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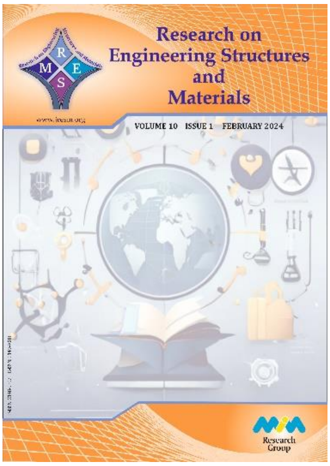

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Review Article

Harnessing microbes for self-healing concrete – A review

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Abstract

This review explores the burgeoning field of microbially enhanced construction materials, with a special focus on self-healing concrete, through the lens of microbial biotechnology. Central to this discourse is the innovative use of bacteria, particularly Bacillus species, to address the pervasive issue of microcracks in concrete, a fundamental material in the construction industry. Traditional remedies, such as chemical admixtures and fiber reinforcements, offer partial solutions; however, self-healing concrete represents a paradigm shift, harnessing the natural calcite-precipitating ability of bacteria to autonomously repair cracks, thereby augmenting structural durability and longevity. Delving into the mechanics, the bacteria, embedded within the concrete matrix, remain dormant until crack formation triggers their metabolic pathways, leading to calcite production that effectively seals the fissures. This bio-mediated repair mechanism not only enhances the structural integrity of concrete but also aligns with sustainable construction practices by minimizing maintenance requirements and material wastage. The review extends beyond self-healing phenomena, encompassing broader applications of microbial technology in construction, including bio-concrete, bio-cement, and soil stabilization methods. These applications underscore the versatility of microbes in enhancing material properties such as compressive strength, tensile resilience, and water impermeability. Empirical evidence underscores the necessity of optimizing bacterial dosages and curing conditions to maximize the self-healing efficiency. Future research trajectories should aim to elucidate the complex interactions between microbial agents and concrete matrices, assess long-term performance, and evaluate the environmental and economic sustainability of microbial interventions in construction. The integration of microbial technology in construction materials heralds a new epoch of innovation, offering robust, sustainable, and resilient solutions to enduring challenges in the industry.

1. Introduction

In the evolving landscape of civil engineering, the quest for durable and sustainable building materials has become paramount, driven by the urgent need to address the environmental and structural challenges of modern construction. Concrete, the cornerstone of global infrastructure development, is under increasing scrutiny due to its vulnerability to degradation and the environmental impact of its production. This paper explores the utilization of microorganisms in construction materials and the methods employed to evaluate their effect on strength. Concrete, being the most extensively used building material in construction projects (1) is not without its challenges, including the presence of micro cracks (2). These micro cracks can reduce the workability of concrete and potentially lead to structural collapse and failure.

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To address these challenges, various approaches have been employed, such as the use of chemical admixtures (3) and fiber concretes (4). Additionally, the concept of self-healing concrete has gained attention, aiming to minimize the need for manual maintenance. One promising self-healing approach involves the utilization of specific bacteria that operate independently within the concrete structure (2). When tensile forces exceed the concrete's strength, fractures occur (5). These fractures can serve as pathways for water ingress and the infiltration of toxic substances (6). Concrete cracks can be classified into two groups: non-hazardous cracks that are visually unappealing but do not pose a safety threat (7), and structural cracks that require careful attention to prevent significant harm to the overall structure. Self-healing concrete methods, such as bacterial reactions within the hardened concrete, have shown promise in mitigating the latter type of cracks (8).

Micro cracks typically range from 0.1 to 0.05 mm in width, and their presence allows the infiltration of water particles, acting as capillaries (9). When water droplets penetrate the cracks, partially or completely unreacted cement hydrates, leading to expansion and subsequent sealing of the crack (10). Recent studies, by Saravanan et al., have delved into the compressive strength development of geopolymer concrete using manufactured sand, highlighting the environmental benefits and performance efficiencies of alternative materials like geopolymer concrete in reducing carbon emissions associated with conventional cement usage (11). Similarly, the work of Chaitanya et al. on the self-healing characteristics of GGBS admixed concrete using Artificial Neural Networks underscores the innovative strategies being developed to enhance concrete's self-healing properties and reduce its carbon footprint (12).

Moreover, Cappellesso et al. (2023) reviewed the efficiency of self-healing concrete technologies, affirming the potential of microbial and chemical methods to extend the service life of concrete structures (13). The research on Ground Granulated Blast Furnace Slag (GGBS) in concrete by Subramanian et al. (2022) also emphasizes the improved flexural behavior and durability of concrete beams reinforced with polymer composites (14). In addition, the investigations into the prediction of self-healing characteristics of concrete with GGBS by M. Chaitanya et al. (2020) and the work of Aleem et al. on the properties of Geopolymer concrete with M-sand provide comprehensive insights into the advancements in concrete technology that contribute to more sustainable construction practices (15,16).

The exploration of self-healing concrete is important, particularly in the context of material costs and the judicious selection of optimal materials tailored to project requirements. Attaining high-quality self-healing concrete necessitates a thorough examination of prior studies to identify effective solutions. Understanding the response and workability of concrete, along with addressing challenges in healing or sealing cracks, remains a dilemma. Furthermore, self-healing concrete is recognized as a viable approach to minimize structural maintenance requirements. Following established methodologies outlined in previous literature can lead to reduced maintenance costs and alleviate environmental impacts associated with traditional repair methods, such as the production and transportation of materials. The field of bacterial concrete has rapidly evolved, offering innovative solutions for enhancing the durability and sustainability of construction materials. This technology is believed to not only promise extended lifespans for concrete structures but also align with environmental sustainability goals. The integration of bacterial spores into concrete matrices for self-healing purposes represents a significant breakthrough in construction material science (17).

This research assumes strategic significance by aiming to comprehensively assess and analyze the impacts of self-healing on key concrete properties, including compressive strength, tensile strength, and water absorption. The insights gleaned from this review are pivotal for researchers, guiding the trajectory of advancements in self-healing concrete. Ultimately, this contributes to the development of infrastructure that is both more resilient and sustainable.

2. Application of Microbes in Construction

Microorganisms, particularly bacteria, have various applications in the construction industry. In other words, bacteria bring a world of possibilities to the construction industry, opening doors to innovative and sustainable practices. These tiny living organisms play a significant role in enhancing various aspects of construction materials and processes, paving the way for a more eco-friendly and resilient future (18).

