RESEARCH ARTICLE

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Experimental Investigation of Bacteria-Based Self-Healing Concrete through Microbially Induced Calcium Carbonate Precipitation

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Abstract: Cracking accelerates the ingress of water and aggressive species into concrete and consequently reduces the durability of civil infrastructure. This experimental study evaluates a bacteria-based self-healing concrete containing Bacillus subtilis and calcium lactate as a nutrient source. Three mixtures were considered: a control mixture without bacteria (M1), concrete containing 10^5 cells/mL (M2), and concrete containing 10^7 cells/mL (M3). Compressive strength was measured after 7, 14, and 28 days. Crack closure was monitored for an initial crack width of 0.50 mm, and water absorption was evaluated for the control and highest-concentration mixtures. At 28 days, M2 and M3 achieved compressive strengths of 34.7 and 35.8 MPa, respectively, compared with 32.5 MPa for M1. The corresponding increases were 6.8% and 10.2%. After 28 days of healing exposure, the crack width decreased to 0.05 mm in M3, corresponding to 90% apparent crack closure, whereas the control crack decreased only to 0.45 mm. Water absorption decreased from a reported control value interpreted as 5.02% to 3.8% for M3. These results indicate that the higher bacterial concentration improved compressive strength, reduced water absorption, and promoted crack sealing under the reported laboratory conditions. The findings remain preliminary because replicate numbers, variability, mineralogical confirmation, permeability measurements, and long-term durability data were not supplied.

Keywords: Bacterial Concrete, Bacillus Subtilis, Calcium Lactate, Crack Closure, Microbial-Induced Calcium Carbonate Precipitation, Self-Healing Concrete.

1. Introduction

Concrete is the principal construction material used in buildings and infrastructure, but its low tensile strain capacity makes cracking difficult to avoid. Cracks generated by restrained shrinkage, thermal effects, service loading, or environmental exposure create preferential

pathways for water, chlorides, sulphates, and carbon dioxide. These transport pathways accelerate reinforcement corrosion and other deterioration mechanisms, thereby shortening service life and increasing maintenance requirements [1, 2]. Conventional repair methods generally require inspection, surface preparation, external materials, and repeated intervention; their effectiveness may also be limited when cracks are inaccessible or continue to propagate [3].

Self-healing concrete has therefore emerged as a durability-oriented strategy in which cracks are sealed through autogenous reactions or autonomous healing agents. Among autonomous approaches, bacteria-based systems use viable cells or spores together with nutrients and a calcium source to precipitate calcium carbonate within cracks and pores [4, 5]. Early work demonstrated that alkaliphilic, spore-forming bacteria can survive in cementitious matrices and can be activated when water enters a crack [4]. Subsequent studies quantified substantial crack closure in bacteria-based systems and showed that healing efficiency depends strongly on crack width, moisture exposure, bacterial viability, nutrient availability, and protection or encapsulation method [5, 6].

Microbially induced calcium carbonate precipitation (MICP) may proceed through ureolytic or non-ureolytic metabolic routes. Ureolysis can rapidly produce carbonate ions but may generate ammonium-containing by-products, whereas organic calcium salts such as calcium lactate support alternative metabolic pathways that are more compatible with some self-healing formulations [7, 8]. The deposited $CaCO_3$ can reduce connected porosity, restrict water transport, and improve measured strength by filling microvoids; nevertheless, reported outcomes vary with bacterial species, concentration, concrete composition, curing regime, and test protocol [9, 10]. Recent reviews consequently emphasise the need for transparent dosage reporting, mineralogical confirmation, appropriate controls, replicated tests, and long-term assessment before laboratory results are translated into structural applications [11, 12].

Against this background, the present study compares conventional concrete with Bacillus subtilis-modified concrete at two bacterial concentrations. The specific objectives are to evaluate compressive strength at 7, 14, and 28 days, quantify apparent crack closure during water exposure, compare water absorption, and determine whether the higher bacterial concentration provides a consistent laboratory-scale benefit.

