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Novel Methods of Analysing Environmental and Biological Benefits of Bacillus Species for Sustainable Green Building: Enhancing the Mechanical Properties and Longevity of M20 Grade Concrete

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Abstract

This research investigates the biological and environmental advantages of using Bacillus subtilis in M20 grade concrete to improve its strength and longevity. Through the application of innovative analytical techniques, the study highlights the role of sustainable green building approaches in minimizing ecological impact while enhancing structural efficiency. It explores the advantages of bio-concrete, which utilizes microorganisms, in enhancing the durability and environmental sustainability of concrete structures. The objective is to assess the strength and durability characteristics of concrete containing varying concentrations of *Bacillus subtilis* and *Bacillus sphaericus* at levels of 10^6, 10^7, and 10^8 CFU/ml. The investigation compares the properties of bio-concrete with those of conventional concrete. It evaluates several long-term performance indicators, including compressive strength, water absorption, sorptivity, acid resistance (H_2SO_4 immersion), and chloride penetration over a period of 90 days. Results indicate that a concentration of 10^7 CFU/ml of *Bacillus subtilis* significantly enhances the durability and lifespan of the concrete. The self-repairing properties of bio-concrete, facilitated by the precipitation of calcium carbonate ($CaCO_3$), are of significant importance, as imaging methods have validated its capacity to autonomously close cracks. This study highlights the advantages of integrating microorganisms into concrete, paving the way for the creation of environmentally friendly construction materials and practices that support sustainability goals. The findings underscore the potential of *Bacillus subtilis* as a sustainable solution for advancing green building practices, offering enhanced durability and reduced environmental impact in concrete applications.

Keywords: *Bacillus Sphaericus*, *Bacillus Subtilis*, Durability, Strength, Structural Efficiency, Sustainable Green Building.

Introduction

The construction sector plays a major role in environmental concerns, highlighting the need for sustainable and environmentally friendly solutions. Utilizing biological interventions like Bacillus subtilis in construction materials offers a promising avenue for improving structural integrity while minimizing environmental footprints. Concrete is highly regarded in the building industry for its extensive mechanical and physical properties, making it fundamental to a wide range of construction activities (1–3). But, the extensive utilization of concrete has raised concerns regarding its ecological impact. Due to micro-crack formation and the large amount of carbon dioxide (CO_2) emissions from cement manufacture, concrete constructions have a significant adverse effect on the environment (4, 5). These micro cracks serve as pathways for air, water, and harmful chemicals, ultimately reducing the strength of the concrete, load-bearing capacity, and overall longevity, which makes it more prone to corrosion (6). If not promptly addressed, these cracks can compromise structural integrity, potentially leading to collapse. Traditional methods that use synthetic polymers for crack repair are not aligned with modern sustainability strategies due to their environmental impact and associated complexities and expenses (7). Consequently, microbiological crack healing has become a promising strategy, integrating sustainable and environmental friendly elements. Studies show that urease-positive bacteria, including Bacillus species, facilitate calcite precipitation by producing urease enzymes. This

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mechanism entails the hydrolysis of urea, resulting in the formation of carbon dioxide and ammonia, which subsequently elevates the concentration and pH of carbonate compounds in the surrounding environment (8). *Bacillus pasturii*, *Bacillus subtilis*, and *Bacillus megaterium* are frequently employed for their self-healing properties additionally (9–13); *Bacillus sphaericus* is increasingly recognized for its potential to enhance concrete strength and promote crack healing (14). During the casting process, these bacteria are introduced into concrete specimens. When cracks appear, bacteria that are exposed to the air produce calcium carbonate ($CaCO_3$), which fills in the voids and facilitates healing. Due to its potential to be a cost-effective and environmentally friendly technique, microbially induced carbonate precipitation, or MICP, has attracted a lot of attention (15). Mineral-producing bacteria have been used in various applications, including limestone monument restoration, wastewater purification, soil contamination remediation, sand strength enhancement, and the reduction of greenhouse gases in landfills (16–19). The incorporation of Bacillus bacteria in concrete has shown significant benefits, particularly in enhancing the material's durability and hardened properties through the promotion of calcite precipitation. This process effectively fills voids and pores, leading to improved overall quality (20–22). This research investigates the impact of different bacterial species, such as *Bacillus subtilis* and *Bacillus sphaericus*, at varying cell concentrations (10^6, 10^7, and 10^8 CFU/ml) on concrete longevity and self-healing mechanisms. The primary aim is to evaluate the durability performance of self-healing concrete using these microbes compared to conventional concrete (CC), with a particular focus on the crack-healing capabilities of the optimized mix. This study aims to explore the potential of Bacillus subtilis as a sustainable biological solution to enhance the mechanical properties, durability, and environmental performance of M20 grade concrete, contributing to the advancement of green building practices.

