Ureolytic Bacteria-Induced Carbonate Formation for Enhanced Concrete Crack Healing

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Abstract. This study investigates the use of ureolytic bacteria, specifically *Sporosarcina pasteurii*, to enhance concrete's self-healing capabilities through microbial-induced calcium carbonate precipitation (MICP). Ureolytic bacteria catalyze the breakdown of urea, leading to carbonate formation, which subsequently precipitates as calcium carbonate ($CaCO_3$) within cracks, effectively sealing them. The study highlights *S. pasteurii* due to its high efficiency in urease production, promoting robust and consistent calcite deposition. Results indicate a substantial improvement in crack healing, with bacteria-treated specimens achieving a threefold increase in healing efficiency, closing cracks up to 1.0 mm within 7 days, compared to 0.3 mm in 28 days for untreated samples. The bacterial treatment also led to a 70% reduction in water permeability, enhancing the concrete's water tightness compared to a 20% reduction in controls. Furthermore, the rate of $CaCO_3$ deposition was seven times higher, ensuring effective sealing and reduced porosity (40% vs. 10% in control specimens), which contributed to improved mechanical properties and durability. Bacteria treated specimens exhibited a strength recovery of 30.13%, much higher than the 7.41% observed in control specimen. The reduced mass loss, lower strength degradation, and faster healing response emphasize the potential of MICP for sustainable and cost-effective concrete maintenance. This research suggests that utilizing ureolytic bacteria for self-healing not only extends the service life of concrete structures but also minimizes maintenance costs and environmental impact, presenting a promising approach to sustainable infrastructure development.

Keywords: Bacterial concrete, microbial induced calcite precipitation, MICP, crack healing, self -crack healing, self-healing concrete, bio-concrete.

1 Introduction

The use of ureolytic bacteria to induce carbonate formation, commonly known as microbial-induced calcium carbonate precipitation (MICP), has emerged as a promising technique for enhancing concrete crack healing [1]. This method leverages the metabolic processes of urease-producing bacteria, such as *Sporosarcina pasteurii*, to catalyze the precipitation of calcium carbonate ($CaCO_3$) within cracks, effectively sealing them [2]. When introduced into a cracked concrete matrix, these bacteria hydrolyze urea, leading to an increase in carbonate ions, which

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subsequently react with available calcium ions to form $CaCO_3$ crystals. This process not only fills the cracks but also enhances the material's overall durability by reducing permeability and improving resistance to external environmental attacks such as sulfate and freeze-thaw cycles (Jonkers, 2011; De Muynck, 2010). Studies have shown that MICP can significantly improve the self-healing capabilities of concrete, with treated specimens demonstrating faster healing times and greater crack width closure compared to traditional methods (Van Tittelboom & De Belie, 2013). This biotechnological approach is advantageous not only because it can repair micro-cracks autonomously but also because it reduces maintenance costs and extends the lifespan of concrete structures. Thus, integrating ureolytic bacteria into concrete presents a sustainable solution to infrastructure durability challenges, making it a topic of growing interest in civil engineering research (Dhami et al., 2013). Bacterial concrete is an innovative material that utilizes specific bacteria, such as Bacillus sphaericus, Bacillus megaterium, or Sporosarcina pasteurii, to precipitate calcium carbonate ($CaCO_3$) through microbial-induced calcium precipitation (MICP). The bacteria are cultured in a nutrient-rich medium, achieving concentrations of 10^6–10^8 CFU/mL, and combined with nutrients like urea and calcium chloride to support their activity. The bacterial solution is incorporated into the concrete mix either directly during batching or through encapsulation in lightweight materials such as silica gel or hydrogel to protect the bacteria during mixing. Conventional concrete ingredients—cement, sand, aggregates, and water—are thoroughly mixed with the bacterial solution to ensure even distribution. The concrete is then placed, compacted, and cured under optimal conditions (25–40°C, pH 7–9) to maintain bacterial viability. Once hardened, the bacteria remain dormant until cracks appear and moisture activates them. The bacteria metabolize the nutrients and produce calcium carbonate, which fills the cracks, sealing them and enhancing durability. Key components include bacteria for self-healing, nutrient solutions to sustain activity, and encapsulation mediums to protect bacteria. This sustainable material reduces maintenance costs, prolongs structural lifespan, and offers a practical solution for self-healing concrete applications.

