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Influence of Bacillus Subtilis Bacteria on Strength and Durability of Concrete with Silica Fume

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Abstract

This study investigates the influence of Bacillus subtilis bacteria on the strength and durability properties of M30 concrete with and without silica fume. The experimental study was conducted on four concrete mix series: conventional concrete (B1), conventional concrete with silica fume (B2), bacterial concrete without any admixtures (B3), and bacterial concrete with silica fume (B4). Silica fume was incorporated at replacement levels of 5% and 10% by weight of cement for the B2 and B4 mix series to evaluate its effect on bacterial activity and concrete performance. The study measured compressive strength, split tensile strength, and water absorption to assess mechanical and durability properties. Results reveal that bacterial concrete (B3 and B4) exhibits improved strength and durability compared to conventional concrete (B1 and B2). Furthermore, silica fume enhances the performance of bacterial concrete due to its pozzolanic action, which refines the microstructure and provides additional nucleation sites for calcium carbonate precipitation by Bacillus subtilis. Among all mixes, B4 with 10% silica fume achieved the highest strength and durability, demonstrating the synergistic effect of bacteria and silica fume. This research highlights the potential of bacterial concrete with silica fume as an innovative material for sustainable construction, offering improved mechanical performance and reduced permeability.

Keywords: Bacillus Subtilis, Bacterial Concrete; Silica Fume; Strength Properties; Durability; Sustainable Construction.

1. Introduction

Bacteria exhibit the highest abundance and metabolic diversity among all life forms on Earth. The process of incorporating bacteria into concrete, referred to as bacterial concrete, enables autonomous crack repair through microbial activity. Ureolytic bacteria are commonly employed in this method due to their high survival rate under harsh conditions [1]. Bacterial growth can be categorized into two distinct phases: the lag phase and the exponential phase. During the lag phase, bacteria synthesize essential proteins required for rapid proliferation, whereas in the exponential phase, the bacterial population grows exponentially. As growth decelerates, bacterial cells reduce their metabolic activity by degrading surplus cellular proteins. Spore-forming bacteria are particularly effective in inducing consistent calcite formation. Numerous researchers have explored bacterial applications in concrete to mitigate crack propagation. For instance, Ghosh et al. [2] introduced thermophilic anaerobic microorganisms at varying concentrations in the water used for concrete mixing. Their findings revealed that a concentration of 10^5 cells/mL resulted in a 25% increase in

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compressive strength compared to conventional concrete. Durability was also enhanced by bacterial mineral precipitation, which improved the concrete's resistance to sulphate attack, alkali degradation, and freeze-thaw cycles [3]. Ureolytic bacteria, such as Bacillus sphaericus, precipitate calcium carbonate ($CaCO_3$) within their microenvironment by hydrolyzing urea into ammonium and carbonate ions. This process elevates the pH and facilitates calcium carbonate deposition in calcium-rich environments [4].

Alkali-resistant, spore-forming bacteria, particularly those from the Bacillus genus, have shown superior performance in producing crack-filling minerals when incorporated into cement paste. Enhanced bacterial growth within cement mortar leads to the formation of a highly impermeable calcite layer on the surface, acting as a filler and significantly improving crack resistance. Additionally, studies involving Bacillus subtilis demonstrated substantial increases in compressive strength when introduced into concrete [5]. The self-healing capabilities of bacterial concrete have also been extensively studied. Researchers created controlled cracks in concrete specimens and measured the degree of damage using the Ultrasonic Pulse Velocity method. Results indicated a correlation between the degree of damage and the self-healing ratio, with the self-healing capacity diminishing as the damage increased [6-8]. Further studies investigated the influence of Sporosarcina pasteurii on the compressive strength and rapid chloride permeability of concrete, both with and without fly ash. Concrete incorporating fly ash and Sporosarcina pasteurii exhibited higher compressive strength, reduced porosity, and lower permeability compared to control specimens [1, 9]. Based on these studies, it is evident that the incorporation of bacteria in concrete not only acts as an effective crack-filling agent but also enhances the strength and durability of the material. This innovative approach holds significant potential for developing sustainable and durable construction materials. No research studies have explored the use of silica fume (SF) in bacterial concrete and examined its impact on the strength and durability characteristics of such concrete.

2. Material and Methods

Figure 1 shows the flowchart of the research methodology through which the objectives of this study were achieved.

