

Increasing structural resilience in high-strength concrete via microbial-based self-healing

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ABSTRACT

Prolonged loading can lead to concrete cracking due to its weak tensile strength, impacting both durability and load-bearing capacity, especially when reinforcing bars corrode. This study investigates the efficacy of microbial-based self-healing in enhancing the performance of high-strength concrete, specifically targeting *Bacillus Pasteurii* and *Bacillus Flexus*. The findings indicate significant improvements in both micro- and macro-properties of high-strength bacterial concrete treated with these strains, surpassing control samples. Concrete infused with *Bacillus Flexus* exhibits a notable increase of 23.75% in compressive strength at 7 days and 12.36% at 28 days, with similar enhancements observed in *Flexus*-treated concrete. The presence of calcite precipitation, confirmed by X-ray diffraction and scanning electron microscopy, contributes to crack healing, achieving closure within 56 days. Microbial concrete from these strains demonstrates superior durability against water, acid, and salt exposure, suggesting the potential of microbial-based self-healing to fortify structural resilience and extend the lifespan of concrete infrastructure.

Keywords: *Bacillus pasteurii*; *bacillus flexus*; high strength concrete; self-healing concrete.

1. INTRODUCTION

Recent advancements in concrete technology have focused on enhancing the durability and longevity of concrete structures. One of the innovative approaches to achieve this is through microbial-based self-healing mechanisms [1]. The formation of tiny fractures in concrete structures is a common issue that can lead to the degradation of material properties. To address this, researchers have explored the use of non-pathogenic bacteria to induce CaCO_3 precipitation, which can repair cracks and enhance the concrete's strength [2]. The application of bacteria such as *Bacillus pasteurii*/*Sporosarcina pasteurii*, *Bacillus subtilis*, and *Bacillus megaterium* has been shown to be effective in sealing cracks through the precipitation of calcium carbonate [3]. The addition of natural zeolite as a mineral additive has been found to enhance the micro-structural properties of concrete, leading to densification and improved interaction within the concrete matrix [4].

The resilience and longevity of concrete structures can be significantly enhanced through the introduction of alkali-resistant, spore-forming bacteria from the genus *Bacillus*. Research indicates that these bacteria can remain viable for extended periods, up to four months, when introduced into cement paste. Moreover, their presence facilitates the production of minerals that effectively fill cracks, suggesting their potential utility in repairing concrete structures [5]. This highlights the promising role of bacterial spores in concrete crack repair applications, offering a sustainable and effective solution for enhancing infrastructure durability. Studies have also investigated the impact of microbiologically induced calcium carbonate precipitation by *Bacillus pasteurii* and *Bacillus sphaericus* on concrete properties [6]. These bacteria have demonstrated the ability to promote calcium carbonate precipitation within the concrete matrix, leading to improved strength and durability.

Furthermore, research into concrete remediation using microorganisms has shown promising results, particularly with *Bacillus pasteurii*, which induces calcite precipitation in portland cement mortar specimens [7].

Bacillus flexus is recognized as a potentially bacterium for concrete applications due to its ability to precipitate calcite and improve concrete's compressive strength. In a study by, *Bacillus flexus* BSKNAU, along with other strains, was isolated from alkaline soil and demonstrated an increase in compressive strength and crack healing in concrete specimens [8]. Similarly, reported that *Bacillus flexus* KC845306, isolated from calcareous sludge, contributed to the improvement of mechanical strength and reduced porosity and permeability of concrete [9, 10]. The bioremediation potential of *Bacillus flexus* was also explored in a study where it was used to restore the compressive strength of mortar degraded by *Thiobacillus thioparus*. The results showed a significant recovery of strength and a denser material due to the precipitation of calcium carbonate [11]. Constructed a bacterial consortium including *Bacillus flexus* MK-FYT-3, which demonstrated the ability to heal concrete cracks and improve its properties, such as increased compressive and tensile strengths and reduced permeability [12].

