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RESEARCH ARTICLE

Crack Healing and Structural Recovery of Concrete Using *Cytobacillus horneckiae*-Induced Carbonate Precipitation

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Abstract Concrete cracking poses a major durability challenge, accelerating reinforcement corrosion and reducing service life. Conventional repair methods such as epoxy injection and cementitious grouting are costly, unsustainable, and often ineffective for microcracks. This study evaluates the potential of *Cytobacillus horneckiae*, a resilient ureolytic bacterium, to promote microbially induced calcium carbonate precipitation (MICP) for sustainable crack remediation. Laboratory experiments assessed carbonate precipitation efficiency under varying conditions of pH, urea concentration, and temperature, alongside controlled crack-healing trials using a combined injection–diffusion method (CIDM). Results showed optimal $CaCO_3$ formation at pH 9, 0.75 M urea, and 35 °C, conditions consistent with concrete pore environments. Image analysis revealed a 90% reduction in surface crack area within 12 days, demonstrating accelerated healing compared with conventional injection methods. Mechanical testing indicated 75% recovery of tensile strength and ultimate load capacity, confirming partial restoration of structural integrity. FTIR spectra identified carbonate functional groups and calcite polymorphs, while SEM–EDX analyses confirmed the presence of Ca and O as dominant elements, validating biogenic $CaCO_3$ deposition within crack voids. Silicon traces reflected interactions with the concrete matrix, while negligible carbon detection was attributed to EDX limitations for light elements. Collectively, the findings highlight the effectiveness of *C. horneckiae*-mediated MICP in sealing cracks and restoring mechanical performance, offering an environmentally friendly alternative to chemical repair agents. However, incomplete depth sealing suggests that further optimization of bacterial delivery strategies is required to achieve long-term durability. This work expands the scope of MICP research beyond the conventional *Sporosarcina pasteurii*, providing new insights into the application of native bacterial strains for sustainable concrete self-healing technologies.

Keywords: Microbially Induced Carbonate Precipitation, *Cytobacillus horneckiae*, Crack Healing, Sustainable Concrete, Structural Restoration.

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Introduction

Concrete is the most widely used construction material in the world, forming the structural backbone of modern infrastructure such as bridges, pavements, tunnels, and buildings. Its widespread adoption is attributed to its affordability, compressive strength, and versatility in diverse engineering applications [1]. Despite these advantages, concrete is inherently vulnerable to cracking, which remains one of the most pressing durability concerns in civil engineering. Cracks can emerge due to thermal stresses, shrinkage, settlement, mechanical overloading, or environmental exposures such as freeze–thaw cycling, carbonation, and sulfate attack [2]. Even microcracks pose serious risks as they permit the ingress of aggressive agents including chlorides, sulfates, and carbon dioxide, which accelerate the corrosion of embedded steel reinforcement, compromise structural capacity, and shorten the service life of infrastructure. The global economic burden associated with concrete repair is significant, with billions spent annually on crack remediation and structural rehabilitation [3] Traditional approaches to crack repair including epoxy injection, cementitious grouting, and polymer patching—offer short-term structural restoration but suffer from multiple limitations. These methods are frequently expensive, labor-intensive, and heavily reliant on petroleum-based or synthetic materials that are environmentally unsustainable. Furthermore, poor bonding between repair agents and the existing cementitious matrix often leads to debonding, premature failure, or unsatisfactory performance in harsh service environments [4]. For instance, epoxy-based repairs have been observed to degrade under sustained moisture exposure and temperature cycling, while cementitious grouts often fail to seal microcracks smaller than 0.2 mm [5]. These shortcomings highlight an urgent need for sustainable and cost-effective alternatives that align with global priorities in green construction and circular economy principles [6].

In response, microbially induced carbonate precipitation (MICP) has emerged as a promising bio-based strategy for concrete crack remediation. MICP exploits the metabolic activity of microorganisms—typically ureolytic bacteria to precipitate calcium carbonate within cracks and pores. During ureolysis, bacterial urease catalyzes the hydrolysis of urea, generating carbonate ions that react with calcium ions to form $CaCO_3$, which fills cracks, bonds fractured surfaces, and reduces permeability. The advantages of MICP are substantial: it requires only low-cost and widely available precursors such as urea and calcium salts, produces biogenic minerals that are chemically compatible with cement matrices, and reduces dependence on synthetic polymers or high-energy processes [7, 8]. Compared with conventional sealants, MICP not only restores mechanical integrity but also contributes to long-term durability and environmental sustainability, making it attractive for adoption in next-generation infrastructure maintenance [9]. Over the past decade, laboratory-scale studies have demonstrated the potential of MICP to recover mechanical strength, reduce permeability, and increase resistance to aggressive exposures. Kulkarni *et al*. [10] reported that MICP-treated mortar recovered up to 90% of its original compressive strength, while Gao and Wang [11] observed significant reductions in water absorption following treatment. Field trials have further validated the applicability of MICP. Limpaninlachat *et al*. [12] successfully treated historic masonry walls in Belgium, while Fan *et al*. [13] demonstrated lower chloride penetration in reinforced beams exposed to marine conditions after bacterial treatment. These outcomes suggest that MICP could become a viable technology for large-scale infrastructure applications.

Recent innovations have also expanded the horizons of MICP research. Encapsulation of bacteria in lightweight aggregates, hydrogels, and polyurethane carriers has been employed to enhance bacterial survivability and improve healing efficiency [14]. Engineered bacterial strains with optimized urease activity and stress tolerance have been reported to accelerate $CaCO_3$ precipitation and enhance sealing efficiency [15]. In parallel, alternative metabolic pathways including acetate-driven carbonate precipitation are being investigated to minimize ammonia generation, a known environmental by-product of ureolysis [5].These developments reflect the growing sophistication of MICP research and its alignment with global efforts to develop environmentally responsible construction practices. Despite these advances, significant gaps remain in the literature. First, most studies focus narrowly on the model organism *Sporosarcina pasteurii*, while little attention has been given to native or non-traditional strains that may offer better resilience in concrete's harsh environment. Second, long-term durability assessments particularly under freeze–thaw cycles, carbonation, sulfate exposure, and cyclic loading remain scarce, yet these factors are critical for infrastructure exposed to natural weathering. Third, while many studies claim environmental benefits, quantitative sustainability analyses such as life-cycle assessment (LCA), energy consumption, or cost-benefit comparisons with conventional methods are seldom presented [16]. Finally, scalability to real-world infrastructure remains a major challenge, as field applications have been limited in scope and rarely explore the feasibility of treating deeper, wider, or horizontally oriented cracks.

