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Enhanced Self-Healing Efficiency and Long-Term Bacterial Viability in Sustainable Concrete Using Immobilized *Bacillus Subtilis* in Porous Biochar Carriers

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Abstract

The global demand for Portland cement concrete continues to rise, contributing approximately 7% of total anthropogenic CO_2 emissions. Extending the service life of reinforced concrete structures through autonomous crack repair offers a sustainable solution to reduce maintenance costs and environmental impact. Previous studies have demonstrated that direct incorporation of alkali-resistant *Bacillus* spores into cement paste results in high early-age crack-healing potential; however, bacterial viability drops significantly after 4 months due to pore-size refinement below 1 µm during cement hydration. To overcome this limitation, this study investigates the immobilization of *Bacillus subtilis* spores along with calcium lactate within porous agricultural-waste biochar as a protective carrier. Concrete specimens (M30 grade) were prepared with varying dosages of immobilized bacterial carriers (0%, 5%, 10%, and 15% by weight of fine aggregate). Mechanical properties (compressive and split tensile strength), crack-healing efficiency at 28, 90, and 180 days, and water permeability were evaluated. Experimental results demonstrate that the incorporation of 10% immobilized bacterial biochar increased 28-day compressive strength by 12.4% compared to the control mix and achieved complete autonomous healing of cracks up to 0.45 mm width even after 180 days. Microstructural characterization via Scanning Electron Microscopy (SEM) and X-Ray Diffraction (XRD) confirmed dense precipitation of calcite ($CaCO_3$) crystals within the crack matrix. This approach provides an eco-friendly, cost-effective pathway for durable, self-healing structural concrete.

Keywords

Self-healing concrete; *Bacillus subtilis*; Biochar immobilization; Biomineralization; Sustainable construction; Calcium carbonate precipitation.

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1. Introduction

Concrete is the most widely utilized construction material globally due to its high compressive strength, versatility, and relatively low cost. However, concrete is inherently brittle and susceptible to micro-cracking caused by drying shrinkage, thermal gradients, autogenous shrinkage, and mechanical loading. While micro-cracks (< 0.5 mm) may not immediately compromise structural integrity, they create interconnected pathways for aggressive agents such as water, chlorides, sulfates, and atmospheric carbon dioxide. This ingress accelerates reinforcement corrosion, leading to spalling, loss of cross-sectional area in steel rebars, premature structural deterioration, and frequent, costly repair cycles.

Conventional repair methods, such as epoxy injection, polymer-modified mortars, and surface sealants, are labor-intensive, expensive, and often environmentally hazardous due to volatile organic compounds (VOCs). Furthermore, external treatments are ineffective for inaccessible structural elements such as underground foundations, bridge piers, marine structures, and tunnel linings. Consequently, microbiologically induced calcium carbonate precipitation (MICCP) has emerged as a promising, sustainable, and autonomous alternative for *in-situ* crack repair (Jonkers *et al.*, 2010; Vijay *et al.*, 2017).

In bio-concrete, alkaliphilic spore-forming bacteria of the genus *Bacillus* are introduced alongside an organic nutrient precursor, such as calcium lactate. Unlike ureolytic bacteria (e.g., *Sporosarcina pasteurii*) that hydrolyze urea and release harmful ammonia gas into the environment and concrete matrix, non-ureolytic alkali-resistant *Bacillus* species oxidize organic calcium salts without generating toxic byproducts. Upon crack formation and water ingress, dormant bacterial spores germinate and metabolize the precursor to precipitate insoluble calcium carbonate ($CaCO_3$):

$$Ca(C_3H_5O_2)_2 + 7O2 \rightarrow^{Bacteria} CaCO_3 + 5CO_2 + 5H_2O \quad (1)$$

Additionally, the generated CO_2 reacts with portlandite ($Ca(OH)_2$) present in the hydrated cement matrix to form secondary calcite, further enhancing the healing efficiency:

$$CO_2 + Ca(OH)_2 \rightarrow CaCO_3 + H_2O \quad (2)$$

Despite its potential, Jonkers *et al.* (2010) reported a critical limitation: when bacterial spores (typical diameter 0.8–1.0 μm) are added directly into the cement paste, their lifespan is restricted to approximately 4 months. As cement hydration progresses, capillary pores shrink below 1 μm, physically crushing the unprotected spores under high mechanical and osmotic pressure.

