

CAPITAL UNIVERSITY OF SCIENCE AND
TECHNOLOGY, ISLAMABAD



**Improving Concrete Durability
with Self-Healing Process
through Microbial Induced
Calcium Precipitation**

by

Hamdan Ullah

A thesis submitted in partial fulfillment for the
degree of Master of Science

in the

**Faculty of Engineering
Department of Civil Engineering**

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In the name of Allah, the most merciful, the most compassionate all praises be to Allah, the lord of the worlds and prayers and Peace be upon Muhammad his servant and messenger.

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(Hamdan Ullah)

Abstract

Concrete is one of the most widely used materials in construction due to its strength, durability, and low cost compared to other materials. However, a major drawback of concrete is the formation and propagation of cracks under tensile stress. These cracks allow harmful substances to penetrate the concrete, which can reduce its durability and lead to degradation. To ensure long-lasting concrete structures, it is crucial to manage the development and spread of microcracks, and self-healing techniques can help mitigate this issue.

Bacillus Subtilis strain of bacteria, was used in this investigation. The chosen strain was found to be the most promising strain in the field based on its urease activity and ability to generate calcite minerals at temperatures higher than 40 °C. One type of culture media, Nutrient Broth is used to grow the strain. In the concrete matrix, dry substances such as calcium nitrate, calcium lactate, yeast extracts, and urea were added as nutrients for the bacterial strains. The material utilized was regular Portland cement CEM-I that complied with ASTM C-150, with an average particle size of 5.74 μm and a specific gravity of 3.05. The main goal of this research is to examine the impact of Bentonite as a carrier material and compare it to other carrier materials. To assess the best technique for immobilization of bacteria in the mixture, light weight aggregates (LWA) were investigated as a carrier material and compared with bentonite. For this investigation, four types of mixes were used. The mix proportion for these four distinct specimen categories included 360 kg/m^3 of ordinary portland cement (OPC) type I, which complies with ASTM C 150-07, 820 kg/m^3 of fine aggregate, 960 kg/m^3 of coarse aggregate, and 17 kg/m^3 of calcium lactate, yeast, urea and calcium nitrate with a water to cement ratio of 0.4 for all concrete mixes.

An experimental study was conducted to investigate the self-healing properties of concrete under various conditions. Bacteria were added to the concrete in 3 ways, such as 1- direct incorporation, 2- immobilization in lightweight aggregates, and 3- with bentonite. The testing plan has been divided into three phases. During the first phase, Cube and prism specimens were mechanically evaluated at 3, 7, 14

and 28 days of curing by compression and flexural test, using ASTM test In the second phase, Pre-cracking specimens of (40×40×160) were made, and one specimen was pre-cracked for each mix at 3, 7, 14, and 28 days. standards C-39 and ASTM-C496/C496M, respectively. In phase three, investigate alterations in concrete microstructure brought on by self-healing, specimens of all four mixes were analyzed using scanning electron microscopy (SEM), in addition to the findings obtained from visual inspection of concrete samples.

The highest crack healing effectiveness was achieved by specimens that included Bentonite as a carrier compound. In early age pre-cracked specimens, specimens using lightweight aggregate (LWA) as a carrier compound were less effective than Bentonite nevertheless, in later days, they demonstrated consistency in their crack healing effectiveness. Specimens mixed directly with bacteria did not demonstrate any benefits in crack healing of concrete. Regardless of the incorporation method, the addition of bacteria “Bacillus Subtilis” led to a small increase in compressive strength, with the LWA technique showing the greatest improvement, according to the trends of compressive strength for all mixes.

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Abbreviations

ASTM	American Society for Testing and Materials
C-S	Calcium-Silicate
CO₂	Carbon dioxide
Ca(OH)₂	Calcium hydroxide
CaCO₃	Calcium carbonate
CaC₆H₁₀O₆	Calcium gluconate
H-Gel	Hydrogel
ITZ	Interfacial transition zone
LWA	Light Weight Aggregate
MICP	Microbial Induced calcium precipitation
Mpa	Megapascal
NB	Nutrient Broth
RC	Reinforced concrete
RS	Reinforcing steel
SEM	Scanning Electron Microscope
SHC	Self Healing Concrete
XRD	X-ray Diffraction

Chapter 1

Introduction

1.1 Background

As a construction material the reinforced concrete (RC) is brittle and is prone to cracking and degradation. Temperature, creep and shrinkage, as well as tensile loading, are a few of the elements that cause cracks in RC structures, which typically appear at a young age. Chloride and carbon dioxide can enter through cracks in concrete, which causes the reinforcing steel (RS) to deteriorate. Concrete is subjected to tensile strains from the corrosion products, which accelerates its aging process and causes larger cracks and spalling [1]. Consequently, the longevity of reinforced concrete buildings has become a major problem and frequently leads to repetitive repairs and rehabilitation. MICP is a potential technique for producing self-healing concrete that has been demonstrated to improve the sustainability and robustness of RC structures [2]. With or without outside help, self-healing concrete auto repairs cracks while the building is in use [3]. Usually, microorganisms that can cause microbial-induced calcium carbonate precipitation (MICP) are added to the concrete mixture to create bio-self-healing concrete. However, the very alkaline composition of the pore solution makes the internal environment of the concrete matrix extremely unsuitable for microbiological viability. Those creatures are killed by the harsh mixing and pouring of concrete. Furthermore, if bacteria are put directly into the concrete matrix, the heat produced by cement

hydration renders them ineffective [4]. That is why it is utmost important to protect them from mixing and harsh concrete environment to function properly. For this purpose, polymeric beads which range in size from 10 micrometer to 1 mm. They could contain nutrients as well as the bacteria or active MICP-producing spores or living cells. In some instances, the concrete matrix is independently supplemented with nutrients, whilst the beads simply carry bacteria or their spores [5]. Under the right circumstances, a number of bacterial species can precipitate calcium carbonates in the soil, lake, and ocean environments [6]. Environmental factors, specifically the surrounding pH, the availability of calcium ions and dissolved inorganic carbon, and the presence of suitable nucleation sites that encourage crystal development, have a significant impact on the degree of calcite formation resulting from microbial activity [7]. The most often researched method for engineering applications is the urea hydrolysis pathway, which is made possible by bacterial urease enzymes [8]. Urease, which is produced by metabolically active bacteria, catalyzes the transformation of urea into carbonate and ammonium, raising the pH and carbonate concentration in the vicinity of the cells. In the process, carbonic acid is created and breaks down into ammonium (NH_4^+) and carbonate (CO_3^{2-}). Calcium ions (Ca^{2+}) are drawn to the negatively charged bacterial cell surfaces and mix with carbonate ions to form calcium carbonate [9]. Many bacterial species have been studied for MICP-based concrete crack repair [10], but only alkali-tolerant strains have shown promise because most bacteria cannot survive in extremely alkaline conditions ($\text{pH} \approx 9$). The most commonly used species among them is *Sporosarcina pasteurii* [11].

However, other bacterial strains are being investigated in continuing research to improve the consistency of concrete fracture healing. For example, *Bacillus pseudofirmus* and *Bacillus cohnii* were directly added to concrete by Jonkers et al. [12], who showed that both species could precipitate calcium carbonate when calcium lactate was present. Hassan et al. used cultures of *Diaphorobacter nitroreducens* and *Bacillus pseudofirmus* in a mortar matrix [13]. They found that carbonate precipitates from both stresses at least partially filled crack widths up to 372 meters. To shield the bio self-healing agents from the high pH concrete environment and prevent additional damage, a variety of techniques have been employed to

encapsulate nutrients and capture bacterial cells or spores. Examples of encapsulating materials include silica sol-gel [14], hydrogels [15], light clay particles [15], recycled glass granules [16], ethylene oxide, and propylene oxide [17]. ethylene oxide, and propylene oxide

However, it might be challenging to continuously inspect for cracks and repair them, particularly when those fractures are in infrastructure like highways, tunnels, or bridges. Many of these infrastructures are in continuous operation, which makes restoration even more challenging, even though infrastructure cracks are difficult to access and demand a significant financial and personnel investment. Additionally, it was reported that in Europe, repairs are made with 50% of the yearly building budget in order to extend the structures' useful lives [18]. In addition to being extremely expensive, most traditional fixes have a ten- to fifteen-year lifespan [19]. In these situations, the best way to fix the fractures is to install an automated healing mechanism that, when activated, restores the concrete structure's functionality without the need for human assistance.

1.2 Research Motivation and Problem Statement

The urgent desire to improve the resilience and sustainability of concrete buildings in construction is the driving force behind this study. Concrete fractures can be repaired using traditional maintenance techniques, adding admixtures (chemicals) but they are expensive, time-consuming, and environmentally harmful.

Self-healing concrete offers a viable way to greatly increase the lifespan of concrete structures while lowering maintenance costs and causing the least amount of environmental damage.

The main difficulty is to create a precise and economical system for repairing cracks in concrete structures through bio self-healing mechanisms.

The achievement of autonomous self-healing activation, cost-effectiveness, and assuring long-term sustainability and durability are major problems. In order to

advance self-healing concrete technology, lower maintenance costs, and reduce the building industry's environmental effect, these issues must be resolved.

1.3 Overall Research Goal and Objectives

The overall aim of this study is to develop and apply self-healing concrete technology to create sustainable, durable structures that autonomously detect and repair cracks, reducing maintenance costs and environmental impact. However, the particular goal of this study is to increase the durability of concrete by using Microbial-Induced Calcium Carbonate Precipitation (MICP), a microbial-based self-healing mechanism that promotes independent crack closure and improve long-term performance.

In order to do this, the efficacy of two distinct bacterial carriers—lightweight aggregate (LWA) and bentonite—in encouraging self-healing in pre-cracked specimens was examined. LWA performed better than bentonite in specimens pre-cracked at 14 and 28 days, perhaps because of improved bacterial survival and shielding, whereas bentonite was found to achieve optimum healing in specimens pre-cracked at 3 and 7 days. All four concrete mixes' compressive strengths were assessed, and the mixes that included LWA performed better mechanically.

Although long-term durability in real structures extends to years, the accelerated evaluation of crack healing in pre-cracked specimens at 3, 7, 14, and 28 days provides an early indicator of self-healing potential. Pre-cracking at 28 days enables assessment in mature concrete, simulating conditions closer to long-term service. The trends of microbial activity and calcium carbonate precipitation observed within these time frames can be correlated with long-term crack closure behavior, making these measurements a reliable proxy for predicting extended performance.

Following are the research question;

- i. What is the effectiveness of bentonite lightweight aggregate as a bacterial carrier in microbial self-healing concrete compared to light weight aggregate carrier materials?

- ii. How does the direct incorporation of bacterial solution influence crack-healing efficiency and durability-related properties of concrete?

1.4 Scope of Work and Study Limitations

The development and evaluation of microbially induced self-healing concrete, or "bio-concrete," in a lab setting was the main goal of this work. A control (Mix 1), direct bacterial addition (Mix 2), and bacterial immobilization utilizing lightweight aggregate (LWA, Mix 3) and bentonite (Mix 4) were the four concrete mixes that were created. Compressive strength was determined for each combination, and crack healing efficiency was evaluated using pre-cracked specimens at 3, 7, 14, and 28 days. The study examined how well bentonite and LWA worked as bacterial carriers to maintain mechanical performance and encourage crack closure.

Limited supply of bacterial broth, the study's limitations include a restricted number of small specimens, identical curing conditions that would not accurately reproduce in-service situations, and a laboratory-only setting without field trials. Furthermore, only two carrier materials were examined, and accelerated testing up to 28 days was used to evaluate long-term durability as a stand-in for longer service life.

1.5 Work Novelty, Research Importance and Real - World Application

By combining *Bacillus cereus* bacteria, varied nutrient compositions, several curing procedures, and diverse bacterial inclusion techniques inside the cementitious matrix, this study offers a thorough and multidisciplinary foundation for the development of self-healing concrete. This study focusses on incorporating a biological healing mechanism directly into the concrete material, in contrast to conventional methods that address fracture repair through chemical additives or external maintenance. Because of its capacity to make spores, which enables it to withstand the

extremely alkaline environment of concrete and remain dormant until favourable conditions exist, the chosen bacterial species was carefully picked. In order to ensure long-term healing performance, various nutrition systems are investigated to stimulate bacterial metabolism at suitable phases of crack formation. In order to comprehend their effects on bacterial viability and mineral precipitation efficiency, the influence of curing regimes—including traditional water curing and alternative curing techniques is also investigated. Pre-cracks are purposefully created in hardened specimens under carefully monitored laboratory circumstances to replicate realistic structural deterioration. Instead of depending only on indirect strength recovery data, these pre-cracks enable a direct assessment of healing efficiency. Before and after the healing process, crack shape, mineral deposition, and internal microstructural changes are observed using sophisticated microscopic inspection techniques. In addition to offering new insights into the healing behaviour of bio-concrete, the combination of controlled crack generation and micro-level observation advances our knowledge of the long-term interactions between biological agents and cementitious materials.

Microbial-induced calcium carbonate precipitation (MICP), a biochemical process started by bacterial metabolic activity within the concrete matrix, is the main healing mechanism examined in this work. *Bacillus cereus* aids in the transformation of accessible calcium sources into calcium carbonate crystals when it is stimulated by moisture and nutrients. These crystals gradually form inside voids, capillary pores, and cracks, effectively blocking the passage of water and hostile substances through the concrete. Continuous calcium carbonate accumulation produces a denser and more refined microstructure, which improves mechanical and durability-related qualities in quantifiable ways. Reduced porosity and pore connectivity greatly reduce permeability, a crucial element influencing the long-term performance of concrete structures, and immediately increase compressive strength. Bio-concrete specimens are subjected to controlled acid attack environments that mimic harsh service conditions frequently found in wastewater, sewerage, and industrial infrastructures in order to further assess durability. Mass loss measures, surface degradation analysis, and residual strength evaluation are used to determine how resistant bio-concrete is to various acidic solutions. The

findings show that the calcium carbonate layer that is produced internally serves as a barrier that prevents chemical degradation and slows the entry of acids.

Bio-concrete is more resistant to acid-induced degradation than normal concrete, demonstrating how microbial activity can improve chemical durability and prolong structural integrity.

This research directly tackles important issues that the worldwide construction industry faces, and its significance goes beyond laboratory-scale experiments. Concrete constructions will inevitably crack as a result of shrinkage, temperature changes, mechanical loading, and exposure to the environment.

These fissures frequently act as entrance routes for acids, sulfates, chlorides, and water, hastening structural deterioration and reinforcement corrosion. Conventional repair techniques are expensive, time-consuming, and sometimes only offer short-term fixes, which results in more frequent maintenance cycles and higher lifespan costs. This study offers a sustainable substitute that reduces human intervention and improves long-term performance by adding self-healing capabilities to concrete. Adoption of bio-concrete has the potential to prolong the service life of vital infrastructure while drastically lowering maintenance and repair costs.

Additionally, lowering the frequency of repairs helps cut down on the amount of materials, energy, and carbon emissions related to cement manufacturing and building. From a sustainability standpoint, this study is in line with international objectives that support robust infrastructure, economical resource use, and ecologically friendly building techniques.

By providing inexpensive, long-lasting, and environmentally friendly solutions for buildings, bridges, tunnels, water-retaining structures, and transportation networks, the practical application of microbial self-healing concrete has the potential to completely transform infrastructure development.

In the end, this research advances intelligent building materials and lays the groundwork for upcoming widespread uses of bio-based self-healing technologies in contemporary civil engineering.

1.6 Thesis Outline

The thesis is divided into the five chapters listed below. The chapters' information are given below:

Chapter 1. The study motivation, problem statement, overall purpose, particular target, research technique, and thesis outline are all included in the introduction section.

Chapter 2. It includes the literature reviews. It also gives the detailed introduction to the self-healing concrete and needs for self-healing concrete. Various parameters of the of the bio concrete are also discussed in this chapter.

Chapter 3. It include the methodology used to carry out. The processes of mix design of concrete, samples preparation and samples testing are the part of this chapter.

Chapter 4. It covers the results and analysis to meet the research objectives.

Chapter 5. It includes the conclusion and recommendations.

References

Chapter 2

Literature Review

2.1 Background

Because of its flexibility in design and relative affordability, concrete is a material that is widely employed in construction across the globe. Concrete, although having many benefits, is prone to cracking because of environmental factors or severe tensile loads. As long as their breadth stays within a predetermined range, these fissures in reinforced concrete (RC) members are accepted and do not indicate a failure. Nevertheless, aggressive materials seep into the concrete through these fractures, degrading it and corroding the reinforcement. These processes impair the concrete structure's mechanical performance and durability, hence shortening its service life. It follows that it is evident how crucial it is to keep an eye on, maintain, and fix concrete cracks. A variety of self-healing processes have been developed over time, although the origins of the phenomenon can be found in old buildings and structures, where it was noticed that white crystalline minerals were filling up cracks [20]. "Autogenous self-healing" refers to the inherent quality of concrete that allows hydrated pozzolanic binders or crystalline ingredients in the right conditions to fill in gaps. The idea that concrete may seal and mend fractures without the need for outside activation led the researchers to create "autonomous self-healing," which involves incorporating engineered components into the concrete that will activate when the fissures begin to show signs of movement.

