MJFAS
Malaysian Journal of Fundamental and Applied Sciences

Basri *et al.* | Malaysian Journal of Fundamental and Applied Sciences, Vol. 21 (2025) 3019–3038

RESEARCH ARTICLE

Sustainable Concrete Restoration Using Microbial-Induced Carbonate Precipitation Technology: Insights from Laboratory and Field Applications

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Abstract Concrete structures are vulnerable to cracking from mechanical, chemical, and environmental stresses, which compromise durability and increase maintenance costs. Conventional repair methods, such as epoxy injection and cementitious grouting, often depend on non-renewable materials and contribute to environmental waste. This study explores Microbially Induced Calcium Carbonate Precipitation (MICP) using *Sporosarcina pasteurii* as a sustainable approach for repairing cracks in cement-based materials. Laboratory experiments were conducted on mortar samples with 1.0 mm predefined cracks, while in-situ trials were performed on cracked cement surfaces at UTMSPACE. Treated samples recovered up to 88.1% of their original tensile strength (19.59 kN compared to 22.24 kN for controls), confirming the structural reinforcement potential of MICP. SEM–EDX analysis showed uniform calcium carbonate deposition along the crack surfaces, while FTIR spectroscopy confirmed biomolecular signatures linked to calcite formation. Water absorption tests revealed a significant reduction in permeability, with secondary absorption rates decreasing from 0.0206 mm/√s to 0.0009 mm/√s, indicating enhanced durability. Field application validated the practicality of the method, achieving visible crack sealing and mineral deposition within one working day per treated area. These results highlight MICP as a viable, eco-friendly alternative to conventional repair strategies, offering both mechanical and environmental benefits. The findings support the integration of MICP into sustainable infrastructure maintenance practices.

Keywords: Microbially-Induced Calcium Carbonate Precipitation, *Sporosarcina pasteurii*, Concrete Crack Repair, Water Absorption Rate, Structural Restoration.

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Received: 19 August 2025
Accepted: 21 Oct. 2025

Introduction

Concrete remains the most widely used construction material globally, forming the structural backbone of buildings, bridges, and transportation infrastructure. Despite its superior compressive strength and long service life, concrete is inherently susceptible to cracking. These cracks are typically caused by thermal stresses, freeze-thaw cycles, shrinkage, chemical reactions (such as alkali-silica reactions), and various environmental exposures. While initially microscopic, such fissures pose significant durability concerns as they facilitate the ingress of aggressive agents like water, chlorides, sulfates, and carbon dioxide. The result is often accelerated corrosion of embedded steel reinforcement, leading to reduced structural capacity, serviceability deterioration, and ultimately, costly repairs or premature structural failure [1]. Globally, the economic burden of concrete repair and rehabilitation is staggering. Billions are spent annually on structural maintenance, with crack remediation constituting a substantial portion of this expenditure. Traditional methods such as epoxy injections, cementitious grouting, and polyurethane sealing have been extensively used due to their immediate efficacy in restoring integrity [2]. However, these methods are not without drawbacks. They often rely on petroleum-based or high-energy materials, generate carbon emissions during production and application, and can leave behind non-biodegradable residues [3]. Furthermore, the performance of these conventional repair materials often varies significantly under harsh environmental conditions such as sustained moisture ingress, freeze–thaw cycles, and chemical exposure which can lead to inconsistent adhesion, premature degradation, and subsequent variability in the structural integrity of repaired concrete. As sustainability becomes a central pillar of modern construction, these limitations highlight the urgency for alternative crack-healing technologies that are not only effective but also environmentally aligned [4]. One such emerging solution is Microbially Induced Calcium Carbonate Precipitation (MICP). MICP is a bio-mediated technique that harnesses the metabolic processes of specific bacteria, most notably *Sporosarcina pasteurii*, to induce the precipitation of calcium carbonate ($CaCO_3$). *S. pasteurii* was selected in this study due to its exceptionally high urease activity, which enables rapid urea hydrolysis and efficient $CaCO_3$ formation. It is a non-pathogenic, well-characterized bacterium that has been widely employed in MICP studies, known for its stability under laboratory and field conditions, ease of cultivation, and high precipitation efficiency. These attributes make it one of the most reliable and effective bacterial species for engineering applications in concrete crack repair. The MICP process involves the hydrolysis of urea by the bacterial urease enzyme, producing ammonium and carbonate ions. [5, 6]. When calcium ions are introduced, the carbonate ions react to form $CaCO_3$, which precipitates and deposits within cracks and pores, effectively bonding and sealing the damaged regions. Unlike synthetic polymers, MICP does not rely on non-renewable materials or high-temperature reactions, making it a low-carbon and sustainable alternative [7]. Moreover, the biogenic nature of the precipitate offers improved compatibility with cementitious matrices, reducing the risk of debonding or incompatibility issues often observed with synthetic fillers.

The benefits of MICP have been well-documented in laboratory settings. Numerous studies have reported significant improvements in both compressive and tensile strength after MICP treatment. For example, Kulkarni *et al*. [8] demonstrated that MICP-treated concrete specimens could regain up to 90% of their original strength after crack formation. Similarly, Gao *et al*. [9] observed a marked reduction in water absorption and permeability, indicating enhanced durability. SEM analyses have consistently confirmed the presence of calcite crystals bridging crack interfaces, while XRD and FTIR spectroscopy have validated the mineralogical identity of the precipitates. Moreover, MICP has been found to increase resistance against sulfate attack and freeze-thaw deterioration, further supporting its applicability in aggressive service environments. Recent advancements have also explored the integration of genetically enhanced bacterial strains to improve precipitation efficiency and environmental tolerance [10]. While laboratory studies establish the foundational efficacy of MICP, translating this success to real-world applications remains a challenge. Field-scale demonstrations are limited but growing. One notable example is the work by De Muynck *et al*. [11], who applied MICP to a historic masonry wall in Belgium. Their results showed not only effective sealing of cracks but also the enhancement of surface strength and reduction in water ingress. Another field study by Kevin *et al*. [12] tested MICP on reinforced concrete beams exposed to marine environments. Over a four-month monitoring period, the treated sections exhibited significantly lower chloride penetration compared to untreated controls, highlighting the potential for MICP in coastal and marine infrastructure. These field validations are essential for demonstrating the robustness and repeatability of MICP under diverse environmental conditions.

Nevertheless, critical gaps remain in the operationalization of MICP for real-world infrastructure applications. Most existing research has been confined to small-scale, laboratory-based experiments, with limited demonstration of efficacy under field conditions. Key challenges including bacterial viability in non-sterile environments, control over mineral morphology and distribution, and process scalability continue to constrain broader implementation [13]. Additionally, while discrete delivery mechanisms such

as injection or spraying have been investigated, the potential of combined approaches, such as the injection-diffusion method, remains underexplored in the context of optimizing calcium carbonate deposition within complex crack geometries [14]. To address some of these challenges, this study investigates the use of a Combined Injection-Diffusion Method (CIDM) to deliver *Sporosarcina pasteurii* and cementation solutions into cracked cement and mortar samples. Unlike single-mode injection or spraying techniques, CIDM integrates pressure-driven delivery with passive diffusion, allowing more effective penetration of bacteria and nutrients into microcrack networks. This method aims to promote more uniform calcium carbonate deposition and stronger bonding across fractured zones . The research comprises both laboratory-controlled experiments and field trials. In the laboratory phase, cracked mortar specimens are inoculated with bacterial solutions, followed by the application of calcium-rich nutrients. The development of calcium carbonate within the crack matrix is monitored using SEM imaging, optical microscopy, and mechanical strength testing. In the field component, MICP treatment is applied to degraded concrete sections of an actual infrastructure element—such as a pavement slab or retaining wall. Performance metrics include visual crack closure, rebound hammer strength, and water permeability before and after treatment. Overall, this study explores Microbially Induced Calcium Carbonate Precipitation (MICP) using *Sporosarcina pasteurii* as a sustainable approach for repairing cracks in cement-based materials. Laboratory experiments were conducted on mortar samples with 1.0 mm predefined cracks, while in-situ trials were performed on cracked cement surfaces at UTMSPACE.