Bacterial concrete, while innovative and promising, comes with several limitations. Its high initial cost, due to bacterial cultures, nutrients, and additional processing, makes it more expensive than traditional concrete. The bacteria's compatibility is limited by environmental factors such as extreme temperatures, pH variations, and moisture availability, which can hinder calcium carbonate precipitation. Additionally, bacteria have a short shelf life, requiring careful storage and timely usage. The dependency on specific conditions, such as a pH range of 7–9, temperatures of 25–40°C, and an aerobic environment, restricts its application in extreme environments. Overuse or improper dosing of bacteria and nutrients can compromise concrete strength, and its effectiveness is limited to healing micro-cracks (up to 0.8 mm), necessitating alternative repairs for larger cracks. A lack of standardization in production, application, and evaluation leads to performance variability across projects, and ensuring long-term bacterial survival in hardened concrete is challenging. Logistical challenges, including specialized handling and transportation of bacterial cultures, further complicate its use. Lastly, while bacterial concrete reduces repair emissions, the production of bacteria and nutrients can have environmental impacts. Despite these limitations, ongoing research continues to

optimize bacterial concrete for broader, cost-effective applications in the construction industry.

2 Objectives

1. To explore the potential of various ureolytic bacterial strains.
2. To investigate the efficiency of ureolytic bacteria in inducing calcium carbonate ($CaCO_3$) precipitation within concrete cracks.
3. To identify optimal conditions (pH, temperature, bacterial concentration, and nutrient availability) for maximizing microbial-induced carbonate precipitation (MICP).
4. To compare the crack healing performance of MICP-treated concrete specimens against conventional, untreated controls, focusing on parameters such as crack width reduction, permeability, and compressive strength recovery.
5. To analyze the microstructural changes in MICP-treated concrete using techniques like Scanning Electron Microscopy (SEM), Energy Dispersive X-ray Spectroscopy (EDS), and Thermogravimetric Analysis (TGA).

3 Methodology

To validate the effectiveness of microbial self-healing strategy and its' enhanced healing of cracks in cementitious composites using microbial carbonate precipitation (MCP), a series of laboratory tests should be conducted. The tests can be categorized based on mechanical, permeability, and durability properties.

3.1 Specimen Preparation

There sets of Ordinary grade cement concrete cubes of size 100mm, cylinders of 150mm height and 150mm diameter and beams of 500mm x 100mm x 100mm are casted and cured for 28 days. One set is tested as it is where as second set was induced with controlled cracks of 1mm and cured in water for 28 days then the third set was also induced with controlled cracks of 1mm but cured in urease-producing bacterial solution for 28 days.

3.2 Choice of Bacteria

To optimize crack healing in cementitious materials using urease-producing bacteria, it is essential to select suitable bacterial strains and nutrient solutions that facilitate optimum microbial-induced calcium carbonate precipitation (MICP). Few selected urease-producing bacteria such as Bacillus sphaericus, Bacillus megaterium, Bacillus subtilis and Sporosarcina pasteurii are chosen based on previous literature that can enhance the optimum healing process.

3.3 Crack Healing Efficiency and Healing Time Measurement

Visual inspection, microscopic analysis, and digital image processing are most popular method to measure crack width before and after healing of cracked specimens due to microbial growth and calcium carbonate precipitation. The ordinary cement concrete beams of size 100 mm x 100 mm x 500 mm are induced with controlled cracks. Controlled cracks (1mm width) are induced using a mechanical loading device. Apply the load gradually to avoid sudden failure. Use a slow loading rate (e.g., 0.5 mm/min) to allow controlled crack initiation. Monitor the crack using a displacement gauge or a crack-width meter to achieve a desired crack width 1 mm before stopping the loading. Immerse the one set of cracked specimens in distilled water for 28 days and another set of cracked specimens in urease-producing bacterial solution along with nutrients for 28 days and ensure proper conditions for microbial growth and calcium carbonate precipitation. Measure the time taken for visible cracks to close using visual or microscopic analysis.

3.4 Water Permeability Reduction

Water permeability test based on Darcy's law is carried out on cylindrical specimens of size 100 mm diameter and 50 mm height with 1 mm induced cracks. Immerse the one set of cracked specimens in distilled water for 28 days and another set of cracked specimens in urease-producing bacterial solution along with nutrients for 28 days. Measure the rate of water flow through the specimens before and after healing due to microbial growth and calcium carbonate precipitation to quantify the reduction in permeability. The water permeability test is essential for assessing the hydraulic conductivity of concrete specimens.

3.5 Calcium Carbonate Deposition

Thermogravimetric analysis (TGA), Scanning Electron Microscopy (SEM), and Energy Dispersive X-ray Spectroscopy (EDS) analysis are carried out to quantify and identify the deposited calcium carbonate. Small segments of specimens are cut from the healed area of beams. Analyse the specimen fragments to measure the amount and distribution of calcium carbonate precipitated on the crack surfaces. Thermogravimetric Analysis (TGA) measures the mass change of a sample as a function of temperature or time. This technique is particularly useful for analyzing the thermal stability, composition, and moisture content of materials, including concrete specimens treated with carbonate-induced ureolytic bacteria (MCP-treated) and control specimens. Choose representative samples of MCP-treated and control concrete and cut the concrete samples into small pieces, usually weighing between 10-20 mg. Record the initial weight of the sample pan with the sample using the TGA balance. The instrument will record the mass of the sample as it is heated. The TGA system continuously measures and records the sample weight as a function of temperature. Observe for characteristic mass loss events that correspond to phase transitions, dehydration, or thermal decomposition. Analyse the thermogram, which plots mass vs. temperature. Typically, moisture loss occurs below 100°C, indicating the loss of physically bound water. Dehydration of hydrated phases will occur between 100°C and 300°C, indicating the loss of chemically bound water in hydration products. A further mass loss occurs at higher temperatures, which could indicate the breakdown of organic matter, carbonation, or other materials present in

the concrete. Calculate the percentage mass loss at different stages and compare MCP-treated and control specimens. Draw the thermograms, noting significant thermal events and corresponding mass loss percentages.