Their goal was to customize new processes that should be more effective than autogenous self-healing. However, a major challenge for its industrial application is the initial cost of various procedures used in this category. Researchers developed a self-healing mechanism known as "Improved autogenous" to get around the aforementioned obstacles. This mechanism adheres to the fundamentals of autonomous self-healing, i.e., manufactured conditions, but its overall operation and healing outcomes are the same as those of autogenous self-healing.

Figure 2.1, which is mostly based on a Venn diagram of self-healing/repairing fractures published by the technical committee TC-075B from the Japan Concrete Institute (JCI) [21], explains the relationships between the several primary self-healing techniques. In addition to summarizing the most recent research on each mechanism and analyzing its benefits and drawbacks, this study attempts to give a succinct overview of the main processes for self-healing in cementitious materials. Prospects for more study on self-healing in engineering practice are suggested, based on the gaps in the current literature.

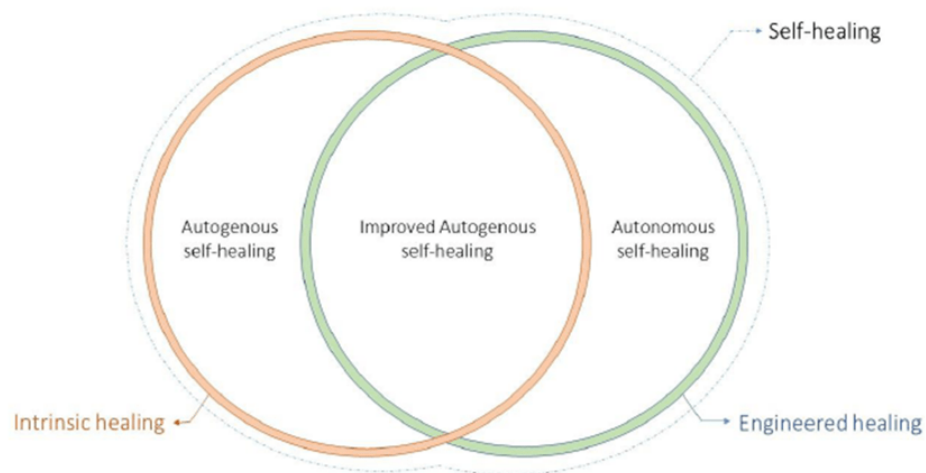


FIGURE 2.1: Schematic diagram illustrating self-healing mechanisms

2.1.1 Autonomous Self-healing

According to RILEM technical committee 221-SHC [22], autonomous self-healing is defined as the process of healing employing material components that are specifically included in concrete for this reason (engineered additions). The primary

autonomous self-healing techniques that have drawn the most attention from researchers in recent decades include the use of bacteria that precipitate calcium carbonate (CaCO_3) when exposed to water, mineral admixtures that can form crystalline or expansive productions in response to a crack, and encapsulations of chemical healing agents.

Some of these methods are not feasible for industrial usage because of their large starting costs, despite the positive results that have been reported. Sometimes, growing bacteria or changing encapsulation methods with materials that should withstand the alkaline and harsh environment of concrete might be more expensive than expected [23]. Nonetheless, researchers in this sector have used cutting-edge techniques, demonstrating the promise of this mechanism.

The rate at which bacteria-based healing has advanced among all autonomous techniques has been remarkable. Researchers have now discovered specific kinds of bacteria that can improve the strength characteristics of concrete in addition to consistently producing self-healing effects in cracks.

Furthermore, in recent years, nanomaterials have been proposed as an appropriate supplementary mechanism for concrete self-healing. Faster hydration rates for cement and other pozzolanic binders, decreased porosity, and enhanced interfacial bonding between hardened cement paste and other components are the primary impacts of nanomaterials on concrete, according to reports.

These benefits also extend to self-healing [24]. However, other applications, including increasing the formulation of C-S-H by nanosilica or giving inner water by nanocalys, have also been demonstrated to have an effective influence on the self-healing process.

2.1.2 Autogenous Self-healing

Since the French Academy of Science made the initial observation of this phenomena in water-retaining structures and pipes approximately 200 years ago, autogenous self-healing can be regarded as the forerunner of self-healing mechanisms [25].

The inherent qualities of regular concrete include the swelling of cement paste, precipitation of calcite, and further hydration of unhydrated cement. Taken together, these characteristics can act as a barrier against the entry of hazardous materials and, in the right circumstances, even restore the mechanical properties of concrete (Figure 2). Nevertheless, the aforementioned features also highlight this mechanism's shortcomings. According to reports, within the first 28 days of concrete, over 80% of the hydration process is finished. Additionally, the production of calcite, the primary cause of autogenous self-healing at later ages, is greatly influenced by the surrounding environment. For instance, in order for carbonates or bicarbonates to react with H^+ ions, water or moisture must be present. The shape of the produced fracture also affects the outcome [26]. All these unknown effective parameters might account for the wide range of repaired fracture width ($5 \mu\text{m}$ to $300 \mu\text{m}$) that researchers have seen as a result of autogenous self-healing.

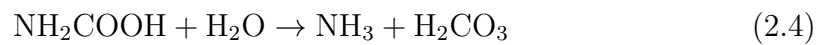
2.1.3 Precipitation of Calcium Carbonate in Concrete Matrix

Microorganisms such as *Bacillus sphaericus* and *Bacillus pasteurii* can produce biominerals through metabolic processes when calcium is available (see Table 1) [13]. These urease-positive bacteria may hydrolyze urea to create calcium carbonate, and they are engaged in the nitrogen cycle. Eqs. 2.1 and 2.2 provide the basic processes needed to cause the precipitation of calcium carbonate [18].

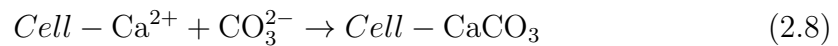
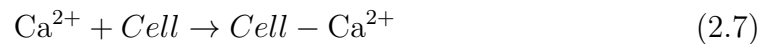
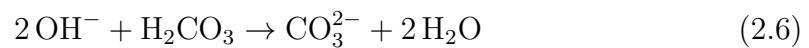
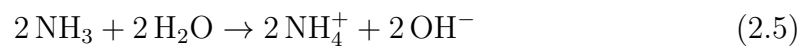


The pH and carbonate content rise as a result of microbial metabolic activity. ApH increase makes it easier for carbon dioxide to convert to carbonate. This precipitate acts as a barrier to prevent corrosive substances from penetrating fissures. Urea hydrolyzes to produce 1 mol of carbamic acid (NH_2COOH) and 1 mol of ammonia (NH_3) (Equation 2.3). Eq. 2.4 illustrates how carbamic acid hydrolysis

simultaneously yields 1 mol of additional ammonium and 1 mol of carbonic acid (H₂CO₃).



Eqs. 2.5 and 2.6 state that carbonate (CO₃²⁻) is produced when hydroxide ions. Positively charged calcium ions can then attach to the negatively charged bacterial cell, as shown by Eq. 2.7.



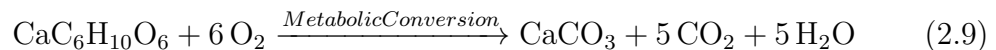
Calcium ions can be added externally, or they can be obtained internally from sources present in the cement structure to complete the final reaction (Eq. 2.4) [14]. When calcium chloride is used as a calcium source, reinforcement bars may deteriorate as a result of chloride ion attack. Therefore, it is advised to apply calcium lactate or calcium nitrate Fig. 2.2.

Even if this strategy has worked, there are still certain issues that need to be resolved. Emission of nitrogen oxide into the environment is the outcome of ureolytic action producing ammonium ions (NH₄⁺). One square meter of concrete is thought to require 10 grams per liter of urea.

Furthermore, by converting to nitric acid, too much ammonium in the concrete matrix raises the possibility of salt corrosion. Therefore, it is advantageous to optimize the amount of urea needed in order to prevent excessive ammonium emissions.

According to this method, organic acids undergo aerobic oxidation, which produces carbon dioxide and carbonate in an alkaline environment.

Calcium carbonate is produced when there is a calcium supply available as a cation. Eq. 25 [15] illustrates the metabolic conversion of calcium lactate to calcium carbonate in the presence of oxygen.



The increase in autogenous healing is attributed to the reaction between the accessible calcium oxide in the concrete matrix and the produced water and carbon dioxide from Eq. 25 [15].

Because there is no ammonium involved, this metabolic conversion is more sustainable than the ureolysis process.

Consequently, in order to minimize expenses, avoid the creation of salt, and achieve the highest possible production of calcium carbonate, the concentration of the calcium source must be regulated. Among the benefits of this technology are its compatibility with the composition of concrete.

The use of nutrients that may quicken the biomineralization process is necessary since calcium carbonate biomineralization proceeds slowly.

Furthermore, it is favored to choose low-risk bacteria that have strong growth rates, enzyme activity, and calcium carbonate precipitation capabilities. On the other hand, uncontrolled superficial biofilm formation and uneven surface might result from bacterial proliferation. Therefore, to avoid bacterial overgrowth and to achieve maximum precipitation. Table 1 provides a summary of the nutrients and microbial strains.

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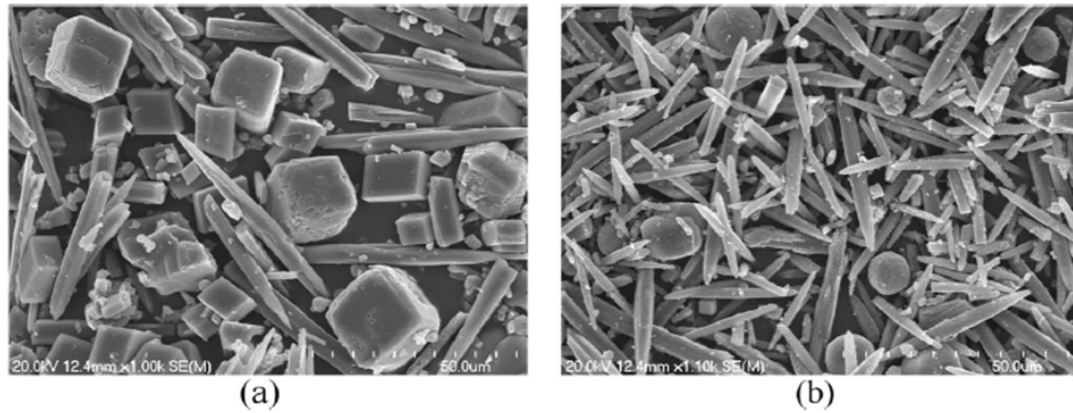


FIGURE 2.2: (a) SEM micrographs of calcite precipitation by *B. sphaericus*.
(b) SEM micrographs of calcite precipitation by *B. Subtilis*

2.2 Embedment of Microorganism in Concrete Matrix

The vascular network in the concrete matrix can be used to introduce the healing agent (nutrients and bacteria), or it can be mixed in during the manufacture of the concrete. The structure of human bone served as the model for the vascular approach. A bone is made up of two components. The trabecular bone is the inner spongy layer, and the compact cortical bone is the outer layer.

As illustrated in Fig. 3a, the vascular approach uses dispersed vascular networks that were previously incorporated into the matrix during the fabrication of the concrete to supply the healing agent from outside the structure. Because of the pressure gradient between the agent source and the crack sites, the healing agent travels through the vessel as the cracks emerge [15] presented a self-healing system in which one or more hollow vascular fibers were used to connect the outside and inner concrete components. In a different study used cylindrical concrete with a porous core and compact exterior layer to replicate vascular networks. The healing agent is distributed via the porous core through the concrete matrix, and it can be activated when a fracture develops in the outer layer of the structure. The vascular network approach appears to be unfeasible because of various drawbacks. First, according to the healing agent should maintain a consistent and prevent

leaks under adverse environmental conditions. Aesthetic problems arise when the healing agent released exceeds the capacity of the crack. Second, it would be challenging to evenly space the vessels during the construction.

Bacteria and nutrients can also be directly implanted in the concrete matrix during the preparation and casting process, as shown in Fig. 3b. In this process, cement and sand are mixed with a solution of dissolved therapeutic ingredients in water. Alkaliphilic bacteria, such those found in *Bacillus* species, are the most preferred species for bio-self-healing concrete because they can tolerate the hard conditions of concrete. These bacteria can survive for hundreds of years without sustenance, according to research [15]. They generate thick membrane spores. In addition, hibernating endospores may tolerate mechanical stressors, chemicals, and variations in the environment.

On the other hand, the direct microbial integration into building materials like concrete has a significant impact on the microbial metabolic activity. High (>11) pH and dry Concrete's state even puts bacteria at risk of dying. Sponges of *Bacillus cohnii* were directly added to the concrete matrix by. Investigations were conducted into the quantity of live cells within the concrete specimen at 9, 22, 42, and 153 days of cure. Up until nine days, the amount of live bacteria in the concrete matrix was roughly stable; however, after 22 and 42 days, it dropped sharply by 80% and 90%, respectively. These findings suggest that the bacteria cells in concrete structures may remain viable for as long as 4 months, or 135 days. As a result, adding immobilized spore-forming bacteria is crucial to extending the duration that bacteria may survive in hostile environments [13].

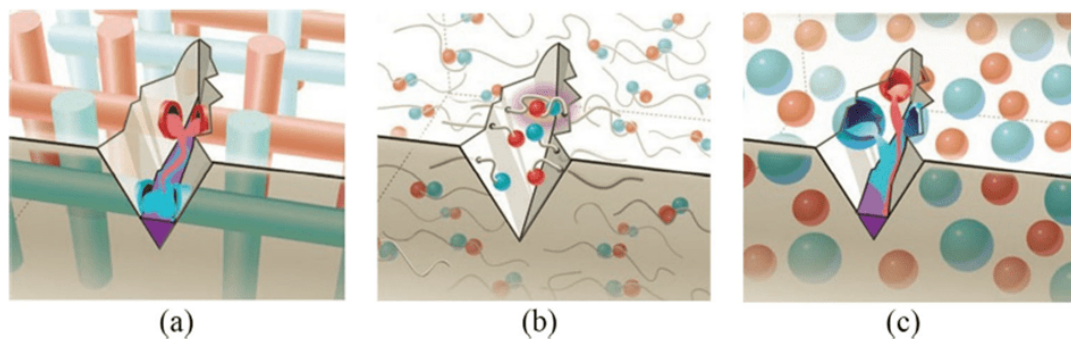


FIGURE 2.3: There are three primary forms of self-healing: (a) vascular, (b) combining with other substances, and (c) encapsulation

Using encapsulation or protective materials like hydrogel, diatomaceous earth, and porous expanded clay particles, several publications have tried to offer bacterial protection. The prolonged survival of bacteria can be enhanced by encasing the healing agent (Fig. 2.3) in tubular or ball-shaped capsules. Microcapsules withstand mechanical forces while preparing concrete. When a fracture forms and the capsule bursts, the healing process will begin. The main difficulties with the encapsulation approach are the preparation of the capsules, their mixing with the aggregate, and the empty space that remains after the activation of the capsules. Recent research has examined the impact of encasing a healing agent within tubular glasses on the management of cracks. Glass and other silicate materials are negatively impacted by alkaline solutions. Glass tube (vessel) walls dissolve in cement-based composites because they are extremely alkaline, which leads to glass corrosion. Reducing the capsule radius or thickening the capsule wall will improve capsule tolerance during mixing. Reducing the radius to thickness ratio, however, might prevent the capsules from activating as soon as a crack emerges. Therefore, it is advised to choose capsules that break easily with age [16]. A ceramic can overcome the drawbacks of a glass capsule if it can stay intact during mixing and perform well in a challenging environment. A greater surface area will result from more integrated capsules.

This could eventually make concrete less workable by reducing the cohesion of binders. It was discovered that using microcapsules to fill pores can result in the formation of bigger pores. In comparison to samples without microcapsules, samples including microcapsules were shown to have worse mechanical characteristics. The voids that form when microcapsules burst are responsible for this. Therefore, it can be concluded that the sole parameters influencing encapsulation effectiveness are the size, properties, and distribution of the capsules inside the concrete matrix. The possibility of immobilizing bacteria in hydrogel has been studied, and embedding bacteria in silica gels have been seen to survive in harsh conditions. It was demonstrated that swelling hydrogel may improve the precipitation of calcium carbonate to fill fractures up to 0.5 mm in width by delivering additional water. In a different study, bacteria were immobilized in polyurethane and silica gel. It was discovered that silica gels preserve bacteria and algae while maintaining their

enzymatic function [16]. Therefore, research into protective particles that can hold onto bacteria for extended periods of time is necessary in order to acquire a sufficient bio-self-healing mechanism to cure the cracks in concrete.

