healing concrete to determine the predominant bacteria used in the process and the optimal number of bacteria administered to achieve a significant outcome. Moreover, this review highlighted the historical evolution of self-healing concrete, the mechanism of self-healing concrete, the biomineralization process, methods for evaluating crack sealing efficiency, and the influence of bacteria in concrete. The objectives of this study are 1. To influence industry experts about the safe application and acceptance of the biomineralization process from a construction perspective; 2. Establish standard quantity and guidelines for bacteria spores, nutrient medium, and self-healing efficiency evaluation of concrete matrices for upscale implementation and field trials; 3. Identifying various challenges in integrating bacteria into existing construction practices ensures seamless conventional concrete mixes, making it a viable and workable solution for the sustainability of roads and infrastructure.

2. Historical Evolution of Self-Healing Concrete

Examining the historical trajectory allows for identifying trends, patterns, and critical milestones. This insight enables researchers and practitioners to recognize recurring themes, breakthroughs, and setbacks, providing valuable lessons for the present and future for applying Self-healing concrete. Moreover, examining the historical context also sheds light on ethical considerations and controversies within the spanning field of biology and construction materials sustainability, illustrated in Figure 1.

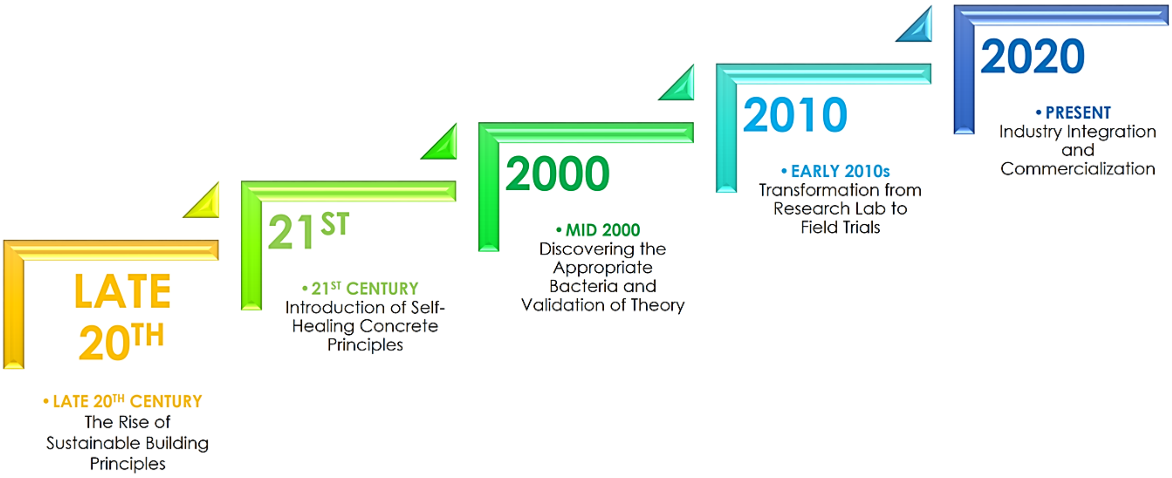

Figure 1. Evolution of Self-Healing Concrete [18-24]

2.1. The Rise of Sustainable Building Principles (Late 20th Century)

In the late 20th century, the rising demand for eco–friendly materials prompted a substantial movement in construction processes toward sustainable approaches. Research on substitute materials and techniques meant to lessen the environmental effect of infrastructure projects. To reduce their adverse environmental impact, engineers emphasized using sustainable materials such as bamboo, recycled steel, and recovered wood [19].

2.2. Introduction of Self-Healing Concrete Principles (Early 21st Century)

In the early 21st century, the generation of ground-breaking research conceptualizing self-healing concrete was identified. Various studies have investigated using bacteria, found in *Bacillus species*—as a self-healing component in concrete [20]. Scientists have been able to preserve the bacteria and enable them to produce calcium carbonate. They are mixed into the concrete mix along with nutrients [17, 18]. It has been shown that this bacteria-induced calcite precipitation process is a potential method of bridging substantial fissures [20]. Furthermore, early research concentrated on identifying suitable bacteria species that could remain dormant in concrete until moisture infiltration stimulated them, such as *Bacillus subtilis, Bacillus sphaericus*, and *Sporosarcina pasteurii.*

2.3. Discovering the Appropriate Bacteria and Validation of Theory (Mid-2000s)

Early research in the mid-2000s revealed bacteria that could survive the alkaline conditions of concrete and produce calcite, including *Bacillus subtilis* and *Sporosarcina pasteurii.* The proof of concept was proven in laboratory trials, which showed that these bacteria could mend fissures when they became activated by moisture. This creative method offers the ability to lessen the environmental impact of concrete repair and maintenance, marking a significant improvement in building materials [20, 21]. Scientists have found that concrete fissures up to 1.8 mm wide can automatically repair themselves by introducing ureolytic immobilized bacteria into concrete matrices [22].

2.4. Transformation from Research Lab to Field Trials (Late 2000s - Early 2010s)

The construction industry has dramatically emphasized the progression of self-healing concrete from successful laboratory tests to field testing and pilot projects. This stage evaluated the practicality of self-healing concrete in building and transportation infrastructure. Studies and advancements in the domain of self-healing concrete have exhibited this substance's capacity to self-heal cracks, prohibit corrosion of rebar, minimize concrete deterioration, lessen the need for costly and time-consuming routine maintenance and repairs, and enhance durability [18, 23] This novel ingredient has been recognized as a potentially effective way to mitigate the deterioration of concrete structures [23].

In Yangzhou City, Jiangsu Province, China, Zhang et al. demonstrated the self-healing technology in a water conveyance gallery of the Mandao River ship lock wall. Researchers vigorously observed cracks in both self-healing and regular concrete. Standard concrete contained fissures that had not healed after 65 days, whereas the self-healing concrete's cracks had several white particles that filled the crack on the surface. The lock chamber was finally delivered for use after 120 days of pouring, and the concrete with microbial self-healing properties completed satisfactorily mending the early cracks before the backwater was navigable [24].

2.5. Industry Integration and Commercialization (Present)

In recent years, guidelines for pursuing self-healing concrete utilizing bacteria stimulation have not been available. The industry has shown a rising interest in implementing sustainable transportation materials such as bio-concrete, and scientific and corporate partnerships seek to increase manufacturing capacity, establish standardized procedures, and explore the financial feasibility of using self-healing concrete in transportation-related projects [25-27].

