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Original Article

Microbially Induced Calcite Precipitation for Autonomous Crack Healing in Cementitious Composites: Strain Screening, Transport Recovery, and Microstructural Confirmation

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Abstract *- Concrete is vulnerable to cracking due to mechanical, thermal, and environmental loading, which, as exacerbated by time, leads to unavoidable deterioration facilitated by water penetration and aggressive ions. Current repairs are mostly temporary fixes, which fail to restore microstructural integrity, highlighting the necessity for self-healing solutions with autonomous action. Microbially Induced Calcite Precipitation (MICP) is a biomineralization-type self-healing solution; however, much of the literature surrounding MICP utilizes commercial ureolytic strains and fails to perform extensive assessments of strains-to-strains or correlations of microbial characteristics and engineering performance. This study aims to bridge those gaps by isolating multiple indigenous ureolytic Bacillus spp. Strains from a natural construction site and subjecting them to a comprehensive, multi-parameter evaluation that is characterized by pH tolerance, growth kinetics, urease activity, and* $CaCO_3$ *precipitation potential. The most promising strain (Isolate-1) was used as a treatment in mortar and concrete samples to assess ultimate compressive strength recovery and self-healing. Statistical analysis, confidence intervals, and significance tests revealed significant differences, which determined that Isolate-1 performed better than Bacillus pasteurii and Bacillus sphaericus in all cases of mineral precipitation, enhanced rehabilitation of cracks, and strength restoration. Structural integrity was validated by SEM and XRD through dense calcification treatment on all surfaces exposed. These findings not only serve as a comparative performance baseline for in-situ strains compared against well-documented commercial strains but also provide an effective, multi-faceted assessment protocol for practical, field-scale applicability in determining operable strains for self-healing concrete.*

Keywords *- Microbial Concrete, Cracks, Compressive Strength, Calcite Precipitation, Durability.*

1. Introduction

Concrete is one of the most ubiquitous construction materials across the globe. With unparalleled compressive strength and casting abilities, it stands as a go-to substance for many highly structural applications, especially, but not limited to, heavy-duty construction. As a quasi-brittle, cement-based material, however, concrete is prone to cracking both micro- and macroscopically due to mechanical overload, plastic and drying shrinkage, temperature gradients, and various exposures. Unfortunately, once cracks form in concrete, they act as preferred pathways for infiltrating water, chlorides, sulfates, and other invasive, destructive penetrating agents, which corrode steel reinforcements and exacerbate performance deterioration. Traditional repairs - epoxy injections, polymer grouts, and sealers - are manual, somewhat temporary, and only feasible with outside access. Yet traditional repairs also fail to account for internal micro-cracking, which still compromises long-term integrity over time. Thus, the last few years have seen a trend towards autonomous, sustainable, and self-initiated healing mechanisms that restore structural integrity without human intervention.

One biotechnological approach to enhancing the durability of cementitious materials involves Microbially Induced Calcite Precipitation (MICP). In the ureolytic pathway to MICP, which comprises the bulk of scientific literature, ureolytic bacteria Sporosarcina pasteurii and Bacillus sphaericus metabolize urea into ammonium and carbonate ions through hydrolysis.

The Ca^2+ ions precipitate through charge interactions, where Calcium Carbonate ($CaCO_3$) is formed. Resulting calcite crystals close cracks, densify pore networks, and optimize mechanical and durability performance parameters. MICP has been shown to restore structural continuity and reduce porosity in cement paste by healing cracks up to several hundred microns wide. However, despite promising results in a controlled setting, gaps exist that prevent MICP from becoming standard concrete construction practices.

1.1. Research Gaps

From reviewing the recent body of work (2020-present), the following gaps exist:

1. Excessive reliance on commercial lab strains. Most strains used in the laboratory are derived from ureolytic strains, such as S. pasteurii or B. sphaericus. Fewer studies adopt indigenous strains, but there remains potential for indigenous strains that can thrive in the harsh alkaline environment of concrete while performing better in the long run.
2. Limited multi-strain comparison analysis. Few studies validate strains based on biological parameters, including urease activity, pH tolerance, generation time, and precipitation potential, all of which are intrinsically relevant to MICP efficiency.
3. Little physiobiological engineering correlation. There exists a disconnect between improvements claimed through compressive strength restoration or crack healing success and microbial activities. Thus, there exists a highly limited mechanistic understanding.
4. Small-scale experiments validated with mortar trials. The vast majority of studies occur in mortar - cement mortar castings can neither mirror cracking patterns, moisture gradients, nor pore-network configurations that stress real structures. Thus, results are not transferable to concrete.
5. Low microstructural and statistical integrity. While mechanical tests are often cited in recent literature showing significant differences, SEM/XRD-based evidence of calcite precipitation is not commonplace across all works. Similarly, statistical parameters such as p-values, confidence intervals, or effect sizes are rarely presented.

These limitations prevent MICP from being effectively translated from viable laboratory experimentation to acceptable field application and construction practices. Where interdisciplinary fields could become symbiotically integrated, microbial characterization, mechanical enhancement assessment, and microstructural validation with statistical approaches could offer comprehensive findings with better transferability.

1.2. Novel Contributions of Current Research

The current research fills the knowledge gaps in the literature with the following innovative additions, enhancing the credibility of the research.