Recent advancements in microbial applications for concrete have highlighted the efficacy of various bacterial strains, such as *Bacillus pseudofirmus* and *Bacillus cohnii*. These bacteria have been shown to significantly enhance concrete's durability through the process of calcium carbonate precipitation. This biogenic mineralization contributes to self-healing of micro-cracks, thereby extending the lifespan of concrete structures. The integration of these specific bacterial strains represents a promising development in sustainable construction materials (19). In the following, the most famous applications of microorganisms and using them in the construction is examined:

2.1. Self-Healing Concrete

In the domain of construction, self-healing concrete emerges as a captivating and groundbreaking innovation. Imagine concrete structures imbued with the extraordinary ability to mend themselves! This remarkable achievement is made possible through the incorporation of specific bacterial strains from the *Bacillus genus* into the concrete mix. These highly adept bacteria remain quiescent within the concrete until the emergence of cracks, at which point they are triggered into action. In a process akin to skilled artisans, they facilitate the production of calcium carbonate, serving as a natural adhesive that adeptly seals the cracks and fortifies the concrete's structural integrity. The enchantment of self-healing concrete extends beyond its capability to diminish maintenance requirements; it also bestows an extended lifespan upon our structures, imbuing them with heightened resilience and reliability as time unfolds (20,21).

2.2. Biodegradable Construction Materials

Microbes have emerged as a significant catalyst in the development of biodegradable construction materials, including bioplastics and biocomposites. The incorporation of these materials signifies a notable stride towards fostering eco-friendliness and mitigating the environmental impact of construction activities. Leveraging the remarkable potential of these microorganisms, we can fabricate bioplastics and biocomposites that epitomize a more environmentally sensitive approach to construction. The intrinsic value of these materials lies in their inherent propensity for natural degradation, leaving behind a minimal ecological footprint while contributing to the reduction of waste in construction projects. Such advancements underscore the crucial role of microorganisms in promoting sustainable practices within the construction industry (22–24).

2.3. Bioconcrete and Biocement

The fascinating world of bioconcrete and biocement unfolds as it harnesses the power of microbes in their production. Bioconcrete ingeniously incorporates bacteria capable of limestone production, fortifying the concrete and enhancing its durability. On the other hand, bio cement relies on microorganisms that induce calcite precipitation within the concrete matrix, bestowing it with heightened strength and resilience. Just imagine the potential of these microbe-powered wonders-construction materials that not only meet our practical needs but also demonstrate a profound commitment to environmental stewardship. Through these advancements, we find ourselves on a path where sustainable construction practices and ecological well-being go hand in hand (18,25,26).

2.4. Soil Stabilization

The other microbial application in construction involves soil stabilization, where certain microbial species come to the rescue, especially in areas with weak or loose soil. These remarkable microorganisms possess the fantastic ability to fortify the soil, rendering it suitable and robust for construction purposes. By promoting microbial-induced calcite precipitation, we can establish a solid foundation for our building endeavors, ensuring stability and longevity in our construction projects. It's like nature's own construction crew working behind the scenes to strengthen the very ground we build upon (27–29).

3. History and Importance of Use of Microbes

In the history of concrete, the introduction of calcium-rich bacteria during the mixing stage has emerged as a significant development. These bacteria play a crucial role in the self-healing process of concrete. When cracks form within the concrete, the introduced bacteria initiate the precipitation of calcium carbonate. This natural process effectively seals the cracks and reinforces the overall structure. Consequently, bacterial concrete exhibits higher strength compared to conventional concrete. By leveraging a biotechnological approach centered around calcite precipitation, it becomes possible to enhance both the strength and durability of structural concrete (30,31).

The use of bacteria in concrete is an innovative method that harnesses the power of microbial activity to improve the performance and longevity of concrete structures. The formation of calcium carbonate through the action of bacteria leads to the creation of a more robust and resilient construction material (32–34). Self-healing concrete can be achieved through three main methods: natural, chemical, and biological. The biological method can be further categorized into three subcategories: bacteria, fungi, and viruses. Among these methods, the utilization of bacteria, particularly specific strains within the concrete, has garnered significant interest (35). Self-healing concrete can be achieved through three main methods: natural, chemical, and biological. The biological method can be further categorized into three subcategories: bacteria, fungi, and viruses. Among these methods, the utilization of bacteria, particularly specific strains within the concrete, has garnered significant interest (35,36). The historical development and increasing interest in using microbes, especially bacteria, in the self-healing process of concrete highlight the potential of biotechnological advancements in construction materials. By incorporating microbial activity, concrete can exhibit improved resilience and longevity, addressing the challenges associated with cracks and structural deterioration (37). The historical development of bio-concrete represents a significant milestone in construction material technology. This evolution from traditional concrete to bio-concrete highlights a shift towards sustainable, self-healing materials, emphasizing the crucial role of microbes in modern construction practices (38).

OPC accounted for approximately 12% of global CO_2 emissions in 2020. Alkali-activated slag (AAS) has emerged as a potentially sustainable alternative to ordinary OPC. Research suggests that producing AAS composites with intelligent properties, enabling maintenance and repair with minimal external assistance, may be a sustainable solution. Additionally, bacteria-based self-repairing represents a promising and sustainable alternative method for repairing and conducting regular maintenance (39).

3.1 Various Types of Bacteria Used in Concrete

Bacterial strains used in concrete are carefully selected for their ability to thrive in high-pH environments. Typically, microorganisms cannot survive in alkaline conditions with a pH value of 10 or higher (40,41). Table 1 provides a list of bacteria capable of withstanding pH levels equal to or greater than 10.

Table 1. Bacteria that can be usable in concrete in the alkaline environment (41)

No.	Type of the Bacteria	Compressive Strength (+ or -)	Application	References
1	*Sporosarcina Pasteurii*	+	Mortar Concrete Fly Ash	(42–46)
2	*Bacillus cereus*	NA	Mortar Concrete	(47–49)
3	*Bacillus flexus*	NA	Mortar	(50–52)
4	*Bacillus megaterium*	+	Concrete Bricks	(51,53–55)
5	*Bacillus sphaericus*	+	Mortar Concrete	(56–61)
6	*Bacillus halodurans*	NA	Concrete with 10% Cement kiln dust	(62,63)
7	*Bacillus cohnii*	+	Mortar	(64,65)
8	*Bacillus pseudofirmus*	-	Cement stone	(66,67)
9	*Bacillus subtilis*	+	Mortar Concrete	(68–71)
10	*Diaphorobacter nitroreducens*	-	Mortar	(61,72)
11	*Shewanella* sp.	+	Mortar	(73)
12	*Escherichia coli*	NA	Mortar Concrete	(74,75)
13	*Bacillus aureus*	+	Rice Husk Ash	(39)

Among these bacteria, those belonging to the *Bacillus* sp., such as *Bacillus subtilis* and *Bacillus megaterium*, are well-known for their ability to thrive in highly alkaline environments. In such conditions, these bacteria produce spores that resemble plant seeds. These spores have robust walls and remain dormant until cracks develop in the concrete, allowing water to penetrate the structure. When exposed to the pH range of 10 to 11.5, typical of highly alkaline concrete, these bacterial spores become active. Apart from *Bacillus* sp., other bacterial species have also been found to survive in alkaline environments (41,76).