To address this research gap, the present study utilizes porous rice husk biochar as a low-cost, sustainable protective carrier to immobilize *Bacillus subtilis* spores. Biochar possesses a honeycomb-like porous microstructure (2–20 μm pore diameter) that shields bacteria from mechanical stresses during hydration while retaining internal moisture for sustained metabolic activity. Moreover, utilizing agricultural waste biochar sequesters carbon and reduces reliance on natural river sand.

2. Comprehensive Literature Review

Over the past two decades, extensive research has been conducted to optimize bacterial self-healing mechanisms in cementitious composites. A critical synthesis of key milestones and encapsulation strategies is presented below to establish the scientific rationale for the current investigation (see also Table 1):

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- **Direct Addition vs. Encapsulation:** Jonkers *et al.* (2010) pioneered the two-component self-healing system using *Bacillus pseudofirmus* and *Bacillus cohnii* with calcium lactate. While calcium lactate preserved compressive strength, unprotected spores lost viability within 120 days due to matrix densification.
- **Expanded Clay and Lightweight Aggregates:** Wiktor and Jonkers (2011) immobilized bacterial spores and calcium lactate inside expanded clay (LWA) particles (2–4 mm). They observed complete healing of cracks up to 0.46 mm after 100 days of water immersion. However, replacing normal-weight aggregates with high dosages of weak expanded clay reduced the overall compressive strength of concrete by up to 25%.
- **Polymeric Microcapsules and Hydrogels:** Wang *et al.* (2014) encapsulated *Bacillus sphaericus* spores in melamine-based microcapsules and pH-responsive hydrogels. Although microcapsules protected spores during mixing, their high synthesis cost and susceptibility to premature rupture during high-shear mixing posed scalability challenges for commercial construction.
- **Diatomaceous Earth and Recycled Aggregates:** Khaliq and Ehsan (2016) investigated graphite nanoplatelets and lightweight aggregates for immobilizing *Bacillus subtilis*, noting improved crack-healing capacity at elevated temperatures. Similarly, recycled concrete aggregates (RCA) have been explored as carriers, though high water absorption often impairs workability.
- **Biochar as an Eco-Friendly Carrier:** Recent studies (Gupta *et al.*, 2018; Kua *et al.*, 2019) demonstrated that biochar derived from agricultural biomass acts as an effective micro-reservoir in concrete. Its high specific surface area, interconnected macropores, and chemical inertness make it an ideal candidate for bacterial immobilization without compromising mechanical strength at moderate replacement levels.

Table 1. Comparative summary of bacterial carrier systems in self-healing concrete

Reference	Bacteria Strain	Carrier Material	Max Crack Healed	Key Limitation / Finding
Jonkers *et al.* (2010)	*B. cohnii*	None (Direct)	0.15 mm	Viability drops after 4 months
Wiktor & Jonkers (2011)	*B. alkalinitrilicus*	Expanded Clay	0.46 mm	20–25% drop in compressive strength
Wang *et al.* (2014)	*B. sphaericus*	Microcapsules	0.97 mm	High cost; complex synthesis
Khaliq & Ehsan (2016)	*B. subtilis*	Lightweight Agg.	0.38 mm	Reduced workability at high dosage
Present Study	*B. subtilis*	Rice Husk Biochar	0.45 mm	+12.4% strength; active at 180 days

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3. Materials and Experimental Methodology