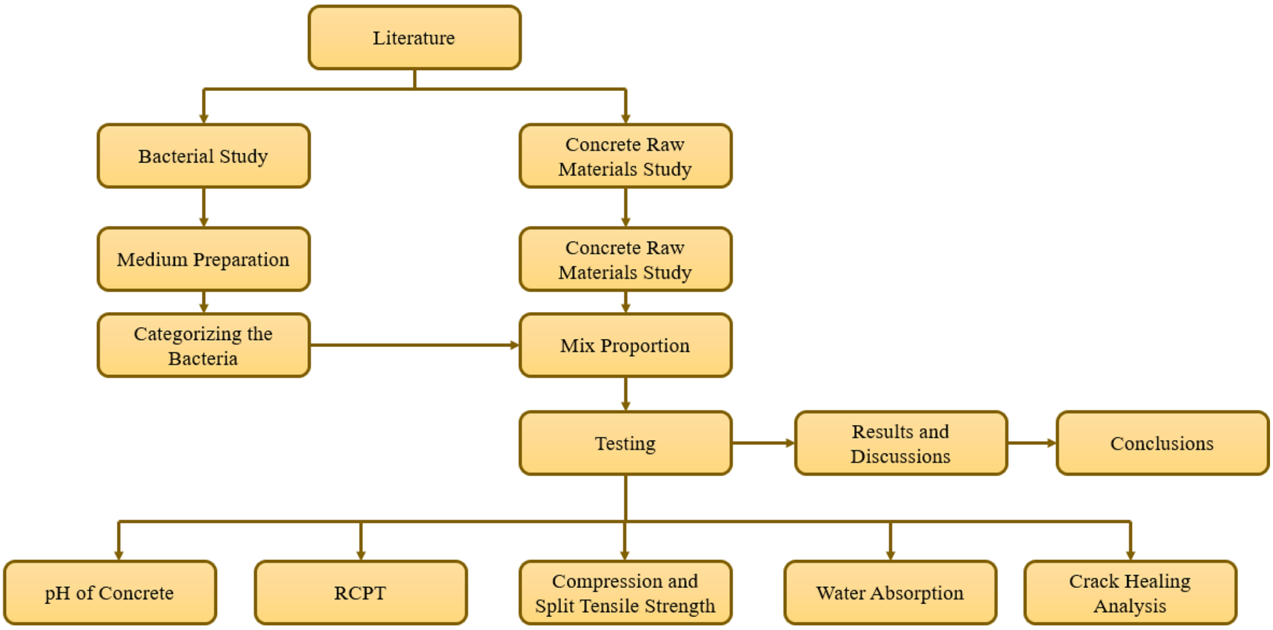

Figure 1. Flowchart of the methodology

2.1. Bacterial Study

The bacteria selected for this study must exhibit the ability to generate calcite in the form of calcium carbonate ($CaCO_3$). Consequently, identifying and isolating the appropriate bacterial strain is critical for achieving the desired outcomes. The process of isolating microorganisms was carried out in two distinct stages: Preparation of Culture Medium: A specialized culture medium was created to facilitate the growth and proliferation of microorganisms under controlled conditions. The composition of the medium was tailored to support bacterial activity and ensure optimal calcite precipitation. Bacterial Population Analysis: The bacterial population in the sample was quantified to evaluate its suitability for the study. This involved determining the density and viability of the bacterial strains capable of inducing calcite formation. By systematically isolating and analyzing the bacterial strains, the study ensures the selection of an appropriate microorganism with the potential to enhance the performance of bacterial concrete.

2.2. Medium Preparation

The essential nutrients required for the growth of microorganisms were prepared. Nutrient agar, a fundamental growth medium, was used as it provides essential sources of carbon, nitrogen, and minerals. Non-fastidious bacteria thrive in this medium due to its balanced composition. The preparation involved sequentially dissolving peptone, yeast extract, and sodium chloride in 250 mL of water. The pH of the solution was adjusted to a range of 7.2 to 7.4 using a 0.1N sodium hydroxide solution. The mixture was then heated in a water bath at 121°C for 15 minutes to liquefy the agar. After sterilization, the medium was cooled to a room temperature of approximately 35°C and poured into sterile Petri plates. Once the medium equilibrated to room temperature, the plates were placed in an incubator at 110°C for 30 minutes to dry the surface of the agar. Subsequently, the nutrient agar solution was reheated to a temperature of 55°C in a water bath to melt it as needed. The melted nutrient agar was poured into 10 sterile Petri plates. For bacterial isolation, six test tubes were labeled and prepared with 9 mL of sterile saline solution in each. The isolation process was conducted as follows:

2.2.1. Serial Dilution

A 1 mL sample of the prepared bacterial suspension was added to the first test tube containing 9 mL of sterile saline solution, creating a 10^{-1} dilution. From this, 1 mL was transferred to the second test tube, creating a 10^{-2} dilution. This process was repeated across all six test tubes to achieve successive dilutions.