In addition to pH, other factors play a role in the biochemical processes involving bacteria, including the concentration of Ca^{+2} ions, the presence of nucleation sites, and the availability of dissolved inorganic carbon (77,78). Bacteria play a crucial role in creating an alkaline environment through various pathways, including autotrophic and heterotrophic processes. Among these pathways, enzymatic hydrolysis of urea, aerobic oxidation of organic carbon, and anoxic oxidation of organic carbon have been extensively studied and recognized as significant contributors to alkalinity generation. These processes are essential for establishing favorable conditions for bacteria to thrive and actively participate in the self-healing process of concrete. Extensive research has demonstrated the pivotal role of bacteria in creating an alkaline environment and promoting the healing capabilities of concrete structures (66,79–89).

The specific strains *Bacillus subtilis* and *Bacillus megaterium*, both gram-positive bacteria, are commonly employed in concrete applications. *Bacillus subtilis* possesses a remarkable ability to form highly resistant dormant endospores as a response to nutrient deprivation and environmental stresses. It is commonly found as a gut commensal in humans and can also be present in the upper layer of soil. On the other hand, *Bacillus megaterium*, besides being prevalent in soil, can be found in various environments, including certain food items like honey, as well as on surfaces such as clinical specimens, paper, and stone (90).

In the context of evaluating bacterial influence on concrete properties, it is imperative to discuss the concept of optical density (OD). Optical density is a quantitative measure of the attenuation of light as it passes through a sample containing particles or solutes. In microbiological assays, this measure is often used to estimate the concentration of bacteria within a culture by assessing the light absorption at a specific wavelength, typically 600 nm (OD_{600}). The attenuation is due to both the scattering and absorption of light by the bacterial cells, which corresponds to their concentration in the culture medium. The relevance of OD measurements in microbial concrete research lies in its ability to correlate bacterial concentrations with the observed effects on concrete's mechanical properties. In self-healing concrete, where bacterial activity is pivotal, an optimal OD value indicates the effective concentration of bacteria required to precipitate calcium carbonate to heal cracks and enhance the structural integrity of the concrete. It is this precise and careful calibration of bacterial density, ascertained through OD measurements, that allows for the targeted improvement of compressive strength and other key concrete properties.

By carefully selecting bacterial strains that can survive and thrive in alkaline environments, concrete applications can harness the self-healing properties of these microorganisms, contributing to the durability and resilience of concrete structures.

4. Results and discussions:

4.1 Compressive Strength

After using *Bacillus cereus* and *Bacillus subtilis*, it is observed that compressive strength of the concrete upsurged in both 7 and 28 days. However, the optimum amount of using these kinds of bacteria (optimum amount of *Bacillus cereus* and *Bacillus subtilis*) is 10^3 CFU/mL and 10^5 CFU/mL respectively (48).

The research utilized Ordinary Portland cement (OPC) with 25 grades along with two different bacterial strains, namely *Bacillus cereus* and *Bacillus subtilis*, in the study. Concrete cubes with dimensions of 150×150×150 mm was used for testing. The data presented in Fig. 1 demonstrates the impact of these bacteria on concrete strength.

It can be observed that the optimal concentration of *Bacillus cereus* resulted in a significant enhancement in concrete strength, with a notable 20% increase at both the 7-day and 28-day curing periods. Similarly, the application of *Bacillus subtilis* showcased even more promising results, exhibiting a remarkable 32% increase in concrete strength at the 7-day mark and a commendable 25% increase at the 28-day stage.

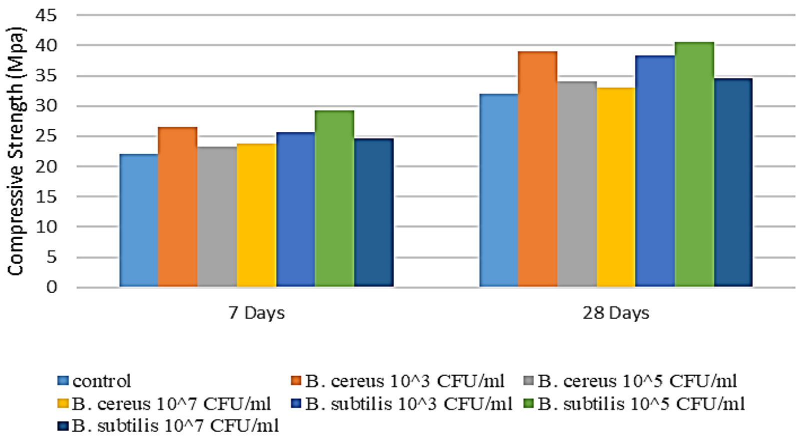

Fig. 1. A comparison about compressive strength of using different types of Bacillus cereus and Bacillus subtilis with different dosages (48)

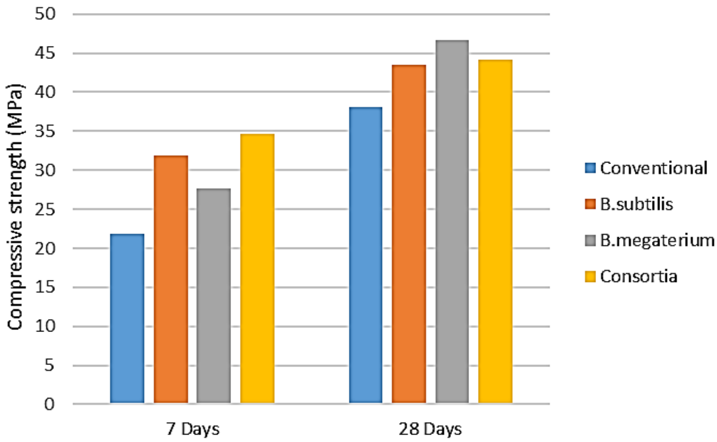

Fig. 2. comparison compressive strength of concrete with different types of bacteria (Bacillus subtilis and Bacillus megaterium) (91)

Based on Figure 2, compressive strength values for four different concrete batches tested at three different curing times: 7 days, 14 days, and 28 days. The concrete utilized is OPC 53 grade, molded into cubes with dimensions of 150mm per side. The chart demonstrates a distinct increase in compressive strength for each batch as the curing time extends from 7 days to 28 days. This suggests that the incorporation of Bacillus sp. Bacteria (specifically Bacillus subtilis and Bacillus megaterium) contributes positively to the strength development of concrete. For instance, Batch 1 shows an appreciable increase in strength at each testing interval, which indicates that the bacterial treatment could be influencing the curing process and improving the concrete's mechanical properties. This pattern is consistent across all batches, confirming the benefit of bacterial additives in concrete mixtures. The data signifies that both early age and longer-term strength properties are enhanced, highlighting the potential for these biological agents to improve construction materials' performance.

