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Performance Evaluation of Bacterial Self-Healing Concrete Using Bacillus Subtilis

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ABSTRACT

This study investigates the influence of Bacillus subtilis as a self-healing agent in M40 grade concrete, focusing on its impact on mechanical properties, crack behavior, and healing efficiency. Concrete specimens were prepared with varying bacterial dosages (0.0%, 0.5%, 1.0%, 1.5%, and 2.0% by cement weight) and evaluated at different curing ages (3, 7, and 28 days). Experimental tests included compressive strength, flexural strength, ultrasonic pulse velocity (UPV), and visual crack observation. Specimens were intentionally cracked using a four-point bending test to simulate structural damage and assess the effectiveness of microbial-induced calcium carbonate precipitation (MICP) in sealing cracks. Results showed that bacterial inclusion enhanced both strength and crack resistance. The 1.5% bacterial dosage consistently performed best across all parameters. At 28 days, this mix demonstrated a substantial increase in compressive and flexural strengths compared to the control. Crack initiation loads increased, and average crack widths decreased notably, indicating improved tensile resistance. Visual analysis of crack patterns revealed finer and more distributed cracks in bacterial mixes, with better confinement and less propagation than in conventional concrete. UPV tests confirmed internal healing by tracking recovery in pulse velocity after 21 days of healing. The 1.5% dosage achieved a significant increase in UPV from 1066 m/s (poste-crack) to 4483 m/s (post-healing) suggesting efficient crack sealing through microbial action. In contrast, the 2.0% dosage showed reduced performance, likely due to oversaturation and uneven bacterial activity. Overall, the incorporation of Bacillus subtilis improved both structural integrity and durability of concrete, with 1.5% emerging as the optimal dosage. This study supports the viability of bio-concrete as a sustainable alternative for improving service life and minimizing maintenance in civil infrastructure. Further research may explore bacterial survival under field conditions and integration with advanced reinforcement techniques.

KEYWORDS: Bacterial Concrete, Bacillus subtilis, Self-Healing Concrete, Pulse Velocity (UPV), Calcium Carbonate Precipitation, Sustainable Construction, Crack Healing

1. Introduction

Concrete remains the cornerstone of modern infrastructure due to its high compressive strength, availability, and versatility. Despite these advantages, its low tensile strength and inherent brittleness make it susceptible to cracking, which compromises durability and serviceability over time [1]. Cracks in concrete not only deteriorate the mechanical properties but also accelerate the ingress of aggressive agents such as water and chlorides, leading to corrosion in reinforced concrete structures. Traditional repair methods are often expensive, time-consuming, and environmentally taxing. To overcome these limitations, researchers have turned to innovative self-healing technologies, particularly microbial-induced calcite precipitation (MICP), which utilizes bacteria to autonomously repair cracks. Non-pathogenic strains such as Bacillus subtilis, Pseudofirmus, and Escherichia coli have shown promising potential in enhancing the compressive strength and durability of concrete through biological healing mechanisms. These bacteria, when embedded in concrete, can survive harsh alkaline conditions and become active upon crack formation and exposure to water, producing calcium carbonate that fills the cracks and restores the structural integrity [2].

The effectiveness of bacterial self-healing concrete has been demonstrated through various experimental studies. For instance, studies have shown that certain bacterial strains can remain dormant within the cement matrix and activate under specific conditions, leading to the precipitation of calcite that seals cracks ranging from 0.02 mm to 0.35 mm [3, 4]. The healing process is further enhanced when optimal concentrations of bacteria and nutrients like calcium lactate are introduced, with some mixes showing nearly 100% crack repair efficiency and a 10–30% increase in compressive strength [1, 5]. Additionally, the inclusion of blast furnace slag (BFS) in concrete not only aids in healing but also improves chloride resistance, especially under wet/dry cycling and marine conditions, where moisture retention helps maintain bacterial activity [6]. Advanced evaluation techniques such as SEM-EDS, acoustic emission, and chloride migration tests have confirmed the formation of calcite and its role in reducing permeability and enhancing durability [7, 8]. Moreover, co-culturing bacteria and engineering strains to thrive in alkaline environments can enhance the self-healing potential, while studies with spore-forming extremophiles such as Bacillus subtilis JC3 have shown the ability to adapt, survive for months, and produce crack-plugging minerals within hardened cement paste [9, 10].

Despite its promise, the application of bacterial self-healing concrete in structural elements like reinforced concrete beams requires further investigation. Current literature has largely focused on small-scale specimens, leaving a gap in understanding the performance of bacterial healing in load-bearing scenarios [11]. The mechanical properties of intact concrete, the influence of bacterial carriers, and the compatibility with alternative binders and fibers need to be optimized to achieve practical application. Studies suggest that while bacterial healing significantly improves strength and durability, it is influenced by variables such as bacterial strain, concentration, curing environment, and crack geometry [12]. The development of standardized models like the Generalized Self-Healing Performance Index (GSPI) could facilitate the assessment of healing efficiency by integrating mechanical, durability, and hydration parameters [11]. Economically, although initial costs for bacterial concrete are higher, the long-term benefits in reduced maintenance, extended service life, and environmental savings are substantial. This makes bacterial self-healing concrete a viable, sustainable, and transformative solution for the construction industry, particularly when addressing challenges in reinforced concrete beam durability [13].

