

BIO-HEALING OF GENERATED CRACKS IN ROCK MASS USING ENZYME

A Dissertation

Submitted in Partial Fulfillment of the Requirements for the Degree of

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IN

GEOTECHNICAL ENGINEERING

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CANDIDATE'S DECLARATION

I hereby declare that the project report entitled **BIO-HEALING OF GENERATED CRACKS IN ROCK MASS USING ENZYME**, submitted to Delhi Technological University in partial fulfillment of the requirements for the award of the degree of Master of Technology (Geotechnical Engineering), represents my own original work. The material presented herein has not been submitted, either in part or in full, to any other university or institution for the award of any degree or diploma.

All sources of information consulted during the preparation of this report have been duly acknowledged. To the best of my knowledge and belief, this report is free from plagiarism and any form of academic misconduct.

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This is to certify that the project report entitled **BIO-HEALING OF GENERATED CRACKS IN ROCK MASS USING ENZYME** , submitted by **Adarsh Anand (Roll No. 24/GTE/14)**, for partial fulfillment of the requirements for the award of the degree of **Master of Technology in Geotechnical Engineering**, is an authentic record of work carried out by him under my supervision and guidance. The work presented in this report is original, and to the best of my knowledge, no part of this report has been submitted elsewhere for the award of any degree.

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ABSTRACT

Rock masses in tunnels, slopes, foundations and underground excavations are highly susceptible to crack development caused by blasting, excavation, weathering and repeated mechanical loading, which compromises their strength, permeability and long-term stability. Conventional repair techniques such as cement grouting and epoxy injection are costly, environmentally damaging and often ineffective for fine or inaccessible cracks. This study investigates Enzyme-Induced Carbonate Precipitation (EICP) as a sustainable, bio-based alternative for healing artificially generated cracks in rock specimens. Rock core samples retrieved from a field borehole were characterized for their physical properties, after which artificial cracks were induced and treated with an enzyme healing solution containing urease, urea and calcium chloride. The treated specimens were cured under controlled conditions to allow calcium carbonate precipitation within the crack network. Post-treatment evaluation included measurements of weight change, density, water absorption, crack width closure and Uniaxial Compressive Strength (UCS) recovery, which were compared against untreated cracked specimens. The results demonstrate that EICP treatment produced measurable healing efficiency, with enzyme-induced calcium carbonate deposits sealing crack surfaces, reducing permeability and restoring a significant portion of the original compressive strength. These findings confirm that enzyme-based bio-healing offers a viable, eco-friendly and cost-effective approach for repairing damaged rock masses in geotechnical and underground engineering applications, with potential for further validation over longer curing durations and

varied rock types. The other serval application of EICP in tunnel stabilization, Sustainable rock engineering and foundation improvement.

Keywords: Bio-healing, Enzyme-Induced Carbonate Precipitation (EICP), Rock mass, Crack healing, Uniaxial Compressive Strength, Calcium carbonate precipitation.

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LIST OF ABBREVIATIONS

Abbreviation	Full Form
EICP	Enzyme-Induced Carbonate Precipitation
MICP	Microbially/Microbial-Induced Carbonate Precipitation
UCS	Uniaxial Compressive Strength
CaCO₃	Calcium Carbonate
CaCl₂	Calcium Chloride
SEM	Scanning Electron Microscopy
XRD	X-Ray Diffraction
UPV	Ultrasonic Pulse Velocity
UTM	Universal Testing Machine
ISRM	International Society for Rock Mechanics
ASTM	American Society for Testing and Materials
DTU	Delhi Technological University
RSM	Response Surface Methodology
DIC	Digital Image Correlation
FEM	Finite Element Modelling
CR	Core Recovery

Chapter 1

INTRODUCTION

1.1 Overview

Rock masses form the basic structural units for many civil construction projects, mining operations and geotechnical engineering applications, such as tunnels, underground caverns, dams, slopes, foundations and transportation infrastructure [1]. Rocks are inherently heterogeneous materials with natural discontinuities like joints, fissures, pores and microcracks, although they are very strong from a compression and durability standpoint. These discontinuities grow larger cracks during excavation, drilling, blasting, seismic activity, weathering, thermal fluctuations and repeated mechanical loading, which severely affect the mechanical stability and durability of rock masses [2]. Crack formation and growth leads to a rise in permeability, degradation of compressive and tensile strength, water infiltration and chemical corrosion, which in turn negatively affects the long-term performance and safety of engineering structures [3]. Traditional methods of repairing cracks involve cement grouting, epoxy injection, or sealing with polymer-based materials, which tend to be costly, damaging to the environment, labor-intensive and hard to apply underground in inaccessible areas [4]. Moreover, these methods are normally passive and fail to react to newly developed cracks after being treated.

Self-healing technologies in the field of crack repairs of cementitious and geological materials have grown into a sustainable and innovative approach in recent years [5]. Bio-healing employs biological agents or the metabolites of these agents to induce

the precipitation of calcium carbonate (CaCO_3) that occurs naturally to fill cracks and restore the integrity of damaged materials [6]. Of all these biological methods, enzyme-induced carbonate precipitation (EICP) has received considerable interest since it eliminates the need for live microorganisms and still possesses the ability of inducing mineral formation [7]. The urease enzymes in the EICP process convert urea into carbonate ions and ammonium ions. The carbonate ions then combine with calcium ions from an external source to form calcium carbonate crystals [8]. These crystals form in the fractures and in the pores, closing fractures, decreasing permeability, increasing stiffness and mechanical properties of the rock mass. Advantages of enzyme-based healing include ease of control, fast reaction time, no need for bacteria to survive, reduced biological contamination risk, and wide applicability over varying environmental conditions, compared with conventional microbial-induced carbonate precipitation (MICP) [9].

Enzyme-induced bio-healing has been gaining more attention in the field of rock engineering applications, aiming to offer a green and economical solution to the problem of long service life of underground structures [10]. Precipitated Calcium Carbonate provides a dense mineral bridge at the surfaces of cracks which provides better interlocking between fractured rock particles and better load transfer mechanisms. Also, the mineralized layer decreases the permeability of the water to the concrete and stops the propagation of cracks due to the hydraulic pressure or weathering processes [11]. It has been observed in various studies that the compressive strength, tensile strength, ultrasonic velocity and durability of various types of rocks such as sandstone, limestone, granite, shale and others can be improved significantly after EICP treatment [12]. Healing efficiency is affected by a

number of parameters including, concentration of enzymes, concentration of urea, source of calcium, curing temperature, pH, treatment time, crack width and mineralogy of the rock. Thus, systematic optimization of these parameters is crucial to achieve optimal healing performance and guarantee successful field implementation.

The present research focuses on the bio-healing of generated cracks in rock mass using enzymes by investigating the effectiveness of enzyme-induced calcium carbonate precipitation in restoring damaged rock specimens. Artificial cracks are created in the controlled laboratory environment, and treated with enzyme chemistry containing urease, urea, and calcium ions to mimic the fracture conditions that might occur in the field. The repaired specimens are tested after healing for mechanical, physical and microstructural properties such as compressive strength test, ultrasonic pulse velocity (UPV) test, water permeability test, scanning electron microscopy (SEM) and X-ray diffraction (XRD). The analyses give a complete overview on the crack closure, mineral deposition, recovery of strength and enhancement of durability through enzyme-induced biomineralization.

1.2 Bio-Healing Technology in Rock Engineering

Bio-healing technology is a new and environmentally friendly method to seal cracks and repair the integrity of rock masses by biologically induced mineral precipitation [13]. Bio-healing is a bio-repair process that involves the use of microorganisms or enzymes that create calcium carbonate (CaCO_3), a naturally occurring mineral, which is used to fill cracks and strengthen damaged rock structures, unlike traditional repair methods which use synthetic chemicals or cementitious grouts [14]. The technique

has attracted considerable interest in geotechnical and mining engineering due to its environmentally friendly nature, which provides a low carbon, non-toxic and sustainable option to address crack propagation in underground structures [15]. Mechanical loading, blasting, excavation, groundwater, freeze–thaw, and seismic activity are all ongoing processes that continually expose rock masses, which are the source of fractures. All these cracks cause a higher porosity and permeability, a lower mechanical strength and make the weathering process faster, which thus affects the stability and service life of engineering structures.

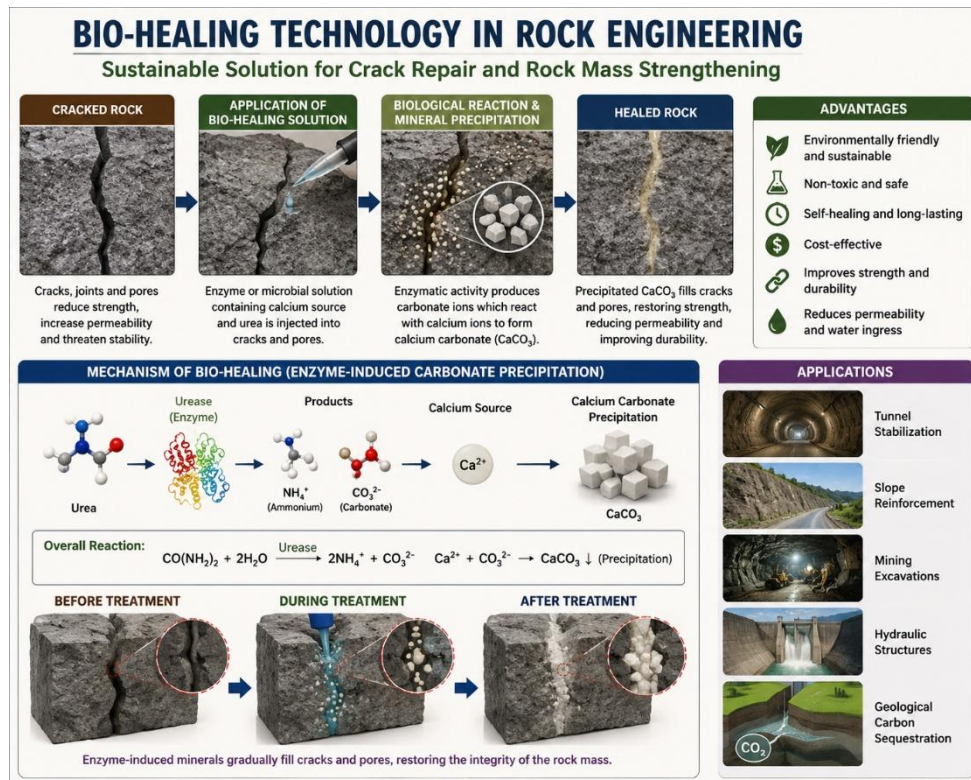
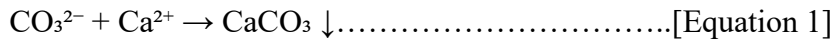