The remediation of concrete using microorganisms has also been investigated, with *Bacillus pasteurii* inducing calcite precipitation in portland cement mortar specimens. The results indicated an increase in compressive strength at lower concentrations of live cells, although higher concentrations and longer curing times led to a decrease in strength due to biomass interference [13, 14]. Research involving *Bacillus cereus* and *Bacillus pasteurii* has shown that the addition of these bacterial cultures to cement mortar can enhance compressive strength due to bio-mineralization of calcium carbonate. The study reported a significant enriching in axial strength and a diminish in Cl⁻ penetration, suggesting improved durability of the concrete [15]. Bacteria's effect on the absorption of water of concrete aggregates was also examined, with two types of bacteria, including *Sporosarcina pasteurii*, reducing water absorption by 20–30%. This reduction was attributed to the deposition of calcium carbonate crystals on the bacteria cell walls within the pores of the aggregates [16].

The integration of microbial adjuvants such as peptone, yeast extract, and *Bacillus Subtilis* into concrete mix designs has emerged as a promising strategy for enhancing the material's properties. Studies have revealed a notable reduction in porosity and a simultaneous increase in strength, dynamic modulus, and durability of concrete specimens treated with these microbial additives. This augmentation in performance attributes can be attributed to the biochemical activities of the incorporated microorganisms, which facilitate the formation of calcium carbonate crystals within the concrete matrix [17]. The morphology of these precipitated calcite crystals exhibits diverse forms, effectively infiltrating and filling cracks within the material, thereby contributing to its overall integrity and resilience [18]. The systematic classification of microorganisms and carriers, along with the evaluation of various immobilization techniques, has been instrumental in elucidating the efficacy of microbial self-healing mechanisms in cement-based composites. By understanding the interactions between different microbial species and their carriers, researchers have been able to optimize the conditions for promoting microbial activity and subsequent crack repair within concrete structures [19].

One notable example is the utilization of *Bacillus subtilis* M9 in biomineralization experiments, where the metabolic activity of the bacteria has demonstrated remarkable results in autonomously healing micro-cracks. These findings underscore the potential of microbial interventions in revolutionizing concrete technology, offering sustainable solutions for improving the performance and longevity of infrastructure systems [20].

The direct incorporation of bacterial self-healing agents has been demonstrated to exert multifaceted influences on the properties of concrete beyond just crack repair. Studies have indicated that the presence of these agents can affect the rheological behavior, hydration kinetics, and pore structure of undamaged concrete [21]. This influence holds the potential to enhance the overall mechanical strength and create a denser pore network within the material. Moreover, advancements in genetic engineering have enabled the development of microbes with enhanced capabilities, leading to the formation of novel mineral phases like gehlenite, further augmenting the material's strength and durability [22]. Research exploring *Bacillus subtilis* natto has shown promising results regarding the repeatability of self-healing cycles and the consequent improvement in concrete strength [23]. The investigations into the enhancement of crack sealing efficacy through the use of lightweight aggregates loaded with healing agents underscore the potential of concrete that repairs itself using microorganisms to significantly improve liquid tightness and durability [24].

A thorough review of microbial crack healing in concrete has underscored the efficacy of microbially induced calcium carbonate precipitation (MICCP) in bolstering the longevity of cementitious constructions, offering promising avenues for widespread implementation at commercial scales [25]. The review highlights how MICCP, facilitated by microbial activity, leads to the deposition of calcium carbonate within concrete cracks, effectively sealing them and enhancing the material's resistance to deterioration over time [26]. An experimental inquiry into the self-healing capabilities of bacteria in sustainable concrete structures has yielded encouraging

results. By employing high-alkaline-tolerant bacteria, researchers successfully retrofitted laboratory-fractured concrete samples, showcasing the potential of bacterial interventions to not only repair but also enhance the compressive strength of concrete elements [27]. These findings collectively contribute to the growing body of knowledge supporting the practicality and efficacy of microbial techniques in fortifying concrete structures against the rigors of environmental stressors and aging processes, paving the way for more resilient and sustainable infrastructure solutions [28].

