5-5-2023

OPTIMIZATION OF THE SELF-HEALING EFFICIENCY OF BACTERIAL CONCRETE USING IMPREGNATION OF THREE DIFFERENT PRECURSORS INTO LIGHTWEIGHT AGGREGATE

Omar Omar
Louisiana State University and Agricultural and Mechanical College

OPTIMIZATION OF THE SELF-HEALING EFFICIENCY OF BACTERIAL CONCRETE USING IMPREGNATION OF THREE DIFFERENT PRECURSORS INTO LIGHTWEIGHT AGGREGATE

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The Department of Engineering Science

by
Omar K. Omar
B.S., Cairo University, 2014
August 2023

ACKNOWLEDGMENTS

The author would like to express his love and appreciation to his parents, Nagat, whose exceptional givingness, persistence, and reliability were inspiring and motivational for the author throughout his life. The departed father, Kamal, who was supportive and admired in his surroundings, inspired many people, including the author. Along with his siblings for their inspiration and encouragement during this time. The author would like to express his deep gratitude to his advisor Dr. Marwa Hassan for the opportunity to join her proficient research team and for her guidance and supervision in conducting the research. Moreover, the author is thankful to all the research team, especially Dr. Momen Mousa, Dr. Gabriel Arce, and Dr. Hassan Noorvand, for sharing their extensive knowledge and experience throughout the research. Additionally, the author would like to express his gratitude to the members of his supervisory committee, Dr. Ayman Okeil and Dr. Ali Kazemian, for providing him with their valuable time and insight.

The author would also like to thank Norris Rosser and Austin Gueho, members of the Louisiana Transportation Research Center (LTRC) concrete laboratory; they were very accommodating and supportive. Furthermore, the author would like to thank Tran-SET for the financial support which made this research possible.

The author would also like to thank all his friends in baton rouge for making this journey easier in different aspects, Especially Ricardo Hungria, Sujata Subedi, Andrea Gavilanes, and Ruwa Abufarsakh, for their help throughout this research.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

NOMENCLATURE, SYMBOLS, AND ACRONYMS

ANOVA	Analysis of Variance
CFU	Colony Forming Unit
C-H	Calcium Hydroxide
C-S-H	Calcium Silicate Hydrate
EDS	Energy Dispersive Spectroscopy
FTIR	Fourier Transform Infrared Spectroscopy
GHG	Greenhouse Gas
HRWR	High-range Water Reducer Admixture
HSD	Tuckey's Honestly Significant Difference
LWA	Lightweight Aggregate
MICP	Microbial Induced Calcite Precipitation
OPC	Ordinary Portland Cement
pH	Potential of Hydrogen
PVA	Polyvinyl Alcohol
RH	Relative Humidity
SE	Secondary Electron
SEM	Scanning Electron Microscopy
STD	Standard Deviation
SMCCS	Simulative Marine Concrete Crack Solution
XRD	X-ray Diffraction Analysis

ABSTRACT

Concrete is the most broadly used construction material; thus, developing sustainable concrete is essential to decrease greenhouse gas (GHG) emissions from concrete production. Implementation of self-healing concrete technologies is a promising approach to enhance the durability and sustainability of the transportation infrastructure. Among these technologies, bacterial concrete has the potential to seal microcracks through microbial induced calcite precipitation (MICP). Bacterial protection is essential to ensure the viability of this technology due to concrete's harsh environment. Additionally, the success of this technology depends on the presence of an adequate mineral precursor compound and nutrient for the bacteria. As such, the main objective of this study was to optimize the healing efficiency of bacterial concrete in subtropical climates through the vacuum impregnation of bacteria into a lightweight aggregate (LWA). To achieve this objective, mortar samples were prepared while incorporating different combinations of precursors (magnesium acetate, calcium lactate, and sodium lactate) and alkali-resistant healing agent Bacillus pseudofirmus bacteria (with and without). In addition, a control sample was prepared without bacteria or precursors for comparative purposes. For each sample, three mortar cubes and three mortar beams were cast and used to evaluate the compressive strength, crack healing efficiency, and flexural strength recovery. The morphology of healing products was also observed in bacteria-containing samples under scanning electron microscopy with energy x-ray dispersive spectroscopy (SEM/EDS). Results showed that self-healing bacterial concrete could be optimized (without significant reduction in mechanical properties) if Bacillus pseudofirmus bacteria at a concentration of 10^8 cells/ml and sodium lactate precursor at a concentration of 75 mM/l are impregnated into lightweight aggregate.

INTRODUCTION

Concrete is the most broadly used construction material due to its remarkable mechanical properties and cost-effectiveness. But it is susceptible to high tensile stresses, which causes cracking. The cracks jeopardize the concrete by increasing the permeability and ingressing harmful substances, which deteriorate the durability of concrete. On the other hand, developing sustainable concrete is essential to decrease greenhouse gas (GHG) emissions from concrete production as concrete emits 8 to 9% of anthropogenic greenhouse gas emissions globally (Brinkman & Miller, 2021). Cement, as one of the main components of concrete, contributes 77% of emissions associated with the concrete industry (Busch et al., 2022). Thus, implementing self-healing concrete technologies is a promising approach to enhance the durability of the transportation infrastructure and reduce the usage of concrete for rebuilding. Among these technologies, as an autonomic self-healing technique, bacterial concrete showed promising results in sealing microcracks through microbial induced calcite precipitation (MICP) (Erşan et al., 2016; Hungria et al., 2021; Omar et al., 2022; Soysal et al., 2020). The MICP requires the presence of bacterial spores, organic precursor, efficient nutrient, and a source of calcium ions (Soysal et al., 2020). The MICP occurs when calcium carbonate precipitation is produced due to the metabolic conversion of organic salts through carbon dioxide production as a by-product of bacterial respiration. The carbon dioxide reacts with water already exists in the concrete environment to form carbonate ions (CO_3). The carbonate ions react with the calcium ions in the cementitious matrix to produce calcium carbonate precipitations.

Several pathways in the literature to acquire the MICP are urea hydrolysis, nitrogen reduction, and conversion of organic salts (Soysal et al., 2020). The urea hydrolysis technique relies on urea, water, nutrient, and a calcium source to precipitate calcium carbonate. The mechanism of this precipitation is activated when urea decomposes into positive ammonium ions and negative carbonate ions. The negative carbonate ions react with the positive calcium ions present in the cementitious matrix. The main disadvantage of this technique is the presence of ammonium in the cementitious matrix, which jeopardize the system the same as acid attacks (PCA, 2002; Wahyudin et al., 2013).

In the nitrogen reduction technique, the reduction of nitrates occurs when nitrate is used for bacteria respiration instead of oxygen (Hassan et al., 2019). Carbon dioxide is produced during the denitrification process in the presence of organic salts. Thereafter, the carbon dioxide combines with water to form carbonate ions. Eventually, these carbonate ions react with the calcium in the cementitious matrix to form calcium carbonate precipitation. This technique effectively hinders reinforced concrete corrosion due to nitrite's presence (Erşan et al., 2016).

On the other hand, the conversion of organic salts is a very effective technique for producing carbon dioxide. It was adopted in several studies, where carbon dioxide produced by bacterial respiration dissolves in water and forms carbonate ions. The cementitious matrix is abundant with calcium ions, which react with the carbonate ions to form calcium carbonate. Simultaneously, carbon dioxide formed could react with the calcium hydroxide in concrete, forming calcite crystals precipitations (Hassan et al., 2019; Hungria et al., 2023; H. M. Jonkers et al., 2010; Omar et al., 2022; Soysal et al., 2020; Wiktor & Jonkers, 2011) This study adopted this technique due to its previous successful contribution, sustainability, and the avoidance of corrosive by-products (Fahimizadeh et al., 2020).

The main challenge of bacterial concrete is protecting the self-healing agent that various encapsulation methods could acquire. Among these encapsulation methods; are polymer microencapsulation (Hilloulin et al., 2015), hydrogel beads encapsulation (J. Y. Wang, Snoeck, et al., 2014), and vacuum impregnation of lightweight aggregates (Alghamri et al., 2016). These methods were valid in increasing self-healing efficiency and strength recovery (H. M. Jonkers et al., 2010; Wiktor & Jonkers, 2011). However, many assumptions only considered the functionality of bacteria in the early ages of concrete samples. Self-healing efficiency was reduced after that due to the crashing of bacterial spores, which reduced the production of minerals (Mors & Jonkers, 2017; Tziviloglou et al., 2017). Thus, vacuum impregnating the self-healing agent into lightweight aggregates was considered one of the most effective protection techniques, especially since aggregate occupies 60 to 75% of the total volume of concrete (Bhavya & Sanjeev, 2017).

The vacuum impregnation of a self-healing agent into porous aggregates improved self-healing efficiency and strength recovery (Alghamri et al., 2016; Wiktor & Jonkers, 2011). This method was valid for sealing cracks up to 0.46 mm (Sisomphon et al., 2011). Thus, vacuum impregnation of bacteria into lightweight aggregates was adopted in this study.

