

A comprehensive study on microbial self-healing concrete for sustainable construction

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ABSTRACT

This study delves into the impact of *Pseudomonas Aeruginosa* bacterial additives on Conventional Concrete (CC) mixes. Four concrete blends (M1-M4) with varying bacterial cell concentrations 10^4 , 10^5 and 10^6 underwent comprehensive analysis to evaluate their strength, durability, workability, and porosity across different curing periods. The research uncovered a consistent enhancement in mechanical properties as bacterial concentrations increased. Notably, compressive, split tensile, and flexural strengths exhibited significant improvements with elevated bacterial cell concentrations, attributed to enhanced cement hydration and mineral precipitation facilitated by bacterial activity. Impact strength tests showcased an enhanced resistance to cracking under impact loads, indicative of improved toughness in bacterial-incorporated mixes. Moreover, porosity tests revealed diminished porosity levels with bacterial incorporation, implying the gradual densification of the concrete structure over time. These findings highlight the promising potential of bacterial concrete for generating high-performance and sustainable construction materials. By harnessing bacterial bio-mineralization capabilities, the construction industry may unlock avenues to enhance concrete properties while simultaneously mitigating environmental impact.

Keywords: *Pseudomonas Aeruginosa*; Workability; Mechanical; Durability properties; Micro analysis.

1. INTRODUCTION

Microbial self-healing concrete utilizes bacteria to autonomously repair cracks, thereby enhancing the durability and sustainability of concrete structures. A study investigated the utilization of alkali-resistant spore-forming bacteria from the *Bacillus* genus. These spores, incorporated into cement paste, maintained viability for four months, producing additional crack-plugging minerals compared to controls. This suggests significant potential for self-healing applications in concrete structures [1, 2]. The encapsulation of bacterial spores within microcapsules has been investigated to protect them from the harsh concrete environment and ensure their viability upon cracking [3]. The breakage of these microcapsules upon cracking releases the spores, which then precipitate calcium carbonate to heal the cracks. This method has shown to significantly reduce water permeability and increase the healed crack width compared to non-bacterial specimens [4].

Hydrogel encapsulation of carbonate precipitating bacteria offers another approach to self-healing in concrete. The hydrogel-encapsulated spores demonstrated superior self-healing capabilities, with a significant reduction in water permeability and the ability to heal larger cracks compared to non-bacterial specimens [5]. The environmental and health risks associated with traditional concrete treatment methods have led to the exploration of microbial self-healing as a more sustainable and long-lasting alternative [6]. This approach not only repairs cracks actively and rapidly but also aligns with environmental sustainability due to its compatibility with concrete compositions and efficient bonding capacity [7].

To enhance the viability of bacteria in the concrete matrix, a protective carrier using low alkali, quick hardening cementitious material has been developed. This narrative microbial self-healing system has shown the potential to achieve near-complete crack closure and improve the compressive strength and water tightness of concrete [8]. The addition of microbial adjuvants such as peptone, yeast extract, and *Bacillus Subtilis* to concrete mix designs has been shown to enhance durability and mechanical properties by reducing porosity and increasing strength [9]. The microbial precipitations within the cracks were identified as calcium carbonate, with various crystal morphologies observed. This finding highlights the effectiveness of *Bacillus subtilis* in facilitating the self-healing process by producing calcium carbonate, which fills and repairs the cracks, thereby enhancing the overall structural integrity of the concrete [10].

The performance of microbial concrete developed using *Bacillus subtilis* has been extensively studied. The results demonstrate significantly enhanced strength and durability, thereby supporting the innovative concept of “Self-Healing Bacterial Concrete”. This advancement suggests a promising future for sustainable and resilient construction materials, capable of autonomously repairing cracks and prolonging structural integrity [11]. This type of concrete can autonomously remediate its cracks, contributing to its durability and sustainability. Practical engineering applications of microbial self-healing concrete have been explored, with the spray-dried fermented bacteria method showing promise for the production of microbial healing agents [12]. The construction experience gained provides valuable insights for the commercialization of this technology [13]. The engineering applications of microbial self-healing concrete have been explored, with the spray-dried fermented bacteria method showing promise for the production of microbial healing agents [14]. The construction experience gained provides valuable insights for the commercialization of this technology. These practical learnings help refine the application processes, optimize the effectiveness of microbial concrete, and pave the way for its broader adoption in the construction industry, ultimately contributing to more durable and sustainable building practices [15].

The sustainability of bacterial concrete has been questioned due to the high cost of substrates. However, ongoing research aims to reduce production costs, making bacterial concrete a viable option for sustainable construction [16]. The incorporation of genetically-enriched microbes into concrete has been investigated, with the genetically modified *Bacillus subtilis* showing improved mechanical strengths and durability [17]. The formation of a novel gehlenite phase within the bio-mortar matrices due to the biochemical activity of the bacteria suggests a new direction for enhancing the structural properties and self-healing activity of concrete [18].

Bacterial spores, specifically from the *Bacillus* genus, demonstrate promise as self-healing agents in sustainable concrete, contributing to extended service life and environmental sustainability through their crack-healing capabilities [19]. Utilizing *Bacillus subtilis*, microbial concrete exhibits remarkable improvements in strength and durability, earning it the title of self-healing bacterial concrete. This innovative material autonomously repairs cracks, eliminating the need for human intervention while enhancing structural integrity and longevity [20].