3. Research Methodology

In pursuing knowledge and exploring complex self-healing mechanisms, authors prioritize the navigation of all publications from 2000 – 2023 in the research database of Science Direct and Google Scholar to generate precise data for reiterating the constraints inhibiting the industry's adoption of self-healing materials. Figure 2 illustrates professionals' growing interest in self-healing from the years 2000 - 2023; 10,546 were included in publications reported in Google Scholar (9050) and Science Direct (1,496). The screening process for publications by the authors illustrated in Figure 3 is restricted to those that begin with the keywords "Self-healing Concrete," "Bacteria," and "Transportation Sustainability" in both databases and published articles between 2019 and 2023. The findings showed that 240 articles were correlated and subjected to initial screening. The authors focus on utilizing bacteria from the Bacillus family, which revealed only 90 out of 240 articles from the selected databases. Only 47 out of 90 articles were synthesized and subject to eligibility requirements, including laboratory-scale experiments of self-healing concrete. The final evaluation navigated 34 out of 47 experimental research, which has been included in the manuscript after eliminating outdated, inconsequential, and duplicated articles. Revealing the research challenges discussed in sections 4 to 6 and containing the research gaps reiterated in section 7.

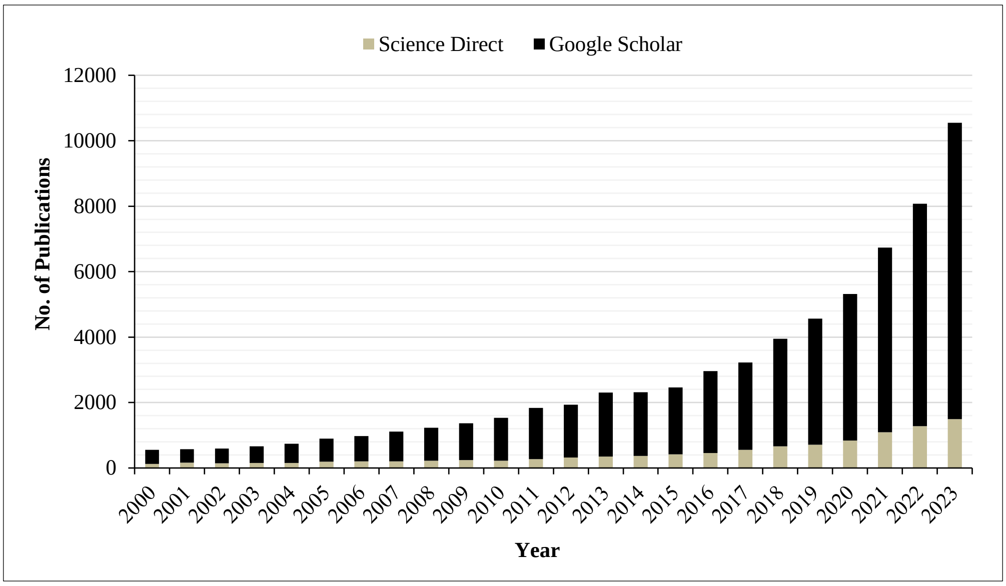

Figure 2. Number of Publication of Self-healing Concrete (Year 2000-2023)

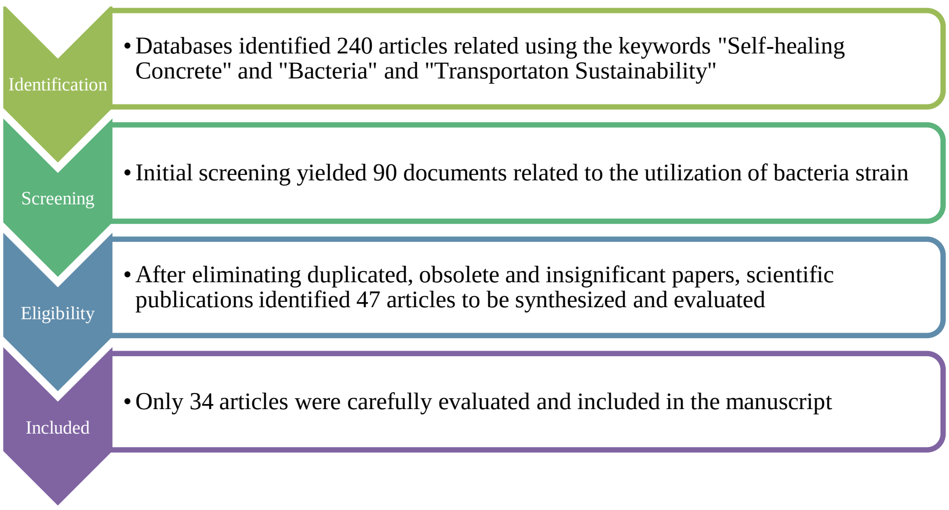

Figure 3. Research Methodology

4. Identification of Bacteria

Identifying bacteria from the Bacillus Family that could endure the extremely alkaline condition of concrete, remain dormant until activated, and produce calcite effectively is the key to using them in self-healing concrete [18, 21]. Bacillus species are known to be capable of producing dormant spores in unfavorable environmental settings. These endospores might live for a very long time. Endospores are extensively distributed in nature, mostly in soil, where they infiltrate dust particles. They are resistant to heat, chemicals, and sunlight [28]. Table 1 showcases the list of Bacteria, bacteria origin, and pathogenicity frequently used for self-healing mechanisms.