Figure 1.1: Bio-Healing technology in rock engineering

Bio-healing is based on the simple principle that minerals can be deposited by biological catalysts in cracks and pores in a natural process [16]. The urease enzyme catalyzes the hydrolysis of urea, producing carbonate ions (CO_3^{2-}) and ammonium

ions (NH_4^+) in EICP. The carbonate ions combine with the calcium ions (Ca^{2+}) to form calcium carbonate when the calcium source (calcium chloride CaCl_2) is added:



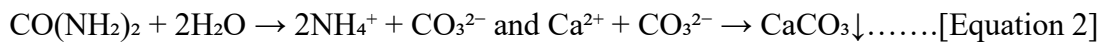
CaCO_3 crystals are precipitated on crack walls and create a dense mineral bond between the broken particles of rock. This mineral deposition resulted in sealing the cracks, lowering the permeability of water, increasing the compressive and tensile strength and improving the durability of the rock mass [17]. Bio-healing has an excellent compatibility with natural geological materials, as the calcium carbonate deposited has mineralogical properties similar to the natural carbonate minerals.

Additionally, enzyme-based bio-healing has several benefits over microbial-induced carbonate precipitation (MICP) such as faster reaction rates, easier control of the process, no requirement of living bacterial cells, less risks of biological contamination and better adaptability to the changes in environmental conditions [18]. The enzyme-assisted bio-healing process has been found to possess these characteristics, making it a promising technology for tunnel rehabilitation, underground mining, rock slopes, hydraulic structures and geological repositories to achieve safer, longer lasting, and more sustainable geotechnical infrastructure.

1.3 Enzyme-Induced Carbonate Precipitation (EICP)

It is an advanced bio-mineralization technology based on the catalytic activity of free urease enzymes for the formation of calcium carbonate (CaCO_3) without the need for living microorganisms [19]. The new technology has proved to be environmentally friendly and has been identified as a potential solution to enhance the engineering properties of soils and rocks, in place of chemical grouting and

Microbially Induced Carbonate Precipitation (MICP). The urease enzyme in EICP converts urea to ammonium and carbonate ions [20]. The carbonate ions react with calcium ions to form calcium carbonate crystals that are insoluble, when a source of calcium, such as calcium chloride (CaCl₂), is added to the reaction medium. The overall reactions are:



Precipitated calcium carbonate is used to fill pores, seal microcracks, and bind mineral grains to increase the mechanical strength, stiffness and durability of rock and soil materials and to significantly decrease permeability [21]. Unlike MICP, EICP does not involve the cultivation and maintenance of bacteria, and this means that there is no worry about microbial survival, transportation of nutrient, bio-clogging, or ecological concerns of introducing living organisms into geological formations [22]. The free enzyme can fill narrow fractures better than bacterial cells, and precipitate minerals in a more uniform manner in thin fractures and low permeability rocks.

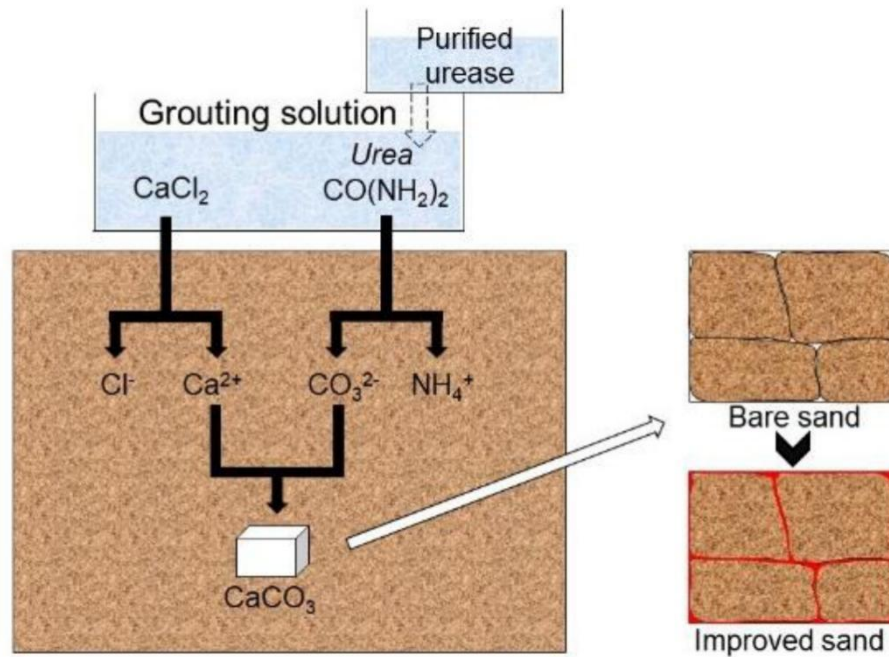


Figure 1.2: Schematic of precipitation process and grouting procedure in the EICP technique [23]

Several parameters are affecting the efficiency of EICP such as urease concentration, urea concentration, calcium ion concentration, pH, temperature, curing time and addition of stabilizing additives like non-fat milk powder or biopolymer which can preserve the enzyme activity [24]. EICP has been shown to have a good potential in rock engineering application for autonomous crack repair, rock mass stabilization, seepage control, slope reinforcement and maintenance of underground infrastructure [25]. During fracturing, the calcium carbonate crystals formed in the fractures restore the structural continuity, increase the uniaxial compressive strength, limit water penetration into the cracks, and enhance weathering and cyclic loading resistance [26]. Due to its environmentally friendly characteristics, ease of use and high healing efficiency, the EICP technology is now widely recognized as a sustainable and eco-

friendly technique for repairing damaged rock masses and increasing the service life of geotechnical and underground engineering structures.

1.4 Applications of Enzyme-Based Bio-Healing in Rock Masses

Crack Healing: Enzyme-based bio-healing repairs microcracks and fractures by precipitating calcium carbonate (CaCO_3) within damaged regions. The mineral deposits bridge crack surfaces and restore the continuity of the rock. This process enhances the structural integrity and prolongs the service life of rock masses.

Strength Enhancement: The precipitation of calcium carbonate acts as a natural cementing material that binds rock grains together. This increases the uniaxial compressive strength, tensile strength, and stiffness of fractured rocks. Consequently, the load-bearing capacity of the rock mass is significantly improved.

Permeability Reduction: Calcium carbonate crystals fill interconnected pores and fractures, reducing the movement of water through the rock. This decreases hydraulic conductivity and minimizes groundwater seepage. Improved impermeability also enhances the long-term durability of engineering structures [27].

Tunnel Stabilization: Bio-healing strengthens rock surrounding tunnels and underground excavations by sealing excavation-induced cracks. This reduces the likelihood of crack propagation and rock falls. As a result, tunnel stability and operational safety are considerably improved.

Slope Reinforcement: The EICP process enhances the cohesion of fractured rock slopes by depositing calcium carbonate within weak zones. This increases slope

stability and resistance to weathering. It also helps reduce the risk of landslides and rockfall hazards.

Foundation Improvement: Enzyme-based bio-healing improves the engineering properties of fractured rock foundations by increasing their strength and stiffness. It minimizes settlement and enhances the load-bearing capacity of foundations. This makes it suitable for supporting heavy civil infrastructure [28].

Dam and Reservoir Seepage Control: The technology seals fractures beneath dams and reservoirs by filling them with calcium carbonate deposits. This effectively reduces water leakage and seepage losses. Improved sealing also enhances the stability and safety of hydraulic structures.

Mining Ground Support: In underground mining, enzyme-based bio-healing reinforces fractured rock surrounding excavations and stopes. The healed rock mass provides better stability and reduces the need for extensive artificial support. This contributes to safer mining operations and lower maintenance costs.

Heritage Stone Conservation: The technique is used to repair weathered cracks in natural stone monuments and historical rock structures. Since it utilizes biologically induced mineral formation, it preserves the original appearance and texture of the stone. This makes it an environmentally friendly conservation method [29].

Sustainable Rock Engineering: Enzyme-based bio-healing offers a green alternative to conventional chemical grouting and cement-based repair methods. It reduces carbon emissions, chemical consumption, and environmental pollution. Therefore, it supports the development of sustainable and eco-friendly geotechnical engineering practices.

1.5 Research Motivation

Cracked rock masses that are induced by excavation, blasting, weathering, seismic activity, and repeated loading are common in rock masses utilized in tunnels and underground openings, dams, slopes, and mining operations. These cracks weaken the strength of the rocks, increase permeability, and pose long-term stability and safety problems for engineering structures. Traditional repair methods like cement grouting and chemical sealants can be detrimental due to environmental pollution, high maintenance, and lack of penetration into fine cracks and durability. These limitations have led to the development of the need for sustainable and effective crack-healing technologies. Enzyme-based bio-healing using Enzyme-Induced Carbonate Precipitation (EICP) has been recognized as a new and innovative method to fill cracks with calcium carbonate to seal fractures and restore the mechanical properties of rocks. The EICP method is considerably quicker than microbial methods and is easier to control and more applicable to difficult geological settings because it is based on free urease enzymes. The aim of this research is to develop an environmentally friendly, sustainable, and effective enzyme treatment to enhance the durability and hydraulic function of broken rocks and increase their longevity and strength.