2.2.2. Plating and Culture Spreading

A 1 mL sample from the sixth test tube (10^{-6} dilution) was pipetted onto the surface of the agar medium. The sample was evenly spread across the surface of the medium using a sterile L-shaped loop. The bacterial growth is shown in Figure 2.

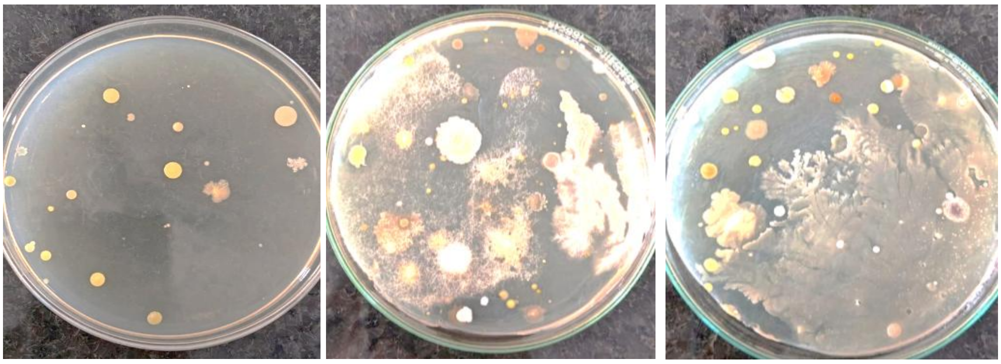

Figure 2. Bacterial growth in agar medium

2.2.3. Incubation

The inoculated plates were incubated at 37°C for 24 hours. Following incubation, bacterial colonies were observed on the agar surface, and their distinct colors and morphological features were used to categorize the bacterial strains. This systematic method ensures accurate isolation and identification of bacteria capable of calcite precipitation for potential use in bacterial concrete applications.

2.3. Categorizing the Micro-Organism

To further categorize the microorganism, motility and MR-VP (Methyl Red-Voges Proskauer) tests were conducted. These tests were performed using bacterial cultures and analyzed through biochemical methods. The results confirmed the presence of *Sporosarcina pasteurii*, also referred to as *Bacillus pasteurii*. The bacterial morphology was visually examined, and the pictorial representation of the bacteria is shown in Figure 3.

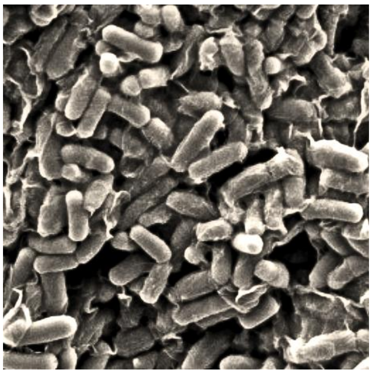

Figure 3. Pictorial view of *Bacillus pasteurii*

The characterization revealed that the bacteria are long rods, gram-positive, and exhibit irregular, dry, and white colony morphology, which is characteristic of *Bacillus pasteurii*. This bacterium is known for its unique ability to solidify organic nitrogen sources through the process of biological cementation, a phenomenon highly beneficial for calcite precipitation in concrete applications [10]. The identification and confirmation of *Sporosarcina pasteurii* highlight its potential as a viable agent for microbial-induced calcite precipitation to enhance the durability and self-healing properties of concrete.

2.4. Materials and Mix Proportions

To achieve M30 grade concrete, the mix design was carried out in accordance with IS 10262:2009. The materials used in this study included Ordinary Portland Cement (OPC) conforming to IS 4031:1988, with a specific gravity of 3.15; river sand, with a specific gravity of 2.31, fineness modulus of 3.06, and passing through a 4.75 mm IS sieve; and coarse aggregate, with a specific gravity of 2.54, fineness modulus of 4.1, and a maximum size of 20 mm. A bacterial concentration of 105 cells/mL was adopted for the study. Silica fume, analyzed as per ASTM C1240, consisted of pure silica in its non-crystalline form. The physical and chemical properties of the cement, sand, and silica fume are summarized in Table 1. The study investigated the strength and durability characteristics of four different concrete mix series: Conventional concrete, Conventional concrete with admixtures, Bacterial concrete without admixtures, and Bacterial concrete with admixtures. The details of the mix proportion are shown in Table 2. The experimental program included compressive strength testing on cubes and split tensile strength testing on cylinders at 28, 56, and 90 days, conducted in accordance with IS 516:1959 and IS 5816:1999, respectively. Durability assessments included a water absorption test (ASTM C642). To evaluate the self-healing capability of the concrete, cracks were introduced into the concrete cubes using a compression testing machine. The crack widths varied between 0.1 mm and 1 mm for all concrete mixes. The healing capacity of bacterial concrete, with and without silica fume, was assessed using an optical image microscope. The crack width was monitored and measured after 3, 7, and 28 days to quantify the self-healing effect. For each mix ratio, 12 cubes and 6 cylinders were cast. This comprehensive testing program enabled the assessment of the mechanical, durability, and self-healing properties of bacterial concrete under various conditions.