Table 1. Commonly Applied Bacteria in Self-Healing Mechanism

Bacteria	Description	Origin
Bacillus Subtilis	*Bacillus subtilis* is a versatile, safe, non-pathogenic Gram-positive bacterium with many biotechnological applications [29]. Since it can produce robust spores, which enable it to withstand harsh environments [30], encompassing the concrete's alkalinity. This spore is commonly considered for use in self-healing concrete [30].	Bacillus subtilis is a widespread soil organism essential to breaking organic materials. Its presence in the soil improves soil health, nutrient cycling, and plant nutrient uptake [31, 32].
Bacillus cohnii	A short rod, aerobic, Gram-positive, Bacilli isolate and migratory [33, 34].	Typically unearthed in waste in landfills or soil [33, 34].
Bacillus megaterium	Bacillus megaterium, the largest known Bacillus species, has dimensions of 4 µm in length and 1.5 µm in width (micrometers; 1 µm = 10−6 m). Usually, Bacillus is found in clusters [27]. For many years, B. megaterium has been a significant industrial organism. It generates many enzymes, including amylases used in baking and glucose dehydrogenase used in glucose blood testing, as well as penicillin amidase, which is used to make synthetic penicillin [35] and AIDS diagnostic [36].	B. megaterium can be found everywhere. It is an endophyte and a common soil bacterium that is also present in various foods, such as honey, dried food, milk, and bee pollen [35, 36]. B. Megaterium thrives in a temperature range of 3 to 45 °C, with 30 °C. It was discovered that specific isolates from an Antarctic geothermal lake could grow at 63 °C [1, 16]. It is a known endophyte that may be used as a biocontrol agent for plant diseases [35].
Bacillus sphaericus	Bacillus sphaericus (Lysinibacillus sphaericus) [37] is an aerobic, mesophilic, Gram-positive bacteria in soil and aquatic environments worldwide. Since certain strains harm mosquito larvae, it is well known for having mosquitocidal qualities. A distinctive spherical spore produced by Bacillus sphaericus is found at one end of the enlarged sporangium. The most potent strains, B. sphaericus 2362, are utilized in commercial solutions like VectoLex® to eliminate nuisance and vector mosquito larvae globally [35, 36], which is efficient against dengue viruses [37].	B. sphaericus is generally found in soil. It can produce endospores that are resistant to harsh chemicals, heat, and U.V. radiation and that can live for extended periods [37]
Bacillus Pasturii	Previously identified as the Gram-positive bacterium Sporosarcina pasteurii [37], One characteristic of the Bacillus class is its capacity to generate endospores under favorable environmental conditions, which increases its chances of survival. Its width and length range from 0.5 to 1.2 and 1.3 to 4.0 microns, respectively. It grows best in basic pH 9–10 conditions as an alkaliphile. It can withstand severe circumstances up to a pH of 11.2 [38].	Bacillus pasteurii is soil-borne, heterotrophic facultative anaerobic anaerobes that need ammonium and urea to grow [38].

The Bacillus family of bacteria is widely utilized in autonomous self-healing mechanisms [6]. These kinds of bacteria can be found in dust, water, and higher concentrations in soil [39]. Research has shown that bacillus spores can also be found in plants and small intestines of humans and animals [39], which could remain dormant and survive in harsh and dry environments for over 200 years [9, 40]. Moreover, these bacteria can still survive in the alkaline environment inside the concrete until they are stimulated by the presence of oxygen and water [7, 11, 14]. The decomposition of bacteria precipitates calcium carbonate. Calcium Carbonate ($CaCO_3$) is a compound that is environmentally safe, highly compatible with concrete, and effectively fills and seals microscopic cracks and voids [11, 14].

Identifying specific bacteria is pivotal in self-healing concrete, enhancing the material's resilience and longevity. Among the significant bacteria implicated, as shown in Figure 4, *Bacillus Sphaericus*, Bacillus subtilis, and Bacillus megaterium have received much interest from experts in recent years. These spores stand out as Gram-positive, versatile microorganisms with various biotechnological applications. Moreover, these bacteria were recognized for their safety and non-pathogenic nature [27, 35, 36]. *Bacillus sphaericus* is renowned for having mosquitocidal effects. Several strains are pathogenic to mosquito larvae and help manage populations of nuisance and vector mosquitoes [41, 42]. The *Bacillus sphaericus* can survive very long by creating endospores that withstand high temperatures, harsh chemicals, and UV rays [37].

The German botanist Fernand Cohn recognized two variants of hay bacillus, or Bacillus subtilis, in 1877. One type could withstand the heat, whereas the other could not resist it. After learning that these latent forms could be transformed into vegetative or actively growing stages, he dubbed the heat-resistant forms "spores" (endospores). Antibiotics that are valuable in medicine, such as bacitracin, are produced by Bacillus subtilis [28]. Bacillus subtilis is a pervasive soil organism essential to breaking organic materials. Its presence in the soil promotes soil health and the cycling of nutrients [31, 32].

Another prevalent bacterium, shown in Figure 4, is a spore-forming bacteria called *Bacillus megaterium*, found in rice paddies, sediments, seas, dried food, milk, and honey [41]. This spore has been an important industrial organism for decades. It produces penicillin amidase used to make synthetic penicillin and several enzymes, such as amylases used in the baking industry and glucose dehydrogenase used in glucose blood tests [35] and AIDS diagnostic [36].

These bacterial protagonists, with distinguished characteristics and applications, are safe [27, 35, 36] and vital ingredients of the concrete paradigm for self-healing. They can endure adverse conditions, thrive in alkaline environments, synthesize urease when activated, and process biomineralization [34]. Studies have confirmed that these Bacillus spores are essential to the state-of-the-art field of self-healing concrete technology.

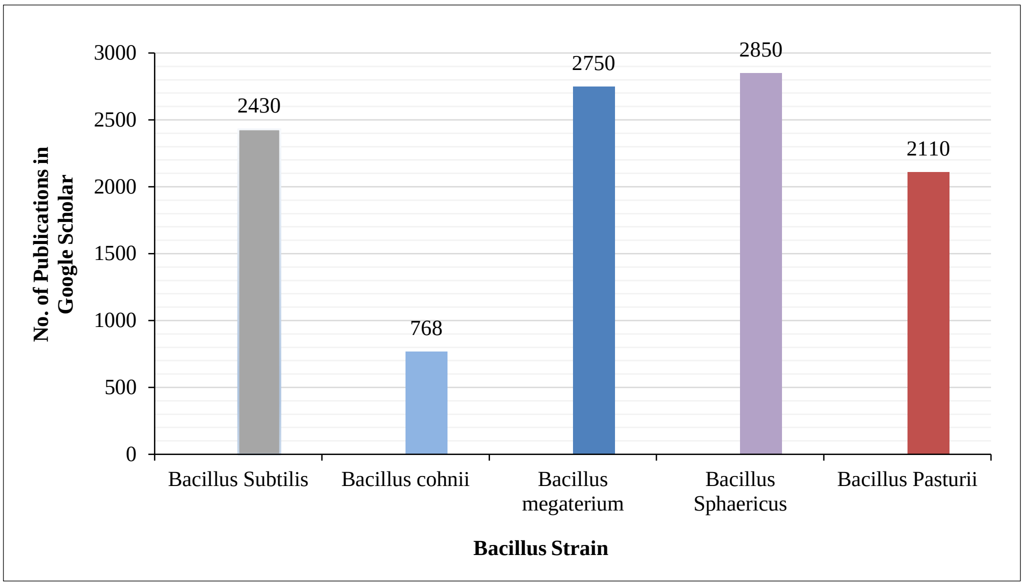

Figure 4. The volume of published articles about the strains of bacteria used in self-healing mechanisms in Google Scholar