2. Materials and Methods

This study employed standard construction materials, carefully selected and characterized in accordance with relevant Indian Standard (IS) codes to produce M40 grade self-healing concrete. The constituents included Ordinary Portland Cement, fine and coarse aggregates, potable water, a chemical admixture, and a biological healing agent, specifically Bacillus subtilis. The binding material used was Ordinary Portland Cement (OPC) of 53 grade [14], procured from Ultratech having physical and mechanical properties are shown in Table 1. Class F fly ash (Table 2) was incorporated as a supplementary cementitious material in accordance with IS: 3812 (Part 1) – 2013 [15]. It also aligns with sustainability goals by reducing cement usage and CO_2 emission. The specific gravity was measured at 2.20. Crushed angular granite aggregates with a maximum size of 20 mm were selected as coarse aggregates. Sieve analysis was conducted to ensure proper gradation, and the results aligned with the standards recommended in IS 383:2016, which governs the specifications for coarse and fine aggregates used in concrete. The particle size distribution indicated a well-graded mix, with 95.5% passing through the 20 mm sieve and 35.73% through the 10 mm sieve. This ensured appropriate interlocking and reduced void content. The specific gravity of the coarse aggregate was recorded at 3.02, confirming its suitability for dense concrete. Manufactured sand, or crushed sand, was utilized as fine aggregate. The material was free from silt and clay and displayed physical characteristics consistent with Zone II classification, as per IS 383:2016. The sieve analysis showed a fineness modulus of 3.52, indicating a moderately coarse material ideal for achieving good workability. The specific gravity was measured at 2.94, within acceptable limits for natural and manufactured sands. The particle size distribution was optimized for a balanced mix, ensuring minimal segregation and improved cohesiveness. Potable water from a municipal supply was used for both mixing and curing. In accordance with IS 456:2000, Clause 5.4, the water used in concrete should be clean and free from harmful contaminants such as alkalis, acids, oils, organic matter, and salts. The water conformed to these standards and ensured effective hydration and strength gain without adversely affecting setting times or long-term durability. A plasticizer named ADMSPLAST, manufactured by CAC/Asian Paints, was incorporated into the mix to improve workability without increasing the water-cement ratio.

Table 1. Physical and Mechanical Properties of Ultratech 53 Grade OPC

Property	*Value*
Fineness (Blaine)	328.0 m^2/kg
Standard Consistency	29.50%
Initial Setting Time	108 minutes
Final Setting Time	226 minutes
Soundness (Le-Chatelier method)	6.5 mm
Compressive Strength (3 Days)	34.4 MPa
Compressive Strength (7 Days)	47.6 MPa
Compressive Strength (28 Days)	61.5 MPa

The self-healing capability of the experimental concrete mix was enabled through the incorporation of Bacillus subtilis, a gram-positive, rod-shaped, endospore-forming bacterium known for its robust survivability under harsh and alkaline conditions commonly found in concrete environments. This microorganism is naturally present in the upper layers of soil and is classified as non-pathogenic, making it safe for handling and incorporation into building materials. In this study, Bacillus subtilis was introduced into the concrete mix in varying concentrations 0.5%, 1.0%, 1.5%, and 2.0% by the weight of cement to investigate the influence of bacterial dosage on crack-healing efficiency. These concentrations were selected based on findings from prior literature that highlight the ability of B. subtilis to induce calcite precipitation through microbial metabolic processes, specifically via the enzymatic conversion of calcium lactate into calcium carbonate, effectively sealing micro-cracks. For the purpose of this study, the bacterial strain was sourced from Katyayani Organics, located in Madhya Pradesh, India. The product used was Katyayani Bacillus Supp 2% Powder, containing a viable cell concentration of 2×10^8 colony-forming units (CFU) per gram, suspended in a talc-based carrier. This specific formulation was chosen for its stability, ease of integration into the dry concrete mix, and consistent microbial viability, making it suitable for experimentation within a controlled laboratory setting.

Table 2. Chemical Composition of C Class Fly Ash

Oxide Compound	*Chemical Formula*	*(%)*
Silicon Dioxide	SiO_2	52.25
Aluminium Oxide	Al_2O_3	27.65
Iron Oxide	Fe_2O_3	11.73
Calcium Oxide	CaO	2.87
Magnesium Oxide	MgO	1.43
Sulfur Trioxide	SO_3	0.27
Loss on Ignition	LOI	1.55
Specific Gravity	—	2.2

The concrete mix shows in Table 3 was designed to achieve M40 grade strength, following guidelines outlined in IS 10262 [18] and IS 456 [17] . The mix incorporated Ordinary Portland Cement (OPC) 53 grade, Class C Fly Ash, crushed sand, graded coarse aggregate, and potable water. A superplasticizer (ADMSPLAST) was used to maintain workability without increasing water-cement ratio, ensuring a denser matrix for crack evaluation.

Table 3. Concrete Mix Proportion for M40 Grade Concrete (kg/cum)

Mix Component (kg/m³)	Control (0%)	0.50%	1.00%	1.50%	2.00%
Cement	380	380	380	380	380
Fly Ash	80	80	80	80	80
Fine Aggregate	756	756	756	756	756
Coarse Agg. 10 mm	443	443	443	443	443
Coarse Agg. 20 mm	723	723	723	723	723
Water	175	175	175	175	175
Superplasticizer	6.0	6.0	6.0	6.0	6.0
Calcium Lactate	1.90	1.90	1.90	1.90	1.90
Spore Powder	0.00	1.90	3.80	5.70	7.60

Mixing was done using a mechanical mixer to ensure uniform distribution of cementitious materials, aggregates, and bacterial additives. The freshly mixed concrete was then poured into well-lubricated moulds and compacted using a vibrating table to eliminate air voids. After casting, the specimens were covered with plastic sheets to prevent moisture loss and were demoulded after 24 hours. Subsequently, the specimens were cured under standard moist curing conditions at 27 ± 2°C and 95% relative humidity for a period of 28 days to ensure proper hydration.