2. METHODOLOGY

2.1. Materials

The physical properties of the cement were evaluated through various tests, yielding significant results. The fineness of the cement was found to be 94.6%, meeting the established standards. With a specific gravity of 3.15 g/cc, the cement demonstrates a density within the expected range. The normal consistency, a vital parameter, was determined to be 28.00%, ensuring optimal workability. Additionally, the initial setting time was recorded at 66 minutes, while the final setting time was observed to be 310 minutes, both aligning with industry standards. When taken as a whole, these findings offer insightful information on the performance and quality of the cement. The mechanical properties of the aggregates were explored, revealing important characteristics for construction purposes. The fine aggregate exhibited a bulk unit weight of 1549.0 Kg/m³, with a relative density of 2.61 and an absorption capacity of 1.36%. Its moisture content measured at 1.88%, while the fineness modulus was determined to be 2.68. On the other hand, the coarse aggregate demonstrated a higher bulk unit weight of 1622.5 Kg/m³, accompanied by a relative density of 2.88 and a lower absorption capacity of 0.89%. Its moisture content was recorded at 0.86%, with a fineness modulus of 2.57. These parameters provide vital insights into the suitability and performance of the aggregates in construction applications. Muraplast SP1 Superplasticizer – High Range Water Reduction was the chemical admixture used. For this research, a dose of 16 oz./cwt of HRWR was used in accordance with other investigations. In order to get the desired consistency and facilitate the concrete's hydration process mixture, tap water had to be added.

2.2. Bacteria isolation, identification, and culturing

Isolating and culturing bacteria such as *Bacillus Flexus* and *Bacillus pasteurii* involves meticulous steps to ensure accurate identification and viable cultures. Initially, samples are collected from environments conducive to *Bacillus* species, such as soil or water. Through serial dilution and spread plate methods, bacterial colonies are isolated on agar plates and then incubated under suitable conditions to encourage growth. Identification begins with observing colony morphology and conducting Gram staining to determine bacterial characteristics. Subsequent biochemical tests and molecular techniques, including PCR and DNA sequencing, aid in precise identification, especially for strains like *Flexus*. Culturing entails inoculating pure cultures into appropriate growth media and maintaining optimal conditions for growth through incubation and periodic subculturing. Ensuring sterility throughout the process is vital to prevent contamination and maintain the purity of cultures, while meticulous labeling and documentation facilitate tracking and further research endeavors. Figure 1 and 2 shows the image of *Bacillus Flexus* and *Bacillus pasteurii*.



Figure 1: *Bacillus flexus*.

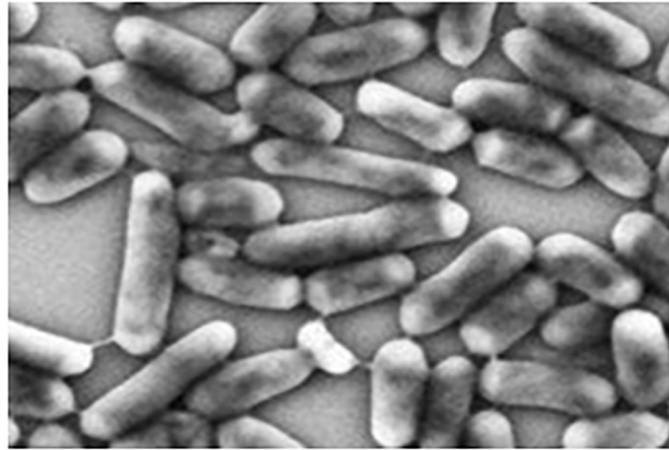


Figure 2: *Bacillus pasteurii*.

2.3. Concrete mix design

Concrete mix design for M60 grade concrete, aiming for a compressive strength of 60 MPa, involves meticulous selection and proportioning of ingredients to meet performance requirements. Incorporating *Bacillus Flexus* and *Bacillus pasteurii*, specialized bacterial agents, can enhance concrete's self-healing capabilities by precipitating calcite in cracks, improving durability. Additionally, the addition of Muraplast SP1 High Range Water Reducing Superplasticizer improves workability and reduces water content while maintaining desired flow properties. For the specific mixture proportions, the batch weights per cubic meter are as follows: Cement (444.3 kg/m³), Fine aggregate (504.5 kg/m³), Coarse aggregate (1101.3 kg/m³), and Water (200.4 kg/m³). These proportions are crucial, with each component's ratio to cement carefully considered to achieve the desired properties. Sample preparation for M60 grade concrete involves accurate measurement and thorough mixing of ingredients to ensure uniformity. Careful attention is given to the incorporation of bacterial agents and superplasticizers to facilitate their dispersion throughout the mix. Test specimens are then prepared using proper compaction techniques and cured under controlled conditions to evaluate strength and durability properties accurately. Through precise mix design and sample preparation, M60 grade concrete incorporating *Bacillus Flexus*, *Bacillus pasteurii*, and Muraplast SP1 can exhibit enhanced self-healing capabilities, improved workability, and superior strength and durability, meeting the demands of challenging construction applications.