1.6 Problem Statement

In geotechnical and underground engineering, rock mass subjected to mechanical loading, blasting, excavation, weathering and seismic activities is always continuously subjected to these activities, which always generate cracks and fractures which will continuously weaken the structural integrity. These defects lead to higher

water permeability, further deterioration, lower load carrying capacity and higher risk of failure of tunnels, slopes, dams and underground excavations. Typical crack repair techniques, such as cement grouting and chemical sealants, are not always effective in fine cracks, are not very durable, need regular maintenance and can lead to environmental issues with the synthetic materials used. Bio-healing technologies in concrete have proven to be promising, but the application of these technologies in rock masses, especially with enzyme-based systems, is not well studied. There is limited knowledge about the ability of the enzymes to heal, the precipitation of calcium carbonate, recovery of strength and durability of enzyme treated cracked rocks. Thus, an effective, sustainable and environmentally-friendly bio-healing technique that restores the mechanical and hydraulic characteristics of damaged rock masses using enzymes would be necessary.

1.7 Aim and Objectives

Aim :

To evaluate the efficacy of use of bio-chemical in Rock Mass for improvement in Engineering properties .

Objectives :

1. To prepare an enzyme-based healing solution containing urease, urea, and calcium chloride for inducing calcium carbonate precipitation through the EICP process.
2. To analyze weight changes associated with cracking and self-healing in rock mass .

3. To assess the recovery of mechanical properties of bio-healed rock specimens using tests such as Uniaxial Compressive Strength (UCS).
4. To evaluate the effectiveness of bio-healing of generated cracks in rock masses and to evaluate its potential as a sustainable crack repair technique.

Chapter 2

Literature review

2.1 Overview

The literature review on bio-healing technologies has shown great advances in the development of sustainable solutions for repairing cracks in geological formations and construction materials. Initially, the studies were concentrated on the microbial induced calcium carbonate precipitation (MICP) for self-healing concrete, which showed significant improvement in the closure of cracks, increase in compressive strength, durability and decrease in permeability of the concrete as a result of biological precipitation of calcium carbonate. Recent research has focused on enzyme induced carbonate precipitation (EICP) that does not require living microorganisms and offers faster reaction rates, improved process control, and increased environmental compatibility. Several studies have been conducted that have shown that EICP is effective in restoring mechanical properties, water ingress and long term durability of damaged materials. Recent studies have further expanded the bio-healing applications to rock engineering areas such as sandstone fracture repair and underground rock stabilization. Few studies have studied the healing efficiency, mechanical recovery with different rock masses and under varying environmental conditions, however, and the studies that do exist do not provide systematic evaluation of enzyme-based healing. Therefore, additional research on healing efficiency, mechanical recovery, and long term durability is needed.

2.2 Literature Review

Zheng et al., (2025) [37] proposed a novel enzyme induced magnesite carbonization (EIMC) method to effectively bio-heal the weathering cracks in sandstone by strengthening bio-cementation. The effect of urea pre-hydrolysis time, L/M ratio (liquid/magnesite) and urea concentration on the bonding and mechanical properties of treated samples was assessed. Under the optimal conditions, the peak tensile stress of 948.45 kPa and the interfacial tensile stress of 608.57 kPa were obtained, which were 18.6% and 23.2% higher, respectively, than the conventional MIMC method. The improved availability of carbonates led to the formation of hydrated magnesium carbonates, which led to a denser microstructure and to better crack healing in sandstone.

Dai et al., (2025) [38] proposed a three-stage injection strategy-based microbial induced calcium carbonate precipitation (TS-MICP) technique for bio-healing rock fracture and leakage of fluid in the underground rock reservoirs. The study compared the effectiveness of sequential injection of bacterial solution and cementation solution for fracture sealing. The experimental results showed that the proposed TS-MICP method had a higher bridging rate (89.5%), higher mechanical strength (1.023 MPa), and higher degree of homogeneity of CaCO_3 precipitation (334.4%) than the conventional MICP methods. Besides, the material utilization rate rose 1.72 times, and consumption of the cementation solution fell by 258.8%, which also proved excellent efficiency and shows great potential for large-scale field work.

Rabie et al., (2025) [40] proposed an enzymatic self-healing method for glass and carbon fibre reinforced concrete (CFRP) by combining Carbonic Anhydrase (CA)

based treatment, and fibre-reinforced composites. Seven concrete mixes with different dosages of glass and carbon fibres were studied and their fresh, mechanical, durability, environmental and self-healing properties compared. Experimental results indicated that whilst incorporation of fibre did decrease the compressive strength, the split tensile strength was significantly increased up to 70% for Glass fibres and 35% for Carbon fibres. The carbon fibres also decreased the water uptake at low doses which shows enhanced durability, while the glass fibres increased the permeability. Although the incorporation of fibre had a higher embodied energy, the CA-based treatment resulted in up to 30% closure of cracks in carbon fibre-reinforced concrete, which is promising for the self-healing capability of the material.

Soleimanbeigi et al., (2025) [41] proposed a microbially induced calcite precipitation (MICP)-based self-healing concrete using *Sporosarcina ureae* and silica fume (SF) to mitigate plastic shrinkage cracking. Six concrete mixes were prepared with and without SF and direct bacterial incorporation (SU) and microencapsulated bacteria (MSU), and mechanical properties, durability, crack healing and microstructural characteristics were compared. The experimental results indicated that the compressive and flexural strength of the resulting composite were superior, the water absorption was decreased, and the formation of CaCO_3 demonstrated crack healing abilities. Cracks up to 0.3 mm did not show any healing, but wider cracks (0.3–1.0 mm) demonstrated very good autonomous healing. The direct bacterial incorporation (SU) was found to outperform microencapsulation (MSU) with respect to the self-healing efficiency and the durability enhancement in concrete pavements, as confirmed by defined crack self healing efficiency (CSHE).

Sumon et al., (2025) [42] proposed a production strategy for *Scopulariopsis brevicaulis* spores optimization for their use in self-healing concrete (SHC) for biomineralization. The study focused on renewable soy-based substrates, fermentation and optimization of important process parameters for spore production. Experimental results showed that soy hull-based solid-state fermentation (SSF) was the best method for obtaining spores, yielding more than 4.5 times the number of spores generated through submerged fermentation. The optimal moisture content was 60-67%, optimal pH was 10-11, and the concentration of NaCl was 15 g/L, whereas the size of the soy hull particles was $>850\text{ }\mu\text{m}$ resulting in a significant increase in the quantity of spores. Optimized process increased the technical and economic feasibility of spore production on a large-scale level of *S. brevicaulis* species, hence its practical application in durable and sustainable self-healing concrete.

Xiao et al., (2025) [43] proposed an EICP approach for crack healing in Portland cement (PC)-based materials as an alternative to microbial-induced carbonate precipitation (MICP). The study examined the feasibility of the urease-based healing, by discussing the stability of the enzyme in an alkaline environment, and by comparing the soaking and injection methods for the repair. Experimental results indicated that the best cure was to soak it in a healing solution with 12U/mL urease and 0.75M urea–CaCl₂, which resulted in complete healing of cracks up to 2 cm in 15 days. The results also showed that urease has a very high sensitivity to pH 12 and a very high efficiency to be used in an external manner to treat EICP for repair of cracks in cementitious materials.

Vedrtnam et al., (2025) [44] proposed a finite element modelling (FEM) framework for modelling the transport, diffusion and chemical reaction kinetic in concrete microstructures containing bacteria-based self-healing agents. A study was performed with X-ray micro-computed tomography (Micro-CT) meshes and numerical solutions of the diffusion–reaction equations with FEniCS to predict calcium carbonate (CaCO_3) precipitation and crack healing. According to simulation results, healing agent diffusion was significantly affected by crack geometry and bacterial activity and occurred in a nonlinear fashion. The model was able to predict the crack closure efficiency under optimal conditions, corresponding to an efficiency of 65.5%, which is very close to the experimental data. The computational framework proposed would be an effective tool to predict the self-healing performance and optimise bacteria encapsulation, healing kinetics and long-term durability of self-healing concrete.

Alshalif et al., (2021) [39] proposed a bio-foamed concrete brick (B-FCB) using *Bacillus tequilensis* to increase the mechanical properties by CO_2 sequestration and enzyme-induced calcium carbonate precipitation. The effects of CO_2 concentration, bacterial concentration, temperature and density were optimized by 2^k factorial design and response surface methodology (RSM). The optimum condition was determined from experimental results of 10% CO_2 , 3×10^7 cells/mL, 27°C and 1800 kg/m^3 density, which yielded compressive strength of 8.22 MPa after 28 days. Proposed bio-foamed concrete achieved a 35.5% improvement in compressive strength compared to conventional foamed concrete and SEM, EDX and XRD analysis revealed improved calcium carbonate precipitation and microstructural densification.

Mohammed et al., (2020) [30] suggested a bio-based self-healing concrete used with non-encapsulated iron-respiring bacteria to seal cracks and enhance the durability of reinforced concrete (RC) structures automatically. The properties of fresh concrete, compressive strength, water absorption, and microstructural characteristics of both CEM I and CEM III concrete mixes with and without the bio-agent were compared. Experimental results indicated that the reduction in water absorption was very significant with the use of the bio-agent of at least 25% reduction in capillary water absorption of CEM III with no reduction in its compressive strength. Bio-mineral crystals were formed within cracks as confirmed by SEM analysis which shows good development of the bio-mineral crystals in the crack areas, and enhanced sealing of cracks, reducing permeability and protecting the steel reinforcement from corrosion.

Algaifi et al., (2020) [31] proposed to use microbial calcium carbonate precipitation (MICP) concrete to enhance crack repair and concrete durability, by using the microorganisms of *Sporosarcina pasteurii* and *Lysinibacillus sphaericus*. The study focused on the influences of pH, urea concentration, calcium ion concentration, and density of bacteria cells on ureolytic activity and the formation of calcium carbonate. Optimized conditions were determined to be pH 9, urea concentration < 333 mM, calcium ion concentration < 150 mM, and cell concentration of 2×10^8 cells/mL to achieve better biomineralization. The results showed that the use of bacterial self-healing can effectively extend the service life of the concrete, as the strength of the bacteria concrete increased by 9–15% compared with the control concrete, and the surface crack of the concrete was completely healed in 70 days.