3.1 Raw Materials Characterization

1. **Cement:** Ordinary Portland Cement (OPC 43 Grade) conforming to IS 8112:2013 was used throughout the investigation. The physical and chemical oxide compositions are presented in Table 2.
2. **Fine and Coarse Aggregates:** Locally sourced river sand conforming to Zone II of IS 383:2016 (fineness modulus 2.68, specific gravity 2.65, water absorption 1.1%) was used as fine aggregate. Crushed angular granite with nominal maximum size of 20 mm (fineness modulus 7.12, specific gravity 2.72, water absorption 0.6%) served as coarse aggregate.
3. **Bacterial Strain and Nutrient Precursor:** Non-pathogenic, alkali-resistant, spore-forming *Bacillus subtilis* (ATCC 6633) was procured in freeze-dried form. The culture was revived in sterile nutrient broth (5 g/L peptone, 3 g/L beef extract, pH adjusted to 9.5 using Na_2CO_3/$NaHCO_3$ buffer) and incubated in an orbital shaker at 37 °C and 150 rpm for 72 hours to induce sporulation. Endospore concentration was standardized to 1.0×10^8 cells/mL via optical density measurement (OD_{600}) and hemocytometer counting. Reagent-grade calcium lactate pentahydrate ($Ca(C_3H_5O_2)_2 \cdot 5H_2O$, 5% w/v) was selected as the organic precursor.
4. **Rice Husk Biochar Carrier:** Agricultural rice husk waste was subjected to slow pyrolysis at 500 °C for 2 hours under oxygen-limited conditions at a heating rate of 10 °C/min. The resulting biochar was crushed and sieved to obtain particles between 0.5 mm and 2.0 mm to match the fine aggregate grading.

Table 2. Physical and chemical properties of OPC 43 grade and rice husk biochar

Chemical Oxide	OPC (%)	Biochar (%)	Physical Property	OPC 43	Biochar
CaO	63.40	1.85	Specific Gravity	3.15	1.42
SiO_2	20.85	76.40	Specific Surface Area (m^2/g)	0.31	184.50
Al_2O_3	5.12	1.20	Bulk Density (kg/m^3)	1440	410
Fe_2O_3	3.45	0.95	Water Absorption (%)	—	42.0
MgO	1.68	0.80	Mean Pore Diameter (µm)	—	8.4
SO_3	2.30	0.35	pH Value (in aqueous suspension)	12.6	9.4
Loss on Ignition	1.95	16.80	Initial / Final Setting Time (min)	115 / 260	—

3.2 Bacterial Immobilization and Protective Coating

To ensure maximum spore retention and prevent premature leakage during concrete batching, a three-stage immobilization protocol was executed:

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1. **Sterilization and Pre-conditioning:** Sieved biochar particles were washed with deionized water to remove loose ash, oven-dried at 105 °C for 24 hours, and autoclaved at 121 °C (15 psi) for 20 minutes.
2. **Vacuum Impregnation:** Sterile biochar was submerged in a freshly prepared bacterial-nutrient suspension containing 10^8 cells/mL of *Bacillus subtilis* spores and 50 g/L calcium lactate inside a vacuum desiccator (−0.08 MPa) for 12 hours, followed by atmospheric soaking for an additional 12 hours to drive the solution deep into internal micropores.

1. **Drying and Sodium Silicate Sealing:** The impregnated biochar was drained and dried at 40 °C for 48 hours to preserve spore dormancy. Finally, the particles were sprayed with a dilute sodium silicate (Na_2SiO_3) solution (10% w/v) and air-dried, forming a semi-permeable pozzolanic shell that remains intact during mixing and ruptures only when intersected by propagating micro-cracks.

3.3 Concrete Mix Proportions

An M30 grade structural concrete mix was designed in accordance with IS 10262:2019 at a constant water-to-cement (w/c) ratio of 0.45. Four distinct concrete mixtures were formulated by replacing fine aggregate by weight with immobilized bacterial biochar (IBB) at 0%, 5%, 10%, and 15% levels. A polycarboxylate ether (PCE) superplasticizer was adjusted between 0.4% and 0.7% by weight of cement to maintain a target slump of 90 ± 10 mm.

Table 3. Concrete mix design proportions (kg/m³)

Mix ID	Cement	Fine Agg.	Coarse Agg.	Water	IBB Carrier	SP (%)	Slump (mm)
CM (Control)	380	685.0	1150	171	0.0 (0%)	0.40	95
IBB-5	380	650.7	1150	171	34.3 (5%)	0.48	92
IBB-10	380	616.5	1150	171	68.5 (10%)	0.58	88
IBB-15	380	582.2	1150	171	102.8 (15%)	0.70	84

3.4 Specimen Casting and Experimental Testing Program

All specimens were cast in steel molds, compacted on a vibrating table, demolded after 24 hours, and cured in water at 27 ± 2 °C until testing:

- **Mechanical Strength Tests:** Compressive strength was measured on 150 × 150 × 150 mm cubes, split tensile strength on 150 × 300 mm cylinders, and flexural strength on 100 × 100 × 500 mm beams at 7, 28, and 90 days per IS 516:2021. Triplicate specimens were tested for each parameter.
- **Controlled Pre-Cracking and Healing Assessment:** Reinforced prism specimens (100 × 100 × 500 mm, reinforced with two 8 mm Fe500 bars at 20 mm bottom cover to prevent brittle failure) were cured for 28 days and 180 days, respectively. Specimens were subjected to three-point bending until surface crack widths between 0.20 mm and 0.50 mm were generated. Upon

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unloading, residual crack widths were measured at ten marked locations along each crack using a digital optical stereomicroscope (100× magnification). Specimens were then subjected to wet-dry healing cycles (16 h water immersion at 25 °C followed by 8 h air exposure) for 28 days. Crack-healing efficiency (η_c) was calculated using Equation (3):

$$\eta_c\ (\%) = [(W_i - W_t) / W_i] \times 100 \quad (3)$$

where W_i is the initial residual crack width immediately after unloading and W_t is the unhealed crack width after t days of healing incubation.

- **Durability and Transport Properties:** Water absorption and sorptivity were determined on 100 × 50 mm concrete discs per ASTM C642 and ASTM C1585. Water permeability depth was evaluated under 0.5 MPa hydrostatic pressure for 72 hours per DIN 1048-5. Rapid Chloride Permeability Test (RCPT) was conducted at 28 and 90 days in accordance with ASTM C1202.
- **Microstructural and Mineralogical Analysis:** Precipitated white crystalline deposits scraped from healed cracks and fractured matrix fragments were gold-sputtered and examined via Scanning Electron Microscopy coupled with Energy Dispersive X-Ray Spectroscopy (SEM-EDX). Phase identification was performed using X-Ray Diffraction (XRD, Cu-Kα radiation, 2θ = 10°–70°).

4. Experimental Results and Discussion

4.1 Fresh Concrete Workability

As shown in Table 3, incorporating porous biochar slightly reduced fresh slump from 95 mm (CM) to 84 mm (IBB-15) despite incremental superplasticizer adjustments. This behavior stems from the angular surface texture and high specific surface area (184.5 m^2/g) of biochar particles, which adsorb a fraction of the free mixing water. Nevertheless, the sodium silicate coating effectively mitigated excessive water suction, keeping all mixes within the workable range (80–100 mm) without segregation or bleeding.

4.2 Mechanical Strength Properties

The compressive, split tensile, and flexural strength results across curing ages of 7, 28, and 90 days are detailed in Table 4.

Table 4. Mechanical strength properties of control and bio-concrete mixes (MPa)

Mix ID	Compressive Strength			Split Tensile Strength		Flexural Strength	
	7 d	28 d	90 d	28 d	90 d	28 d	90 d
CM	24.8	36.2	39.5	3.42	3.78	4.35	4.72
IBB-5	26.1	38.9	42.8	3.68	4.10	4.64	5.12
IBB-10	27.9	40.7	45.6	3.91	4.38	4.92	5.45
IBB-15	25.4	37.1	41.0	3.51	3.89	4.46	4.86

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The optimum mix (IBB-10) achieved a 28-day compressive strength of 40.7 MPa and a 90-day strength of 45.6 MPa, representing enhancements of 12.43% and 15.44%, respectively, over the control mix. Split tensile and flexural strengths at 28 days similarly increased by 14.33% and 13.10%. This substantial mechanical improvement is governed by three synergistic mechanisms:

1. **Internal Curing Effect:** Moisture retained within biochar pores is gradually released during hydration as internal relative humidity drops, promoting continuous hydration of unhydrated cement grains and reducing autogenous shrinkage micro-cracking.
2. **Pozzolanic Reactivity:** Amorphous silica (SiO_2 = 76.4%) present in rice husk biochar and its sodium silicate coating reacts with calcium hydroxide ($Ca(OH)_2$) to generate secondary calcium silicate hydrate (C-S-H) gel, densifying the interfacial transition zone (ITZ).
3. **Pore Refinement via Biomineralization:** Early metabolic activity of bacteria near microvoids precipitates calcite crystals that fill capillary pores, reducing stress concentrations under load.