Cytobacillus horneckiae represents a promising but underexplored candidate for MICP-based concrete repair. Originally isolated from harsh environments such as spacecraft clean rooms, this Gram-positive, spore-forming bacterium exhibits exceptional adaptability and tolerance to extreme conditions [17]. Recent experiments demonstrated that *C. horneckiae* can induce significant $CaCO_3$ precipitation, enabling slope stabilization under rainfall conditions and reducing soil erosion dramatically, with precipitation volumes comparable to those achieved using laboratory-grade media [18]. Its ability to thrive under nutrient-limited and variable conditions suggests it may be especially well-suited for application in the alkaline, heterogeneous environment of cracked concrete. The use of such native strains offers two critical advantages: enhanced environmental adaptability, which increases the likelihood of long-term survivability and activity, and greater sustainability, since native bacteria reduce reliance on engineered or imported strains. To date, no study has systematically examined the application of *C. horneckiae* for crack remediation in concrete. Addressing this knowledge gap is particularly relevant in advancing bio-based construction technologies toward real-world scalability. Accordingly, the present study aims to investigate the capacity of *C. horneckiae* to induce $CaCO_3$ precipitation for the repair of cracked concrete. Specifically, the research evaluates the mechanical recovery of treated specimens, quantifies improvements in permeability resistance, and explores the durability of MICP-treated samples under environmental stressors. By situating this investigation within the broader discourse on sustainability, the study also seeks to assess the cost and environmental implications of employing native bacteria in practical crack remediation. In doing so, this research contributes to filling a critical gap in the existing literature on MICP and concrete self-healing. It advances the knowledge frontier from laboratory model organisms to native strains with inherent resilience, providing insights into a potentially scalable and environmentally responsible approach to infrastructure maintenance. The findings are expected to inform both academic research and engineering practice, aligning with international efforts to create more durable, low-carbon, and sustainable infrastructure systems.

Materials and Methods

Preparation of *Cytobacillus horneckiae*

The ureolytic bacterium *Cytobacillus horneckiae* was preserved in glycerol stock at –80 °C and reactivated for experimental use following standard revival protocols used for ureolytic bacteria [19]). Under aseptic conditions, the frozen stock was streaked onto UV-sterilized nutrient agar plates containing 30 g/L UV-treated urea and incubated at 32 °C for 24–48 h. Non-inoculated plates served as sterility controls. A single well-isolated colony from the second streak was then inoculated into 25 mL of nutrient broth (13 g/L) supplemented with 10 g/L ammonium chloride and 30 g/L UV-sterilized urea, adjusted to pH 8.0. The culture was incubated at 32 °C with shaking at 125 rpm for 24–48 h until turbidity indicated active bacterial growth. To produce sufficient biomass for biomineralization, stepwise sub-culturing was conducted at a 1:9 inoculation ratio, first into 50 mL and subsequently into 125 mL of fresh broth, under identical conditions. This scaling-up procedure ensured bacterial adaptation, high cell density, and reproducible metabolic activity for subsequent MICP applications, consistent with approaches used in previous studies on urease-producing strains [20].

Carbonate Precipitation Capacity and Bacterial Growth Monitoring

The carbonate precipitation capacity of *Cytobacillus horneckiae* was evaluated to determine its effectiveness in inducing calcium carbonate ($CaCO_3$) formation under controlled conditions. A 2.5 mL aliquot of actively growing bacterial culture was aseptically introduced into 22.5 mL of fresh nutrient broth containing urea in a 50 mL conical flask. The mixture was incubated at 32°C with shaking at 125 rpm for 24–48 hours to promote further bacterial activity, as observed through increased turbidity. Following incubation, 5 mL of the culture was added into a sterilized Falcon tube containing 45 mL of a 0.5 M cementation solution, consisting of UV-sterilized 74 g of calcium chloride and 30 g of urea dissolved in 1 L of autoclaved distilled water. After 30 minutes of static contact, visible white precipitates formed, indicating the occurrence of calcium carbonate precipitation. The precipitated $CaCO_3$ was separated via vacuum filtration and dried in a 100°C oven (Memmert GmbH, Germany). The dry mass was measured using an analytical balance (GR200, Muhibbah Saintifik Sdn Bhd, Selangor, Malaysia), with repeated measurements taken to ensure accuracy. These results were used to quantify the carbonate-forming ability of S. pasteurii in simulated conditions. To monitor bacterial growth and urease activity potential, the optical density at 600 nm (OD_{600}) was measured using a UV-Visible spectrophotometer (GENESYS™ 20, Thermo Fisher Scientific, MA, USA). Equal volumes (2 mL each) of nutrient broth and bacterial suspension were prepared under aseptic conditions, and OD_{600} values were recorded. Higher OD_{600} readings reflected greater bacterial density, which correlates with increased carbonate precipitation capability through enhanced urease activity.

Mortar samples preparation and Controlled Cracking

Mortar samples were prepared using Ordinary Portland Cement (OPC), river sand, and water. A mixture comprising 1700g of sand and 640 g of cement was dry-mixed in a large container to ensure uniformity, followed by the gradual addition of 255 mL of water over 5 minutes to achieve a consistent and workable mix. The fresh mortar was then cast into lightly oiled plastic cube molds (50 mm × 50 mm × 50 mm) and cylindrical molds (100 mm height × 50 mm diameter), with compaction carried out using a concrete vibrating table (MTM Precision Sdn Bhd, Selangor, Malaysia) to eliminate entrapped air. To create a predefined crack, a 1 mm × 25 mm × 25 mm aluminum foil strip was embedded in one cube mold during compaction. After setting for 2 hours, the foil was carefully removed to form a 1.0 mm artificial crack. All specimens were left to cure in molds at room temperature for 24 hours, then demolded and labeled for identification as shown in Figure 1. Curing was continued by fully submerging the samples in a water tank for 7 days. Following the curing period, samples were dried under sunlight to eliminate residual surface moisture. Controlled cracking was induced in one of the cylindrical specimens using a compression machine (NL 000 X/006, NL Scientific Instruments Sdn. Bhd., Selangor, Malaysia) by applying a 0.10 kN load over 9 seconds following method from Choi *et al*. [21]. The mechanically induced cracks in cylindrical specimens were further analyzed using ImageJ software to quantify surface damage.

