INVESTIGATION OF SELF-HEALING CHARACTERISTICS OF BACTERIA-BASED CONCRETE

This technical brief summarizes the findings of a CIAMTIS-funded project focused on developing and evaluating bacteria-based self-healing concrete (BBSHC) systems using microbial-induced calcite precipitation (MICP). The research addresses the critical challenge of crack formation and durability loss in concrete by integrating encapsulated bacterial spores that autonomously heal microcracks under favorable conditions.

INTRODUCTION

Concrete is the most extensively used construction material due to its strength and versatility [1], with typical structures lasting over 50 years [2]. However, issues like thermal stresses, autogenous shrinkage [3], and corrosion [4] lead to cracking, compromising structural durability. Cracks facilitate moisture ingress, accelerating steel rebar corrosion and potentially causing failure [4]. The cost of repairing concrete structures often exceeds initial construction costs—$60–80/m³ for production versus $147/m³ for repairs [5]. Additionally, about 20% of repairs fail within 5 years and 55% within 10 years [6], raising concerns about sustainability. In nations like the USA, Germany, and South Korea, repair and maintenance costs increasingly outweigh new construction investments [7,8]. Environmentally, cement production and aggregate extraction are major contributors to CO_2 emissions [9], while construction and demolition waste comprises 59% of total waste, 40% of which ends up in landfills [10]. Conventional repair techniques using resins or epoxies are costly, environmentally unfriendly, and unsuitable for internal cracks [6,11].

Bacterial-based self-healing concrete provides a promising alternative by promoting in-situ calcite precipitation at crack sites, improving durability while reducing carbon emissions and maintenance costs [12–14]. This mechanism uses dormant bacterial endospores that activate under favorable conditions and precipitate calcite by consuming nutrients [15,16]. Bacillus species, especially alkaliphilic and gram-positive strains, are ideal due to their resilience in harsh environments [15,17]. To protect bacterial viability, spores are commonly immobilized in carriers rather than directly added to the concrete [18]. Various carriers have been explored. Polyurethane foam offered porosity and nucleation sites [19,20], and polyurethane-based systems performed better than silica gel [21]. Sodium alginate beads proved effective; Shahid et al. [22] observed improved compressive strength and healing with 2–3% bacterial culture. Calcium alginate beads used in marine settings led to calcite formation of 1 mm³ per 30 beads [23]. Hydrogels improved bacterial viability, healing cracks up to 0.5 mm and reducing permeability by 80% [24–26]. Microencapsulation also showed promise using ethyl cellulose microcapsules, enhanced healing versus unencapsulated bacteria [27-30], and melamine microcapsules containing *Pseudomonas* spores achieved 48–80% healing efficiency, compared to 18–50% without encapsulation [15]. Zhang et al. [28] used core-shell capsules as fine aggregate substitutes, maintaining bacterial viability for over 200 days without affecting concrete strength.

Despite these advances, high costs and complexity hinder widespread use. Natural inorganic carriers like diatomaceous earth have been investigated for their porosity and urease activity, healing cracks up to 0.17 mm [31]. LECA has emerged as a preferred carrier for its ability to heal cracks up to 0.46 mm [32], with enhancements seen when used with graphite nanoplatelets (up to 0.81 mm) [33] or expanded perlite (up to 1.24 mm) [34,35]. Yan et al. [36] emphasized that ideal carriers must be porous, chemically inert, and capable of rupturing upon cracking. LECA satisfies these criteria and has gained traction in recent studies [37–39]. Environmental conditions, particularly freeze-thaw cycles, affect bacterial healing performance. Freeze-thaw action causes internal damage due to water expansion [40]. Yan et al. [41] reported reduced healing efficiency under such conditions due to bacterial degradation, while Liu et al. [42] found that bacteria-treated recycled aggregate concrete improved freeze-thaw resistance by filling pores with calcite. However, most studies focus on controlled environments, with limited attention to real-world freeze-thaw durability. Additionally, the lack of standardized methodologies, variations in bacterial strains, carrier types, spore concentrations, and crack-inducing techniques make comparison and optimization difficult. Wide cracks may also result in bacterial washout during curing. Further research is needed to establish optimal spore dosage, encapsulation techniques, and long-term environmental effects.