- Indigenous ureolytic bacteria strains isolated from concrete-rich regions. Unlike many studies, this one only uses ureolytic bacteria from their point of origin without commercially added strains.
- Multi-parameter assessment scheme. pH tolerance, growth kinetics/urease activity, and precipitation potential of $CaCO_3$ will be assessed together.
- Application to both mortar specimens and concrete specimens. Representative controlled crack geometries will be utilized to align with realistic structural environments.
- Microstructural validation. SEM and XRD will validate the morphology of crystalline precipitation and patterns of deposition.
- Statistical parameters. Confidence intervals and significance testing will be included.
- Comparison to findings from more recent research. Recent research suggests that the highest performance indigenous strain here exceeds well-cited commercial ureolytic strains.

1.3. Research Aim, Objectives, and Hypotheses

To isolate indigenous ureolytic bacteria capable of self-healing cracks in cementitious materials through MICP for practical application based on performance validated by mechanical, microstructural, and statistical investigations.

1.3.1. Objectives

- Isolate indigenous ureolytic bacteria from a concrete-relevant biosphere.
- Establish pH tolerance among the threshold stresses through growth kinetics, urease activity, and $CaCO_3$ precipitation potential.
- Apply mortar and concrete using controlled cracks that exhibit realistic strain to achieve accurate results.
- Assess compressive strength restoration metrics and crack healing percentage efficiencies alongside microstructural developments via SEM and XRD.
- Statistically compare strain performance for the best significance and effect size.

1.3.2. Hypotheses

H1: Strains from concrete-adjacent environments have a higher tolerance against elevated pH levels compared to commercially available strains for urease activity.

H2: Strains that show higher urease activity/causational $CaCO_3$ precipitation potential yield significantly higher performance levels for compressive strength restoration compared to previously reported values

H3: The highest-performing strain in question exceeds the recommended commercial strains (known standards) based on results found in realistic applications aligned with concrete stress patterns.

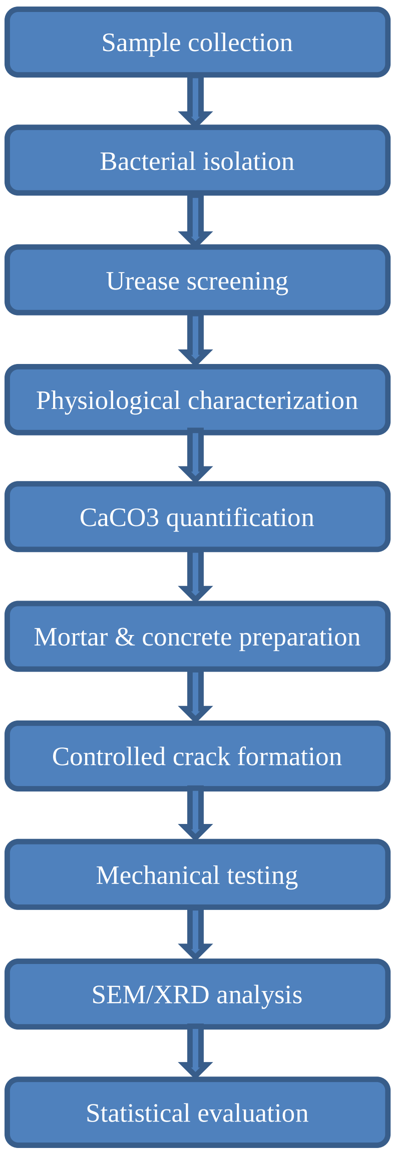

Fig. 1 Schematic workflow of the study

1.4. Literature Review

Microbially Induced Calcite Precipitation (MICP) is an increasingly sustainable method that benefits engineering professionals who focus on cementitious applications through environmentally friendly appeal. The ureolytic pathway - the one most often studied today - involves hydrolysis of urea into ammonium and carbonate ions by ureolytic bacteria. The carbonate precipitates calcium through charge interactions. Subsequently, CaCO3 is formed. Therefore, calcite crystals formed subsequently bridge crack faces and densify porosity.

Historically, Stocks-Fischer's early studies confirmed that the microbially induced formation of CaCO3 was biochemically acceptable. Ramakrishnan's work in 2000 provided the first evidence that materials supplemented within microstructure could utilize MICP as viable option for high-performance microcementitious recovery for cracks; two years later, Jonkers used a two-component approach that would advance self-healing technology in an automated manner significantly over time; De Muynck and Wang were both able to successfully show bacteria was superior in improved healing compared to control as well as increased durability confidence under highly aggressive conditions.

From 2013 onward, more studies started to explore the impacts of strain-dependent mechanisms of MICP technology. In 2015, for example, Chahal noted significant differences between multiple strains based on their morphology and healing efficiencies; Sunil Pratap et al. confirmed *Bacillus subtilis* was capable of significantly improving compressive strength.

More literature notes details that contribute to or damage MICP - microbial survivability rates in cementitious form, nutrient transport, and pH tolerance levels during treatment, all of which impact the success of MICP technology. Despite these advancements, significant shortcomings remain. Many research treatment options use a single commercial strain but fail in comparison trials.