1.1. Problem Statement

Many researchers have evaluated the vacuum impregnation of the self-healing agent into porous aggregate in terms of mechanical properties and self-healing efficiency. However, a few studies compared the efficiency of different mineral precursors in concrete samples with 50% replacement in terms of self-healing efficiency. The present study will evaluate the effect of three mineral precursors, namely, magnesium acetate, calcium lactate, and sodium lactate, side by side with *Bacillus pseudofirmus* bacteria regarding self-healing efficiency and mechanical

properties. It’s worth noting that sodium lactate has never been used in previous relevant studies as a precursor. Furthermore, the findings' validation was investigated by scanning electron microscopy with energy x-ray dispersive spectroscopy (SEM/EDS) for the morphology of healing products.

1.2. Research Objectives

Aiming to resolve the previously stated problem statement, the following is the study's objective: (1) determine the best mineral precursor which can be associated with *Bacillus pseudofirmus* bacteria; (2) assess the effect of the precurors with and without bacteria on the self-healing efficiency and mechanical properties of the mortar samples; (3) comparing the self-healing efficiency and strength recovery of the samples after subjecting the samples to a self-healing regime of wet/dry cycles to allow the self-healing reactions to take place; (4) characterize the morphology of the self-healing products after the self-healing regime, especially for the bacteria containing samples using (SEM) images; (5) Further validation of the results by doing EDS point analysis and mapping to quantify the self-healing products.

1.3. Research Approach

To achieve the previously stated objectives, the study was performed in three phases consisting of the following tasks:

<u>Phase 1: Vacuum impregnation of the self-healing agent into fine lightweight aggregates</u>

Task 1: Preparing the self-healing agents

The self-healing agent consisted of *Bacillus pseudofirmus* bacteria, yeast extract as a nutrient, and three mineral precursors: magnesium acetate, calcium lactate, and sodium lactate. *Bacillus pseudofirmus* bacteria were suspended at 10^8 cells/ml in a solution of 0.48 g/l (1.74 mM/l) yeast extract and the mineral precursor

(magnesium acetate, sodium lactate, or calcium lactate) at a concentration of 75mM/l.

Task 2: Vacuum impregnation set-up

The three combinations of self-healing agents were impregnated into expanded lightweight clay aggregate using a vacuum chamber. At first, the lightweight aggregate was oven-dried at a temperature of 100°C for 24 hours. The aggregate was placed in the vacuum chamber at constant pressure inside the chamber of 21 in. of mercury (71,114 Pascal). The solution was then added to the chamber for impregnation.

Task 3: Preparation of mortar specimens

The seven sets included three mortar samples prepared using three precursor types with bacteria, three mortar samples with the three precursors but without bacteria, and one sample without bacteria or any precursor to serve as a control sample. Three replicates of mortar cubes and three replicates of mortar beams were cast per set to evaluate the compressive and flexural strength, respectively.

Phase 2: Evaluation of the mechanical properties of the specimens

Task 4: Conducting compressive strength tests on mortar cubes

The compressive strength was assessed after 28 days of curing. The effect on compressive strength of the presursors with and without the bacteria was evaluated; against the control sample.

Task 5: Conducting flexural strength tests on mortar beams

After 28 days of curing, the mortar beams were subjected to a flexural strength test to evaluate the effect of the precursors and bacteria on the flexural strength, along with inducing microcracks.

Phase 3: Wet/dry cycles and crack width measurements

Task 6: Conducting wet/dry cycles on the cracked beams

A self-healing regime of wet/dry cycles was conducted to facilitate the self-healing reaction in the cracked beams. The wet/dry cycles simulated the subtropical climate conditions. These cycles consisted of 16 hours of immersion in water and 8 hours of dry conditions in a 100°F room.

Task 7: Using a Light microscope for crack monitoring and crack measurements

During the wet/dry cycles, using a light microscope, the cracked beams were monitored at zero-day (before the self-healing regime), 3 days, 7 days, 14 days, and 28 days. Afterward, the images were used for crack measurements, using Image J software to compare the self-healing efficiency of the samples.

Task 8: Flexural strength recovery tests

By the end of the wet/dry cycles self-healing regime, the specimens were subjected to three point-bending tests to compare the strength recovery of different samples.

Phase 4: Self-Healing products characterization

Task 9: SEM analysis for the morphology of self-healing products

The samples were cut with a diamond blade saw to get the mid-span part containing the self-healing products. Small sections were extracted and placed in an oven for 24 hours at 38°C to avoid moisture. Afterward, the specimens were

sputter-coated with a platinum film to remove any charging and ensure good conductivity. Eventually, the morphology of the self-healing products for the bacteria-containing samples was investigated.

Task 10: EDS point analysis and EDS mapping

To further validate the results, EDS point analysis was conducted on one specimen from each mortar sample to quantify the self-healing products in the bacteria-containing samples against the control samples. As well as, EDS maps were obtained to assess the chemical composition of the healing products of the bacteria-containing samples.

2. LITERATURE REVIEW

Concrete is the most broadly used construction material, but the usage and production of concrete can have significant negative impacts on the environment. For instance, the greenhouse gas emissions caused by cement production include carbon dioxide, the release of chemical pollutants into waterways during manufacturing, and the landfill and environmental degradation generated due to discarding concrete and concrete processing waste. Thus, Self-healing concrete was introduced as an innovative material that has the potential to revolutionize the construction industry. The importance of self-healing concrete lies in its ability to repair cracks and fissures that can occur over time due to environmental factors such as temperature changes, humidity, and the loads applied to the structure.

2.1. Self-healing Concrete Mechanisms

The different self-healing mechanisms help improve concrete structures' durability and longevity, reducing the need for maintenance and repairs and extending their service life. There are two main self-healing mechanisms: a) Autogenous mechanism. b) Autonomous mechanism (Susanto et al., 2021). Autogenous healing is a passive process that does not require the addition of any special materials or substances to the concrete mix. This makes it a cost-effective and sustainable option for improving the durability and longevity of concrete structures. It is important to note that autogenous healing is a slow process and is most effective for small cracks. Larger cracks or significant damages may require additional interventions, such as adding microencapsulated healing agents or applying polymers (Hong et al., 2020). Alternatively, autonomic self-healing is based on embedding unconventional engineered additions in the matrix to provide a self-healing function (De Belie et al., 2018).

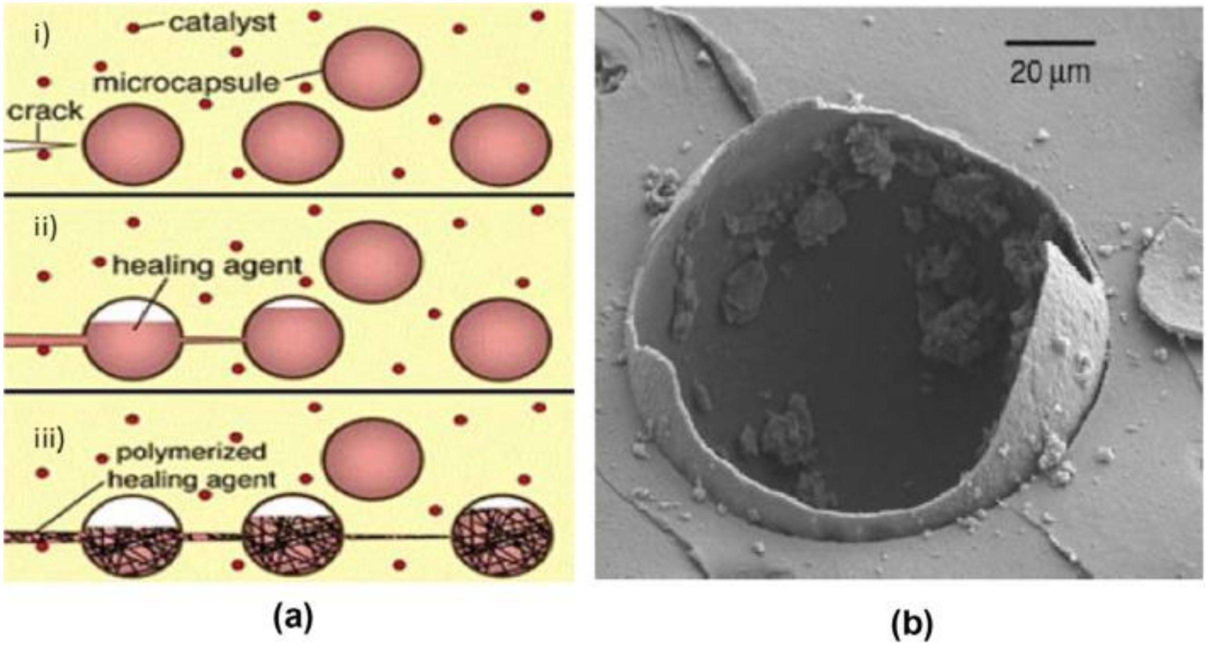


Figure 2.1. (a) Self-healing process by microcapsules approach: (i) Formation of cracks in the cementitious matrix; (ii) process of releasing healing agent; (iii) process of crack healing and (b) ESEM image displaying a ruptured microcapsule (Vijay et al., 2017).