Figure 1. Mortar samples with precast crack preparation

MICP Treatment Method for Crack Repair

Crack remediation was carried out using a combined injection–diffusion method (CIDM), adapted from established MICP protocols [6], as illustrated in Figure 2. The treatment materials included an actively growing broth culture of *Cytobacillus horneckiae*, a 0.5 M cementation solution, pre-cracked cube and cylindrical mortar specimens, sterilized sponge pieces, and sterile syringes (3 mL). The cementation solution was prepared by dissolving 74 g/L calcium chloride and 30 g/L urea in autoclaved distilled water, followed by UV sterilization to prevent microbial contamination. Each treatment cycle consisted of two sequential steps. First, 1 mL of the *C. horneckiae* suspension was carefully injected into the surface cracks using a sterile syringe to promote bacterial colonization within the fissures. Immediately afterward, 2.5 mL of cementation solution was applied using a sterilized sponge placed directly over the crack, allowing passive diffusion of calcium and urea into the fracture network. This dual-step procedure was designed to couple microbial inoculation with nutrient supply, thereby enhancing localized $CaCO_3$ precipitation and promoting crack sealing, as similarly demonstrated in sponge-assisted MICP delivery systems [22]. A total of six cycles were conducted per day, with each cycle consisting of bacterial injection, nutrient application, and a drying interval of approximately 30–45 minutes to facilitate stable carbonate deposition. Treatments were applied daily for 12 consecutive days. At the end of the treatment period, specimens were air-dried and visually inspected for evidence of $CaCO_3$ deposition within the crack voids to assess sealing efficiency.

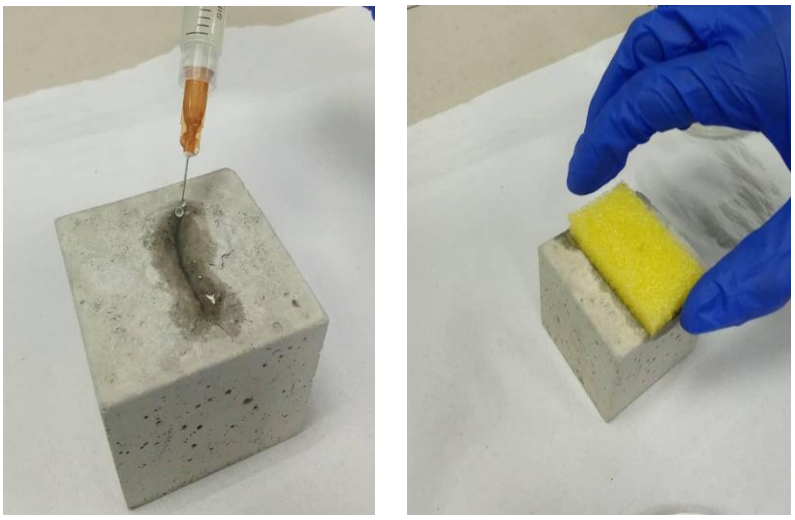

Figure 2. MICP treatment using Combined Injection Diffusion Method (CIDM)

Splitting tensile strength

The mechanical performance of mortar specimens was assessed using the splitting tensile strength test, a standard method for evaluating the tensile capacity of cementitious materials. Both MICP-treated and control (uncracked) cylindrical specimens were cured under identical conditions and measured for dimensional accuracy with a digital caliper prior to testing. The tests were performed using an automatic compression machine (NL 4000X/013, NL Scientific Instruments Sdn. Bhd., Selangor, Malaysia). Each specimen was positioned horizontally between the machine's loading platens, with bearing strips placed along the contact surfaces to ensure uniform load distribution, following ASTM E4 guidelines. A monotonic load was applied at a controlled rate of 0.05–0.08 MPa/s until specimen failure occurred, typically within 1–2 minutes. The peak load (P) at failure was recorded, and the splitting tensile strength (f_{st}) was calculated using the following equation:

$$f_{st} = \frac{2D}{\pi LD}$$

,

where P is the maximum load at failure (N), L is the specimen length (mm), and D is the specimen diameter (mm). The results were used to compare the tensile performance of MICP-treated specimens with that of untreated controls, providing insights into the extent of structural integrity restored by biomineralization.

Fourier-Transform Infrared Spectroscopy (FTIR)

Fourier-Transform Infrared Spectroscopy (FTIR) was performed to characterize the chemical composition of MICP-treated concrete, with emphasis on calcium carbonate ($CaCO_3$) formation. FTIR enables identification of functional groups and mineral phases, thereby providing insights into the chemical processes occurring within the concrete matrix and the microbial precipitates. Concrete specimens treated with Cytobacillus horneckiae were finely ground into powder prior to analysis. Spectra were recorded in the range of 4000–400 cm^{-1}, where characteristic molecular vibrations are typically observed. Particular attention was given to the carbonate bands (~1416 cm^{-1}) corresponding to CO_3^{2-} groups in $CaCO_3$. Additional peaks of interest included the broad O–H stretching vibration (~3390 cm^{-1}), associated with calcium silicate hydrate (C–S–H) and calcium hydroxide [$Ca(OH)_2$], as well as the Si–O stretching region (~990 cm^{-1}), which reflects interactions between $CaCO_3$ and the silicate phases of the cement matrix. This analysis provided qualitative confirmation of functional groups and mineral phases, supporting the role of MICP in enhancing carbonate precipitation and its potential contribution to concrete crack repair.

SEM-EDX Analysis

Scanning Electron Microscopy (SEM) coupled with Energy Dispersive X-ray Spectroscopy (EDX) was employed to investigate the morphology and elemental composition of calcium carbonate ($CaCO_3$)

precipitates within MICP-treated concrete cracks, following approaches widely used in previous MICP studies [11]. Concrete specimens were sectioned from regions containing visible precipitates, cleaned, UV-treated to minimize contamination, and sputter-coated with a thin gold layer to ensure conductivity. SEM imaging was conducted using a Hitachi TM3000 microscope at an accelerating voltage of 5 kV. Micrographs were obtained at ×1000 and higher magnifications to examine the morphology, spatial distribution, and integration of $CaCO_3$ crystals within the cementitious matrix. EDX analysis was subsequently performed on selected regions exhibiting prominent $CaCO_3$ deposits using EDAX APEX software. Elemental spectra and mapping confirmed the presence of calcium (Ca), carbon (C), and oxygen (O) as the dominant constituents of the precipitates, with silicon (Si) also detected as part of the cement matrix. Elemental maps and counts-per-second (CPS) plots verified the localization of $CaCO_3$ within crack voids. Quantitative analysis of the Ca/C ratio confirmed the predominance of calcium carbonate as the principal crystalline phase, consistent with earlier observations of biogenic calcite formation in MICP-treated cementitious materials [4]. The combined SEM–EDX results provided complementary microstructural and compositional evidence, demonstrating that Cytobacillus horneckiae effectively facilitated the deposition of $CaCO_3$ within cracks and contributed to the healing of the concrete matrix.