This study addresses these gaps by investigating the self-healing performance of *Bacillus subtilis*, *Bacillus sphaericus*, and *Bacillus megaterium* immobilized in LECA using vacuum impregnation. The study also examines the influence of LECA in combination with polyvinyl acetate (PVA) fibers and air-entraining admixture (AEA) on mechanical and healing

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performance. Additionally, alginate beads were tested as an alternative encapsulation method. Long-term healing was monitored through ultrasonic pulse velocity (UPV), surface crack analysis using ImageJ, and visual inspection. Self-healing precipitates were characterized using SEM, XRD, and FTIR. The research aims to determine optimal bacterial dosages, encapsulation methods, and curing conditions to improve the practical application of self-healing concrete.

METHODOLOGY

This study utilized ASTM Type I cement, natural aggregates, and lightweight expanded clay aggregates (LECA) as bacterial carriers. LECA was vacuum-impregnated with spores of *Bacillus subtilis*, *Bacillus sphaericus*, and *Bacillus megaterium*, and nutrient media (calcium lactate and yeast extract), then sealed with a PVA coating, as shown in Fig. 1 (left). Three bacterial dosages (0.2, 2, and 20 µL/g LECA) were tested, with 20 µL/g selected for further evaluation based on superior self-healing results.

Six concrete mixes were developed as shown in Table 1: three without bacteria (one control and two reference mixes) and three mixes incorporating different bacterial strains. Some mixes included air-entraining admixture and PVA fibers to enhance mechanical strength and durability. All specimens were cured for 7 days, then pre-cracked using an Instron UTM by applying tensile load via embedded steel rods to induce stable cracks (0.1–0.80 mm) as shown in Fig. 1 (right).

Self-healing was assessed through ultrasonic pulse velocity (UPV) testing and surface crack analysis using ImageJ, conducted every 28 days up to 224 days. Mechanical testing included compressive and flexural strength at 7 and 28 days, strength regain after preloading, and freeze-thaw durability per ASTM C666. Water absorption and Sorptivity were evaluated using ASTM C1585. Microstructural analyses using SEM-EDS and XRD were carried out for characterization of the healing compound. Additional mixes used alginate beads as bacterial carriers, prepared with sodium alginate and calcium lactate, and dried via cement coating, freeze-drying, or oven drying, as shown in Table 2. These beads were added to concrete at 3% by cement weight. Mixes prepared are described in Table 3.

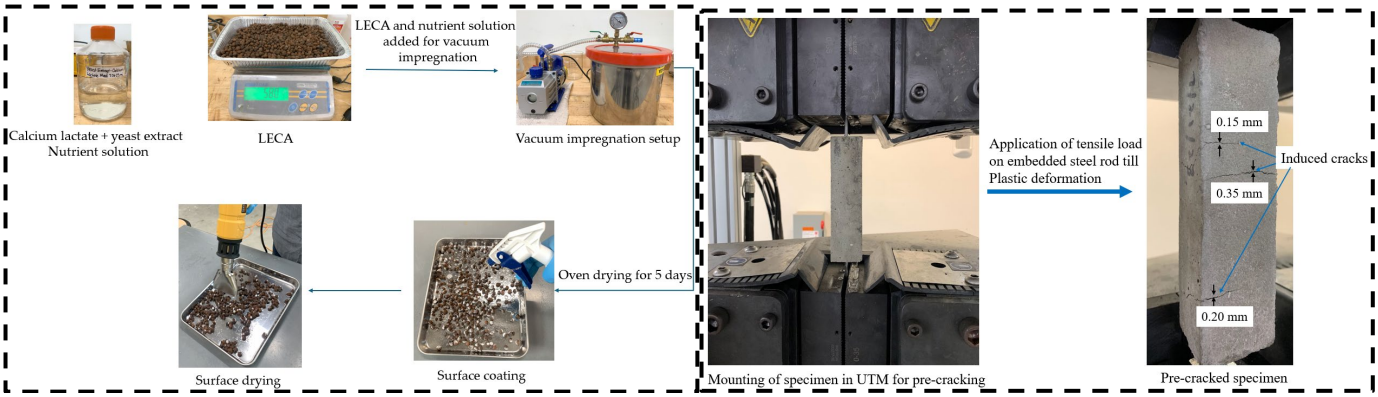


Fig. 1. Flowchart of the immobilization process of LECA (left), Pre-cracking of concrete specimens (right)