Pungrasmi et al., (2019) [33] proposed the freeze-drying microencapsulation method of *Bacillus sphaericus* spore in sodium alginate to protect the spores of *B. sphaericus* for the biological self-healing concrete based on microbial-induced calcium carbonate precipitation (MICP). The present study compared three different bacterial survival, metabolic activity and the crack healing performance of the extrusion, spray drying and freeze-drying processes. The experimental results indicated that the freeze-drying method gave 100% viability of spores, which is higher than the extrusion method (93.8%) and the spray drying method (79.9%). Spore activity (urea decomposition) and the ability to significantly enhance the crack healing efficiency of the mortar specimens were also found to be the best for freeze-dried spores. The results showed that freeze drying is a successful microencapsulation method to improve the self-healing ability of concrete for long-term use and also the viability of the bacteria.

Luo et al., (2018) [32] explored a new self-healing concept in which fungi are used as a self-healing agent to promote calcium mineral precipitation to fill the cracks in concrete. An initial screening of different species of fungi has been conducted. Cured concrete plate was covered with fungal growth medium. Mycelial discs were aseptically placed in the middle of the plates. The results showed that, due to the dissolving of $\text{Ca}(\text{OH})_2$ from concrete, the pH of the growth medium increased from its original value of 6.5 to 13.0. *Trichoderma reesei* (ATCC13631) were able to germinate into hyphal mycelium and developed as well in all pH conditions with or without concrete.

Dakhane et al., (2018) [34] proposed a method for autonomous repair of cracks in concrete based on EICP technique with urease derived from plants, which reduces the cost of the technique compared to conventional methods. To investigate the effect of the various CaCl_2 concentrations, pre-cracked mortar beams were treated with the appropriate CaCl_2 -based EICP solutions. The results of the experiment showed that calcium carbonate formed within the cracks, which was verified by the results of X-ray diffraction (XRD) and thermal analysis. The flexural strength and fracture toughness of mortar treated with 0.5 M CaCl_2 were obtained, and were about 33% higher and 2 times greater, respectively, than those of moist cured mortar. Moreover, digital image correlation (DIC) analysis results indicated that the crack propagation was effectively reduced, which verified the improvement of mechanical properties and crack-healing ability of concrete by EICP.

Xu et al., (2018) [36] suggested a calcium sulphoaluminate cement-based carrier for the protection of alkaliphilic bacterial spores for microbial induced calcium carbonate precipitation (MICP) in self-healing concrete. The study focused on optimizing the carrier composition for the most acceptable compatibility with bacteria and the concrete matrix with regard to sustaining the activity of bacteria in the carrier for long duration. Experimental results showed that cracks could be sealed to almost 100% within 28 days for cracks up to 417 μm . This was achieved showing that the self-healing system had 130% compressive strength recovery and 50% water tightness recovery when compared with plain mortar. These results have validated the proposed bacterial carrier that improved the healing, durability and service life of concrete structures.

Zhang et al., (2016) [35] suggested a high throughput screening approach to select high efficient mineralization bacteria for microbial self-healing of concrete crack. A study was carried out to screen bacterial strains for their calcium precipitation activity (CPA) and it was found that *Bacillus* sp. was present. The highest CPA (94.8%) was obtained with the strain H4, which was the most efficient. Experimental studies found that the carbon and nitrogen sources of lactate (25 mM) and nitrate (18 mM) were the best options, whereas the bacterial activity was higher at a pH of 9.5–11.0. The results indicate that the suitable spore concentration and concentrations of calcium ions for bacterial growth and calcium carbonate precipitation were 4.0×10^7 spores/mL and < 60 mM, respectively, which indicates that the *Bacillus* sp. is suitable for this purpose. H4 - high performance self-healing concrete applications.

Castanier et al. (1999) explained the role of microorganisms in calcium carbonate precipitation and limestone formation. The authors demonstrated that microbial metabolic activities promote calcite formation, which can enhance the cementation and durability of geological materials. This work significantly contributed to the understanding of biomineralization mechanisms used in modern MICP and EICP techniques.

Pedersen (1997) investigated the occurrence of microorganisms in deep granitic rock formations. The study demonstrated that microbial communities can survive and remain active even under extreme subsurface conditions. These microorganisms influence mineral dissolution, precipitation, and geochemical reactions within rock masses. The findings highlighted the possibility of utilizing microbial processes for

future applications in rock stabilization, underground engineering, and bio-mediated crack repair.

Lama and Vutukuri (1978) carried out experimental studies on fractured rock masses and observed that the presence of joints considerably reduces the overall strength of rock specimens. Their investigation focused on the mechanical behavior of jointed rocks under laboratory loading conditions. The study reported that discontinuities act as weak planes within the rock mass and alter stress distribution during loading. It was also found that the spacing and orientation of joints significantly influence the compressive and shear behavior of rock specimens.

2.3 Research Gaps

After going through a detailed research literature available on the Bio Healing of Rock Mass using An Enzymes the following research gaps are observed:

- Limited research is available on enzyme-based self-healing of rock mass .
- Most existing studies focus only on crack closure, with little attention to parameters such as compressive strength after enzyme healing.
- There are insufficient studies examining weight change due to healing.
- There are insufficient studies examining density change due to healing.

Chapter 3

Research Methodology

This study's approach is based on an experimental assessment of the ability of enzyme-induced calcite precipitation (EICP) to repair rock fractures. Since rock cracking is a normal and inevitable process brought on by mechanical forces, shrinkage, and environmental changes, it is crucial to simulate these fractures in a lab setting in order to comprehend how they mend. Because autonomous healing cannot be fully replicated by conventional repair techniques, experimental protocols must be created to simulate actual fracture creation and healing under carefully monitored circumstances.

3.1 Rock Sample Collection and Preparation

The first part of the research consists of collecting and preparing rock samples and obtaining reliable and reproducible experimental results. The type of rock sampled for representative purposes depends on its engineering importance and is chosen from a quarry or a geological field site because the mineralogical composition is relatively uniform. Rock blocks are only picked if they are not cracked, weathered or contaminated in order to reduce variations in experimental results. The specimens collected are then taken to the lab and cut to a standardized cylindrical or cubic shape according to the International Society for Rock Mechanics (ISRM) or ASTM guidelines. The specimens for compressive strength are usually cylindrical in shape and have a diameter of about 50 mm and a length of about 100 mm.

After cutting, the specimen surfaces are ground and polished to obtain smooth and parallel ends, ensuring uniform stress distribution during mechanical loading. Dust and loose particles created during the machining process are then removed from the samples by clean and deep washing with distilled water. The specimens are then oven dried at $105 \pm 5^{\circ}\text{C}$ for 24 hours to a constant weight after which moisture which can influence enzyme activity and mineral precipitation is removed. Each specimen is cooled in a desiccator, then given a unique ID number. Lastly, the size, weight, density, porosity, and water absorption of each rock sample is determined and documented as initial properties prior to artificial crack formation and bio-healing treatment.

3.2 Preparation of Enzyme Healing Solution

Enzyme healing solution is ready to be used for enzyme induced calcium carbonate precipitation (EICP), which is a bio-mineralisation process that seals cracks in rock masses by the precipitation of calcium carbonate (CaCO_3). The process of EICP is similar to microbial-induced carbonate precipitation (MICP) except that it uses the enzyme urease directly, and no microorganisms are needed, thus it can provide rapid and controlled mineralization. The healing solution contains urease enzyme and the substrate, urea ($\text{CO}(\text{NH}_2)_2$), the calcium source, calcium chloride (CaCl_2), and a buffer solution to keep the pH of the reaction within the optimum range of 7.5 to 9.0, at which urease is most catalytically active. The urease enzyme is first dissolved in distilled water, then urea and calcium chloride are added in predetermined concentrations, usually 0.5 - 1.0 M urea, 0.5 - 1.0 M CaCl_2 and 5 - 20 g/L enzyme urease. The solution is dissolved with the magnetic stirrer, and the impurities are

filtered out before the solution is poured into the cracks formed. The amount of each component is optimized to promote calcium carbonate precipitation and to allow uniform penetration into fractured rock matrix.

3.3 Enzyme Injection into Generated Cracks

Once the enzyme healing solution is prepared it is fed into the created cracks of the rock specimens to start the enzyme induced calcium carbonate precipitation (EICP) process. The cracked samples are first flushed with compressed air and then flushed with distilled water to clean up any free particles or dust that would block the healing solution from penetrating the crack. The specimens are then dried in controlled laboratory conditions to allow for good uptake of enzyme solution. The prepared EICP solution was carefully applied into the artificial rock cracks using a squeeze bottle to ensure uniform distribution and effective penetration. The treated specimens were then left under ambient laboratory conditions to allow calcium carbonate precipitation and crack healing . After they have been injected, the treated rock specimens are under cured under controlled environmental conditions, usually at 25 to 30°C and relative humidity of 70 to 90%, for three to 28 days. The urease enzyme catalyzes the hydrolysis of urea during curing, creating carbonate ions which combine with calcium ions to form calcium carbonate (CaCO_3) crystals in the crack voids. The precipitated crystals slowly close off the gap between the crack surfaces, decrease permeability and return mechanical integrity to the rock mass. The enzyme solution can be re-injected after a specific period to achieve maximum mineral precipitation and/or crack sealing efficiency . The efficacy of the injection operation

is then checked by crack closure measurements and compressive strength . This controlled injection method provides a uniform biomineralization throughout the fracture network and provides environmentally friendly method to repair damaged rocks.

3.4 Bio-Mineralization Process

The bio-mineralization process is the core mechanism responsible for healing the generated cracks in the rock mass through Enzyme-Induced Calcium Carbonate Precipitation (EICP). Following injection of the enzyme healing solution into the fractured rock, the urease enzyme catalyzes the hydrolysis of the urea to ammonia and carbon dioxide gases. Ammonia formed reacts with water, leading to a rise in the pH of the solution, and carbon dioxide is then transformed into bicarbonate and carbonate ions. The higher the alkalinity, the more carbonate ions are generated which readily react with calcium ions provided by calcium chloride to form an insoluble calcium carbonate crystal (CaCO_3). These crystals form on the crack surfaces and slowly fill in the spaces between the faces of the cracks, forming strong mineral bridges between the crack faces. During precipitation, the deposited calcium carbonate causes the width of cracks to be closed up considerably, reduces the permeability and increases the cohesion and stiffness of the damaged rock. The mineralized crystals enhance the bond between neighbouring rock particles, creating the rock structure continuity and mechanical strength. It is generally believed that the bio-mineralization process can be divided into five sequential steps: (i) hydrolysis of urea, (ii) generation of carbonate ions, (iii) precipitation of calcium carbonate, (iv) filling of cracks by progressive mineralization and (v) mineral bond formation. The

efficacy of the crack healing is influenced by enzyme concentration, calcium ion availability, pH, temperature, curing time and crack geometry. The bio-mineralization process is an environmentally friendly, sustainable and efficient self-healing process, greatly enhancing the durability, strength and long-term stability of fractured rock masses through continual deposition of calcium carbonate in the fracture network.