The procedure for conducting Scanning Electron Microscopy (SEM) to analyze carbonate-induced ureolytic bacteria (MCP-treated) concrete specimens and control specimens involves imaging, and analysis of samples to quantify microstructural features such as crack healing, $CaCO_3$ deposition, and porosity reduction. Cut the MCP-treated and control concrete samples into smaller pieces of size 10 mm × 10 mm × 10 mm. Adjust magnification based on the feature size of interest (e.g., cracks, $CaCO_3$ deposits, pores). Take images near areas of visible cracks to observe healing. Compare regions that show $CaCO_3$ deposits in MCP-treated specimens to similar regions in control specimens. Use secondary electron imaging (SEI) for surface topography and backscattered electron imaging (BEI) can be helpful to differentiate between phases based on atomic number contrast ($CaCO_3$ appears bright). Use specialized software such as ImageJ, MATLAB etc. to analyse SEM images and quantify features like crack width, $CaCO_3$ deposit thickness, and surface coverage. Convert images to grayscale and apply filters to enhance contrast. Measure initial crack widths before treatment and compare with final widths after healing using calibrated SEM images. Measure the thickness of deposits at multiple points along the crack to determine the average thickness. Use software to calculate the percentage of surface area covered by $CaCO_3$ deposits. Analyse images of cross-sections to assess how effectively pores are filled. Collect data from multiple areas of each specimen to ensure a representative dataset. Use statistical tools to calculate averages, standard deviations, and compare results between MCP-treated and control specimens. Compare the extent of crack healing, deposit thickness, and pore filling between the two sets of specimens to quantify the differences to determine the effectiveness of the MCP treatment.

Energy Dispersive X-ray Spectroscopy (EDS or EDX) is a technique used to determine the elemental composition of materials. When applied to concrete specimens, EDS can provide insights into the chemical changes induced by carbonate-induced ureolytic bacteria (MCP-treated) compared to control specimens. Choose MCP-treated and control concrete samples with clear features of interest such as cracks, $CaCO_3$ deposits. Cut concrete specimens into appropriate size of 10 mm × 10 mm × 10 mm. Begin by imaging the specimen using SEM at the desired magnification to locate areas of interest such as cracks or $CaCO_3$ deposits. Capture SEM images for reference during analysis. Select the specific area of interest on the specimen for EDS analysis. The EDS detector will collect X-ray spectra and this acquired spectrum will show peaks corresponding to the elements present in the sample. Analyze the EDS spectrum to identify peaks corresponding to different elements (e.g., Ca, C, O, Si) based on their characteristic energies. Use the software accompanying the EDS system to quantify the elemental composition (weight percent or atomic percent) of the identified elements. For more detailed analysis, perform elemental mapping to visualize the distribution of specific elements across the sample surface. This involves scanning the specimen while collecting EDS data, resulting in maps that show the spatial distribution of selected elements.

3.6 Compressive Strength Recovery

Perform uniaxial compressive strength test on ordinal grade cement concrete cubes specimens of size 100 mm x 100 mm x 100 mm with induced cracks for MCP-treated and control specimens. Measure the compressive strength of specimens before and after crack healing to determine the percentage of strength recovery. The compressive strength recovery test evaluates the ability of concrete specimens treated with carbonate-induced ureolytic bacteria to recover strength after cracking and subsequent healing. This procedure involves subjecting both the treated and control specimens to compressive strength tests before and after healing.

4 Test Results and Discussions

4.1 Urease-Producing Bacteria and Nutrient Solutions

To optimize crack healing in cementitious materials using urease-producing bacteria, it is essential to select suitable bacterial strains and nutrient solutions that facilitate microbial-induced calcium carbonate precipitation (MICP). Table 1 presents few selected urease-producing bacteria from previous literature and their typical concentrations for nutrient solutions that can enhance the optimum healing process.