2.2.1 Performance of Bio Concrete

Compressive strength and durability are two of concrete's most important characteristics. It is necessary to assess how biomineralization affects these characteristics. size of pores, cracks, and their dispersion negatively affects the qualities of concrete and, as a result, the service life of concrete structures. Reducing absorption, permeability, and diffusion—the three main ways that fluids and gases are transported into concrete—will increase the durability of concrete. The impact of bio-based healing agents on concrete permeability and water absorption has been assessed in a number of research. It has been demonstrated that adding bio-based elements to concrete lowers permeability and water uptake, as indicated in Table 2. Using immobilized *B. sphaericus* cells, a study [12] examined the impact of calcium carbonate production on permeability and found that specimens containing bacteria encapsulated in polyurethane had permeability values six times lower than control samples. Furthermore, it was discovered that immobilizing *B. sphaericus* in diatomaceous earth had a substantial impact on water absorption, with bacterial specimens absorbing 50% less water than those without bacteria [21]. Additionally, penetration tests showed that the water absorption coefficient of treated specimens was six times lower than that of untreated samples over a 168-hour period, indicating that treatment with *B. sphaericus* effectively rendered concrete waterproof, as reported in [16]. The production of freshly precipitated calcium carbonate as a result of bacterial metabolic activity is thought to be responsible for these effects. The durability and service life of concrete structures can be significantly increased by biologically induced sealing of pores and cracks, according to current research.

The compressive strength of mortar can be reduced by 15% to 34% when encapsulated *B. sphaericus* is added, according to [12]. But according to [17], adding

B. sphaericus to cube mortar can increase compressive strength in as little as 7 and 28 days. as the cell concentration was 5×10^6 cells/mm³, the application of bio-based agent boosted the mortar's compressive strength; however, as the cell concentration rose to 5×10^8 cells/mmmm³, the mortar's compressive strength declined. The effect of *Sporosarcina pasteurii* on the compressive strength of mortar specimens was studied for seven and twenty-eight days [20].

Highest concentration of immobilized *S. pasteurii* on porous glass beads was shown to significantly raise the mortar specimen's compressive strength by 24%. Additionally, as cell concentration increased from 6.1×10^7 cells/cm³ to 3.1×10^9 cells/cm³, compressive strength improved.

The reported impact of bio-based healing agents on concrete strength is still variable, in contrast to durability-related data. The mortar's compressive strength decreased between 15% to 34% when encapsulated *B. sphaericus* was added, according to [12]. On the other hand, research [17] found that mortar cubes' compressive strength increased seven and twenty-eight days after *B. sphaericus* was added. The bio-based treatment improved compressive strength at a bacterial concentration of 5×10^2 cells/mm³; but, strength decreased when the concentration was increased to 5×10^1 cells/mm³. In [20], the effect of *Sporosarcina pasteurii* on mortar compressive strength was assessed at 7 and 28 day curing ages.

The greatest dosage of immobilized *S. pasteurii* on porous glass beads resulted in a significant 24% increase in compressive strength, according to the results. Additionally, as the bacterial concentration rose from 6.1×10^2 cells/cm³ to 3.1×10^3 cells/cm³, a gradual improvement in strength was noted. Additionally, the impact of immobilized ureolytic and denitrifying bacteria on compressive strength was documented by in their study of protective materials. The addition of *B. sphaericus* decreased the concrete's compressive strength by 60% and 63%, respectively, at curing ages of 7 and 28 days. Comparably, applying the denitrifying bacterium *Diaphorobacter nitroreducens* reduced compressive strength at both testing ages; however, immobilizing *D. nitroreducens* with granular activated carbon or expanded clay resulted in a minor improvement. Conversely, the immobilization of *B. sphaericus* in metakaolin and zeolite had a negative impact on compressive

strength. The brittle character of precipitated calcium carbonate may be the cause of these contradictory results.

The observed heterogeneity in strength performance is also probably caused by variations in nutrient supplies, culture media, and environmental factors.

TABLE 2.1: Microbial agent's impact on water absorption, permeability, and compressive strength

Microorganism	Effect on Compressive Strength		Effect on Durability			References
	Effect	Time(day)	Permeability	Water absorption		
<i>Bacillus sphaericus</i>	N	28	P	–		Wang et al. (2014c)
	N	90				
	P	3	–	P		Achal et al. (2011)
	P	7				
	P	21				
	–	–	–	P		Wang et al. (2012b)
	P	7	P	–		Achal et al. (2013)
	P	28				
<i>S. pasteurii</i>	–	–	P	–		Wang et al. (2014b)
	–	–	P	–		Wang et al. (2012a)
	P	7	–	–		Bang et al. (2010)
<i>Bacillus cohnii</i>	P	28				
	P	28	–	P		Chahal et al. (2012)
	P	7	–	–		Sierra-Beltran et al. (2014)
<i>Bacillus cohnii</i>	P	28				
	P	56				

Table 2.1 continued from previous page

Microorganism	Effect on Compressive Strength		Effect on Durability			References
	Effect	Time(day)	Permeability	Water absorption		
<i>Bacillus pseudofirmus</i>	N	3	–	–		Jonkers et al. (2010)
	N	7				
	N	28				
<i>Diaphorobacter nitroreducens</i>	N	7	–	–		Erşan et al. (2015a)
	N	28				

N Negative effect and P Positive effect

2.2.2 Effect of Bacteria Encapsulation on Crack Width

If the sealing can be kept in place for the duration of a system, efficient self-healing is attained. Bacteria must therefore survive in order to function properly. However, there can be some difficulties with bacterial survival if bio-chemical are directly keep inside in concrete. Self-healing has only been achieved in young samples. According to research, and untreated bacterial spores have a two-month lifespan when added directly to the mixture [26]. This could be due to a variety of factors, such as the cement matrix's extreme pH values during the early stages of concrete (high alkalinity) and the carbonation phase of mature concrete (high acidity), as well as the components of the concrete mixture and the cement's hydration. Long-term exposure of spores to high alkaline conditions can significantly reduce the bacterial response. Furthermore, the effect or mixing strength of aggregates may affect some spores during mixing. Pores can eventually diminish by up to $0.5\mu\text{m}$ when cement hydration reduces the matrix porosity. Typically, bacterial cells are bigger than the pores [11]. Therefore, late-stage pore reduction in concrete structures will significantly minimize or completely prevent cell germination. One method to get around this limitation is to protect the bacteria

without changing the characteristics of the concrete or the bacterium's ability to precipitate [27].

Because cement materials have autogenous healing qualities, self-healing fissures strengthen concrete constructions. Nevertheless, earlier studies [13] show that the crack width, which can be fixed by autogenous self-healing, is only roughly 0.2 mm. Because hazardous ions can seep into the concrete, an increase in crack width can lead to a decline in durability. On the possible essential fracture width for corrosion onset, there is, however, disagreement [14]. Therefore, in order to strengthen concrete, cracks with widths more than 0.2 mm must be repaired using an efficient self-healing method.

Several encapsulation techniques have been attempted to enlarge the healed fissures in the concrete specimens and shield the bacteria from the high alkalinity of the cement matrix. Rubber particles [16] healed a crack width of 0.86 mm, but melamine-based microcapsules [17] completely restored a maximum crack width of 0.85-0.97 mm in polymeric material encapsulation. In a similar vein, the maximum fully healed fracture widths for biomass obtained from waste sawdust and nanomaterials like GNP were 0.7 mm and 0.81 mm, respectively. Encapsulation in lightweight aggregates was also used to mend cracks wider than 0.1 mm. A maximum crack width of 0.79 mm was healed 100% of the time using expanded perlite. The authors' chosen bacterial carrier type is shown in Table 3, along with the maximum fully healed crack width that corresponds to it [28].

TABLE 2.2: Bacterial carrier types and the greatest crack width that can be fully healed

Material	Bacterial	Maximum Healed	References	
type	carrier	Cracked width 100% (mm)		
		Hydrogel	0.5	Wang et al 2014
			0.34	Trenson G
			0.25	Wang et al 2018
Polymers	Sodium Algininate	0.28	Wiboonluk 2019	
	Calcium Algininate	0.4	Palin et al 2018	
	Rubber particle	0.87	Xu et al 2019	
	Melamine	0.85-0.97	Wan et al 2014	
LWA	LWA	0.61	Khaliq et al 2016	

Table 2.2 continued from previous page

Material	Bacterial	Maximum Healed	References
	EC	0.46	Wiktor et al 2016
		0.15	Jonkers 2011
		0.37	Ersan et al 2016
	EP	0.79	Zhang et al 2017
	Ceramsite	0.25	Xu et al 2018
Nanomaterial	GNP	0.81	Khaliq et al 2016
Special	DE	0.15-0.17	Wang et al 2012
Minerals		1.8	Huynh et al 2017
	Zeolite	0.1	Bhasker et al 2017
Cementitious	CSA	0.394	Xu et al 2018
Biomass	Biochar	0.7	Gupta et al 2018
		0.2	Kua et al 2019

On the other hand, the fully healed crack width of EC varied widely (0.15-0.46mm). The maximum fracture width of 1.8 mm in the case of specific minerals was fully repaired by DE [25] Comparing this repaired crack width to other encapsulating materials, it was the largest. The effect of certain bacterial carriers on bacterial spores was explored, however the repaired crack width was not [29]. The authors' chosen bacterial carrier type is shown in Table 3, along with the maximum fully healed crack width that corresponds to it.

TABLE 2.3: Illustrates how various factors affect the microbially driven formation of calcium carbonate, as shown by the sealed crack width

Microorganisms type	Cell Concentration (Cells/ml)	Optimal PH	Optimum Temp PH	°C	Crack Width Sealed (mm)	Ref
<i>Sporosarcina pasteurii</i> and native Lysinibacillus sphaericus	2×10^8	13-Jul	9	28	0.4	Algaifi et al 2020
<i>Bacillus pasteurii</i> , <i>Bacillus subtilis</i>	-	14-Aug	13	28	0.81	Jogi et al 2020
<i>Bacillus sphaericus</i>	109	9-12.5	12.5	28	0.15-0.17	Wang et al 2012

Table 2.3 continued from previous page

Microorganisms type	Cell Concentration (Cells/ml)	Optimal PH	Optimum Temp PH	°C	Crack Width Sealed (mm)	Ref
<i>Lysinibacillus sphaericus</i>	107	9	9	30	0.4	Algaifi et al 2018
<i>Bacillus subtilis</i>	2.8×10 ⁸	Highly alkaline	Highly alkaline	28	0.37	Kashif et al 2020
<i>Bacillus mucilaginosus</i>	109	Highly alkaline	Highly alkaline	18-22	0.4-0.6	Qian et al
<i>Bacillus subtilis</i>	105	8.5-10	10	-	0.5	Rao et al 2013
<i>Bacillus subtilis</i>	108	7	7	37	-	Durga et al 2019
<i>Bacillus sphaericus</i> and <i>Bacillus subtilis</i>	105	10-13.5	13-13.5	37	-	Ganesh et al 2019

2.3 Test Method to Assess the Effectiveness of Self-healing

Researchers have used a variety of experimental methods to study concrete's self-healing behavior, especially to look at how crack width affects healing efficiency. Measuring the degree of crack closure, figuring out the recovery of specific engineering qualities, and doing qualitative investigations to describe the type of mineral precipitates created during the healing process are just a few of the methods commonly used to assess self-healing.

When combined, these evaluations make it possible to comprehend the connections between material recovery, fracture sealing, and the underlying healing processes. Additionally, the analysis of healing products contributes to the understanding of the mechanisms underlying performance restoration and damage repair [30].

Structural evaluations are carried out at several observation scales, such as the macro-, micro-, and nanostructural levels, in order to develop thorough performance standards for hardened concrete. Together, these scales give a trustworthy way to assess the effectiveness of self-healing. Each scale offers unique but related information about material behavior. Therefore, a variety of assays intended to capture changes occurring across different structural levels are used to evaluate the efficacy of healing mechanisms.

In order to evaluate the effectiveness of self-healing on a large scale, this study conducted a number of experimental investigations. The recovery of mechanical qualities, enhancements in durability-related performance, and the production of mineral precipitates by the healing agents were the main objectives of these experiments. Durability restoration is one of these elements that is commonly seen as a crucial sign of effective self-healing.

Water permeability, fluid flow properties, sorptivity, water absorption capacity, gas permeability, and resistance to chloride ion penetration are important durability-related metrics that are frequently investigated. Comparing healed specimens with pre-cracked or untreated reference samples is a useful and instructive way to understand healing activity [31].

On the other hand, the assessment of mechanical property recovery offers information about the concrete's structural reaction following cracking, which is crucial for comprehending the healed elements' capacity to support loads. In order to ascertain the degree of mechanical restoration attained through healing, these evaluations usually entail assessing compressive strength, tensile strength, and flexural capacity.

The first stage in evaluating and improving the healing process is crack closure, which is the most basic indicator of self-healing. Because of their accessibility and the ease with which healing progress can be tracked, surface fractures are often investigated in experimental experiments. Several analytical methods have been used to measure bacterial activity linked to healing processes in addition to mechanical and visual assessments. These techniques include thermogravimetric

analysis, spectrophotometry, oxygen profile measurement, and total ammoniacal nitrogen measurement to assess urea decomposition. When combined, these methods offer insightful information about the functioning of bacteria and how they contribute to self-healing behavior.

2.3.1 Comparative Assessment of Test Method

Concrete self-healing performance is assessed using a variety of testing methods, including mechanical, durability-based, physicochemical, and microscopic, each with unique benefits and drawbacks [12]. Optical-based techniques like light microscopy and optical polarization microscopy (OPM) are frequently used to quantify crack healing. Because light microscopy is inexpensive, simple to use, and requires little sample preparation, it is frequently used. It makes it possible to observe surface fractures directly and more clearly than with the unaided eye, which makes it possible to track the closure of cracks over time. This technique also makes it easier to examine comparatively broad surface areas and yields accurate results for crack widths that are usually between 0.2 and 0.7 mm [13].

However, because it does not allow for the assessment of interior fracture closure or the identification of the chemical nature of the healing products, its relevance is mostly limited to surface-level investigation. Furthermore, because of the restricted field coverage, it might be necessary to take several pictures to fully capture the specimen's surface [14].

The chemical processes involved in self-healing are often studied using thermogravimetric analysis (TGA), especially when it comes to detecting reaction products created during healing [16]. This method works well for measuring mass changes associated with hydration and carbonation processes as well as for identifying phase shifts. By evaluating weight loss at particular temperature ranges, TGA also helps to differentiate the role of therapeutic medicines [17]. Despite these benefits, samples must be completely dried for TGA to remove interference from physically bonded water. Moreover, it needs to be supplemented by other analytical methods like X-ray diffraction (XRD) in order to produce a definitive

mineralogical identification [18]. The type of the carrier gas, sample mass, heating rate, and other experimental factors can all have an impact on how reliable TGA results are.

When researching physiologically induced self-healing processes, OPM is especially useful [19]. By distinguishing between calcium carbonate that precipitates biologically and carbonate that is chemically produced, this technique provides information about the metabolic activities of bacteria in the crack environment [32]. However, its relevance for large-scale or uniform evaluation of self-healing performance is limited by the absence of comprehensive datasets.

Permeability-based tests, including as water permeability, water absorption, and chloride penetration assessments, are widely used to evaluate the recovery of durability qualities [18]. By measuring the decrease in water flow via healed cracks, water permeability testing offers a quantitative assessment of crack sealing effectiveness [20].

In a similar vein, water absorption tests evaluate concrete's capillary suction capability, which indicates how much healed fissures prevent moisture intrusion. Resistance to vigorous ion transport is assessed using chloride penetration tests, such as fast chloride migration techniques [21].

However, because uneven fracture geometry can greatly affect results, the efficacy of these tests is heavily dependent on the technique employed to create cracks. Furthermore, since water uptake may happen through the entire matrix, reference comparisons with uncracked specimens are necessary for water absorption measurements [22]. Creating sufficiently large and consistent cracks without sacrificing specimen integrity is a real difficulty in chloride penetration testing [25].

Compressive strength, flexural strength, and split tensile strength tests are frequently used to assess the recovery of mechanical properties as a result of self-healing [12]. These techniques offer a clear indicator of strength restoration after healing and are well-established, scalable, and appropriate for industrial applications. However, a number of variables, such as specimen handling, curing conditions, and mixing ratios, affect mechanical test results [13]. More significantly,

durability and transport qualities may still be affected even when strength is restored, so strength recovery by itself does not accurately reflect self-healing efficiency [11]. Correlating strength values from retrieved cores with the real in-situ performance of concrete structures is another area of uncertainty.

Advanced material analysis techniques like X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FT-IR), and scanning electron microscopy (SEM) are commonly used for detailed characterization of healing goods [9]. SEM provides important insights into microstructural changes during healing by enabling high-resolution observation of crystalline deposits developed within cracks. However, it is challenging to evaluate the uniformity of healing product distribution because the accuracy of SEM observations depends on the image resolution and the chosen observation region [18]. Although FT-IR can quickly identify functional groups and vibrational modes linked to hydration and carbonate compounds, its efficacy may be limited by its sensitivity to moisture content and inability to detect small deposits [19]. By comparing diffraction patterns with reference databases, XRD is especially helpful for detecting crystalline phases like calcite [32]. However, it is still difficult to quantify calcium carbonate using XRD, because phases that are present in very small amounts may go unnoticed, particularly in finely powdered samples [19].