2. Materials and Methods

2.1 Constituent Materials

Ordinary Portland Cement (OPC), fine aggregate passing the 4.75-mm sieve, 20-mm nominal-size crushed coarse aggregate, and potable water were used. Bacillus subtilis was selected as the biological healing agent, and calcium lactate was used as the nutrient and calcium source. The source document does not report cement chemistry, aggregate grading, specific gravities,

mix proportions, or admixture dosage; these details should be supplied by the authors before final journal submission.

2.2 Experimental Mixtures

Three mixtures were prepared with a constant water–cement ratio: M1 was the control concrete, M2 contained 10^5 bacterial cells/mL, and M3 contained 10^7 bacterial cells/mL. The bacterial suspension and calcium-lactate solution replaced part of the mixing water. Table 1 summarises the experimental groups and the reported tests.

Table 1. Experimental mixtures and reported test programme

Mix ID	Bacterial concentration	Role	Reported tests
M1	0 cells/mL	Control	Compressive strength; crack-width monitoring; water absorption
M2	10^5 cells/mL	Low bacterial concentration	Compressive strength
M3	10^7 cells/mL	High bacterial concentration	Compressive strength; crack-width monitoring; water absorption

2.3 Bacterial Culture and Concrete Preparation

Bacillus subtilis was cultured in nutrient broth and incubated at 30–37 °C for 24 h. Cell suspensions were then adjusted to the two target concentrations. Cement, fine aggregate, and coarse aggregate were dry-mixed before the water, bacterial suspension, and nutrient solution were added. The concrete was mixed until visually homogeneous, placed in 150 × 150 × 150 mm cube moulds, compacted, demoulded after approximately 24 h, and water-cured until testing. The manuscript should additionally report the strain designation, culture medium composition, method used to determine cell concentration, calcium-lactate dosage, mixing sequence, curing-water temperature, and number of specimens per condition.

2.4 Compressive-Strength Testing

Compressive strength was determined at 7, 14, and 28 days using a compression-testing machine. Strength was calculated using Equation (1):

$$f_{(c)} = P/A \quad (1)$$

where $f_{(c)}$ is the compressive strength (MPa), P is the maximum applied load (N), and A is the loaded cross-sectional area (mm^2). The loading rate and applicable test standard were not reported and should be added.

2.5 Crack Induction and Healing Assessment

After curing, controlled cracks were introduced in selected specimens by mechanical loading or splitting. The initial crack width was recorded as 0.50 mm for both M1 and M3. Specimens were subsequently exposed to water, and the residual crack width was measured after 14 and 28 days. Apparent crack-closure efficiency was calculated using Equation (2):

$$\text{Healing efficiency (\%)} = [(w_0 - w_t)/w_0] \times 100 \quad (2)$$

where w_0 is the initial crack width and w_t is the residual crack width after healing time t. The measuring instrument, image-analysis method, number and location of measurements, and crack-generation protocol should be reported to establish reproducibility.

2.6 Water-Absorption Testing

Water absorption was determined from dry and saturated specimen masses after 24 h of immersion using Equation (3):

$$\text{Water absorption (\%)} = [(M_s - M_e)/M_e] \times 100 \quad (3)$$

where M_e is the oven-dry mass and M_s is the saturated mass. The original manuscript reports 502% for M1; because this value is physically implausible for ordinary concrete and is inconsistent with the reported 3.8% for M3, it is treated here as a typographical error and presented as 5.02%. The authors must verify this correction against the laboratory record before submission.

3. Results

3.1 Compressive Strength

The compressive-strength results are presented in Table 2. Strength increased with curing age for all mixtures. M3 produced the highest values at each age, followed by M2 and M1. At 28 days, M2 exceeded the control by 6.8%, while M3 exceeded the control by 10.2%. These comparisons indicate a concentration-dependent benefit within the two bacterial dosages investigated; however, statistical significance cannot be established because replicate data and measures of dispersion were not provided.