Material and Methods

53 grade of Ordinary Portland Cement (OPC) with specific gravity 3.13 (22–26). Due to price and scarcity of natural fine aggregate, M-sand of 4.75 mm size with 2.65 specific gravity and crushed granite aggregate of a nominal 20 mm size, with specific gravity of 2.7 is utilized (27). For this investigation, water available within the campus is used for mixing and curing with pH value ranges from 6.5-7.5 (28). Calcium acetate is used with bacterial solution which is used as nutrient source for bacteria.

Preparation of Bacterial Culture

The glassware that was used to cultivate microorganisms was autoclave-sterilized. In 1000 milliliters of distilled water, 28.0 grams of nutritional agar was dissolved to create the subculturing medium. Peptone (10 g), beef extract (10 g), sodium chloride (5 g), and agar (20 g) comprise the nutritional agar. After dissolving the media components in distilled water, the conical flask was wrapped with sterile cotton and covered with aluminum foil. After that, the prepared media was once more sterilized in an autoclave set at 121°C for 15 mins while applying 15 lbs of pressure. Following sterilization, the media was allowed to cool to a temperature range of 40–45°C before being carefully poured into petri dishes. The petri dishes were subsequently exposed to UV light for 30 minutes to ensure further sterilization. Once the agar medium solidified, the bacterial culture was streaked onto the surface. After completing the inoculation, the petri dishes were incubated at 37°C for 24 hours. The maintenance of stock cultures of *Bacillus sphaericus* and *Bacillus subtilis* was done on nutrient agar slants. Streaking of culture was done on agar slants with an inoculation loop and are incubated at 37°C. The preservation of slant cultures is done under refrigeration for a temperature of 4°C to 5°C after growth until further use. The stock culture was carefully maintained and continuously observed for the prevention of contamination. The microbial cells are thoroughly mixed with concrete during the preparation of concrete mixture. So, the cells are intactly immobilized in the concrete during solidification process and the chances of leakage are very very less or almost nil. Further the micro-organisms used in this study is *Bacillus subtilis* and *Bacillus sphaericus*, it is commonly known as saprophytic organism present in soil. They don’t have pathogenicity against humans and animals. Since the micro-organisms were the environmental isolate, they won't make any unexpected adverse effects during their environmental interactions.

Mix Proportion

The mix proportion was derived for the M20 grade concrete is 1:1.59:2.96 with water by cement ratio of 0.45 (29). Consider 10% of water is substituted by the bacterial solution. The mix quantity of materials required for 1 m^3 of concrete is shown in the table. The conventional concrete (CC) is compared to the CC with *Bacillus subtilis* (10^6) (CC+BS1), *Bacillus Subtilis* (10^7) (CC+BS2), *Bacillus subtilis* (10^8) (CC+BS1), *Bacillus sphaericus* (10^6) (CC+BSp1), *Bacillus sphaericus* (10^7) (CC+BSp2), and *Bacillus sphaericus* (10^8) (CC+BSp3). The mix proportions for the conventional and bacterial concrete were represented in the Table 1.