Results and Discussion

Carbonate Precipitation Capacity and Bacterial Growth

The growth and biomineralization capacity of *Cytobacillus horneckiae* under varying conditions are summarized in Table 1. The OD_{600} of the pure isolate reached 0.987, confirming high cell density and active growth after isolation from agricultural wastewater. This result is consistent with Moqsud and Gochi (2024), who reported that *C. horneckiae* is highly adaptable and resilient under fluctuating environmental conditions, often outperforming commonly used model strains such as *Sporosarcina pasteurii*. The strong growth performance of the isolate therefore establishes a solid foundation for its role in biomineralization and crack-healing applications. The effect of pH on calcium carbonate precipitation showed that mineralization increased with alkalinity, from 0.194 g at pH 7 to 0.235 g at pH 9. This trend supports the understanding that weakly alkaline environments enhance urease activity and promote carbonate nucleation, a phenomenon similarly reported by Soleimanbeigi *et al*. [23]. Since the pore solution of concrete is typically highly alkaline (pH ~12–13), the ability of *C. horneckiae* to achieve peak performance at alkaline conditions suggests compatibility with real cementitious environments.

Urea concentration was also found to influence precipitation yield. Increasing the concentration from 0.50 M to 0.75 M enhanced $CaCO_3$ formation from 0.3103 g to 0.3344 g, with the highest yield (0.3401 g) recorded at 0.78 M. This increase reflects the role of urea as a substrate for urease-mediated hydrolysis, consistent with previous findings that optimized urea dosing enhances carbonate precipitation efficiency [24]. However, the plateau observed beyond 0.75 M suggests potential substrate inhibition and excessive ammonium production, a limitation highlighted by Xiong *et al*. [25].Therefore, 0.75 M appears to be the most effective concentration, balancing efficiency with environmental sustainability. Temperature exerted a significant effect on precipitation yield, which increased from 0.2382 g at 25 °C to 0.2839 g at 35 °C. This is consistent with previous studies showing that ureolytic bacteria exhibit optimum enzymatic activity between 30–37 °C [10]. The finding that *C. horneckiae* achieved maximum precipitation at 35 °C highlights its suitability for application in tropical climates, although further validation under fluctuating or extreme field conditions would strengthen its practical relevance.

Taken together, the results confirm that *C. horneckiae* demonstrates strong growth potential and robust biomineralization performance under conditions relevant to construction materials. The highest efficiency was observed at pH 9, urea concentration of 0.75 M, and temperature of 35 °C. These findings are in agreement with established MICP literature but also highlight the novelty of employing *C. horneckiae*, a native strain with proven adaptability, as an alternative to conventional model organisms. The outcomes not only validate its role as a promising candidate for MICP-based concrete crack repair but also emphasize the broader potential of native isolates in developing sustainable and eco-efficient biomineralization strategies [26].

Table 1. Growth and biomineralization performance of Cytobacillus horneckiae under varying conditions with supporting references

Condition	Parameter Tested	Experimental Setting	Measured Response	Observation	Supporting References
Growth	OD_{600} (after isolation)	Pure *C. horneckiae* culture	0.987	High cell density confirming active growth	[6]
pH effect	Precipitate weight (g)	pH 7	0.194	Moderate precipitation	[11]
	Precipitate weight (g)	pH 8	0.201	Slight increase	-
	Precipitate weight (g)	pH 9	0.235	Maximum precipitation, optimal alkaline condition	[27]
Urea concentration	Precipitate weight (g)	0.50 M	0.3103	Baseline precipitation	[28]
	Precipitate weight (g)	0.75 M	0.3344	Higher yield, optimal substrate availability	[29]
	Precipitate weight (g)	0.78 M	0.3401	Plateau, possible substrate inhibition	[30]
Temperature effect	Precipitate weight (g)	25 °C	0.2382	Lower activity	[31]
	Precipitate weight (g)	30 °C	0.2441	Moderate activity	
	Precipitate weight (g)	35 °C	0.2839	Optimum activity	

Surface Observations

The healing progression of mortar cracks treated with *Cytobacillus horneckiae* was quantitatively assessed using ImageJ software. The initial average crack width of 1.172 mm served as the baseline for performance evaluation. Over 12 days of treatment, progressive $CaCO_3$ deposition resulted in a reduction of the crack area from 34.346 mm² to 3.393 mm² (Figure 3 and 4), corresponding to a surface sealing efficiency of approximately 90.1%. These findings confirm that *C. horneckiae* can rapidly induce surface-level crack closure through microbially induced calcium carbonate precipitation (MICP).

The efficiency of surface sealing can be attributed to microbial ureolysis, which produces carbonate ions that react with calcium to form $CaCO_3$ crystals. Precipitation occurred predominantly at the crack surface, where oxygen and nutrients were abundant, resulting in dense carbonate deposition. Conversely, limited healing was observed in deeper regions of the crack, reflecting restricted bacterial migration and nutrient diffusion. Such depth-dependent repair behavior has been consistently reported in previous MICP crack-healing studies, where surface closure often precedes internal crack sealing [32, 33]

Figure 3 MICP crack repair progress using *Cytobacillus horneckiae* bacteria

Compared to previous investigations utilizing direct injection or immersion techniques, the CIDM approach demonstrated enhanced efficiency in crack healing. For instance, Jiang *et al*. [34] reported only partial crack filling after 28 days using conventional injection methods, whereas the CIDM-based treatment in this study achieved complete surface sealing in just 12 days. This accelerated healing is attributed to the hybrid delivery mechanism of CIDM, which integrates pressure-driven injection with passive diffusion, improving penetration and retention of bacterial and nutrient solutions within the crack matrix. The dual-mechanism delivery ensures more uniform distribution and sustained microbial colonization, thereby expanding the spatial extent of $CaCO_3$ precipitation and enhancing repair efficiency. Although the observed 90% surface repair demonstrates strong potential for impermeability enhancement, the lack of complete depth sealing raises questions about long-term durability. Surface sealing may temporarily protect against ingress of chlorides, sulfates, and moisture, but without internal healing, cracks remain vulnerable to cyclic loading, freeze–thaw damage, carbonation, and sulfate attack. Previous studies emphasize that durable crack remediation requires continuous mineral growth throughout the entire fissure, ensuring structural integrity under prolonged exposure to aggressive environments [9]. Addressing this limitation will be essential for advancing MICP as a practical repair strategy.