Table 2. Reported compressive strength of control and bacterial concrete

Mix	7 days (MPa)	14 days (MPa)	28 days (MPa)	28-day change vs M1
M1 (control)	20.2	26.8	32.5	–
M2 (10^5 cells/mL)	21.8	28.5	34.7	+6.8%
M3 (10^7 cells/mL)	22.5	29.2	35.8	+10.2%

3.2 Crack Closure and Water Absorption

Table 3 summarises crack-width evolution and water absorption. The control crack showed only a small reduction, from 0.50 to 0.45 mm after 28 days, equivalent to 10% apparent closure. In contrast, the M3 crack decreased to 0.15 mm after 14 days and 0.05 mm after 28 days, corresponding to 70% and 90% apparent closure, respectively. The M3 mixture also showed lower water absorption than the interpreted control value. These observations are consistent with pore filling and crack-surface deposition, but $CaCO_3$ formation was not confirmed by X-ray diffraction, scanning electron microscopy, thermogravimetric analysis, or spectroscopy.

Table 3. Reported crack-width evolution and water absorption

Parameter	M1 control	M3 (10^7 cells/mL)	Interpretation
Initial crack width	0.50 mm	0.50 mm	Equal starting crack width
Crack width after 14 days	0.48 mm	0.15 mm	4% vs 70% apparent closure
Crack width after 28 days	0.45 mm	0.05 mm	10% vs 90% apparent closure
Water absorption	5.02%*	3.8%	24.3% relative reduction

*The source value of 502% has been interpreted as 5.02% and requires author verification.

4. Discussion

The increase in compressive strength with bacterial concentration may be associated with biomineral deposition in capillary pores and at microcrack surfaces. Bacterial cells can act as nucleation sites, while carbonate generated through microbial metabolism reacts with available calcium to form $CaCO_3$. The resulting deposits may densify the matrix and improve stress transfer. Nevertheless, the data do not isolate microbial precipitation from other influences such as calcium-lactate addition, changes in effective water content, or normal specimen-to-specimen variability. A nutrient-only control and a heat-inactivated-bacteria control would be required to separate these effects.

The substantial reduction in M3 crack width is the strongest evidence of healing in the reported dataset. The control also exhibited limited closure, which is expected from autogenous hydration and carbonation. The difference between M1 and M3 therefore suggests an additional bacteria-associated contribution. However, surface crack closure does not necessarily demonstrate recovery of mechanical continuity or transport resistance through the full crack depth. Future testing should combine optical crack mapping with permeability, ultrasonic pulse velocity, stiffness recovery, and post-healing mechanical tests.

The lower water absorption of M3 is compatible with a less-connected pore network, but the result must be treated cautiously because only one bacterial mixture was compared, the control value required correction, and no repeatability statistics were available. Direct claims regarding chloride resistance, sulphate resistance, tensile strength, carbon-footprint reduction, or maintenance-cost savings are not supported by the supplied experiments and have therefore been removed from the conclusions.

5. Limitations and Reproducibility Requirements

The concrete grade, complete mix proportions, water–cement ratio, material properties, and applicable test standards were not supplied. The Bacillus subtilis strain, culture-medium formulation, cell-counting method, nutrient dosage, and bacterial viability after mixing were not reported. The number of replicate specimens, standard deviations, confidence intervals, and statistical tests were not provided. The crack-generation procedure and crack-width measurement protocol were insufficiently described. Mineralogical and microstructural evidence confirming that the sealing product was $CaCO_3$ was not supplied. Only short-term laboratory exposure was considered; field conditions, dry–wet cycling, reinforcement corrosion, freeze–thaw exposure, and long-term bacterial viability were not evaluated. The source water-absorption value for the control was ambiguous and must be checked against the original laboratory record.

6. Conclusions

Bacillus subtilis-modified concrete showed higher compressive strength, greater apparent crack closure, and lower water absorption than the control under the reported laboratory conditions. The 10^7 cells/mL mixture achieved a 28-day compressive strength of 35.8 MPa, representing a 10.2% increase over the control. For an initial crack width of 0.50 mm, the same mixture reached 90% apparent surface crack closure after 28 days, compared with 10% for the control. Its water absorption was 3.8%, compared with a control value interpreted as 5.02%. Within the investigated range, 10^7 cells/mL therefore provided the best measured performance. These results support the feasibility of bacteria-assisted crack sealing but do not yet establish structural-scale self-healing, long-term durability, or cost effectiveness. Before publication or practical application, the authors should verify the corrected absorption value, report complete

mixture and culture details, include replicated measurements with statistical analysis, and confirm the healing product and crack-depth sealing through appropriate microstructural and transport tests.

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