The microbial self-healing concrete represents a significant enhancement in sustainable construction technology. The various methods of bacterial incorporation and encapsulation have demonstrated promising results in improving the durability and self-healing capabilities of concrete, with the potential to diminish maintenance costs and environmental impact [21, 22].

2. MATERIALS AND METHODS

2.1. Cement

Cement holds a pivotal position within concrete structures, influencing their overall performance. The key criterion guiding cement selection is its ability to enhance the microstructure of concrete. Several factors warrant consideration during this process, including compressive strength across different stages of curing, fineness, heat generated during hydration, alkali content, as well as the proportions of tricalcium aluminate (C3A), tricalcium silicate (C3S), and dicalcium silicate (C2S) present. Additionally, ensuring the compatibility of bacteria with the chosen cement is paramount. This holistic approach guarantees the efficacy and durability of the concrete, ensuring it meets the stringent demands of various construction applications.

The physical properties of the cement are as follows: Fineness measures at 320 m²/kg, surpassing the minimum requirement of 225 m²/kg. Initial setting time is 35 minutes, meeting the minimum standard of 30 minutes. Final setting time falls within the acceptable range at 430 minutes, not exceeding the maximum limit of 600 minutes. The standard consistency stands at 27%. Soundness, as per IS: 12269-2013, records 1.0 mm, well below the maximum allowance of 10 mm. Heat of hydration measures 266 kJ/kg at 7 days. Compressive strength at 3 days, 7 days, and 28 days stands at 37.5 MPa, 48.0 MPa, and 62.0 MPa respectively, all exceeding the required minimums. Specific gravity is noted at 3.15.

2.2. Fine aggregate

The test results conducted in accordance with IS:383-1970 standards reveal the following: Deleterious materials such as those passing 75 μ and including shale, coal, lignite, clay lumps, and other substances, all fall well below the maximum permissible limits, with traces or nil values recorded. Relative density, water absorption, fineness modulus, moisture content, and bulk density all meet or exceed the specified standards, indicating the material's suitability for its intended use. With a relative density of 2.60, water absorption at 1.48%, and a bulk density of 1570 kg/m³, the material demonstrates favorable characteristics.

2.3. Coarse aggregate

According to BIS: 383-1970 standards, the test results for various parameters are as follows: Deleterious material is absent, complying with the maximum permissible limit of 2%. Specific gravity is recorded at 2.84, surpassing the minimum requirement of 2.6. Water absorption is at 0.485%, well below the maximum limit of 3%. The fineness modulus is 5.13. Crushing value is slightly higher at 33%, exceeding the maximum limit of 30%. Impact value and abrasion value are within the acceptable range at 14.3% and 19.4% respectively. Flaky particles and elongated particles are both below the maximum limit of 15%, at 8% and 7% respectively. Traces of chloride and sulphate are detected, well below the maximum permissible limit of 0.4%.

2.4. Bacteria

The type species of the *Pseudomonas* genus, *Pseudomonas Aeruginosa* (PA), is a gram-negative, aerobic (and occasionally facultative anaerobic) bacillus with unipolar motility that is known to be an opportunistic pathogen for both people and plants. To help with identification, PA secretes different pigments under certain circumstances, such as pyocyanin (blue-green), pyoverdine (yellow-green and fluorescent), and pyorubin (red-brown). In vitro, its characteristic smell and pearly look serve as its primary identifiers for identification. Verifying pyocyanin synthesis, fluorescence, and growth ability at 42°C are frequently necessary for clinical confirmation. Because PA is a facultative anaerobe that can adjust to different oxygen concentrations, it may survive in microaerobic or anaerobic conditions, such as thick lung mucus in cystic fibrosis patients. Because of its adaptation, it can survive in harsh environments and develop gelatinous mats that are occasionally mistaken for "algae". The capacity of PA to survive in oxygen-poor conditions is essential to its pathogenicity and variety of lifestyles. Figure 1 shows the image of *Pseudomonas Aeruginosa*.



Figure 1: *Pseudomonas Aeruginosa*.

2.4.1. Growth of bacteria in concrete

The potential for bacterial growth within concrete poses a question, primarily due to its high alkalinity, which typically inhibits bacterial survival. However, specific alkaliphilic bacteria have demonstrated the ability to thrive in such harsh concrete environments. To safeguard these bacteria from the elevated pH levels, immobilization of bacterial cells is commonly practiced. Polyurethane serves as a notable immobilizing agent for this purpose. Additionally, supplementary measures like incorporating trace nutrients and silica gel are employed to shield bacteria when exposed to the alkaline conditions prevalent in concrete. These strategies ensure the viability and efficacy of bacteria-based applications within concrete structures despite the challenging alkaline environment.