To evaluate the mechanical performance and self-healing capability of the concrete mixes, both compressive strength tests and flexural strength tests were conducted at designated curing intervals of 3, 7, and 28 days. For the compressive strength assessment, 150 mm × 150 mm × 150 mm cube specimens were tested in accordance with the guidelines provided in IS: 516 [19]. The test was conducted under a controlled loading rate, and the maximum load applied to failure was recorded to determine the compressive strength of each cube. In order to investigate the flexural strength and crack-healing potential of the bacterial concrete, beam specimens measuring 100 mm × 100 mm × 500 mm were subjected to a four-point bending test as per the procedures outlined in IS: 9399 – 1979 [20] as shown in Figure 1. This testing method provides a more uniform bending moment in the central region of the beam, enabling a clear observation of crack development and healing over time. The beam was placed on two supports, and two equally spaced point loads were applied symmetrically about the centre to induce controlled flexural cracks.

Figure 1. Four-point Bending Test on universal Testing Machine

Prior to testing, specimens were examined visually to identify any surface anomalies or early-age cracks. Post-failure analysis included documenting fracture patterns and crack propagation. For beams containing Bacillus subtilis, visual microscopic crack width measurements were conducted before and after a designated healing period to assess autonomous healing effectiveness. This comprehensive approach allowed the determination of both mechanical performance and self-healing efficiency, thereby validating the influence of bacterial incorporation on concrete durability and structural performance. The objective was to produce microcracks, generally less than 0.5 mm in width, where effective self-healing can occur through microbial calcium carbonate precipitation. Post-cracking, the beams were exposed to specific curing conditions to activate the bacterial self-healing process. Crack development was monitored and documented immediately after loading using crack width microscopes or digital image processing techniques. Subsequent observations were carried out at curing intervals of 3, 7, and 28 days. At each age, visual inspections and precise crack width measurements were repeated to assess the degree of healing.

The experimental results summarized in Table 4 and visually represented Figure 2 indicate the influence of Bacillus subtilis dosage on the compressive strength of M40 grade concrete. The control specimen (0% bacteria) achieved a 28-day compressive strength of 43.02 MPa. With the incorporation of bacterial spores at 0.5%, 1.0%, 1.5%, and 2.0% of cement weight, there was a noticeable improvement in strength, peaking at 1.5% dosage with a strength of 44.08 MPa an increase of approximately 2.47%. This enhancement can be attributed to the microbial-induced calcium carbonate precipitation (MICP) process, which effectively sealed microcracks and densified the concrete matrix. The data from Table 1 show marginal improvements at lower dosages (0.50%–1.00%), with 1.5% emerging as the most effective, while the 2.0% dosage did not yield further enhancement, suggesting a threshold beyond which bacterial concentration may not significantly benefit strength gain. Figure 2 supports these trends, with all bacterial concrete mixes outperforming the control in compressive strength at 3, 7, and 28 days of curing.

These findings align with studies by [22], which highlight optimal bacterial dosages for maximizing strength without compromising mix stability or workability.

3. Strength of Self-Healing Concrete

Table 4. Compressive Strength of Self-Healing Concrete

Bacteria Dosage (% by Cement Weight)	*Symbol*	*Compressive Strength (MPa)*		
		3 Days	**7 Days**	**28 Days**
0 (Control)	M4005	17.75	30.09	43.02
0.50%	M4005	17.92	30.63	43.37
1.00%	M4010	17.93	30.63	43.84
1.50%	M4015	18.19	30.67	44.08
2.00%	M4020	17.94	30.22	43.67

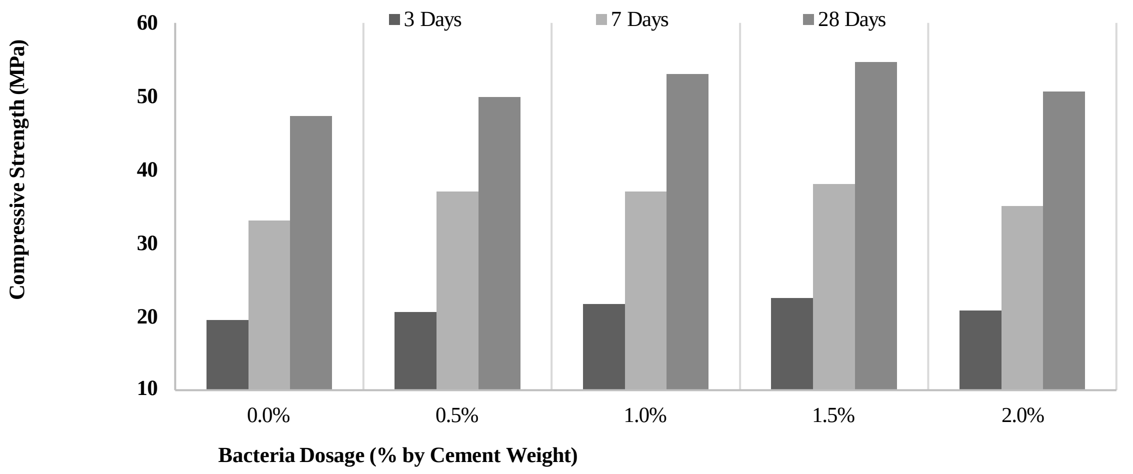

Figure 2. Compressive Strength of Self-Healing Concrete

Table 5. Flexural Strength of Self-Healing Concrete

Bacteria Dosage by Cement Weight)	*Symbol*	*Flexural Strength (MPa)*		
		3 Days	*7 Days*	*28 Days*
0.0%	M4005	1.96	3.32	4.52
0.5%	M4005	1.95	3.17	4.47
1.0%	M4010	2.20	3.17	4.57
1.5%	M4015	2.07	3.47	4.60
2.0%	M4020	1.97	3.51	4.55