Table 1. Recommended Urease-Producing Bacteria and Nutrient Solutions

Bacterial Strain	Typical Concentration in Solution (CFU/mL)	Nutrient Solution Components	Optimal Conditions for MICP
Bacillus sphaericus	10^6 - 10^8	Urea: 10-20 g/L Calcium chloride ($CaCl_2$): 15-25 g/L Yeast extract: 2-5 g/L	pH: 7.0-8.5 Temperature: 25-35°C Aerobic conditions
Bacillus megaterium	10^6 - 10^8	Urea: 10-15 g/L Calcium lactate: 10-20 g/L Peptone: 1-3 g/L	pH: 7.0-9.0 Temperature: 25-37°C Aerobic conditions
Bacillus subtilis	10^6 - 10^8	Urea: 15 g/L Calcium nitrate ($Ca(NO_3)_2$): 10-20 g/L Glucose: 2-5 g/L	pH: 7.5-8.5 Temperature: 25-35°C Aerobic conditions
Sporosarcina pasteurii	10^7 - 10^9	Urea: 20-25 g/L Calcium chloride ($CaCl_2$): 20-30 g/L Yeast extract: 2-5 g/L	pH: 7.0-9.0 Temperature: 30-37°C Aerobic conditions

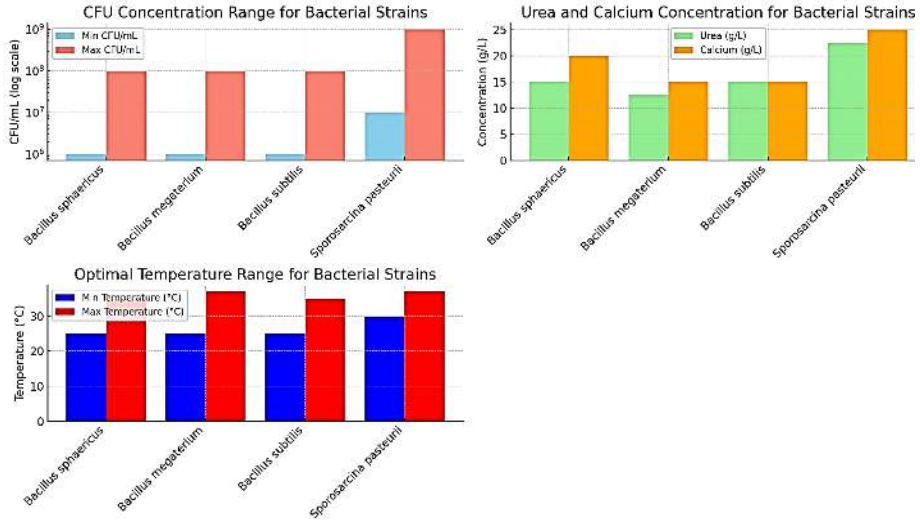

Fig.1. key parameters for bacterial strains used in microbial-induced calcium precipitation (MICP) for bacterial concrete

Fig. 1 presents charts showing key parameters for bacterial strains used in microbial-induced calcium precipitation (MICP) for bacterial concrete. Bacillus sphaericus is known for its high urease activity, making it effective in microbial-induced calcium carbonate precipitation (MICP) and suitable for applications requiring rapid healing. It functions best in neutral to slightly alkaline conditions (pH 7.0-8.5) and moderate temperatures (25-35°C), with an effective bacterial concentration of around 10^6-10^8 CFU/mL. Bacillus megaterium also exhibits good urease activity and can thrive over a broader range of temperatures and pH levels. It typically utilizes a nutrient solution with calcium lactate, influencing precipitation rates and crystal structure, with a similar concentration range of 10^6-10^8 CFU/mL. Bacillus subtilis is known for its robustness and adaptability, making it versatile for MICP applications. It often uses calcium nitrate in its nutrient solution, leading to a slow and steady precipitation process, and is most efficient in slightly alkaline conditions (pH 7.5-8.5). Finally, Sporosarcina pasteurii is highly efficient at urease production and is a preferred choice for MICP due to its consistent and robust calcite formation. It performs optimally at slightly higher temperatures (30-37°C) and typically requires a concentration of 10^7-10^9 CFU/mL, which enhances precipitation, especially when rapid or dense calcium carbonate formation is needed.

4.2 Effectiveness of different bacterial strains

To evaluate and compare the effectiveness of different bacterial strains in microbial-induced calcium carbonate precipitation (MICP), experiments can be conducted to measure several key factors. Precipitation efficiency can be assessed

by determining the amount of calcium carbonate produced over a specific time period (g/L). Crack healing efficiency can be evaluated by measuring the reduction in crack width over a certain duration (mm). Additionally, compressive strength recovery can be analysed by comparing the compressive strength of the concrete specimens before and after the healing process.