2.4.2. Self-healing mechanism

Microcracks ranging from 0.05 to 0.1 mm in width experience complete sealing during dry and wet cycles, facilitated by secondary hydration of unreacted or partially reacted cement particles within the concrete matrix. Capillary action draws water into these microcracks, initiating a process where the expansion of hydrated cement particles, due to the formation of calcium silicate hydrates and calcium hydroxide, leads to self-healing. Additionally, the reaction involving atmospheric carbon dioxide (CO₂) and (CaOH)₂ particles present in the concrete matrix produces various CaCO₃ minerals, further contributing to self-healing. Rapid sealing of freshly formed surface cracks is vital for durability, preventing the ingress of water and aggressive chemicals into the concrete matrix. While traditional perceptions view bacteria, especially acid-producing strains, as detrimental to concrete, recent studies have identified specific beneficial bacteria, such as ureolytic species, capable of repairing cracks or cleaning concrete surfaces. External application of bacteria on concrete surfaces has been explored in previous research, while autogenous repair necessitates an intrinsic healing agent. Bacteria can serve as one component in an autogenously healing system, acting as catalysts for the metabolic conversion of suitable organic or inorganic components into filler materials, such as bio minerals like calcite or apatite. These dense minerals effectively block cracks, enhancing resistance to water ingress.

3. METHODOLOGY

The design process for bacterial concrete mixes entails meticulous calculation of constituent proportions, including cement, coarse and fine aggregates, water, and bacteria. The objective is to formulate a blend that exhibits desired characteristics both in its fresh and hardened states. Given the absence of a standardized code for bacterial concrete, the mix design methodology outlined in the Indian Standard IS: 10262-2019 for Conventional Concrete (CC) was adhered to. In this study, M25 ordinary grade concrete was specifically chosen as the control

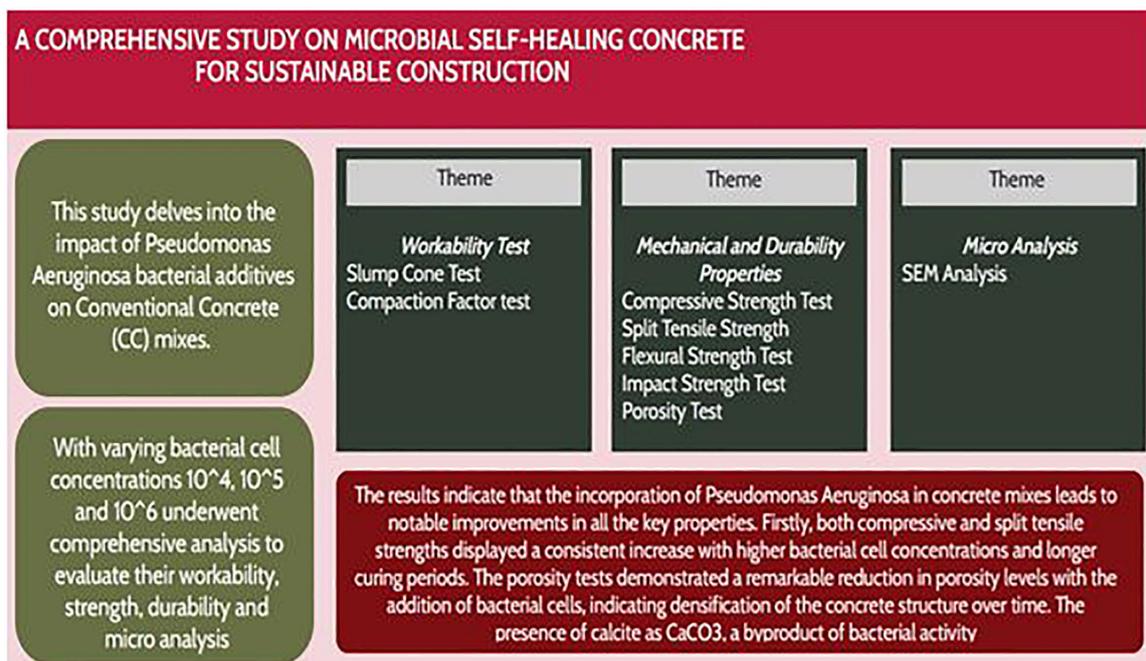


Figure 2: Graphical representation of methodology.

mix for the investigation. By following established guidelines for conventional concrete and adapting them to accommodate bacterial additives, a structured approach is ensured, facilitating the development of reliable and effective bacterial concrete compositions.

Following material weighing, mixing procedures were conducted utilizing an ordinary drum-type concrete mixer for approximately 3 minutes. Cast-iron moulds were employed for specimen casting, with a coating of oil applied inside to ease removal. Concrete deposition occurred in three equal-height layers, each compacted using a table vibrator. Control specimens were crafted devoid of microbial inclusion, while bacterial concrete specimens varied in cell concentrations for each bacterium utilized. A minimum of three specimens were cast per mix for every test conducted. Across all mixes, specimens were meticulously crafted to investigate strength and durability-related properties, ensuring a comprehensive assessment of the performance of the bacterial concrete formulations under scrutiny. Figure 2 shows the graphical representation of methodology.

4. RESULTS AND DISCUSSION

4.1. Slump cone test

The Figure 3 showcases the impact of bacterial additives on the slump cone test of the concrete mixes. Conventional SCC (Self-Compacting Concrete) without bacterial additives (M1) exhibited a slump value of 96 mm. Introducing *Pseudomonas Aeruginosa* in mixes M2, M3, and M4 led to increased slump values, with concentrations of 10^4 , 10^5 , and 10^6 cells respectively, resulting in 99 mm, 102 mm, and 105 mm slumps respectively. This trend suggests a correlation between bacterial cell concentration and workability, with higher concentrations generally yielding higher slump values. The increment in slump values with increasing bacterial cell concentration implies potential improvements in the flowability and ease of placement of the concrete mixes.