In general, no single test technique is adequate to fully assess concrete's capacity for self-healing. To gain a trustworthy and comprehensive understanding of self-healing mechanisms and efficacy, a multi-technique approach that incorporates mechanical evaluation, durability testing, visual assessment, and microstructural characterisation is necessary [32].

2.4 Summary

With an emphasis on bacteria-based healing mechanisms, this chapter offers a thorough analysis of self-healing concrete. Its importance stems from the thorough identification and comparison of standardized experimental techniques used to evaluate the effectiveness of bacterial healing agents and the different materials

utilized to encapsulate microorganisms within concrete matrices. The most popular encapsulating materials for transporting and safeguarding microorganisms are polymers and lightweight aggregates, according to numerous prior research. Apart from these materials, research indicates that diatomaceous earth has shown excellent healing properties, allowing for the mending of fissures as wide as about 1.8 mm.

Furthermore, it has been demonstrated that the use of lightweight aggregates and nanoparticles improves the mechanical performance of concrete when compared to alternative encapsulating techniques, resulting in increased strength and structural integrity.

Concrete's vital durability qualities have also been successfully restored by bacteria-based mending therapies. These enhancements include improved resistance to chloride ion penetration and decreased water absorption, both of which are crucial markers of long-term durability. Researchers most commonly used the recovery of mechanical properties, such as compressive and flexural strength, among the many macrostructural evaluation approaches.

Measurements of mineral precipitation within fissures and durability evaluations were frequently added to this method. Light microscopy (LM) has become the most used viewing and quantification method for assessing bacterial activity and crack-healing. On the other hand, thermogravimetric analysis (TGA) was found to be the least used technique, probably because of its difficulty and restricted availability.

Some research used microstructural analyses in addition to macrostructural tests to further increase the dependability of experimental results. Scanning electron microscopy (SEM) was the most widely used method at the microstructural level to confirm the creation of healing products, closely followed by X-ray diffraction (XRD).

The main purpose of these techniques was to verify the existence and makeup of crystalline precipitates produced by bacterial action. The usage of encapsulated microorganisms at the nanostructural scale has not yet been investigated, which

highlights a major research gap in the reviewed literature. This gap indicates a chance for future studies to look at innovative encapsulation techniques that can improve material performance and healing efficiency even more.

Appropriate experimental techniques were chosen to assess the efficacy of recently created bacteria-based therapeutic agents based on the findings of this thorough literature review. Laboratory-scale durability studies, such as water permeability and water absorption assessments, are suggested to evaluate the capacity of healed cracks to withstand fluid penetration. These tests offer vital information about how concrete tightness can be restored after healing. Furthermore, TGA and LM can be used to measure the degree of crack closure and visually record the healing process. When combined, these methods provide for a comprehensive assessment of the suggested bacterial system's capacity for self-healing. Microstructural characterisation techniques including as SEM, XRD, and Fourier-transform infrared spectroscopy (FT-IR) can be used to detect and evaluate the mineralized products generated within the cracks in order to validate and support the macroscopic results. Overall, the concrete mix design, specimen preparation, and testing methodology chosen as the main research framework for this thesis have been greatly influenced by the results of this literature study. The complete study technique that was created based on these observations is presented and discussed in the following chapter.

Chapter 3

Experimental Program

3.1 Background

Using the idea of self-healing concrete, microbially induced calcium carbonate precipitation (MICP) is a viable method for creating long-lasting and sustainable reinforced concrete (RC) structures. Throughout its useful life, this kind of concrete may self-heal cracks. Adding MICP-active bacteria to the concrete mixture is the most popular technique for creating bio-based self-healing concrete. However, because of the extremely alkaline pore solution, the internal concrete environment presents serious obstacles to microbiological survival. Bacterial cells may also be further harmed by the mechanical pressures involved in mixing and placing concrete. Because of this, mixing and casting self-healing concrete requires more caution.

This chapter provides a detailed explanation of the materials, concrete preparation process, specimen information, and testing processes.

3.2 Research Methodology

Following is the schematic diagram of research methodology.

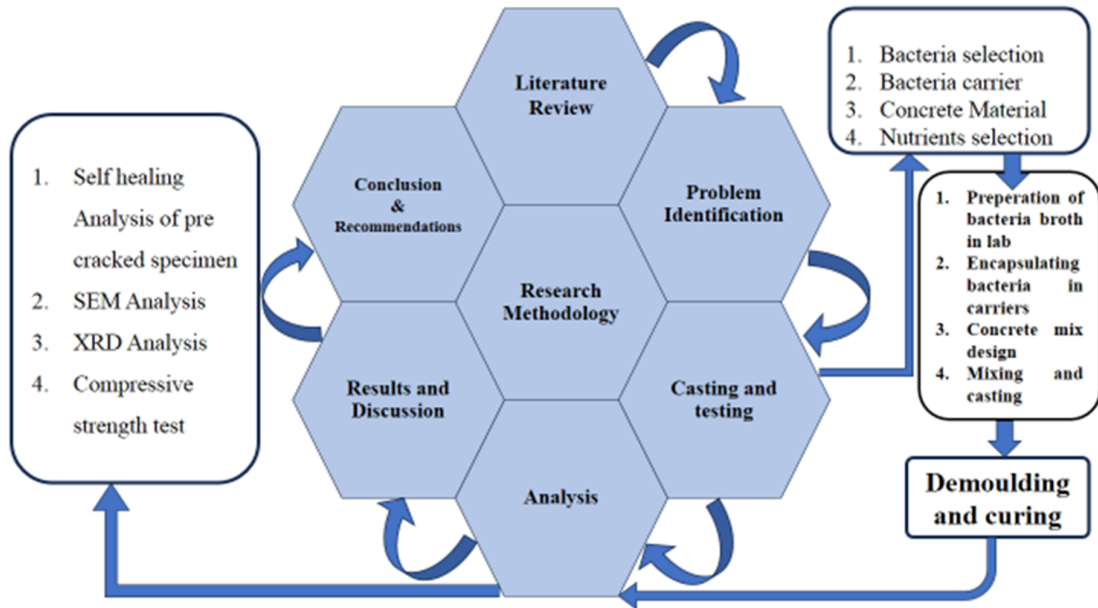


FIGURE 3.1: Research Methodology

3.3 Urease Producing Bacterial Strain

Bacillus Subtilis strain of bacteria, was used in this investigation [33]. In Doha, Qatar, a strain of Bacillus subtilis was identified from the soil. The chosen strain was found to be the most promising strain in the field based on its urease activity and ability to generate calcite minerals at temperatures higher than 40 °C. There have been further examples of these strains' vitality, prolonged growth, and precipitation of calcium carbonate. This bacteria is imported from Qatar University. The strains were kept at -80 °C in 30% glycerol in the Qatari Mineral Precipitating Strains Bank [33]. To obtain live, fresh, and pure cells, they were struck on solid Luria-Bertani (LB) media and recovered.

3.3.1 Growth Media

The bacterial strain was grown using Nutrient Broth (NB) as the culture medium. 5 g/L NaCl, 10 g/L tryptone, and 5 g/L yeast extract made up the NB. In order to encourage spore production, prepared bacterial solutions were treated under carefully monitored laboratory settings before being incorporated into concrete.

Fig. 3.2 displays the *B. subtilis* spore suspension. Based on the cell concentration determined by an optical density (OD) test performed using a spectrophotometer, the necessary volume of bacterial solution for the concrete mixture was computed. For calibration, the growth medium served as the blank reference. The baseline was first established by measuring 0.5 mL of the blank solution at a wavelength of 600 nm.



FIGURE 3.2: Bacteria solution and spectrophotometer

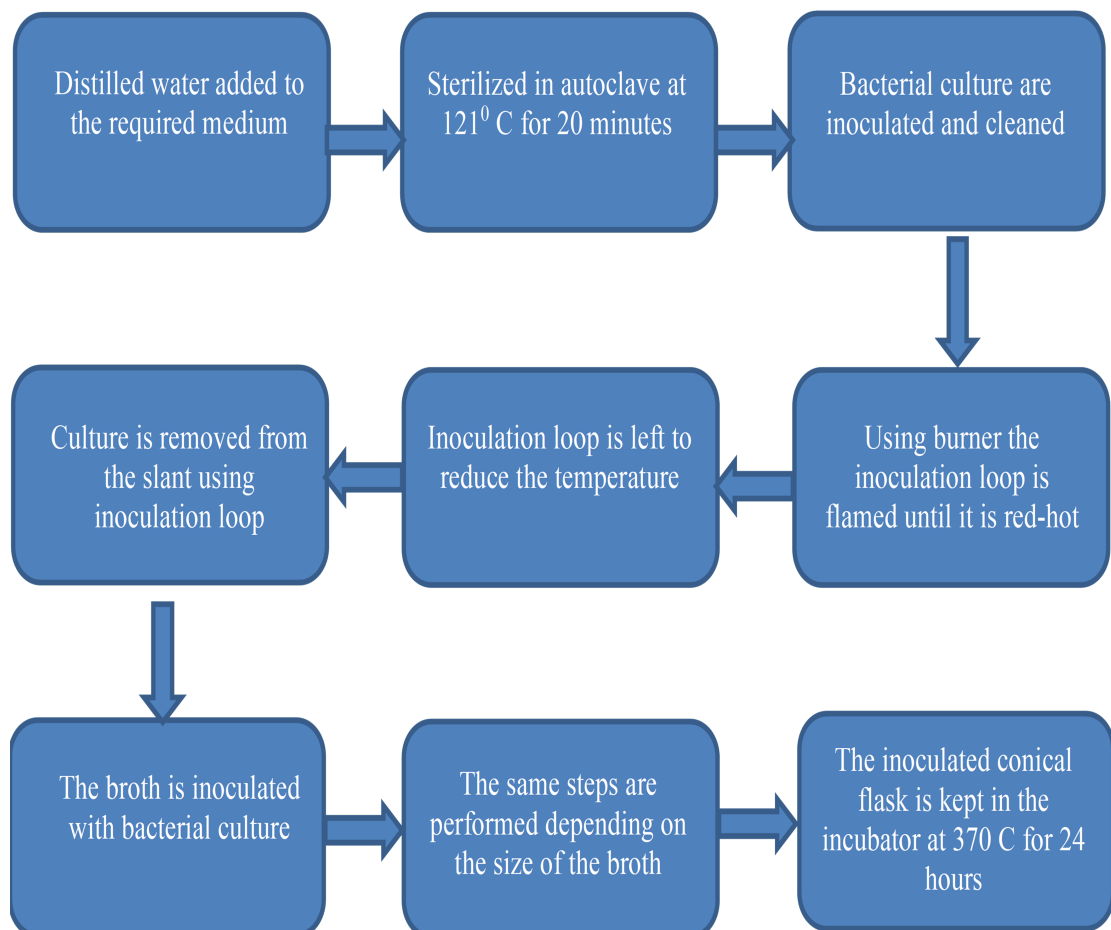


FIGURE 3.3: Bacteria cultivation process [34]

Following calibration, the OD of 0.5 mL of the bacterial suspension was measured at 600 nm. The bacterial concentration was then estimated using the empirical relationship $Y = 8.59 \times 10^7 X^{1.3627}$ (Ramachandran et al., 2001), where X represents the OD₆₀₀ reading and Y denotes the number of bacterial cells per milliliter. Using this method, the bacterial concentration was calculated as 2.8×10^8 cells/mL. Based on these results, the spore dosage in the concrete mixtures was maintained at 3×10^8 cells/cm³ [33].

3.3.2 Nutrients

In the concrete matrix, dry substances such as calcium nitrate, calcium lactate, yeast extracts, and urea were added as nutrients for the bacterial strains. The chosen set of nutrients has previously been used by a number of researchers.



FIGURE 3.4: Calcium nitrate, Calcium lactate, Yeast extracts and Urea

3.4 Concrete Constituent Materials

The material utilized was regular Portland cement CEM-I that complied with ASTM C-150, with an average particle size of $5.74 \mu\text{m}$ and a specific gravity of 3.05. Table 3.1 provides an overview of CEM-I chemical and physical characteristics. According to ASTM C128 [35], fine aggregate with a specific gravity of 2.73 and a fineness modulus of 2.69 was obtained from a local quarry in Pakistan. A maximum size of 16 mm for coarse aggregate was obtained from Margalla, Pakistan. According to ASTM C33, the coarse aggregate's specific gravity and fineness

modulus were 2.71 and 2.77, respectively. To prevent the production of hazardous ammonia, bacteria were fed calcium lactate, calcium, nitrate, yeast extract, and urea [36].

TABLE 3.1: Properties of cement

Chemical Composition Component	Content (% by mass of cement)
CaO	61.70
SiO ₂	21.00
Al ₂ O ₃	5.04
Fe ₂ O ₃	3.24
SO ₃	1.51
MgO	2.56
Loss on ignition	1.83
Mechanical Properties	
Compressive Strength (MPa)	
3 days	27.38 MPa
7 days	33.64 MPa
28 days	47.27 MPa
Physical Properties	
Initial and Final setting time (minutes)	175 min and 375 min

3.4.1 Bentonite

The main goal of this research is to examine the impact of Bentonite as a carrier material and compare it to other carrier materials. As a result, assessing bentonite characteristics is crucial. Fig. 3.2 illustrates Bentonite physical properties.



(a) Bentonite particles



(b) Bentonite powder

FIGURE 3.5: Bentonite

TABLE 3.2: Material parameters of Bentonite provided by supplier are listed

Properties of bentonite	
Grade	NB (Nano bentonite) -25
Particle size	25 nm
Composition	90% Montmorillonite
Surface area	600-800 m ² /g
Lamella Thickness Index	1.0-1.5 nm per layer

To determine Bentonite maximal absorption capacity, absorption experiments were also performed on them. This test helped in figuring out how much bentonite was needed as a bacterial carrier molecule. Filter paper was used to filter Bentonite after they had been immersed in water for 24 hours. Bentonite saturated weight was determined, and then it was oven-dried and weighed once more to determine water absorption. This test revealed that the water absorption of bentonite was 81%.

3.4.2 Light Weight Aggregate

To assess the best technique for immobilization of bacteria in the mixture, light weight aggregates (LWA) were investigated as a carrier material and compared with bentonite. Figure 3.6 illustrates the LWA that was utilized in this investigation to incorporate bacterial spores.



FIGURE 3.6: Light weight Aggregate (pumice)

Similar to Bentonite, a water absorption test was performed on LWA to ascertain the precise quantity that should be utilized. Five hundred grams of LWA were saturated with water in order to assess the water absorption in the light-weight aggregate [37]. They were surface dried after twenty-four hours, and their saturated weight was determined. After being dried for 24 hours in an oven, the dry weight of these aggregates was determined. According to this test, LWA's water absorption was 23.3%.

3.5 Mix Proportion

Four distinct types of concrete mixes were prepared for this study. In addition to 820 kg/m³ of fine aggregate, 960 kg/m³ of coarse aggregate, and 17 kg/m³ of additives such as calcium lactate, yeast, urea, and calcium nitrate, each mix included 360 kg/m³ of ordinary Portland cement (OPC) Type I, which complies with ASTM C 150-07.

Every mix was created with a goal compressive strength of 30 MPa and a water-to-cement ratio of 0.4. The ASTM C 191-11 and ASTM C 187-11 standards were used to determine the cement's initial and final setting times as well as its usual consistency.

There were no bacterial spores in the control mix, known as "Mix 1." In "Mix 2," the bacterial solution and mixing water were combined without the use of a carrier material to transfer bacterial spores directly into the concrete.

In "Mix 3," bacteria were immobilized on lightweight aggregate (LWA)), which replaced 8% of the coarse aggregate by volume, which was submerged in the bacterial solution for a full day to guarantee complete saturation before being mixed into the concrete.

The bacteria in "Mix 4" were immobilized using bentonite, which replaced 8% of the fine aggregate by volume, which was also submerged in the bacterial solution before being mixed with the concrete. Table 3.3 provides a summary of the precise

ratios for each batch of concrete. To ensure homogeneity, the mixing process and time were maintained for all mix types [38].

TABLE 3.3: Mix Proportion of all Formulations

Specimen code	Unit	Mix 1	Mix 2	Mix 3	Mix 4
Cement	Kg/m ³	360	360	360	360
Fine Aggregate	Kg/m ³	820	820	820	754
Coarse Aggregate	Kg/m ³	960	960	883	960
Water cement Ratio		0.4	0.4	0.4	0.4
Calcium lactate	Kg/m ³	17	17	17	17
Calcium nitrate	Kg/m ³	17	17	17	17
Yeast	Kg/m ³	17	17	17	17
Urea	Kg/m ³	17	17	17	17
Bacteria Immobilization	Kg/m ³	None	Direct	By LWA (8% of coarse agg.)	By Bentonite (8% of fine agg.)

3.5.1 Mixing of Concrete Matrix

Below are the schematic diagram which was adopted for the mixing of concrete in a mechanical mixer. Special care taken while mixing because bacteria life is prone to danger when harsh mixing take place.