3. RESULTS AND DISCUSSION

3.1. Compressive strength test

The compressive strength results of different concrete mixes at 7, 14, and 28 days reveal notable variations in performance. Conventional concrete (M1) exhibited respectable strengths, with values of 27.26 MPa, 40.23 MPa, and 68.23 MPa at 7, 14, and 28 days, respectively. Introducing *Bacillus Pasteurii* (M2) into the mix enhanced strength across all curing periods, with values of 42.86 MPa, 60.18 MPa, and 71.39 MPa at 7, 14, and 28 days, respectively. Similarly, *Bacillus Flexus* (M3) demonstrated improved compressive strength compared to conventional concrete, recording 44.03 MPa, 62.36 MPa, and 73.61 MPa at the same respective intervals. The results suggest that incorporating bacterial agents like *Bacillus Pasteurii* and *Bacillus Flexus* positively impacts concrete strength development. This enhancement can be attributed to the bacteria's ability to precipitate calcite, contributing to the self-healing properties of the concrete matrix. The observed increase in compressive strength indicates potential applications in structures requiring improved durability and longevity. Figure 3 shows the graphical representation of compressive strength test.

3.2. Flexural strength test

Results for the various concrete mixes' flexural strengths at 7, 14, and 28 days show changes in performance with time. Conventional concrete (M1) displayed moderate flexural strengths, with values of 4.51 MPa, 4.961 MPa, and 5.4571 MPa at 7, 14, and 28 days, respectively. Incorporating *Bacillus Pasteurii* (M2) into the mix led to improvements in flexural strength across all curing periods, with values of 4.71 MPa, 5.181 MPa, and 5.6991 MPa at the same respective intervals. Similarly, *Bacillus Flexus* (M3) exhibited enhanced flexural strength compared to conventional concrete, recording 4.77 MPa, 5.247 MPa, and 5.7717 MPa at the corresponding intervals.

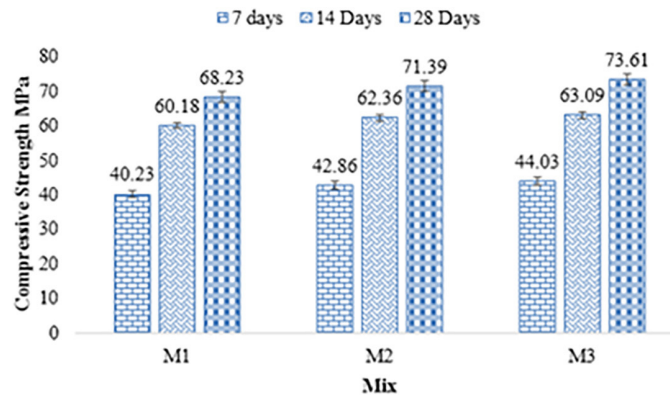


Figure 3: Displays a test results for compressive strength.

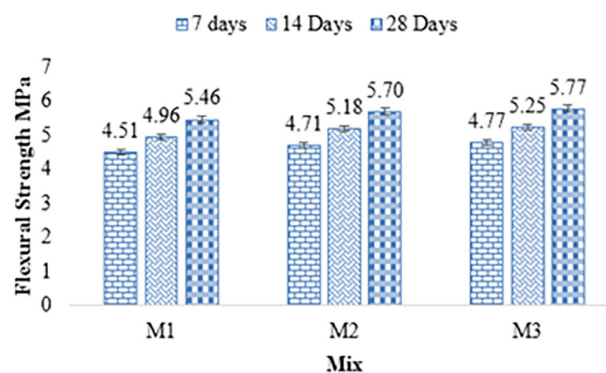


Figure 4: Displays a test results for flexural strength test.