3.5 Mechanical Performance Evaluation

Uniaxial Compressive Strength (UCS) Test

It is conducted to evaluate the compressive strength recovery of enzyme-treated rock specimens after the bio-healing process. The cylindrical samples are made to meet the ASTM D7012 or ISRM requirements and tested for compression failure in a Universal Testing Machine (UTM) under axial compression. The maximum compressive load that the specimen can withstand is measured and UCS is then determined as the ratio of the maximum load to the original cross-sectional area. The recovered compressive strength of the healed specimens is compared with the compressed strength of the specimens that were intact and cracked but not healed to quantify the effectiveness of calcium carbonate precipitation in recovering the structural integrity and load-bearing capacity of the rock mass.

3.6 Healing Efficiency Analysis

The healing efficiency analysis is conducted to quantitatively assess the efficiency of EICP in repairing the generated cracks in rock specimens. Healing performance is evaluated by comparing the physical and mechanical properties of bio-healed

samples with the physical and mechanical properties of cracked and intact rock samples which have not undergone the healing process.

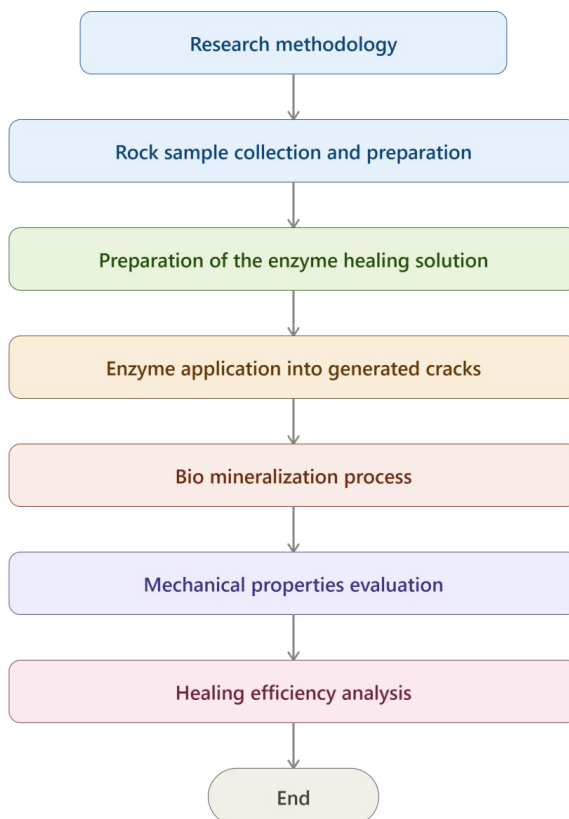
The crack-healing efficiency based on crack closure is calculated as

$$H = \frac{W_i - W_f}{W_i} \times 100 \dots \dots \dots [\text{Equation 3}]$$

where:

- H = healing efficiency (%)
- W_i = initial crack width (mm)
- W_f = final crack width after enzyme treatment (mm)

Flow Chart of Methodology



Chapter 4

Data Collections

Experimental results of the study of enzyme-induced carbonate precipitation (EICP) for bio- healing of cracks in the rock specimens are presented and discussed in this chapter. The performance of the treated specimens is then compared with the untreated specimens of cracked specimens of the same length, after curing for 28 days, to assess the effectiveness of the healing process. The mechanical tests used to determine mechanical performance are the Uniaxial Compressive Strength (UCS) test.

4.1 Sample Collection

The rock core specimens used in the present study were retrieved from Borehole No. 27 (Project: CREHS Pvt. Ltd., Location: Mudhol-B), drilled to a depth of 6 metres by Esscon Engineering Services Pvt. Ltd. The borehole was located at Latitude 18°12'17.472" N and Longitude 77°20'11.07" E, with the water table recorded as nil. The recovered core was logged as rock throughout the drilled depth, with core recovery (CR) values ranging from 26 to 43 cm across the four sampled runs. The specimen ranged from fresh to slightly weathered in condition and odour observation ranged from the light grey to dark grey consistent with typical granite mineralogy.

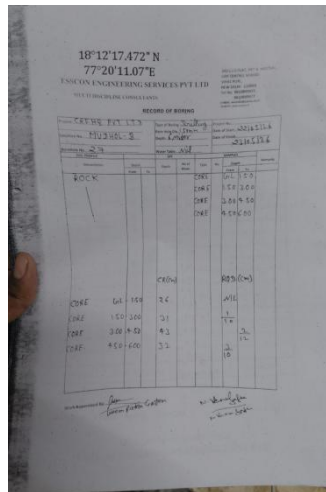


Figure 4.1: Borehole log showing the drilling details and rock core recovery from Borehole No. 27 (Mudhol-B)



Figure 4.2: Rock core specimens collected from the borehole for the present study

4.2 Sample Prepration

The recovered rock cores were cut into standardized cylindrical specimens using a diamond core-cutting machine to achieve a length-to-diameter ratio suitable for uniaxial compressive strength testing, in accordance with ISRM/ASTM guidelines. The cut ends of each specimen were checked for smoothness and parallelism using a steel ruler and marked for identification prior to further testing.



Figure 4.3: Sample preparation of rock core specimens using a core cutting machine.

4.3 Physical Characterization of Rock Specimens

Twenty Rock core specimens (S1-S20) were prepared and characterized prior to crack induction and enzyme treatment. All specimens were observed to be intact before crack induction, with a fine to medium-grained texture and a relatively uniform mineral distribution. The specimens ranged from fresh to slightly weathered

in condition, and colour observations ranged from light grey to dark grey, consistent with typical granite mineralogy.

Table 4.1: Colour Description and Shape of Granite Specimens

Sample	Colour Description	Shape
S1	Dark grey	Cylindrical
S2	Dark grey	Cylindrical
S3	Grey	Cylindrical
S4	Grey	Cylindrical
S5	Light grey	Cylindrical
S6	Grey	Cylindrical
S7	Dark grey	Cylindrical
S8	Grey	Cylindrical
S9	Light grey	Cylindrical
S10	Grey	Cylindrical
S11	Dark grey	Cylindrical
S12	Grey	Cylindrical
S13	Light grey	Cylindrical
S14	Grey	Cylindrical

S15	Dark grey	Cylindrical
S16	Grey	Cylindrical
S17	Grey	Cylindrical
S18	Light grey	Cylindrical
S19	Grey	Cylindrical
S20	Dark grey	Cylindrical

Table 4.2: Dimensions and Mass of Rock Specimens

Sample	Length (cm)	Diameter (cm)	Mass (kg)	Volume (cm ³)
1	10.0	5.0	0.49	196.35
2	10.2	5.1	0.626	208.37
3	9.5	4.75	0.494	168.35
4	10.5	5.25	0.646	227.30
5	9.5	4.75	0.496	168.35
6	9.6	4.8	0.5	173.32
7	10.0	5.0	0.572	196.35
8	9.5	4.75	0.492	168.35
9	10.2	5.1	0.52	208.37
10	9.0	4.5	0.478	143.14
11	9.8	4.9	0.515	184.80

12	10.1	5.0	0.535	198.31
13	9.7	4.8	0.505	175.53
14	10.3	5.1	0.55	210.41
15	9.6	4.8	0.485	173.72
16	10.4	5.2	0.59	220.7
17	9.9	4.9	0.52	186.7
18	10.0	5.0	0.545	196.3
19	9.5	4.8	0.495	171.89
20	10.2	5.1	0.525	208.13

Before Healing: The dry rock specimen's mass and dimensions are measured to calculate its density.

After Healing: The EICP-treated specimen is dried, and its mass and dimensions are measured again to determine the healed density.

Table 4.3: Density of Rock Specimens Before and After Enzyme-Induced Healing

Sample	Density Before Healing (g/cm ³)	Density After Healing (g/cm ³)
S1	2.49	2.50
S2	3.01	3.01
S3	2.93	2.94
S4	2.84	2.84
S5	2.94	2.95
S6	2.87	2.87

S7	2.91	2.92
S8	2.92	2.93
S9	2.49	2.49
S10	3.33	3.35
S11	2.78	2.78
S12	2.72	2.73
S13	2.87	2.88
S14	2.61	2.62
S15	2.79	2.80
S16	2.67	2.67
S17	2.78	2.79
S18	2.77	2.78
S19	2.87	2.87
S20	2.51	2.52

A consistent increase in density of approximately 0.01-0.2 g/cm³ was observed across all specimens after healing, reflecting the deposition of calcium carbonate within the crack network and pore spaces during the EICP treatment.

Table 4.4: Water Absorption of Rock Specimens

Sample	Dry Mass (kg)	Saturated Mass (kg)	Water Absorption (%)	Porosity (%)

S1	0.49	0.501	2.24	5.60
S2	0.626	0.64	2.24	6.72
S3	0.494	0.505	2.23	6.53
S4	0.646	0.661	2.32	6.60
S5	0.496	0.507	2.22	6.53
S6	0.5	0.511	2.2	6.33
S7	0.572	0.585	2.27	6.62
S8	0.492	0.503	2.24	6.53
S9	0.52	0.532	2.31	5.76
S10	0.478	0.489	2.3	7.68
S11	0.515	0.527	2.33	6.49
S12	0.535	0.547	2.24	6.05
S13	0.505	0.516	2.18	6.27
S14	0.55	0.563	2.36	6.18
S15	0.485	0.496	2.27	6.33
S16	0.59	0.603	2.2	5.89
S17	0.52	0.532	2.31	6.43
S18	0.545	0.557	2.2	6.11
S19	0.495	0.506	2.22	6.40
S20	0.525	0.537	2.29	5.76

The specimens exhibited a water absorption in the narrow range of 2.18% to 2.36% .