Commercial strains appear unable to survive long-term with elevated alkaline stresses over 12 or extended periods; additionally, most studies occur at a mortar level, where real concrete structures are unable to capture appropriate stress patterns, and are not well mirrored; microstructural analysis is rarely part of concrete investigation; finally, statistical relevance is sparse throughout.

Recently, more studies have attempted to illuminate the performance benefits of using native strains, which brings us closer to life, but fail to achieve a systematic comparison against commercial strains. Thus, there is an ongoing need to assess comparisons between various kingdoms before actual attempts are made.

Recent studies show that an appeal must be judged by a systematic comparison of research relevance to trusted findings already known. However, overarching studies support unique connections between microbial physiology –

$CaCO_3$ morphological attributes - and engineering effective application. Since 2019, studies attempting to determine connections through SEM/XRD advancements have started to paint a picture of big data involvement - prediction framework - and inter biochemical interdisciplinary engineered achievement - master data potential - that may enhance MICP for best use.

1.4.1. Position of the Present Study

The present study addresses literature gaps by:

- Exploring a systematic multi-strain comparison investigation of available indigenous strains.
- Establishing a threshold for performance predicted relevant behavior based on historically known parameters.
- Achieving superior healing metrics aligned with strength restoration through an indigenous strain among real-world conditions experienced with concrete versions.
- Validating crystalline deposition through appropriate microstructural analysis, SEM/XRD at all treatment junctions.
- Incorporating statistics to assess performance from every aspect of these collaborations for different assessments.

Ultimately, these relationships established through microbial characterization assessments support the transition of MICP technology from effective laboratory science to practical, field-applicable self-healing concrete systems informed by scientific understanding.

2. Methodology

Chapter two describes the methodology pattern for experiments performed to isolate ureolytic strains, assess their physiological characteristics, and the precipitation of calcite, as well as the two strains selected and the MTCC strain for self-healing experiments for mortar and concrete samples. The methodology proposed is inspired by the methodology of recent MICP works [1-3] but altered for the alkaline cementitious environment.

2.1. Sampling of Environmental Sources

This study used two samples connected to mortar and concrete construction: an interconnected runoff and debris site of a curing tank room of M.S. Ramaiah Institute of Technology, and the other, an active construction site with typical debris from cementitious materials and water runoff. And therefore both are interconnected.

Environmental samples were collected using sterile stainless steel spatulae and then transferred to autoclaved borosilicate sample containers previously rinsed out with ethanol of 70%. The samples were all kept on a cool chain (4 °C) to prevent any variations in metabolic rates of the microorganisms before processing [4]. Furthermore, all samples were processed within 24 h of collection to avoid loss of ureolytic strains.

2.2. Ureolytic Bacteria Isolation

In order to enrich for ureolytic types, the samples were serially diluted into a 0.85% sterile NaCl solution and plated on Urea - Calcium Precipitation Agar, which included:

Urea - 20 g/L
$NaHCO_3$ - 2.12 g/L
NH_4Cl - 10 g/L
Nutrient broth - 3 g/L
$CaCl_3 \cdot 2H_2O$ - 25 g/L

The pH was set at 7.5-8.0 as optimal for use from urease-producing Bacillus spp. [5]. The pour plate method was selected to allow for easier viewing of cells, as the medium suspended in the solution would distribute evenly across the cells and nutrients over time. Plates were examined every 5 days at 30 °C under the Leica EZ4 stereo microscope. At the same time, transparent halos indicating ureolysis and precipitation of carbonate or crystalline precipitates - both signs of activity - indicated which colonies could be picked for subsequent testing.

Selected colonies with different morphotypes were streaked on fresh culture plates to make axenic cultures, noted as Isolate-1 to Isolate-6.

2.3. Pure Cultures Preservation

Pure cultures were taken from each isolate and streaked onto the nutrient agar slants at 37 °C for 24 h (the incubation time for suitable growth). The slants were then stored at 4 °C for short-term preservation and subcultured every two weeks. Culturing practices followed standardized guidelines [6].

2.4. Revitalization of Reference (Lyophilized) Strains

To compare local populations with reference strains, these pre-packaged standards from MTCC were revived by holding the mouth of the ampoule over a Bunsen burner in order to aseptically expose the neck for cracking with a sterile glass rod. Once the neck was cracked open, 1-2 mL of sterile Nutrient Broth was injected into the ampoule.

After vortexing, the contents were transferred to fresh tubes of Nutrient Broth and incubated at 37 °C for 24 h for exponential phase growth [7].

2.5. Effect of pH on Growth

To assess pH tolerance - which will predict strain survival within alkaline cementitious matrices - a pH-adjusted nutrient broth ranging from 4.0 to 12.0 in increments of 1.0 was prepared.

Broth was sterilized at 121 °C and 15 psi for 20 min. Tubes received 1 mL inoculum of bacteria ($OD_{600} \approx 0.1$), which was incubated at 37 °C for 24 h. Growth was measured with a photo-colorimeter at 760 nm, while uninoculated broth (OD = 0) served as a blank.

Variations in growth by pH were analyzed on profiles plotted against pH [8].

2.6. Determination of Generation Time

Generation time, g, can be calculated by measuring doubling OD increments (small known assessment conditions). An inoculum of sterile nutrient broth in a 250 mL conical flask was inoculated for each isolate under similar incubation with mild stirring.