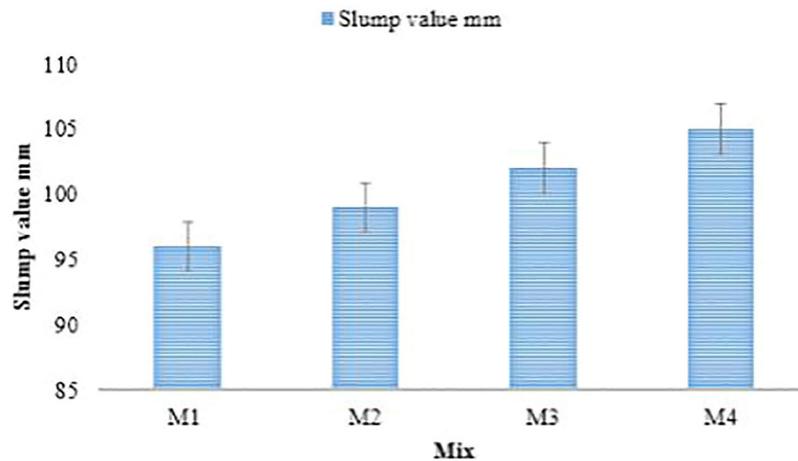


Figure 3: Slump cone test.

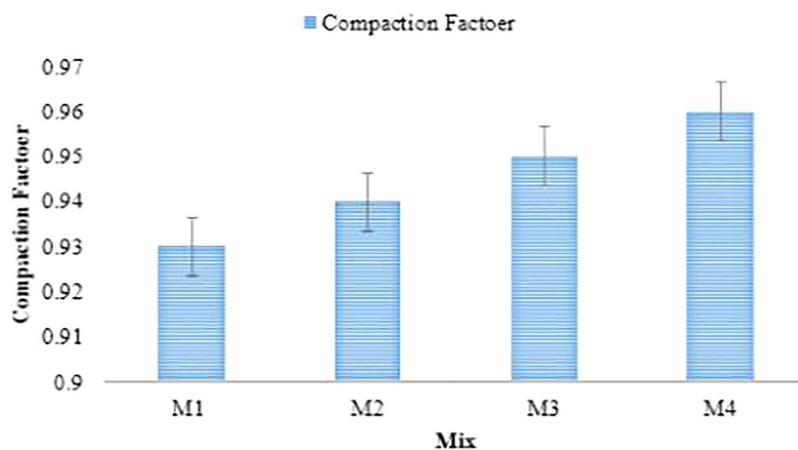


Figure 4: Compaction factor test.

4.2. Compaction factor test

The Figure 4 showcases the impact of bacterial additives on the compaction factor of the concrete mixes. The control mix, M1, featuring Conventional SCC without bacterial augmentation, exhibited a compaction factor of 0.93. Introducing *Pseudomonas Aeruginosa* into mixes M2, M3, and M4 at concentrations of 10^4 , 10^5 , and 10^6 cells respectively resulted in incremental improvements in compaction factor, with values of 0.94, 0.95, and 0.96 respectively. This progression suggests that higher bacterial cell concentrations tend to enhance the compactability of the concrete mixes, leading to improved compaction factors. The observed trend indicates the potential of bacterial additives to positively influence the workability and density of the concrete, which could contribute to enhanced strength and durability.

4.3. Compressive strength test

The compressive strength results demonstrate the influence of bacterial additives on the concrete mixes over different curing periods. Control mix M1, comprising Conventional SCC without bacterial incorporation, exhibited compressive strengths of 19.79 MPa, 28.53 MPa, and 32.53 MPa at 7 days, 14 days, and 28 days respectively. Introduction of *Pseudomonas Aeruginosa* in mixes M2, M3, and M4 at concentrations of 10^4 , 10^5 , and 10^6 cells respectively, led to progressive improvements in compressive strength across all curing durations. At 28 days, M4 exhibited the highest strength at 38.16 MPa, followed by M3 and M2, with strengths of 36.26 MPa and 33.84 MPa respectively. These findings suggest that higher bacterial cell concentrations enhance the compressive strength of the concrete mixes, potentially due to bacteria aiding in mineral precipitation and improving cement hydration. The Figure 5 showcases the impact of bacterial additives on the compressive strength test of the concrete mixes.

4.4. Split tensile strength test

The split tensile strength results highlight the impact of bacterial additives on the concrete mixes across different curing periods. Mix M1, representing Conventional SCC without bacterial inclusion, demonstrated split tensile strengths of 1.47 MPa, 2.15 MPa, and 2.71 MPa at 7 days, 14 days, and 28 days respectively. Introducing *Pseudomonas Aeruginosa* in mixes M2, M3, and M4 at concentrations of 10^4 , 10^5 , and 10^6 cells respectively, led to progressive enhancements in split tensile strength across all curing durations. At 28 days, M4 exhibited the highest split tensile strength at 4.48 MPa, followed by M3 and M2 with strengths of 3.81 MPa and 3.36 MPa respectively. These findings suggest that higher bacterial cell concentrations contribute to improved split tensile strength in the concrete mixes, likely attributed to bacterial activities promoting cementitious bond formation and aggregate interlock. The Figure 6 showcases the impact of bacterial additives on the split tensile strength test of the concrete mixes.