Table 1: Mix proportion

Concrete mix ID	Cement (kg)	Fine Aggregate (kg)	Coarse Aggregate (kg)	Water (l)	Bacterial Solution (l)	Cell Concentration (CFU/ml)
CC	186	413.33	656.84	1221.64	-	-
CC+BS1	186	413.33	656.84	1099.48	122.16	10^6
CC+BS2	186	413.33	656.84	1099.48	122.16	10^7
CC+BS3	186	413.33	656.84	1099.48	122.16	10^8
CC+BSp1	186	413.33	656.84	1099.48	122.16	10^6
CC+BSp2	186	413.33	656.84	1099.48	122.16	10^7
CC+BSp3	186	413.33	656.84	1099.48	122.16	10^8

The long-term compressive strength of the cubes was evaluated by conducting tests on cube specimens of size 10 cm^3 at 90 days. The rate of load applied is 4.5 kN/sec (30). The specimens tested were shown in Figure 1A. The cubes were casted with dimensions of 10 cm^3 (31). After the procedure of removing the molds, the cubes were submerged in water for a period of 28 days. The cubes subjected to oven drying for 24 hours at a temperature of 110°C until their mass stabilized. The cube's weight after drying was reported as (W_1). Subsequently, the cube was submerged in water for a period of 24 hours at a temperature of 21°C. The weight was noted as W2. It is expressed as the percentage, calculate by (W_2- W_1)/ W_2 x 100. The water absorption of the specimen was shown in Figure 1B. The test specimens underwent the sorptivity test, with diameter and thickness measurements of 10 cm and 5 cm, respectively (32). The specimens were sealed with wax and are being protected from water by a tape. The absorption at a specific time was determined using equation given below.

Absorption (I) in mm = Change of mass/ (Exposed area of specimen x Density of water)

As shown in Figure 1C, the specimens were immersed in water, with only 2 mm of their surface area exposed. A total of six hours were spent recording weights at various intervals: sixty seconds, five minutes, ten minutes, twenty minutes, thirty minutes, and sixty minutes. Thereafter, measurements were taken every day for a maximum of eight days. Acid resistance test of the concrete specimen were studied on cube specimen size of 10 cm^3 at the age of 90 days (33). For this test, the cube specimens were immersed in water diluted sulphuric acid (H_2SO_4) for 90 days continuously is shown in the Figure 1D. The H_2SO_4 solution was 5% of 0.01 N and the pH was maintained as 2.1. The cube specimens were removed from the acid solution and their surfaces were thoroughly cleaned. Then, loss of weight of the specimens was noted. The test was conducted in the RCPT setup, the cylindrical specimens of size (10 cm dia and 5 cm thick) was used (34). Place the specimen between two chambers of the test cell. Fill one chamber side with 3% of NaCl solution and the opposite side with 0.3M NaOH solution. Apply a constant 60V DC potential across the specimen for 6 hours. The NaCl side should be attached to the positive terminal and the NaOH side to the negative terminal. The RCPT setup was shown in the Figure 1E. The overall charge that has flowed through the specimen can be calculated using the equation [1].

$$Q = 900\ (I_0 + 2I_{30} + 2I_{60} + \cdots + 2I_{330} + I_{360}) \ldots\ldots\ldots\ldots \quad [1]$$

Q represents Charge passed through the specimen (Coulombs) and I_0 represents current immediately following the application of voltage (in amperes) and Current at 't' minutes after voltage is applied (in amperes), respectively.

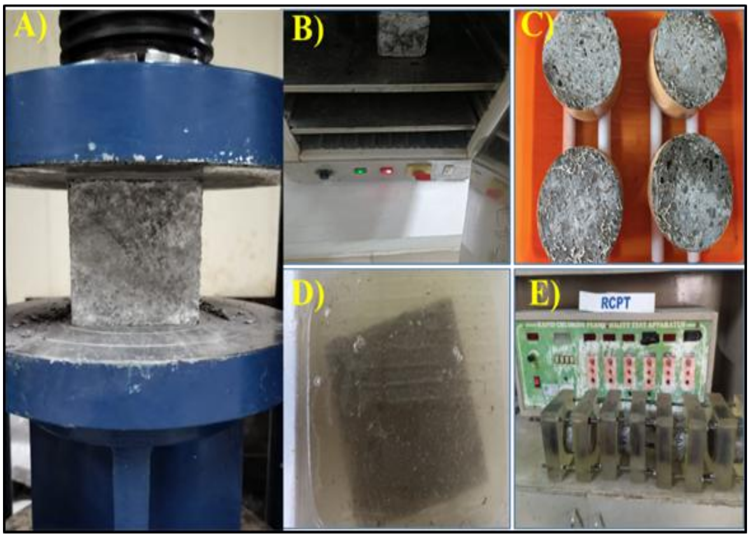

Figure 1: Experimental Setup
A) Compressive Strength Test B) Water Absorption C) Sorptivity D) Acid Resistance E) RCPT