The specimens exhibited a porosity in the narrow range of 5.60% to 7.68% .

Table 4.5: Initial and Final Weight of Rock Specimens Following Crack Induction

Sample	Initial Weight (kg)	Final Weight (kg) (After Healing)
1	0.490	0.491
2	0.626	0.628
3	0.494	0.495
4	0.646	0.646
5	0.496	0.498
6	0.500	0.500
7	0.572	0.575
8	0.492	0.493
9	0.520	0.520
10	0.478	0.480
11	0.515	0.515
12	0.535	0.536
13	0.505	0.505
14	0.550	0.551

15	0.485	0.487
16	0.590	0.590
17	0.520	0.521
18	0.545	0.547
19	0.495	0.495
20	0.525	0.527

The weight reduction of approximately 1-1.5% was observed across all specimens following artificial crack induction, attributable to the loss of fine rock particles generated during the cracking process.

4.4 Preparation of the Enzyme Healing Solution

The enzyme healing solution was prepared using jack bean meal (as the source of urease enzyme), urea, calcium chloride (CaCl_2), and distilled water, mixed in the proportions given in Table 4.6. The dry components were dissolved in distilled water using a syringe, and the pH of the solution was monitored using a digital pH meter to maintain conditions favourable for urease activity.

Table 4.6: Dosage of Components used in the Enzyme Healing Solution

Component	Quantity
Urea (0.75 M)	4.50 g
Calcium Chloride, CaCl_2 (anhydrous, 0.75 M)	8.33 g

Urease Enzyme (Jack Bean Meal source)	0.50 - 1.00 g
Distilled Water	Made up to a total volume of 45-50 ml



Figure 4.6: Chemicals used in the EICP treatment process.



Figure 4.7: Distilled water used in the project.

4.5 Crack Characterization and Healing Efficiency

Artificial cracks were induced in each of the twenty specimens under controlled laboratory conditions. The Artificial crack are created by the help of UTM machine. The initial crack width was measured using a crack bore gauge prior to treatment in all 20 specimen. The enzyme healing solution was then injected into the cracks and the specimens were cured under ambient laboratory conditions. After the healing period, the final crack width was re-measured to evaluate crack closure and healing efficiency.



Figure 4.8: Surface crack observed on the cylindrical rock specimen.



Figure 4.9: Measuring of Crack width with bore gauge .



Figure 4.10: Surface crack observed on the cylindrical rock specimen.



Figure 4.11: Measurement of crack width in the rock specimen.

Table 4.7: Initial and Final Crack Width and Healing Efficiency of Rock Specimens

After EICP Treatment

Sample	Initial Crack Width (mm)	Final Crack Width (mm)	Healing Efficiency (%)
S1	0.3	0.1	66.6
S2	0.4	0.2	50.0
S3	0.3	0.1	66.6

S4	0.2	0.1	50.0
S5	0.4	0.1	75.0
S6	0.3	0.1	66.6
S7	0.3	0.1	66.6
S8	0.4	0.2	50.0
S9	0.2	0.1	50.0
S10	0.5	0.2	60.0
S11	0.3	0.1	66.6
S12	0.4	0.1	75.0
S13	0.5	0.2	60.0
S14	0.3	0.1	66.6
S15	0.3	0.1	66.6
S16	0.4	0.1	75.0
S17	0.2	0.1	50.0
S18	0.5	0.2	60.0
S19	0.3	0.1	66.6
S20	0.4	0.1	75.0

The healing efficiency , ranged from approximately 50% to 75% across the twenty specimens. These results indicate that enzyme-induced calcium carbonate precipitation was effective in substantially reducing the surface crack width of the

treated Rock specimens within the curing period 28 days , confirming the viability of EICP as a crack-healing mechanism in rock mass.

4.6 Uniaxial Compressive Strength (UCS) Test and Strength

Recovery

The tests were carried out on the healed rock specimens using a computer-controlled compression testing machine (HEICO, 2000 kN capacity) interfaced with a digital load indicator. Each cylindrical specimen (50 mm diameter, 100 mm height) was placed centrally between the loading platens and loaded at a constant pace rate of 5.2 kN/sec until failure. The peak load and peak stress were recorded automatically by the digital control panel for each specimen.



Figure 4.12: Rock specimen placed in the compression testing machine for UCS testing.



Figure 4.13: UCS test setup on the digital control panel showing sample type, dimensions and pace rate.



Figure 4.14: UCS test result displayed on the digital control panel.

Table 4.8: Uniaxial Compressive Strength Before and After Enzyme-Induced Healing

Sample	Initial UCS (MPa)	Healed UCS (MPa)	Strength Recovery (%)
1	55.45	64.12	15.64
2	53.32	67.45	26.5
3	98.24	115.32	17.39
4	86.36	103.42	19.75
5	96.24	115.32	19.83
6	60.78	70.84	16.55
7	62.45	73.56	17.79
8	80.98	93.62	15.61
9	72.42	84.58	16.79
10	70.18	81.35	15.92
11	86.42	100.65	16.47
12	90.54	105.68	16.72
13	94.38	109.52	16.04
14	76.25	90.52	18.71
15	82.37	96.45	17.09
16	84.15	98.28	16.79
17	78.94	91.53	15.95
18	96.45	112.58	16.72

19	90.54	105.68	16.72
20	92.6	107.45	16.04

Table 4.9: Rock Strength Classification Criteria (Deere and Miller, 1966)

UCS Range (MPa)	Rock Strength Classification (Deere and Miller, 1966)
25 - 50	Moderately Strong Rock
50 - 100	Strong Rock
100 - 200	Very Strong Rock
> 200	Extremely Strong Rock

Table 4.10: Rock Strength Classification of Healed Specimens (Deere and Miller, 1966)

Sample	Classification After Healing
1	Strong Rock
2	Strong Rock
3	Very Strong Rock
4	Very Strong Rock
5	Very Strong Rock
6	Strong Rock
7	Strong Rock

8	Strong Rock
9	Strong Rock
10	Strong Rock
11	Very Strong Rock
12	Very Strong Rock
13	Very Strong Rock
14	Strong Rock
15	Strong Rock
16	Strong Rock
17	Strong Rock
18	Very Strong Rock
19	Very Strong Rock
20	Very Strong Rock

According to the Deere and Miller (1966) rock strength classification based on uniaxial compressive strength, the specimens prior to healing (55.45-98.24 MPa) were classified as Strong Rock. After 28 days of bio-healing treatment, the UCS increased to a range of 64.12-115.32 MPa, resulting in nine of the twenty specimens (Samples 3, 4, 5, 11, 12, 13, 18, 19 and 20) being upgraded to the Very Strong Rock category, while the remaining specimens showed a marked strength recovery while continuing to be classified as Strong Rock.

Chapter 5

Conclusion

5.1 Summary of Findings

This chapter summarizes the major findings of the research on the bio-healing of generated cracks in rock masses using Enzyme-Induced Carbonate Precipitation (EICP). The study examined efficacy of the urease-induced calcium carbonate precipitation for strengthening the naturally weathered rock specimens artificially cracked. The study investigated the effectiveness of the urease induced calcium carbonate precipitation to restore the mechanical strength, durability and structural integrity of the artificially cracked rock specimens. To study the healing performance, a systematic method of experimentation was adopted and consisted of specimen preparation, generation of crack, enzyme treatment, controlled curing, mechanical testing. The key findings are summarised below.

Density and Weight Changes: The bulk density of the specimens showed only a marginal increase after healing, rising from a pre-treatment range of 2.49-3.33 g/cm³ to a post-treatment range of 2.50-3.35 g/cm³. Similarly, the specimen weights increased marginally after healing around 0.1-0.4 g consistent with the deposition of calcium carbonate (CaCO₃) crystals within the induced cracks although the change is small.

Crack Closure and Healing Efficiency: The EICP treatment was effective in visibly reducing the surface crack width of all specimens. Initial crack widths ranging from

0.2 mm to 0.5 mm were reduced to a narrow range of 0.1-0.2 mm after treatment, corresponding to a healing efficiency of between 50% and 75%,. Specimens with a smaller initial crack width (0.2 mm) generally recorded lower healing efficiencies (~50%), whereas specimens with wider initial cracks (0.4-0.5 mm) achieved healing efficiencies of up to 75%, indicating that the enzyme solution was able to penetrate and precipitate calcite effectively even in comparatively larger crack openings within the 28-day curing period.

Mechanical Strength Recovery: The Uniaxial Compressive Strength test results demonstrated a clear and consistent improvement in mechanical performance following bio-healing. The average UCS increased from 80.45 MPa (untreated) to 94.40 MPa (healed), with individual specimens showing a strength recovery ranging from about 15.6% to 26.5%, and an average recovery of approximately 17.5% across all twenty specimens. Sample 2 recorded the highest strength recovery (26.5%), while the recovery for most specimens was consistently clustered between 15% and 20%, indicating a reliable and repeatable healing response across specimens of varying initial strength.

Rock Strength Classification: Based on the Deere and Miller (1966) classification, all specimens were classified as Strong Rock prior to healing (initial UCS 55.45-98.24 MPa). After the 28-day bio-healing treatment, nine of the twenty specimens (Samples 3, 4, 5, 11, 12, 13, 18, 19 and 20) were upgraded to the Very Strong Rock category, while the remaining eleven specimens continued to be classified as Strong Rock but with a markedly improved strength margin. This upgrade in classification for nearly

half of the tested specimens highlights the practical engineering significance of the strength gains achieved through enzyme-induced bio-healing.