Materials and Methods

Preparation of *Sporosarcina pasteurii*

The urease-producing bacterium *Sporosarcina pasteurii DSM 33* was prepared following method from Kulkarni *et al*. [8]. The bacterium was preserved in glycerol stock at -80°C, was revived to prepare active cultures for experimental use. Under aseptic conditions, the glycerol stock was streaked onto UV-sterilized nutrient agar plates supplemented with 30 g/L of UV-treated urea. The plates were incubated at 32°C for 24–48 hours to allow colony formation, and non-inoculated control plates were used to confirm sterility. A single, well-isolated colony from the second streak plate was selected and inoculated into 25 mL of sterilized nutrient broth (13 g/L) containing 10 g/L of ammonium chloride and 30 g/L of UV-sterilized urea, with the pH adjusted to 8.0. The culture was incubated at 32°C and shaken at 125 rpm for 24–48 hours to promote bacterial growth, indicated by increasing turbidity. To ensure sufficient biomass for biomineralization, the culture was gradually scaled up using a stepwise sub-culturing method. A 1:9 transfer ratio was applied, first into 50 mL and subsequently into 125 mL of fresh broth under identical growth conditions. This process ensured bacterial adaptation and optimized cell density for subsequent MICP applications.

Carbonate Precipitation Capacity and Bacterial Growth Monitoring

The carbonate precipitation capacity of *Sporosarcina pasteurii* 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 total of 648.6 g of cement and 1621.6 g of sand were dry-mixed in a large container to achieve uniform distribution. Subsequently, 259.5 mL of water was gradually added over five minutes while mixing to obtain a homogeneous and workable mortar. The fresh mortar was then cast into lightly oiled plastic

molds to form two specimen geometries: cubes (50 mm × 50 mm × 50 mm) and cylinders (100 mm height × 50 mm diameter). Compaction was carried out using a concrete vibrating table (MTM Precision Sdn Bhd, Selangor, Malaysia) to eliminate entrapped air and ensure good consolidation. Two types of cracks were introduced: controlled cracks in cube specimens and uncontrolled cracks in cylindrical specimens. Controlled cracks were created by embedding a 1 mm × 30 mm × 30 mm aluminium foil strip at the mid-depth of one cube mold during compaction. After two hours of initial setting, the foil was carefully removed to form a uniform 1.0 mm artificial crack, ensuring consistent crack geometry across all cube samples. In contrast, uncontrolled cracks were generated in cylindrical specimens using a compression machine (NL 000 X/006, NL Scientific Instruments Sdn. Bhd., Selangor, Malaysia). A load of 0.10 kN was applied over nine seconds to induce cracking along the specimen surface, producing irregular patterns that simulate realistic field crack development. All specimens were left to cure in molds at room temperature for 24 hours, demolded, and labeled for identification as shown in Figure 1. Curing was then continued by submerging the specimens fully in a water tank for seven days. After curing, the samples were dried under sunlight to remove residual surface moisture. The mechanically induced cracks in the cylindrical specimens were analyzed using ImageJ software to quantify crack geometry, including width, length, and estimated area, as summarized in Table 1. Cylinder 1 and Cylinder 2 exhibited distinct crack characteristics due to controlled variations in the applied compressive load during cracking. Cylinder 1 was subjected to a slightly higher load, resulting in wider and darker cracks, which indicate larger crack openings and greater surface damage. In contrast, Cylinder 2 experienced a lower applied load, leading to narrower and lighter cracks, corresponding to less severe surface damage. These intentional variations were introduced to simulate different levels of crack severity, enabling the evaluation of the MICP treatment's performance under varying crack conditions that resemble real field scenarios. Cracked and uncracked specimens were subsequently transported to the Environmental Laboratory at Universiti Teknologi Malaysia (UTM) for treatment and further testing.

Figure 1. Mortar samples with precast crack preparation

Table 1. Average crack measurements for cube specimens analyzed using Image J.

Specimen	Crack Width (mm)	Crack Length (mm)	Estimated Crack Area (mm²)
Cube 1	1.172	25	29.30
Cube 2	1.138	25	28.45
Cube 3	1.154	25	28.85
Cylinder 1	0.340	41	13.94
Cylinder 2	0.420	44	18.48
Cylinder 3	0.480	46	22.08

MICP Treatment Method for Crack Repair

The crack repair process using Microbially Induced Calcium Carbonate Precipitation (MICP) was performed using a combined injection and diffusion method (CIDM) as illustrated in Figure 2. The materials prepared for the treatment included *Sporosarcina pasteurii* broth culture, a 0.5 M cementation solution, cracked cube and cylindrical mortar specimens, a 3 mL syringe, and sterilized sponge pieces. Each treatment cycle began by injecting 1 mL of the active S. pasteurii culture directly into the cracks using the syringe, ensuring thorough bacterial penetration into the fissures. This was followed by the application of 2.5 mL of the cementation solution, dispensed onto a sponge and placed over the crack to allow passive diffusion of calcium ions. The procedure combined bacterial inoculation with nutrient delivery, enhancing calcium carbonate precipitation within the crack voids. A total of six treatment cycles were performed, with each cycle consisting of bacterial injection followed by nutrient application and a drying interval of 30–45 minutes to ensure stable deposition. This process was repeated daily over 14 consecutive days. Upon completion, treated specimens were visually inspected, and the extent of calcium carbonate formation within the cracks was recorded to assess the sealing efficiency.

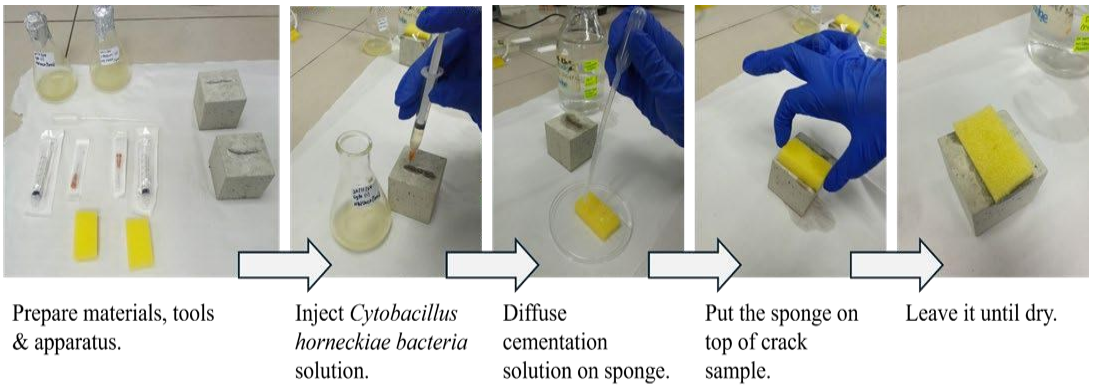


Figure 2. MICP treatment using Combined Injection Diffusion Method (CIDM)

Splitting Tensile Strength

The mechanical performance of the mortar samples was evaluated using the splitting tensile strength test. Both treated and control (uncracked) cylindrical specimens were cured according to protocol and measured for dimensional accuracy using calipers prior to testing. Testing was conducted using an automatic compression machine (NL 4000X/013, NL Scientific Instruments Sdn. Bhd., Selangor, Malaysia). Each specimen was placed horizontally between the loading platens, with bearing strips applied on both contact surfaces in accordance with ASTM E4 standards. A uniform load was applied at a rate of 0.05–0.08 MPa/s until failure occurred within 1–2 minutes. The peak load (P) at failure was recorded, and splitting tensile strength f_{st} was calculated using the standard equation:

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

where *P* is the maximum load (N), *L* is the length (mm), and *D* is the diameter (mm) of the specimen. Results were used to compare the structural integrity restored by MICP treatment relative to uncracked specimens.

Water Absorption Rate Test

Water permeability was assessed following ASTM C1585 (2020) using a sorptivity test. Treated and control samples were preconditioned at 50 ± 2°C for 72 hours, followed by storage at 23 ± 2°C in sealed containers for at least 15 days. On the testing day, samples were weighed with 0.01 g precision using a Shimadzu, Japan ELB3000 balance. Lateral surfaces were sealed, exposing only one circular surface for water contact. Tap water was maintained at 1–3 mm above the sample base during testing. Mass gain was recorded at intervals of 1, 5, 10, 20, 30, and 60 minutes during the first hour, then hourly for 6 hours and daily up to 7 days. Water absorption (mm) was calculated based on mass gain, surface area, and water density. Results were used to determine initial and secondary absorption rates, allowing comparison of permeability reductions due to MICP treatment.