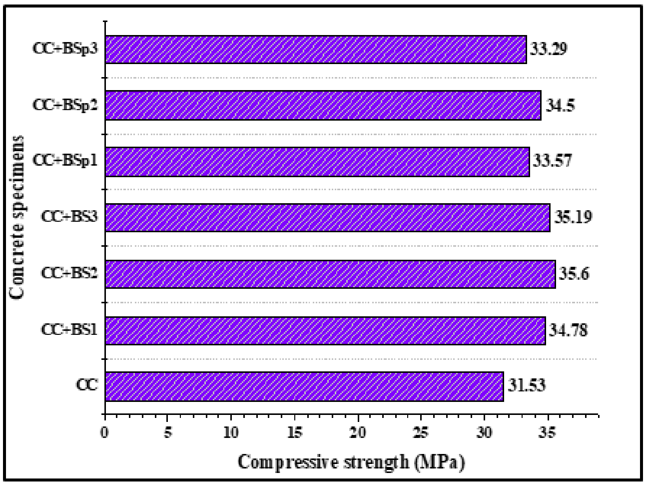

Figure 2: Long-Term Compressive Test Results

Results and Discussion

Long-Term Compressive Strength

Figure 2 displays the 90-day long-term compressive strength of the bacterial concrete mixes and conventional concrete. The development of M20-grade bacterial concrete mixes, which contain two distinct bacteria with varying cell concentrations, was observed in terms of their compressive strength.

The findings from the long-term evaluation of compressive strength distinctly demonstrate the advantages of bacterial concrete compared to conventional concrete. Notably, the bacterial concrete sample designated CC+BS2 achieved a remarkable peak compressive strength of 35.6 MPa. This indicates that the integration of bacteria into concrete not only enhances its durability but also significantly boosts its structural strength. The key factor contributing to this enhancement is the biological generation of calcium carbonate ($CaCO_3$), which serves as a binding agent, effectively filling micro-cracks and increasing the density of the concrete matrix. In contrast, an M25 grade concrete mix, supplemented with a *Bacillus subtilis* concentration of 10^5 cells/ml, attained a compressive strength of 25.35 MPa after 28 days of curing. This result is considerably lower than that of the CC+BS2 sample, highlighting the potential for improved strength through higher bacterial concentrations or alternative mix designs. This observation suggests that bacterial concrete not only achieves superior initial strengths but also continues to develop strength over time, potentially providing enhanced long-term structural integrity (35). The research indicates that bacteria-enhanced concrete presents significant potential for use in applications that demand durable and resilient structures, particularly in settings where longevity is essential.

Water Absorption

Water absorption test results of the conventional concrete were compared to the different bacterial concrete with different bacterial cell concentrations, as shown in Figure 3.

The results of the study reveal that specimens treated with bacteria showed a marked decrease in water absorption after a curing period of 90 days, particularly in comparison to standard concrete samples. The various concrete mixes identified as CC (control concrete), CC+BS1, CC+BS2, CC+BS3, CC+BSp1, CC+BSp2, and CC+BSp3 exhibited different levels of water absorption. The absorption rates recorded for these mixes were 2.9%, 1.84%, 1.76%, 1.8%, 1.89%, 1.82%, and 1.85%, respectively. Notably, the CC+BS2 sample demonstrated the lowest water absorption, surpassing the performance of the other samples in terms of water resistance. This reduction in water absorption is linked to the activity of bacteria that promote microbial-induced calcium carbonate precipitation (MICP).