2.1.1. Autogenous Mechanism

The central concept of autogenous self-healing in cementitious materials, is the presence of air and water. That allows the hydration of non-hydrated cement particles. These reactions result in self-healing products filling the cementitious matrix microcracks (Susanto et al., 2021). The autogenous healing of concrete was inspired by the damaged skin of living organisms and trees, which can be healed by themselves. Autogenous healing was noticed likely for the first time by the French Academy of Science in 1836 in the structures that can repair themselves after sustaining damage (Hearn, 1998). It was observed at first in the water-retaining structures and pipes. The difference between self-healing and self-sealing was demonstrated, as the former indicates that the strength was wholly recovered. In contrast, the latter reveals that cracks were closed without gaining any strength recovery (Wu et al., 2012).

There are various ways of sealing the microcracks and preventing further damage, such as the following:

A- Formation of self-healing products such as calcium carbonate and calcium hydroxide (Van Tittelboom & De Belie, 2013).

B- The hydration of unreacted cement (Van Tittelboom & De Belie, 2013).

C- Swelling of C-S-H near the crack flanks (Mihashi & Nishiwaki, 2012).

2.1.1.1. Formation of Calcium Carbonate

The formation of calcium carbonate results when the calcium hydroxide is liberated and dissipated in the area surrounding the cracks. Calcium ions from cement hydration react with carbon dioxide, forming self-healed crystals which grow in the surrounding area of the cracks, then filling these cracks. The chemical reactions to develop calcite occurred as in Equation 2-1 (Aliko-Benítez et al., 2015). Equation (1) shows the equilibrium between water, carbon dioxide, and carbonic acid. When carbon dioxide dissolves in water, it can react with water to form carbonic acid (H_2CO_3), which can then dissociate to form hydrogen ions (H^+) and bicarbonate ions (HCO_3^-). Bicarbonate ions can further dissociate to form more hydrogen ions and carbonate ions (CO_3^{2-}). This reaction is essential in regulating water's acidity (pH) and plays a role in ocean acidification. Equation (2) shows the equilibrium between calcium ions (Ca^{2+}) and carbonate ions (CO_3^{2-}) at high pH values ($pH>8$). In this case, the carbonate ions are in excess, so they react with the calcium ions to form calcium carbonate ($CaCO_3$), which is insoluble and can precipitate out of the solution. Equation (3) shows the equilibrium between calcium ions and bicarbonate ions at lower pH values ($7.5<pH<8$). In this case, the bicarbonate ions are in excess,

so they react with the calcium ions to form calcium carbonate and release a hydrogen ion (H^+), lowering the water's pH.

Equation 2-1

$$H_2O + CO_2 \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3{}^- \rightleftharpoons 2H^+ + CO_3{}^{--} \quad (1)$$

$$Ca^{++} + CO_3{}^{--} \rightleftharpoons CaCO_3\ (pH\ of\ water > 8) \quad (2)$$

$$Ca^{++} + HCO_3{}^- \rightleftharpoons CaCO_3 + H^+\ (7.5 < \ pH\ of\ water < 8) \quad (3)$$

2.1.1.2. Hydration of Unreacted Cement

The hydration of unreacted cement is a natural self-healing mechanism in concrete, and is considered a form of autogenous healing. When concrete is mixed, some of the cement particles may not undergo full hydration during the curing process. However, if cracks form in the concrete, moisture can penetrate into the unhydrated cement particles near the crack and cause them to continue to react and generate more calcium-silicate-hydrate (C-S-H) gel. This process is limited by the availability of unhydrated cement particles near the crack and by the amount of moisture that can penetrate into the crack (Li & Yang, 2007; Van Tittelboom & De Belie, 2013).

2.1.1.3. Swelling of C-S-H near the Crack Flanks

The swelling of C-S-H near the crack flanks occurs because C-S-H is a hygroscopic material, meaning it has a natural affinity to absorb moisture from the surrounding environment. When C-S-H absorbs moisture, it swells, and this swelling can cause the crack flanks to close up, reducing the width of the crack and potentially improving the material's ability to resist further cracking (Alderete et al., 2019; Mihashi & Nishiwaki, 2012).

Moreover, crack width is a crucial factor in determining whether autogenous healing will occur(Neville, 2002). Edvardsen (Edvardsen, 1999) stated that 25% to 50% of cracks with 200 μm width were completely healed after 49 days of water exposure concerning their water pressure.

2.1.2. Autonomous Mechanism

There are various applications of autonomous mechanisms: a) Vascular self-healing, which simulates the network system in the human body at which a network of tubes flows a healing agent to the cracks in the cementitious matrix (Gardner et al., 2014; Souradeep & Kua, 2016; Susanto et al., 2021). b) Modifying autogenous healing using cementitious materials, such as microorganisms, fibers, fly ash, and fine silica to seal the microcracks (Basheer et al., 2001; Juliafad et al., 2019; Susanto et al., 2021; W. Zhang et al., 2020). C) Encapsulating healing agents by using microcapsules containing bacteria and other self-healing agents, which start to react when the concrete cracks and the capsules break, produces self-healing products due to this reaction (Alghamri et al., 2016; Hungria et al., 2021; Soysal et al., 2020).

2.2. Bacterial Concrete

Bacteria based self-healing concrete, as one of the autonomous mechanism applications, is based on microbially induced calcite precipitation (MICP). MICP involves a metabolic conversion of organic salts through carbon dioxide production as a by-product of bacterial respiration. Carbon dioxide dissolves in water in the concrete environment and produces carbonate ions. On the other hand, the ionization process produces calcium ions, which readily react with carbonate ions to precipitate the calcium carbonate's self-healing product, which seals the microcracks (De Belie, 2016; H. M. Jonkers et al., 2010; Soysal et al., 2020). Among the different techniques of MICP is the metabolic conversion of organic salts through alkali-resistant bacteria respiration, which

produces carbon dioxide as a byproduct (Wiktor & Jonkers, 2016). Self-healing using bacteria can increase the service life of concrete structures, increase cost-effectiveness, and improve environmental sustainability due to reduced cement production by avoiding rebuilding concrete structures and reducing carbon dioxide emissions (Alemu et al., 2022a; Lee & Park, 2018). The bacteria-containing systems can resist acids and cause less water absorption (Alemu et al., 2022a; Italia et al., 2016), reduce the chloride permeability and the porosity of mortar samples (Italia et al., 2016), and increase electrical resistance (Tayebani & Mostofinejad, 2019). These contributions are due to the calcite precipitation of MICP (Chang et al., 2015). In contrast, there are some shortcomings of using bacterial concrete, such as its vital for microcracks only, reduction of compressive strength due to embraced capsules (Jin et al., 2018), the sensitivity of bacteria to the harsh environment such as high alkalinity, and high temperatures (Alemu et al., 2022b; Jin et al., 2018; Lee & Park, 2018).

Among the different microbial applications in the construction industry bacterial concrete was introduced to self-heal the cracks formed on the concrete (Bashir, 2016; Mote & Ghodke, 2018). There are four main methods by which bacteria are introduced in the healing agents' formation : (i) By introducing bacteria with the other healing agents in the concrete's water to produce calcite (Mohammad et al., 2019). (ii) By injecting bacteria into the concrete matrix through a vascular network system in which the vascular network breaks after inducing cracks, the bacteria start to react and grow in the crack's place (Seifan et al., 2016). (iii) The encapsulation of bacteria, such as hydrogel beads (Hungria et al., 2021; Soysal et al., 2020). (iv) The protection of bacteria from the harsh environment of concrete by incorporating expanded clay (Omar et al., 2021).

2.2.1. Methods of Protection of Self-healing Agent

2.2.1.1. Vacuum Impregnation of Lightweight Aggregate

Vacuum impregnation of bacteria into lightweight aggregate; is one of the promising protection techniques, especially that aggregate occupies 60% to 75% of the total volume of concrete. Tziviloglou et al. (Tziviloglou et al., 2016)impregnated healing agent containing bacteria into lightweight aggregate and added it to mortar mixtures. The healing agent consists of *Bacillus* alkaliphilic bacteria, calcium lactate as a mineral precursor, and yeast extract as a nutrient. Three different mixtures were compared in terms of mechanical properties and water tightness: the sample without lightweight aggregate (LWA), the sample with LWA but without bacteria, and the sample with LWA and bacteria. The lightweight aggregates break after inducing cracks in the mortar beams allowing the healing agent to react with oxygen and water to form calcium carbonate precipitates. The recovery of liquid tightness was evaluated by using water permeability tests. Water immersion and wet-dry cycles were the two different healing regimes compared in the study. The compressive strength of the mortar samples did not significantly differ between the samples with and without a healing agent. Similarly, the recovery of water tightness did not differ substantially when they were immersed in water. On the other hand, the recovery of water tightness increases significantly by adding the healing agent in case of wet/dry cycles.