Material Characterization Using FTIR, SEM, and EDX

The formation and chemical composition of calcium carbonate precipitates produced by *Sporosarcina pasteurii* were characterized using Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron

Microscopy (SEM), and Energy-Dispersive X-ray Spectroscopy (EDX). FTIR analysis was performed with an ALPHA II spectrometer (Bruker) to detect functional groups associated with carbonate compounds and microbial metabolites. Dried samples were scanned within the 4000–400 cm^{-1} range at a 4 cm^{-1} resolution over 16 scans. Spectral peaks corresponding to hydroxyl (O–H), amine (N–H), carbonyl (C=O), and carbonate (CO_3^{2-}) groups confirmed the presence of calcium carbonate and bio-organic residues involved in the precipitation process. SEM and EDX analyses were performed to investigate the morphology and composition of calcium carbonate precipitates formed through microbial treatment. Samples for SEM/EDX were collected after the tensile strength test, ensuring that the observed microstructure represented realistic post-mechanical conditions. Following testing, visible $CaCO_3$ deposits from the crack voids and fracture surfaces were carefully scooped and collected for analysis. The collected materials were oven-dried at 40 °C for 24 hours to remove residual moisture before being mounted on aluminum stubs and sputter-coated with gold to enhance conductivity.Surface morphology of the mineral deposits was examined using a TM4000 SEM (Hitachi). Samples were cleaned, dried, mounted on conductive stubs, and coated with gold to enhance image resolution. Micrographs taken at magnifications ranging from 150x to 5000x revealed compact and uniformly distributed calcium carbonate crystals filling the crack voids. The crystal structure suggested effective bonding between the precipitate and the mortar surface, indicating successful repair. Elemental composition was further assessed using EDX analysis integrated with SEM. The spectra showed dominant peaks for calcium and oxygen, while carbon was present only in trace amounts, likely due to environmental contamination. Elemental mapping confirmed a homogeneous distribution of Ca and O across the treated regions, verifying the purity and uniformity of the precipitated $CaCO_3$.

In-situ Field Application of MICP Treatment

An in-situ application of Microbially Induced Calcium Carbonate Precipitation (MICP) was carried out to evaluate the field implementation of the treatment protocol. The selected site was located at UTMSPACE, Johor Bahru (Latitude: N 1° 33.506′, Longitude: E 103° 38.302′), where two visibly cracked cement surfaces were identified and marked for treatment. The same combined injection and diffusion method (CIDM) used in the laboratory was applied in the field. Each treatment involved the preparation of 50 mL of *Sporosarcina pasteurii* broth culture and 1 L of a 0.5 M cementation solution, consisting of UV-sterilized calcium chloride and urea dissolved in autoclaved distilled water. For each crack, 5 mL of the bacterial culture was injected directly into the fissures using a 5 mL syringe. Immediately following the injection, 12.5 mL of the cementation solution was applied by saturating sterilized sponge strips and placing them over the treated area to promote calcium ion diffusion into the cracks. Each treatment cycle was followed by a resting period of 30–45 minutes to allow for microbial activity and precipitation. Each crack area received six consecutive treatment cycles within the same day, with one full day allocated per crack. Including preparation, injection, and drying intervals of 30–45 minutes per cycle, the estimated duration of treatment per crack was approximately 4 to 6 hours. The complete in-situ procedure was completed over a span of two days. Upon completion of the treatment cycles, the repaired surfaces were documented and prepared for subsequent analysis alongside the laboratory samples.

Results and Discussion

Carbonate Precipitation Capacity and Bacterial Growth

The carbonate precipitation ability of *Sporosarcina pasteurii* was confirmed through visible mineral formation and quantitative analysis. Precipitation was observed within 30 minutes of introducing the bacterial culture to the 0.5 M cementation solution, producing a final dried precipitate mass of 0.55 g. The pH of the supernatant measured at 8.05 further supported active ureolysis, which increases alkalinity and promotes calcium carbonate ($CaCO_3$) formation. These findings align with Carter *et al*. [15], who noted that *S. pasteurii* thrives in mildly alkaline environments and precipitates significant amounts of $CaCO_3$ within similar timeframes under optimal urea and calcium chloride concentrations. Optical density (OD_{600}) analysis of the culture yielded a value of 0.989, indicating a high cell density. This is critical, as previous studies Li *et al*., [16] have shown that urease activity and carbonate precipitation scale positively with viable cell concentration up to saturation limits. High bacterial density ensures sufficient production of carbonate and ammonium ions through urea hydrolysis, a prerequisite for rapid nucleation and mineral growth. Furthermore, the relatively fast onset of precipitation and substantial mass suggests that the bacterial cells likely possessed active nucleation sites, consistent with observations by Intarasoontron *et al.* [17] on cell-surface-induced mineralization .

Surface observations

The surface healing and crack closure of both cube and cylindrical mortar specimens treated with Microbially Induced Calcium Carbonate Precipitation (MICP) using the Combined Injection and Diffusion

Method (CIDM) were visually monitored and are presented in Figure 3 for cube sample and Figure 4 for cylindrical sample. The healing process was evaluated and analysed using J software over the 14-day period (Figure 5), revealing a progressive and systematic deposition percentage of calcium carbonate within the cracks. Mineralization was observed to initiate at the lower regions of the fissures and gradually advanced upward, a pattern likely influenced by gravity-assisted sedimentation of the bacterial solution and calcium source. This directional healing is consistent with earlier MICP studies, such as Shen *et al*. [18], which also noted that $CaCO_3$ tends to accumulate first at the base of vertical cracks before filling laterally and upwards. By Day 5, untreated control specimens showed little to no change in surface appearance, with cracks remaining visibly open and unaltered. In contrast, the MICP-treated specimens exhibited early signs of crack bridging, with partial filling and a reduction in crack width. By Day 10, significant crack coverage was observed in the treated samples, indicating sustained microbial activity and ongoing mineral deposition. By Day 14, the cracks were fully sealed, and the surface was uniformly coated with white calcite precipitates, confirming the completion of the healing cycle.

Figure 3. MICP crack repair progress using *Sporocarcina Pasteurii* bacteria

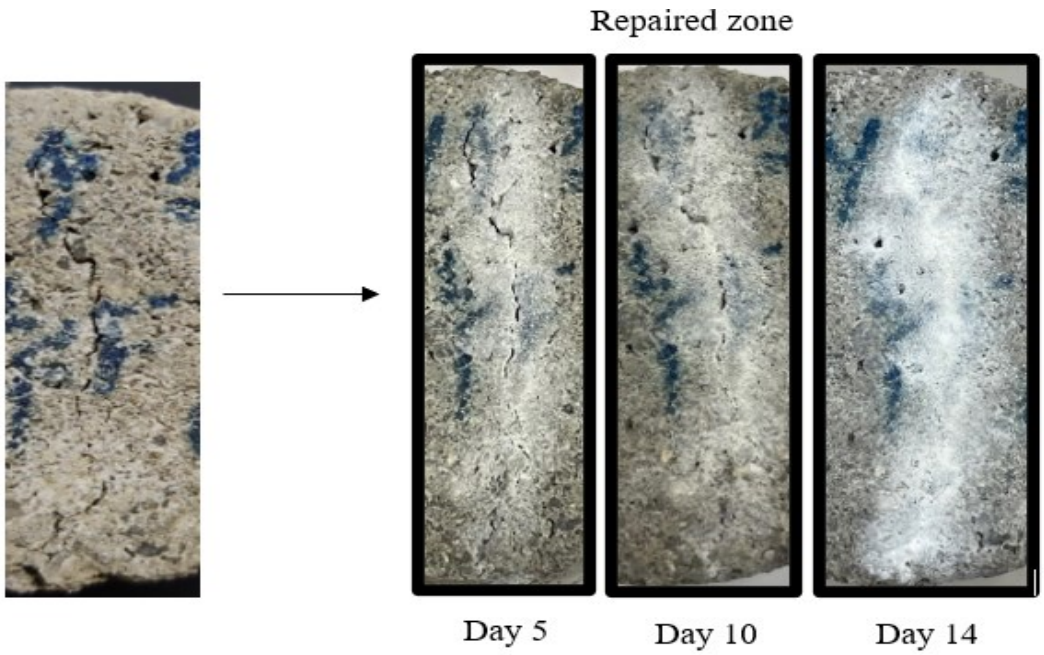


Figure 4. Surface changes of samples with CIDM at 5-day, 10-day and 14-day intervals, for cylindrical mortar