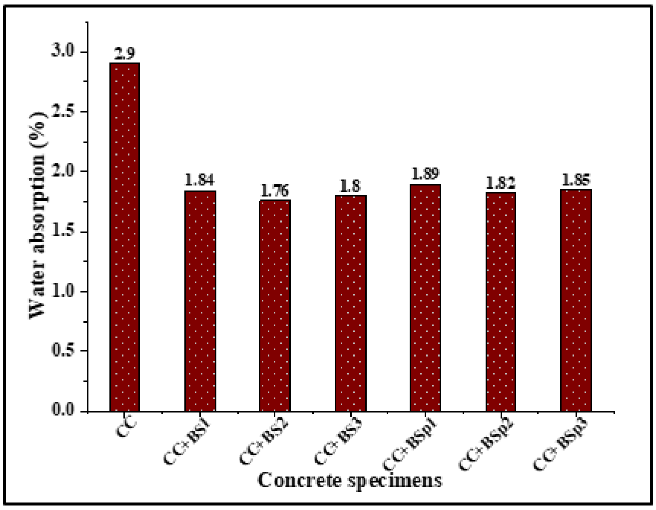

Figure 3: Water Absorption Test Results

MICP facilitates the creation of bacterial compounds that effectively seal the pores on the concrete surface, thereby serving as a barrier against water infiltration. This mechanism of pore blockage significantly enhances the water resistance of the concrete, with bacterial-treated samples showing a 50%–80% decrease in water absorption for specimens aged between 28 and 90 days. As a result, the incorporation of bacteria plays a crucial role in improving water retention, which is advantageous for increasing the durability of concrete, especially in conditions where water resistance is critical (36).

Sorptivity

The research outlines the results of sorptivity tests conducted on conventional concrete (CC) and bacterial concrete, which was treated with various bacterial species and cell concentrations, as illustrated in Figure 4. After a period of 90 days, the sorptivity rates (expressed in mm/min$^{0.5}$) for the different concrete mixes were recorded as follows: 0.122 for CC, and 0.099, 0.096, 0.102, 0.105, 0.098, and 0.107 for the bacterial concrete samples. These findings reveal that the bacterial concrete samples demonstrated lower sorptivity compared to the conventional concrete. The significance of the reduced sorptivity in bacterial-treated concrete lies in its measurement of the rate at which water is absorbed through capillary action. The lower sorptivity values observed in the bacterial concrete suggest enhanced resistance to water penetration, which can contribute to improved durability of the concrete. Consequently, the bacterial-treated concrete samples exhibited superior performance in minimizing water absorption, likely attributed to the pore-blocking effects of microbial-induced calcium carbonate precipitation (MICP), leading to a denser and more water-resistant concrete structure (37).