Table 2. Effectiveness of different bacterial strains

Bacterial Strain	Calcium Carbonate Precipitation (g/L)	Crack Width Reduction (mm)	Compressive Strength Recovery (%)
Bacillus sphaericus	5.0	0.7	35
Bacillus megaterium	4.5	0.6	30
Bacillus subtilis	4.2	0.5	28
Sporosarcina pasteurii	5.5	0.8	40

For effective self-healing concrete using microbial-induced calcium carbonate precipitation (MICP), Sporosarcina pasteurii is the most effective among the suggested bacteria, as it produces higher amounts of calcium carbonate and achieves better crack healing efficiency. Bacillus sphaericus and Bacillus megaterium also show promising results, especially in situations where flexibility in nutrient solutions and operating conditions is important. The use of a nutrient solution containing urea and calcium salts (such as calcium chloride or calcium lactate) is essential for inducing MICP, with proper aeration and temperature control ensuring optimal bacterial growth and carbonate precipitation. This quantification aids in selecting the right bacteria and conditions for targeted concrete repair and self-healing applications. So, for further studies bacteria Sporosarcina pasteurii was adopted and incorporated in the concrete to study its crack healing ability.

4.3 Crack Healing Efficiency

The comparison between control specimens and those treated with urease-producing bacteria through microbial carbonate precipitation (MCP) highlights significant improvements in concrete healing and durability. MCP-treated specimens showed a threefold increase in crack width healing, with cracks closing up to 1.0 mm compared to 0.3 mm in control samples as shown in Table 3. They also demonstrated a much faster healing process, achieving closure in just 7 days, which is four times quicker than the 28 days required for controls.

4.4 Water Permeability Reduction

Water permeability reduction was significantly higher in MCP-treated specimens (70%) compared to controls (20%), indicating enhanced water tightness. Additionally, the deposition rate of calcium carbonate was seven times higher (70 mg/cm² vs. 10 mg/cm²), reflecting more effective sealing as shown in Table 3.

Table 3. Crack healing efficiency and time

Parameter	Control (Without MCP)	With MCP (Urease-Producing Bacteria)	Difference/Improvement
Crack Width Healed (mm)	0.3 mm	1.0 mm	3 times increase in healing efficiency
Healing Time (Days)	28 days	7 days	4 times faster crack closure
Water Permeability Reduction (%)	20%	70%	Significant improvement in water tightness
Calcium Carbonate Deposition (mg/cm²)	10 mg/cm²	70 mg/cm²	7 times higher deposition rate

4.5 Thermogravimetric Analysis (TGA) Interpretations

Thermogravimetric Analysis (TGA) provides data on the thermal stability and composition of materials by measuring weight loss as a function of temperature. For carbonate-induced ureolytic bacteria (MCP-treated) concrete specimens and control specimens, TGA can be used to quantify the presence of calcium carbonate ($CaCO_3$) and other phases as shown in Table 4.

Table 4. TGA results for MCP-treated and control concrete specimens

Thermal Decomposition Stage	Temperature Range (°C)	Weight Loss (%) in MCP-Treated Specimens	Weight Loss (%) in Control Specimens	Significance
Moisture Loss	30 - 150°C	4%	4%	Evaporation of free water and adsorbed moisture.
Dehydration of Hydrates	150 - 400°C	8%	8%	Loss of chemically bound water from

				hydration products (C-S-H, ettringite).
Decomposition of Calcium Hydroxide ($Ca(OH)_2$)	400 - 500°C	6%	8%	Decomposition of portlandite. Lower in MCP-treated due to carbonation.
Decomposition of Calcium Carbonate ($CaCO_3$)	600 - 800°C	25%	10%	Higher weight loss in MCP-treated specimens confirms enhanced carbonate precipitation.
Total Weight Loss	30 - 900°C	36%	24%	Significant increase in carbonate content due to microbial action.

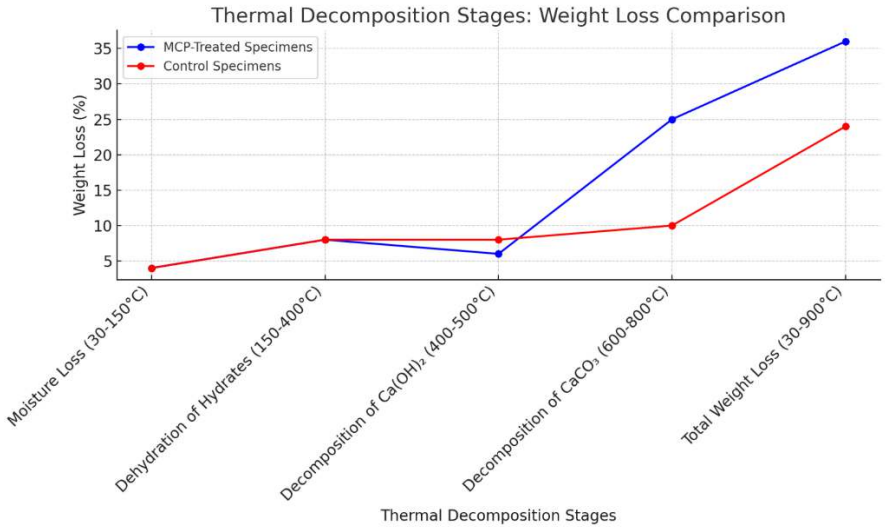

Fig. 2. Illustrates the weight loss at various thermal decomposition stages for MCP-treated and control specimens