5. Biomineralization Process

Biomineralization is how organisms create mineralized components with superior qualities and hierarchical structures, such as bones and teeth. Materials for complex tissue regeneration can be designed and built with the influence of the fundamental processes and mechanisms of biomineralization. Precisely, by using artificial materials inspired by biology to replicate the roles of biomolecules or stabilize intermediary mineral phases involved in biomineralization, the creation processes of minerals can be partially mimicked [43].

The mechanisms underlying the healing process in self-healing concrete involve the activation of specific bacteria within the concrete matrix, triggering a sequence of biological and chemical reactions illustrated in Figure 5. Dormant biological healing agents are activated when concrete cracks allow water and air gasses to infiltrate the structure, as shown in Figure 5-a, and the spores then start synthesizing calcium carbonate by breaking down nutrients in the concrete matrix, which helps to heal cracks. The newly generated calcite crystals fill the crack naturally, blocking water, harsh chemicals, and other harmful substances from permeating the concrete [18, 23] shown in Figure 5-b.

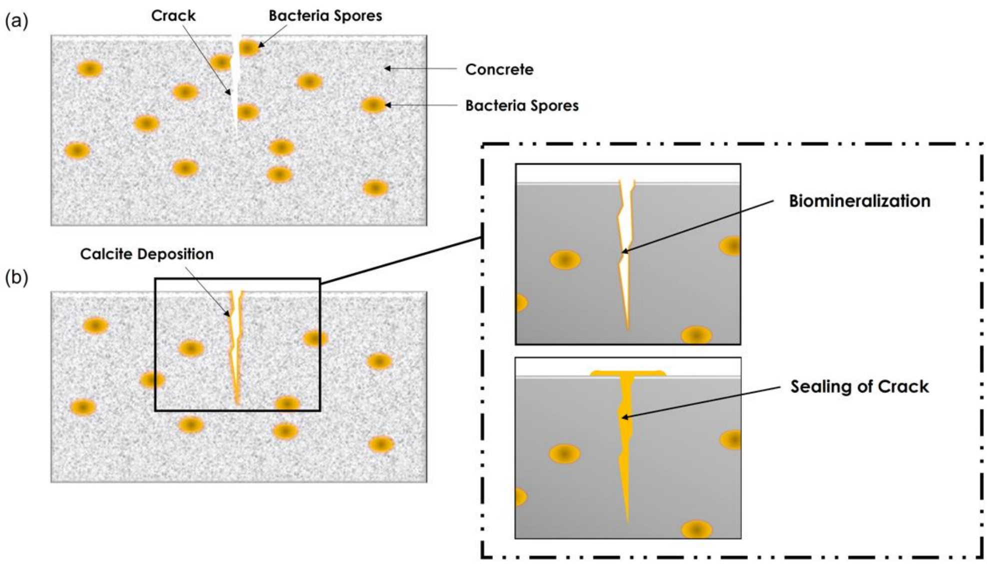

Figure 5. Schematic Illustration of Self-Healing Mechanism: a) crack formation in concrete, b) Bacteria-induced biomineralization process sealing of the crack

Concrete micro-cracks and micropores can be sealed by Calcium carbonate crystals produced by bacteria-incorporated materials, making them effective in sealing cracks. A physiologically driven mineralization reaction can precipitate $CaCO_3$ in the presence of a calcium supply. In this Biomineralization process, carbonate is created extracellularly by microbes through a variety of metabolic pathways, including dissimilatory nitrification, urea hydrolysis, oxygenic and anoxygenic photosynthesis, minimization of calcium sulfate, and utilization of organic acid [20].

Biomineralization yields calcium carbonate, which lowers the porosity of concrete by 32–48% and plugs gaps of varying sizes [11]. The biomineralization process from bacteria dormancy and crack augmentation is listed below, and this bacterial activity is deemed environmentally beneficial and non-hazardous.

5.1. Dormancy and Activation

Bacteria incorporated in concrete matrices are dormant or spore-like organisms that stay inert within the concrete mixture. These bacteria are usually " asleep " until certain stimuli cause them to become active. They may survive in the complex alkaline environment of the concrete matrix in their dormancy, which prevents them from using up all of their energy rapidly [44-46].

5.2. Moisture Activation

Concrete cracks allow water to penetrate the material, creating paths for moisture to enter. The hibernating bacteria in the fractures are activated when they encounter moisture. The bacteria respond biologically to this moisture, a significant environmental indication that turns them from dormant to active. The bacteria then break down micronutrients in the concrete matrix to create calcium carbonate, contributing to the healing of cracks. Concrete's ability to restore itself relies on moisture activation, which starts the healing process by igniting dormant biological healing agents [44, 46-48].

5.3. Bacteria Nutrients and Carrier

Nutrient combinations are essential for the growth and metabolism of bacteria. Vital minerals like iron, magnesium, zinc, and others are considered micronutrients. The primary macronutrients include carbon, nitrogen, phosphorus, sulfur, oxygen, and hydrogen. Organic materials, inorganic chemicals, and the environment are among the different sources from which bacteria get these nutrients [49, 50]. Bacteria can have varying nutritional necessities depending on their taxonomy and ambient suitability. For instance, although some bacteria are heterotrophs and rely on organic matter for sustenance, others are autotrophs and can generate nutrition from inorganic compounds [51]. Moreover, bacteria can receive nutrients and energy through photosynthesis, the breakdown of chemical compounds, and the decomposition of waste and lifeless organisms [49, 50, 52].