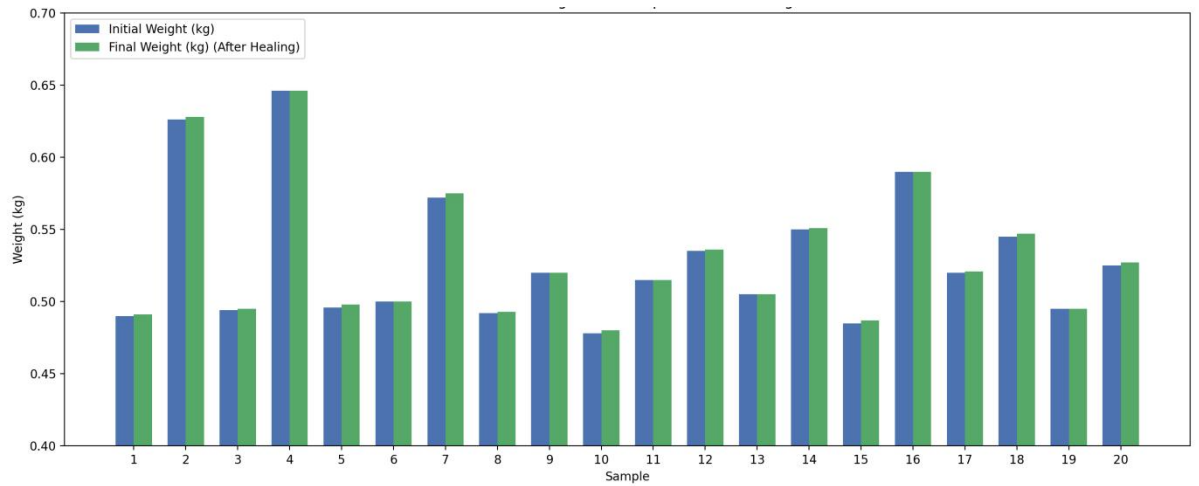


Figure 5.1 : Initial and Final Weight of Rock Specimens Following Crack Induction

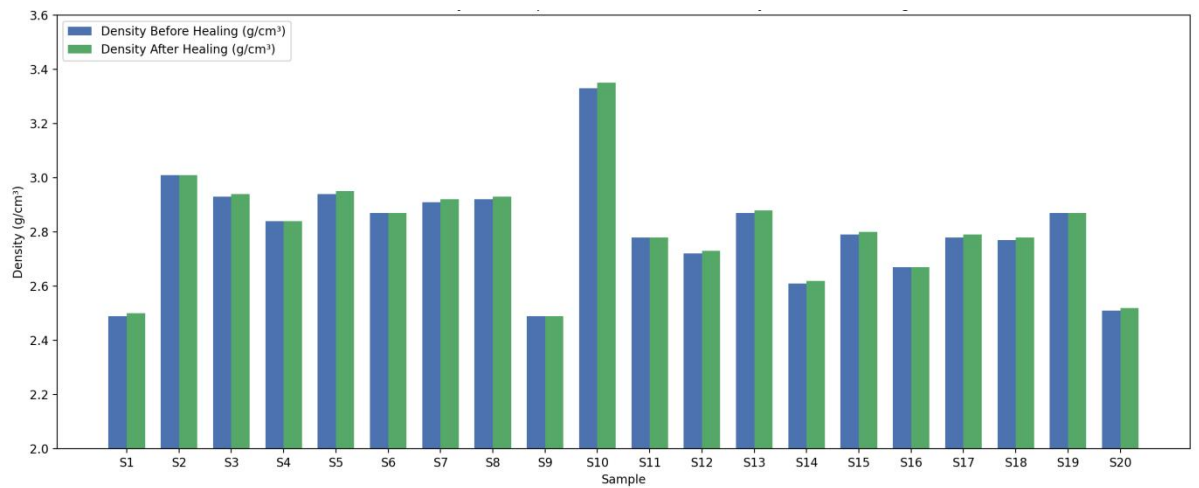


Figure 5.2 : Density of Rock Specimens Before and After Enzyme-Induced Healing

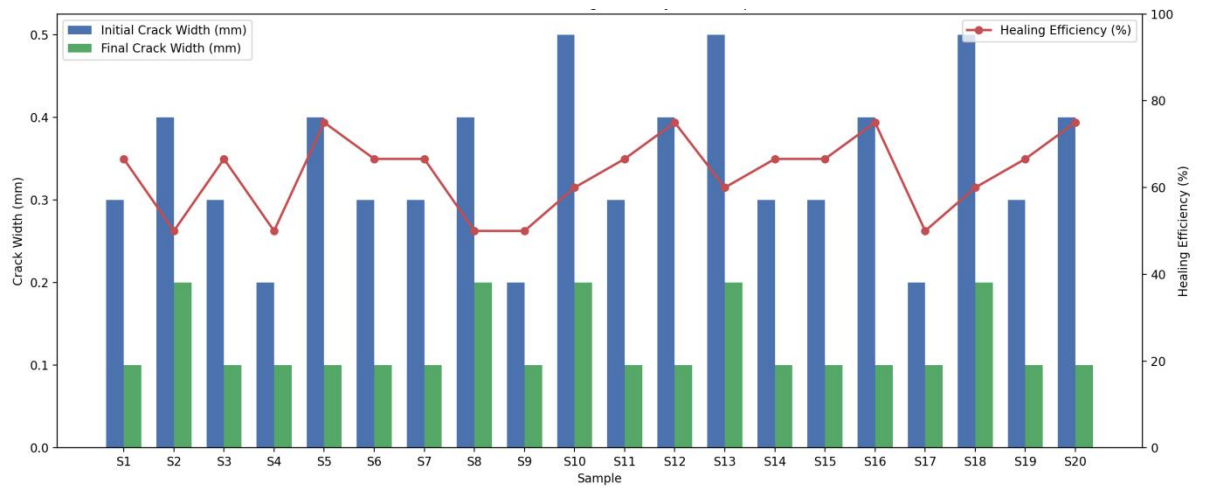


Figure 5.3 : Initial and Final Crack Width and Healing Efficiency of Rock Specimens After EICP Treatment

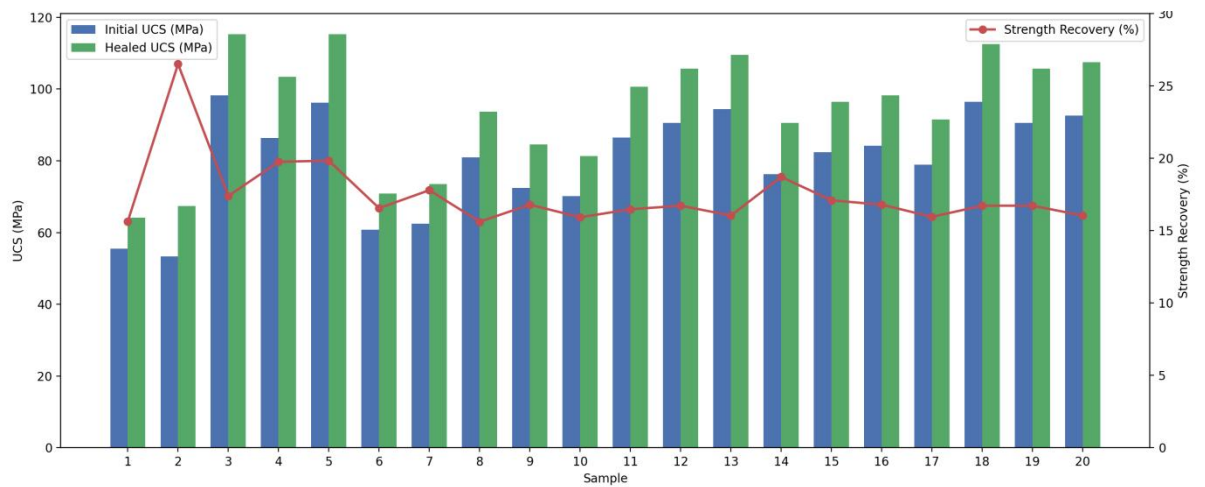


Figure 5.4 : UCS before/after healing

5.2 Scope For Future Work

- The study can be extended by using different dosages of healing agents .

- Advanced tests such as microstructural analysis can be performed to study healing products.
- Long terms performance data like permeability have to be evaluated.
- Currently, the healing effect was observed for a short duration. Future work can involve testing the samples over months .

Reference

1. Zheng, Xiangcou, Shuying Wang, Feng Yang, and Junsheng Yang. "Journal of Rock Mechanics and Geotechnical Engineering." (2023).
2. Dong, Zhi-Hao, Xiao-Hua Pan, Chao-Sheng Tang, and Bin Shi. "Microbial healing of nature-like rough sandstone fractures for rock weathering mitigation." *Environmental Earth Sciences* 81, no. 15 (2022): 394.
3. Kashif Ur Rehman, Sardar, Faisal Mahmood, Mohammed Jameel, Nadia Riaz, Muhammad Faisal Javed, Abdelatif Salmi, and Youssef Ahmed Awad. "A biomineralization, mechanical and durability features of bacteria-based self-healing concrete—a state of the art review." *Crystals* 12, no. 9 (2022): 1222.
4. Šovljanski, Olja, Ana Tomić, Tiana Milović, Vesna Bulatović, Aleksandra Ranitović, Dragoljub Cvetković, and Siniša Markov. "Construction Biotechnology: Integrating Bacterial Systems into Civil Engineering Practices." *Microorganisms* 13, no. 9 (2025): 2051.
5. Zhang, Jianwei, Xinwei Guo, Yang Yang, Peikun Wang, Yu Song, and Xiangpeng Yu. "Research on microbially induced calcite precipitation (MICP) based on gene vector construction optimization technology for stone relic crack repair." *Biogeotechnics* (2026): 100227.
6. Wilson, Uwemedimo Nyong, Abubakar Sanusi Abdullahi, Mohammed Abdulkareem Adisa, and Samson Olalekan Odeyemi. "Microbial induced calcite precipitation on macrostructural properties of concrete: a review." *Journal of Infrastructure Preservation and Resilience* 6, no. 1 (2025): 42.