Also, it is observed that concrete batches treated with Bacillus megaterium initially demonstrate lower compressive strength at the 7-day mark compared to those treated with Bacillus subtilis and the combined consortia. This initial lag suggests that Bacillus megaterium may have a slower start in the bio-mineralization process which contributes to the concrete's strength. However, as the curing period extends to 28 days, a remarkable phenomenon occurs: the Bacillus megaterium-treated batches exhibit superior compressive strength compared to their Bacillus subtilis counterparts and the consortia.

The reasons behind this trend could be multifold. It is possible that Bacillus megaterium engages in a more gradual but ultimately more effective calcium carbonate precipitation process, which is a critical factor in concrete strengthening. The delay in strength gain could also be attributed to the specific metabolic pathways of Bacillus megaterium that may take longer to kickstart but result in more robust crystal formation over time. Another aspect to consider is the possibility that Bacillus megaterium could be more effective at pore-filling within the concrete matrix, which becomes evident only in the later stages of curing.

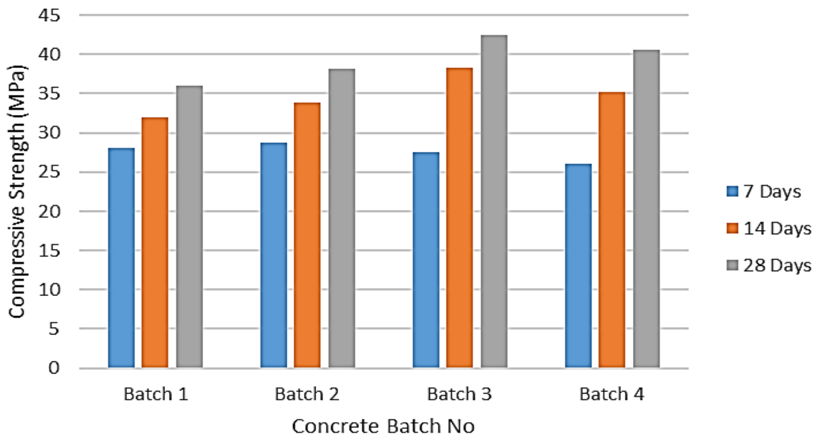

Fig. 3. Compressive strength of using Enterococcus faecalis, Bacillus sp. and mix of Enterococcus faecalis and Bacillus sp. per MPa (92)

In this experiment, the compressive strength of concrete was evaluated over an extended curing period by examining four distinct batches: Batch 1 (control), Batch 2 (*Enterococcus faecalis*), Batch 3 (*Bacillus* sp.), and Batch 4 (combination of *Bacillus* sp. and *Enterococcus faecalis*). The primary objective was to assess the impact of these bacterial compositions on the concrete's strength development. The cube specimens used in the study were 150mm ×150mm ×150mm, and Portland composite cement (PCC) was utilized. The presented graphical representation (Fig. 3) displayed the results obtained from the experiment, with Batch 3 (*Bacillus* sp.) demonstrating the most favorable and optimal outcomes in terms of compressive strength. Notably, the concrete's strength noticeably increased after 7 and 14 days of curing, with Batch 3 exhibiting the highest strength values among all the batches. The inclusion of *Bacillus* sp. at a 5% concentration proved to be effective in enhancing the concrete's compressive strength within the specified time frame. Through this analysis, valuable insights were gained regarding the influence of different bacterial additives on the concrete's performance, contributing to a deeper understanding of their role in enhancing the material's mechanical properties.

Fig. 4 presents the results of the compressive strength test, revealing a consistent enhancement in the strength of the various concrete samples over the curing duration. After 28 days of curing, the compressive strengths of the concrete specimens with different concentrations of *Bacillus subtilis* (0, 10^5, 10^7, and 10^9 cell/ml) were measured at 26.2 MPa, 29.9 MPa, 27.4 MPa, and 26.8 MPa, respectively. Notably, all the tested concretes exhibited higher compressive strengths than the target strength, with all samples incorporating different concentrations of *B. subtilis* outperforming the control sample.

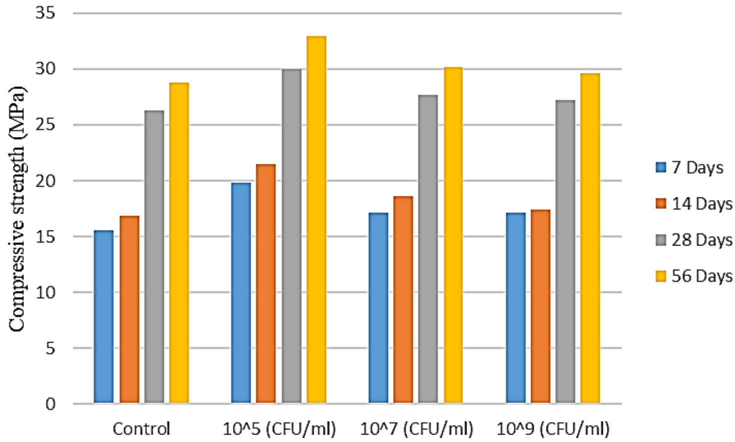

Fig. 4. Compressive strength of concretes with different concentrations of Bacillus subtilis (93)

The percentage increases in strength relative to the control sample were 14%, 5%, and 2% for *B. subtilis* concentrations of 10^5, 10^7, and 10^9, respectively, after 28 days of curing. Similarly, after 56 days of curing, the percentage increases in strength compared to the control sample were 13%, 4%, and 1% for *B. subtilis* concentrations of 10^5, 10^7, and 10^9, respectively, consistent with the 28-day curing results. The obtained results underwent statistical analysis using Analysis of Variance (ANOVA), confirming that both bacterial concentrations and curing duration significantly influenced the compressive strengths of the concrete samples ($p < 0.05$). This analysis highlights the critical role of these factors in determining the performance and quality of the concretes. It is worth noting that for this research, Portland cement (32.5 N) was used as the binder material.

Fig. 5 presents a comparison of different mixes, denoted as Mix 1 to Mix 5, with varying culture densities (OD_{600}) of 0, 0.107, 0.2, 0.637, and 1.221 respectively. The graphical representation highlights the relationship between compressive strengths and optical densities across various curing periods. The control specimen or Mix 1 exhibited compressive strengths of 20.1, 31.8, and 38.9 MPa at the age of 28 days, as per the designed strengths. Notably, all bacterial groups displayed an increase in compressive strength upon the addition of *B. subtilis*, with the highest values recorded in Mix 4 at 23.7, 35.6, and 42.5 MPa respectively.