Bacteria are added to the concrete mixture as admixture through a carrier network or specific protective agent. The bacteria have been sustained during the mixing process and are dormant until they are activated by the carrier system, which acts as a protective refuge for them. Usually, the carrier is made from materials that protect the bacteria from the abrasive properties of the concrete mix and offer a regulated release mechanism if cracks appear. In self-healing concrete, organic substances like gelatin, chitosan, and alginate, as well as inorganic substances like zeolites, silica, and clay minerals, are frequently used as carriers of bacteria. Considering that the performance of concrete varies depending on the type of area and structure, the practical use of concrete must be factored in while adopting a carrier. Every structure has unique characteristics in specific performance areas and the carriers' final varying effects [46, 53, 54]. Bacteria can flourish in a dynamic environment by using a diverse range of nutritional methods to stimulate the precipitation of calcium carbonate. Moreover, to improve the effectiveness of self-healing to a certain degree, it is necessary to create carriers for microbial self-healing materials with various unique components [13].

5.4. Calcium Carbonate Synthesis

Calcium carbonate synthesis is a form of biomineralization where living organisms produce minerals through biological processes. The bacteria generate calcium carbonate as a by-product of their metabolic activity, contributing to the healing of concrete cracks [15, 55, 56]. It has been revealed that calcium chloride and sodium carbonate/bicarbonate produced during microbial decomposition [57] are crucial for the crystallization (vaterite or calcite phase) of particles of Calcium carbonate [56].

5.5. Crack Sealing and Augmentation

Calcite crystals that develop due to biomineralization are the crack's genetic sealant. This capping mechanism prevents water, harsh chemicals, or other toxic materials from infiltrating the concrete. The existence of unique, intertwined calcite crystals suggests that the calcite crystals are growing in an uneven mesangial matrix manner, a process known as calcite sealing [58]. The closure mechanism enables the restoration of the material's structural integrity, making it a vital phase in the self-healing process of concrete [18, 44].

5.1.1. Methods in Identifying Crack Sealing Efficiency

5.1.1.1. Sorptivity

Sorptivity is essential for grasping the durability of concrete materials, especially regarding water intrusion and possible moisture-related damage. Concrete with a low sorptivity coefficient is more resistant to absorbing water. Still, a high sorptivity coefficient suggests the presence of a highly linked pore structure, which may result in increased water absorption and possible damage [59, 60].

According to the investigation of Sumathi et al. [34], wet and dry curing procedures make the structure more compact as sorptivity reduces when bacteria are present in the concrete mixture. The sorptivity value dropped because of the pores' and crevices' improved infill capacity, which led to more calcium silicate hydrate gel and higher matrix healing efficiency, which can also be seen in several studies.

5.5.1.2. Water Absorption

The ability of concrete to absorb water significantly impacts the performance and longevity of concrete structures, especially regarding water intrusion and possible moisture-related damage. The water absorption test determines the concrete sample's capacity to absorb water, and the findings are reported as a percentage of the sample's original weight [59, 61]. The ASTM C1585-13 Standard Test Method measures the rate at which hydraulic cement concretes absorb water [62]. This technique aims to ascertain whether unsaturated concrete is susceptible to water seeping through. Concrete absorbs water at a different pace near the surface than when a sample is obtained from the inside [62]. Concrete is said to be more water-resistant and long-lasting, lowering its water absorption rate [59, 61].

Research by Sumathi et al. [34] has shown that self-healing concrete lowers water absorption because of the biomineralization process [55]. This process results in the filling of the concrete's micropores and voids, which is caused by the growth of bacteria that permits the accumulation of excessive calcium carbonate precipitation within the pores [34].

5.5.1.3. Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) is a sophisticated imaging method that uses electron beams to see the specimens at very high magnifications. Compared to optical microscopy, SEM offers far more detailed, high-resolution images with a significantly more comprehensive depth of field. The surface morphology and topography of the calcite crystals produced by bacteria and the surrounding concrete matrix can be captured in detail using SEM, making it an invaluable tool for researching the impacts of biomineralization in concrete. As a result, self-healing concrete's performance can be enhanced, and researchers' understanding of its mechanics can be deepened [63, 64]. Research has shown that SEM images have observed the dense formation of calcium carbonate crystals in a rhombohedral shape and ettringite fibers [34].

5.5.1.4 X-ray Diffraction (XRD)

X-ray powder diffraction (XRD) is a straightforward analytical method that can reveal unit cell dimensions and is mainly used to identify the phase of crystalline material. The average bulk composition is established after homogenizing and finely pulverizing the examined material [65]. Moreover, XRD is the only non-destructive and precise laboratory method for determining characteristics, including chemical composition, crystal structure, orientation, crystallite size, lattice strain, preferred orientation, and layer thickness. Thus, a wide range of materials, including solids, thin films, and nanomaterials, can be analyzed using XRD [66]. Studies have demonstrated that XRD can evaluate the self-healing characteristics of a mixture of bacteria-caused calcite (Ca), aragonite (A.R.), and vaterite (Va) through mineral precipitation [34].

6. Influence of Bacteria in Concrete Structures

Integrating bacteria into cement-based materials can benefit different aspects of the structure, especially regarding self-healing concrete. Studies show that mineralizing bacteria can strengthen concrete's structure and enhance durability [40, 67]. Concrete pores can be sealed with bacterial precipitation, lowering pore volumes and increasing compressive strength [67]. Furthermore, the bacteria's deposition of calcium carbonate can strengthen the damaged area, contribute to the repair of the structural integrity of the concrete, and increase the concrete's load-bearing capacity [67]. Table 2 illustrates favorable outcomes observed in diverse experiments involving the incorporation of bacteria.