The results suggest that the inclusion of bacterial agents like *Bacillus Pasteurii* and *Bacillus Flexus* positively influences the flexural strength development of concrete. This enhancement may be attributed to the bacteria's role in promoting calcium carbonate precipitation, which contributes to the self-healing and strengthening of the concrete matrix. The observed increase in flexural strength highlights the potential of these bacterial agents for enhancing the durability and structural integrity of concrete elements subjected to bending stresses. The flexural strength test is graphically represented in Figure 4.

3.3. Ultrasonic pulse velocity test

The results of the Ultrasonic Pulse Velocity (UPV) tests for the different concrete mixes at 7, 14, and 28 days demonstrate variations in the propagation of ultrasonic waves through the specimens. Conventional concrete (M1) exhibited UPV values of 4.01 km/s, 4.19 km/s, and 4.31 km/s at various curing periods. Introducing *Bacillus Pasteurii* (M2) into the mix led to slightly higher UPV values across all testing periods, with measurements of 4.12 km/s, 4.39 km/s, and 4.55 km/s at the same respective intervals. Similarly, *Bacillus Flexus* (M3) showed improved UPV results compared to conventional concrete, recording 4.17 km/s, 4.46 km/s, and 4.64 km/s at the corresponding intervals. The observed trends suggest that incorporating bacterial agents like *Bacillus Pasteurii* and *Bacillus Flexus* may positively influence the UPV characteristics of concrete. This enhancement could be attributed to the bacteria's role in promoting cementitious material deposition and improving the overall integrity of the concrete matrix. The increase in UPV values indicates potential improvements in the material's homogeneity and structural quality. Figure 5 shows the graphical representation of ultrasonic pulse velocity test.

3.4. Compressive strength test for sulphate attack section after healing

The compressive strength results of various concrete mixes after 28 days of exposure to sulfate attack indicate significant variations in performance. Conventional concrete (M1) exhibited a compressive strength of 63.91 MPa before sulfate attack, which decreased to 67.72 MPa after exposure. In contrast, concrete mixes incorporating *Bacillus Pasteurii* (M2) and *Bacillus Flexus* (M3) demonstrated improved resistance to sulfate attack, with compressive strengths of 66.87 MPa and 68.95 MPa before exposure, respectively. After exposure, these

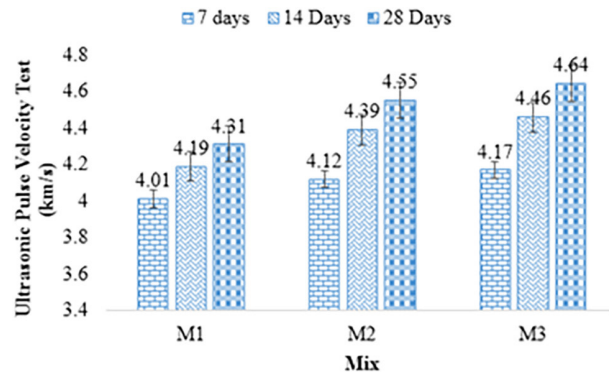


Figure 5: Displays a test results for ultrasonic pulse velocity test.

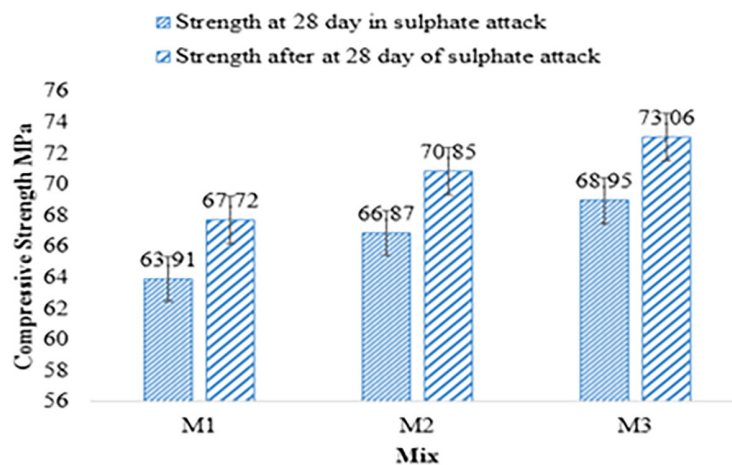


Figure 6: Shows compressive strength after sulphate attack test.