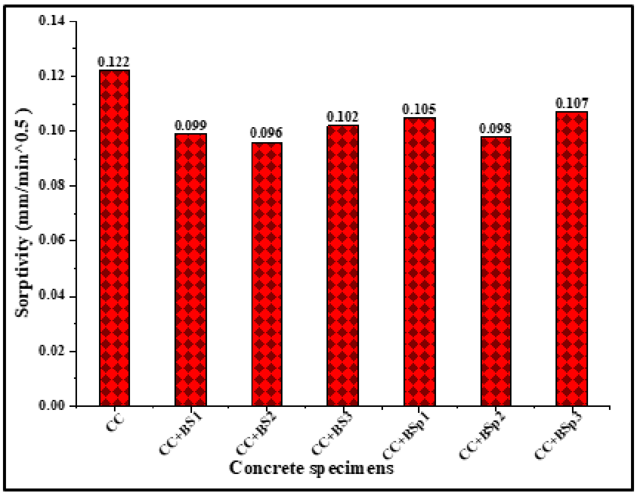

Figure 4: Sorptivity Test Results

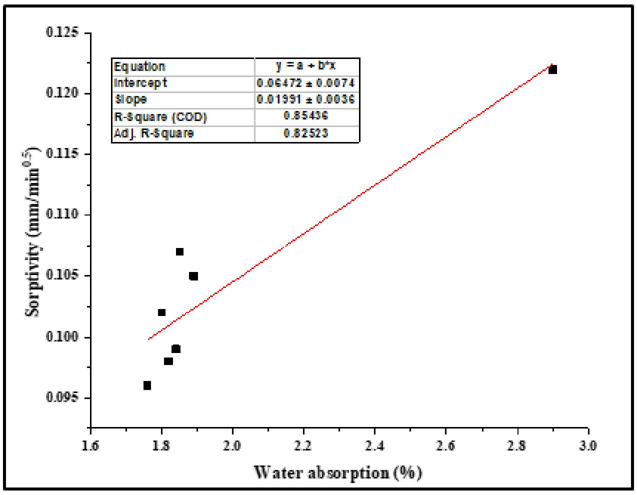

Figure 5: Relationship between Water Absorption and Sorptivity

Relationship between Water Absorption and Sorptivity

Following a 90-day curing period, the results of the sorptivity and water absorption tests were evaluated. Figure 5 illustrates the correlation between water absorption and sorptivity.

From the test results, the relationship equation [2] is found to

$y = 0.06472 + (0.01991)\,x$ ………….. [2]

Where, y is the Water absorption (%)

x is the Sorptivity (mm/min$^{0.5}$)

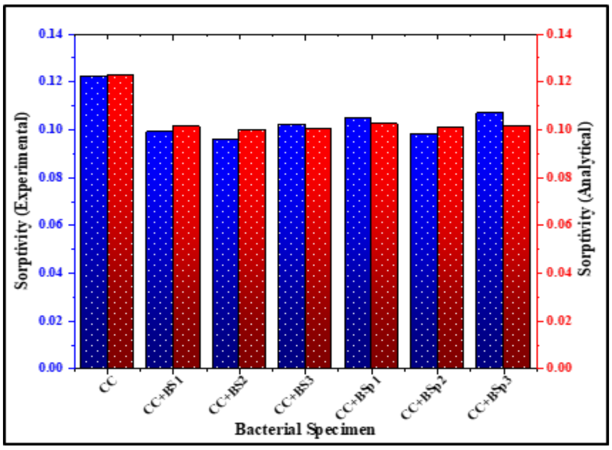

Figure 6: Experimental and Analytical Value for Sorptivity Test Results

The validation of sorptivity results is achieved through a comparison of experimental and analytical values were shown in the Figure 6. This study indicates a notable discrepancy between these two sets of values. Throughout all trials conducted, the results were consistently similar. Specifically, the sorptivity results for the CC sample revealed no difference, with both experimental and analytical values recorded at 0.122. Further analysis identified the greatest deviations in sorptivity values between analytical calculations and experimental results in the CC+BSp3 combination, which exhibited a 5.09% increase in experimental values relative to the analytical figures. Conversely, the CC+BS3 combination demonstrated the smallest deviations, with a 1.41% increase in experimental values compared to the analytical results.

Acid Resistance Test

The results of the acid resistance test, as shown in Figure 7, indicate that concrete specimens treated with bacteria demonstrate a significantly greater resistance to acid attack than traditional concrete. Specifically, conventional concrete experienced a weight loss of 3.95%, while the bacterial concrete mix labeled CC+BS2 exhibited a markedly lower weight loss of just 1.28%. This observation underscores the superior durability of bacterial concrete in acidic environments, as all bacterial-treated samples showed reduced weight loss compared to the untreated control. Furthermore, the bacterial concrete mixes maintained a higher level of structural integrity than conventional concrete when subjected to acid exposure. This enhanced resistance to acid is likely attributed to the development of MICP crystals within the concrete, which effectively seal micro-pores and enhance the material's impermeability to acid penetration. In summary, the findings suggest that bacterial concrete, especially the CC+BS2 mix, provides a significant benefit in settings where concrete is exposed to acidic conditions, as it better preserves both its weight and structural strength. (38). Therefore, the introduction of bacteria in to the concrete can strengthen its resistance and enhances its acid resistance.