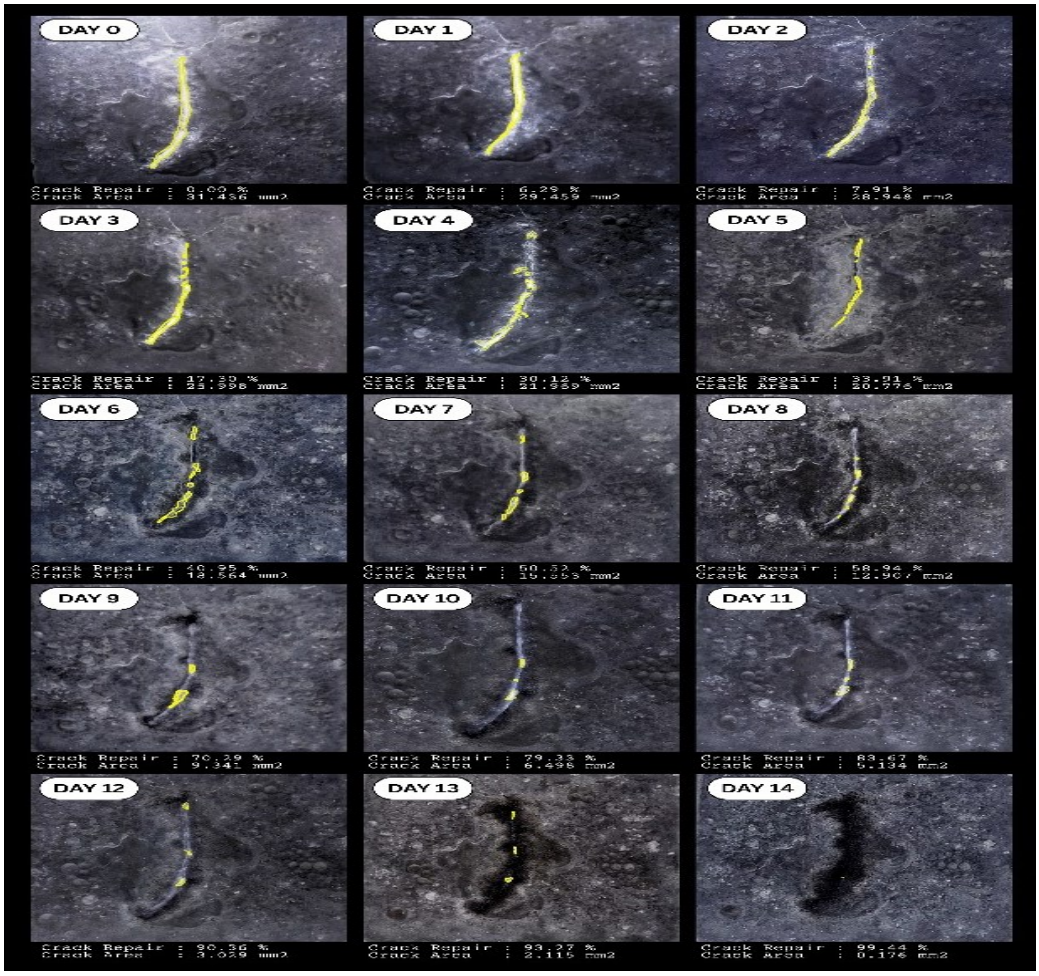


Figure 5 The crack repair progress percentage captured by ImageJ software

Compared to previous investigations utilizing direct injection or immersion techniques, the CIDM approach demonstrated enhanced efficiency in crack healing. For instance, Xiang *et al*. [19] reported partial crack filling over a 28-day period using conventional injection methods. In contrast, our CIDM-based treatment achieved full surface healing in half the time. This accelerated healing can be attributed to the hybrid nature of the CIDM, which combines pressure-driven injection with passive diffusion, thereby improving the penetration and retention of bacterial and nutrient solutions within the crack matrix. The dual-mechanism delivery ensures more uniform distribution and sustained microbial colonization, enhancing the spatial extent of $CaCO_3$ precipitation [20]. Furthermore, the effectiveness of CIDM in healing both cube and cylindrical specimens illustrates its adaptability to different geometries and crack orientations. The uniform sealing achieved at both macro- and micro-scales also indicates that the technique is suitable for addressing complex crack networks, which are typically more challenging to treat using traditional methods. The visual evidence of progressive healing, accompanied by complete mineral coverage by Day 14, underscores the robustness of CIDM as a self-healing strategy. In terms of environmental performance, this study advances the application of MICP by minimizing chemical waste and reducing the need for synthetic repair materials. Unlike epoxy or polymer-based systems that may pose environmental hazards, MICP is inherently sustainable and compatible with cementitious matrices. Moreover, the success of CIDM in facilitating surface-level restoration and internal crack sealing without the need for invasive or high-energy processes further supports its viability for in-situ repair applications. [21].

Splitting Tensile Strength

The splitting tensile strength test was conducted to assess the mechanical restoration achieved through Microbially Induced Calcium Carbonate Precipitation (MICP) treatment. Two cylindrical mortar specimens, one uncracked (control) and one cracked and subsequently treated were evaluated before and after repair, as depicted in Figure 3(a) and Figure 3(b). The results, summarized in Figure 6, reveal a distinct difference in both ultimate load capacity (F, kN) and calculated splitting tensile strength (f_s, N/mm²) between the two specimens. The uncracked control sample exhibited an ultimate load-bearing capacity of 22.24 kN and a corresponding tensile strength of 2.83 N/mm². In comparison, the MICP-treated sample reached 19.59 kN and 2.49 N/mm², respectively, indicating a recovery of approximately 88.1% of the original structural strength. This result demonstrates that the MICP process was effective in re-establishing mechanical integrity despite the presence of prior damage. The partial restoration in tensile strength is consistent with findings from Nasser *et al*. [22], who reported recovery rates of 80–90% in MICP-treated mortar, emphasizing that even with minor strength deficits, the structural continuity and durability of the material can be substantially enhanced. The observed reduction in load capacity may be attributed to heterogeneity in mineral deposition and limited bonding at the crack interface factors also highlighted in studies by Shi *et al*. [23] and Omoregie *et al*[24], which showed that inconsistencies in calcite growth and surface adhesion can slightly diminish the tensile performance of repaired zones. Nonetheless, the 88% recovery achieved in this study is mechanically significant and supports the interpretation that MICP can serve as an effective self-healing mechanism. The precipitation of calcium carbonate within the crack voids not only bridges the fractured surfaces but also increases the internal cohesion of the concrete matrix. This is in line with observations by Bandyopadhyay *et al.* [25], who demonstrated that bacterial urease activity enables crystal formation that mimics natural cementitious bonds. Moreover, the use of the combined injection and diffusion method (CIDM) likely contributed to the uniform distribution of bacteria and nutrients, enhancing mineral formation and crack closure throughout the depth of the fissure. This approach improves upon conventional injection methods by addressing internal damage zones that are otherwise inaccessible. In summary, while the MICP-treated specimen did not fully match the mechanical performance of the uncracked control, the high retention of tensile strength and load-bearing capacity confirms the viability of MICP as a sustainable and functionally reliable repair strategy. These findings reinforce the broader potential of bio-based crack healing technologies in extending the service life and resilience of concrete infrastructure.

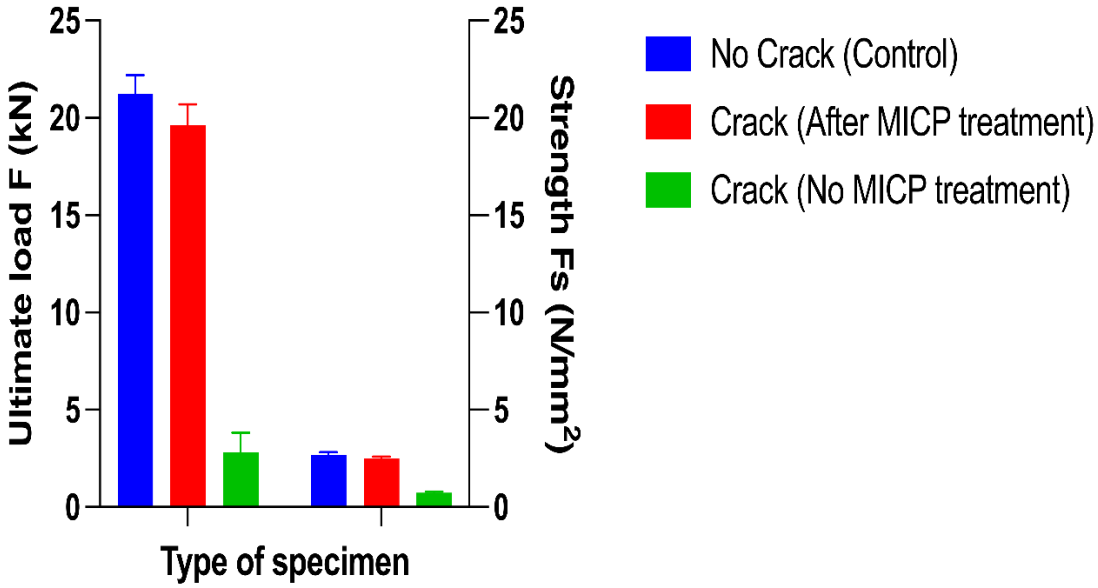


Figure 6. Comparison splitting tensile strength between no crack (control), cracked (after MICP treatment), cracked (No MICP treatment)