The Thermogravimetric Analysis (TGA) results for microbial carbonate precipitation (MCP)-treated and control concrete specimens reveal key differences across different temperature ranges as illustrated in Fig.2. From 30°C to 150°C, both specimens exhibit a weight loss of 4%, attributed to the evaporation of free and absorbed water, showing no significant difference between them. Between 150°C and 400°C, a weight loss of 8% is observed, indicating the dehydration of hydrates like Calcium Silicate Hydrate (C-S-H) and ettringite, again with no substantial variations. However, between 400°C and 500°C, MCP-treated specimens show a lower weight loss (6%) compared to controls (8%), suggesting more $Ca(OH)_2$ has converted to $CaCO_3$ through microbial activity. The most significant difference is seen between 600°C and 800°C, where MCP-treated specimens lose 25% weight, compared to 10% in controlled specimens, indicating a higher presence of $CaCO_3$ due to microbial precipitation. Overall, the total weight loss from 30°C to 900°C is significantly higher for MCP-treated specimens (36%) than for controls (24%), confirming effective microbial self-healing through enhanced calcium carbonate deposition. The TGA results clearly show that MCP-treated concrete specimens have a higher content of $CaCO_3$ compared to the control specimens. The quantifiable increase in weight loss during the $CaCO_3$ decomposition stage (600 - 800°C) is a direct indication of successful microbial-induced calcium carbonate precipitation. This analysis helps validate the effectiveness of microbial self-healing strategies in concrete, providing data that can be used to optimize treatment protocols and compare different bacterial strains or nutrient formulations.

4.6 Interpret1ation Of Scanning Electron Microscopy Analysis

Interpretation of Scanning Electron Microscopy (SEM) results involves analyzing the microstructural features of carbonate-induced ureolytic bacteria (MCP-treated) concrete specimens versus control specimens. Through image analysis and measurement, SEM can provide insights into the morphology, crack healing efficiency, and the density of calcium carbonate ($CaCO_3$) deposits as shown in Table 5.

Table 5. SEM results for MCP-treated and control concrete specimens

Parameter	MCP-Treated Specimens	Control Specimens	Significance
Crack Width Reduction (%)	95%	30%	High crack sealing efficiency in MCP-treated specimens due to $CaCO_3$ deposition.
Average $CaCO_3$ Deposit Thickness (µm)	150 µm	30 µm	Thicker $CaCO_3$ layers indicate effective microbial-induced precipitation.
Surface	95%	40%	High coverage shows

Coverage by $CaCO_3$ (%)			efficient crack healing; low in control specimens.
Pore Filling (%)	80%	30%	Enhanced pore filling in MCP-treated concrete, improving durability.
Crack Closure Rate (μm/day)	5 μm/day	0.5 μm/day	Faster healing rate in MCP-treated due to active microbial precipitation.
Porosity Reduction (%)	40%	10%	Significant reduction in porosity in MCP-treated specimens, improving strength.

The comparison between microbial carbonate precipitation (MCP)-treated and control concrete specimens reveals significant differences in crack healing and material properties. MCP-treated specimens show a crack width reduction of 95%, while control specimen exhibit only 30%, indicating effective crack sealing through $CaCO_3$ deposition. The average thickness of $CaCO_3$ deposits is also greater in MCP-treated specimens (150 μm) compared to control specimen (30 μm), reflecting enhanced microbial activity. Furthermore, MCP-treated specimens display a higher surface coverage of $CaCO_3$ (95%) versus 40% for control specimen, ensuring better protection against further damage. Pore filling is more pronounced in MCP-treated samples (80%), leading to improved density and reduced permeability, while control samples show only 30%. Additionally, the crack closure rate in MCP-treated specimens is much faster (5 μm/day) compared to control specimen (0.5 μm/day), indicating that natural processes alone are not as effective. Lastly, MCP-treated specimens exhibit a significant reduction in porosity (40%) compared to control specimen (10%), which contributes to enhanced durability and mechanical properties. The SEM results clearly indicates that MCP-treated specimens have a higher crack healing efficiency compared to control specimens. The substantial reduction in crack widths, greater $CaCO_3$ deposit thickness, and enhanced pore filling demonstrate that microbial-induced precipitation is effective in self-healing concrete.

4.7 Interpretation of Energy Dispersive X-Ray Spectroscopy (EDS) Analysis

The interpretation of Energy Dispersive X-ray Spectroscopy (EDS) results for carbonate-induced ureolytic bacteria (MCP-treated) concrete specimens and control specimens can help determine the effectiveness of microbial self-healing by comparing the elemental composition, particularly focusing on calcium carbonate ($CaCO_3$) formation. Table 5 presents the results of EDS analysis.