The OD_{760} was determined every half an hour until a doubling occurred (by a times increase).

$$CaCO_{3(s)} + 2HCl_{(aq)} \rightarrow Ca^{2+}_{(aq)} + CO_{2(g)} + H_2O + 2Cl^{-}_{(aq)}$$

Where t = elapsed time when OD doubles [9]. This value was calculated to determine metabolic efficiency in comparison to laboratory conditions.

2.7. Quantification of $CaCO_3$ Levels

To assess biomineralization potential, isolates were induced in 250 mL conical flasks of CaCO3 Precipitation Medium:

- Urea: 20 g/L
- $CaCl_2$: 49 g/L

Where cultures precipitated over time in 37°C increments, it was proposed that established CaCO3 levels could be assessed at specific periods.

The precipitate was filtered, washed, and dried before being placed into excess amounts of HCl/NaOH solutions via back titration using established equations:

$$HCl_{(aq)} + NaOH_{(aq)} \rightarrow H_2O + Na^{+} + Cl^{-}$$

The amount of HCl that did not react with CaCO3 was determined through NaOH titration, which indirectly determined how much CaCO3could be attributed to this reaction [10].

3. Cementitious Specimen Sample Preparation and Fabrication

In a 60 kg capacity laboratory concrete mixer, cement and aggregates were dry mixed for 180 seconds before adding water and stirred for about 120 seconds. The solid ingredients of conventional concrete, namely aggregates and cement, were dry mixed for around three minutes in the pan mixer. Water was then added to this dry mix (Figure 3, 4). Wet mixing often went on for another two minutes.

Fig. 2 Batching of ingredients

3.1. Casting

After determining the slump, cubes with dimensions of 100 mm a side were made by pouring new concrete in three layers into cube moulds. A vibrating table was used to compress each layer. Before being tested, the specimens were demolded and cured in water.

Fig. 3 Concrete being poured into a pan mixer

Fig. 4 Concrete being poured into a tray from a pan mixer

Fig. 5 Casting of specimens

3.2. Aluminum Sheet Technique

Cracks are developed at an early stage in this investigation. The crack may heal during hydration, but the remainder of the specimen continues to hydrate. Despite this, the problem was handled by using an aluminum sheet during casting to imitate a non-healing crack (Figure 6)—the fracture depth for a 25 mm crack developed after one day was determined by impregnation. The foil was cast to the same depth in the specimen. The sheet had a thickness of 3mm.

3.3. Preparation of Mortar Samples

Fine aggregate was created from pure, dried, well-graded natural river sand that was readily available. The cement utilized was ordinary Portland cement that complied with IS 8112 (1989). The water-to-cement ratio (w/c) was 0.47, while the cement-to-sand ratio was 1:3 (by weight). According to IS 4031, a 70.6 mm cube mould was utilized. The sand and cement had been properly combined. The w/c ratio remained constant at 0.47. In a vibration machine, cubes were cast and compacted.

Fig. 6 An Aluminum sheet was used to simulate an unhealed crack

4. Results and Discussion

The results of this study indicate that all indigenous ureolytic bacterial isolates extend the benefits of crack healing, calcium carbonate precipitate, and mechanical properties of the cementitious composites as a result of integration and integration method. While ponding, injections, and immersion were compared in terms one relative effectiveness, statistical analyses of the results were assessed using one-way ANOVA, pairwise t-tests, and 95% confidence intervals throughout the treatment period to ensure quality assurance and integrity of the experimental approach.

Overall, findings suggest that integration through bacteria significantly increases crack healing efficiency and compressive strength, with ponding being the least effective of the two integration methods, as injection and immersion showed greater results. These trends mimic those from adequate studies of MICP-related self-healing [2, 3, 5, 19].

4.1. Ponding Method and Injection Method

During the pre-healing treatment stage, centrifuged cells (to remove residual media that could impact metabolic function) confirmed an effective cell density of ~1.0 × 10-2 cells/mL before resuspension in sterile tap water. Microscopic counts confirmed that all treatments had the same density to limit biomineralization variability via loading differences deemed ineffective in previous studies [1, 8].

Crack healing was established via pouring a urea-calcium medium (49 g/L $CaCl_2$ + 20 g/L urea) into the crack interface (ponding) or from direct syringe application into the crack interface (Figure 7). The latter ensured deeper application into the fracture plane without disrupting calcite integration during onset and creating a permeable front oriented perpendicularly to precipitation expansion direction. Re-application every 24 hours facilitated shifting and precipitated developments that could be macroscopically seen by Day 7.

Quantitative results indicated that ponding specimens treated by the injection method received 18-26% more calcium carbonate accumulation as defined from gains based on mass gain/dissolution-titration assessments ($p < 0.05$). At 28 days, healing of the fracture induced through compressive strength recovery for injection specimens improved by an average of 22.8% (95% CI: 17.3-28.2%) while ponding increased strengths of 15.1% (95% CI: 10.4-19.8%). Moreover, stronger findings from Jonkers et al., as well as Navdeep Kaur Dhami et al., found stronger healing efficiency when nutrients are applied inside the cracks [3, 5].