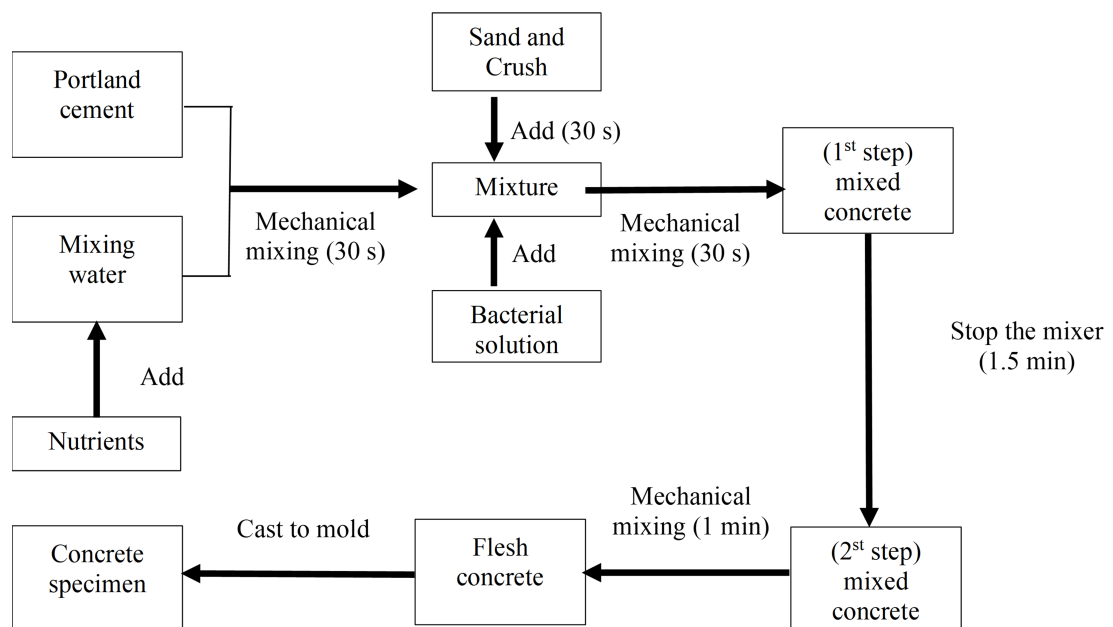


FIGURE 3.7: Procedure for concrete mixing

3.5.2 Casting and Testing Regimes

Every specimen was prepared in compliance with ASTM guidelines. Four distinct mix patterns were used to cast fifty prism specimens ($40 \times 40 \times 160$ mm) and forty cubical specimens (50×50 mm). The specimens were demolded and immersed in water to cure until their specified testing ages after a 24-hour period.

There were three stages to the experimental program [39]. The cube specimens' mechanical characteristics were assessed in the first phase at curing ages of 3, 7, 14, and 28 days. Compressive strength testing were conducted in accordance with ASTM C496/C496M and ASTM C39 standards, respectively.

Two specimens of each mix were examined for compressive strength at each age, and the average result was reported [40]. Total specimen tested for compressive strength is 32 Nos. Specimens were taken out of the water and any surface moisture was cleaned off before testing. Each specimen was placed in the testing apparatus, as shown in Fig. 3.8, and the standard technique was followed to apply the load at the designated rate.

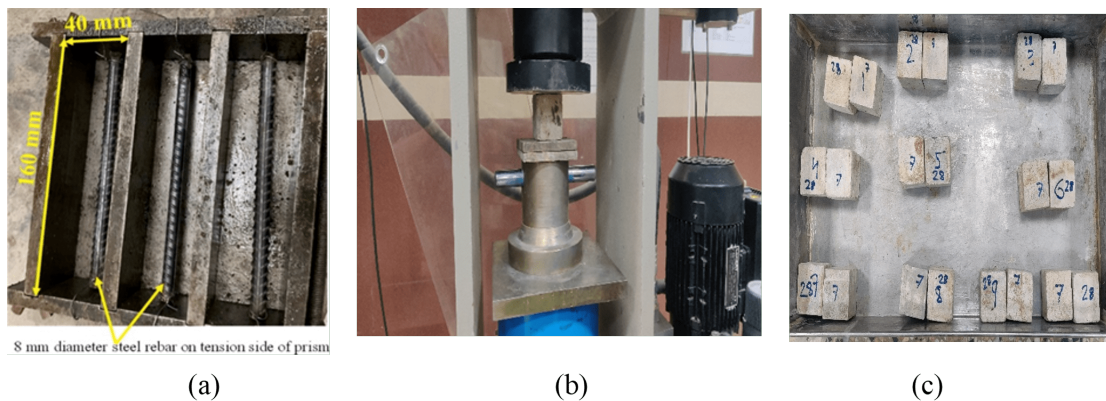


FIGURE 3.8: (a) Prism mold (b) compression testing machine (c) sample specimens

Pre-cracked specimens ($40 \times 40 \times 160$ mm) were created in the second phase, with three specimen from each mix pre-cracked at 3, 7, 14, and 28 days of curing. To start cracking, the specimens were subjected to controlled compressive force while being attentively observed. The applied load was stopped as soon as noticeable cracks formed. Out of three specimen for each mix at 3, 7, 14 and 28 days, one

specimen is selected, for the purpose of later observing self-healing, cracks that were about 1 mm wide were chosen, marked, and measured at various sites. As seen in Fig. 3.9, the initial crack widths at the time of crack formation were recorded using a crack width measuring microscope with an accuracy of 0.02 mm.

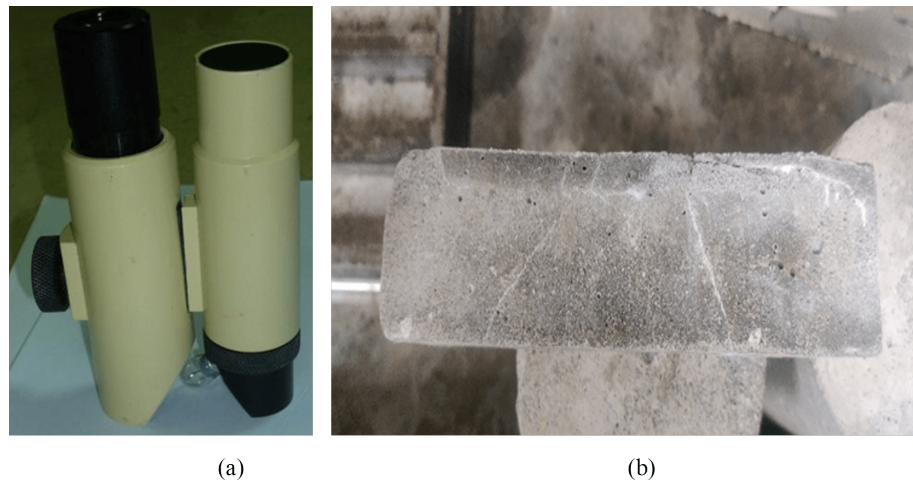


FIGURE 3.9: (a) crack measuring microscope, (b) crack healing through MICP

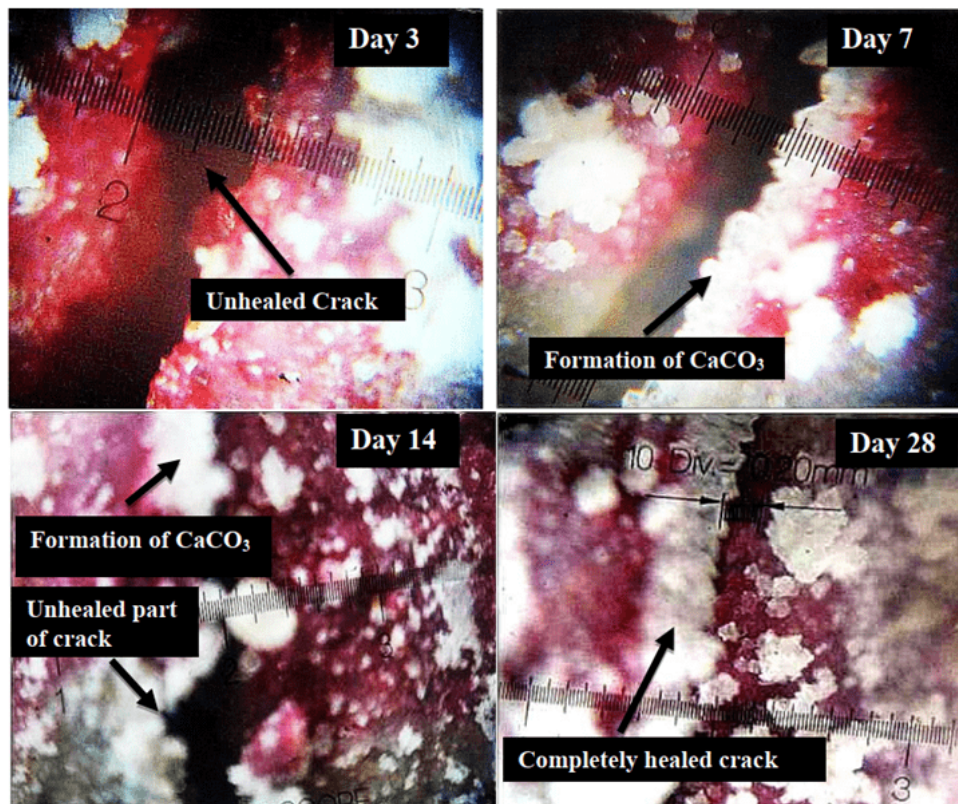


FIGURE 3.10: A closer look at the cracks reveals a calcium carbonate-producing self-healing process.

The pre-cracked specimens were put back under strictly regulated curing conditions after the initial measurements were recorded. The specimens were taken out

and the crack widths were measured once more using the crack width microscope at the specified ages of 3, 7, 14, and 28 days. Visual examinations were also carried out, as shown in Fig. 3.10. The effectiveness of the concrete's self-healing was assessed by calculating the difference between the initial and subsequent readings.

In phase three, scanning electron microscopy (SEM) was used to examine the microstructural alterations in concrete brought about by self-healing for each of the four mix types, supplementing the findings from ocular examinations of the specimens. To assess the development of the self-healing process, SEM studies were carried out at 7 and 28 days of healing. In order to confirm the development of calcium carbonate in the samples and to have a better understanding of the self-healing mechanism, the compounds generated within the fractures were further analyzed using X-ray diffraction (XRD).

Compressive strength tests, SEM, XRD, and visual evaluations of crack healing were all documented. To assess the efficacy of the various approaches, these results were further examined and contrasted. Chapter 4 presents a thorough analysis and comparison of these findings.

Chapter 4

Results and Discussions

4.1 Background

The studies carried out to assess the efficacy of self-healing in each concrete mix are presented and interpreted in this part. The results include the self-healing concrete samples' compressive strength, water absorption, resistance to acid attack, measurements of crack widths, visual observations of cracks, microstructural characterization using SEM, and XRD analysis of the mineral composition of the healing products. Additionally, the correlation between the relevant compressive strength and self-healing at various curing ages is investigated.

4.2 Self-healing Analysis

A crack width measuring microscope was used to examine pre-cracked specimens at intervals of three, seven, fourteen, and twenty-eight days in order to assess the efficacy of the self-healing process. There was noticeable crack closure, especially in the specimens from Mixes 2, 3, and 4. A significant accumulation of calcium carbonate (CaCO_3) crystals took place inside the fissures, as shown in Fig. 4.1. The development of these CaCO_3 crystals was mainly responsible for the decrease

in fracture width in the specimens containing bacteria. In a similar vein, [18] documented that the bacterial transformation of calcium lactate into calcium carbonate produced this therapeutic chemical.

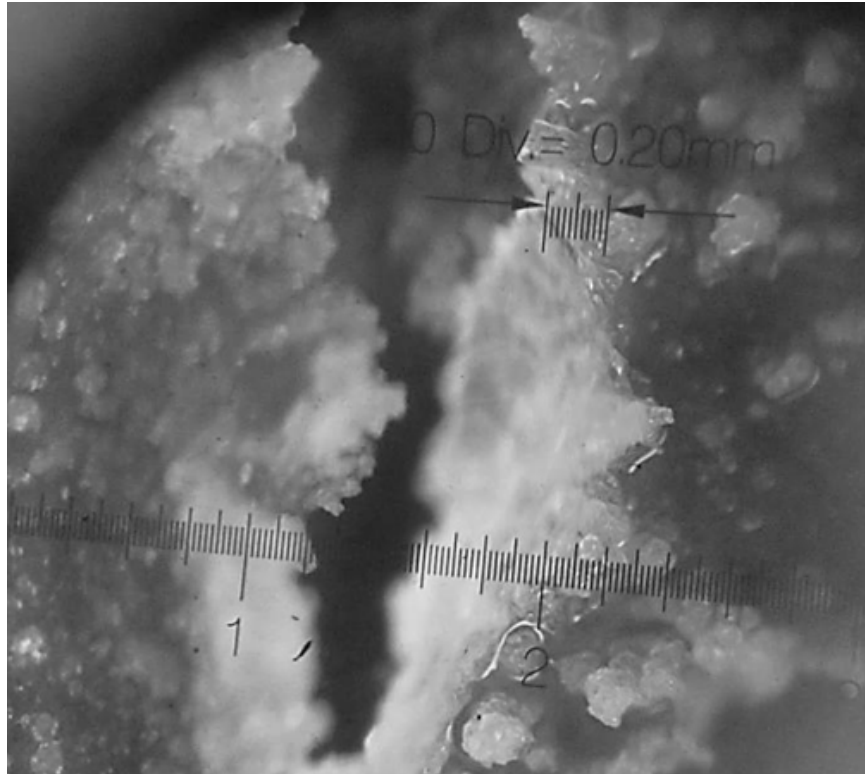


FIGURE 4.1: Measurement of crack width using a crack measuring microscope

The difference between the initial and healed crack widths at various predetermined dates is used to calculate the healing measure, which is expressed in millimeters. The effectiveness of crack healing for each mix as a function of time is shown in Fig. 4.2. Crack healing was noticeable when specimens of all integrated procedures were pre-cracked after three days of curing, particularly after seven days. The maximum healing as a function of time is displayed in Fig. 4.2 for Mix 4 samples that use Bentonite as a carrier chemical. The bentonite particle size is the cause of Mix 4's increased healing. Bentonite small size allows it to function as a filler material and ensures that it is distributed uniformly throughout the mixture [19]. Bentonite particles are saturated with bacterial medium, which ensures that there is bacteria medium available at the crack site for the synthesis of healing compounds and causes a uniform distribution of bacteria by colloidal dispersion in the mix. When these self-healing bacteria enter through cracks and come into touch with water, they become activated and begin converting calcium

lactate into calcium carbonate, which is insoluble in water. This process causes the fractures to repair.

Specimens with LWA as a carrier material come after Mix 4 specimens. It becomes challenging to guarantee that LWA is distributed in the mix as uniformly as Bentonite since its particle size is larger than Bentonite. A larger LWA makes it more difficult for LWA to enter the interparticle gaps, where Bentonite nanoparticles allow for easy penetration. This characteristic of LWA reduces the effectiveness of the self-healing process in concrete by impeding the equal and even dispersion of bacteria in the mix. All of the samples that included bacteria had better healing outcomes when compared to control samples, as seen in Fig. 4.2. For a variety of reasons, controlled concrete samples devoid of microorganisms also shown minor fracture healing. In the early stages of development and during the first mixing process, certain cement particles are not fully hydrated. These particles undergo continuous hydration, which produces expansive hydration products that ultimately cause fissures to heal. The carbonation of calcium hydroxide is another factor contributing to crack healing in the control samples. In this process, calcium hydroxide is converted into calcium carbonate [19].

The availability of carbon dioxide (CO_2) dissolved in the infiltrating water is the main factor controlling calcium carbonate formation in the control specimens. However, there is a limit to the amount of carbon dioxide that can dissolve in water, which also limits the amount of calcium carbonate that can be produced by the carbonation process. Additionally, because portlandite ($\text{Ca}(\text{OH})_2$) dissolves in water, the water that is added for the carbonation process not only supplies carbon dioxide but also dissolves In controlled samples, calcium hydroxide precipitated, reducing the amount of calcium hydroxide available for conversion to calcium carbonate in concrete samples.

The self-healing mechanism in bio-concrete is distinct because it involves both bacterial activity and the presence of calcium lactate, which together promote the precipitation of calcium carbonate within cracks. Bacterial spores that are dormant become active when they come into touch with water seeping through the cracks. The concrete's calcium lactate, calcium nitrate, yeast, and urea are then

transformed by the bacteria into insoluble calcium carbonate. Furthermore, CO_2 is produced as a byproduct of bacterial metabolism, which aids in the carbonation of calcium hydroxide and the subsequent production of calcium carbonate. In bio-concrete, these two processes take place concurrently, boosting the overall amount of healing compound generated [20].