DATA SUMMARY

Table 1. Mix proportioning for LECA encapsulated bioconcrete (per m^3 concrete)

Materials	Unit	CM	CMN	R1	R2	MSb	MSp	MMe
Cement	kg	483	483	483	483	483	483	483
Natural Coarse aggregate	kg	755	755	604	604	604	604	604
Fine aggregate	kg	933	933	933	933	933	933	933
LECA	kg	0	0	151	151	151	151	151
w/c ratio	-	0.45	0.45	0.45	0.45	0.45	0.45	0.45
Amount of spores (per gram of LECA)	µL	20	-	-	-	20	20	20
AEA	L	-	-	-	1.45	1.45	1.45	1.45
PVA fibers	%	-	-	-	1	1	1	1

Table 2. Mix proportioning for alginate beads encapsulated concrete (per m^3 concrete)

Materials	Unit	Cement coating	Freeze-drying	Oven drying

Cement	Kg	483	483	483
Natural Coarse aggregate	Kg	755	755	755
Fine aggregate	Kg	933	933	933
Na-Alginate dry beads	Kg	14.3	14.3	14.3
Spores	µL	20	20	20
w/c ratio	-	0.45	0.45	0.45

Table 3. Description of control, reference and alginate encapsulated concrete mixes

Mix ID	Description
CM	Control mix (no beads, no bacteria, no nutrients)
RM-AB	Reference mix with only non-encapsulated alginate beads
NM	Mix with only nutrient media (no bacteria or beads)
BSM	Bacillus subtilis encapsulated in alginate beads + nutrients
BSPM	Bacillus sphaericus encapsulated in alginate beads + nutrients
BMM	Bacillus megaterium encapsulated in alginate beads + nutrients

RESULTS AND DISCUSSION

1. Bioengineered concrete with LECA as a bacterial carrier

1.1. Self-healing assessment of multiple bacterial strains encapsulated in LECA

The self-healing performance of concrete mixes incorporating *Bacillus subtilis* (MSb), *Bacillus sphaericus* (MSp), and *Bacillus megaterium* (MMe) immobilized in LECA was evaluated over 224 days through surface crack analysis and ultrasonic pulse velocity (UPV) testing. All bacterial mixes exhibited superior crack healing compared to the control mix (CM) and reference mix (R1). *Bacillus megaterium* demonstrated the highest self-healing efficiency, fully sealing cracks up to 0.45 mm within 28 days. This superior performance is attributed to its protective exosporium, broader pH tolerance, larger cell size, and higher calcite precipitation potential. ImageJ-based surface analysis showed MMe achieved the highest healing efficiency (66.87%), followed by MSp (60.75%) and MSb (57.45%), as shown in Fig. 1(a). UPV testing confirmed internal crack healing, with efficiency trends consistent with surface analysis, though absolute values were lower due to limited healing of deeper cracks. At 224 days, MMe again led with 71.03% healing efficiency, followed by MSp (53.38%) and MSb (41.96%), as shown in Fig. 1(b, c). The CM and R1 mixes exhibited minimal healing due to direct bacterial addition and absence of spores, respectively.