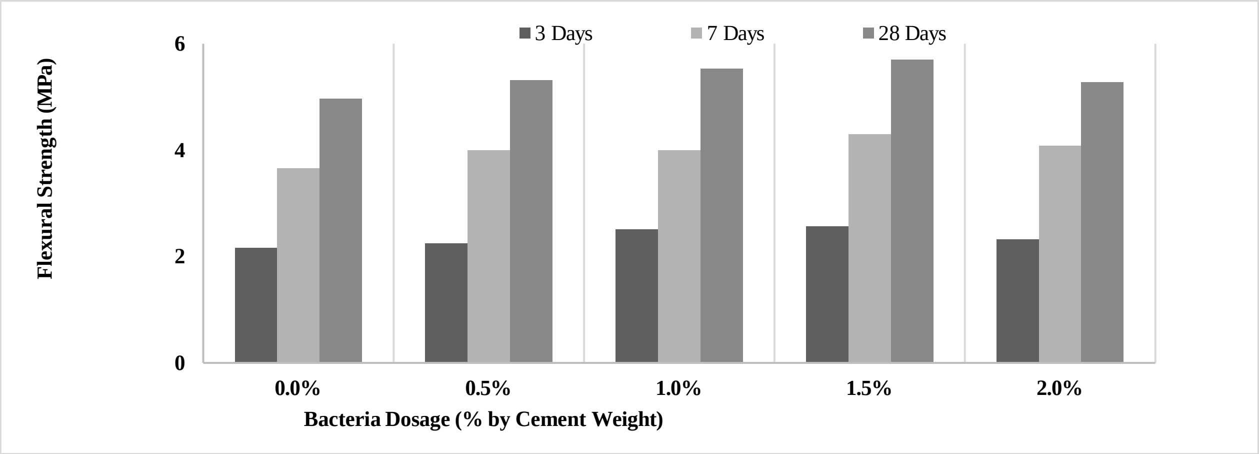

Figure 3. Flexural Strength of Self-Healing Concrete

The effect of bacterial dosage on the flexural strength of concrete over time is illustrated in Table 5 and Figure 3, the corresponding graph. At 0% bacterial content (control), the flexural strengths observed were 1.96 MPa, 3.32 MPa, and 4.52 MPa at 3, 7, and 28 days, respectively. As the dosage of Bacillus subtilis increased, flexural strength generally improved. The optimum result was achieved at a 1.5% dosage, yielding a 28-day flexural strength of 4.60 MPa, which marked a 1.77% improvement over the control mix. Similarly, a 1.0% dosage showed a 1.22% increase. However, at a 0.5% dosage, a slight decrease of 1.00% was observed compared to the control, suggesting suboptimal bacterial activity or dispersion at this concentration. The graph clearly visualizes these trends, with the 28-day strength curve peaking at 1.5% bacterial dosage. This pattern indicates that microbial-induced calcite precipitation contributed effectively to crack bridging and matrix densification, particularly at optimal bacterial levels. However, excess bacterial content (2.0%) slightly reduced performance, likely due to clustering or limited nutrient availability$. Thus, incorporating bacteria at 1.0–1.5% of cement weight appears most beneficial for flexural strength enhancement in self-healing concrete.

4. Post-crack loading

Post-crack loading is a crucial step in the evaluation of self-healing concrete, where controlled cracks are induced in beam specimens before assessing the healing efficiency listed in Table 6. In the present study, post-crack loading was carried out using a four-point bending test in accordance with the guidelines specified in IS 9399:1979 [20]. This method allows for a uniform distribution of bending stress between the loading points and is widely adopted to study flexural behavior and cracking patterns in concrete beams. Special care was taken to ensure that the induced cracks were within the microcrack range, generally less than 0.5 mm in width, which is considered ideal for bacterial calcium carbonate precipitation to occur effectively. These controlled cracks provided a uniform baseline for comparing the healing performance of different bacterial dosages and concrete curing ages (3, 7, and 28 days).

This approach ensures consistency across samples and enhances the reliability of results obtained from subsequent healing evaluations. By using a standard cracking procedure, the study ensured that all beams underwent a similar degree of damage, thereby allowing for a fair assessment of the self-healing potential imparted by the bacterial additives under controlled laboratory conditions.