mixes maintained higher strengths, with values of 70.85 MPa for M2 and 73.06 MPa for M3. These results highlight the beneficial effects of incorporating bacterial agents like *Bacillus Pasteurii* and *Bacillus Flexus* in concrete mixes for enhancing sulfate attack resistance. The observed increase in compressive strength after sulfate exposure suggests that these bacteria contribute to the formation of CaCO_3 , which fills pores and cracks in the concrete matrix, thus mitigating sulfate-induced deterioration. This improvement in durability is promising for applications in sulfate-rich environments, such as marine structures and wastewater treatment facilities. Figure 6 shows the graphical representation of compressive strength after sulphate attack test.

3.5. Compressive strength test for chloride attack section after healing

The assessment of concrete's resistance to chloride attack revealed notable differences among the mixes, highlighting the efficacy of microbial interventions in enhancing durability. *Bacillus Flexus* (M3) exhibited the highest strength both before and after exposure to chloride attack. At 28 days of exposure, M3 demonstrated a substantial strength of 66.80 MPa, outperforming *Bacillus Pasteurii* (M2) at 64.78 MPa and conventional concrete (M1) at 61.92 MPa. After exposure, M3 maintained superior strength at 70.78 MPa, followed by M2 at 68.64 MPa and M1 at 65.61 MPa. The superior performance of *Bacillus Flexus* (M3) can be attributed to its ability to promote microbially induced calcium carbonate precipitation (MICCP) within the concrete matrix. This process facilitates the formation of calcium carbonate crystals, which fill cracks and pores, effectively reinforcing the structure and impeding chloride ingress. *Bacillus Pasteurii* (M2) also demonstrated enhanced strength compared to conventional concrete, suggesting its effectiveness in mitigating chloride attack, albeit to a lesser extent than M3. The results underscore the potential of microbial techniques, particularly *Bacillus Flexus* (M3), in improving concrete's resistance to chloride attack. By harnessing microbial activity, concrete structures can achieve greater durability and longevity, reducing maintenance costs and environmental impact. Figure 7 shows the graphical representation of compressive strength after chloride attack test.

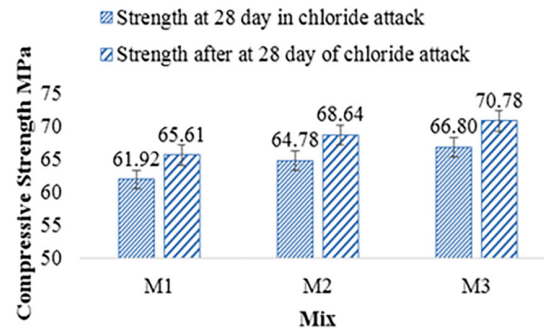


Figure 7: Shows compressive strength after chloride attack test.

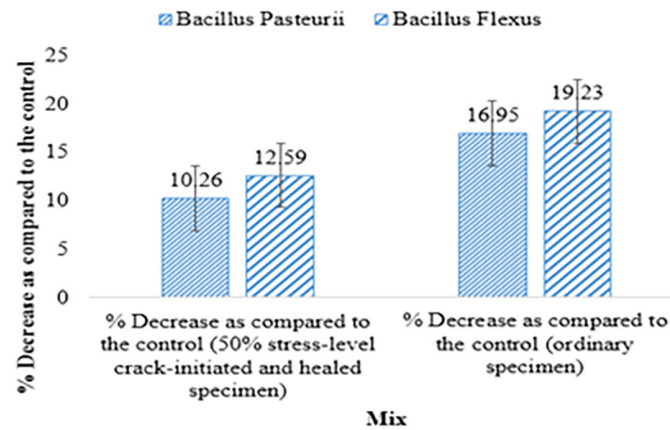


Figure 8: Shows percentage decrease in water absorption as compared to the control.