Sisomphon et al. (Sisomphon et al., 2011)impregnated expanded clay with sodium monofluorophosphate as a self-healing agent encapsulated in the cement matrix in the blast furnace slag mortar. In this study, the absorption rate of LWA was up to 22% in one day after water immersion which remained almost constant in atmospheric air. In contrast, the absorption rate changed to 41% by weight after 30 min of water immersion under vacuum pressure. For

coating the self-healing system, two types of cement were used with a cement-to-LWA ratio of 0.3. The results from freezing and thawing tests showed that the samples containing the self-healing agent acquired better performance. The capillary water absorption was reduced as well compared to the control sample.

Wiktor and Jonkers (Wiktor & Jonkers, 2011) studied a self-healing agent consisting of a bio-chemical agent impregnated in expanded clay. The self-healing agent is formed of bacteria and calcium lactate. Two mortar specimens were prepared. After cracking the samples, the self-healing agent reacted with water and oxygen to produce calcite carbonate precipitations which sealed the microcracks. The cracked control and bacteria-based specimens were immersed in an open bucket filled with tap water. The bucket was opened to allow oxygen and carbon dioxide diffusion during self-healing. Photographic imaging monitored the cracks weekly to quantify the self-healing efficiency during the healing regime. Five cracks were observed for each specimen. Cracks up to 0.46 mm were closed for bacterial concrete specimens. Alternatively, the cracks for control specimens with 0.18 mm width were sealed after 100 days of the self-healing regime. Thus, it was concluded that using a bio-chemical self-healing agent could increase durability in wet environments.

Kim et al. (Kim et al., 2013) investigated the microbiological precipitation of calcium carbonate by using normal weight aggregate and lightweight aggregates. Two types of bacteria were used: *Sporosarcina pasteurii* and *Bacillus sphaericus*. The scanning electron microscope (SEM) images, energy dispersive spectroscopy (EDS) spectra, and X-ray diffraction (XRD) analysis were used to evaluate the chemical composition and the distribution of calcium carbonate crystals formed with and without bacteria. It’s worth noting that the concentration of bacteria increased exponentially through the first 20 hours, and the rate decreased after 2 days. For

normal-weight concrete, sand and crushed gravel were the used aggregates. While for the lightweight concrete, fine and coarse bottom ash from a boiler system of a coal firing was the utilized aggregate. After mixing the concrete samples, they were exposed to surface treatment using a liquid medium containing bacteria. The liquid medium used in this study contained: 10 g/L of ammonium chloride, 3 g/L of nutrient, 2.1 g/L of sodium bicarbonate, 10 g/L of urea, and 26 g/L of calcium acetate, in addition to the bacteria provided through the sterilized loop. The bacteria were allowed to grow in the appropriate sealed medium to avoid contamination, containing urea at 30°C for 7 days. Four different surface treatment methods were applied on the normal and lightweight concrete: water, medium with *S. pasteurri*, medium with *B. sphaericus*, and cell-free medium. The cylinder concrete with 100 X 200 mm specimens were split into two to prepare the cylinder specimens for surface treatment. A plastic film covered all the cylinders to avoid liquid pervasion except the top surfaces. The top surfaces were polished and exposed to the liquid medium. Afterward, the specimens were stored for 14 days in a curing room at 20°C. The liquid medium was removed, and the treated specimens were kept for 14 days under the same conditions. The results showed that using the medium with *S. pasteurii* will result in smaller and denser calcium carbonate crystals than the cell-free medium. On the other hand, normal and lightweight concrete specimens treated with *B. sphaericus* showed the lowest weight increases per unit area and the highest density of calcium carbonate among all the treatment methods.

Alghamri et al. (Alghamri et al., 2016) impregnated LWA with a self-healing agent, a sodium silicate solution. The diameter of the LWA varies from 4 mm to 8mm. The LWA was oven dried for 3 days and then kept in the vacuum chamber for 24 hours. The absorption rate was evaluated

in the case of immersion and impregnation processes. The aggregates were immersed in a sodium silicate solution for different durations (1,2, and 3 days).

On the other hand, specimens LWA were introduced to the vacuum chamber under pressure as low as -0.7 bar for one hour. Afterward, the self-healing agent solution was inserted in the chamber. The solution level was maintained at 2 cm above the aggregates to ensure full submersion. The results showed that the optimum absorption rate was 31% which occurred after using the vacuum chamber for one hour. Two LWA mixtures were prepared; namely, the control (CN) and the other one contained the impregnated aggregates (SHM). The digital microscope image analysis was used to monitor the cracks at different time intervals to quantify self-healing efficiency. The evaluation of crack depth was evaluated by using ultrasonic pulse velocity. The results after 28 days of the self-healing regime revealed the following: 1) The sample containing the impregnated aggregate (SHM) acquired 80% strength recovery, five times more than the control sample. 2) Some enhancement in the capillary water absorption occurred in the SHM sample compared to the control. 3) The sorptivity index was reduced by 50% in the sample with impregnated aggregate compared to the control sample.

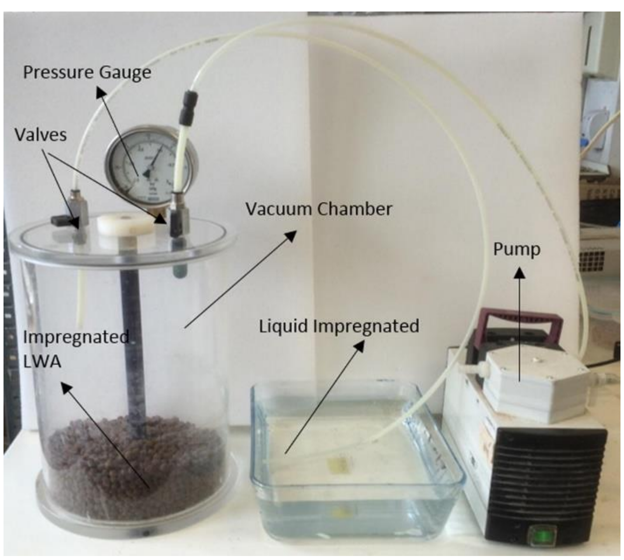


Figure 2.2. Vacuum impregnation of lightweight aggregate set-up (Alghamri et al., 2016).

2.2.1.2. Hydrogel Beads

Encapsulation of Hydrogels is one of the most convenient encapsulation methods of bacteria for general practical occasions. Water is an essential factor for bacterial survival. Thus, a continuous source of water supply is required for crack healing of bacterial concrete (J. Wang et al., 2015). The water supply in the case of the hydrogels includes a limited time of immersion in water compared to the other self-healing techniques. The water immersion for hydrogels was a 1hr wet cycle and 11 hrs dry cycle (J. Y. Wang, Snoeck, et al., 2014a), while the other methods included 16 hrs wet cycle and 8 hrs dry cycle (J. Y. Wang, Soens, et al., 2014).

Various studies applied the encapsulation of hydrogels. Fahimizadeh et al. (Fahimizadeh et al., 2020) investigated the survivability and retention of a self-healing agent, *Bacillus pseudofirmus*, grown into the nutrient broth. Besides, the evaluation of the pre-cracked mortar specimens' survivability of bacterial spores after the encapsulation process. For this investigation, *Bacillus pseudofirmus* was allowed to grow under 30°C and 9.7 pH. On the other hand, sodium alginate with 37 % mannuronic acid and 63 % guluronic was used, to prepare the calcium alginate capsules. The growth medium of *B. pseudofirmus* of 100 ml of nutrient broth contained 5 g/L peptones and 3 g/L meat extract. To adjust the pH value of this solution to 9.7, 1M NaOH was used then sterilization by autoclaving took place for 15 min at 121°C.

As aforementioned, calcium carbonate precipitation is the main self-healing product. To facilitate the formation of calcium carbonate, 90 mL of nutrient broth was supplemented with 10 mL of sterile 0.5 M calcium lactate to have a final concentration of 0.05 M for calcium lactate. Afterward, the bacteria spores suspension was achieved using a basal salt media at 100 mL with the *Bacillus pseudofirmus* at a 1% ratio kept under 30°C for 7 days. The encapsulation method

adopted was the ionic gelation of alginate after a reaction with calcium ions. The solution used for encapsulation consisted of sodium alginate and nutrient broth dissolved in distilled water; then, autoclaving took place at 121°C for 15 min. The alginate mixture was left to cool down overnight, then added using a syringe pump to 200 mL of a sterile 0.2 M calcium lactate. The calcium lactate was stirred using a magnetic stirrer. Calcium alginate capsules were allowed to stir for 20 min to ensure that cross-linking was acquired. Mortar beams were cast with dimensions 25 mm X 25 mm X 100 mm, and glass fiber was added to the initial layer to avoid complete cracking for the monitored samples. The capsules containing the self-healing agent were added to the middle section of the samples. The middle section had a paste of 5% calcium alginate capsules by the sample volume. The samples prepared were the following: capsules with no additives (O), capsules containing only spores (S), capsules containing only nutrients (N), capsules containing nutrients and spores (NS1), and capsules containing nutrient and increased spores (NS2). The results showed that most bacteria spores survived encapsulation and were kept in capsules under cement mixing conditions. The calcium alginate did not need to be damaged to start the precipitation of calcium carbonate because the precipitation started when exposed to water and oxygen in the presence of calcium ions. The samples containing calcium alginate showed self-healing for the micro-cracks after 56 days of wet-dry cycles. On the other hand, calcium alginate capsules reduced the flexural strength of mortar samples but increased the flexural strength recovery of cement mortars up to 39.6%.