The compressive strengths of Mix 1 at a curing period of 120 days were determined to be 30.6, 35.8, and 47.1 MPa, while Mix 4 exhibited superior performance compared to the other mixes with recorded values of 35.2, 43.2, and 57.2 MPa for the specified design strengths. Moreover, it illustrates the increase in strengths on the 28th day for different culture densities. It is evident that Mix 4, with a culture density of OD_{600}=0.637, displays a notable improvement across all design strengths. This can be attributed to the enhanced formation of mineral deposits within the internal structures of the concrete. Same results reported from Nivedhitha et. Al while they used the *Bacillus subtilis* for self-healing concrete (95).

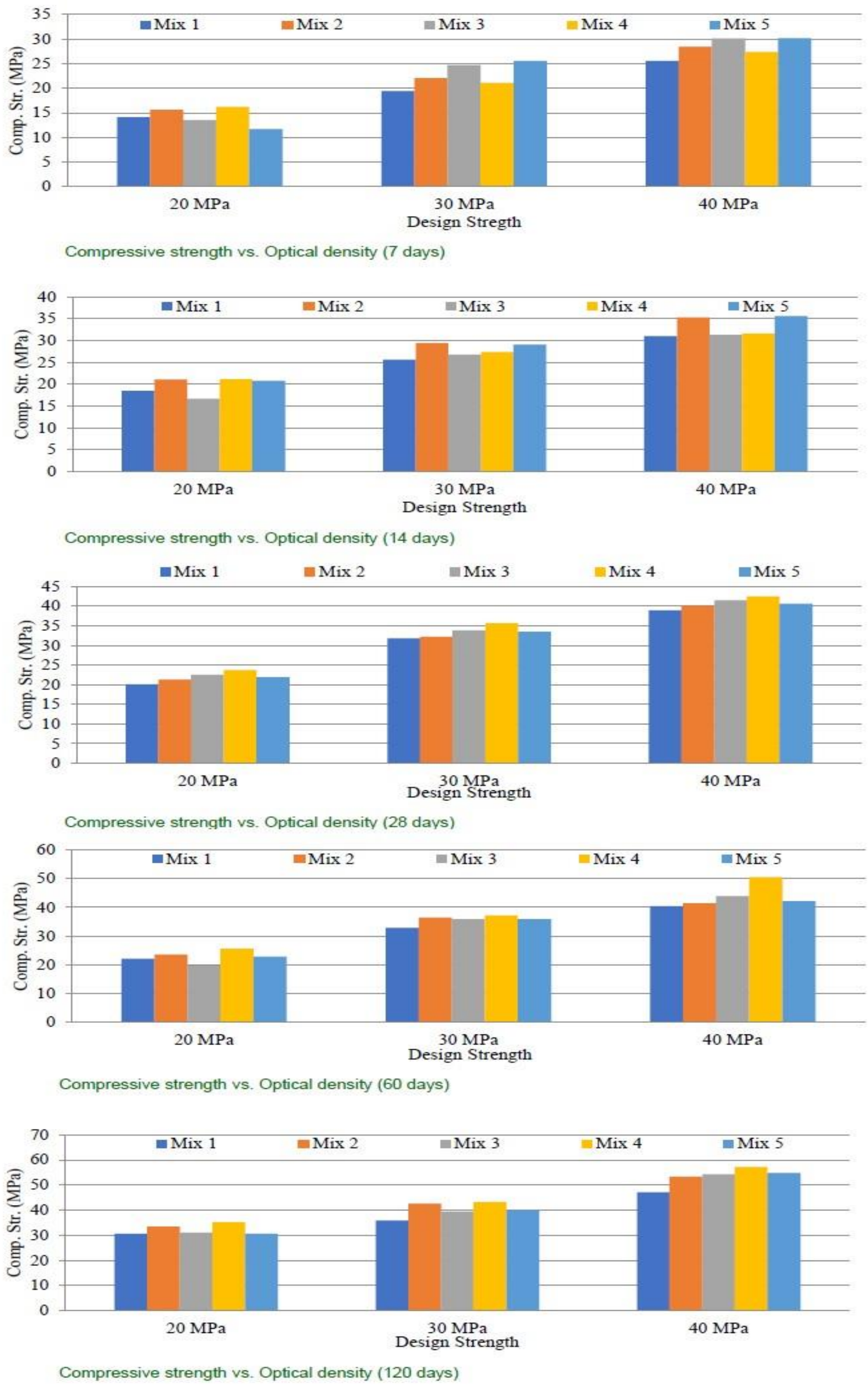

Fig. 5. Comparison of Compressive Strength and Optical Density of Bacillus subtilis (94)

In the domain of microbial-enhanced concrete, the introduction of *Bacillus subtilis* is of paramount significance, which could be elaborated in the context of the experimental observations discussed in Figure 2. Notably, this strain has demonstrated a consistent ability to increase the compressive strength across all bacterial groups. This enhancement is attributable to the unique properties of *Bacillus subtilis* that include promoting better cohesion in the cement matrix, instigating biogenic mineral precipitation, and fostering synergistic interactions within the microbial community embedded in the concrete. Such cohesive interactions are believed to reduce the porosity of concrete, thus densifying the matrix and enhancing the mechanical properties. Furthermore, *Bacillus subtilis* facilitates calcium carbonate precipitation, not merely as a crack-filling agent but as a means to bind concrete constituents more effectively, thereby improving compressive strength. Its ability to stimulate other beneficial bacteria within the concrete also contributes to a more robust and uniformly healed material. The intricacies of these interactions and their influence on the mechanical properties of concrete provide valuable insights into the biological mechanisms underpinning the improved durability and structural integrity of the construction material.

4.2 Tensile Strength

The inclusion of bacteria in concrete demonstrates a parallel effect on the tensile strength, mirroring the notable improvements observed in compressive strength. Tensile strength is an important measure of a material's ability to resist breaking under tension and is crucial for the overall structural integrity of concrete. Fig. 6 displays the outcomes of the tensile strength tests conducted on the concrete samples.

The results indicate that the presence of bacteria has a substantial and successful impact on the tensile strength of the concrete, particularly after a curing period of 7 and 28 days. It is worth noting that the tensile strength values are measured in megapascals (MPa). These findings further support the efficacy of bacteria in enhancing the tensile properties of the concrete over time. Fig. 7 presents a comparative analysis of several concrete mixes, labeled as Mix 1 to Mix 5, with each mix associated with different culture densities represented by the optical density at 600nm (OD_{600}) values.