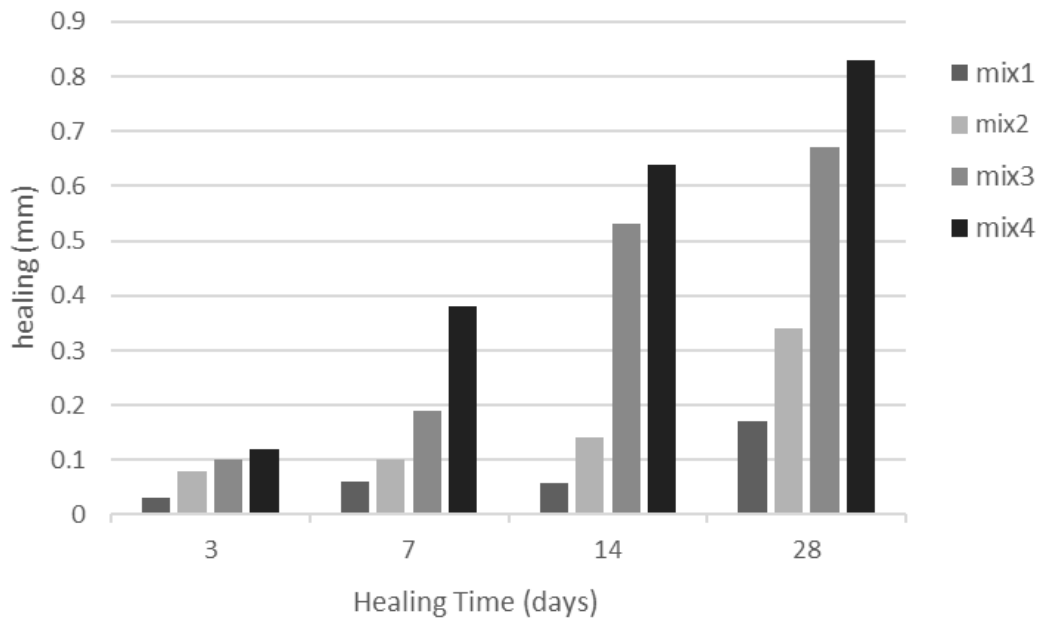


FIGURE 4.2: Healing of cracks in specimens that were pre-cracked at 3 days of first 12 specimens

As seen in Fig. 4.3, crack healing in specimens pre-cracked after 7 days of curing exhibits a similar tendency to that of specimens pre-cracked at 3 days. Mix 4 specimens, which contain bentonite as a carrier chemical, exhibit significantly more healing than specimens prepared using any other procedure. As was previously said, Bentonite efficient behavior results from its tiny size, which guarantees complete and uniform dispersion of the bacteria-carrying particles in the mixture. Because of their small size, Bentonite particles can enter the spaces between other particles, enabling the presence of bacteria the self-healing process.

Because LWA is larger than bentonite, it cannot fit through the tiny openings, which restricts its ability to disperse self-healing bacteria throughout the concrete matrix uniformly. After 28 days, Mix 4's cracks had healed to a maximum of 0.81 mm. The Mix 3 specimens, which included LWA, showed a 0.61 mm crack

healing. Mix 2 samples, which were made by directly introducing bacteria into the concrete mix, displayed 0.37 mm of healing. Comparing the outcomes obtained from specimens that were pre-cracked for three and seven days, however, shows that self-healing has somewhat decreased in specimens of all mix types.

Since a large portion of the cement in concrete is already hydrated, there is a general decrease in the production of additional hydrated products, which results in a decrease in self-healing. The overall decline in bio-concrete specimens can also be attributed to a minor decline in bacterial survival viability in concrete when denser microstructure development begins.

Denser microstructures at this stage have the least impact on Mixes 3 and 4, as carrier chemicals may better shield bacteria from the pressure being applied and the pressure imposed by microstructure development is not yet considerable.

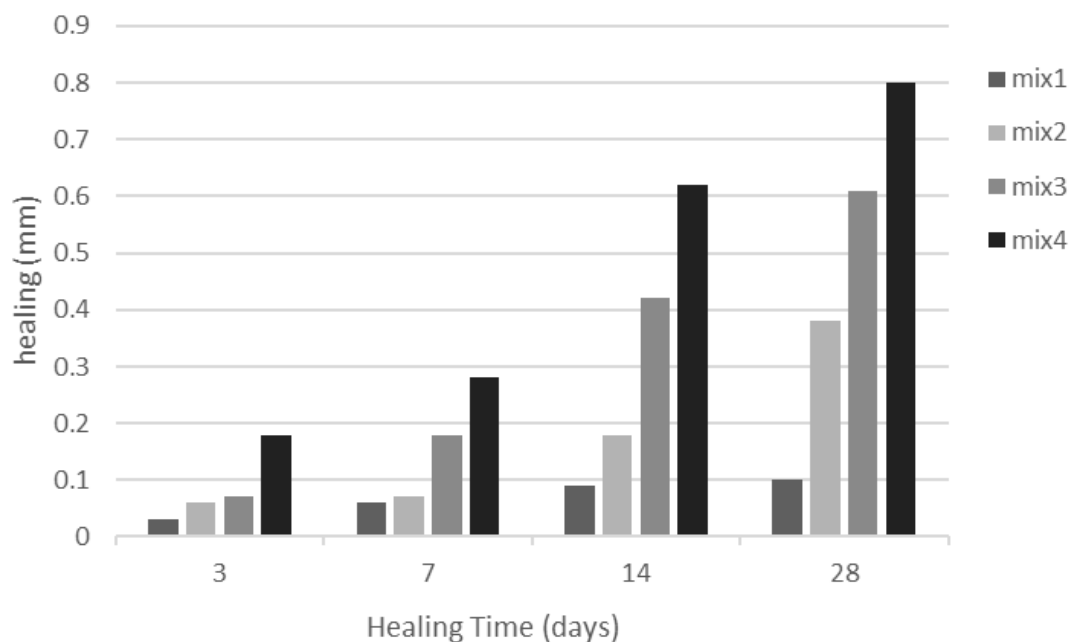


FIGURE 4.3: Healing of cracks in specimens that were pre-cracked at 7 days of second 12 specimens

Figure 4.4 shows the healing that was seen in specimens that had already been fractured after 14 days of cure. At 14 days of curing, Mix 3 specimens exhibit the greatest amount of healing in pre-cracked samples. When samples were pre-cracked at 3 and 7 days of curing, Mix 4 showed the greatest healing; however,

when samples were pre-cracked at 14 days of curing, Mix 4 was less effective than Mix 3.

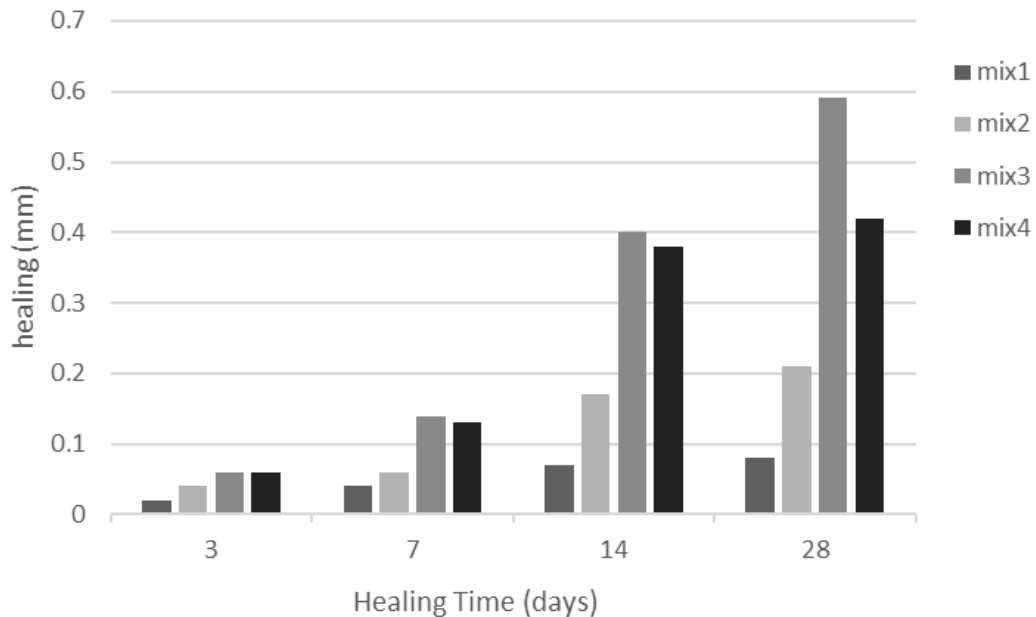


FIGURE 4.4: Healing of cracks in specimens that were pre-cracked at 14 days of third 12 specimens

This shift in Mix 4's behavior may be the consequence of the concrete's ongoing hydration reactions, which continue to create a dense microstructure. The carrier compounds with integrated microorganisms are under pressure from the concrete's thick microstructure. The viability of bacterial survival in specimens that had already been fractured at 7 days was not significantly impacted by this pressure. But in specimens that were pre-cracked at 14 days of cure, this pressure was raised to the point where it significantly impacted each specimen's ability to mend itself. Microstructural pressure has the biggest impact on Mix 2 and Mix 4 specimens. However, it appears that this pressure has the least impact on LWA. When exposed to multiaxial loading, bentonite is poor [21], which means that it cannot give bacteria better support than LWA. As a result, the bacteria in Mix 4 are eliminated and annihilated, which slows down the healing process compared to samples that were pre-cracked at 3 and 7 days. The poor microstructure, which is not completely formed until just seven days of casting and becomes more compact and mature at fourteen days, is the cause of this decrease in self-healing.

The Mix 2 specimens, which had bacteria directly integrated, also showed a shift in the self-healing process; the 14-day healing value dropped from 0.37 mm to 0.21 mm. The reduction in the ability of bacteria to survive in concrete under pressure is the cause of the decline in crack healing of Mix 2 samples, that emerged as a result of the development of a thick microstructure during the mixing phase [1].

The breakdown of calcium lactate in the concrete mix was the cause of this decline in self-healing activity, according to [2]. In contrast, 4.86% of the cement weight was utilized in this study to guarantee that the bacteria had adequate access to calcium lactate, whereas 1% of the cement weight was used in his study. Additionally, only Mix 2 specimens showed this sharp decline in bacterial activity, indicating that the bacteria's removal rather than calcium lactate breakdown is to blame.

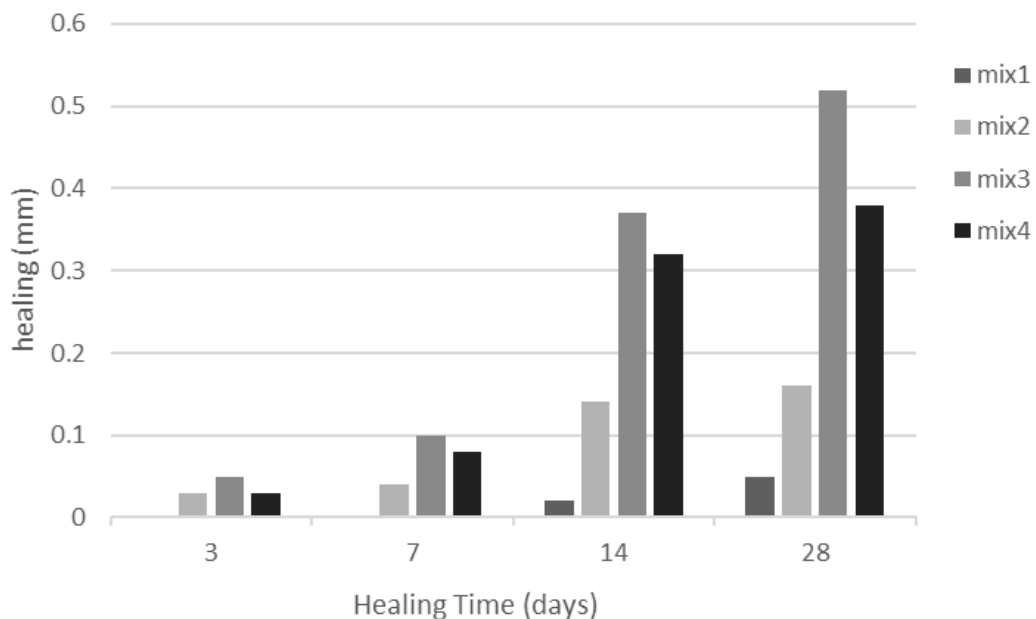


FIGURE 4.5: Healing of cracks in specimens that were pre-cracked at 28 days of forth 12 specimens

After 28 days of cure, the results of measures of self-healing in precracked samples are shown in Fig. 4.5. As can be observed, combination 3 with LWA exhibits the highest healing of 0.52 mm, which is greater than that of any other combination. Specimens in Mix 4 with bentonite exhibit much less healing than those at 3 and 7 days after pre-cracking. The healing of Mix 4 specimens was 0.38 mm, greater than the healing of Mix 2 specimens, which was 0.15 mm.

This demonstrates that while Mix 4 still significantly improves healing with Bentonite - incorporated bacteria but Mix 3's healing effect is greater. The dense microstructure that forms in the concrete after 28 days of curing, which is comparable to samples pre-cracked at 14 days of curing, is once again responsible for the decrease in healing of Mix 4 specimens at 28 days pre-cracking.

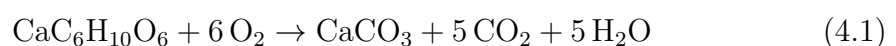
4.2.1 Microstructure Analysis

To investigate alterations in concrete microstructure brought on by self-healing, specimens of all four mixes were analyzed using scanning electron microscopy (SEM), in addition to the findings obtained from visual inspection of concrete samples. SEM analysis was done at 7 and 28 days of healing to compare the self-healing process.

The primary goal of this study was to produce crystals based calcium carbonate, to heal cracks and increase durability of concrete. Calcite, aragonite, and vaterite are the three distinct kinds of calcium carbonate crystals that can form [3]. Calcite is the most stable form of calcium carbonate among the three.

SEM pictures of all four mixtures at a resolution of 2 μm taken seven days prior to specimen cracking are displayed in Fig. 4.6. The formation of orthorhombic calcite crystals is depicted in Fig. 4.6. When compared to mixes using other methods, Mix 4, which used Bentonite as a carrier chemical, demonstrated the highest production of calcium carbonate (CaCO_3).

Two components of the calcium lactate-based healing system—bacteria and lactate—combine to create this CaCO_3 . Although CaCO_3 can also form in controlled samples, the formation of CaCO_3 crystals is catalyzed by the presence of bacteria and calcium lactate. Higher amounts of CaCO_3 crystals grow, and their shapes are identical and match those reported by [3] and [4]. The following equation illustrates the chemical process by which bacteria produce calcium carbonate.



As previously mentioned, concrete that contains bacteria is not the only material that produces CaCO_3 . Controlled concrete sample also exhibit the presence of CaCO_3 .

However, compared to bacteria-incorporated specimens, the process of CaCO_3 crystal production in controlled specimens is very different. According to equation 4.2, the carbonation of calcium hydroxide, one of the main hydration products of cement, is what causes the formation of CaCO_3 in Mix 1 specimens.

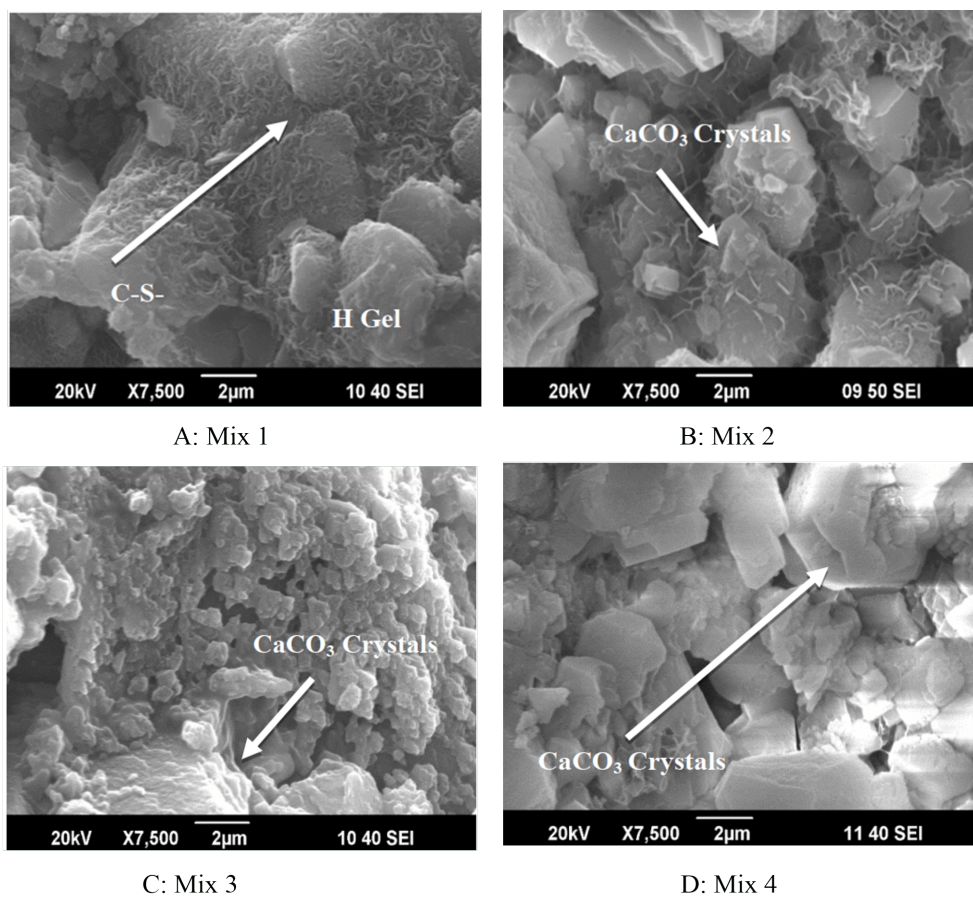
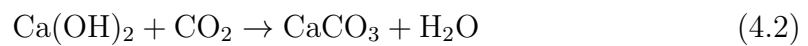


FIGURE 4.6: Analysis of 7-day pre-cracked samples using a scanning electron microscope

However, compared to those created by bacterial action, the carbonation process in Mix 1 produces CaCO_3 crystals relatively slowly. Furthermore, less CO_2 is available for the carbonation process because the production of calcium carbonate in controlled specimens is caused by the presence of carbon dioxide (CO_2) dissolved

in the injected water. Additionally, because portlandite ($\text{Ca}(\text{OH})_2$) dissolves in water, it is mixed with water whenever it comes into touch with it, reducing the amount of calcium hydroxide that can be converted to CaCO_3 on the contact surface. However, because bio-concrete contains bacteria and calcium lactate, the process is different. Calcium lactate is directly converted by bacteria into calcium carbonate, which is insoluble in water. CO_2 is created as a byproduct of this metabolic process, and it then combines with calcium hydroxide on the spot and prevents it from washing off. Consequently, more calcium carbonate is produced [5].