Table 1. Physical and chemical properties of raw materials used

Chemical Properties	**SiO_2**	**Al_2O_3**	**CaO**	**Fe_2O_3**	**MgO**	**SO_3**	**K_2O**	**Na_2O**	**LOI**
Cement	20.8	5.1	63.27	3.87	2.26	2.12	-	-	2.52
Sand	93	1	0.4	0.5	0.8	0.5	-	-	3.8
Silica Fume	89.62	1.12	0.8	1.46	0.5	-	0.5	0.5	5.5
Physical Properties	**D_{10}**	**D_{50}**	**D_{90}**	**Specific Gravity**	**Fineness Modulus**				
Cement	2.56	4.32	6.92	3.15	3.06				
Sand	30	70	475	2.31	4.1				
Silica Fume	-	0.15	-	2.2	-				

Table 2. Mix Proportion details

Mix series	**Mix ID**	**OPC (kg/m^3)**	**FA (kg/m^3)**	**SF (kg/m^3)**	**CA (kg/m^3)**	**w/c ratio**
B1 (conventional Mix)	Ref	420	560	-	1119	0.45
B2 (conventional Mix with silica fume)	B2-5%	420	560	28	1119	0.45
	B2-10%	420	560	56	1119	0.45
B3 (Bacterial concrete without admixtures)	B3	420	560	-	1119	0.45
B4 (Bacterial concrete with silica Fume)	B4-5%	420	560	28	1119	0.45
	B4-10%	420	560	56	1119	0.45

3. Results and Discussion

3.1. pH of Concrete

The pH levels of fresh concrete were analyzed at various silica fume (SF) and bacterial dosages, as depicted in Figure 4. The test results revealed that the control concrete mix (B1) exhibited a pH of 10.58. With the addition of 5% SF, a 10% reduction in the alkalinity of the concrete was observed. Further increases in SF concentration led to additional reductions in alkalinity. This decrease in alkalinity can be attributed to the pozzolanic reaction initiated by the inclusion of SF, wherein calcium hydroxide (CH) is consumed to form additional calcium silicate hydrate (C-S-H). This reaction enhances the strength and durability of the concrete by increasing the binding phases. However, the reduction in free CH levels slightly lowers the alkalinity. Despite this reduction, the overall pH of the concrete remains within the range of 8 to 10, which is sufficient to maintain a protective environment for steel reinforcement, preventing corrosion. Studies indicate that *Bacillus subtilis* bacteria can grow within a pH range of 4.8 to 9.2, with optimal growth observed at lower pH values [11]. In the B3 mix (conventional concrete with bacteria), a 5.94% increase in pH was noted compared to the control mix (B1). This rise in alkalinity is attributed to the bacterial urease enzymes, which hydrolyze urea into ammonia and carbon dioxide. This reaction increases the pH and facilitates carbonate precipitation, which contributes to the self-healing properties of bacterial concrete [8, 12, 13]. However, in bacterial concrete mixes containing SF (B4 with 5% SF and B5 with 10% SF), the alkalinity levels were reduced by 8.92% and 13.91%, respectively, compared to the bacterial-only mix (B3). This reduction in pH further supports bacterial growth by providing an environment closer to the optimal range for bacterial activity. Hence, the results demonstrate that the inclusion of SF not only improves the mechanical properties of concrete through pozzolanic reactions but also promotes bacterial growth by lowering the pH, thereby enhancing the efficiency of bacterial self-healing mechanisms.