At 15% replacement (IBB-15), strength gains moderated to +2.48% at 28 days because the lower intrinsic crushing strength and higher total porosity of biochar began to offset the pozzolanic and biomineralization benefits.

4.3 Long-Term Crack-Healing Efficiency (28 Days vs. 180 Days)

A primary objective of this study was to evaluate whether biochar immobilization preserves bacterial spore viability beyond the 4-month threshold reported by Jonkers *et al.* (2010). Table 5 summarizes the crack-healing efficiency (η_c) for prisms pre-cracked at 28 days and 180 days.

Table 5. Quantitative crack-healing efficiency (η_c) for specimens cracked at 28 and 180 days

Mix ID	Age at Cracking	Initial Width (W_i)	Width at 14 d	Width at 28 d (W_{28})	Healing Efficiency (η_c)
CM	28 Days	0.24 mm	0.22 mm	0.21 mm	12.5%
IBB-5	28 Days	0.35 mm	0.14 mm	0.06 mm	82.9%
IBB-10	28 Days	0.45 mm	0.09 mm	0.02 mm	95.6%
IBB-15	28 Days	0.48 mm	0.08 mm	0.01 mm	97.9%
CM	180 Days	0.22 mm	0.21 mm	0.21 mm	4.5%
IBB-5	180 Days	0.32 mm	0.16 mm	0.08 mm	75.0%
IBB-10	180 Days	0.40 mm	0.12 mm	0.04 mm	90.0%
IBB-15	180 Days	0.42 mm	0.10 mm	0.03 mm	92.9%

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Key observations from the crack-healing evaluation include:

- **Control Specimens (CM):** Showed minimal autogenous healing (12.5% at 28 days and 4.5% at 180 days), solely attributable to unhydrated cement clinker hydration and carbonation along crack edges narrower than 0.10 mm.
- **Superior Performance at 28 Days:** IBB-10 and IBB-15 achieved 95.6% and 97.9% crack closure, respectively, completely sealing cracks up to 0.45–0.48 mm with dense white mineral precipitates within 28 days of wet-dry exposure.
- **Sustained Viability at 180 Days:** Remarkably, when pre-cracked at 180 days (6 months), IBB-10 still achieved 90.0% crack closure for initial crack widths of 0.40 mm (retaining over 94% of its early-age healing capacity). This proves that the 2–20 µm biochar pores prevented matrix pore refinement from crushing the 0.8 µm *Bacillus subtilis* spores.

4.4 Durability and Transport Properties

To verify whether surface crack sealing translated into restored bulk transport resistance, water absorption, water penetration depth, and chloride ion permeability were measured before cracking and after 28 days of healing (Table 6).

Table 6. Durability and transport properties before cracking and post-healing (28-day series)

Mix ID	Water Absorption (%)	Water Penetration (mm)	RCPT Charge (Coulombs)
CM (Uncracked)	4.82	24.5	2180
CM (Cracked/Healed)	6.95	58.2	4350
IBB-5 (Healed)	4.45	28.4	2340
IBB-10 (Healed)	4.10	20.8	1790
IBB-15 (Healed)	4.68	22.1	1920

In the cracked control mix, water penetration depth escalated from 24.5 mm to 58.2 mm, and RCPT charge passed reached 4350 Coulombs ("High" chloride permeability). Conversely, healed IBB-10 specimens exhibited a water penetration depth of just 20.8 mm—a 64.3% reduction compared to cracked control concrete and even lower than uncracked control specimens (24.5 mm). Similarly, RCPT charge dropped to 1790 Coulombs, shifting the concrete durability classification into the "Low" chloride ion penetrability category per ASTM C1202. This confirms that biogenic $CaCO_3$ precipitation bridges deep internal crack networks rather than forming merely a superficial crust.

4.5 Microstructural and Mineralogical Characterization (SEM-EDX and XRD)

Scanning Electron Microscopy (SEM) of the healed crack interface in IBB-10 revealed tightly packed, rhombohedral and trigonal-prismatic crystals (15–45 µm in size) firmly anchored to the hydrated cement paste and bridging across the fracture walls. Imprints of rod-shaped bacterial cells (~1.5 µm

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length) were embedded directly on the crystal facets, serving as nucleation sites for heterogeneous crystal growth.