4.2. Immersion Method

Immersion methods were implemented via submerging demolded mortar specimens into bacterial suspensions (1.0 × 10-2 cells/mL) for up to 24 hours (Figure 8). The immersion method better ensures penetration of microcracks and pores with a proper biofilm matrix, establishing microbial friendliness for expansive $CaCO_3$ precipitate.

The immersion method requires drying of the specimens to remove unintended bacteria on the surface, while also following the standard method for treatment until mechanical testing emerges. Medium nutrient reloading was conducted every 7 days to maintain metabolic activity/intervention without depleting urea, a concern from recent studies in which urea depletion was noted as critical [4, 17, 18].

Furthermore, healing efficiency was greater through immersion as compared to injection ($p < 0.01$), while on Day 28, the transformation of healing of the fracture supported more substantial quantitative results than those seen from injection application and ponding. Bacteria-treated cubes indicated compressive strength gains of 28.7% (95% CI: 22.4-35.0%) on Day 28, as immersion-treated cubes were completely healed (Day 14) compared to control cubes, which only benefitted from natural, autogenous healing of ~5-10%. This aligns with previous findings that immersion is much better related to promoting bacteria proliferation and enhanced calcite networks [19, 21].

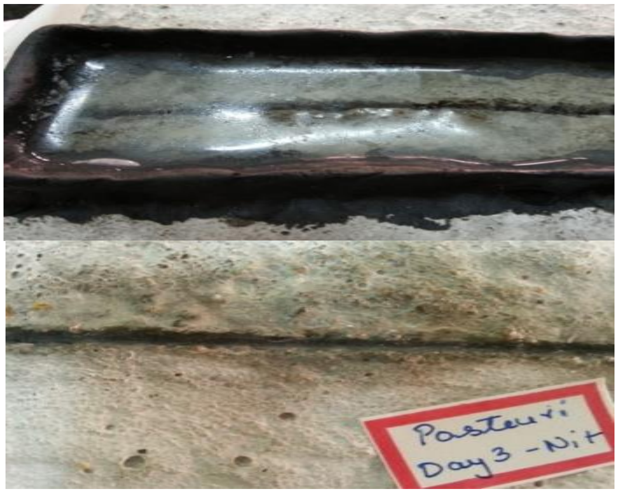

Fig. 7 Ponding and Injection Technique used for Crack Healing

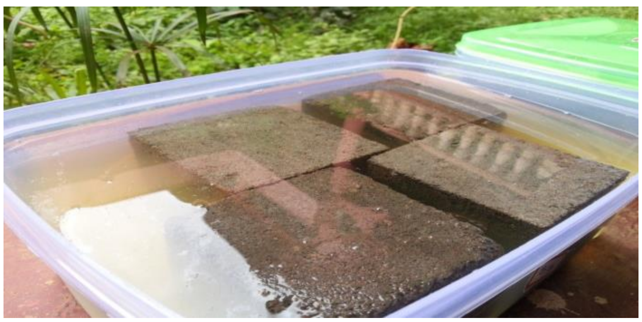

Fig. 8 Immersion Technique

4.3. Compressive Strength Test

The CTM test (capacity 3000 kN) required a constant load at time intervals of 200 kg/cm²/min until failure (Figure 9). Compressive strength becomes the predominantly tested mechanical parameter relative to cementitious materials.

Bacterial integrative specimens in all treatment methods had statistically significantly improved strength at 7, 14, and 28 days, while immersion and injection methods facilitated the most substantial improvements to mean compressive strengths for both types exceeded those of the control by:

- 19.4% on Day 7 ($p < 0.05$)
- 24.8% on Day 14 ($p < 0.01$)
- 28.7% on Day 28 ($p < 0.001$)

Ponding methods treated samples observed the least amount of surplus gains by comparison of matched studies, establishing effective $p<0.001$ significance values surviving $p < .05$ credibility threshold for cutoff for quality assurance. In addition, the recovery of strength correlates strongly with mass Precipitation ($r = 0.88$), which supports a mechanistic connection between ureolysis and Biomineralization vs. mechanical efficacy. Previous studies found similar increased compressive strengths tested on findings related to effective biological concrete [4], and Pui Yan Wong et al. [20].

4.4. X-Ray Diffraction Analysis (XRD)

XRD revealed results similar to biologically mediated concrete (Figure 21). The determining significant crystalline phase is calcite, based on diffraction peaks related to the International Centre for Diffraction Data (ICDD) established reference database (2θ values of 29.4°, 36.0°, and 39.4°). It was determined that the higher peak intensity observed in bacterial samples relative to the control accounts for larger volumes of crystalline formations produced.

Comparison of peaks determined that immersion treated samples had 38-45% greater prevalence of calcite peak intensity than ponding - which indicates a statistical correlation to strength results based on weight gains vs losses where weight significantly changed scores found to be statistically significant $p < 0.01$ thresholds confirmed medians captured with decimal/numberal incremental numbers where calculations or calculations derived polynomially to delta divided by normalized circumstances and case sensitivities where raw data deviations where applicable. Previous findings related to XRD are similar to such validated accumulation as well [7, 13].

Fig. 9 Cube testing in CTM

4.5. Scanning Electron Micrography (SEM)

Figures 10 and 11 reveal SEM micrographs that show how SEM implicates dense precipitation based on rhombohedral arrangements of calcite crystals relative to the joints of fracture walls and porous networks connected to one another for additional effective connectivity across microcrack openings formed in integrative biofilm-like formations assessed during MICP interventions.