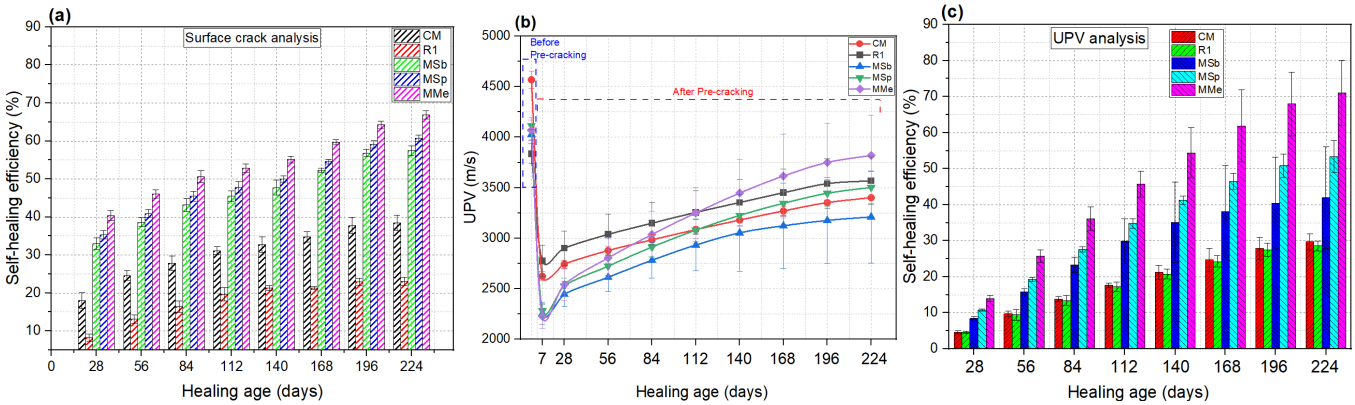


Fig. 2. Self-healing of various bioengineered concrete mixes through (a) surface crack analysis, (b) UPV measurements, (c) UPV analysis

1.2. Mechanical Performance of Bacterial Concrete

Concrete with 100% natural aggregates (CMN) showed the highest compressive strength at both 7 and 28 days. Partial replacement of coarse aggregate with 20% lightweight expanded clay aggregate (LECA) in all other mixes (R1, R2, MSb, MSp, MMe) led to a strength reduction due to LECA's porous nature. However, mixes incorporating PVA fibers (R2 and bacterial mixes) exhibited improved strength retention compared to R1, due to fiber bridging action. Among bacterial mixes, MMe showed the smallest reduction in strength, attributed to its superior self-healing and calcite precipitation

capabilities. After inducing microcracks through pre-loading, bacterial mixes showed significant restoration in compressive strength, with MMe achieving the highest recovery (3.83% at 7 days, 2.71% at 28 days). Non-bacterial mixes (CMN, R1, R2) showed minimal recovery due to limited or no biomineralization. The bacterial restoration was attributed to effective calcite precipitation, matrix densification, and improved interfacial bonding facilitated by the bacterial metabolic activity. Similar to compressive strength trends, CMN had the highest flexural strength, followed by R2 and the bacterial mixes. LECA inclusion reduced strength, but PVA fibers mitigated this loss by preventing brittle failure. At 28 days, MMe had the lowest reduction in flexural strength (2.89%), followed by MSp (3.48%) and MSb (4.53%), reinforcing the superior healing and crack-bridging capacity of *Bacillus megaterium*.

1.3. Freeze-Thaw Resistance

Concrete specimens were subjected to 300 freeze-thaw cycles following ASTM C666 to evaluate durability based on mass loss and Relative Dynamic Elastic Modulus (RDEM); the results are shown in Fig. 3 (a, b). Among all mixes, MMe exhibited the lowest mass loss (1.16%) and highest RDEM retention (93.87%), indicating superior freeze-thaw durability. This performance surpassed MSp and MSb and significantly outperformed non-bacterial mixes R1 and CMN.

Mass Loss Trend (300 cycles): R1 > CMN > R2 > MSb > MSp > MMe
RDEM Loss (%): R1 (16.66%) > CMN (15.13%) > R2 (13.74%) > MSb (10.47%) > MSp (8.54%) > MMe (6.13%)

The enhanced durability of bacterial mixes was due to microbial-induced calcite precipitation during thawing, which sealed microcracks and densified the concrete matrix. PVA fibers and air-entraining admixture (AEA) in R2 also mitigated damage by distributing freeze-induced stresses and improving matrix cohesion. Additionally, polymer-coated LECA contributed to a stronger interfacial transition zone (ITZ), further enhancing resistance.