Table 2. Experimental outcomes observed in various studies using different Bacteria spores

Bacteria ID	Origin/Bacteria ID	Qty of Bacteria in Concrete Mixture	Nutrient/Medium	Bacteria Carrier	SHC Analyses	Results	References
Bacillus Subtilis	ATCC 11774	1.9×10^7 cells/cm^3 of concrete.	Lablemco powder (1.0 g/l), peptone (5 g/l), yeast extracts (2.0 g/l), and sodium chloride (5.0 g/l)	Impregnated in RCA	Determine elements such as "Aragonite" or other forms such as calcite, aragonite, and Vaterite through FESEM and XDS analysis.	There is conclusive proof of bio-mineralization brought on by microbial metabolism, as calcite precipitation-filled fissures that heal in 28 days. Developed fissures in the specimens carrying immobilized B. subtilis demonstrated consistent crack repair with comparatively calcite precipitation higher.	Khushnood et al. (2020) [6]
Bacillus Subtilis		10^7 CFU/ml	Nutrient Agar	Direct Application 6L/cu.m with Calcium Lactate 18kg/cum	Subjected to 3-point bending test analysis	Reconfigure the reinforced concrete beam's fracture and deflection parameters.	Shashank, et al. (2022) [13]
Bacillus cohnii	Not specified	(10^5-10^{10} cells/ml)	Luria Bertani (LB) media at pH 7.0, 37 ◦C for 16h	Direct mixture with calcium lactate (2 wt% of cement).	They were calculated using TGA (Thermal Gravimetric Analysis) at temperatures between 550^0 and 800^0 Celsius. The amount of $CaCO_3$ was confirmed using EDS	Strengthens concrete, heals cracks, occupies spaces, and lessens the material's permeability.	Sakar et al. (2023) [11]
Bacillus cohnii	Dumping area at Tamil Nadu, India.	10^5 cells/mL	Basal media	Direct application in the concrete mix, 5% Bacterial Solution, and 95% Nutrient Solution	XRD (Calcium, Argonite, Veritite), SEM, Sorptivity	The authors confirmed the presence of calcium carbonate in concrete.	Sumathi et al. (2020) [34]
Bacillus megaterium	Not specified	2.8×10^8 cells/ml	Nutrient Agar	Calcium lactate 18kg/m^3		Significant reduction in crack width	Rajawat et al. (2023) [14]
Bacillus megaterium	Not specified	2.8×10^8 cells/ml	Nutrient Agar	Light Weight Aggregate 6L/cu.m with Calcium Lactate	Only a tri-axial loading test was conducted	It seems to be unaffected by multiple axial stress	Rajawat et al (2023), and Jenson [14, 39]
Bacillus megaterium	Not specified	2.8×10^8 cells/ml	Nutrient Agar	Exfoliated Graphite Nano-Platelets (xGnP) 6L/cu.m with Calcium Lactate	-	Demonstrated an increased crack healing	Rajawat et al. (2023) [14]
Bacillus Sphaericus	Not specified	10^7 CFU/ml	Nutrient Agar	Direct Application 6L/cu.m with Calcium Lactate 18kg/cum	Subjected to 3-point bending test analysis	Based on observations, it was determined that the use of bacteria enhances the self-healing and fracture behavior of concrete	Shashank, et al. (2022) [13]
Fusarium oxysporum	(ATCC MYA-1198)	Not Specified	Potato Dextrose Agar (PDA) and PDB broth solution	Mycelium fibers	SEM/EDS, FTIR	The authors concluded that pH and healing time are related. In 2.38 days, cracks less than pH 9 healed by 0.15 m, while cracks over pH 12 healed in 16.71 days.	Zhang et al (2021) [7]
Bacillus Pasturii	BCRC11596 (Taiwan)	Not Specified	-	Light Weight Aggregates, natural shale burned around 1100-1200 Celsius	Upon observing urease activity, calcium carbonate was formed. XRD and FESEM	The calcium carbonate crystal healed the crack, measuring less than 0.1 mm on the 14th day. Filled the fissure was observed after 91 days of curing,	Chen et al (2019) [21]

Bacteria affiliated with the Bacillus family in concrete mixtures have the most potential to minimize crack width and enhance the structure's longevity [14]. Sumathi et al. [34] and Sakar et al. [11] experimented directly integrating 10^5 – 10^9 cells/mL of *Bacillus Cohnni* into the concrete mix. Sakar et al. [11] conducted an experiment showing a relationship between self-healing concrete strength, porosity, and calcium carbonate deposition. Sakar et al. [11] blended *Bacillus Cohnii* and calcium lactate to generate calcium carbonate in the concrete mixture; the integration enhanced the concrete strength through the microbial metabolism of aerobic bacteria, which plugged up voids and reduced the permeability of materials. Moreover, Sumathi et al. [34] reported that the direct addition of *Bacillus Cohnni* to the concrete mix had increased the compressive strength by about 15.81% in full-wet curing and 12.8% in wet-dry curing of the specimen compared to the conventional mix after 28 days. Strength recovery was also reported to be successful in full-wet curing, rising around 60%, 85%, and 98% for 3, 7, and 14 days, respectively. Research demonstrated that a 0.285 mm crack width was 90% repaired at the end of 28 days, and introducing bacteria to concrete brings a decrease in the water absorption and sorptivity of the concrete mixture [13]. Therefore, Sumathi et al. [34] hypothesized that full-wet curing might result in notable self-healing efficiency of materials. However, Zhang et al. [7] concluded that healing time and pH level correlate. In 2.38 days, cracks less than pH 9 healed by 0.15 m, while cracks over pH 12 healed in 16.71 days. Future research must investigate the survivability of spores that can withstand high pH levels and hostile environments to enhance long-term and repetitive crack healing.

Furthermore, Shashank et al. [13] observed that the direct addition of *Bacillus Subtilis* and *Bacillus Sphaericus* yielded significantly higher strength properties than conventional concrete mixtures. Also, a study has observed beam samples' enhanced fracture and deflection attributes. Additionally, Khushnood et al. [6] and Shashank et al. [13] have concluded the presence of biomineralization using *Bacillus Subtilis* and continuous healing of fractures in concrete samples, as examined by FESEM and XRD analysis [6] an 85% maximum strength recovery was achieved on the 3rd day in pre-cracked samples by utilizing a combination of recycled concrete aggregate (RCA) and 50% bacteria-suspended fine aggregates. Bacteria impregnated with RCA effectively restored a crack width of 1.1 mm [6], and the experiment concluded that RCA was an effective carrier of microbial cells for interim crack mending and strength restoration [6]. However, concrete in roads and infrastructures endure repeated loads and fatigue, which require long-term sealing and multiple-strength recovery. Therefore, research suggests that the efficacy of encapsulation solutions utilizing various carriers should be assessed in the self-healing process to achieve substantial outcomes in repetitive crack sealing and enhancement of concrete mechanical properties [14].