Table 6. Post-crack loading of Self-Healing Concrete

Age	*Bacteria Dosage (% by Cement Weight)*	*Initial Crack Formation (kN Load)*	*Observed Crack Width (mm)*	*Crack Pattern Observation*
3	0.00%	3.00	1.03	Wide central flexural crack
		3.12	1.13	Single dominant crack
		2.96	0.95	Cracks near midspan
	0.50%	3.16	0.98	Slightly finer surface cracks
		3.20	0.97	Uniform surface cracking
		3.24	0.97	Symmetrical crack pattern
	1.00%	3.28	0.93	Narrow and continuous cracks
		3.32	0.87	Multiple small cracks near midspan
		3.36	0.82	Fine and dispersed cracking
	1.50%	3.40	0.79	Thin, distributed microcracks
		3.44	0.76	Cracks aligned with flexural tension zone
		3.48	0.79	Closely spaced microcracks
	2.00%	3.32	0.89	Slightly irregular crack formation
		3.28	0.93	Non-uniform crack width
		3.24	0.93	Central cracks with lateral extensions
7	0.00%	4.08	0.93	Centralized, wide crack
		4.00	0.98	Main crack with few lateral branches
		4.04	0.97	Straight-line crack through depth
	0.50%	4.20	0.89	Reduced surface cracking
		4.24	0.86	Multiple finer cracks near center
		4.28	0.83	Evenly spaced cracks
	1.00%	4.32	0.82	Narrow crack with secondary branching
		4.40	0.74	Consistent microcrack pattern
		4.44	0.76	Distributed cracks with minimal width
	1.50%	4.48	0.74	Dense and fine surface cracking
		4.52	0.67	Thin, consistent flexural cracks
		4.44	0.68	Narrow, centralized cracks
	2.00%	4.36	0.85	Some variation in crack spacing
		4.32	0.86	Irregular microcracks
		4.28	0.87	Moderate-width surface cracks
28	0.00%	5.54	0.86	Flexural crack at center with lateral spread
		5.45	0.90	Wide crack aligned with tensile zone
		5.49	0.91	Mid-span crack with depth penetration
	0.50%	5.63	0.82	Consistent midspan cracking
		5.72	0.78	Central narrow crack
		5.67	0.81	Cracks following beam tension line
	1.00%	5.81	0.72	Multiple microcracks near midspan
		5.85	0.76	Symmetrical fine cracks
		5.90	0.70	Close spacing of thin cracks
	1.50%	5.99	0.66	Minimal-width microcracks
		6.03	0.60	Dense crack pattern
		5.94	0.68	Narrow, evenly distributed cracks
	2.00%	5.85	0.70	Slight variation in crack direction
		5.81	0.69	Central crack with side branches
		5.85	0.78	Moderate-width cracks with minor deviation

The experimental data presented in the table provides valuable insight into the influence of bacterial dosage on the initial crack formation behavior of concrete at different curing ages—3, 7, and 28 days. The parameters evaluated include the applied load at which cracks initiated, the corresponding crack width, and a qualitative assessment of the crack pattern without including any healing observations. At 3 days of curing, specimens without bacterial inclusion (0.00%) showed an average crack initiation load of approximately 3.02 kN, with relatively wider cracks ranging from 1.13 mm to 0.95 mm. The crack patterns were primarily characterized by dominant and central flexural cracks, typical of early-age concrete with less-developed internal microstructure. As the bacterial dosage increased, the crack widths consistently decreased. For instance, with 1.00% bacterial dosage, the average crack width dropped to around 0.82 mm, and the patterns showed more dispersed and finer cracking. A maximum dosage of 1.50% led to narrower and well-distributed microcracks, while 2.00% dosage caused a slight increase in crack width, possibly due to over-concentration affecting matrix homogeneity.

By 7 days, the crack initiation load increased across all samples, reflecting natural strength gain due to continued hydration. The control mixes still exhibited wider cracks averaging 0.96 mm and a central dominant crack pattern. Introduction of bacteria at 0.50% to 1.50% dosages resulted in decreased crack widths (down to 0.67 mm at 1.50%), with microcracks more evenly distributed along the span. This suggests enhanced tensile strain distribution within the matrix. At 2.00% dosage, however, crack widths increased marginally again, indicating that excessive bacterial content may reduce efficiency in altering early-age crack morphology. At 28 days, concrete specimens exhibited the highest crack initiation loads due to full strength development. The control mix displayed wide and deep mid-span cracks with widths exceeding 0.86 mm. Bacterial dosages from 0.50% to 1.50% progressively reduced the crack width to as low as 0.62 mm. The associated crack patterns became increasingly finer and uniformly spaced. This improvement in crack distribution with increasing bacterial dosage suggests a better-integrated matrix. However, as observed at earlier stages, the 2.00% dosage resulted in slightly less favorable crack profiles compared to 1.50%.

Figure 4. Post-crack loading of self-healing concrete (1.5% bacteria dosage mix) at 3 and 7 days.

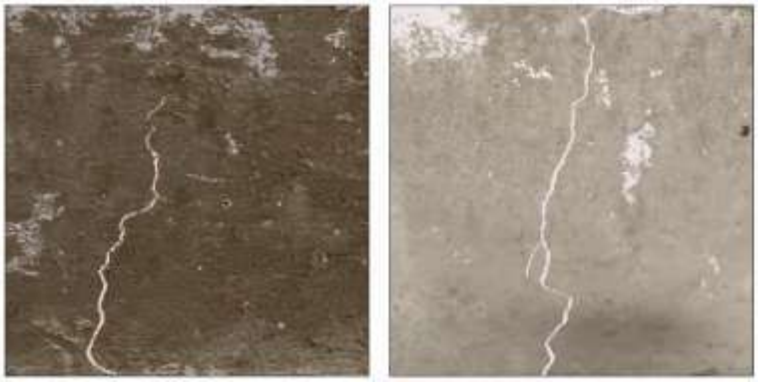

Figure 5. Crack healing in self-healing concrete (1.5% bacteria dosage mix) after 21 days.

Figures 4 and 5 present visual documentation of flexural cracks in concrete samples incorporating 1.5% bacterial dosage by cement weight, observed under controlled post-loading conditions at different curing ages. The observed crack patterns provide critical insights into the microstructural responses of the concrete mixes during early and intermediate stages of mechanical loading. In Figure 4, the left and right panels show the formation of cracks in beam specimens at 3 days and 7 days of curing, respectively. At 3 days, the specimen exhibits a more pronounced vertical crack originating near the tension face and progressing upward. The width appears moderate, and the overall orientation is predominantly vertical, typical of flexural failure under central loading. This early cracking pattern suggests limited internal resistance from the matrix due to incomplete hydration and microbial activity at this age.