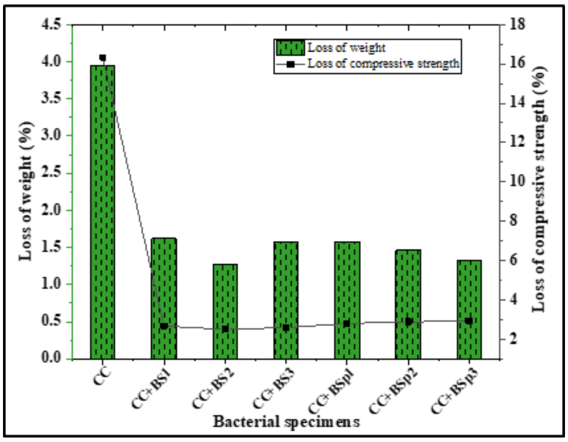

Figure 7: Acid Resistance Test Results

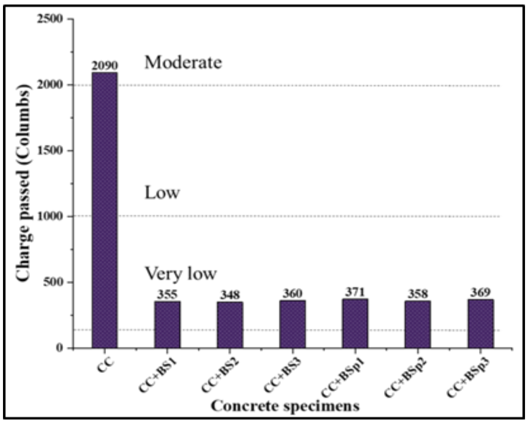

Figure 8: RCPT Results

Rapid Chloride Permeability Test (RCPT)

The conventional concrete and bacterial specimens were undergone to the Rapid chloride permeability test (RCPT) after 90 days of curing (34). The RCPT results were represented in the Figure 8. The test findings indicate that concrete specimens treated with bacteria show a marked reduction in chloride permeability when compared to traditional concrete. The incorporation of bacteria into the concrete facilitates microbial-induced calcium carbonate precipitation (MICP), which aids in the development of calcium carbonate crystals within the concrete's pores. This crystallization effectively fills the pores, minimizing voids and forming a barrier that limits the ingress of chloride ions. While conventional concrete exhibited a "moderate" level of chloride ion penetration, the bacterial-treated concrete revealed a "very low" level of penetration. This difference implies that the MICP process in bacterial concrete results in a more durable and impermeable structure. Furthermore, the rapid chloride permeability test (RCPT) results for M20 grade bacterial-treated specimens were significantly lower than those of conventional concrete. Reduced chloride permeability is vital for improving the durability of concrete, as it lessens the likelihood of corrosion in embedded steel reinforcements, thereby prolonging the lifespan of structures, especially in chloride-rich environments such as marine areas. (39).

Self- Healing – Imaging Techniques

Cracks in concrete can occur due to various reasons, such as mechanical stress, thermal expansion, shrinkage, and freeze-thaw cycles. To evaluate the crack-repairing potential of bacterial concrete, a refined mix was selected based on experimental findings. This mix included *Bacillus subtilis* at a concentration of 10^7 CFU/ml to investigate its self-healing properties. Upon the introduction of *Bacillus subtilis* into the concrete, it reacts with water and atmospheric CO_2, initiating a process known as Microbial-Induced Calcite Precipitation (MICP). During this process, the bacteria promote the formation of calcite (calcium carbonate) crystals within the cracks. These crystals effectively fill the gaps, restoring the structural integrity of the concrete and potentially improving its durability. This natural repair mechanism presents significant benefits, particularly in prolonging the lifespan of concrete structures and minimizing the frequency of maintenance or repairs. The visible crack and after healing mechanism was shown in the Figure 9.