Exploring different application approaches, Rajawat et al. [14] evaluated the direct application, implantation, and encapsulation of 2.8×10^8 cells/mL Bacillus Megaterium in the concrete mixture. Employing exfoliated graphite nanoplatelets (xGnP) as the bacteria carrier component exhibited higher efficacy of crack healing, which repaired around 0.81mm crack width after 28 days, concerning Lightweight Aggregate (LWA) implantation and direct spore integration with fracture mending widths of 0.65 mm and 0.38 mm, respectively. Rajawat et al. [14] concluded that the direct application of Bacillus Megaterium into specimens had an insignificant effect on concrete crack healing and suggested the use of different carriers and encapsulation to promote the long-term viability of bacteria spores in the concrete mix.

Research has demonstrated that applying bacteria from the Bacillus family in concrete can autonomously heal cracks higher than 1.0 mm [6, 7, 11, 13, 14] and improve concrete's durability in abrasive environments [6, 7, 11], making it suitable for use in various construction projects, including roads and infrastructure. However, experts must address all associated challenges to assess the suitability of bacteria in a large-scale construction project with an uncontrolled environment, including identifying the optimal quantity of bacteria in the concrete mixture to produce higher self-healing efficiency without compromising the concrete durability. In Table 2, Sumathi et al. [34] harnessed a smaller quantity of bacterial spores, about 10^5 cells/m. However, the minimal number of spores demonstrated superior results. Sakar et al. [11] evaluated different quantities of spores with different amounts of calcium lactate. 10^5 - 10^8 cells/mL of endospores inhibited an increase of 40% and 60% in compressive strength on the 3rd day and 28th day, respectively. However, no significant strength increment is observed between a higher number of cells from 10^8-10^{10} cells/mL. Sakar et al. [11] also concluded that the strength of concrete is decreased with an increased amount of nutrient medium calcium lactate added to the mixture. Assessing the spores' origin and growing media is necessary to standardize the self-healing materials. Although nutrient mediums such as yeast, meat extract, peptone, and sodium chloride [6] are now readily accessible, future studies should assess bacterial growth using various media to enable industry experts to propagate and inoculate on a broad spectrum for field and up-scale implementation without compromising the durability and mechanical properties of the structure.

Indeed, guaranteeing calcite production is crucial for developing self-healing concrete [18, 44]. Analyzing self-healing efficiency in laboratory settings requires using FESEM and SEM, two microscopic imaging tools for calcite formation detection [6], covered in Chapter 5.5.1. Besides, discerning the amplification of the biomineralization process employing sorptivity and water absorption tests [34], Tri-axial loading test, and split tensile test for fracture behavior analysis and strength recovery of materials [13] are crucial to the self-healing concrete's standardization. Most studies examined the calcite deposition in pre-cracked material using X-ray diffraction (XRD) and field emission scanning electron microscopy (FESEM) [6, 7, 34]. Sakar et al. [11] utilized Mercury Porosimetry Intrusion (MIP) to measure the crack diameter, ascertain the material's pore size distribution and the degree of biomineralization-induced crack refinement [11], Sakar et al [12] emphasized that concrete deteriorates by fissures larger than 200 mm which could be reduced to 87% by biomineralization after 28 days, as per experimental results [12]. However, evaluation procedures mainly focus on the accumulation of calcite. The preliminary biological test must be performed before the concrete mix to confirm the biomineralization process and examine if the bacteria can still survive the harsh environment of concrete [13, 21]. Chen et al. [21] performed urease test to examine the Bacillus Pasturii survivability, reactivation, and bacterial activity after being stored in lightweight aggregates. The experiment concluded that Bacillus spores could be regerminated after being dormant in its carrier. Moreover, the study concluded that the urease-containing Bacillus pasteurii strain will still be released after activation to undergo microbial-induced-calcite-precipitation activity [19].

Bacteria boost the self-healing capacity of concrete, which could enhance its durability. Bacillus Cohnni, Bacillus Subtilis, Bacillus Sphaericus, Bacillus Megaterium, Bacillus Pasturii and other spores from Bacillus., have been found to minimize crack width and improve structure longevity. However, since concrete experiences residual loads and fractures, evaluation of multiple strength recovery and long-term sealing is necessary. Experts must address all the challenges to evaluate the suitability of bacteria in large-scale implementation and field application.

7. Research Challenges

Identifying and addressing research gaps is crucial for advancing the field of self-healing concrete using bacteria. Allocating resources can be achieved by prioritizing research fields according to identified gaps. To maximize the impact of research endeavors, researchers might concentrate their time, resources, and efforts on areas where there is an actual need for additional exploration, as suggested below.

7.1. Durability and Long-Term Performance

Self-healing properties in laboratories and fields have shown indications of short-term efficacy. Long-term investigations are required to evaluate self-healing concrete's resilience and continuous performance. It is crucial to comprehend how the material behaves in changing environments [68-70].

7.2. Bacterial Viability and Activation Efficiency

Enhancing the bacterial viability and activation efficiency in the concrete matrix is still challenging. Research should maximize bacterial dormancy, activation stimuli, and nutrition availability parameters to ensure reliable and consistent healing recovery over time. Today, there is no standard for the number of bacteria, even on a laboratory scale; upscale implementation field trials must be obtained to examine the efficacy of the self-healing mechanism in an uncontrolled environment. To improve the chances of bacterial survival, encapsulation of bacteria in protective materials [14] such as hydrogels, expanded clay, perlite, diatomaceous earth, and alginate beads [71], a combination of fibers and bacteria, with natural fibers being more beneficial to the concrete matrix and holds excellent significance [72]. Moreover, they ensure that the necessary nutrients are sufficient for calcite precipitation and bacterial metabolism. Research suggested that the survivability of bacteria at high temperatures (up to 50 °C) and high humidity (up to 100%), typical conditions in many regions of the world, must be observed. Furthermore, parametric studies are required to establish the ideal concentrations of nutrients and particles in concrete [16].

7.3. Scaling Up Production and Cost-Effectiveness

The transition from laboratory-based investigations to large-scale manufacturing presents obstacles concerning preserving bacteria, economic feasibility, and harmonizing with traditional concrete production procedures. Significant studies are required to create scalable manufacturing techniques that are practically and economically feasible for broad industry adoption in sustainable construction. The application cost of bacteria concrete can be higher, which is prevalent because of nutrients, transportation, and preservation of bacterial viable cells [9, 71].