4.5. Flexural strength test

The flexural strength results from Figure 7 illustrate the impact of bacterial additives on the concrete mixes over different curing periods. Mix M1, representing Conventional SCC without bacterial inclusion, exhibited flexural strengths of 2.78 MPa, 3.86 MPa, and 4.28 MPa at 7 days, 14 days, and 28 days respectively. Introducing *Pseudomonas Aeruginosa* in mixes M2, M3, and M4 at concentrations of 10^4 , 10^5 , and 10^6 cells respectively led to

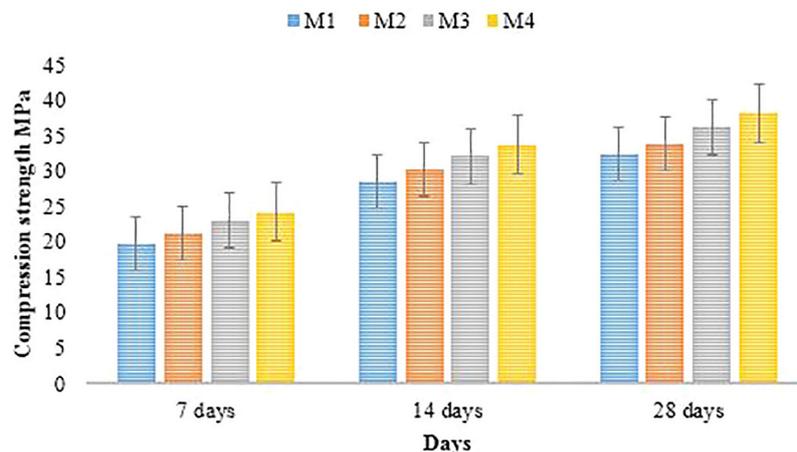


Figure 5: Compressive strength test.

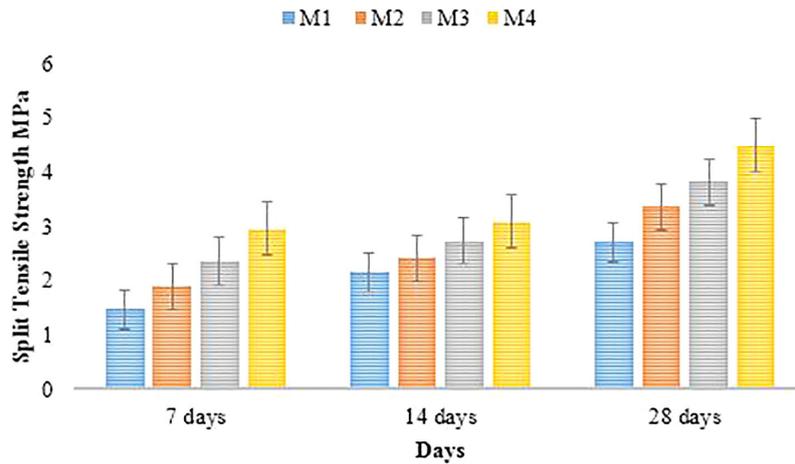


Figure 6: Split tensile strength test.

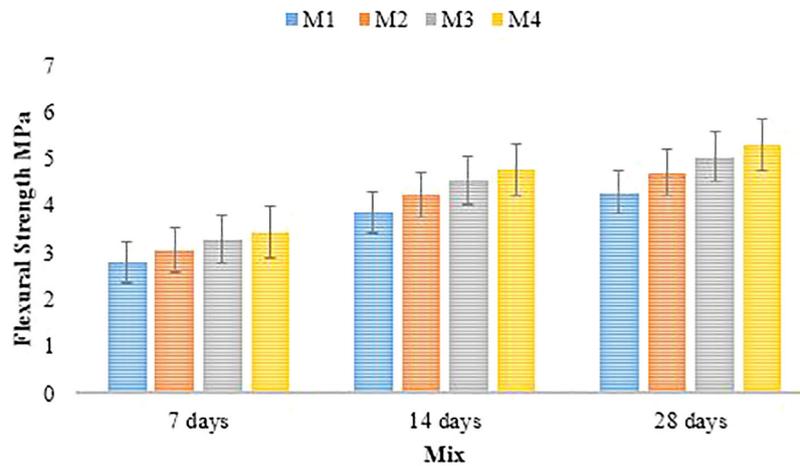


Figure 7: Flexural strength test.

progressive enhancements in flexural strength across all curing durations. At 28 days, M4 exhibited the highest flexural strength at 5.31 MPa, followed by M3 and M2 with strengths of 5.04 MPa and 4.71 MPa respectively. These findings suggest that higher bacterial cell concentrations contribute to improved flexural strength in the concrete mixes, potentially due to enhanced cementitious matrix formation and fiber-matrix interaction facilitated by bacterial activity.