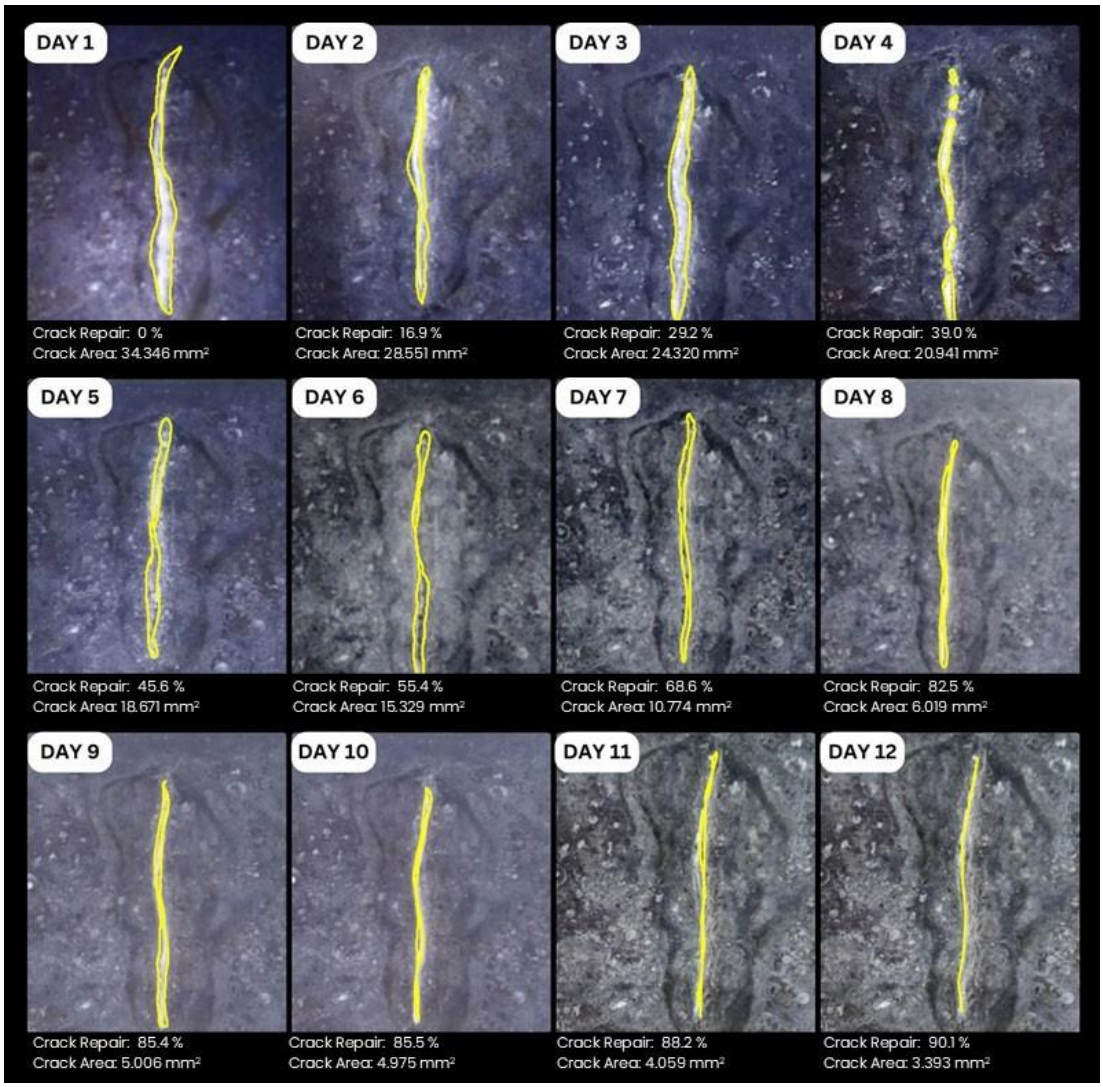

Figure 4 The crack repair progress percentage captured by ImageJ software

From a sustainability perspective, the results highlight the promise of MICP as a bio-based alternative to conventional crack repair techniques such as epoxy injection or cementitious grouting. Unlike chemical sealants, MICP uses relatively low-cost substrates and produces $CaCO_3$ compatible with the host matrix, reducing risks of material incompatibility. However, one limitation is the generation of ammonium as a by-product of ureolysis, which may have environmental implications if not managed appropriately [35]. Future optimization should therefore integrate nutrient formulations with reduced ammonia release and evaluate the overall carbon footprint compared with conventional repair materials, as recommended in recent sustainability assessments [36].

Splitting Tensile Strength

The experimental findings demonstrated that Microbially Induced Calcium Carbonate Precipitation (MICP) using *Cytobacillus horneckiae* effectively restored the structural performance of cracked mortar specimens, as reflected in both ultimate load capacity and tensile strength recovery (Figure 5). The uncracked control specimen sustained the highest ultimate load of 31 kN with a corresponding splitting tensile strength of 8 N/mm², representing the intact structural benchmark. In stark contrast, the cracked specimen without MICP treatment registered only 3 kN and 1 N/mm², respectively, confirming the severe deterioration of both load-bearing capacity and tensile resistance once cracks formed. Remarkably, the cracked specimen treated with *C. horneckiae* achieved 23 kN ultimate load and 6 N/mm² tensile strength, representing approximately 74–75% recovery of the original structural integrity. This outcome is significant, as it underscores the ability of bacterial mineralization to not only seal cracks but also restore

a substantial proportion of the mechanical properties essential for long-term durability. The partial but robust recovery observed here is in line with other MICP studies, which commonly report strength restoration in the range of 70–90% depending on the bacterial strain, treatment protocol, and crack geometry [37]. In particular, our results are consistent with the recovery efficiencies documented by Nasser *et al*. [4] and Omoregie *et al*. [5], who emphasized that although complete restoration is rarely achieved due to incomplete penetration and heterogeneous calcite precipitation, the recovered strength is mechanically meaningful for practical applications. In the present study, the performance of *C. horneckiae* is particularly notable, given that it is a relatively underexplored species compared with the widely studied urease bacterstrain *Sporosarcina pasteurii*. The comparable efficiency suggests that *C. horneckiae* possesses sufficient ureolytic activity to induce substantial calcite deposition, thereby expanding the range of microbial candidates available for sustainable construction biotechnology.