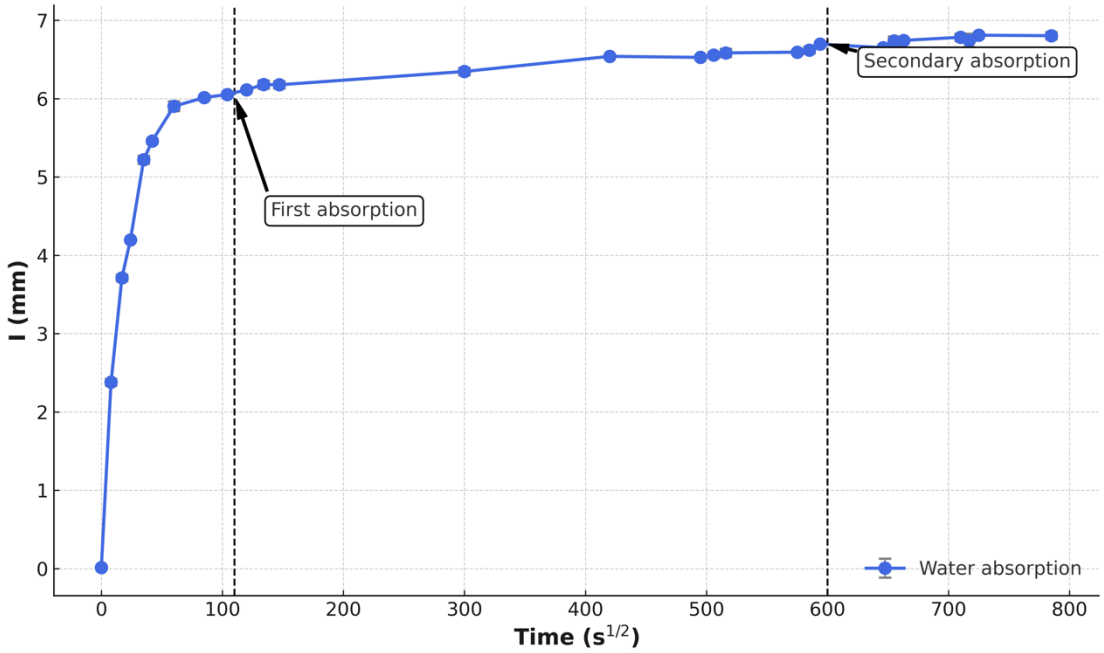
Water Absorption Analysis and Permeability Assessment

The water absorption performance of MICP-treated cracked mortar was evaluated against uncracked mortar to assess the impact of bacterial treatment on permeability. The absorption characteristics for both specimen types, illustrated in Figure 7 reflect the distinct differences in microstructural porosity and surface sealing capacity. The treated sample demonstrated a two-phase absorption profile characteristic of capillary transport in porous media. During the initial phase, the treated cracked mortar showed a relatively steep slope (0.0206 mm/√s), with a moderate-to-strong linear correlation (R^2 = 0.8069), suggesting rapid water ingress through accessible surface pores and partially filled microcracks. This behavior is consistent with early-stage absorption in MICP-treated specimens reported by Yehya and Rice [26], who observed that partially healed cracks exhibit transient high permeability before mineral saturation stabilizes flow. Transitioning into the secondary absorption phase, the absorption rate significantly decreased to 0.0009 mm/√s, with the correlation coefficient rising to 0.9825. This strong linearity indicates that water uptake became progressively limited as the microstructure approached saturation, likely due to the deposition of calcium carbonate narrowing or sealing internal capillary pathways. The reduction in slope confirms the efficacy of microbial precipitation in improving water resistance, although minor residual flow was still evident suggesting incomplete sealing of microcracks or inconsistent mineral deposition within deeper fissure zones. Similar outcomes were noted by Chen *et al*. [27], where MICP treatments achieved substantial but not total impermeability, with final absorption rates remaining slightly above those of intact concrete.

The uncracked mortar exhibited a distinctly different absorption behavior, characterized by higher predictability and overall lower permeability. The initial absorption rate was steeper (0.03607 mm/√s), and the correlation coefficient was nearly perfect (R^2 = 0.9932), indicating a highly uniform capillary-driven uptake limited to surface porosity. As the saturation point was approached, the secondary absorption rate dropped sharply to 0.0007466 mm/√s, accompanied by a strong correlation (R^2 = 0.9762), reaffirming the integrity of the specimen and the minimal pathways available for continued ingress. Compared to the treated cracked sample, the uncracked mortar absorbed less water overall and transitioned more sharply between the initial and secondary phases. These results underscore the natural impermeability of intact concrete, where pore connectivity is minimal and tortuosity inhibits sustained water transport. These findings align with baseline expectations established in the work of Lekundayo [28], which emphasized the inherently low sorptivity of high-quality, uncracked mortar. The comparative analysis between treated and uncracked mortars highlights several key observations. Both samples demonstrated a clear two-phase absorption pattern, but the treated cracked mortar exhibited greater variability in the initial stage, as indicated by its lower R^2 value. This deviation likely reflects residual microcracks or heterogeneities in bacterial or nutrient distribution during the MICP treatment process. Although the secondary absorption rates for both samples were similar in order of magnitude, the treated mortar still exhibited slightly higher water uptake, suggesting partial not complete recovery of impermeability.

Nevertheless, the reduction in absorption rate from 0.0206 mm/√s to 0.0009 mm/√s in the treated mortar demonstrates the effectiveness of the MICP process in sealing capillary voids. When compared to values reported for untreated cracked concrete (often exceeding 0.005 mm/√s in secondary absorption), the treated specimens performed favorably, reaffirming the contribution of *Sporosarcina pasteurii*-mediated calcite precipitation in permeability mitigation. Studies by Jongvivatsakul *et al*. [29] and Gao *et al*. [30] similarly noted that MICP treatments result in 60–80% reductions in water absorption compared to their untreated cracked counterparts. However, the marginal difference between the treated and uncracked specimens in the secondary phase highlights the limitations of the current treatment configuration. Variability in bacterial colonization, crack depth, and mineral uniformity may explain the incomplete sealing effect [31]. The presence of residual unfilled pathways, particularly in deeper crack networks or at the interface between the precipitate and matrix, could sustain low levels of water ingress, limiting the complete restoration of barrier properties [32]. The water absorption data confirms that MICP treatment enhances the resistance of cracked mortar to water penetration, offering a bio-based alternative to traditional sealants. While not fully restoring impermeability to the level of uncracked concrete, the treatment significantly reduces capillary action and slows the rate of water ingress an essential feature for increasing the service life of repaired structures exposed to moisture-driven deterioration mechanisms. Future improvements in treatment efficiency may be achieved through the optimization of bacterial concentration, nutrient composition, and treatment duration. Multicycle or staged nutrient delivery, or integration with additional sealing agents, may further enhance crack closure and minimize residual flow paths. The use of imaging techniques such as micro-CT or 3D profilometry could also assist in verifying sealing continuity and improving treatment targeting in heterogeneous crack geometries.