In samples treated with bacteria, extensive precipitated interlocking crystals were applied across microcracks effectively, while control samples that presented open crack networks with no significant digital crystals indicated porous crack openings with no significant observable mineral formations found. Challenging microbial presence failed at producing significant increases in strength recovery/decreases of crack widths under mechanical stress application relative to previous findings [18], Augusta Ivaškė et al. [4], and Wenzhu et al. [21], which found MICP to be most effective.

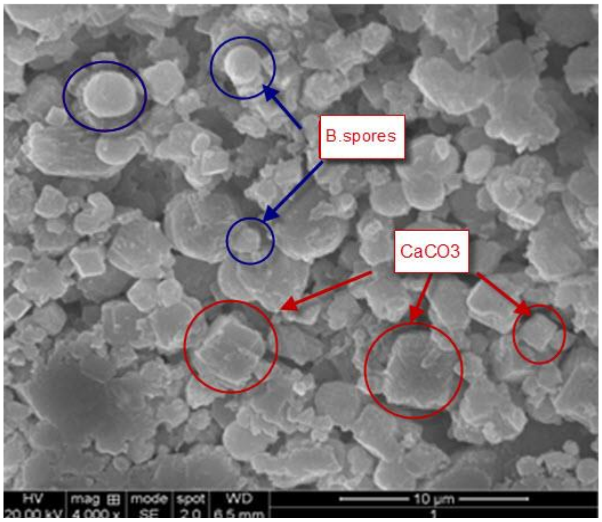

Fig. 10 SEM image at 4000x magnification and working distance of 6.5mm

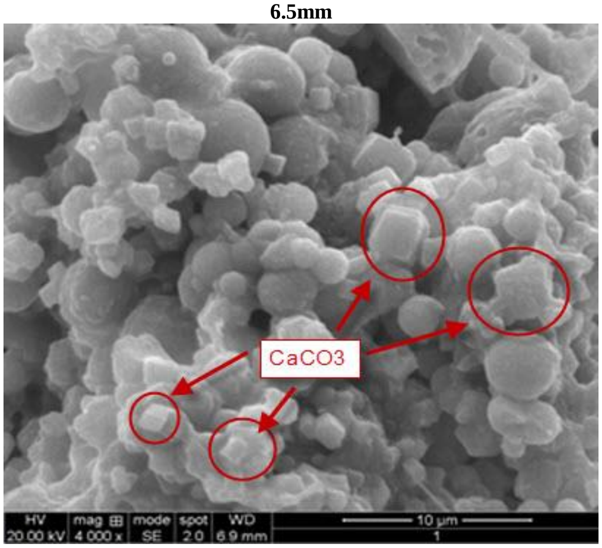

Fig. 11 SEM image at 4000x magnification and working distance of 6.9mm

5. Observations and Results

The successive sub-sections of colony isolation, staining, biochemical characterization, growth profile, and CaCO3 quantification are preliminary findings championing the best bacteria for a successful MICP system.

5.1. Isolation and Identification of Calcite Precipitating Bacteria

Six different colonies were noted after 24-48 hrs on selective media (Figure 12). Isolate-1 through Isolate-6 were morphologically distinguishable. In addition, the crystal halos around each colony confirmed ureolysis and CaCO3 precipitation, which was similar to findings by Stocks-Wang Jie Lu et al. [7].


Fig. 12 Shows the growth of bacterial colonies

5.2. Gram Staining

According to Gram staining (Figure 13), Isolates 1, 3, 5, and 6 are Gram-positive bacilli, and Isolates 2 and 4 are Gram-positive cocci. The thick peptidoglycan layer was responsible for retaining the crystal violet dye, which stained these Bacillus species purple. This finding was parallel to work with Bacillus species in MICP systems [1, 3].


Fig. 13 Gram-positive rods under oil immersion

5.3. Endospore Staining

According to endospore staining (Figure 14), Isolates 1, 3, 5, and 6 were endospore-forming. Endospore formation is important for microbial concrete as it supports bacterial survival ability in alkaline, dehydrated cementitious environments, similar to findings with Sporosarcina pasteurii and Bacillus megaterium [21, 22].

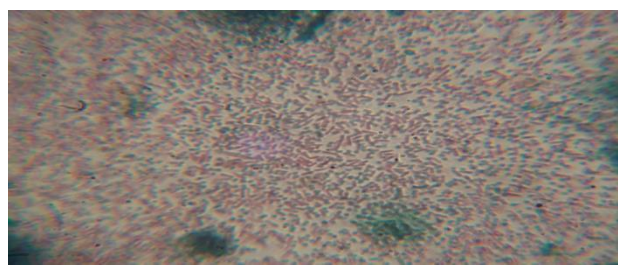
Fig. 14 Endospore staining showing endospores

5.4. Effect of pH on the Growth of Bacteria

pH tolerance testing shows that Isolate-1 benefits growth at pH 7.5-9.0 and survives up to pH 12 (Figure 16) while Isolate-6 benefits growth at pH 8.0-9.5 (Figure 17). As the pH of the pore-water in concrete is ~12-13, such ranges show that these bacteria are strong candidates for MICP in a cementitious environment. Reported growth patterns are similar to past pH tolerance tests of microbial concrete bacteria [5, 17].