7.4. Market and Industry Acceptance

The biggest problem is that experts involved in construction are uninformed of bioprocesses. Bacillus bacteria are frequently misinterpreted as being harmful to health. This gap indicates that human psychology is the main factor opposing their acceptance. Experts must advocate bacteria's pathogenicity, which would benefit both the building society and business [11].

7.5. Alternative Bacteria Species and Integration Methods

Research into various bacterial species possessing superior characteristics, including increased calcite production or resilience, may result in progress. Furthermore, looking into other ways to integrate bacteria more effectively and consistently into concrete mixes is imperative for practical implementation. Proposed mixtures of fibers, glass fiber, steel fiber, or synthetic non-corrosive steel fiber and bacteria can impact concrete properties and be advantageous [72].

7.6. Standardization and Certification

The adoption and implementation of self-healing concrete are hampered by the absence of standard operating procedures and guidelines for its manufacture and use. Creating industry-accepted guidelines, certification processes, and legislative frameworks is necessary to close this gap and guarantee the dependability and security of self-healing concrete in building projects. To produce, test, and apply self-healing concrete in a consistent and validated manner and eventually enable its integration into construction processes, upscale implementation and field trial are significant [18, 25, 70].

7.7. Integration with Existing Road and Infrastructure

The integration and compatibility of self-healing concrete with current infrastructure should be investigated. For practical implementation, it is essential to comprehend how this novel material interacts with conventional concrete and evaluate whether it is for retrofitting, patching, and repairing the existing roads and structures.

8. Conclusion and Recommendation

One of the main problems of roads, infrastructure, and structural concrete is that cracks will inevitably emerge due to environmental causes, fatigue, and material degradation. This problem might be addressed by the self-healing ability of bacteria in concrete structures. Bacteria use chemical and biological processes to help produce calcium carbonate, which efficiently seals and repairs fissures. When a crack forms, biomineralization occurs through water and oxygen ingress, triggering the activation of dormant bacteria containing proper nutrients. Bacteria precipitate calcium carbonate, which gives concrete a unique ability for self-repair. This mechanism strengthens the concrete's structural integrity and lessens the effects of water intrusion, chemical ingress, and reinforcing steel corrosion. Incorporating bacteria into concrete structures is a ground-breaking method for improving the durability, longevity, and sustainability of construction materials and infrastructure. However, there are numerous constraints that this mechanism must overcome. Solving these challenges will significantly impact the construction industry and the preservation of roads and infrastructure.

The quantity of Bacillus to be added to the concrete mixture is an integral part of the standardization of the self-healing mechanism. 10^5 - 10^8 cells/mL of spores experienced improvement in mechanical properties and self-healing efficiency. Various Bacillus strains, such as *Bacillus sphaericus*, *Bacillus subtilis*, and *Bacillus megaterium*, are typically utilized in self-healing. Although it has been demonstrated in multiple publications that these bacteria can rapidly mend concrete cracks, Upscale implementation and industry adoption of guidelines are still inhibited, attributable to policies and standard operating procedures that allow for its widespread distribution, particularly the mass propagation of bacteria. Perhaps people are still worried about a potential outbreak. These soil-found bacteria are non-pathogenic and are typically used as antibiotics and medicines. Negative stereotypes regarding the pathogenicity of this strain hamper the use of these innovative materials. Closing this gap will require industry-accepted standards, certification procedures, public understanding, and guaranteed safety of the self-healing process.

Calcium carbonate (Calcite) was produced as a by-product of biomineralization and is used as an autonomous crack and pore sealer for concrete. Self-healing analyses such as the water absorption test, sorptivity test, SEM, XRD, and EDS are frequently used to examine calcite deposition in concrete. It has been found, nevertheless, that only a few studies have discussed urease activity before bacterial propagation. The ability of the spores to generate calcium carbonate can be determined initially by urease activity. Researchers need to conduct a urease test to verify that the spores have undergone the biomineralization process before the mass propagation of bacteria for large-scale projects.

Studies have proven the improved mechanical properties and sealing effectiveness of bacterial concrete. However, roads and infrastructures are subjected to massive stress and fatigue, and it is essential to enhance their regenerative strength capacity after the initial healing. The viability of the bacteria must be improved through encapsulation pathways using protective materials like hydrogels, lightweight aggregate, alginate beads, fibers, and biochar, which can store nutrients, oxygen, and water for long durations. Moreover, integrating steel fibers as bacteria carriers will probably improve materials' stress-bearing capacity and self-healing efficiency since fibers have been utilized to minimize cracks and combat residual stresses and fatigue.

Laboratory experimentation must shift to a large-scale implementation to demonstrate the sustainability of these innovative materials. Large-scale implementation initiatives are needed to develop optimized manufacturing of bacteria products or admixtures that are practically and economically feasible for broader industry adoption.

Indeed, the influence of bacteria in concrete extends beyond mere crack repair. It holds the potential to revolutionize maintenance practices, reducing the need for frequent manual repair and contributing to long-term cost savings. The adaptability of bacteria to existing construction practices ensures seamless integration into conventional concrete mixes, making it a feasible and practical solution for the sustainability of transportation materials.

Introducing bacteria into concrete represents a radical shift in which materials are active in maintenance and repair. This bio-inspired method opens new possibilities for developing infrastructure by combining biology and construction. The impact of bacteria in concrete structures stands out as a ground-breaking solution with enormous potential for the long-term sustainable development of our built environment as we traverse the complex roads and infrastructure challenges.

9. Declarations

9.1. Author Contributions

Conceptualization, R.D.R. and M.P.D.G.; methodology, R.D.R.; software, A.D.L.C.; validation, R.D.R. and M.P.D.G.; formal analysis, R.D.R.; investigation, R.D.R.; resources, R.D.R.; data curation, R.D.R. and M.P.D.G.; writing—original draft preparation, R.D.R.; writing—review and editing, M.P.D.G.; visualization, R.D.R. and M.P.D.G.; supervision, A.D.L.C.; project administration, M.P.D.G.; funding acquisition, A.D.L.C. All authors have read and agreed to the published version of the manuscript.

9.2. Data Availability Statement

Data sharing is not applicable to this article.

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9.4. Conflicts of Interest

The authors declare no conflict of interest.

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