(a)



(b)

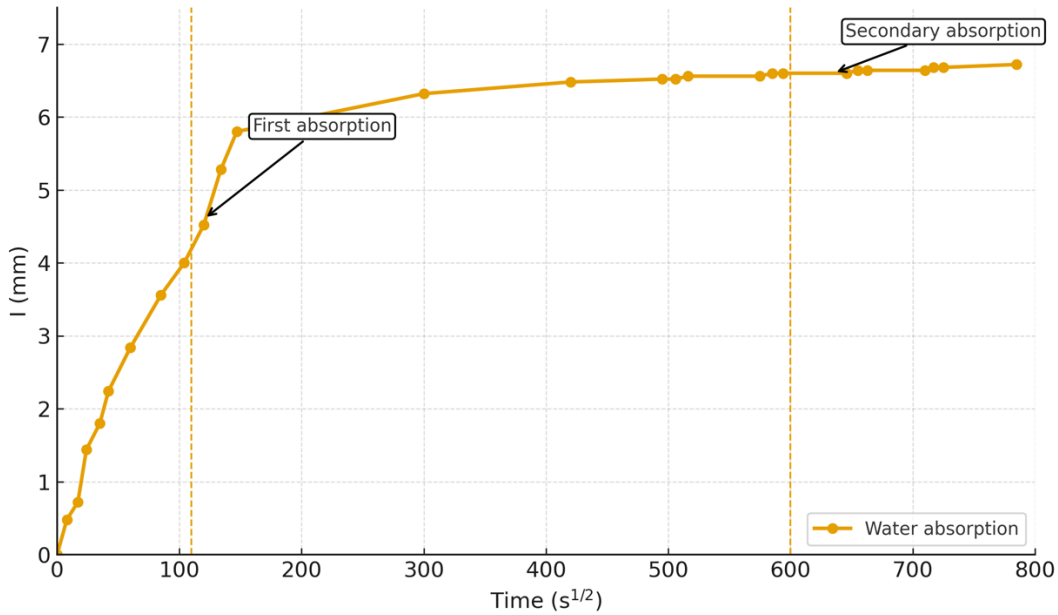


Figure 7. Water absorption rate of (a) MICP treated cube mortar and (b) Control (uncracked cube mortar)

FTIR Characterization of Functional Groups in $CaCO_3$ Precipitates

The Fourier Transform Infrared (FTIR) spectroscopy was performed to identify the functional groups associated with the calcium carbonate precipitate produced by *Sporosarcina pasteurii*. The spectral profile, shown in Figure 8, reveals the simultaneous presence of organic biomolecules and inorganic carbonate both of which are essential to the microbial-induced precipitation mechanism. A broad absorption band observed at 3305.29 cm^{-1} corresponds to O–H and N–H stretching vibrations, typically found in hydroxyl and amine groups. These functional groups are prevalent in proteins and polysaccharides, which form part of the bacterial cell membrane and extracellular polymeric substances (EPS). Their presence in the precipitate confirms active microbial involvement in the mineralization process. This is in agreement with previous studies which demonstrated that bacterial EPS not only supports microbial viability but also provides chemically active sites for calcium binding and nucleation [33–35]. Peaks detected at 2514.35 cm^{-1} and 2319.29 cm^{-1} are indicative of asymmetric CO_2 stretching vibrations. These are likely residual signatures from urease-catalyzed urea hydrolysis a critical biochemical step that increases pH and facilitates calcium carbonate supersaturation. Similar peaks were reported by Ojha *et al*. [36], who noted that the temporary entrapment of CO_2 within the biofilm microenvironment is essential for carbonate precipitation to initiate effectively. Additional absorption features at 1757.05 cm^{-1} and 1664.40 cm^{-1} represent carbonyl (C=O) stretching and the amide I band, respectively. These bands are typically associated with protein secondary structures, particularly enzymes like urease. Their presence corroborates the catalytic role of microbial proteins in accelerating carbonate formation. Krajewska *et al*. [37] also linked amide I bands to active ureolytic processes, confirming that microbial proteins not only hydrolyze urea but also enhance surface reactivity for mineral deposition.

The 1085.65 cm^{-1} peak, attributed to C–O stretching vibrations, suggests the presence of polysaccharides. These are a key component of bacterial EPS and act as scaffolds or templates for mineral nucleation. Recent findings by Niu *et al*. [38] emphasized that such functional groups improve the mechanical adhesion of calcium carbonate crystals within crack voids and influence the morphology of precipitated minerals. This contributes directly to the sealing and bonding strength observed in MICP-treated concrete. Additional minor bands between 2000–2100 cm^{-1} and at 1397.24 cm^{-1} suggest the presence of alkynes and aromatic or carboxyl-related groups, which may originate from bacterial metabolites or amino acid residues. Although not primary agents in mineralization, these functional groups may regulate local chemical environments and support the formation of stable crystal phases. Crucially, well-defined peaks at 742.81 cm^{-1}, 711.29 cm^{-1}, and 872.45 cm^{-1} confirm the presence of calcite—a stable and mechanically favorable polymorph of calcium carbonate. The peak at 872.45 cm^{-1}, corresponding to out-of-plane C–H bending, is a hallmark of calcite lattice vibrations. This aligns with previous FTIR studies by Zhang *et al*. [39] and Zhu *et al*. [40], which found that calcite formation, as

opposed to vaterite or aragonite, results in more durable and cohesive crack sealing in concrete matrices. Taken together, the FTIR spectrum provides molecular-level evidence that *S. pasteurii* facilitated the precipitation of structurally significant calcium carbonate through ureolytic activity. The detection of proteins, polysaccharides, and stable carbonate phases confirms the dual biological and mineral nature of the repair. Moreover, the spectral consistency with prior studies strengthens the conclusion that MICP is not only an environmentally benign process but also a functionally robust solution for restoring the integrity of cracked concrete through biochemical self-healing.

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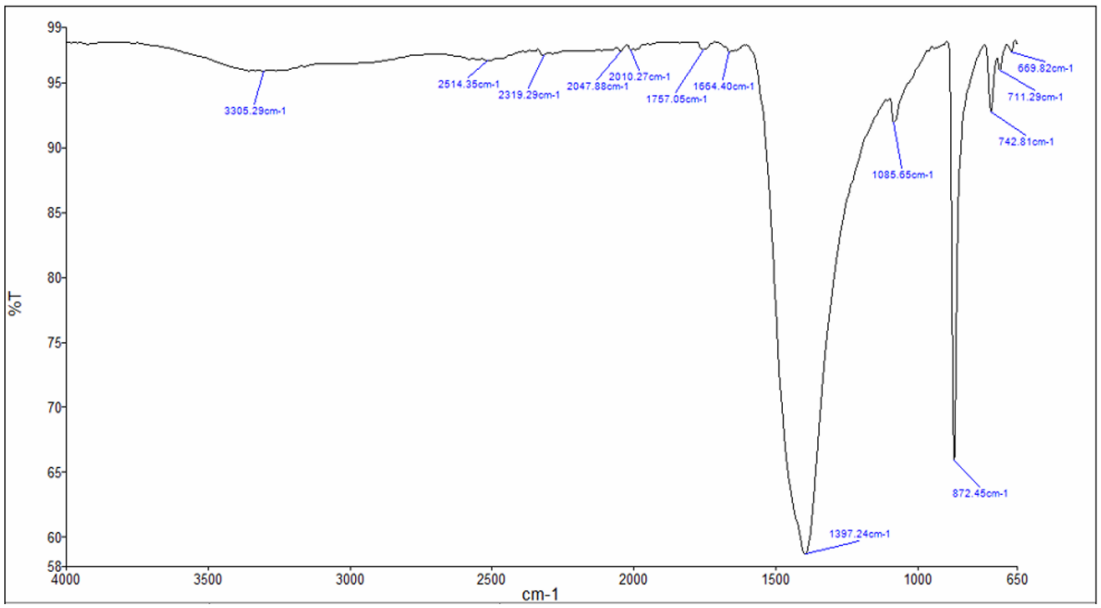

Figure 8. Fourier Transformed Infrared Spectroscopy (FTIR) spectrum

Morphological and Elemental Characterization of MICP-Treated Specimens Using SEM–EDX

The morphological and compositional features of calcium carbonate precipitates formed through Microbially Induced Calcium Carbonate Precipitation (MICP) were characterized using Scanning Electron Microscopy (SEM) and Energy-Dispersive X-ray Spectroscopy (EDX). SEM imaging provided direct evidence of mineral accumulation within the cracked regions of treated specimens. At 150× magnification Figure 9 (a), a homogeneous and continuous layer of mineral deposits was observed across the previously damaged surface. The presence of a cohesive mineral film bridging the fissures implies effective bacterial colonization and mineral deposition along the crack walls. This observation is consistent with Huang *et al*. [41], who reported similar surface morphologies in concrete specimens treated with ureolytic bacteria, indicating the sealing capability of biologically derived calcium carbonate. Higher magnifications revealed more intricate microstructural features. At 500× maginification, Figure 9 (b), granular clusters of calcium carbonate were observed, forming interconnected networks along the crack plane. These features suggest not only surface coverage but also subsurface penetration of the mineral into the void structure. Such clustered morphologies were also reported by Dong *et al*. [42], who linked this growth pattern to enhanced fracture bridging and reduced permeability in MICP-repaired specimens.