4.6. Impact strength test

The impact strength results from Figure 8 illustrate the impact of bacterial additives on the concrete mixes over different curing periods. The impact strength results highlight the effectiveness of bacterial additives in enhancing the resistance of concrete mixes to cracking under impact loads. At 28 days, Mix M1, representing Conventional SCC without bacterial incorporation, exhibited an impact strength of 34 MPa at first crack and 36 MPa at final crack. Introducing *Pseudomonas Aeruginosa* in Mixes M2, M3, and M4 at concentrations of 10^4 , 10^5 , and 10^6 cells respectively led to progressive improvements in impact strength. Mix M4 demonstrated the highest impact strength, reaching 39 MPa at first crack and 42 MPa at final crack. These results suggest that higher bacterial cell concentrations contribute to enhanced impact resistance in concrete mixes, potentially due to improved matrix densification and crack-bridging mechanisms facilitated by bacterial activity.

4.7. Porosity test

The porosity Figure 9 demonstrate the impact of bacterial additives on the porosity levels of concrete mixes over different time intervals. Mix M1, representing Conventional SCC without bacterial incorporation, exhibited

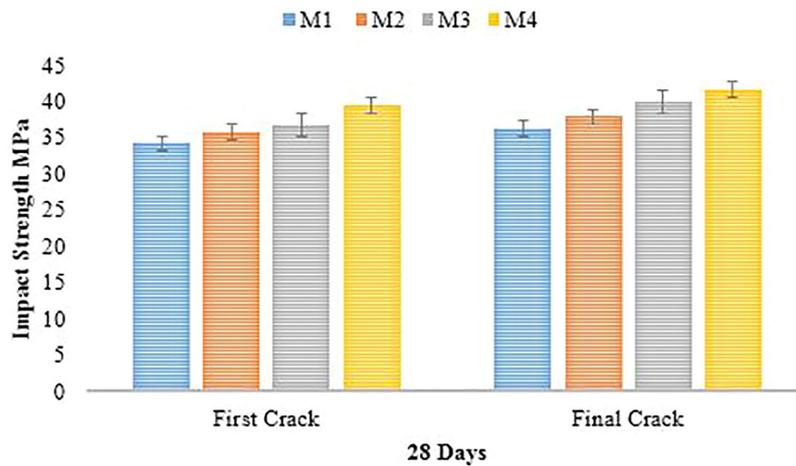


Figure 8: Impact strength test.

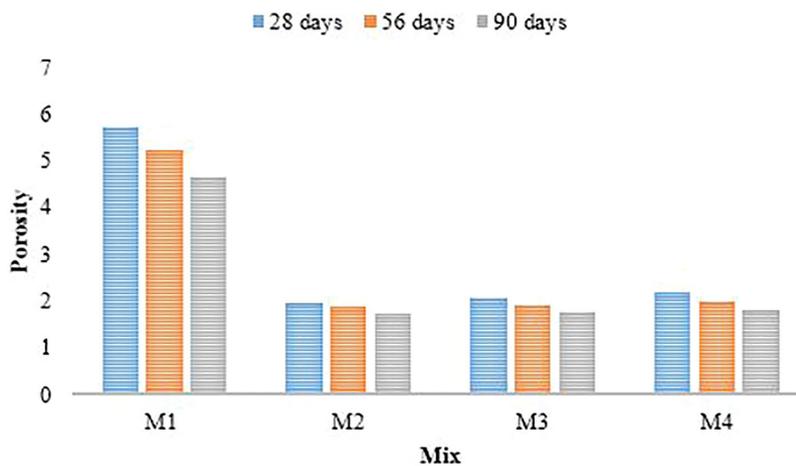


Figure 9: Porosity test results.

porosity values of 5.719, 5.225, and 4.655 at 28 days, 56 days, and 90 days respectively. Introducing *Pseudomonas Aeruginosa* in Mixes M2, M3, and M4 at concentrations of 10^4 , 10^5 , and 10^6 cells respectively resulted in substantial reductions in porosity. At 90 days, Mix M4 displayed the lowest porosity at 1.805, followed by M3 and M2 with porosity values of 1.767 and 1.729 respectively. These findings suggest that higher bacterial cell concentrations contribute to decreased porosity in concrete mixes over time, potentially due to bacterial activities promoting the formation of denser cementitious matrices and reducing voids within the structure.

4.8. SEM analysis

The comparison between the fracture surfaces of control concrete and bacterial concrete composites can be elucidated through the examination of their respective structural morphologies at high magnification levels. In the scanning electron micrograph of the control concrete specimen, limited individual crystals or no signs of CaCO_3 crystals are discernible. Conversely, in the bacterial concrete specimen (M4), a significant amount of individual crystalline calcium carbonates with bacteria is evident. Additionally, scanning electron micrographs of bacterial concrete specimens (M2 and M3) display the presence of crystalline CaCO_3 with bacteria, albeit in slightly lesser amounts compared to the M1 specimen. Notably, pores are partially filled with material growth facilitated by the bacteria, resulting in a reduction in pore volume. This phenomenon enhances material strength and augments the durability of the concrete. The presence of calcite crystalline structures within the pores, attributed to microbiologically produced calcite, further reinforces this effect.