Mechanistically, the restoration can be attributed to the precipitation of calcium carbonate crystals within the crack network, which acted as mineral bridges connecting fractured surfaces. This biomineralization increased cohesion across the crack plane and limited further propagation under load. Urease activity by *C. horneckiae* hydrolyzed urea into ammonium and carbonate ions, elevating the local pH and creating favorable conditions for calcite nucleation [38]. As crystals grew, they gradually filled voids and bonded with the surrounding cementitious matrix, enhancing tensile resistance. Nonetheless, the incomplete recovery (relative to the control specimen) highlights limitations such as uneven bacterial distribution, localized variations in pore structure, and restricted penetration depth of the treatment solution. Similar limitations were reported by Soleimanbeig *et al*. [39], who noted that inconsistencies in crystal growth and adhesion at deeper crack regions often reduce overall efficiency. From an engineering perspective, achieving ~75% recovery of both ultimate load and tensile strength is highly significant. It implies that MICP treatment can extend service life and reduce the need for repeated maintenance, thereby lowering lifecycle costs of infrastructure. Moreover, the approach aligns with the principles of sustainable and green engineering, as it reduces reliance on energy-intensive or chemical-based repair materials. Recent studies have highlighted that even partial restoration of tensile strength considerably enhances durability against subsequent environmental and mechanical stresses [40]. Therefore, while the repaired specimens did not fully match the intact benchmark, the high degree of mechanical restoration supports the viability of MICP as a reliable bio-based crack healing strategy.

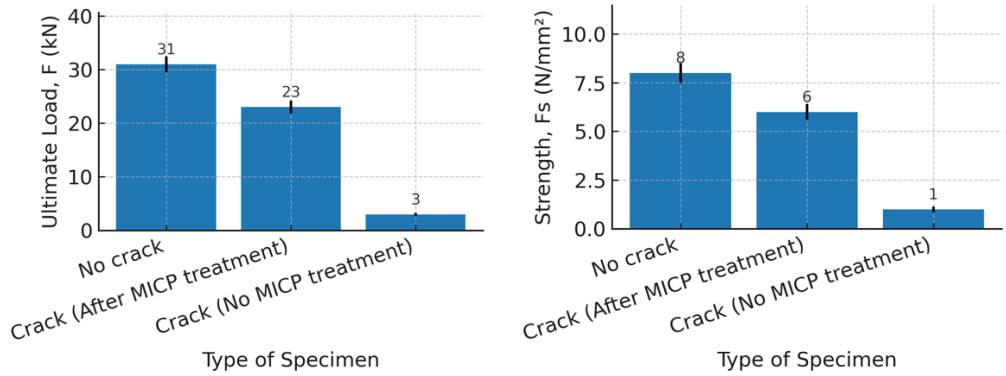

Figure 5 Comparison splitting tensile strength and ultimate load between no crack (control), cracked (after MICP treatment), cracked (No MICP treatment)

FTIR Analysis

Fourier Transform Infrared (FTIR) spectroscopy was performed to identify the functional groups associated with the calcium carbonate precipitate produced by *Cytobacillus horneckiae*. The spectral profile, shown in Figure 6, indicates the co-existence of organic biomolecules and inorganic carbonate, both of which are integral to the microbial-induced calcium carbonate precipitation (MICP) process. A broad absorption band observed at 3371.56 cm^{-1} corresponds to O–H stretching vibrations, commonly associated with hydroxyl groups in bacterial extracellular polymeric substances (EPS) and cement hydration products. The peak at 2925.86 cm^{-1} represents C–H stretching, likely originating from aliphatic components of proteins and polysaccharides within the bacterial EPS. These organic functional groups suggest microbial activity in the precipitate formation, consistent with previous reports that highlighted the role of EPS in providing nucleation sites for mineral crystallization [41]. Notable peaks at 2584.37 cm^{-1} and 2318.61 cm^{-1} indicate asymmetric CO_2 stretching, which are residual signatures of urease-catalyzed urea hydrolysis. This is a crucial step in MICP, as CO_2 generation and subsequent pH elevation create favorable conditions for $CaCO_3$ supersaturation and precipitation. Such peaks have also

been observed by Ojha *et al*. [42] who emphasized the importance of temporary CO_2 entrapment in biofilms to initiate carbonate crystallization.

The absorption bands at 1793.76 cm^{-1} and 1634.12 cm^{-1} correspond to C=O stretching and the amide I band, respectively. These are associated with protein secondary structures, particularly urease and related enzymes that catalyze urea hydrolysis. Their presence provides strong evidence of microbial involvement in the mineralization process. Similar findings were reported by Dhami *et al*. [43], linking amide I bands to ureolytic enzymatic activity that accelerates $CaCO_3$ precipitation. The strong carbonate absorption bands observed at 1412.51 cm^{-1}, 1000.99 cm^{-1}, and 873.16 cm^{-1} are characteristic of calcite, the most stable polymorph of calcium carbonate. The band at 1412.51 cm^{-1} corresponds to asymmetric stretching of carbonate (CO_3^{2-}), while the 873.16 cm^{-1} peak indicates out-of-plane bending vibrations specific to calcite. Additional lattice vibrations observed at 775.78 cm^{-1}, 712.25 cm^{-1}, and 694.53 cm^{-1} further confirm the predominance of calcite as the precipitated phase. The dominance of calcite, rather than metastable forms such as vaterite, implies enhanced mechanical stability and durability of the MICP treatment, consistent with findings by Krajewska [44]. Together, these FTIR results confirm that *C. horneckiae* facilitated the precipitation of $CaCO_3$ via ureolytic activity, with clear evidence of both organic microbial components (proteins, polysaccharides) and inorganic carbonate phases. The detection of calcite as the principal polymorph underscores the potential of this bacterium to generate mechanically robust and durable mineral deposits within cracks. Moreover, the alignment of these spectral features with prior studies reinforces the conclusion that MICP represents a biologically driven yet structurally effective solution for sustainable concrete crack repair.

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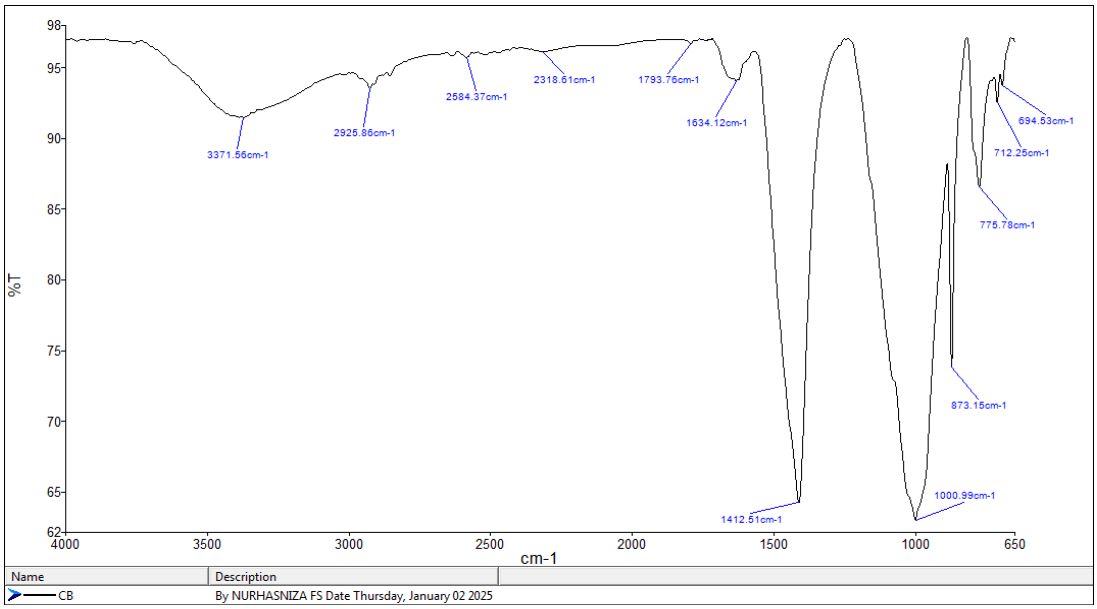