At 1500× magnification Figure 9 (c), the growth of rounded and polygonal $CaCO_3$ aggregates became prominent, suggesting progressive stages of biomineral development. These morphologies reflect an advanced state of nucleation and crystal consolidation, likely resulting from the repetitive 14-day bacterial and nutrient treatment cycle. Similar morphologies have been identified in earlier studies in such formations with high-efficiency microbial healing processes in cementitious matrices [43–45]. Finally, at 5000× magnification Figure 9 (c), well-defined rhombohedral crystals characteristic of calcite were distinctly visible. These structures appeared densely packed and geometrically regular, indicating phase-pure mineral growth. Rhombohedral crystal forms are a well-established signature of microbial calcite precipitation [46, 47]. The presence of such defined crystals supports the conclusion that the MICP treatment not only sealed cracks but also contributed to microstructural reinforcement by forming a durable mineral phase within the fracture. The evolution from diffuse, early-stage deposits to

consolidated crystal networks across increasing magnifications reinforces the effectiveness of the Combined Injection and Diffusion Method (CIDM). This technique ensured that both bacterial culture and cementation solution reached and reacted within the entirety of the crack void, enabling uniform and progressive mineralization.

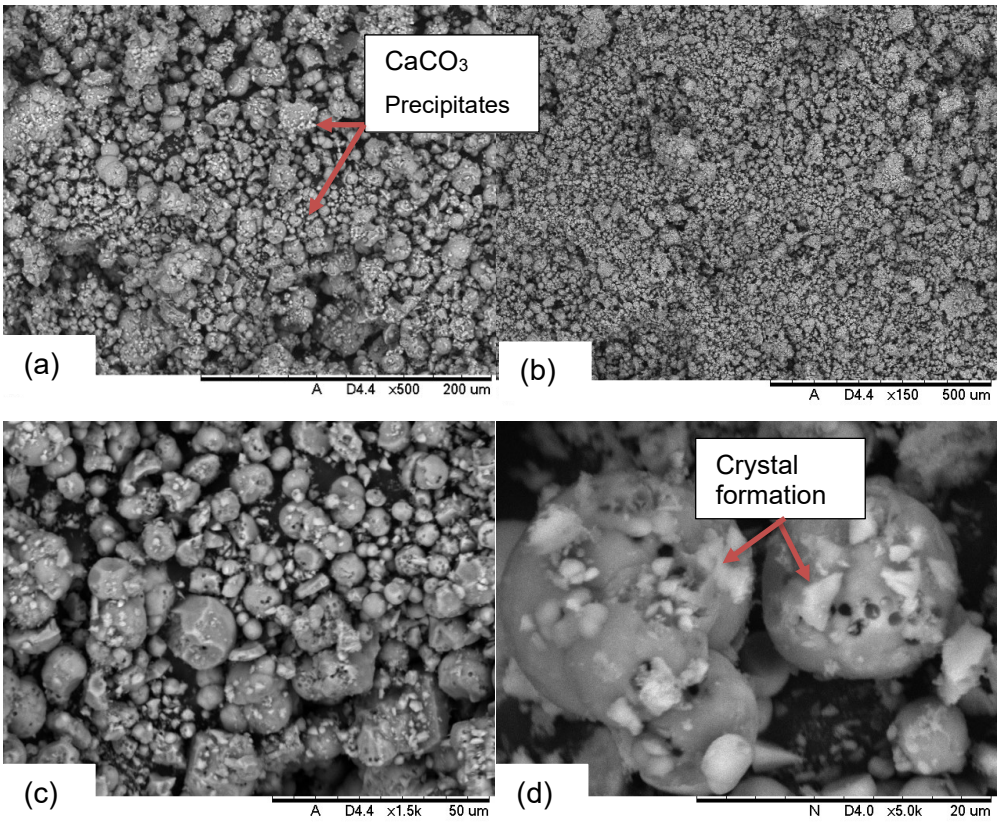

Figure 9. SEM images illustrates the surface morphology of the calcium carbonate formed for the crack repair

To validate the chemical identity and purity of the precipitated material, EDX analysis was conducted on the mineral deposits observed in SEM. Spectral data from Figure 10 revealed a dominant presence of calcium (Ca) and oxygen (O), which accounted for 40.52% and 59.46% by weight, respectively. Atomic percentages followed a similar trend, with Ca at 21.38% and O at 78.59%. These ratios strongly correspond to those reported for calcium carbonate ($CaCO_3$), particularly the calcite polymorph [48]. The detection of carbon (C) was negligible (0.01% by weight, 0.02% atomic), which may be attributed to instrumental limitations in detecting light elements or to surface preparation artefacts such as coating interference or beam-induced volatilization. Importantly, the near absence of extraneous elements (e.g., heavy metals or silicates) confirms the purity of the mineral phase and suggests minimal contamination from the cement matrix or external sources. Elemental mapping further confirmed the uniform distribution of Ca and O across the treated region, with no signs of aggregation or compositional inhomogeneity. This homogeneity is critical for the mechanical performance of the repair, as patchy or localized mineralization may lead to stress concentration and premature failure. Similar uniformity in elemental composition has been associated with improved bonding and long-term durability in studies by Sun *et al*. [49] and Huang *et al*. [50]. The consistency between the EDX findings and SEM morphologies supports the interpretation that the precipitate was phase-pure, well-structured calcium carbonate formed through biologically driven processes. The purity and distribution of Ca and O, combined with the distinct rhombohedral crystal habit observed via SEM, confirm that the predominant mineral phase is calcite thermodynamically the most stable form of $CaCO_3$. This contrasts with vaterite or aragonite, which are less stable and often observed in uncontrolled or early-stage MICP.

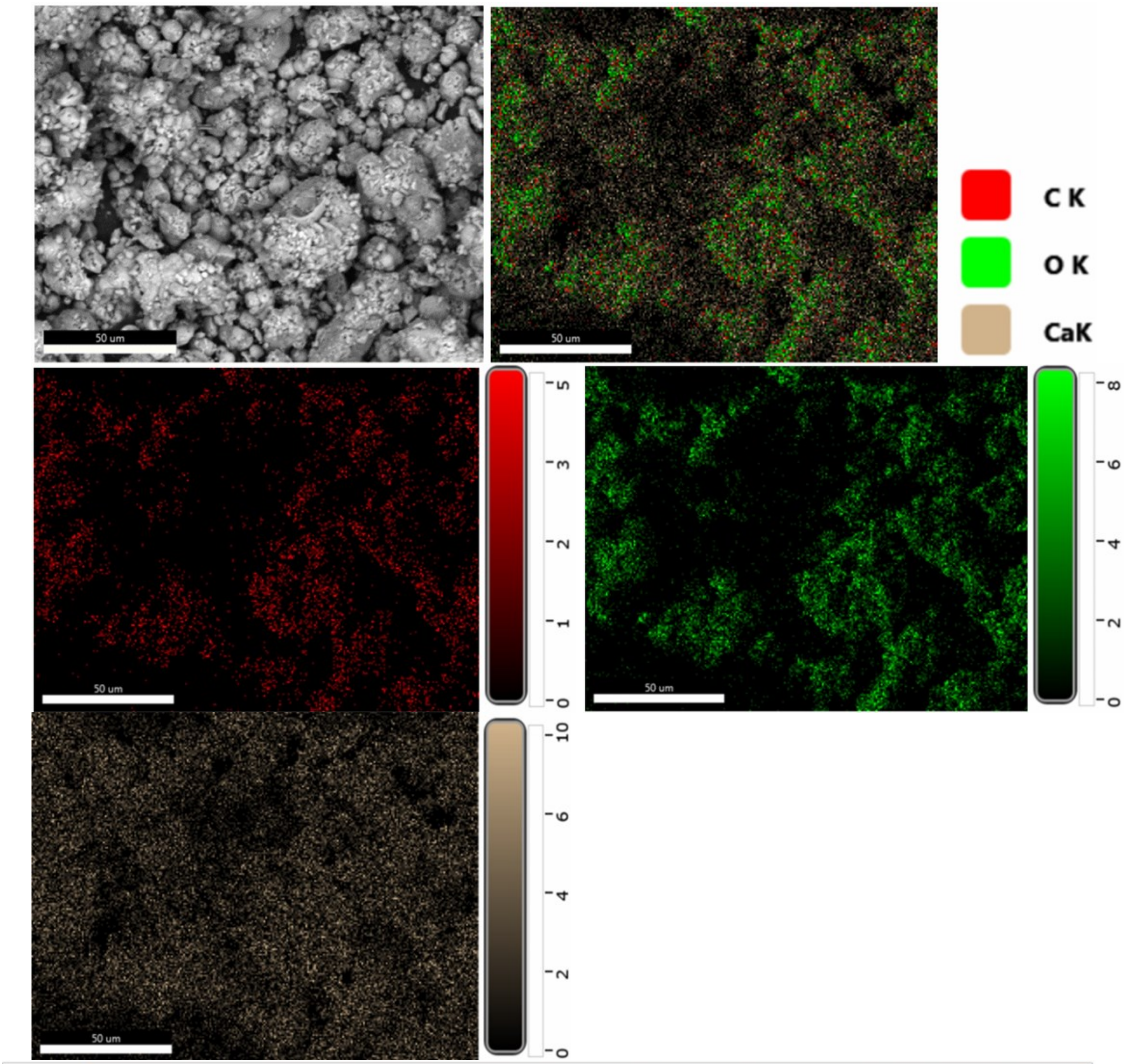

Figure 10. Energy Dispersive X-ray (EDX) results for elemental, (a) composition and (b) mapping