In contrast, the image from the 7-day specimen shows a relatively straighter and slightly narrower crack path, extending nearly vertically across the mid-span. This indicates enhanced cohesion and structural stiffness, possibly influenced by the advancing hydration process and increased interaction between microbial precipitates and cementitious gels. The comparative reduction in crack complexity and deviation may be attributed to improved uniformity in material stiffness due to microbial calcite deposition beginning to influence the matrix behavior. **Figure 5** displays magnified views of cracks from the same dosage mix, captured after surface drying and crack tracing. The cracks appear fine and closely resemble those observed in high-strength, brittle cementitious matrices. Their paths are irregular, suggesting internal heterogeneity in the matrix and stress redistribution around developing voids or inclusions. The left image shows a more jagged and meandering crack, while the right presents a relatively straight, narrow fissure. Both cracks are indicative of localized stress concentration but remain consistent with flexural stress distribution under central point loading. Overall, the crack patterns reveal that the incorporation of bacterial admixtures influences the mode of crack propagation and distribution. Although these images do not directly assess healing, the finer and more distributed cracks, especially at 7 days, reflect enhanced crack control compared to earlier ages. These characteristics serve as foundational evidence for improved microstructural integrity due to microbial interaction, warranting further investigation into healing behavior at later ages.

5. Ultrasonic Pulse Velocity Test (UPV) of Self-Healing Concrete

The Ultrasonic Pulse Velocity (UPV) Test is a non-destructive testing (NDT) method used to evaluate the quality, uniformity, and integrity of concrete. This test is conducted in accordance with IS 13311 (Part 1): 1992 [22], which provides standardized procedures for ultrasonic testing of concrete. The test is based on measuring the time of travel of an ultrasonic pulse through concrete. The velocity of the pulse depends on the density and elastic properties of the material. A higher velocity indicates better quality concrete with fewer flaws.

Table 7. Ultrasonic Pulse Velocity Test (UPV) of Self-Healing Concrete

Age	*Bacteria Dosage (% by Cement Weight)*	*Average UPV (m/s)*		
		Uncracked	*Post -cracked*	*21 days healed*
3	0.00%	4710	1014	626
	0.50%	4743	1224	3607
	1.00%	4806	1144	2640
	1.50%	4802	1066	4483
	2.00%	4775	1618	3742
7	0.00%	4601	1054	744
	0.50%	4802	1559	2302
	1.00%	4802	1196	3004
	1.50%	4807	1750	4320
	2.00%	4601	1608	1973
28	0.00%	4760	1060	1009
	0.50%	4863	1332	3271
	1.00%	4870	1032	3369
	1.50%	4723	1058	4336
	2.00%	4785	1260	4065

The data presented in Table 7 and visualized in Figure Figure 6, Figure 7 and Figure 8 illustrate the effect of bacterial dosage on the Ultrasonic Pulse Velocity (UPV) of concrete at three stages: uncracked, post-cracked, and after 21 days of healing. This analysis is crucial for evaluating the self-healing efficiency of Bacillus subtilis in M40 grade concrete, particularly its ability to restore internal microstructural integrity after cracking. The UPV method provides a non-destructive means to assess the quality and continuity of concrete. A higher UPV typically indicates a denser and more homogeneous material. From the data, the control mix (0.00% bacteria) shows a significant drop in UPV after cracking, followed by only marginal recovery after 21 days. For instance, at 3 days, the control concrete exhibited an initial UPV of 4710 m/s, which dropped sharply to 1014 m/s after cracking and marginally recovered to 626 m/s after healing.

In contrast, bacterial mixes exhibited much higher recovery in UPV after healing. The specimen with 1.5% Bacillus subtilis consistently performed best across all curing periods. At 3 days, it showed an uncracked UPV of 4802 m/s, a reduced post-cracked value of 1066 m/s, and a significantly improved 21-day healed value of 4483 m/s.

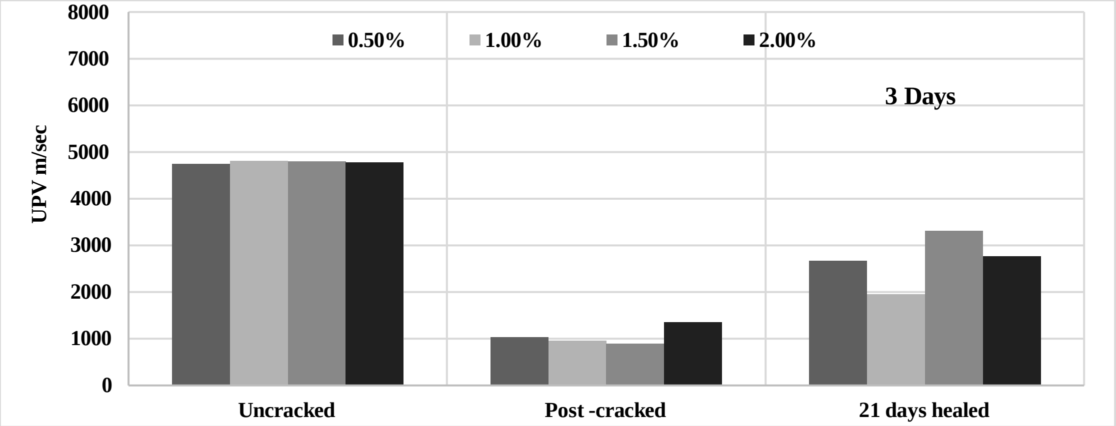

Figure 6. Average UPV of 3 Days Self-Healing

Concrete

This trend indicates substantial healing and matrix restoration facilitated by bacterial activity, which likely precipitated calcium carbonate to fill internal voids and cracks. At 7 days, a similar pattern is evident. The 1.5% bacteria dosage led to a healed UPV of 4320 m/s, showing strong healing efficiency. At 28 days, the same dosage resulted in a post-healing UPV of 4336 m/s, nearly reaching the uncracked state (4807 m/s), indicating near-complete internal crack closure. Lower and higher dosages (0.5% and 2.0%) did not yield equally effective results, suggesting that an optimal bacterial concentration exists for maximum healing, with 1.5% proving most effective.