1.4. Sorption Resistance

Sorption tests measured water ingress to assess pore connectivity and sealing effectiveness. CMN showed the best performance (lowest sorption depth: 1.79 mm; sorptivity index: 0.0121 mm/√s), as shown in Fig. 3 (c). Reference mixes R1 and R2, containing porous LECA, exhibited significantly higher sorption (4.20 mm and 4.38 mm), reflecting increased permeability. Bacterial mixes using LECA as carriers MSb, MSp, MMe and a new combined strain mix (MCombo), demonstrated reduced sorption due to MICP. The MCombo mix, incorporating equal portions of all three bacteria, yielded the best durability improvement among bacterial variants (sorption depth: 2.62 mm; sorptivity index: 0.0178 mm/√s), suggesting synergistic effects from multiple strains.

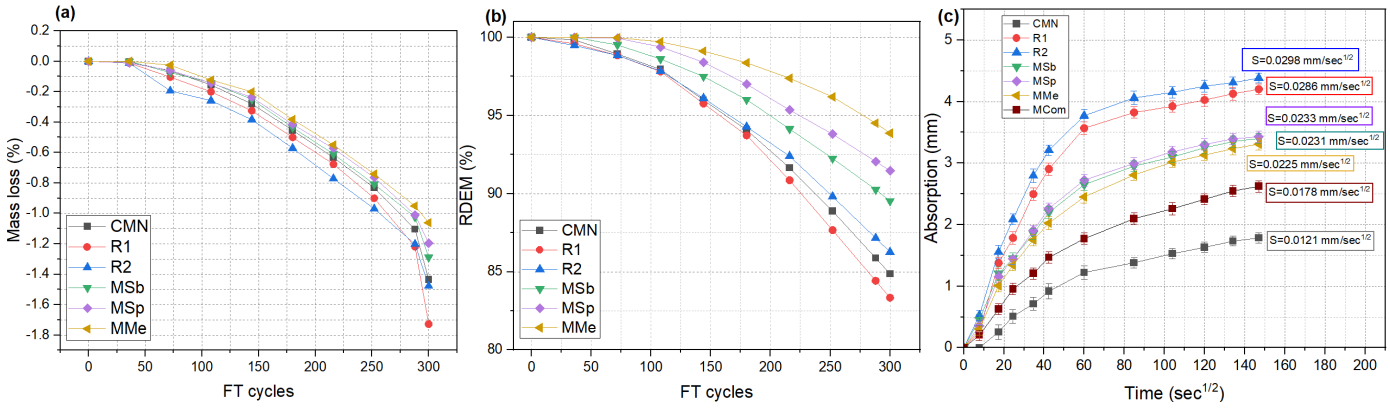


Fig. 3. Freeze-thaw durability (a) rate of mass loss, (b) RDEM, (c) water absorption and Sorptivity index

1.5. Microstructural Analysis

SEM-EDS and XRD analyses confirmed that calcite was the primary self-healing precipitate in all bacterial concrete mixes (MSb, MSp, MMe). SEM images showed rhombohedral calcite crystals within cracks, while EDS spectra identified calcium, carbon, and oxygen as dominant elements. *Bacillus megaterium* (MMe) produced the highest calcium content (39.08%), indicating greater calcite formation compared to other strains.

XRD results supported these findings, showing the strongest calcite peaks for MMe, followed by MSp and MSb. Lower portlandite peaks indicated calcite as the dominant mineral phase. These microstructural results aligned with the superior mechanical and durability performance of MMe mixes, demonstrating that enhanced calcite precipitation by *Bacillus megaterium* led to more effective crack sealing and matrix densification through microbially induced calcium carbonate precipitation (MICCP).

2. Bioengineered concrete with Alginate beads as a bacterial carrier

2.1. Self-healing assessment

The self-healing performance of concrete incorporating Bacillus subtilis, Bacillus sphaericus, and Bacillus megaterium encapsulated in alginate beads was evaluated using three methods: cement-coating (CC), freeze-drying (FD), and oven-drying (OD). Oven-dried beads showed the most effective and sustained crack healing. Full healing of 0.30 mm cracks occurred within 28 days, and partial healing of larger cracks (up to 0.40 mm) was observed by day 56–84. Freeze-dried beads provided moderate performance, with healing up to 0.35 mm in some cases. Cement-coated beads showed the least healing, often plateauing after 28 days. Among all combinations, oven-dried Bacillus megaterium mixes demonstrated the most extensive and prolonged healing.