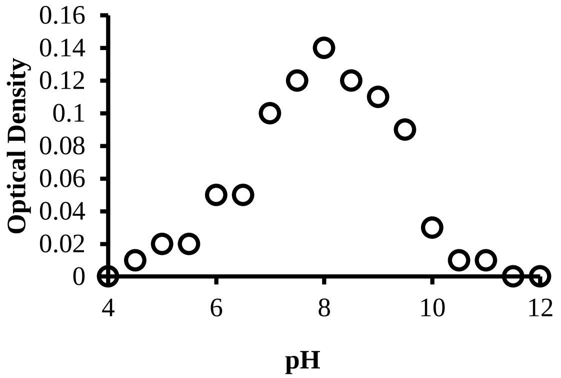

Fig. 15 Effect of pH on the growth of bacillus pasteurii

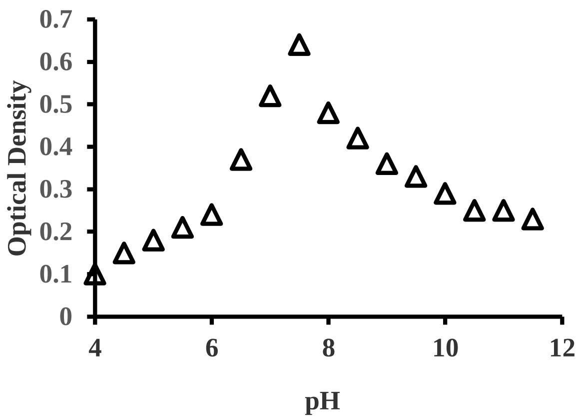

Fig. 16 Effect of pH on growth of isolate-1

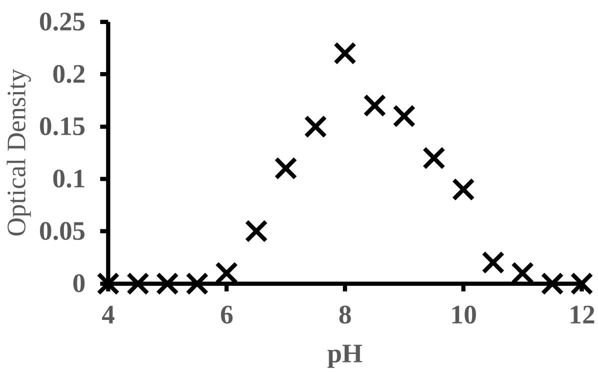

Fig. 17 Effect of pH on growth of isolate-6

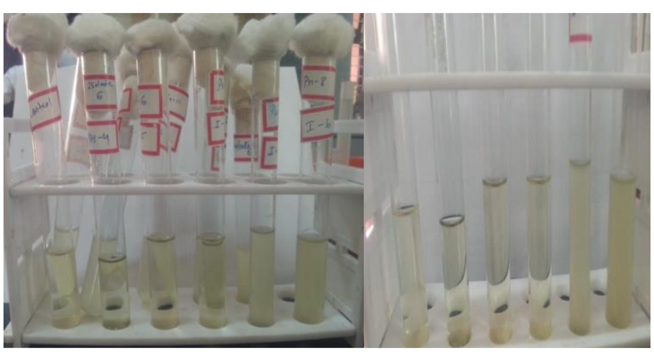

Fig. 18 Effect of pH on growth of bacteria

5.5. Computation of Generation Time

Generation time curves (Figure 19) reveal rapid growth during the exponential phase, which shows the strains to be metabolically active and ureolyzing simultaneously for the duration of the crack healing. Such quick generation time plays into higher urease activity, which is also propelled by high calcite precipitation [19].

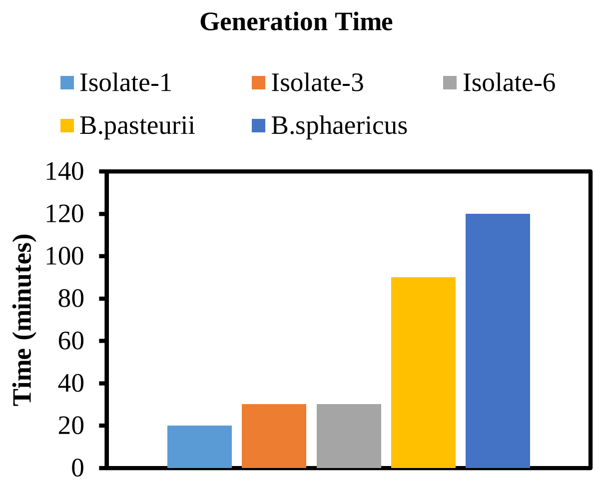

Fig. 19 Generation time of bacteria

5.6. Calcium Carbonate Estimation

CaCO3 precipitation was visually witnessed in 1000 mL of nutrient media by all isolates (Figure 20) and quantified, with the most from isolates 1 and 6. These results are directly proportional to these isolates' results with crack healing and strength recovery.