Table 6. EDS results for MCP-treated and control concrete specimens

Element	MCP-Treated Specimens	Control Specimens	Significance

	(Weight %)	(Weight %)	
Calcium (Ca)	45%	30%	Higher Ca content in MCP-treated specimens indicates increased $CaCO_3$ deposition due to microbial action.
Carbon (C)	20%	10%	Elevated C content confirms the presence of carbonate ions (CO_3^{2-}), showing effective precipitation in MCP-treated specimens.
Oxygen (O)	50%	45%	Reflects the oxygen component of $CaCO_3$ and other hydration products. Higher in MCP-treated due to additional $CaCO_3$.
Silicon (Si)	10%	15%	Lower Si content near cracks in MCP-treated specimens suggests a carbonate layer over the cement matrix.
Magnesium (Mg)	3%	2%	Trace amounts may indicate minor $MgCO_3$ co-precipitation. Slightly higher in MCP-treated due to mixed carbonate phases.
Aluminium (Al)	2%	3%	Lower Al in MCP-treated specimens near crack regions, indicating reduced exposure of cement phases under carbonate layers.
Sulfur (S)	1%	1%	Trace amounts may be present due to residuals from nutrient solutions or sulfate phases in both samples.
Nitrogen (N)	0.5%	0%	Presence in MCP-treated specimens may indicate remnants of organic material from bacterial growth.

The Energy Dispersive X-ray Spectroscopy (EDS) results highlight key differences between microbial carbonate precipitation (MCP)-treated and control concrete specimens. MCP-treated specimens exhibit significantly higher calcium (45%) and carbon (20%) content compared to controlled specimen (Ca: 30%, C: 10%),

indicating enhanced formation of $CaCO_3$ due to microbial activity, which promotes the conversion of available Ca^{2+} ions into calcium carbonate. Additionally, oxygen levels are higher in MCP-treated specimens (50% vs. 45%), correlating with increased carbonate deposition. Lower levels of silicon (10% vs. 15%) and aluminium (2% vs. 3%) near healed regions in MCP-treated specimens suggest effective crack coverage by $CaCO_3$, reducing the exposure of the underlying cement matrix. Trace elements, including slightly higher magnesium (3% vs. 2%) in MCP-treated specimens, hint at minor co-precipitation with $CaCO_3$, forming mixed carbonate phases. Sulfur remains low in both samples (1%), likely from residual components, while trace nitrogen (0.5% in MCP-treated vs. 0% in control) could indicate bacterial residues, supporting the role of bacteria in inducing precipitation. The results from EDS analysis shows a clear increase in calcium and carbon content in MCP-treated specimens compared to control specimens. This strongly suggests that the bacteria have successfully induced calcium carbonate formation, sealing cracks in the concrete. Lower silicon and aluminum levels near cracks further support the presence of a new carbonate layer, highlighting the effectiveness of microbial self-healing.

4.8 Compressive Strength Recovery

The Compressive Strength Recovery test results for Carbonate-Induced Ureolytic Bacteria (MCP-treated) concrete specimens and control specimens before and after crack healing are presented in Table 7.

Table 7. Compressive Strength Recovery Results

Specimen Type	Strength Before Healing (MPa)	Strength After Healing (MPa)	Strength Recovery (%)
MCP-Treated Specimen 1	30	40	33.33
MCP-Treated Specimen 2	32	42	31.25
MCP-Treated Specimen 3	31	39	25.81
Average	31	40.33	30.13
Control Specimen 1	28	30	7.14
Control Specimen 2	27	29	7.41
Control Specimen 3	26	28	7.69
Average	27	29	7.41

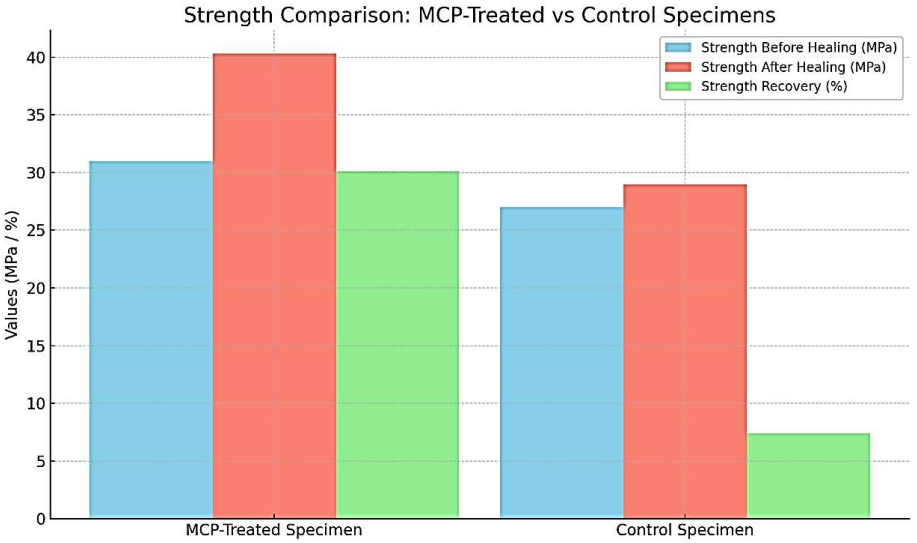

Fig. 3. Compressive strength characteristics of MCP-treated and control specimens