Another study was conducted for the self-healing of marine concrete at a low temperature by Plain et al. (Palin et al., 2016). They encapsulated the calcium alginate beads with bacterial spores and mineral precursors. The solution containing the precursor consisted of 1.5% weight/volume of sodium alginate, 0.48 g/l of yeast extract, 6.4 g/l of magnesium acetate, and 7

X 10^8 bacteria spores per liter. The droplets of the solution were introduced to a gelling solution of 6.4 g/l calcium acetate. The gelling solution and precursor solution reaction result in the cross-polymerization producing calcium alginate beads. The beads were extracted from the calcium acetate solution after 30 min, washed with demineralized water, and left to dry. Another kind of beads were produced for the control sample without calcium alginate and bacteria. An oxygen consumption test was conducted using an oxygen meter to evaluate the oxygen concentration for 14 days. Aseptic conditions were maintained during the test because the sterilization using autoclaving produced alginate depolymerization. In another regard, the swelling was evaluated by determining the diameter of the bacteria-based beads during the 6 days immersion in the simulative marine concrete crack solution (SMCCS) at 8°C. The beads consisted of calcium alginate encapsulated bacterial spores, and mineral precursor compounds that decreased the oxygen concentration for this specific solution (SMCCS) at 8°C, facilitating the production of calcite alginate. The beads swelled to a maximum diameter of 3 mm. The estimates concluded that 30 beads with a 1 mm diameter could produce 1 mm^3 of calcium carbonate over 2 weeks, which is a main self-healing product. These attributes to the beads' potential as a self-healing concrete in marine environments under low temperatures.

Soysal et al. (Soysal et al., 2020) applied the hydrogel-encapsulated bacteria method using two bacteria strains at different dosages by weight of cement in concrete. *Bacillus Pseudiformus* and *Diaphorobacter Nitroreducens* bacteria strains at a concentration of 10^7 cells/ml were encapsulated in a solution consisting of sodium alginate, yeast extract, and magnesium acetate tetrahydrate. The healing agent was pumped from the nozzle of the BUCHI B-390 device to prepare the hydrogel beads. The droplets of the healing agent were introduced to a solution of 0.1M $CaCl_2$ gelling put through a 200 rpm agitation rate. This solution was stirred with a

magnetic stirrer and left to cure for 30 minutes. After preparing the hydrogel beads, the beads were added to the concrete samples by weight of cement at different portions. For each bacteria strain, three different alginate bead concentrations by weight of cement were prepared. Polymer fibers were added to the mixture design to avoid the sudden failure of samples by increasing the ductility of samples. After curing the concrete samples and evaluating their mechanical properties, a self-healing regime of wet-dry cycles consisted of 8 hrs dry cycle followed by 16 hrs of wet cycle every day conducted during the evaluation of self-healing efficiency for 28 days. The compressive strength, modulus of elasticity, and self-healing efficiency were evaluated. The main results showed that increasing the dosage of microcapsules reduced the compressive strength. There was no significant difference in the modulus of elasticity of the samples before the self-healing regime. In addition, self-healing efficiency was evaluated at different time intervals during the self-healing regime. Images were taken using a light microscope at the following time intervals: 0,3,7,14 and 28 days, during the wet-dry cycles. The sample with the highest concentration of hydrogel beads for the two bacteria strains (i.e., *Bacillus pseudiformus* and *Diaphorobacter Nitroreducens*) acquired the highest self-healing efficiency with 57%. On the other hand, the sample containing 3% of hydrogel beads by weight of cement without bacteria showed significantly high self-healing efficiency, which showed the potential of hydrogel beads to act as a water reservoir to increase the self-healing efficiency.

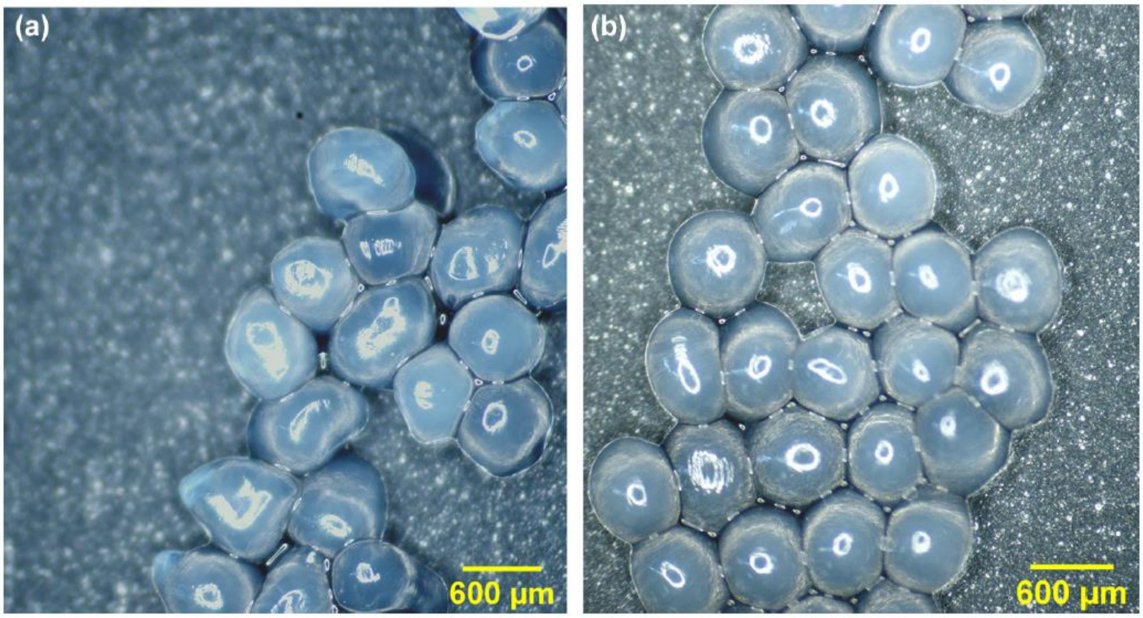


Figure 2.3. Calcium alginate beads encapsulating (a) *Bacillus pseudofirmus*, and (b) *Diaphorobacter nitroreducens* (Soysal et al., 2020).

2.2.2. Factors Affecting the Self-healing Process

2.2.2.1. Bacteria Selection

The concrete matrix is highly alkaline due to the production of calcium hydroxide, which is responsible for the hydration of Portland cement. The capillary water of fresh concrete has a pH value between 11 and 13. Thus, alkaliphilic bacteria should be added to the bacterial concrete, to withstand the high alkalinity in the concrete matrix (H. M. Jonkers et al., 2010). Based on previous studies (Andalib et al., 2016; Jonkers et al., 2010; Krishnapriya et al., 2015; J. Wang et al., 2012, 2014; J. Y. Wang, Snoeck, et al., 2014), *Bacillus* bacteria were effective in high alkalinity in the concrete matrix. Table 2.1(Souradeep & Kua, 2016) below shows the positive impacts of using *Bacillus* species, for instance, the effectiveness in sealing microcracks, enhancing the compressive strength at an early age (7 days), and formation of calcite crystals precipitations. Thus, one of the *Bacillus* types bacteria was adopted in this study.

Table 2.1. The Bacterial Species Performance in the Previous Studies of Self-Healing (Souradeep & Kua, 2016)

Species of microorganism	Mechanism	Major findings	References
Bacillus pasteurii and *Pseudomonas aeruginosa*	Urease metabolism	More effective as a crack sealant but less effective in increasing compressive strength. Higher efficiency in shallow cracks due to higher availability of oxygen.	(Ramachandran et al., 2001)
Bacillus pasteurii	Polyurethane (PU) immobilized, urease metabolism	Immobilization technique retains high cellular metabolism and protects the cells against the harsh environment. Higher bacteria cell concentration per crack helps to regain higher early (7 days) compressive strength.	(Bang et al., 2001)
Bacillus sphaericus	Ureolytic decomposition of calcium nitrate	Concrete permeability reduced. Crack widths between 150 and 170 μm completely healed.	(J. Y. Wang et al., 2012)
Bacillus pseudofirmus and *Bacillus cohnii*	Metabolism of calcium lactate	20–80 μm calcium crystals were seen on crack surfaces. Performance is limited to young concrete.	(Jonkers, 2011)

The survival rate of bacteria species is a crucial factor in the success of the MICP process. Sharma et al., (Sharma et al., 2017)evaluated three different alkaliphilic bacteria species regarding their survivability in the bacterial concrete application. Three bacteria strains of *Bacillus* bacteria were *Bacillus pseudofirmus*, *Bacillus halofurans*, and *Bacillus cohnii*. The bacteria strains with yeast extract as a nutrient and calcium lactate as a mineral precursor were incorporated into cement, which was added to the mortar cubes' matrix. The survival rate of bacteria was quantified for the three bacteria strains along with the calcite precipitation of the samples. The sample containing *Bacillus pseudofirmus* bacteria showed the highest survivability and calcium carbonate presence after conducting Fourier transform infrared (FTIR) spectroscopy

analysis. Similarly, Jonkers et al., (Jonkers et al., 2010) studied the survival rate of incorporated *Bacillus pseudofirmus* bacteria spores using the microbiological dilution-to-extinction method. The results showed the viability of most spores was up to 4 months. Due to these attributes, *Bacillus pseudofirmus* bacteria were selected as the strains of alkaliphilic *Bacillus* bacteria in this study.