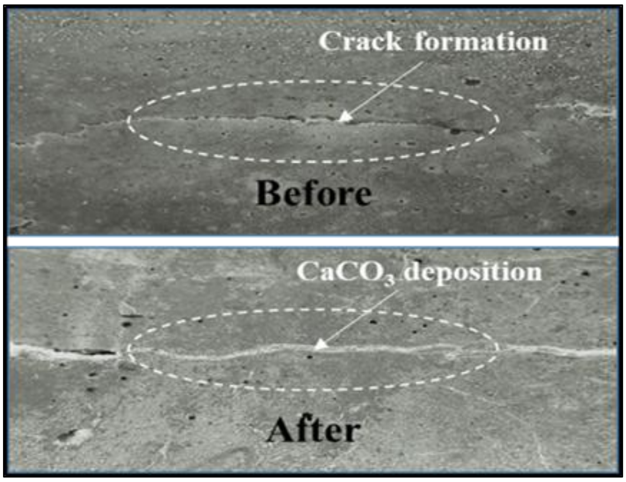

Figure 9: Self-Healing Concrete

Therefore, the integration of microbial concrete into mainstream construction presents a sustainable alternative to traditional methods, enhancing durability and reducing maintenance needs. For practical implementation, challenges such as bacterial survival, nutrient delivery, and material compatibility must be addressed, with encapsulation methods offering promising solutions. Standardized testing, regulatory approval, and cost considerations are critical to its adoption. Sustainability metrics like Life Cycle Assessment (LCA) can help quantify its environmental benefits. Future advancements may include genetically enhanced bacteria, smart self-healing systems, and improved scalability through pilot projects. Industry training and clear guidelines will be essential to facilitate its widespread use.

Conclusion

The addition of *Bacillus subtilis* to M20 grade concrete shows remarkable enhancements in strength, durability, and ecological sustainability, underscoring its viability as a transformative approach for sustainable construction. This research aims to explore the improved longevity of the bacterial concrete samples when compared to conventional concrete materials. The study investigated two separate bacterial species, specifically *Bacillus subtilis* and *Bacillus sphaericus*, at varying cell concentrations (10^6, 10^7, and 10^8 CFU/ml). The durability characteristics, such as the ability to withstand compression over a longer time period, the capacity to absorb water, the rate of absorption, resistance to acid, and the ability to resist the passage of electric current, were evaluated after a 90-day period of curing. These findings pave the way for further research and application of biological solutions in advancing sustainable construction and promoting environmentally friendly building practices. The test findings allow for the following inferences to be made:

The long-term compressive strength of specimens containing bacteria was found to be higher than that of conventional concrete. Specifically, the concrete specimens with CC+BS2 exhibited a strength of 35.6 MPa, due to the presence of $CaCO_3$ precipitation. Incorporating bacteria into concrete contributes to a reduction in sorptivity and water absorption by facilitating the development of CSH gel and MICP, thereby improving the durability of concrete structures. When the experimental and analytical values of the relationship between water absorption and sorptivity were evaluated, from test results comparable outcomes were observed. Among all the concrete samples, those with *Bacillus subtilis* bacteria had higher long-term compressive strength, less water absorption, less chloride ion penetrability, and less weight loss from acid attack than those with *Bacillus sphaericus* bacteria and regular concrete. The chloride ion penetration against concrete specimens with bacteria results in lesser penetration compared to those without bacteria. As per ASTM C 1202, all the bacterial concrete specimens come under the category “very low”. The optimal mixture, containing *Bacillus subtilis* at a cell concentration of 10^7 CFU/ml, was applied to create cracks in a beam. Visual inspection clearly demonstrated that the bacterial concrete effectively filled the cracks through the action of MICP. The effective incorporation of *Bacillus subtilis* into concrete applications has the potential to revolutionize the construction industry by merging practical functionality with environmental sustainability.

Abbreviations

BSp: *Bacillus sphaericus*, BS: *Bacillus subtilis*, $CaCO_3$: Calcium carbonate, CC: Conventional concrete, CSH: Calcium Silicate Hydrate, MICP: Microbially Induced Calcite Precipitation, OPC: Ordinary Portland Cement, RCPT: Rapid Chloride Penetration Test.

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Author Contributions

Pooja Damodaran: Ideology, Methodology, Conceptualization, Experimental Investigation, Original draft writing, Result analysis, Editing, Lakshmi Thangasamy: Manuscript draft formation, Result analysis, Validation, Technical Check, Final original drafting, Editing.

Conflict of Interest

The authors of this work state that they have no conflicts of interest about its publication.

Ethics Approval

Not applicable.

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