Self-healing efficiency (SHE) was significantly higher in bacterial mixes than in the control (13.74% SHE at 28 days). Oven-dried beads achieved the highest SHE improvements, with BMM-OD reaching 2.76 times the control, followed by BSPM-OD (2.32) and BSM-OD (1.86). Freeze-dried beads showed strong but slightly lower improvements (up to 2.63), while cement-coated beads had the lowest gains (up to 2.14), likely due to issues with delayed bacterial activation and uneven distribution. Across all methods, Bacillus megaterium outperformed the other strains, confirming its superior viability and calcite-forming ability in cementitious environments. The results highlight that both encapsulation techniques and bacterial strain selection are critical for maximizing bio-concrete's self-healing performance.

2.2. Mechanical Performance and Self-Healing Restoration of Bio-Concrete with Alginate Beads

The incorporation of 3% alginate beads, whether empty or bacteria-encapsulated, led to a slight reduction in both 7-day and 28-day compressive strength compared to the control mix (CMN). The reduction is attributed to the soft, porous nature of the beads, which weakened the interfacial transition zone (ITZ) and disrupted matrix density**.** 28-day strength reductions ranged from 4.3% for cement-coated beads to 8.3% for freeze-dried beads. BMM consistently showed slightly better strength retention across all methods. All modified mixes exhibited reduced flexural strength at both ages. Cement-coated mixes again performed best, while freeze-dried mixes saw the largest decrease up to 15.5% at 28 days, due to structural discontinuities and poor bead integrity. Oven-dried mixes fell in between.

Pre-cracked specimens cured for 28 days showed no healing in control and reference mixes (RM-AB, NM), with slight post-healing strength losses. Significant recovery in bacterial mixes, especially with oven-dried encapsulation: BMM-OD restored 4.07% of compressive strength, followed by BSPM-OD and BSM-OD. Cement-coated and freeze-dried bacterial mixes also showed healing, but to a lesser extent.

CONCLUSION AND IMPLEMENTATION

This study evaluated the self-healing performance of concrete using three bacterial strains, Bacillus subtilis, Bacillus sphaericus, and Bacillus megaterium, immobilized in lightweight expanded clay aggregates (LECA) and alginate beads. LECA proved to be an effective carrier, protecting bacteria and nutrients and enabling crack healing for up to 224 days. Among all strains, Bacillus megaterium consistently demonstrated the highest healing efficiency, mechanical strength recovery, and freeze-thaw durability, healing cracks up to 0.45 mm and achieving over 70% healing efficiency. Mechanical strength was slightly reduced by LECA inclusion but improved with the addition of PVA fibers and bacteria. Microstructural analyses (SEM, EDS, XRD) confirmed that calcite precipitation was the primary healing mechanism.

Alginate beads were also tested in three encapsulation forms: oven-dried, freeze-dried, and cement-coated. Oven-dried beads, especially those containing Bacillus megaterium, showed the most effective healing (up to 95% crack closure). While all alginate-based mixes experienced minor reductions in strength (3–8%), cement-coated beads maintained better structural performance. The inclusion of nutrient media led to only slight additional strength loss, while bacterial encapsulation promoted measurable compressive strength restoration in cracked specimens, further confirming the role of microbial-induced calcite precipitation in autonomous healing.

Based on these findings, Bacillus megaterium in combination with LECA or oven-dried alginate beads is recommended for use in durable, self-healing concrete, particularly in freeze-thaw environments. Future research should explore performance under hot and arid conditions, optimize encapsulation techniques, and conduct pilot-scale field trials. Life cycle assessments and studies on multi-strain synergy (as seen with the MCombo mix) are also encouraged to further develop bio-concrete as a resilient and sustainable material for real-world infrastructure.

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