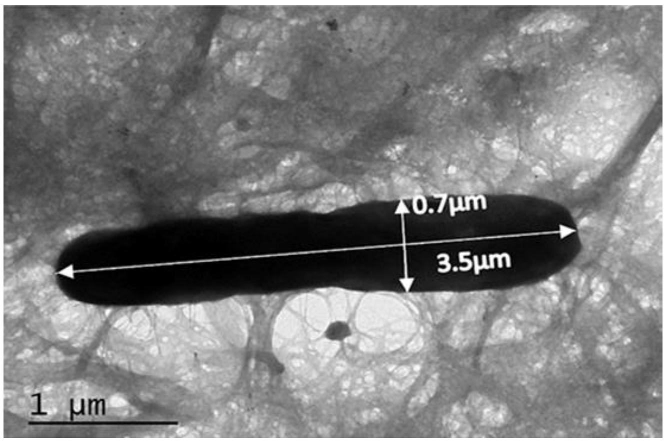


Figure 2.4. *Bacillus pseudofirmus* bacteria under Transmission electron microscope (TEM).

2.2.2.2. Bacteria Concentration

The amount of bacteria added to the concrete matrix affects the sealing performance. Wang et al. (J. Y. Wang, Snoeck, et al., 2014) encapsulated the bacterial spores into hydrogels with 10^9 spores/ml concentration and then added them to the polymer solution. Eventually, this self-healing agent was used to evaluate bacterial-based self-healing in concrete development. The results showed that the samples with cracks of 0.3 mm healed by 80 to 90 %. In comparison, the samples with cracks of 0.3 to 0.7mm were healed by 30 to 50% in 28 days. In another study by R. Andalib et al. (Andalib et al., 2016), *Bacillus Megaterium* was used at five different concentrations from 10^6 to 5 X 10^6 cfu/ml (where cfu is equivalent to colony-forming unit) were introduced to structural concrete. The results showed that the bacterial suspension concentration

at 3 X 10^6 was ideal for enhancing the concrete characteristics and the compressive and flexural strength of structural concrete. Moreover, the results revealed the impact of the optimum bacteria concentration on the concrete strength. A different concentration of 10^8 spores/L was impregnated in lightweight aggregate by Rajczakowska et al. (Rajczakowska et al., 2019). The results showed that the recovery after 28 days was up to 69%. Increasing the concentration to more than 10^8 spores/L will not significantly increase the self-healing efficiency because of the bacteria's ability to reproduce (Abdullah et al., 2018). Another study was conducted to evaluate the effect of increasing the bacteria concentration on the compressive strength of bacterial concrete. Shaikh and John (Shaikh & John, 2017) assessed the variation in compressive strength after using *Bacillus subtilis* bacteria as a self-healing agent. The main results shown in Table 2.2 (Shaikh & John, 2017) reveal that *Bacillus Subtilis* bacteria increased the compressive strength of conventional concrete by 21.33% for 28 days and 25.78% after 90 days. Alternatively, the optimum concentration for improving the compressive strength was 10^5 cells/ml; when the bacteria cells increased more than this level, the compressive strength reduced. Thus, a concentration of 10^8 was adopted in this study to achieve optimum self-healing efficiency. Nonetheless, mechanical properties could be reduced compared to normal concrete.

Table 2.2. The effect of bacteria concentration on the compressive strength of bacterial concrete (Shaikh & John, 2017).

Concrete	3 days	7 days	28 days	56 days	90 days
Conventional Concrete	14.4	21.8	36.5	41.9	42.3
Bacteria 10^3 cells/ml	16.4	25.8	44.6	51.7	53.3
Bacteria 10^5 cells/ml	17.4	27.8	46.4	54.3	57
Bacteria 10^7 cells/ml	14.6	24.5	40.6	46.9	47.8

2.2.2.3. Mineral Precursor Selection

The precursor selection is crucial in the formation of an effective self-healing agent. The addition of calcium lactate as a mineral precursor is auspicious (Omar et al., 2022). Irwan et al.(Irwan et al., 2016) used calcium lactate at concentrations of 0.001 mol/l, 0.005 mol/l, and 0.01 mol/l added to two bacteria strains. Calcium lactate with 0.005 mol/l showed the best compressive strength for the two bacteria strains. Alternatively, the lowest water penetration was achieved at a concentration of 0.01mol/l. In the same regard, Vijay et al.(Vijay & Murmu, 2019) studied the effect of different concentrations of calcium lactate, namely 0.5%,1%,1.5%,2%, and 2.5%. Calcium lactate was added to the concrete mixtures compared to other samples which contained cultured *Bacillus subtilis* mixed with concrete. The compressive strength increased by 12% with the addition of 0.5% of calcium lactate in concrete. In conclusion, calcium lactate increases compressive strength and reduces water penetration in the cementitious material matrix.

Magnesium acetate was adopted in some studies (Hungria et al., 2021; Soysal et al., 2020). The results showed an enhancement in self-healing performance without a sufficient increase in mechanical properties. Hungria et al. used a combination of magnesium acetate and yeast extract added to *Bacillus pseudofirmus* bacteria into hydrogel beads and added to the mortar matrix. Different concentrations were used for magnesium acetate. The results showed that the best performance in terms of self-healing efficiency was for the samples with the highest concentration of magnesium acetate. On the other hand, in some studies, sodium lactate broth was a carbon source for bacteria growth (Glatz & Anderson, 1988; Houtsma et al., 1996). The effect of sodium lactate as a precursor in bacterial concrete has not been evaluated.

3. OPTIMIZATION OF THE SELF-HEALING EFFICIENCY OF BACTERIAL CONCRETE USING IMPREGNATION OF THREE DIFFERENT PRECURSORS INTO LIGHTWEIGHT AGGREGATE

3.1. Introduction

Concrete is the most common construction material as it is inexpensive, durable, and robust. However, concrete is well known to be susceptible to surface cracks because of its low tensile strength. The existence of microcracks is inevitable in ordinary concrete; if the microcracks develop substantially, they may form a continuous network, which increases the permeability of the concrete. This allows the ingress of aggressive substances decreasing its durability as well as corrosion problems(Wiktor & Jonkers, 2011). On the other hand, concrete produces 8% to 9% of anthropogenic greenhouse gas emissions worldwide (Brinkman & Miller, 2021), and cement contributes 77% of emissions associated with concrete production (Busch et al., 2022).Thus, extending the service life of the concrete by using self-healing techniques could reduce the use of concrete for rebuilding and accordingly reduce the harmful emissions associated with it. As such, different researchers have adopted autonomic self-healing, which is a process that includes the use of engineered additives to improve self-healing efficiency(Alghamri et al., 2016; Rooij et al., 2011). The following techniques were used in previous studies as autonomic self-healing techniques: bacterial concrete, chemical encapsulation, mineral admixtures, a chemical in glass tubing, and intrinsic self-healing with small self controlled crack width(Arce et al., 2019; Erşan et al., 2016; Hungria et al., 2021; Li & Herbert, 2012; Schreiberová et al., 2019). Bacterial concrete is an autonomic self-healing technique that showed promising results in sealing microcracks through microbial-induced calcite precipitation (MICP) (Erşan et al., 2016; Hungria et al., 2021; Soysal et al., 2020). The main components of the self-healing process by MICP are the bacterial spores, the type of organic precursor, the efficiency of the nutrients, and a source of

calcium ions (Soysal et al., 2020). MICP involves a metabolic conversion of organic salts through carbon dioxide production as a by-product of bacterial respiration. The carbon dioxide produced dissolves in water in the concrete environment and produces carbonate ions. On the other hand, the ionization process produces calcium ions, which readily react with carbonate ions to precipitate the calcium carbonate's self-healing product, which seals the microcracks. Calcium carbonate precipitation could be increased from the reaction of carbon dioxide produced from bacterial respiration with the calcium hydroxide (CH) in the cementitious matrix to form calcium carbonate crystals (De Belie, 2016; Jonkers et al., 2010; Soysal et al., 2020). CH produced from cement hydration promotes the self-healing of cement-based materials (Silva et al., 2021). Bacteria species in the cementitious matrix are essential for the successful self-healing process. Consequently, several studies considered different species of bacteria to evaluate self-healing efficiency. For instance, *Bacillus subtilis* (Sisomphon et al., 2011), *Bacillus megaterium* (Jose et al., 2018), *Bacillus cohnii* (Kumari et al., 2016), and Bacillus sphaericus (Muynck & Verstraete, 2008) were used and accomplished successful self-healing. A study was conducted by Sharma et al. (Sharma et al., 2017) to evaluate three different strains of alkaliphilic Bacillus bacteria (Bacillus pseudofirmus, Bacillus halofurans, and Bacillus cohnii). Besides the bacterial species, calcium lactate as a mineral precursor and yeast extract as a nutrient were added to the cementitious material. Bacillus pseudofirmus showed the highest survival rate and delivered the best calcite precipitation results as a healing product as it deposed 230 mg/l after seven days (Sharma et al., 2017). Generally, the two main factors for bacteria survival are: (i) the ability of bacteria to adapt to the alkaline environment with a pH level between 8.5 and 11; and (ii) its ability to survive during curing despite internal compression (Joshi et al., 2017). Given these attributes, *Bacillus pseudofirmus* bacteria were selected in this study. The integration between