SEM analysis of 28-day pre-cracked specimens is displayed in Fig. 4.7. Comparing Mix 2 to Mix 3, it is evident that Mix 3 exhibits greater calcium carbonate crystal formation in specimens that were pre-cracked at 28 days. Comparing Mix 2 to samples that were pre-cracked at 7 days of curing, the amount of CaCO_3 appears to be significantly lower. Concrete samples that are cured over 28 days acquire a better microstructure and become denser. As a result, pressure is applied to self-healing bacteria and pore diameters are reduced. Bacteria are eliminated as a result, leaving fewer bacteria in specimens to produce calcium carbonate.

In 7-day pre-cracked samples, Mix 4 showed much higher crystal formation than Mix 3, as seen in Fig. 4.6. Nevertheless, as Fig. 4.7 illustrates, bentonite can no longer effectively cover bacteria, which results in a much lower development of calcium carbonate crystal in Mix 4 as compared to Mix 3's CaCO_3 crystal. These findings show that LWA gives bacteria the best cover in samples that were pre-cracked at 28 days of curing. According to [5], bentonite does not give microorganisms greater cover and is ineffective when applied under multi-axial loads. Therefore, the healing efficiency of Mix 4 samples declines as the hydration reaction completeness increases and the pore size falls.

However, because LWA offers resistance against the pressure created in samples as a result of microstructure development, it offers cover during the mixing phase and better protection for spores in the samples. The difference between CaCO_3 production with and without a carrier component is consistent with the patterns observed in the findings of the study conducted by [1]. The creation of calcium

carbonate with a comparable crystalline structure is confirmed by the SEM pictures, which also show crystals that are similar to those seen and reported by [4].

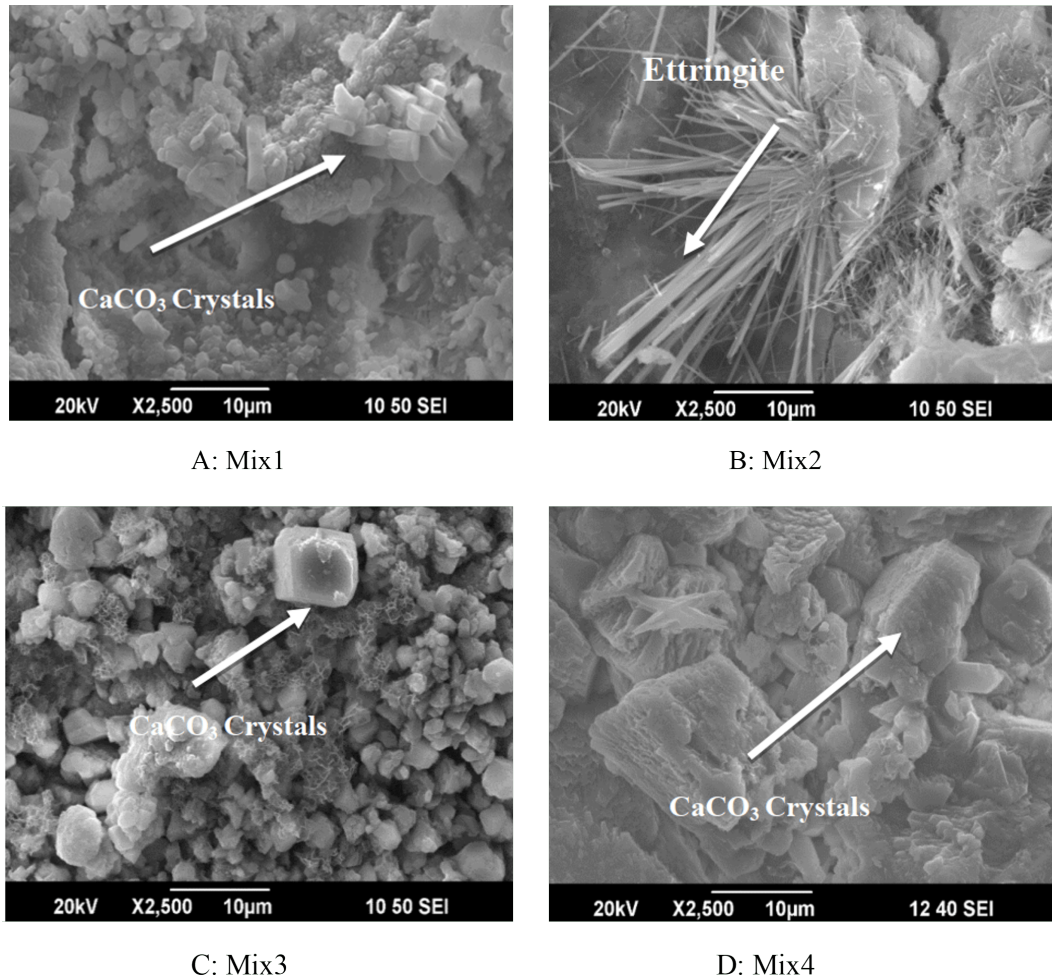


FIGURE 4.7: Analysis of 28-day pre-cracked samples using a scanning electron microscope

4.2.2 X-Ray Diffraction Analysis

The healing chemicals that formed in fractures were exposed to XRD analysis in order to better understand the self-healing mechanism and confirm the production of calcium carbonate in the samples. The sample was obtained by carefully scratching the healing product that had developed inside the cracks and then placing it in the XRD instrument. Because of its strong $K\alpha$ and $K\beta$ lines and high thermal conductivity, copper (Cu) was chosen as an X-ray target. It can also be kept cool easily. A wavelength of 1.54 \AA was used to capture the data, and Fig. 4.8

displays the many representative peaks that were found. The figure shows sharp needle-like peaks were found during the XRD process. This is because a mixture of compounds from the concrete surface was also present in the sample, which was scraped off the fracture surface.

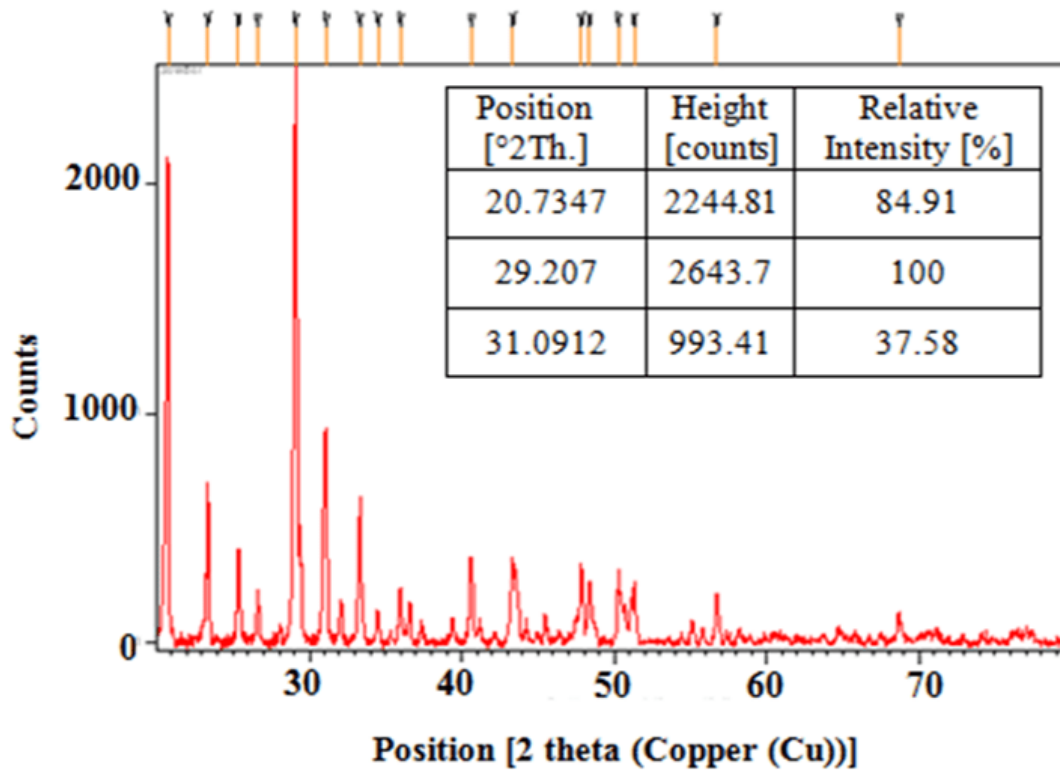


FIGURE 4.8: XRD examination of the healing chemical generated in the cracks

Since *Bacillus subtilis* is a bacterium that forms calcite [1], the XRD data were contrasted with the calcite reference cards. The 2θ (2θ) value of 29.2070 yielded the highest peak, which is quite near to the 2θ of 29.455 of pure calcite that [4] observed. The powder's impurities from the scratching off process may be the cause of the small variation in the 2θ value. This demonstrates that the substance created in the crack is naturally calcium carbonate and is consistent with the findings of earlier research.

4.3 Compressive Strength Analysis

Fig. 4.9 shows the self-healing specimens' measured compressive strength. It is evident that every method of bacterial integration raises the mix's compressive

strength.

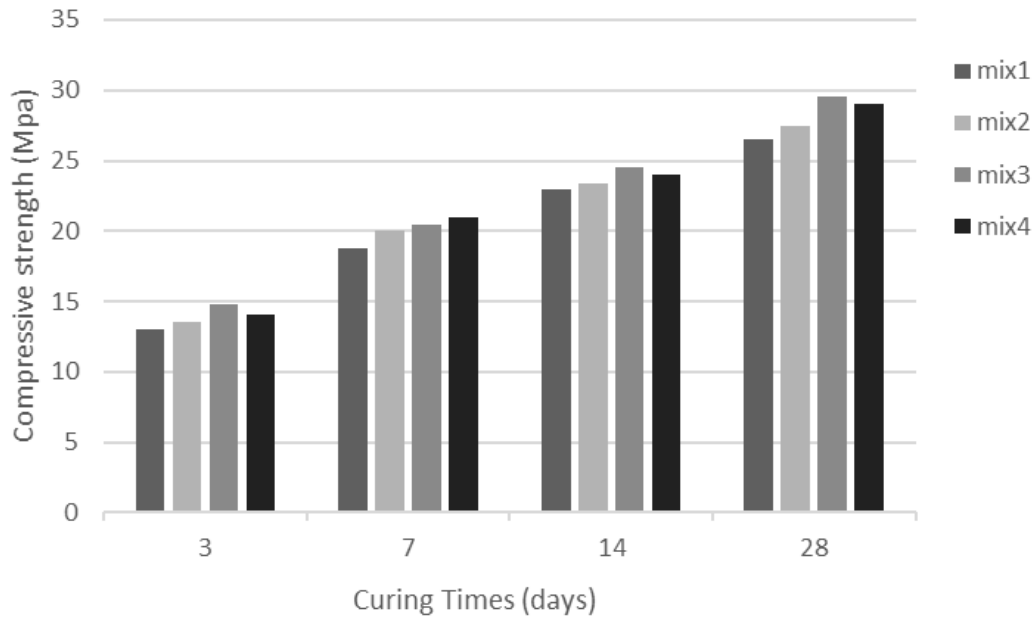


FIGURE 4.9: Development of compressive strength using various methods for incorporating bacteria

Comparing samples with LWA as a carrier chemical for bacterial inclusion to controlled concrete specimens, the former demonstrated a maximum strength of 29.43 MPa and a 12% increase in compressive strength. The results of the study on self-healing conducted [2] are consistent with the rise in compressive strength, confirming that self-healing is a contributing factor to the increase in compressive strength. Since LWA is smaller than regular-sized coarse aggregates, this increase in compressive strength can be explained. Because of the improved concrete matrix packing and compaction that resulted, these specimens were significantly stronger than controlled ones.

The compressive strength of specimens containing Bentonite increased by 9.8%. The addition of Bentonite is responsible for this increase in compressive strength. Bentonite functions similarly to a filler material because it is nanosized and suspends evenly throughout the mixture. By enabling the filling of porous and crystalline microstructure within the interfacial transition zone (ITZ), small Bentonite sizes help lessen the production of weak ITZ in concrete. Higher compressive strength results from a denser and more compact mortar matrix caused by a decrease in ITZ. According to [21], bentonite particles also serve as crack arrestors,

preventing and rerouting the creation and spread of cracks. Thus, Bentonite improves concrete's compressive strength in a variety of ways.

Concrete's compressive strength increased when microorganisms were directly incorporated.

The inclusion of microorganisms that produce calcite in the mixture is what caused this improvement. Because calcium lactate is supplied as an organic precursor and calcium carbonate is continuously produced by bacteria, the internal structure of concrete becomes more compacted, which leads to expansion of compressive strength. The direct introduction of bacteria produced this improvement, which is consistent with the outcomes obtained by [5]. But after careful It is clear from a comparison [1], results obtained via direct bacterial introduction that there is no difference in strength when *Bacillus Pasteurii* is introduced at a rate of 7.2×10^7 cell/cm³. This demonstrates that *B. Subtilis* is a superior option over *B. Pasteurii* in terms of compressive strength because its addition greatly raises the concrete's compressive strength.

4.4 Discussion

The study's experimental findings unequivocally show that adding bacteria-based self-healing mechanisms to concrete greatly improves its mechanical performance, longevity, microstructural integrity, and capacity to cure cracks. This study offers a thorough grasp of how self-healing efficacy changes over time and under various microstructural settings by combining several bacterial insertion strategies with varied curing ages and carrier materials. The results show that the age at which cracks are introduced, the process of bacterial integration, and the capacity of carrier materials to shield bacterial spores from the growing densification of the concrete matrix all have a significant impact on the efficacy of self-healing.

According to the crack-healing research, at all curing ages, all mixtures including bacteria showed significantly more healing than the control specimens. This demonstrates that the primary mechanism causing fissure closure in bio-concrete

is biologically induced calcium carbonate precipitation. When cracks were introduced at early ages (3 and 7 days), Mix 4 (bentonite as a carrier) obviously obtained the maximum healing effectiveness, according to ocular observations and crack-width measurements.

The nanoscale particle size of bentonite, which permits uniform dispersion throughout the cementitious matrix and enables effective transport of bacteria and nutrients to crack areas, is responsible for this excellent performance. Because bentonite can function as both a filler and a bacterial carrier, it can occupy interparticle gaps, which increases bacterial activation when water seeps through the cracks. Therefore, during the early phases of curing, fissures are efficiently sealed by the quick production of calcium carbonate crystals.

On the other hand, Mix 3 specimens including lightweight aggregate (LWA) demonstrated better healing performance than bentonite-based mixes when cracks were introduced at later ages (14 and 28 days). This behavioral change emphasizes how important microstructural evolution is in controlling the effectiveness of self-healing. The concrete matrix gets denser as hydration goes on, which reduces pore size and puts buried bacterial carriers under compressive stresses. Although bentonite is quite efficient in the early stages, it becomes less effective in preserving bacterial spores in a mature microstructure and shows low resistance under multiaxial loading conditions. As a result, Mix 4's bacterial viability declines at later curing ages, which lowers the amount of calcium carbonate produced and the effectiveness of healing. On the other hand, because of its bigger size and internal porosity, LWA offers superior mechanical shielding, allowing bacteria to survive under higher microstructural pressure. These results show that the choice of carrier material needs to be in line with the anticipated stage of fracture formation in actual structures.