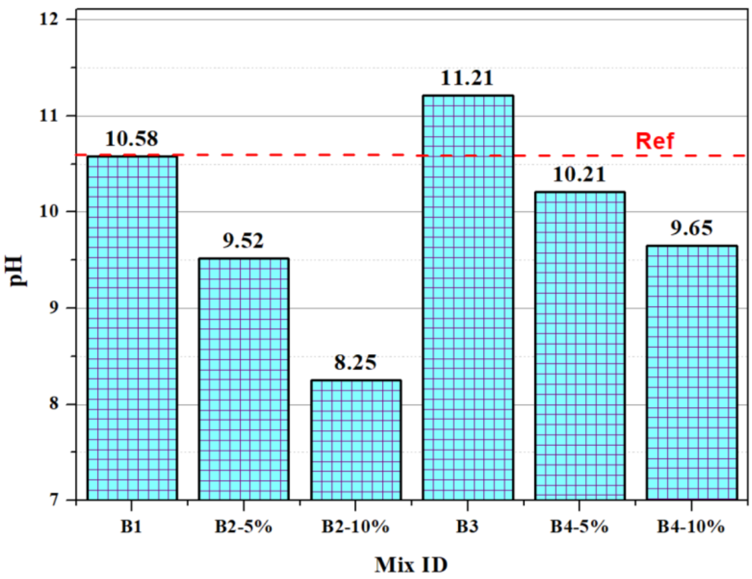

Figure 4. pH level of fresh concrete of different concrete mix

3.2. Compressive and Split Tensile Strength

The compressive strength of concrete cubes at 14, 28, 56, and 90 days is shown in Figure 5. The results indicate that the highest compressive strength was achieved in bacterial concrete containing 10% silica fume (SF), with an increase of 13.6% compared to the control mix (B1) at 90 days. Strength values for all mixes were observed to increase with age, irrespective of the testing days. The B3 mix, which included bacteria but no SF, showed a marginal increase of 0.76% in compressive strength compared to the control mix, indicating that the addition of bacteria alone has a minimal effect on improving concrete strength. However, the incorporation of SF at 5% and 10% significantly enhanced the compressive strength, with a 9.09% increase observed for bacterial concrete containing 10% SF. This improvement can be attributed to the high surface area of SF, which enhances pozzolanic reactivity, producing additional calcium silicate hydrate (C-S-H) and contributing to strength development [14].

The results align with previous literature findings [3, 4], where higher SF concentrations were reported to improve the early-age strength of concrete. However, only a slight increase in strength was observed between 56 and 90 days, suggesting that the pozzolanic reaction reaches a plateau at later ages. When compared to conventional concrete, the bacterial concrete with 10% SF exhibited 12.12% and 8.82% higher compressive strength than mixes without and with SF, respectively. Similarly, Figure 6 also illustrates the split tensile strength results for all concrete mixes at 28, 56, and 90 days. The trends were consistent with those observed in the compressive strength tests. While bacterial incorporation had no significant impact on split tensile strength, a slight increase was noted, with no reduction in strength observed. The observed improvement in split tensile strength can be attributed to the presence of SF, which contributes to better bonding within the mix. These findings confirm that the addition of SF, particularly at 10%, plays a critical role in enhancing both compressive and split tensile strength, while bacterial inclusion contributes minimally to strength improvement but facilitates durability and self-healing properties.