bacteria and precursor in the material matrix is crucial to creating a product effective in crack healing. Thus, the selection of precursor can significantly affect the self-healing process. The addition of calcium lactate as a mineral precursor is auspicious as it increases the compressive strength and reduces the water penetration in the specimen (Irwan et al., 2016; Vijay & Murmu, 2019). Alternatively, magnesium acetate was used with yeast extract as a precursor-nutrient combination in previous studies, and the results showed enhancement in the self-healing performance (Soysal et al., 2020). As a vital carbon source, sodium lactate broth was used in bacterial strains growth (Glatz & Anderson, 1988; Houtsma et al., 1996). However, the effect of sodium lactate as a precursor in bacterial concrete has not been evaluated in the literature. Accordingly, this study included three mineral precursors: magnesium acetate, calcium lactate, and sodium lactate.

3.2. Objective and Scope

The objective of this study was to optimize the crack healing efficiency of bacterial concrete in subtropical climates through the vacuum impregnation of bacteria into a lightweight aggregate (LWA). To achieve this objective, three sets of mortar specimens were prepared, including three precursor types (magnesium acetate, calcium lactate, and sodium lactate) with bacteria. In addition, three mortar samples were prepared with the three precursors but without bacteria, and one more control sample was prepared without bacteria or precursor for comparative purposes. Mortar is a mixture of cement, sand, water, and other additives. In terms of analysis, mortar samples can be subjected to various tests to assess their strength, composition, and other properties. Compressive strength, flexural strength, crack width measurements, and scanning electron microscopy with energy x-ray dispersive spectroscopy (SEM/EDS) were conducted for all the mortar specimens prepared in this study.

3.3. Background

3.3.1. Microbial-Induced Calcite Precipitation Pathways

In general, MICP occurs through one of the following pathways: urea hydrolysis, nitrogen reduction, or conversion of organic salts (Soysal et al., 2020). In the urea hydrolysis technique, the rate of calcium carbonate precipitation depends on the availability of urea, nutrient, water, and a calcium source. The calcite precipitation occurs when urea decomposes into carbonate ions and ammonium ions. The carbonate interacts with the calcium ions in the cementitious matrix. However, the presence of ammonium ions endangers the concrete structure, in a similar way to acid attacks (PCA, 2002; Wahyudin et al., 2013). The second pathway is nitrogen reduction through biological means when bacteria consume nitrate instead of oxygen. Carbon dioxide is produced from the process of denitrification of organic salt. Afterward, carbon dioxide in aqueous media produces carbonate, which reacts with calcium ions to produce calcium carbonate (Soysal et al., 2020).The third pathway is the conversion of organic salts to carbon dioxide through bacterial respiration. The conversion of carbon dioxide to calcite precipitation occurs in the presence of water and an alkaline environment. This study adopted this approach because it is sustainable and does not include corrosive by-products (Fahimizadeh, Abeyratne et al., 2020).

3.3.2. Bacterial Encapsulation Techniques

Previous studies adopted different encapsulation techniques for self-healing agent protection. The various encapsulation techniques improved concrete’s self-healing efficiency and strength recovery (Alghamri et al., 2016; Jonkers et al., 2010; Soysal et al., 2020; Wiktor & Jonkers, 2011). However, many authors reported that the functionality of bacterial mineral production of a two component healing agent was observed in the early ages of concrete specimens. That raised the hypothesis that most bacterial spores were crushed, decreasing the viability and the

production of minerals (Mors & Jonkers, 2017; Tziviloglou et al., 2017).Thus, bacterial impregnation into porous aggregates was proposed as one of the most effective protection techniques, especially that aggregate occupies 60% to 75% of the total volume of concrete (Bhavya & Sanjeev, 2017). Alghamri et al. (Alghamri et al., 2016) studied vacuum impregnation into lightweight porous aggregate by impregnating sodium silicate as a healing agent. A vacuum chamber at low pressure conditions of up to 0.7 bar (20.7 in. of mercury) was used to impregnate the lightweight aggregate. After introducing the self-healing agent solution in the chamber, the aggregate remained in the chamber for 1 h to acquire optimum absorption, which is the maximum amount of the solution that the aggregate can absorb while still maintaining its strength. SEM analysis of cracked concrete specimens demonstrated that lightweight aggregate impregnated with sodium silicate enhanced calcium silicate hydrate gel formation in the cracks, thus enhancing self-healing. For the mechanical properties, the efficiency of strength recovery for the specimens containing impregnated lightweight aggregate with a self-healing agent solution was five times greater than that of the control specimens (Alghamri et al., 2016). In another study, Wiktor and Jonkers (Wiktor & Jonkers, 2011) evaluated the impregnation of lightweight aggregate. Bacterial spores and calcium lactate were used as two component biochemical self-healing agents, impregnated into expanded porous clay. Wiktor and Jonkers concluded that the biochemical self-healing agent (containing calcium lactate, yeast extract, and bacterial spores) could be successfully applied to improve the self-healing capacity of concrete as the maximum healing efficiency increased up to double its value as compared with the control samples.

Similarly, vacuum impregnation of a self-healing agent into fine lightweight aggregate was conducted in several previous studies for better protection of the self-healing agent (Alghamri et

al., 2016; Wiktor & Jonkers, 2011). Cracks up to 0.46mm were sealed by impregnating bacterial spores and mineral precursor into porous clay particles (Sisomphon et al., 2011). The vacuum impregnation of a self-healing agent into fine lightweight aggregates technique of vacuum impregnation of a self-healing agent into fine lightweight aggregates was adopted in this study given its contribution to maintaining the protection of the self-healing agent through the entire incubation period.

3.3.3. *Advancements Based on Previous Studies*

Based on the literature review, there is a general agreement that impregnating bacteria into lightweight aggregates is a practical self-healing approach. Yet, this study is expected to address identified knowledge gaps in the literature as follows:

- None of the previous studies evaluated the use of sodium lactate as a precursor in bacterial concrete. Thus, the first evaluation of sodium lactate as a precursor in bacterial concrete was conducted in this study to determine if it is commensurate with a worthy precursor for converting organic salts metabolic pathway.
- A number of studies considered comparing samples with LWA replacement in relation to self-healing efficiency. This study compared the self-healing efficiency of 50% LWA as sand replacement samples by using *Bacillus pseudofirmus* bacteria, yeast extract as a nutrient, and three different precursors, namely magnesium acetate, calcium lactate, and sodium lactate, to compare their healing efficiency and to examine their effects on compressive strength, flexural strength, and the strength recovery.
- The morphology of healing products was evaluated in bacteria-containing samples under scanning electron microscopy with energy x-ray dispersive spectroscopy (SEM/EDS).

3.4. Experimental Program

3.4.1. Experimental Matrix

Seven sets of mortar samples were prepared (three replicates of mortar cubes and three replicates of mortar beams per set), as shown in Table 3.1. The seven sets included three mortar samples prepared using three precursor types with bacteria, three mortar samples with the three precursors but without bacteria, and one sample without bacteria or any precursor to serve as a control sample.

Table 3.1. Description of Sample Sets

Sample ID	Description
Control	Control with 50% of LWA as a sand replacement (no nutrient, no precursor, and no *Bacillus pseudofirmus* bacteria)
N+MgA	Nutrient + magnesium acetate precursor + 50% of LWA as a sand replacement (without *Bacillus pseudofirmus* bacteria)
N+MgA+BP	Nutrient + magnesium acetate precursor + 50% of LWA as a sand replacement + Bacillus pseudofirmus bacteria
N+CaL	Nutrient + calcium lactate precursor + 50% of LWA as a sand replacement (without *Bacillus pseudofirmus* bacteria)
N+CaL+BP	Nutrient + calcium lactate precursor + 50% of LWA as a sand replacement + *Bacillus pseudofirmus* bacteria
N+NaL	Nutrient + sodium lactate precursor + 50% of LWA as a sand replacement (without *Bacillus pseudofirmus* bacteria)
N+NaL+BP	Nutrient + sodium lactate precursor + 50% of LWA as a sand replacement + *Bacillus pseudofirmus* bacteria

Note: LWA = lightweight aggregate.