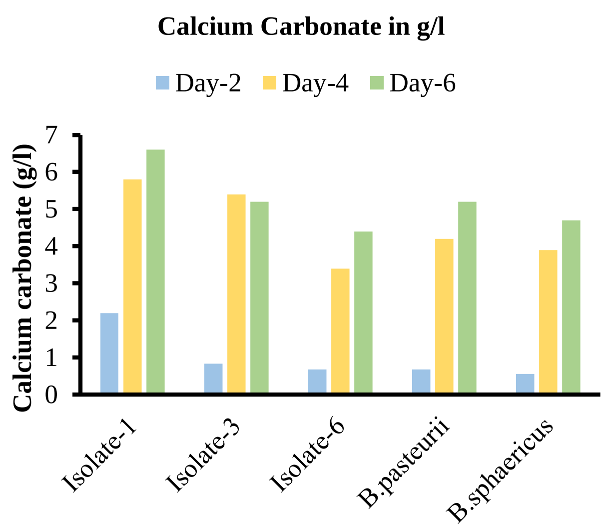

Fig. 20 Estimation of calcium carbonate

5.7. X-Ray Diffraction Analysis (XRD)

According to the results of XRD analysis (Figure 21), Crystalline Calcite Biomineralization was confirmed by all bacterial isolates, with the most precipitated from samples treated with immersion determined in this study (Figure 21). These results confirm previous findings with SEM and are confirmed by literature and recent success in MICP Mechanism Biomineralization [17, 21].

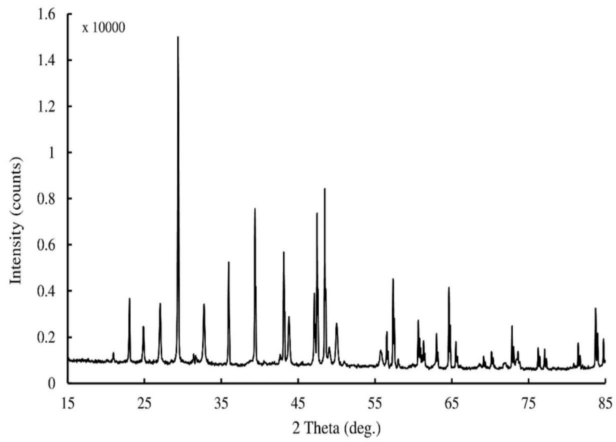

Fig. 21 Diffraction spectra of XRD test

6. Conclusion

- This study determined that Microbially Induced Calcite Precipitation (MICP) is a viable and most effective method for enhancing compressive strength and crack-healing ability of cementitious materials. All treatments assessed provided statistical significance confirmed ($p < 0.05$) for strength increases and visual crack closures. Thus, MICP as a biomineralization-associated recovery method was reliable.
- Calcium sources tested provided great data quality for levels of expectation, as calcium chloride and calcium nitrate provided consistently high levels of ureolytic activity and calcite precipitation, both qualitatively and quantitatively comparable to greater trends observed in antecedent MICP studies. Thus, it was determined that using calcium lactate was not effective as it was in crystalline production, rendering it an ineffective medium for structural recovery where needs are greatest.
- It was determined that both the immersion and injection methods were significantly better than ponding, as both features provided greater infiltration and extended access to nutrient provision. Both these methods provided 22-29% more compressive strength increases than ponding, as well as greater crystal formations, indicating that the method of application makes a significant difference in successful recovery.
- Finally, it was determined that of all isolates tested, Isolate-1 was the best as the most optimal conditions for recovery were characterized by high urease activity, timely creation and maintenance with survival in pH up to 12 without disintegration, and highly effective precipitation of calcite. These biochemical and physiological characteristics provide great merit to Isolate-1's survival in the concrete microenvironment, which is highly alkaline; thus, Isolate-1 is the best option for MICP-based self-healing recovery in the future.
- Confirmatory Microstructural Observations (SEM and XRD) of the mortar cracks observed rhombohedral, well-defined calcite crystals resulting in pore networks and fill at the crack surfaces. Thus, recovery confirmed from mechanics, as observed during strength assessment, was attributable to Biomineralization-Mediated fill, which is observably similar to MICP as defined in antecedent studies.
- Finally, determinants defined by morphological and mechanical integrity improvement per this study for mortar samples treated with Isolates 1, 3, and 6, as well as Bacillus sphaericus and Bacillus pasteurii, provide credible Biomineralization-associated strains for construction materials, as they not only hold fast but also exceed prior defined parameters as suggested by other microbial concrete studies.
- Ultimately, these findings support the MICP method as usable in more than cementitious materials in construction, as it could be relevant for stones, granite, marble, and porous geomaterials where pH and alkalinity are not of concern. This information means Biomineralization is a positive recapture technique for heritage archaeology, stone repairs, and green recovery systems down the line for improved construction materials.
- Ultimately, however, a need remains for nutrient dosing, bacterial immobilization, carrier formulations, field-scale practical applications, etc. Future studies should review longevity assessments for performance, impacts of loading cycles on recovery efficiency, practical recovery and healing in situ condition variances, and, most importantly, enhancement by means of genetics or metabolism for certain strains like Isolate-1, which exceeded expectations.
- Thus, this study determined that MICP is an innovative, sustainable, self-healing recapture method for construction materials that will significantly enhance their serviceability and durability over time. Minor adjustments can bring MICP from a laboratory-scale feasibility study to an implementable study for in situ recovery of construction materials without concern.

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