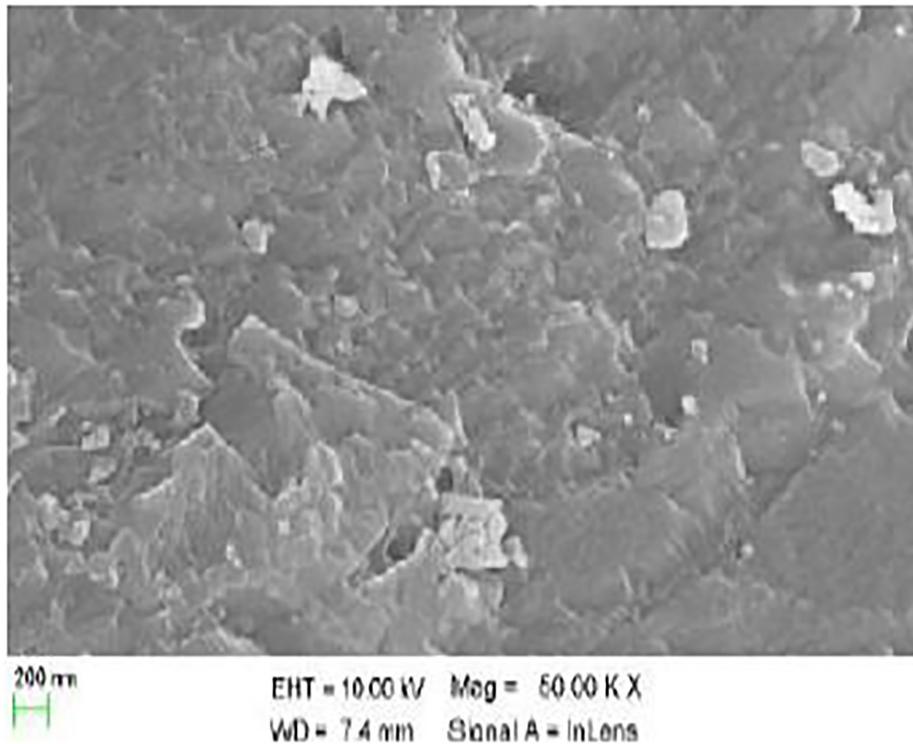


Figure 10: SEM image of M1 mix.

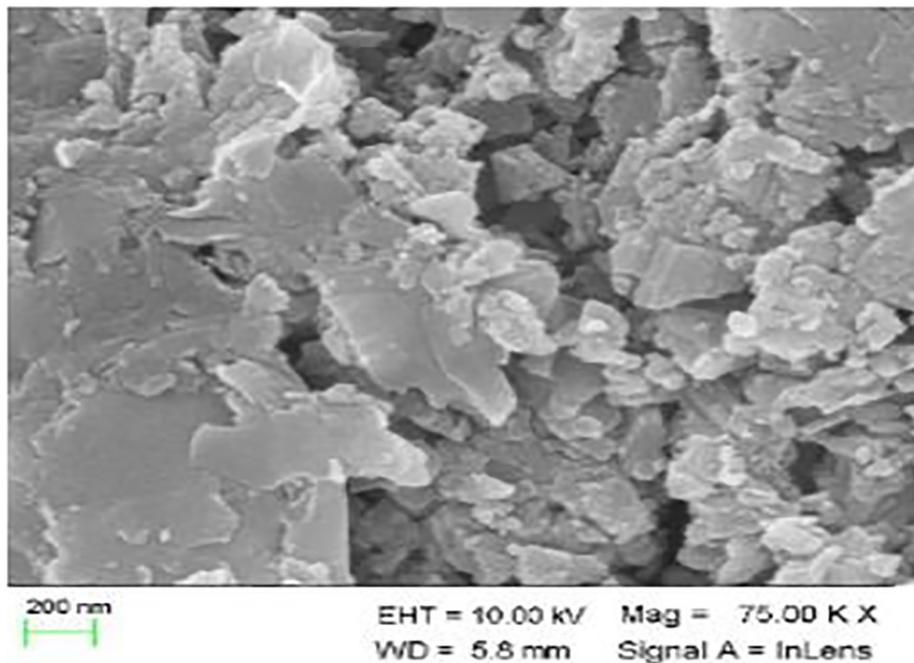


Figure 11: SEM image of M2 mix.

The spherical crystals of CaCO_3 , known as calcites, play a crucial role in filling the pores in cement composites, thereby enhancing strength and durability characteristics. Ultimately, the incorporation of bacteria leads to the formation of calcite structures, which contribute significantly to the mechanical properties and longevity of the concrete. SEM analysis revealed that bacterial concrete exhibits fewer voids compared to conventional concrete, with a dense arrangement of CaCO_3 crystals, predominantly in the form of calcite. This closer packing contributes to the improved mechanical properties and durability of the material. Figure 10 to 13 shows the SEM images of mix M1 to M4.