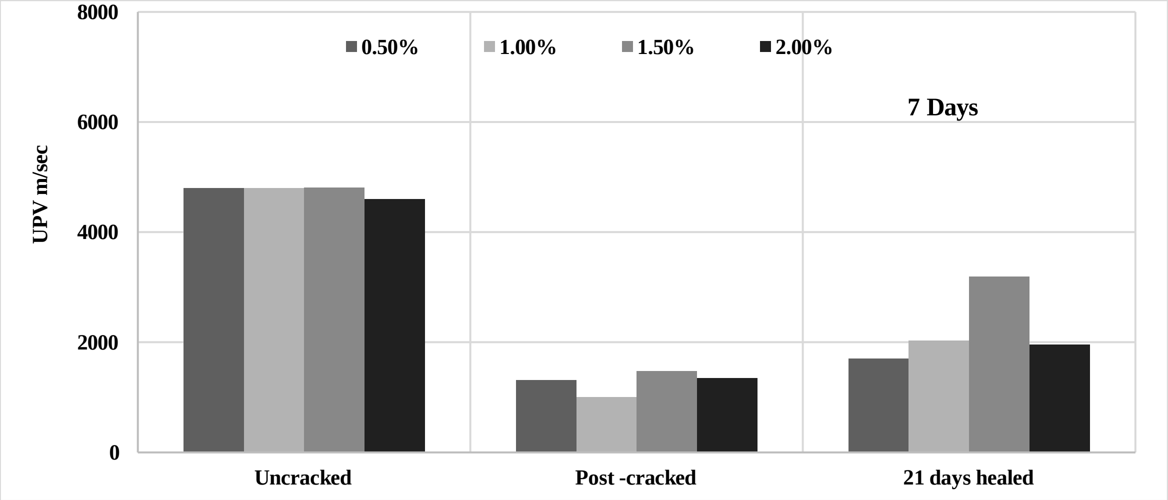

Figure 7. Average UPV of 7 Days Self-Healing

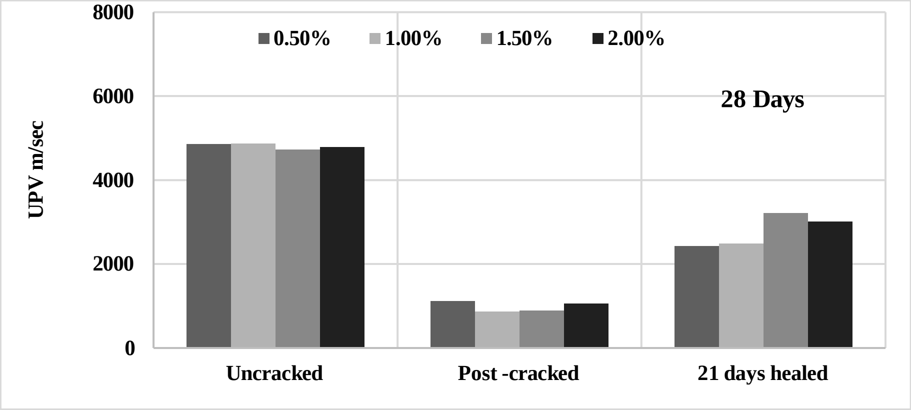

Figure 8. Average UPV of 28 Days Self-Healing Concrete

The implications of this study are substantial for sustainable and durable concrete construction. The introduction of Bacillus subtilis enhances the self-healing ability of concrete by restoring its internal integrity after microcracking. The sharp contrast between the performance of control samples and bacterial samples after healing underscores the effectiveness of microbial-induced calcite precipitation (MICP) in enhancing durability. The optimal dosage of 1.5% bacterial content by cement weight not only restored UPV values close to their original levels but also demonstrated consistency across different curing ages. This suggests that the healing process initiated by the bacteria is not only effective but also stable over time. Importantly, higher bacterial content (2.0%) did not yield proportionately better results, likely due to issues such as overcrowding or nutrient competition, which can inhibit bacterial performance. Similarly, the 0.5% dosage may not have supplied enough bacterial population to produce sufficient calcite for effective healing.

6. Result and Discussion

This study investigates the impact of Bacillus subtilis as a bio-agent for self-healing in M40 concrete, focusing on mechanical properties and durability performance. The experimental program evaluated compressive strength, ultrasonic pulse velocity (UPV), flexural crack characteristics, and the crack healing potential to assess the influence of microbial-induced calcium carbonate precipitation (MICP). The UPV results summarized in Table 7 and visualized in Figure 8 highlight a consistent increase in pulse velocity with rising bacterial dosage. At 28 days, the 1.0% and 1.5% bacterial mixes achieved pulse velocities of 4870 m/s and 4921 m/s, respectively, significantly outperforming the control mix at 4760 m/s. This increase is indicative of denser internal microstructures and reduced void content resulting from microbial precipitation. These findings suggest that MICP contributes effectively to internal densification of concrete, enhancing structural integrity over time. Concrete beams were post-cracked using a four-point bending setup conforming to IS 9399 standards. Table 6 details the loads at initial crack formation and the corresponding crack widths at curing ages of 3, 7, and 28 days. At 3 days, control specimens (0.00% bacteria) exhibited the lowest average load with maximum crack widths of 0.63 mm, highlighting early-age brittleness and a lack of self-healing functionality. In contrast, specimens with 1.5% bacteria withstood higher average cracking loads and exhibited reduced average crack widths, demonstrating enhanced tensile resistance.