3.6. Water absorption percentage reduction relative to the control

Concrete specimens incorporating *Bacillus Pasteurii* and *Bacillus Flexus* demonstrated significant improvements in crack healing compared to control specimens. When subjected to a stress level resulting in a 50% crack initiation and subsequent healing, the percentage decrease in crack size was 10.26% for *Bacillus Pasteurii* and 12.59% for *Bacillus Flexus*, compared to the control. Additionally, when compared to ordinary specimens without bacterial agents, both *Bacillus Pasteurii* and *Bacillus Flexus* exhibited even greater reductions in crack size, with percentage decreases of 16.95% and 19.23%, respectively. These results underscore the effectiveness of *Bacillus Pasteurii* and *Bacillus Flexus* in promoting crack healing and improving the durability of concrete structures. The observed reductions in crack size indicate enhanced self-healing properties attributed to the bacteria's ability to precipitate calcium carbonate, which fills and seals cracks in the concrete matrix. This enhancement in crack healing is promising for increasing the service life and resilience of concrete infrastructure, particularly in environments prone to mechanical and environmental stresses. The percentage decrease in water absorption as compared to the control is graphically represented in Figure 8.

4. RECOMMENDATIONS FOR FUTURE STUDY

For the sustainable building industry, more research on high-strength concrete made by mixing waste materials and microbial self-healing agents in the substitution is advised. It is advised that more study be done to evaluate the self-healing capabilities of bio-concrete in real-world building projects and to encourage its use in marine environments, cyclic loading scenarios.

5. CONCLUSION

In this comprehensive study, we investigated the effects of incorporating bacterial strains, *Bacillus Pasteurii* (M2) and *Bacillus Flexus* (M3), on the physical properties and durability of concrete compared to conventional concrete (M1). The properties evaluated included flexural strength, compressive strength, ultrasonic pulse velocity (UPV), and resistance to sulphate attack. Our results indicate that the addition of *Bacillus Pasteurii* (M2) and *Bacillus Flexus* (M3) resulted in notable improvements in both flexural and compressive strength of concrete

at different curing periods. At 28 days, *Bacillus Flexus* (M3) exhibited the highest compressive strength, with values of 73.61 MPa, representing a significant enhancement compared to conventional concrete (M1) at 68.23 MPa. Similarly, in terms of flexural strength, *Bacillus Flexus* (M3) demonstrated superior performance, further emphasizing its effectiveness in enriching the physical properties of concrete. Moreover, the resistance of concrete to sulphate attack was evaluated, revealing that concrete specimens incorporating *Bacillus Pasteurii* (M2) and *Bacillus Flexus* (M3) exhibited enhanced resistance compared to conventional concrete (M1). The compressive strength after 28 days of sulphate attack for M2 and M3 was higher than M1, indicating their ability to mitigate the detrimental effects of sulphate exposure on concrete durability. The study assessed the self-healing capability of concrete specimens incorporating bacterial strains. It was observed that *Bacillus Pasteurii* (M2) and *Bacillus Flexus* (M3) contributed to the healing of cracks, with both strains demonstrating a decrease in crack-induced strength loss compared to control specimens. This highlights the potential of microbial technology in promoting autonomous repair mechanisms within concrete structures, thereby enhancing their longevity and serviceability. Additionally, the ultrasonic pulse velocity (UPV) test was conducted to assess the structural integrity of concrete specimens. The results revealed that concrete mixes containing *Bacillus Pasteurii* (M2) and *Bacillus Flexus* (M3) exhibited higher UPV values compared to conventional concrete (M1) at all curing periods. This suggests that the incorporation of bacterial strains led to improved homogeneity and reduced porosity within the concrete matrix, resulting in enhanced structural integrity and resistance to deterioration. The findings of this study demonstrate the promising potential of bacterial strains, particularly *Bacillus Pasteurii* (M2) and *Bacillus Flexus* (M3), in enriching the physical properties and durability of concrete. Their ability to improve flexural and compressive strength, resistance to sulphate attack, self-healing capability, and structural integrity signifies a significant advancement in the field of concrete technology.

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