7. Tegegn, Yoseph Sisay. "The Role of Microorganisms in Bio-cement Production: An Extended Review." *Engineering and Applied Sciences* 9, no. 6 (2024): 147-159.
8. Beskopylny, Alexey N., Evgenii M. Shcherban', Sergey A. Stel'makh, Alexandr A. Shilov, Andrei Chernil'nik, Diana El'shaeva, and Vladimir A. Chistyakov. "Analysis of the current state of research on bio-healing concrete (bioconcrete)." *Materials* 17, no. 18 (2024): 4508.
9. Tegegn, Yoseph Sisay. "The Role of Microorganisms in Bio-cement Production: An Extended Review." *Engineering and Applied Sciences* 9, no. 6 (2024): 147-159.
10. Li, Yong, Shengya Zhou, Fankai Liu, Zhiyu Dong, Xiangtai Fan, Zhi Ge, Chong Li, and Hongzhi Zhang. "Enzyme-Induced Carbonate Precipitation (EICP) for Soil Stabilization: A Review of Mechanisms, Applications, and Future Challenges." *Geotechnics* 6, no. 2 (2026): 53.
11. Sidiq, Amir. "Healing efficiency of cementitious materials for durable and intelligent transport infrastructures." PhD diss., RMIT University, 2024.
12. Abbas, Ali, Khalid Abdalla, Thomas Chalklen, Laurence de Lussy Kubisa, David Tann, Davor Ivankovic, Liv Andersson, and Silvia Kazi. "Bioconcrete as an alternative sustainable construction material." In *MATEC Web of Conferences*, vol. 409, p. 15001. EDP Sciences, 2025.
13. Dong, Zhi-Hao, Xiao-Hua Pan, Chao-Sheng Tang, and Bin Shi. "Microbial healing of nature-like rough sandstone fractures for rock weathering mitigation." *Environmental Earth Sciences* 81, no. 15 (2022): 394.

14. Zheng, Xiangcou, Shuying Wang, Feng Yang, and Junsheng Yang. "Journal of Rock Mechanics and Geotechnical Engineering." (2023).
15. Kashif Ur Rehman, Sardar, Faisal Mahmood, Mohammed Jameel, Nadia Riaz, Muhammad Faisal Javed, Abdelatif Salmi, and Youssef Ahmed Awad. "A biomineralization, mechanical and durability features of bacteria-based self-healing concrete—a state of the art review." *Crystals* 12, no. 9 (2022): 1222.
16. Abdelwahed, Mai M., Amany M. Reyad, Ahmed Abd-Alazim, and Gehad Mokhtar. "Immobilization of bacterial spores using eggshells nanoparticles and their effective role in concrete bio-healing process: novel approach." *BMC biotechnology* (2025).
17. Konwarh, Rocktotpal, Suresh Babu Palanisamy, and Pavan Kumar Jogi. "A mini review on prospects and challenges of harnessing fungi for concrete-crack healing." *Material Science Research India* 17, no. 2 (2020): 117-128.
18. Tegegn, Yoseph Sisay. "The Role of Microorganisms in Bio-cement Production: An Extended Review." *Engineering and Applied Sciences* 9, no. 6 (2024): 147-159.
19. Kavazanjian, Edward, and Nasser Hamdan. "Enzyme induced carbonate precipitation (EICP) columns for ground improvement." In *IFCEE 2015*, pp. 2252-2261. 2015.
20. Ahenkorah, Isaac, Md Mizanur Rahman, Md Rajibul Karim, Simon Beecham, and Christopher Saint. "A review of enzyme induced carbonate precipitation (EICP): the role of enzyme kinetics." *Sustainable Chemistry* 2, no. 1 (2021): 92-114.

21. Saif, Ahsan, Alessia Cuccurullo, Domenico Gallipoli, Céline Perlot, and Agostino Walter Bruno. "Advances in enzyme induced carbonate precipitation and application to soil improvement: A review." *Materials* 15, no. 3 (2022): 950.
22. Chandra, Alok, and K. Ravi. "Application of enzyme-induced carbonate precipitation (EICP) to improve the shear strength of different type of soils." In *Problematic Soils and Geoenvironmental Concerns: Proceedings of IGC 2018*, pp. 617-632. Singapore: Springer Singapore, 2020.
23. Cui, Ming-Juan, Han-Jiang Lai, Tung Hoang, and Jian Chu. "One-phase-low-pH enzyme induced carbonate precipitation (EICP) method for soil improvement." *Acta Geotechnica* 16, no. 2 (2021): 481-489.
24. Ahenkorah, Isaac, Md Mizanur Rahman, Md Rajibul Karim, and Simon Beecham. "Optimisation of chemical constituents on enzyme-induced carbonate precipitation in test-tube and soil." *Geotechnical Research* 8, no. 3 (2021): 66-84.
25. Wang, Hengxing, Linchang Miao, Xiaohao Sun, Linyu Wu, and Guangcai Fan. "Experimental study of enzyme-induced carbonate precipitation for high temperature applications by controlling enzyme activity." *Geomicrobiology Journal* 39, no. 6 (2022): 502-514.
26. Martin, Kimberly K., Hamed Khodadadi Tirkolaei, and Edward Kavazanjian Jr. "Mid-scale biocemented soil columns via enzyme-induced carbonate precipitation (EICP)." *Soils and Foundations* 61, no. 6 (2021): 1529-1542.
27. Zheng, Xiangcou, Shuying Wang, Feng Yang, and Junsheng Yang. "Journal of Rock Mechanics and Geotechnical Engineering." (2023).

28. Li, Yong, Shengya Zhou, Fankai Liu, Zhiyu Dong, Xiangtai Fan, Zhi Ge, Chong Li, and Hongzhi Zhang. "Enzyme-Induced Carbonate Precipitation (EICP) for Soil Stabilization: A Review of Mechanisms, Applications, and Future Challenges." *Geotechnics* 6, no. 2 (2026): 53.
29. Almajed, Abdullah, Hamed Khodadadi Tirkolaei, and Edward Kavazanjian Jr. "Baseline investigation on enzyme-induced calcium carbonate precipitation." *Journal of Geotechnical and Geoenvironmental Engineering* 144, no. 11 (2018): 04018081.
30. Mohammed, Hazha, Montserrat Ortoneda-Pedrola, Ismini Nakouti, and Ana Bras. "Experimental characterisation of non-encapsulated bio-based concrete with self-healing capacity." *Construction and Building Materials* 256 (2020): 119411.
31. Algaifi, Hassan Amer, Suhaimi Abu Bakar, Abdul Rahman Mohd Sam, Mohammad Ismail, Ahmad Razin Zainal Abidin, Shafinaz Shahir, and Wahid Ali Hamood Altowayti. "Insight into the role of microbial calcium carbonate and the factors involved in self-healing concrete." *Construction and Building Materials* 254 (2020): 119258.
32. Luo, Jing, Xiaobo Chen, Jada Crump, Hui Zhou, David G. Davies, Guangwen Zhou, Ning Zhang, and Congrui Jin. "Interactions of fungi with concrete: Significant importance for bio-based self-healing concrete." *Construction and building materials* 164 (2018): 275-285.
33. Pungrasmi, Wiboonluk, Jirapa Intarasoontron, Pitcha Jongvivatsakul, and Suched Likitlersuang. "Evaluation of microencapsulation techniques for MICP

- bacterial spores applied in self-healing concrete." *Scientific reports* 9, no. 1 (2019): 12484.
34. Dakhane, Akash, Sumanta Das, Hannah Hansen, Sean O'Donnell, Farouq Hanoon, Aimee Rushton, Carlos Perla, and Narayanan Neithalath. "Crack healing in cementitious mortars using enzyme-induced carbonate precipitation: Quantification based on fracture response." *Journal of Materials in Civil Engineering* 30, no. 4 (2018): 04018035.
 35. Zhang, J. L., R. S. Wu, Y. M. Li, J. Y. Zhong, X. Deng, B. Liu, N. X. Han, and F. Xing. "Screening of bacteria for self-healing of concrete cracks and optimization of the microbial calcium precipitation process." *Applied Microbiology and Biotechnology* 100, no. 15 (2016): 6661-6670.
 36. Xu, Jing, and Xianzhi Wang. "Self-healing of concrete cracks by use of bacteria-containing low alkali cementitious material." *Construction and Building Materials* 167 (2018): 1-14.
 37. Zheng, Cheng-Tao, Xiao-Hua Pan, Xiao-Zhao Li, Chao-Sheng Tang, and Bin Shi. "Practical bio-healing of rock weathering cracks using an enzyme-induced magnesite carbonization." *Journal of Rock Mechanics and Geotechnical Engineering* 17, no. 6 (2025): 3662-3675.
 38. Dai, Qi-Chen, Xiao-Hua Pan, Chao-Sheng Tang, and Bin Shi. "A bio-healing method for underground long rock fractures with high bridging rate." *Journal of Rock Mechanics and Geotechnical Engineering* (2025).
 39. Alshalif, Abdullah Faisal, J. M. Irwan, Husnul Azan Tajarudin, N. Othman, A. A. Al-Gheethi, S. Shamsudin, Wahid Ali Hamood Altowayti, and Saddam Abo

- Sabah. "Optimization of bio-foamed concrete brick strength via bacteria based self-healing and bio-sequestration of CO₂." *Materials* 14, no. 16 (2021): 4575.
40. Rabie, Mohamed, Ali Bahadori-Jahromi, and Ibrahim G. Shaaban. "Optimisation of glass and carbon fibre-reinforced concrete with external enzymatic self-healing: an experimental and environmental impact study." *Buildings* 15, no. 19 (2025): 3455.
 41. Soleimanbeigi, Alireza, Parham Hayati, Jafar Sobhani, and Parvaneh Saffarian. "Synergistic effects of *Sporosarcina ureae* and silica fume on self-healing and plastic shrinkage cracks in bio-green concrete pavement." *Results in Engineering* 26 (2025): 105526.
 42. Sumon, Ahsanul Kabir, and Lu-Kwang Ju. "Factors affecting production of *Scopulariopsis brevicaulis* spores for use in self-healing concrete." *Bioprocess and Biosystems Engineering* 48, no. 9 (2025): 1481-1494.
 43. Xiao, Xi, Yifei Chen, and En-Hua Yang. "Study of crack healing in Portland cement-based mixtures via enzyme-induced carbonate precipitation (EICP)." *Construction and Building Materials* 490 (2025): 142437.
 44. Vedrtnam, Ajitanshu, Kishor Kalauni, and M. T. Palou. "Finite element simulation of bacterial self-healing in concrete using microstructural transport and precipitation modeling." *Scientific Reports* 15, no. 1 (2025): 15809.