Energy Dispersive X-Ray Spectroscopy (EDX) of the precipitated crystals yielded elemental weight percentages of Calcium (Ca): 38.4%, with carbon (C) and oxygen (O) as the other dominant elements and only trace amounts of Si and Mg originating from the cement matrix. The measured Ca content is close to the theoretical value for pure calcite (≈ 40.0 wt% Ca), indicating that the precipitates are essentially $CaCO_3$ of biogenic origin.

X-Ray Diffraction (XRD) patterns of the deposits scraped from the healed cracks showed the strongest reflection at $2\theta \approx 29.4°$ (104 plane), accompanied by secondary reflections at approximately 36.0°, 39.4°, 43.2°, 47.5°, and 48.5°. These peaks match the standard pattern of calcite (JCPDS No. 05-0586), confirming calcite as the dominant crystalline phase. Together, the SEM, EDX, and XRD results verify that the crack-filling material is dense, well-crystallized $CaCO_3$ nucleated on bacterial cells, which explains the crack closure in Table 5 and the improved transport properties reported in Table 6.

5. Cost-Benefit and Environmental Sustainability Analysis

For widespread adoption in civil infrastructure, self-healing concrete must balance initial material costs against lifecycle maintenance savings and carbon footprint reductions. Table 7 presents an economic and embodied carbon evaluation per cubic meter (1 m^3) of M30 concrete based on current Indian construction market rates.

Table 7. Material cost and embodied carbon (e-CO_2) comparison per m^3 of M30 concrete

Parameter	CM (Control)	IBB-5	IBB-10	IBB-15
Initial Material Cost (INR/m^3)	4,850	5,280	5,710	6,140
Initial Cost Premium (%)	—	+8.87%	+17.73%	+26.60%
Estimated Service Life (Years)	50	68	80	82
Lifecycle Cost (INR/m^3/year)	97.00	77.65	71.38	74.88
Net Embodied Carbon (kg CO_2e/m^3)	342.0	311.5	281.0	250.5

Although incorporating 10% immobilized bacterial biochar (IBB-10) increases initial production cost by approximately 17.73% (primarily due to bacterial culturing and calcium lactate), extending the structural service life from 50 to 80 years and eliminating periodic crack-injection repairs reduces the annualized lifecycle cost by 26.4%. Furthermore, because biochar acts as a stable carbon sink (~0.85 kg CO_2e sequestered per kg of biochar) and replaces mined river sand, IBB-10 reduces net embodied carbon by 17.8% (61.0 kg CO_2e/m^3) compared to conventional M30 concrete.

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6. Conclusions and Future Scope

Based on the experimental, microstructural, and sustainability investigations conducted in this study, the following conclusions are drawn:

1. **Long-Term Spore Protection:** Immobilizing *Bacillus subtilis* spores and calcium lactate within porous rice husk biochar (2–20 µm pores) coated with sodium silicate effectively prevents spore crushing during cement hydration, extending functional bacterial viability well beyond 180 days.
2. **Optimum Mix Dosage:** Replacing 10% of fine aggregate by weight with immobilized bacterial biochar (IBB-10) is the optimum mix proportion, increasing 28-day and 90-day compressive strengths by 12.43% and 15.44%, respectively, through internal curing and ITZ densification.
3. **Autonomous Crack Healing:** IBB-10 achieved 95.6% crack closure for crack widths up to 0.45 mm at 28 days and maintained 90.0% crack closure for crack widths up to 0.40 mm when cracked at 180 days.
4. **Enhanced Durability:** Biomineralized calcite precipitation reduced water penetration depth by 64.3% and lowered chloride ion penetrability into the "Low" class (1790 Coulombs), substantially protecting embedded steel reinforcement from corrosion.
5. **Economic and Environmental Viability:** Despite a 17.73% upfront material cost premium, IBB-10 lowers annualized lifecycle costs by 26.4% and reduces net embodied carbon by 17.8%, offering a scalable green technology for durable civil infrastructure.

Future Scope: Future investigations should evaluate the self-healing performance of biochar-immobilized bacterial concrete under aggressive marine splash zones, freeze-thaw cycles, and sustained cyclic fatigue loading in full-scale reinforced concrete structural elements.

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