Figure 6. Fourier Transformed Infrared Spectroscopy (FTIR) spectrum

SEM and EDX Analysis

The SEM micrographs (Figure 7) provide direct evidence of microbially induced calcium carbonate ($CaCO_3$) precipitation within the cracks of concrete treated with *Cytobacillus horneckiae*. At lower magnifications (Figures 8a and 8b, ×150 and ×1.5k), the surface appears densely covered with fine, granular deposits, suggesting widespread nucleation sites facilitated by bacterial activity. The relatively uniform dispersion of particles across the crack surface indicates effective bacterial colonization and EPS-mediated nucleation, which has been reported in earlier studies as a critical step for initiating $CaCO_3$ precipitation on heterogeneous concrete substrates [45]. At higher magnifications (Figures 8c and 8d, ×5k), the precipitates are observed as irregular and angular crystallites, consistent with early-stage calcite formation. The presence of these micro-crystals suggests rapid nucleation but limited crystal growth, a feature typical of MICP where the precipitation rate is governed by ureolysis kinetics and local supersaturation conditions [46]. The irregular morphologies also point to spatial confinement within the crack micro-environment, restricting crystal development into larger faceted structures. This phenomenon has also been noted in studies where limited diffusion of ions within cracks resulted in finer, clustered $CaCO_3$ morphologies (Cheng *et al*., 2023).

The identification of discrete $CaCO_3$ crystals (Figure 8c) confirms that *C. horneckiae* was able to promote carbonate precipitation effectively, thereby contributing to crack infilling. However, the fine powdery and loosely bound nature of the precipitates suggests that sealing is primarily concentrated at the surface level rather than forming dense interlocking crystals within deeper zones of the crack. This observation aligns with the surface-sealing trend also reported by Fan *et al*. [47], where microbial precipitation improved impermeability but did not achieve complete structural restoration.

Overall, the SEM results validate the capacity of *C. horneckiae* to induce $CaCO_3$ precipitation and initiate crack sealing. The fine, irregular morphology of the crystals highlights the rapid, surface-driven nature of the repair mechanism, which enhances impermeability but may limit long-term mechanical reinforcement. To overcome this limitation, future work should investigate delivery enhancements—such as encapsulated bacteria, bio-carriers, or pressure-assisted injection to enable deeper ion penetration and promote the growth of more cohesive crystal networks, thereby ensuring both sealing efficiency and structural durability.

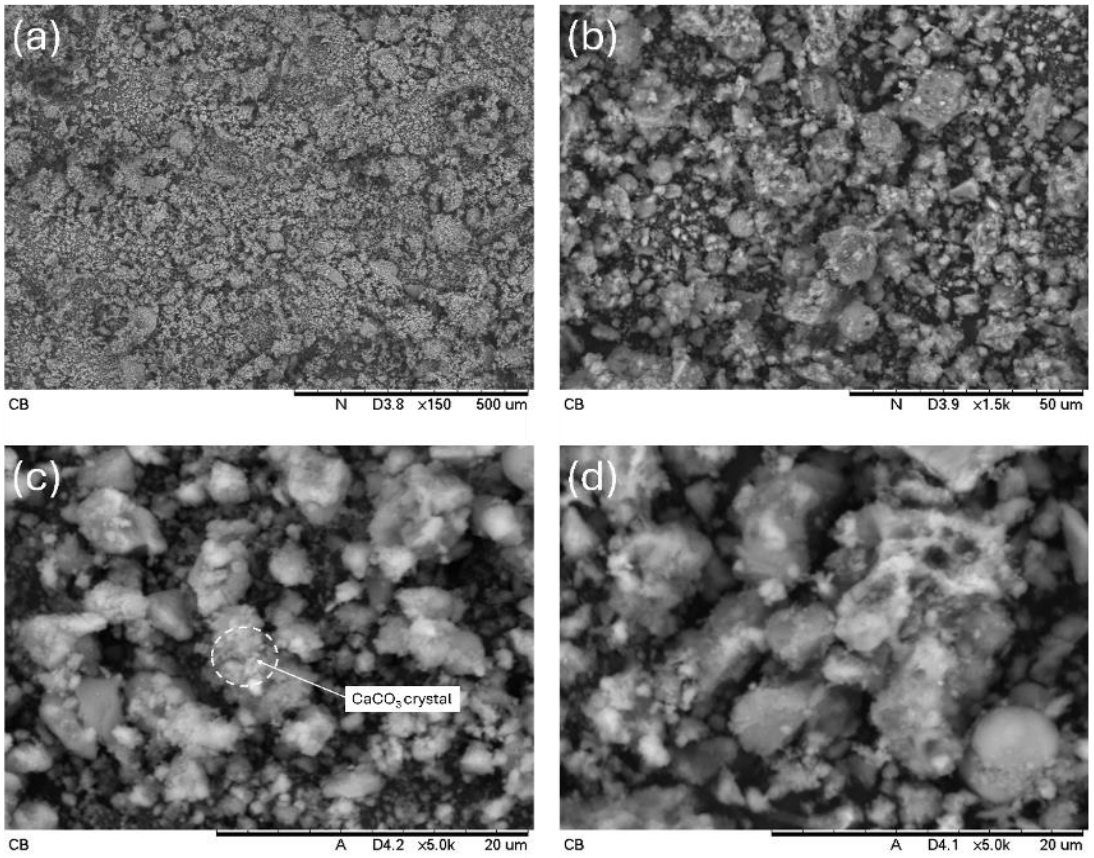

Figure 7. SEM images of white precipitates extracted from the treated crack, showing fine, granular precipitates at varying magnifications

The elemental composition of the precipitates analyzed by Energy Dispersive X-ray Spectroscopy (EDX) (Figure 8; Table 2) provides strong evidence of calcium carbonate ($CaCO_3$) formation induced by *Cytobacillus horneckiae*. Oxygen (O) was the most abundant element, with a weight percentage of 66.87% and an atomic percentage of 82.74%. This dominant oxygen signal, coupled with the presence of calcium (Ca) and trace carbon (C), confirms that the precipitate was primarily composed of carbonate phases. The relatively high oxygen concentration is characteristic of $CaCO_3$ crystallization, as previously reported in microbial biomineralization studies [48]. Calcium accounted for 28.91% by weight and 14.28% by atomic fraction, further supporting the dominance of calcium carbonate within the mineralized matrix. The elevated calcium content highlights the efficiency of microbial ureolysis in mobilizing calcium ions from the cementation solution and incorporating them into stable carbonate lattices. This observation aligns with earlier findings by Lekundayo [49], who demonstrated that bacterial urease activity accelerates Ca^{2+} capture and subsequent $CaCO_3$ deposition within crack voids.