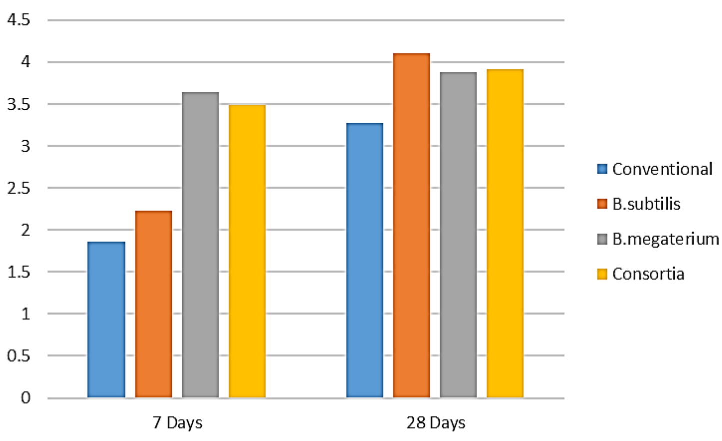

Fig. 6. Comparison tensile strength of concrete with different types of bacteria (Bacillus subtilis and Bacillus megaterium) (91)

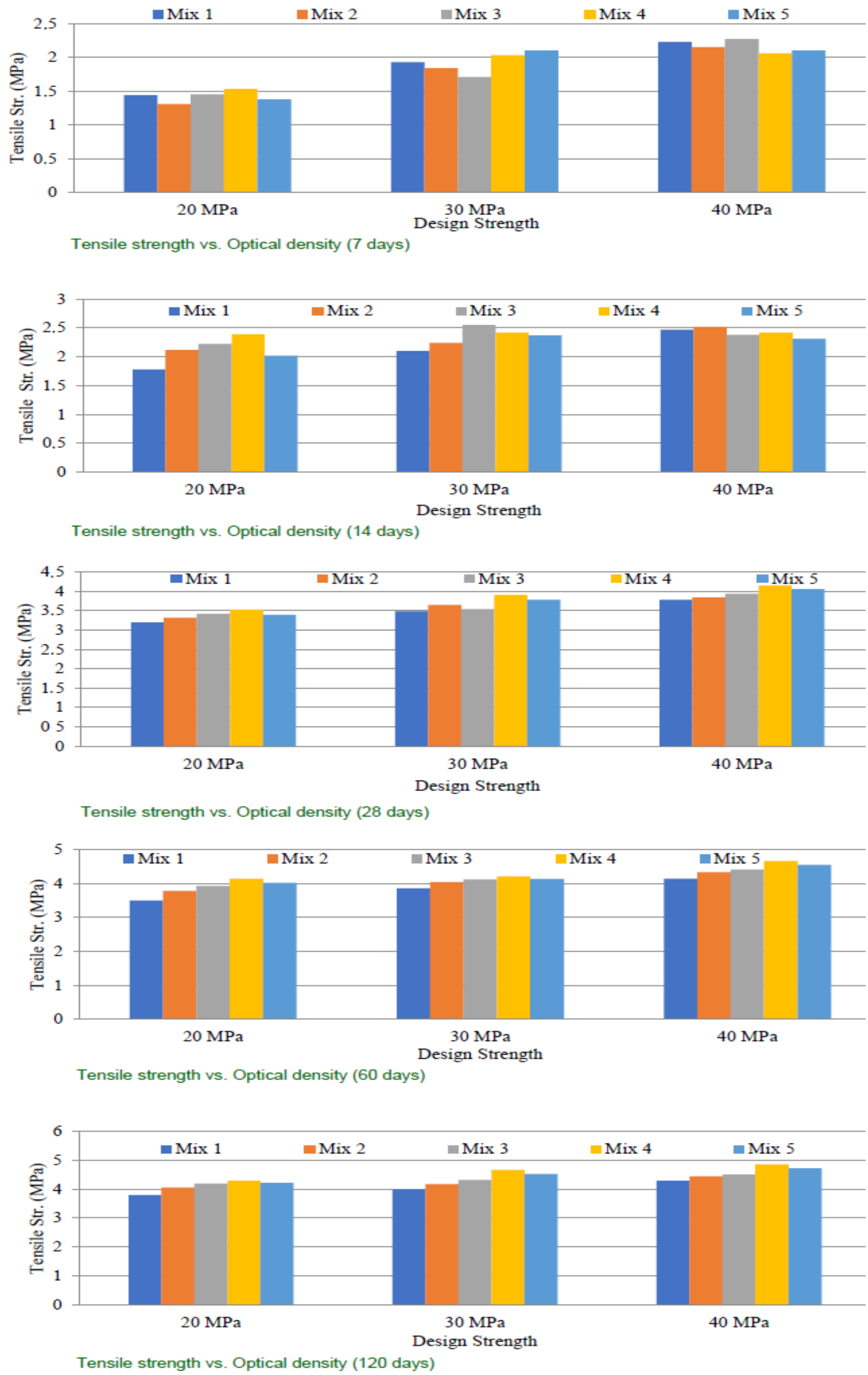

Fig. 7. Comparison of Tensile Strength and Optical Density of Bacillus subtilis (94)

The evaluated mixes correspond to OD_{600} values of 0, 0.107, 0.2, 0.637, and 1.221, respectively, enabling a thorough examination of how varying culture densities influence the performance and properties of the concrete. At a curing period of 28 days, mix 1 exhibited split tensile strengths of 3.2 MPa, 3.5 MPa, and 3.8 MPa for the specified design strengths. Notably, mix 4 demonstrated the highest increase in split tensile strength, reaching values of 3.5 MPa, 3.9 MPa, and 4.2 MPa, respectively, indicating a significant enhancement in its tensile properties compared to the other mixes. Moreover, after an extended curing period of 120 days, Mix 4 exhibited substantial improvements ranging from 13% to 18% in split tensile strength across all grades. This extended curing duration facilitated further development and strengthening of the concrete's tensile capabilities. The results underscore the effectiveness of Mix 4 in enhancing split tensile strength, suggesting its potential for applications requiring improved tensile performance in various construction projects. For this research, specimens were prepared using ordinary Portland cement (OPC) with strength class 42.5 N.