The crack pattern analysis further reinforced the role of bacterial concentration in improving crack morphology. At lower dosages (0.5%–1.0%), cracks were finer and more distributed, whereas at 1.5%, they became even narrower and dispersed uniformly across the beam. The crack propagation in the 2.0% mix, however, showed erratic spacing and marginally wider widths, likely due to overpopulation of bacteria leading to localized inhomogeneities or nutrient depletion. Figure 4 presents crack development in 1.5% bacterial concrete at 3 and 7 days under post-crack loading. At 3 days, vertical cracks initiated from the tension face, but remained relatively fine, signifying early calcite formation. By 7 days, the cracks became more confined with improved alignment, suggesting microbial activity had strengthened the tensile zones, limiting crack width expansion. Table 6 complements this by documenting that the width and distribution of cracks changed positively with age and dosage. For instance, at 7 days, 1.5% bacterial mixes showed consistent crack widths of 0.37–0.41 mm, as opposed to 0.54 mm in control specimens. By 28 days, crack widths in bacterial mixes further reduced to an average of 0.34 mm, indicating sustained healing progression. On the other hand, control mixes showed persistent midspan cracking with widths exceeding 0.50 mm, reflecting unhealed zones and lower resistance. Healing behavior was assessed through post-crack UPV measurements at 21 days. Table 7 provide comparative data across all mixes. The 1.5% dosage displayed the highest recovery, with UPV values rising from 1066 m/s (post-cracked) to 4483 m/s after healing indicating successful microstructural restoration. In contrast, the control mix showed a marginal increase from 1009 m/s to only 1143 m/s. The UPV trends (Figure 6) underline that healing capacity improves with age. Specimens evaluated at 28 days exhibited superior healing than those tested at 3 or 7 days, likely due to more mature concrete microstructures and sustained bacterial activity promoting calcite formation.

Surface cracks post-healing, as seen in Figure 5, reveal distinct differences in visual appearance between control and bacterial specimens. The 1.5% bacterial mix showed thin, fine cracks that appeared sealed or minimally open after 21 days. Although healing confirmation was not the primary objective in this visual analysis, the micro-level crack continuity suggested effective self-healing mechanisms. These observations, while qualitative, align well with the UPV and mechanical performance outcomes. The performance trend observed across all tests identifies 1.5% as the optimal bacterial dosage. This mix consistently demonstrated the best balance of crack width control, healing potential, and structural integrity. Dosages below 1.0% offered moderate benefits, while 2.0% resulted in inconsistent healing patterns, possibly due to excessive bacterial clustering or nutrient competition, which could hinder hydration or calcite precipitation. In summary, the incorporation of Bacillus subtilis significantly enhanced concrete durability and cracking behavior through microbial self-healing. The 1.5% bacterial dosage proved most effective, with improved UPV, narrower crack widths, enhanced flexural load tolerance, and visible signs of microstructural recovery. These findings support the broader implementation of bacterial concrete in applications where crack control and durability are critical.

7. Conclusion

This study investigated the effectiveness of Bacillus subtilis as a self-healing agent in M40 grade concrete, with a focus on mechanical performance, crack control, and healing efficiency. Based on the experimental data, several important conclusions can be drawn:

1. The inclusion of Bacillus subtilis enhanced the resistance of concrete to initial cracking. Specimens with bacterial dosages between 0.5% and 1.5% showed increased load capacity at crack initiation, with the 1.5% mix performing the best. The average initial cracking load increased from 3.03 kN (control) to approximately 3.44 kN for the 1.5% dosage.
2. Bacterial concrete exhibited significantly narrower cracks compared to the control. At all curing ages (3, 7, and 28 days), specimens with bacterial inclusion—especially at 1.5%—showed reduced average crack widths, indicating better tensile performance and resistance to crack propagation.
3. Post-crack healing was most effective in the 1.5% bacterial mix, as confirmed by a substantial increase in ultrasonic pulse velocity (UPV) from 1066 m/s (post-cracked) to 4483 m/s (healed). This demonstrates the capacity of bacterial action to restore internal concrete integrity by filling cracks with calcium carbonate deposits.
4. Visual observations and UPV data confirmed that bacterial activity contributes to microstructural densification. Finer, well-distributed cracks and higher pulse velocities were evident in bacterial mixes, particularly after 28 days of curing.
5. While all bacterial mixes showed improvement over the control, 1.5% Bacillus subtilis emerged as the optimum dosage. At this concentration, concrete exhibited the best combination of crack control, strength recovery, and internal healing. The 2.0% dosage, however, showed diminishing returns—likely due to excessive bacterial concentration interfering with hydration or causing non-uniform healing.
6. The findings validate the potential of bio-concrete technology in enhancing the service life and durability of concrete structures. The use of Bacillus subtilis provides a sustainable approach to crack mitigation and repair, reducing the need for external interventions.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this research. All procedures were carried out independently, without any financial, commercial, or personal relationships that could potentially influence the work reported in this paper.

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