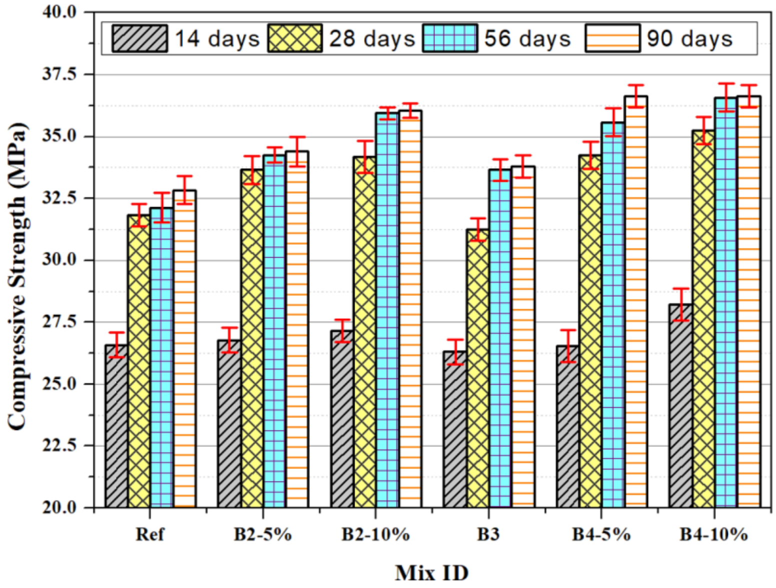

Figure 5. Compressive strength

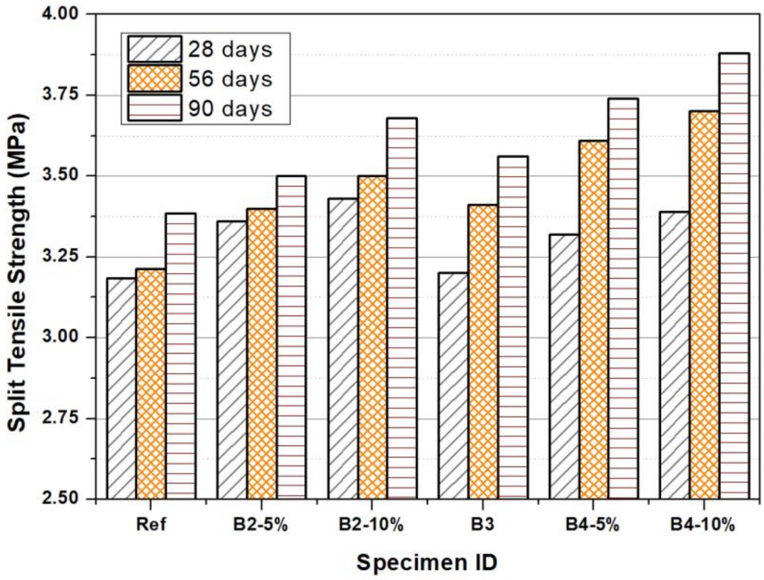

Figure 6. Split Tensile Strength

3.3. Water Absorption

The percentage of water absorption for each concrete mix designation is illustrated in Figure 7. The results indicate that the highest water absorption was observed in the control concrete mix (B1). A significant reduction in water absorption was noted with the increase in silica fume (SF) concentrations. In the B2 series, the mix with 10% SF substitution exhibited the lowest water absorption, demonstrating the effectiveness of SF in reducing permeability. For bacterial concrete (B3), a progressive reduction in water absorption was observed over time. A 12% reduction in water absorption was recorded between 3 days and 28 days for the B3 specimen, indicating that bacterial activity plays a key role in blocking pores through calcite precipitation. This MICP reduces the connectivity of pores, thus lowering water permeability. The reduction in water absorption with SF addition can be attributed to the densifying effect of the mineral admixture. Silica fume reacts with calcium hydroxide to form additional calcium silicate hydrate (C-S-H), which refines the pore structure. In addition, bacterial growth further contributes to pore blocking, leading to minimal water absorption [10]. The lowest water absorption value, 5.1%, was recorded for the B4-10% SF mix at 28 days. This result highlights the synergistic effect of silica fume and bacterial incorporation in reducing permeability. These findings suggest that bacterial concrete with SF substitution is highly suitable for water-retaining structural applications, as it significantly minimizes water absorption while enhancing durability.