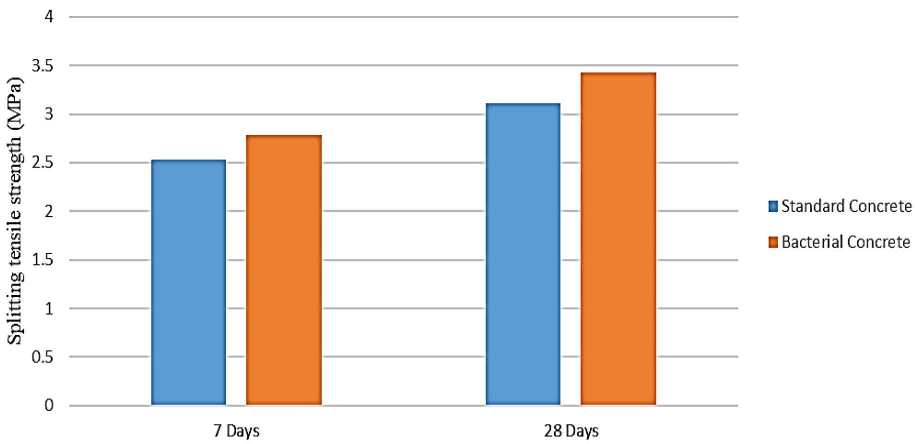

Fig. 8. splitting tensile strength of standard and Bacillus megaterium bacteria concrete. (96)

According to Fig 8, the addition of *Bacillus megaterium* into the concrete mixture results in a noticeable improvement in splitting tensile strength. The concrete samples with *Bacillus megaterium* showed an increase of 9.88% at 7 days and 10.28% at 28 days, compared to the standard concrete samples.

4.3 Water Absorption

Water absorption is a significant and widely recognized test for concrete. In this study, we will delve into the water absorption test while incorporating bacteria and examine their impact on the concrete's strength and the rehabilitation of micro cracks. This investigation aims to shed light on the potential benefits of utilizing bacteria in enhancing concrete properties and durability.

Fig. 9 illustrates the distinctions between Ordinary Portland Cement (OPC) and the addition of a pozzolan replacement, specifically calcined clay concrete (CC), with varying dosages of *Bacillus subtilis*. The incorporation of bacteria in calcined clay concrete led to a notable reduction in water absorption capacity compared to regular concrete and calcined clay concrete. Over a 28-day curing period, the inclusion of *B. subtilis* in calcined clay concrete resulted in water absorption reductions of 18.30%, 17.38%, and 13.59% for 10%, 15%, and 20% calcined clay replacements, respectively. These findings indicate that the water absorption of the specimens decreased in bacterial-infused CC when compared with specimens without bacteria. The study utilized OPC grade 43 and casted M25 grade of concrete cubes with dimensions of 150×150×150 mm.

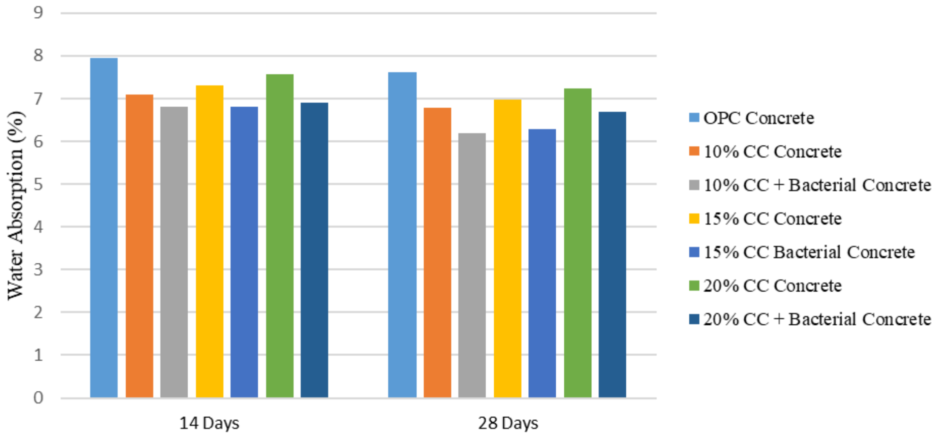

Fig. 9. Analysis of water absorption on calcined clay concrete and OPC in different dosages of bacteria. (97)

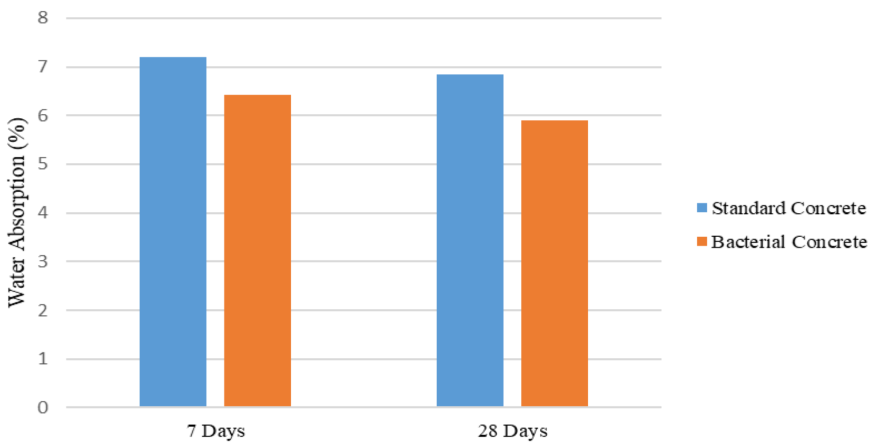

Fig. 10. Water absorption of standard and Bacillus megaterium concrete. (96)

In Fig. 10, the study utilized Ordinary Portland Cement (OPC) of grade 43, and *Bacillus megaterium* at a concentration of 10^8 CFU/mL was employed. The specimens were of size 150mm x 150mm x 150mm. The results demonstrate that when *B. megaterium* is incorporated into the concrete mix, it leads to a reduction in water absorption both at 7 and 28 days, by 5.25% and 7.35%, respectively, as compared to the control samples.

Based on the data presented in Table 2, a comparison was made regarding the impact of various bacterial types on water absorption. The results indicate that *Sporosarcina pasteurii* exhibited the highest effectiveness in reducing water absorption, achieving a remarkable reduction of up to 70%. On the other hand, other *Bacillus* sp. showed a water absorption reduction ranging from 45% to 50%. Consequently, it can be inferred that the selection of bacterial species does not significantly influence the water porosity variation.

Table 2. Water absorption of different bacteria after 28 days of curing. (98)

Type of the Bacteria	Researchers	Results of water absorption after 28 days
B. sphaericus	De Muynck et al. (99), Achal et al. (100)	The concrete sample exhibited a reduction of 45–50% compared to the controlled sample.
B. subtilis	Reddy et al. (101), Muhammad et al. (102), Pei et al. (103)	The concrete sample showed a reduction of nearly 50% compared to the controlled concrete sample.
B. megaterium	Dhamia et al. (53)	The concrete sample exhibited a decrease of 46% compared to the controlled concrete sample.
Sporosarcina pasteurii	Achal et al. (104), Pei et al. (105)	The concrete sample showed a reduction of 50-70% compared to the controlled concrete sample.
B. pseudofirmus	De Muynck et al. (99), Maheshwaran et al.	The concrete sample exhibited a 50% reduction compared to the controlled concrete sample.