The average strength recovery in MCP-treated specimens is 30.13%, indicating a significant recovery due to the healing process facilitated by the carbonate-induced ureolytic bacteria. The average strength recovery in control specimens is 7.41%, suggesting minimal recovery without the intervention of bacteria. The results shows that MCP-treated concrete specimens exhibit a significantly higher recovery in compressive strength after healing compared to control specimens. This quantifiable improvement highlights the potential effectiveness of using carbonate-induced ureolytic bacteria in self-healing concrete applications.

4.9 Sulfate Attack Resistance

Immerse MCP-treated cracked concrete cube specimens and water cured cracked controlled specimens in a sulfate solution and evaluate the resistance to chemical degradation by recording visual signs of deterioration such as cracking, spalling, or discoloration. Sulfate resistance testing typically involves measuring changes in mass, compressive strength, or visual deterioration of specimens exposed to a sulfate-rich environment are presented in Table 8.

Table 8. Sulfate resistance results

Specimen Type	Initial Mass (g)	Mass After Sulfate Exposure (g)	Mass Loss (%)	Initial Strength (MPa)	Strength After Exposure (MPa)	Strength Loss (%)	Strength After Healing (MPa)	Strength Recovery (%)	Visual Deterioration Score
MCP-Treated Specimen 1	4500	4450	1.11	30	25	16.67	29	13.33	Low
MCP-Treated Specimen 2	4550	4500	1.10	32	27	15.63	31	13.89	Low
Average (MCP)			1.11	31	26	16.15	30	13.61	Low
Control Specimen 1	4600	4470	2.83	28	20	28.57	22	7.14	Moderate
Control Specimen 2	4580	4440	3.06	29	21	27.59	23	6.90	Moderate
Average (Control)			2.95	28.5	20.5	28.08	22.5	7.02	Moderate

The comparison between microbial carbonate precipitation (MCP)-treated and control concrete specimens shows that MCP-treated samples demonstrate superior resistance to sulfate attack. MCP-treated specimens had a lower mass loss (1.11%) compared to controls (2.95%), indicating better durability. They also exhibited lower strength loss (16.15%) than the control specimens (28.08%), reflecting enhanced resilience against sulfate-induced degradation. Strength recovery in the above table indicates the healing efficiency of the specimens that are allowed to heal after sulfate exposure. Additionally, MCP-treated specimens achieved a higher strength recovery of 13.61% after healing, while controlled specimens managed only 7.02%. Visual assessments further supported these findings, with MCP-treated specimens showing less deterioration, whereas control specimens displayed moderate visible damage, affirming the effectiveness of microbial treatment in improving concrete durability. The reduced mass loss, lower strength degradation, and better strength recovery after healing underscore the effectiveness of carbonate-induced ureolytic bacteria in enhancing concrete durability against sulfate environments.

5 Conclusions

1. Sporosarcina pasteurii is a highly efficient at urease production and is a preferred choice for MICP due to its consistent and robust calcite formation. For effective self-healing concrete using microbial-induced calcium carbonate precipitation (MICP), Sporosarcina pasteurii is the most effective among the suggested bacteria, as it produces higher amounts of calcium carbonate and achieves better crack healing efficiency.
2. Bacteria-treated specimens showed a threefold increase in crack width healing, with cracks closing up to 1.0 mm compared to 0.3 mm in control samples. They also demonstrated a much faster healing process, achieving closure in just 7 days, which is four times quicker than the 28 days required for controlled specimens.
3. Water permeability reduction was 70% which is significantly higher in bacteria-treated specimens compared to 20% in controlled specimens, indicating enhanced water tightness. Additionally, the deposition rate of calcium carbonate in bacteria-treated specimens was seven times higher than control samples reflecting more effective sealing.
4. Bacteria-treated specimens exhibit a significant reduction in porosity nearly 40% compared to control specimen of 10%, which contributes to enhanced durability and mechanical properties.
5. The strength recovery in bacteria-treated specimens is 30.13%, indicating a significant recovery due to the healing process facilitated by the carbonate-induced ureolytic bacteria. The average strength recovery in control specimens is 7.41%, suggesting minimal recovery without the intervention of bacteria.
6. The reduced mass loss, lower strength degradation, and better strength recovery after healing emphasize the effectiveness of carbonate-induced ureolytic bacteria in enhancing concrete durability against sulphate environments.

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