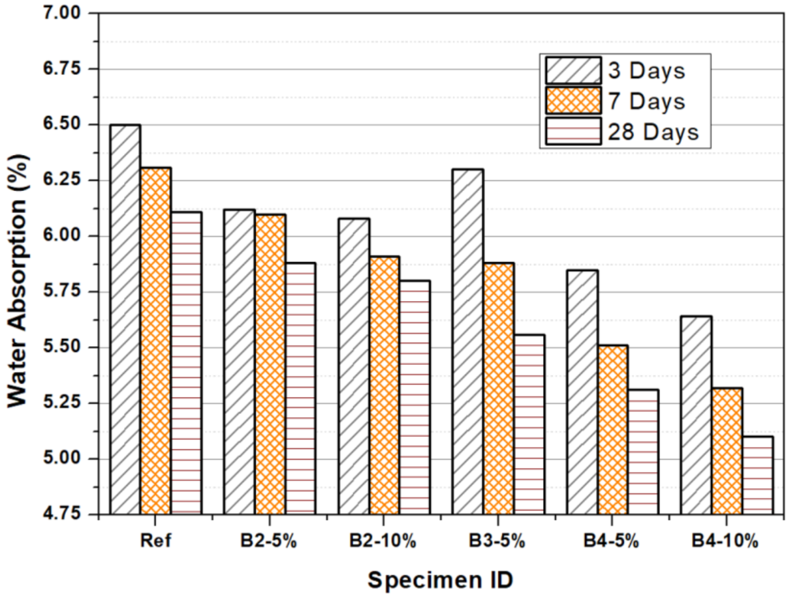

Figure 7. Percentage water absorption

3.4. Crack Healing Analysis

Crack healing analysis, which examines the percentage of crack healing over time, is illustrated in Figure 8. The formation of white calcite precipitation on the surface of the cracks, as shown in Figure 9, indicates MICP. This process led to a noticeable reduction in crack width over time. The reduction in crack width was quantified as the crack healing percentage. After 3 days of incubation, the crack width reduction began. Initially, the maximum crack width was 0.9 mm, which was completely healed by 28 days. Notably, the healing percentage was significantly higher for bacterial concrete mixes containing SF. For instance, at 7 days, the crack healing percentage for the B4 mix with 10% SF was 138% higher than that of conventional bacterial concrete, while the B4 mix with 5% SF exhibited a 77.77% higher healing percentage. Complete crack healing (100%) was observed within 7 days for the bacterial concrete mix containing 10% SF. The results also indicate that higher SF concentrations in the mix accelerated the healing process. This enhanced healing efficiency can be attributed to the synergistic effects of SF and bacterial activity. The high packing density achieved with SF reduces the pore size, promoting a favorable environment for bacterial growth and calcite precipitation. Furthermore, the lower pH values in SF containing concrete enhance bacterial activity, further accelerating the healing process [15]. These findings demonstrate that bacterial concrete with higher SF concentrations exhibits superior self-healing capabilities, making it highly suitable for applications where crack repair and durability are critical.

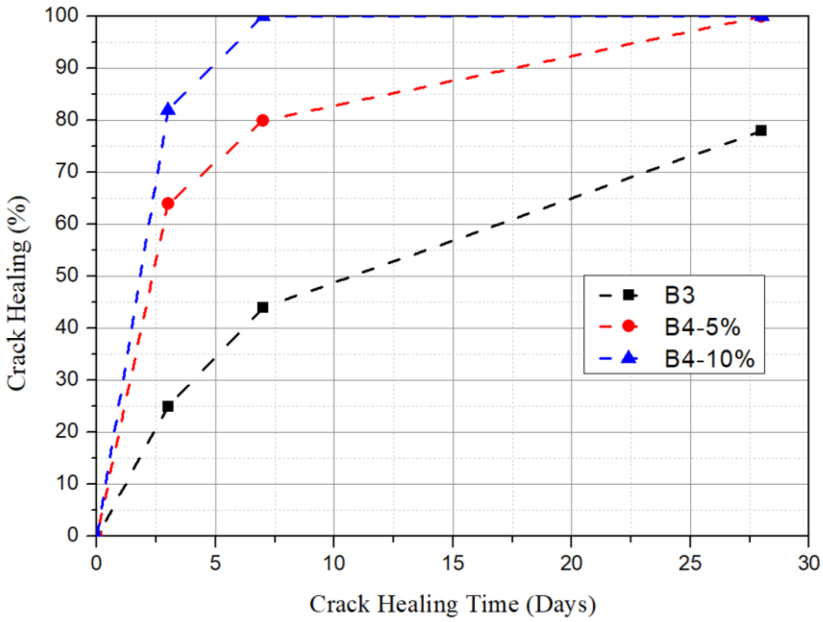

Figure 8. Percentage healing of cracks with respect to time

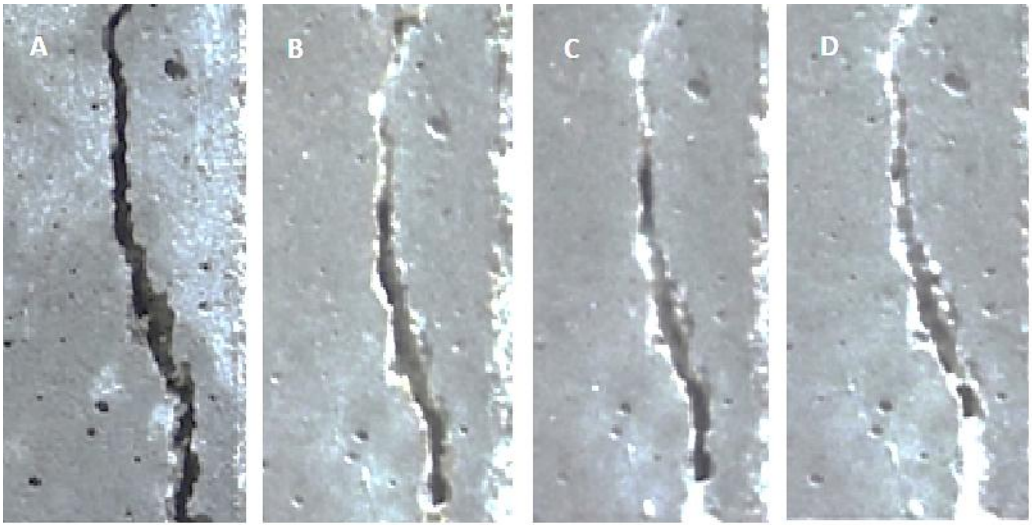

Figure 9. (a) 1st of crack, (b) 3rd day of crack healing, (c) 7th day of crack healing, (d) 28th day of crack healing for B3 mix