Recent studies, as explored in 'Calcium Carbonate Precipitation by Different Bacterial Strains for Concrete Repair', have demonstrated the significant role of bacterial strains like *Bacillus sphaericus* and *Bacillus pasteurii* in enhancing concrete's compressive strength and reducing its water absorption. These strains facilitate the biomineralization process, leading to calcium carbonate formation that not only aids in sealing micro-cracks but also improves the overall structural integrity of the concrete. This innovative approach promises a new dimension in prolonging the durability of concrete structures, particularly in environments subjected to extensive wear and tear (106).

5. Discussion and Recommendation for Future Studies

The exploration into microbial concrete has laid a foundation for innovation within the construction industry, yet it is clear that we have only begun to unearth the full spectrum of its capabilities. Future research directives must be comprehensive and multifaceted to span the depths of this burgeoning field.

Firstly, the scope of microbial agents employed in concrete must be broadened. Diverse microorganisms, including fungi and algae, should be investigated for their unique biogenic processes that may yield more robust and resilient self-healing concrete formulations. Uncovering alternative biological pathways could pave the way for concrete that is not only self-repairing but also possesses enhanced mechanical properties.

Longitudinal studies are imperative to ascertain the long-term viability and efficacy of microbial concrete. Such studies should rigorously evaluate the performance of these innovative materials against the rigors of environmental stresses encountered in situ, such as freeze-thaw cycles, corrosive chemical exposures, and abrasion. Understanding how microbial concrete withstands such conditions will inform the engineering of materials that can endure through decades rather than just years.

Further, there is an essential need to refine the delivery and maintenance of microbial concentrations within the concrete matrix. Innovative techniques, such as advanced encapsulation or the development of biofilms, must be optimized to balance the microbial activity with structural integrity. These methods should ensure the longevity of microbial viability and consistent healing action throughout the concrete's lifecycle.

Additionally, a thorough ecological and economic analysis is essential to evaluate the sustainability of microbial concrete. This encompasses a complete lifecycle assessment that addresses the environmental impacts from production to end-of-life recycling or disposal. The integration of such assessments will ensure that the advancement in construction materials technology is in concert with our sustainability objectives, minimizing the carbon footprint while maintaining cost-efficiency.

In the vein of scientific inquiry, there is an urgency to decode the biochemical intricacies of microbial calcite precipitation. Understanding the molecular mechanisms that govern this process could lead to the optimization of microbial consortia, tailored for enhanced self-healing efficiency. Investigating how Bacillus subtilis and other bacteria interact with varying concrete compositions will allow for broader application and more customized material solutions.

The path to harnessing the full potential of microbial technologies within construction materials is complex, demanding a confluence of expertise from disciplines such as microbiology, material science, and civil engineering. Collaborative research efforts must converge to innovate resilient, self-sustaining, and environmentally benign construction materials. As we stride toward this goal, we must also ensure that our scientific and engineering pursuits are scalable and transferable to practical applications, cementing the legacy of microbial concrete as a pillar of sustainable development in the construction industry.

6. Conclusion

The study meticulously explores the innovative integration of microbial technologies in the construction sector, specifically through the development of self-healing concrete utilizing bacterial agents. The investigation has illuminated the significant role of bacteria, notably the Bacillus species, in autonomously repairing microcracks that compromise the structural integrity of concrete. This self-healing mechanism, driven by the microbial-induced calcite precipitation process, not only seals cracks but also contributes to the long-term durability and strength of the concrete infrastructure.

Our comprehensive analysis has revealed that the incorporation of bacteria like Bacillus cereus and Bacillus subtilis leads to marked improvements in the compressive strength of concrete within the initial seven days of curing. This enhancement persists and becomes more pronounced with prolonged curing periods, underlining the enduring benefits of microbial action on the concrete's mechanical properties. The optimal bacterial dosages and specific curing conditions emerge as critical factors that significantly influence the efficacy of the self-healing process, necessitating further research to establish standardized protocols for practical applications.

Furthermore, the study extends beyond the immediate realm of self-healing concrete to explore broader applications of microbial interventions in construction materials. This includes the development of bioconcrete and biocement, which leverage microbial activities for material fortification and sustainability. The soil stabilization techniques enhanced by microbial action also present a promising avenue for improving the foundational stability of construction sites, particularly in geotechnically challenging environments.

The empirical evidence gathered points to a substantial reduction in water absorption rates in microbially treated concrete, a factor that contributes to the material's resilience against environmental degradation and extends its service life. The nuanced interplay between bacterial concentration, culture density, and environmental conditions in dictating the performance of microbial concrete underscores the complexity of this innovative technology.

The integration of microbial technologies in the construction industry heralds a transformative era in material science, where sustainability, durability, and self-repairing capabilities become intrinsic characteristics of construction materials. The potential of bacteria-infused concrete to revolutionize building practices is immense, offering a sustainable solution to the perennial challenges of material degradation and environmental impact. However, the path to widespread adoption and optimization of these technologies are paved with challenges that necessitate meticulous research, standardized testing protocols, and a deeper understanding of microbial ecology within construction materials.

The dynamic interplay of bacterial agents within the concrete matrix heralds a complex ecosystem, whose understanding is pivotal for the optimization of material properties and engineering applications. Future investigations must delve into the nuanced mechanisms of bacterial interactions, with an emphasis on real-time dynamics and long-term impacts on structural resilience and durability. Moreover, the potential for an engineered synergy between diverse microbial species and concrete formulations beckons a new frontier in construction material science. To translate this pioneering research into tangible engineering practices, it is imperative to devise strategies for the scaled-up application that address economic feasibility, environmental considerations, and compatibility with existing construction methodologies. The pathway forward is collaborative and interdisciplinary, requiring concerted efforts from microbiologists, material scientists, and civil engineers, to harness the full spectrum of benefits offered by microbial technologies in construction.

While this study has provided valuable insights into the potential of microbial concrete, particularly focusing on self-healing properties and the role of Bacillus species, it acknowledges certain limitations. The experimental conditions under which the microbial concrete's performance was evaluated might not fully replicate the complex environmental conditions encountered in real-world applications. The longevity and sustainability of the microbial-induced healing process under varied climatic and loading conditions remain to be comprehensively understood. Additionally, while the study explored a range of bacterial species, the ecological impact of introducing these microorganisms into construction materials warrants further investigation to ensure that no adverse environmental consequences arise. Economic feasibility, scalability of the microbial application process, and the potential for microbial resistance development in the concrete matrix are aspects that need to be addressed in future research. These limitations underline the necessity for continued and extensive investigation into the dynamics of microbial concrete to optimize its application in the construction industry and to fully understand its environmental implications.

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