The microstructural and elemental features observed in this study are highly consistent with previous MICP research, confirming both the reproducibility and reliability of bio-mediated crack healing. Likewise, the high Ca/O ratio observed in the EDX analysis is nearly identical to that found in the work of Omoregie *et al*. [51], who demonstrated that phase-pure calcite formation is critical for long-term stability and chemical compatibility with concrete substrates. Moreover, the absence of organic or amorphous phases

in the EDX spectra suggests that the repair process reached full mineral maturity by the end of the 14-day treatment cycle. This is particularly significant when compared to early-stage carbonate capacity studies, such as those by Liu *et al*. [52], where insufficient carbonic capacity led to weak interface bonding and reduced mechanical recovery. In contrast, the current study's SEM–EDX data illustrate a fully formed, spatially integrated mineral network with high phase purity and structural coherence. Together, the SEM and EDX analyses confirm that the MICP treatment, delivered through the CIDM protocol, produced dense, uniformly distributed, and chemically pure calcium carbonate precipitates within the repaired crack zones. The observed morphologies and elemental profiles correspond to phase-pure calcite with optimal microstructural integration. These findings not only validate the biochemical functionality of *S. pasteurii* in concrete crack repair but also reinforce the structural performance gains observed in mechanical tests. Compared with prior studies, the results achieved here demonstrate a high level of consistency, reliability, and applicability affirming the feasibility of MICP as a robust and sustainable strategy for cementitious infrastructure rehabilitation.

In-Situ MICP Crack Repair

The in-situ application of Microbially Induced Calcium Carbonate Precipitation (MICP) was carried out at UTMSPACE to evaluate the field performance of the Combined Injection and Diffusion Method (CIDM) under uncontrolled environmental conditions. Two cement surfaces with visible cracks were selected for treatment and repaired over the course of two consecutive days one surface per day Figure 11. Each surface received six full treatment cycles, including microbial injection, cementation solution diffusion via sponge contact, and resting intervals, completed within approximately 4 to 6 hours per location. Visual inspection immediately following treatment revealed distinct mineral deposits forming along the treated cracks. The presence of white crystalline material consistent with calcite was observed, closely resembling the biomineral formations seen in laboratory-treated specimens. This confirmed that the core MICP mechanisms, urease-mediated urea hydrolysis, calcium ion availability, and localized pH elevation remained active in the open-air environment. The observable reduction in crack width and the partial or complete infill of fissures demonstrated the viability of field-scale MICP implementation for near-surface crack remediation. Notably, the field treatment yielded visible mineralization and partial sealing within a single working day per crack substantially faster than the 14-day laboratory protocol. This rapid response may be attributed to the nature of surface cracks in the field, which were shallower and more exposed to air. Higher evaporation rates and increased air contact could have accelerated supersaturation and carbonate precipitation. Similar field observations were reported by Lu *et al*. [14], who found that MICP reaction kinetics can vary significantly under field conditions depending on substrate geometry, ambient temperature, and drying rates.

The delivery technique, involving manual syringe-based bacterial injection followed by nutrient diffusion through saturated sponge strips, was effective and logistically simple. The approach did not require pressurized systems or automated dosing, making it especially applicable for sites with limited access to equipment or power sources. This supports the practical scalability of CIDM for real-world repairs, particularly in remote, resource-constrained, or emergency-response contexts. Comparable strategies using low-tech delivery mechanisms have also been recommended in recent applied MICP field studies [50, 53]. While initial observations confirmed mineral formation and crack coverage, long-term performance under natural weathering conditions remains unverified. Environmental exposure to moisture cycling, freeze-thaw events, ultraviolet radiation, and physical abrasion may affect the adhesion, cohesion, and structural integrity of the bio-deposited calcite. Extended monitoring and durability testing are therefore essential to evaluate the persistence of the repair under service conditions. Several researchers, including Kalawole *et al*. [54] and Jiang *et al*. [55], have emphasized the need for long-term durability validation in MICP applications before adoption in structural-grade repairs. The field trial demonstrated that CIDM-based MICP treatment is a viable, fast-acting, and low-tech method for sealing shallow cracks in cementitious surfaces. The observed in-situ calcium carbonate precipitation and visible sealing effects confirm that the key biochemical processes function effectively outside controlled laboratory settings. These results underscore the potential of MICP as a sustainable, environmentally friendly alternative to conventional repair materials for surface-level crack remediation. However, future work must focus on performance validation under prolonged field exposure to fully establish the method's structural reliability and lifecycle impact

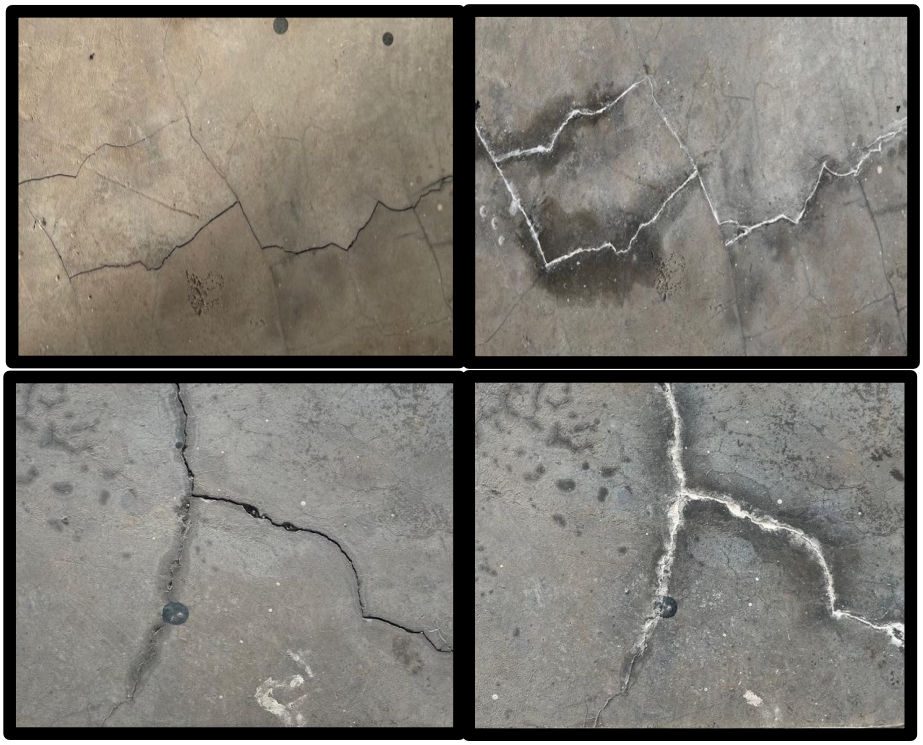

Figure 6. Surface changes of two selected cement cracked in situ before and after MICP treatment

Conclusion

This study demonstrated the effectiveness of Microbially Induced Calcium Carbonate Precipitation (MICP) using *Sporosarcina pasteurii* for crack repair in cementitious materials. The treatment achieved visible crack sealing within 14 days in laboratory conditions and within a single day under in-situ field application. Treated specimens recovered approximately 88% of their original tensile strength, confirming the structural benefit of bio-induced calcium carbonate deposition. Water absorption tests showed a significant reduction in permeability, indicating improved durability, though slightly higher intake compared to uncracked mortar suggests partial, but effective sealing. Chemical and microstructural analyses supported these findings: FTIR confirmed the presence of functional groups associated with biomineralization and calcite formation, while SEM–EDX revealed densely packed crystals with high calcium and oxygen purity. The practical feasibility of the Combined Injection and Diffusion Method (CIDM), along with consistent performance across scales, highlights MICP as a promising bio-based solution for surface-level concrete crack remediation. Future research should focus on long-term durability, deeper crack penetration, and treatment optimization to further enhance field performance and scalability.

Conflicts of Interest

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

Acknowledgment

The authors would like to acknowledge UTMSPACE Research Funding and Universiti Teknologi Malaysia for the funding provided through Potential Development Fund (PDF) research grant (SP-PDF2402) and UTM Matching grant scheme (Grant No: Q.J130000.3022.04M98).

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