3.5. Rapid Chloride Permeability Test

The ability of concrete to resist chloride ion ingress is a crucial factor in determining its durability, particularly in structures exposed to deicing salts or marine environments. Figure 10 presents the test results on the rapid chloride permeability of different concrete samples. The lowest chloride ion penetration was observed in bacterial concrete incorporating silica fume, showing a 27.5% reduction compared to conventional concrete. The results for samples B2 and B3 demonstrate that the inclusion of silica fume and bacterial activity significantly contributed to minimizing chloride penetration. Concrete containing silica fume and Sporosarcina pasteurii exhibited excellent resistance to rapid chloride penetration.

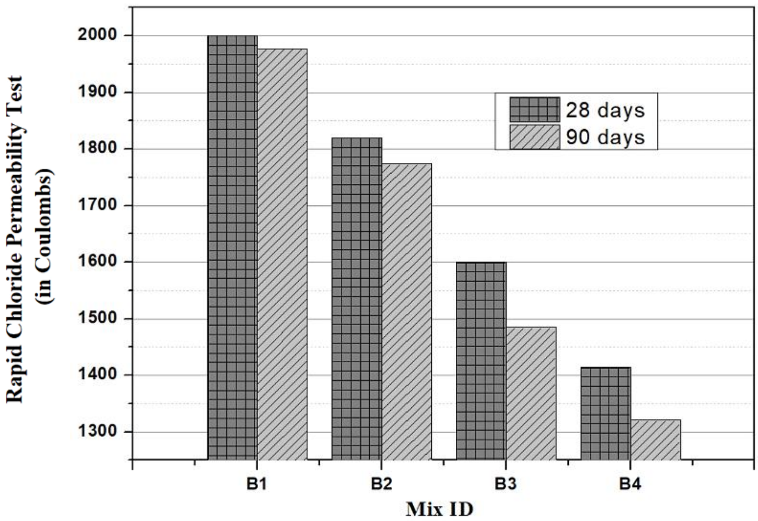

Figure 10. Rapid Chloride Permeability

4. Conclusions

The integration of bacterial concrete technology with supplementary cementitious materials, such as silica fume (SF), presents a transformative approach to enhancing the mechanical, durability, and self-healing properties of concrete. The experimental results from this study demonstrate that the addition of ureolytic bacteria, particularly *Sporosarcina pasteurii*, significantly enhances the self-healing ability of concrete through microbial-induced calcite precipitation (MICP). The inclusion of SF further amplifies these effects by refining the microstructure, reducing porosity, and lowering the pH, which optimizes conditions for bacterial activity and growth.

- Bacterial concrete with 10% SF achieves the highest compressive and split tensile strength, with a notable improvement of 13.6% in compressive strength compared to the control mix at 90 days.
- Water absorption tests revealed that bacterial concrete with 10% SF exhibited the lowest absorption rates, highlighting its superior impermeability and potential for water-retaining applications.

- Crack healing analysis showed that 100% healing was achieved within 7 days for bacterial concrete containing 10% SF, demonstrating the accelerated self-repair capability enabled by the synergistic interaction between bacteria and SF.

This research underscores the potential of bacterial concrete infused with silica fume as an innovative, eco-friendly solution for sustainable construction practices. By addressing challenges such as crack formation, permeability, and long-term durability, this approach not only enhances structural performance but also reduces maintenance costs and extends the lifespan of concrete structures. Future work can explore further increases in SF dosage and improve the scalability of this technology for large-scale applications and assess its performance under diverse environmental conditions, paving the way for next-generation smart materials in civil engineering.

5. Declarations

5.1. Author Contributions

Conceptualization, K.K.P. and M.A.; methodology, D.Q.; validation, D.Q. and A.A.; formal analysis, M.A.; investigation, K.K.P.; resources, K.K.P.; data curation, M.A.; writing—original draft preparation, K.K.P.; writing—review and editing, M.A.; visualization, K.K.P.; supervision, A.I.A.; project administration. All authors have read and agreed to the published version of the manuscript.

5.2. Data Availability Statement

The data presented in this study are available in the article.

5.3. Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

5.4. Conflicts of Interest

The authors declare no conflict of interest.

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