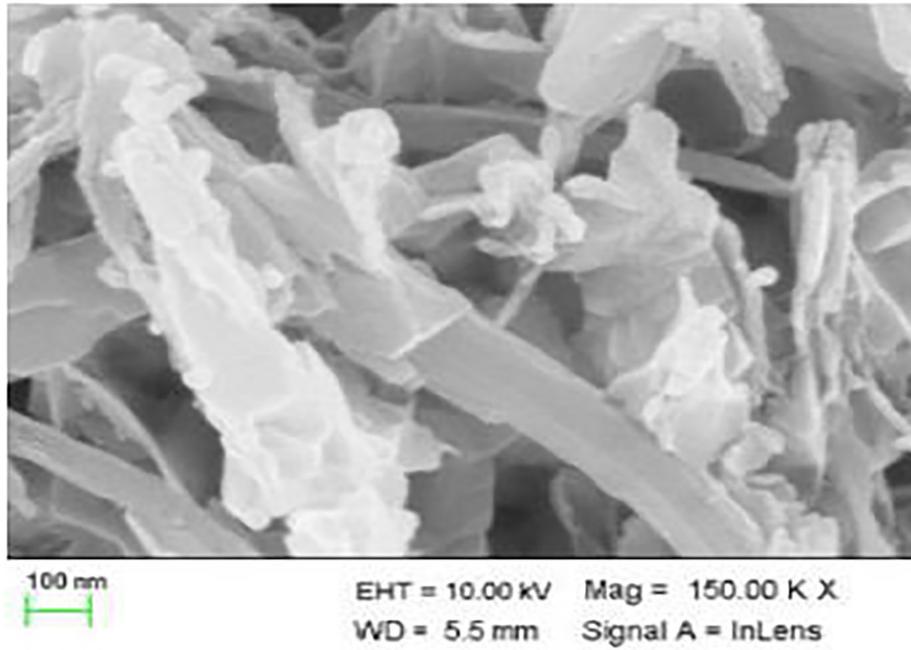


Figure 12: SEM image of M3 mix.

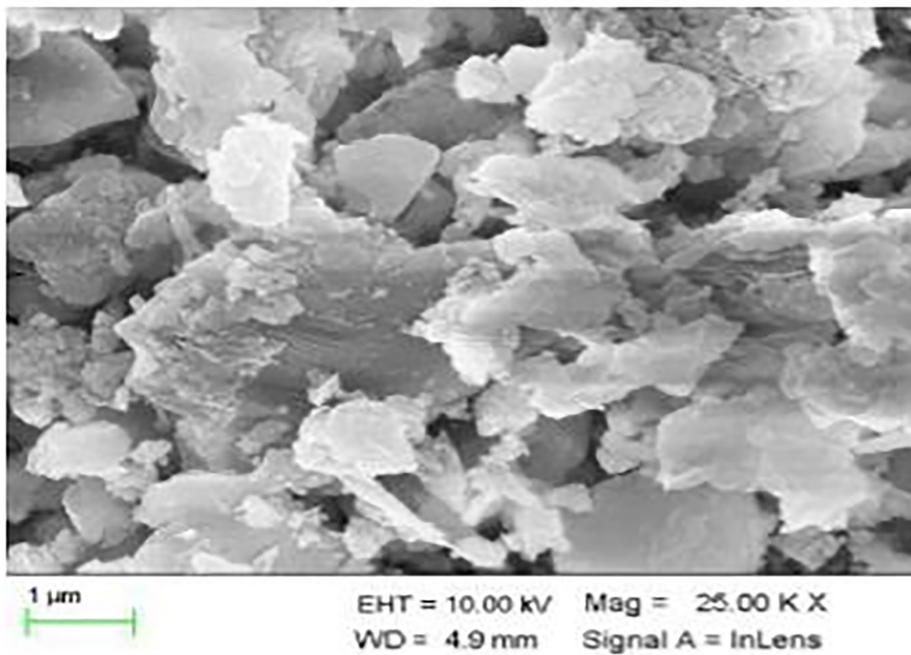


Figure 13: SEM image of M4 mix.

5. CONCLUSION

The investigation into bacterial concrete mixes has provided valuable insights into the influence of bacterial additives on various properties of concrete. The study employed different concentrations of *Pseudomonas Aeruginosa* in Conventional concrete mixes (M1-M4) and evaluated their performance in terms of strength, durability, workability, and porosity over different curing durations. The results indicate that the incorporation of *Pseudomonas Aeruginosa* in concrete mixes leads to notable improvements in several key properties.

Both compressive and split tensile strengths displayed a consistent increase with higher bacterial cell concentrations and longer curing periods. This suggests that bacterial activity contributes to enhanced cement hydration and mineral precipitation, resulting in denser and stronger concrete matrices. The flexural strength results also showed significant improvements with increasing bacterial concentrations, indicating enhanced fiber-matrix interaction and crack resistance. Notably, the impact strength tests revealed a substantial increase in the resistance to cracking under impact loads, further highlighting the effectiveness of bacterial additives in improving the mechanical performance of the concrete mixes.

The porosity tests demonstrated a remarkable reduction in porosity levels with the addition of bacterial cells, indicating densification of the concrete structure over time. This reduction in porosity is crucial for enhancing the durability of concrete by minimizing ingress of harmful agents such as water, chlorides, and sulfates, thereby improving resistance to deterioration mechanisms. SEM analysis of bacterial concrete specimens unveiled the presence of distinct calcite crystals within the concrete matrix. Elevated levels of calcium confirmed the presence of calcite as CaCO_3 , a byproduct of bacterial activity. The deposition of calcite acts as a barrier against harmful substances, enhancing the impermeability of the concrete and consequently improving its durability and resistance to environmental degradation.

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