The progressive completion of cement hydration is consistent with the observed decrease in healing efficiency at later curing ages across all mixes. The ability to maintain self-healing through hydration decreases as hydration products occupy accessible pore spaces. Furthermore, bacterial survival and metabolic activity are physically restricted by the matrix's densification. Because bacterial spores are

subjected to mechanical pressure and chemical stress in the absence of a protective carrier, this effect is most noticeable in cases where bacteria were directly added to the mix (Mix 2). When compared to carrier-based methods, direct bacterial integration is less successful for long-term self-healing, as demonstrated by Mix 2's dramatic healing reduction at older ages.

The crack-healing data are highly supported by the SEM microstructural investigation. Microbial-induced mineral precipitation is the main healing mechanism, as demonstrated by the existence of distinct calcium carbonate crystals in bacteria-incorporated specimens. In line with their superior fracture closure, Mix 4 specimens showed the highest density of calcite crystals at early ages. The precipitated calcium carbonate's form and crystalline structure closely match those described in earlier research, demonstrating the biological healing process' dependability. The carbonation of calcium hydroxide was the main cause of the limited and sluggish calcium carbonate production seen in the control specimens. The amount of calcium carbonate that can be formed is limited by the solubility of portlandite and the availability of dissolved carbon dioxide.

The long-term benefits of LWA as a bacterial carrier are further demonstrated by the SEM analysis of 28-day pre-cracked specimens. LWA-based specimens maintained a comparatively higher amount of crystal growth, but bentonite-based specimens demonstrated a noticeable decrease in calcium carbonate production at later ages. This suggests that LWA offers bacteria long-term protection as the microstructure develops, enabling ongoing healing activity even in the presence of decreased pore connectivity. These results highlight the fact that both bacterial activity and the mechanical compatibility of the carrier material with the changing concrete matrix control long-term self-healing capability.

The XRD study unequivocally shows that calcium carbonate, mostly in the calcite phase, is the main healing component generated within cracks. The suggested biochemical healing mechanism is supported by the close agreement between the recorded diffraction peaks and standard calcite reference values, which validates the SEM data. Impurities introduced during sample extraction, which is anticipated when healing products are mechanically scraped off crack surfaces, can be

the cause of the modest differences in peak positions. The conclusion that calcium carbonate synthesis takes place in situ inside the cementitious matrix is further supported by the lack of distinct needle-like peaks, which further suggests that the healing products are a combination of chemicals produced from concrete.

When compared to control concrete, the compressive strength data show that all bacterial insertion techniques result in a discernible strength increase. LWA-based specimens showed the largest gain in compressive strength, followed by bentonite-based specimens and direct bacterial inclusion. Several synergistic mechanisms, including as better particle packing, refinement of the interfacial transition zone, and pore filling by calcium carbonate, are responsible for this strength gain. While prolonged bacterial activity further improves microstructural densification, the smaller aggregate particles in LWA-based mixes help to improve matrix compaction. The filler action of nanoscale particles improves strength in bentonite-based mixes by reducing porosity and acting as crack arrestors. The findings also demonstrate that *Bacillus subtilis* outperforms bacterial species described in previous research in terms of improving compressive strength.

Overall, the results of this study unequivocally show that bacteria-based self-healing concrete is a practical and successful method for improving mechanical performance, durability, and crack resistance. The findings emphasize how crucial it is to choose the right bacterial carriers based on the concrete microstructure's age and the anticipated period of crack formation. Long-term durability and healing are better assisted by mechanically robust carriers like LWA, while early-age cracking is best treated with fine carrier materials like bentonite. These discoveries support the development of intelligent and sustainable building materials and offer a solid basis for the real-world application of bio-concrete in infrastructure.

Chapter 5

Conclusion and Recommendation

5.1 Background

This study compares the effectiveness of various self-healing methods in terms of their ability to repair cracks. The effectiveness of these methods was examined in relation to their impact on microstructure, calcium carbonate formation, compressive strength, and crack healing.

To see how different procedures affected the microstructure, SEM examination was done. To ascertain its chemical makeup, the healing component found in the specimens was also exposed to XRD. The results of the experiments were utilized to establish the connections between the approaches for incorporating bacteria and the reported compressive strength and crack healing.

5.2 Conclusion

The following conclusions are made in light of the findings of this study:

- i. The highest crack healing effectiveness was achieved by specimens that included Bentonite as a carrier compound. These specimens showed uniform distribution and protection of bacteria at samples that had already been

cracked at an early age of 3 and 7 days. Nevertheless, these specimens showed a marked reduction in fracture healing when pre-cracked subsequently.

- ii. In early age pre-cracked specimens, specimens using lightweight aggregate (LWA) as a carrier compound were less effective than Bentonite nevertheless, in later days, they demonstrated consistency in their crack healing effectiveness.
- iii. Specimens mixed directly with bacteria did not demonstrate any benefits in crack healing of concrete.
- iv. Regardless of the incorporation method, the addition of bacteria "Bacillus Subtilis" led to a small increase in compressive strength, with the LWA technique showing the greatest improvement, according to the trends of compressive strength for all mixes.

5.3 Recommendation

The complicated nature of biological and physicochemical interactions within the cementitious matrix calls for more research, even though this study has greatly improved our understanding of self-healing mechanisms in bacteria-based concrete and made clear the role of recently developed carrier materials in increasing crack-healing efficiency. Numerous interrelated factors, including as bacterial kind, concentration, nutrition availability, carrier characteristics, curing conditions, and environmental exposure, affect the self-healing process in bio-concrete. Although the current study has shown encouraging durability improvement and crack-sealing capability, the precise order of biological activities and how they interact with cement hydration products are still unclear. Microbial metabolism, ion transport, and microstructural limitations control the multi-stage precipitation of calcium carbonate inside fissures and pores. To develop a thorough theoretical model of bio-mediated self-healing, these mechanisms must be more thoroughly characterized using sophisticated analytical techniques. In order to better anticipate healing effectiveness under various service conditions, future research should concentrate

on identifying and measuring the distinct mechanisms involved in bacterial survival, activation, and mineral precipitation within concrete.

The tuning of bacterial content inside the concrete matrix to enhance self-healing efficiency without sacrificing mechanical performance is one of the most important areas for future research. While overly high concentrations may negatively impact workability, early-age strength, or long-term durability, insufficient bacterial dosage may lead to restricted calcium carbonate production and poor fracture sealing.

To determine the ideal bacterial concentration that simultaneously increases compressive strength and decreases permeability and facilitates effective biologically induced calcium carbonate precipitation, systematic experimental programs should be carried out. Because nutrient imbalance or deficit can severely restrict bacterial activity, these studies should take into account the relationship between bacterial concentration and nutrient supply.

Additionally, as localized clustering may result in uneven healing performance, the impact of bacterial distribution and homogeneity within the concrete matrix should be investigated. To provide trustworthy design guidelines for bacterial dose in real-world applications, numerical modeling and statistical optimization techniques can be used in addition to laboratory-scale studies.

Gaining a better knowledge of the bacterial activation process and creating efficient control methods to manage self-healing behavior throughout the service life of concrete buildings constitute another crucial research avenue. Until environmental factors like moisture intrusion, oxygen availability, or crack formation activate them, bacteria in bio-concrete systems usually remain dormant. The exact circumstances needed for reliable and prompt activation are still unknown, though.

Future studies should focus on figuring out the crucial thresholds for bacterial activation as well as how outside variables like temperature swings, frequent wet-dry cycles, and chemical exposure affect microbial activity. The dependability and predictability of self-healing performance could be greatly increased by creating

regulated activation mechanisms, such as time-delayed nutrient release or encapsulation technologies. Furthermore, long-term experimental research is necessary to assess the longevity and resilience of bacteria-based self-healing concrete in practical service circumstances. These studies should evaluate the long-term survivability of bacteria, the stability of precipitated calcium carbonate, and the ability to heal after several cracking incidents. Adoption of bio-concrete in large-scale infrastructure projects, where longevity, dependability, and lifecycle performance are critical, depends on an understanding of its long-term behaviour.

Bibliography

- [1] N. De Belie and W. De Muynck, “Crack repair in concrete using biodeposition,” in *Proceedings of the International conference on concrete repair, rehabilitation and retrofitting (ICRRR)*, Cape Town, South Africa, 2008, pp. 291–292.
- [2] M. De Rooij and E. Schlangen, “Self-healing phenomena in cement-based materials,” Tech. Rep., 2011, draft of state-of-the-art report of RILEM technical committee.
- [3] J. J. De Yoreo and P. G. Vekilov, “Principles of crystal nucleation and growth,” *Reviews in Mineralogy and Geochemistry*, vol. 54, no. 1, pp. 57–93, 2003.
- [4] Federal Highway Administration, “Preventive strategies in the united states, report by cc technologies laboratories, inc. to federal highway administration (fhwa), office of infrastructure research and development,” Federal Highway Administration, Tech. Rep. FHWA-RD-01-156, 2001.
- [5] J. Gadea, A. Rodríguez, P. Campos, J. Garabito, and V. Calderón, “Lightweight mortar made with recycled polyurethane foam,” *Cement and Concrete Composites*, vol. 32, no. 9, pp. 672–677, 2010.
- [6] P. Ghosh, S. Mandal, B. Chattopadhyay, and S. Pal, “Use of microorganism to improve the strength of cement mortar,” *Cement and Concrete Research*, vol. 35, no. 10, pp. 1980–1983, 2005.

-
- [7] S. G. Gupta, C. Rathi, and S. Kapur, “Biologically induced self healing concrete: A futuristic solution for crack repair,” *International Journal of Applied Sciences and Biotechnology*, vol. 1, no. 3, pp. 85–89, 2013.
- [8] F. Hammes, N. Boon, J. de Villiers, W. Verstraete, and S. D. Siciliano, “Strain-specific ureolytic microbial calcium carbonate precipitation,” *Applied and Environmental Microbiology*, vol. 69, no. 8, pp. 4901–4909, 2003.
- [9] E. A. Herrington, “X-ray diffraction measurements on some of the pure compounds concerned in the study of the portland cement,” *American Journal of Science*, vol. 13, no. 5, pp. 467–479, 1927.
- [10] P. Hewlett, *Lea’s chemistry of cement and concrete*. Butterworth-Heinemann, 2003.
- [11] D. Homma, H. Mihashi, and T. Nishiwaki, “Self-healing capability of fibre reinforced cementitious composites,” *Journal of Advanced Concrete Technology*, vol. 7, no. 2, pp. 217–228, 2009.
- [12] H. M. Jonkers and E. Schlangen, “A two component bacteria based self healing concrete,” in *Concrete Repair, Rehabilitation and Retrofitting*, vol. 1, 2009, pp. 119–120.
- [13] H. M. Jonkers, A. Thijssen, G. Muyzer, O. Copuroglu, and E. Schlangen, “Application of bacteria as self-healing agent for the development of sustainable concrete,” *Ecological Engineering*, vol. 36, no. 2, pp. 230–235, 2010.
- [14] P. K. Mehta and P. J. Monteiro, *Concrete: microstructure, properties, and materials*. New York: McGraw-Hill, 2006.
- [15] S. K. Ramachandran, V. Ramakrishnan, and S. S. Bang, “Remediation of concrete using microorganisms,” *ACI Materials Journal*, vol. 98, no. 1, pp. 3–9, 2001.
- [16] M. S. Rao, V. S. Reddy, M. Hafsa, P. Veena, and P. Anusha, “Bioengineered concrete—a sustainable self-healing construction material,” *Research Journal of Engineering Sciences*, vol. ISSN 2278–9472, 2013.

-
- [17] H.-W. Reinhardt and M. Jooss, “Permeability and self-healing of cracked concrete as a function of temperature and crack width,” *Cement and Concrete Research*, vol. 33, no. 7, pp. 981–985, 2003.
- [18] H. Schlangen, H. Jonkers, S. Qian, and A. Garcia, “Recent advances on self healing of concrete,” in *FraMCoS-7: Proceedings of the 7th International Conference on Fracture Mechanics of Concrete and Concrete Structures*, Jeju Island, Korea, May 2010.
- [19] H. G. Schlegel, *General microbiology*. Cambridge University Press, 1993.
- [20] H. Sixuan, “Multifunctional graphite nanoplatelets (gnp) reinforced cementitious composites,” Master in Civil Engineering, National University of Singapore, 2012.
- [21] N. Ter Heide, “Crack healing in hydrating concrete,” Masters in Civil Engineering, Delft University of Technology, 2005.
- [22] K. Van Tittelboom, N. De Belie, W. De Muynck, and W. Verstraete, “Use of bacteria to repair cracks in concrete,” *Cement and Concrete Research*, vol. 40, no. 1, pp. 157–166, 2010.
- [23] M. S. Vekariya and J. Pitroda, “Bacterial concrete: New era for construction industry,” *International Journal of Engineering Trends and Technology*, vol. 4, pp. 4128–4137, 2013.
- [24] J. Wang, H. Soens, W. Verstraete, and N. De Belie, “Self-healing concrete by use of microencapsulated bacterial spores,” *Cement and Concrete Research*, vol. 56, pp. 139–152, 2014.
- [25] J. Wang, K. Van Tittelboom, N. De Belie, and W. Verstraete, “Use of silica gel or polyurethane immobilized bacteria for self-healing concrete,” *Construction and Building Materials*, vol. 26, no. 1, pp. 532–540, 2012.
- [26] V. Wiktor and H. M. Jonkers, “Quantification of crack-healing in novel bacteria based self-healing concrete,” *Cement and Concrete Composites*, vol. 33, no. 7, pp. 763–770, 2011.

- [27] E. Mekonnen, S. Hailu, W. K. Hareru, and K. Tegegn, “Engineering experimental study on the mechanical and durability properties of concrete by partial replacement of cement with microbially induced calcite precipitation (micp) bio-cement,” *Discover Applied Sciences*, vol. 7, no. 10, p. 1064, 2025.
- [28] L. K. Youssa Tchamou and C. Xue, “Effect of MICP on reinforcement corrosion of cement concrete exposed to the marine environment,” *Discover Civil Engineering*, vol. 2, no. 1, pp. 1–16, 2025.
- [29] H. F. Basri, D. Saravanan, F. I. Yusri, A. I. Omoregie, A. Rajasekar, S. M. Jamil, and N. Abd Aziz, “Crack healing and structural recovery of concrete using *Cytobacillus horneckiae*-induced carbonate precipitation,” *Malaysian Journal of Fundamental and Applied Sciences*, vol. 21, no. 6, pp. 2889–2904, 2025.
- [30] H. F. Basri, D. Saravanan, S. M. Sani, A. I. Omoregie, A. Rajasekar, S. M. Jamil, and T. Ouahbi, “Sustainable concrete restoration using microbial-induced carbonate precipitation technology: Insights from laboratory and field applications,” *Malaysian Journal of Fundamental and Applied Sciences*, vol. 21, no. 6, pp. 3019–3038, 2025.
- [31] G. M. Castro, G. El Mountassir, and R. Lunn, “Development of a treatment strategy applicable to field conditions for the repair of vertical fractures in concrete using MICP,” in *Proceedings of the 2025 International Conference on Bio-mediated and Bio-inspired Geotechnics (ICBBG)*. Center for Bio-mediated and Bio-inspired Geotechnics, May 2025, pp. ICBBG2025–57.
- [32] M. Sierra-Beltran and H. Jonkers, “Bio-based mortar for concrete repair,” in *Proceedings of 14th International Conference on Structural Faults and Repair*, 2012, pp. 1–8.
- [33] M. G. Sohail, “Bio self-healing concrete using micp by an indigenous *Bacillus cereus* strain isolated from qatari soil,” *Construction and Building Materials*, vol. 328, p. 126943, 2022.

-
- [34] N. Shaheen, “Feasibility assessment of newly isolated calcifying bacterial strains in self-healing concrete,” *Construction and Building Materials*, vol. 362, p. 129662, 2023.
- [35] A. Almajed *et al.*, “State-of-the-art review of the applicability and challenges of microbial-induced calcite precipitation (micp),” *Crystals*, vol. 11, p. 370, 2021.
- [36] R. M. R. Pannem *et al.*, “The effect of fly ash aggregates on the self-healing capacity of bacterial concrete,” *Ain Shams Engineering Journal*, 2023.
- [37] C.-S. Tang *et al.*, “Factors affecting the performance of microbial-induced carbonate precipitation (micp) treated soil: a review,” *Environmental Earth Sciences*, vol. 79, p. 94, 2020.
- [38] E. Kalkan, “A review on the microbial induced carbonate precipitation (micp) for soil stabilization,” *International Journal of Earth Sciences Knowledge and Applications*, vol. 2, no. 1, pp. 38–47, 2020.
- [39] H. Doostkami *et al.*, “Self-healing capability of conventional, high-performance, and ultra high-performance concrete with commercial bacteria characterized by means of water and chloride penetration,” *Construction and Building Materials*, vol. 401, p. 132903, 2023.
- [40] U. N. Wilson, A. S. Abdullahi, M. A. Adisa, and S. O. Odeyemi, “Microbial induced calcite precipitation on macrostructural properties of concrete: A review,” *Journal of Infrastructure Preservation and Resilience*, vol. 6, no. 1, p. 42, 2025.