Silicon (Si) was detected at a lower concentration (4.22 wt%), which is most likely derived from the concrete matrix rather than from bacterial activity. The incorporation of Si signals suggests that the precipitates were formed in intimate contact with the underlying cementitious substrate, where silicate minerals may have been partially embedded in the carbonate layer. Such substrate–precipitate interactions have also been documented in previous studies, where bacterial carbonate overlayers incorporated trace amounts of cementitious components during crack sealing [50]. Interestingly, the carbon (C) signal appeared negligible (0.00 wt%, 0.01 at%). While this may seem counterintuitive for a carbonate phase, it is important to recognize that EDX often underestimates light elements such as carbon due to detector limitations and surface charging effects [51]. The absence of a strong carbon peak does not negate the presence of $CaCO_3$ but instead reflects the dominance of heavier Ca and O atoms in the spectra. This interpretation is reinforced by the SEM observations of granular $CaCO_3$ deposits, confirming that carbonate crystallization was indeed the primary process.

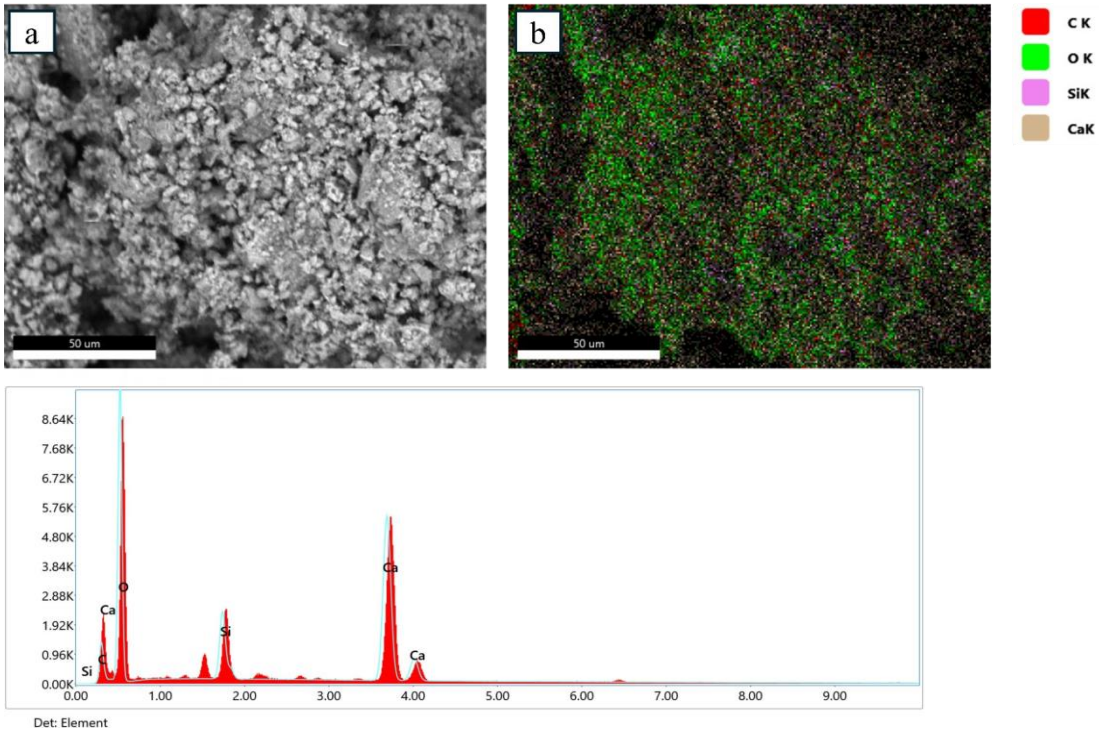

Figure 8. Energy Dispersive X-ray (EDX) results for elemental, (a) composition, (b) Element overlay (c) Sum spectrum

Table 2. Elemental composition of precipitates formed after MICP treatment in the crack

Element	Weight %	Atomic %	Error %
C K	0.00	0.01	99.99
O K	66.87	82.74	10.31
Si K	4.22	2.97	5.61
Ca K	28.91	14.28	2.63

Conclusion

This study demonstrated the effectiveness of *Cytobacillus horneckiae* in promoting microbially induced calcium carbonate precipitation (MICP) for concrete crack repair. Laboratory investigations confirmed that carbonate precipitation was strongly influenced by environmental parameters, with optimal conditions achieved at pH 9, 0.75 M urea concentration, and 35 °C. Under these conditions, the bacterium facilitated rapid calcium carbonate formation, leading to a 90% reduction in surface crack area within 12 days. Mechanical testing further revealed that MICP treatment restored approximately 75% of the tensile strength and ultimate load capacity, indicating significant but partial recovery of structural integrity. Microstructural and compositional analyses provided additional validation. FTIR spectra confirmed the presence of calcite polymorphs, while SEM–EDX analyses revealed fine, irregular $CaCO_3$ crystals composed predominantly of calcium and oxygen, with minor silicon incorporation from the concrete matrix. These results establish *C. horneckiae* as an effective biomineralizing agent capable of sealing cracks through surface-level precipitation. However, the predominance of shallow deposition highlights a key limitation: incomplete sealing of crack depth, which may affect long-term durability. Overall, this work expands the scope of MICP-based repair by introducing *C. horneckiae* as a novel bacterial candidate for sustainable crack remediation. While the findings underscore its potential as an environmentally friendly alternative to conventional repair methods, further optimization is required to enhance bacterial penetration, improve crystal cohesion, and ensure long-term durability under service conditions. Future research should focus on delivery strategies such as encapsulation, bio-carriers, or pressure-assisted injection, as well as long-term performance testing under variable environmental stresses.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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