3.4.2. Vacuum Impregnation of Self-Healing Agent

3.4.2.1. Self-Healing Agents Preparation

Bacillus pseudofirmus bacteria, yeast extract as a nutrient, and three mineral precursors, namely, magnesium acetate, calcium lactate, and sodium lactate, were the main components of the three biochemical self-healing agents. *Bacillus pseudofirmus* bacteria were suspended at 10^8 cells/ml in a solution of 0.48 g/l (1.74mM/l) yeast extract, and the mineral precursor (magnesium acetate, sodium lactate, or calcium lactate) at a concentration of 75 mM/l was added to deionized water and was stirred gently by a magnetic stirrer. The three combinations of self-healing agents were impregnated into expanded lightweight clay aggregate using a vacuum chamber.

3.4.2.2. Vacuum Impregnation Set-up.

After preparing the solution, the expanded clay fine lightweight aggregate was oven dried at a temperature of 100°C and 50% relative humidity (RH) for 24 h. Then, the dried aggregate was placed in the vacuum chamber; see Figure 3.1. The vacuum chamber was connected to a pump to provide a constant pressure inside the chamber of 21 in. of mercury (71,114 Pascal). The solution was then added to the chamber to maintain a 20 mm or higher level above the aggregate to ensure proper immersion of lightweight aggregate in the solution. The aggregate was allowed to impregnate while submerged in the solution. To determine the optimum submergence time, the three combinations of self-healing agents were impregnated into the aggregate for three periods, namely, 30 min, 60 min, and 120 min. For each case, the absorption percentage of the aggregate was calculated as follows:

$$Absorption\ (\%) = (A - B)/(B) \qquad (1)$$

where,

A=Mass of the wetted surface-dry specimen,

B=Mass of the oven-dry specimen

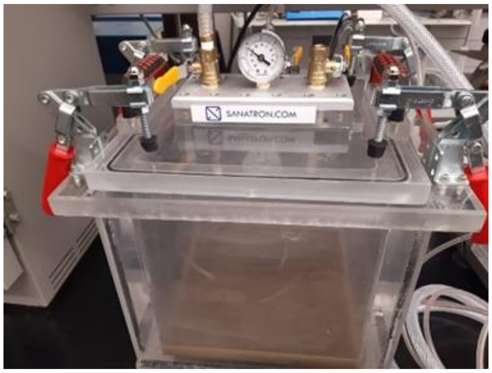


Figure 3.1. Fine lightweight aggregate submerged in the solution
inside the vacuum impregnation chamber.

The absorption percentage values for magnesium acetate with bacteria after 30 min, 60 min, and 120 min were 23.9 %, 24.5%, and 24.7%, respectively. The values for calcium lactate with bacteria were 23.1% ,24.3%, and 24.8 % respectively. The values for sodium lactate with bacteria were 24.8%, 25.5%, and 25.9 %, respectively. Based on these values, the absorption percentage after 60 min for the three precursors was found adequate because the results did not show a significant increase in absorption percentage after 60 min. As such, the impregnated aggregate in this study was submerged in the self-healing agent solution for 60 minutes. The procedures of vacuum impregnation of the self-healing agent were similar to a previous study (Omar et al., 2021).

3.4.3. Mortar Mix Design

The mortar mixture proportions are shown in Table 3.2 Ordinary Portland Cement (OPC) Type I was utilized in the mortar mixture. A water-cement ratio of 0.45 was selected, and a sand-binder ratio of 1.85. Polyvinyl Alcohol (PVA) fibers were added to enhance post-cracking load carrying capacity and prevent sudden failure of the beam specimens. PVA fibers had a diameter of 1.49 mm, a length of 8.13 mm, a tensile strength of 1600.4 MPa, a maximum elongation of 5.7%,

Young's modulus of 40 GPa, and a density of 999.6 kg/m3. The mortar mixture included an equal proportion (by mass) of fine lightweight aggregate and concrete sand. The nominal maximum aggregate size of concrete sand and fine lightweight aggregates were 4.65 mm and 2.36 mm, respectively.

Moreover, the specific gravity of concrete sand and fine lightweight aggregate were 2.62 and 1.65, respectively. A high-range water reducer admixture (HRWR) was added with the same amount to all the prepared mixtures to enhance workability. Mortar mixing was performed according to the ASTM C109 standard. A lime saturated water tank was used for curing at room temperature in the laboratory.

Table 3.2. Mortar Mixture Proportions

Cement (Kg/m^3)	Fine Lightweight Aggregate (Kg/m^3)	Concrete Sand (Kg/m^3)	Water (Kg/m^3)	Fibers (Kg/m^3)	HRWR (ml/ m^3)
475.9	637.6	637.6	214.1	6.5	1.5

3.4.4. Specimen Preparation

Three mortar beams with dimensions (38.1 mm x 38.1 mm x 152.4 mm) and three mortar cubes with dimensions (50.8 mm x 50.8 mm x 50.8 mm) were cast for each mixture according to ASTM C305 to evaluate compressive strength, flexural strength, crack healing efficiency, and to conduct SEM-EDS analysis.

3.4.5. Compressive Strength Test

After 28 days of curing, the three mortar cubes were used to evaluate the effect of bacteria cells and mineral precursors on the compressive strength according to ASTM C109. The specimens were tested using a hydraulic press at a constant loading rate of 124.11 MPa/min.

3.4.6. *Flexural Strength Test*

After 28 days of curing, a three-point bending test was conducted on all the beams. A MARK 10 ESM 1500 equipment was utilized, and the load was applied at a displacement rate of 0.2 mm/min, as included in a previous study (Fahimizadeh et al., 2020). The distance between the two supports was 15.24 cm. The test was conducted twice for each beam. The three-point bending test was concluded on the pristine beams after 28 days of curing, and the cracked beams were tested again after applying the healing regime. To determine their flexural strength, the following equation was used:

$fs = 3PL/(2\,bd^2)$ (2)

where,

fs= Flexural strength (MPa),

P= Peak load (Kg),

L= Span length (mm),

b= Specimen width (mm), and

d = Specimen height (mm)

All the beams were subjected to a healing regime of 28 wet-dry cycles. Each cycle of the healing regime was allocated as 8 hours of dry air at a relative humidity of 50% and 16 hours of immersion in an opened bucket filled with tap water. The bucket containing the samples was kept open to allow oxygen and carbon dioxide diffusion during the healing regime, similar to a previous study (Wiktor & Jonkers, 2011). At the end of the 28 wet-dry cycles, the three-point bending test was conducted again on the cracked beam to calculate the flexural strength recovery by the end of the healing regime, as shown in Equation 3:

$FSR = (\frac{fs_f}{fs_i}) \times 100$
(3)

where,

FSR = Flexural Strength Recovery (%),

fs_i = Initial flexural strength (MPa),

fs_f= Flexural Strength after finishing the healing regime (MPa)

3.4.7. Crack-Healing Quantification

After cracking the pristine beam in the first three-point bending test, the water was able to ingress through the cracks to activate the bacterial spores and start the healing process. The healing process was monitored at five-time intervals for each sample, day-zero (before the beginning of the wet-dry cycles), 3 days, 7 days, 14 days, and after 28 days of wet-dry cycles. The self-healing was monitored using a stereo microscope with a digital camera attached. The self-healing imaging covered the three cracked faces (one bottom face and two sides). The images of the cracks were processed by using the Image J processing program with 30 width measurements per image with approximately 10,000 measurements for all the samples.

3.4.8. SEM-EDS Analysis and Characterization of Self-Healing Products

After 28 days of the healing regime, the microstructure of one beam from each mortar sample was examined through SEM-EDS to testify the characterization of self-healing products. A Quanta™ 3D Dual Beam™ FEG FIB - Scanning Electron Microscope (SEM) with EDAX Pegasus Energy Dispersive Spectroscopy (EDS/EBSD) detectors were utilized. To prepare the samples for SEM, the samples were cut with a diamond blade saw to get the mid-span part surrounding the crack. Thus, a small section of the beam was extracted, and it contained part of the sealed crack. The small sections were placed in an oven for 24 hours at a temperature of 38°C to avoid any moisture. Afterward, the specimens were mounted onto metal stubs using carbon tape and sputter-coated with a platinum film to remove any charging and ensure good

conductivity. Eventually, the specimens were used in SEM-EDS analysis to investigate the morphology of the healing products. Besides, an EDS maps of one specimen from each mortar sample was obtained to assess the chemical composition of the healing products.

3.5. Results and Analysis

3.5.1. Compressive Strength

Figure 3.2 presents the compressive strength test's average and standard deviation values. An Analysis of Variance (ANOVA) showed statistically significant differences between the samples' average compressive strength at a significance level of 0.05. Consequently, the Tuckey's Honest Significant Difference (HSD) Test was used to examine the significance of the difference between the average values. In general, the output of this statistical test is a letter for each sample (or each mean value), where samples sharing at least one letter are statistically similar, while samples that do not share any letters are statistically different at 0.05 significance level. As an example, in Figure 3.2, Sample Control and N+MgA+BP are statistically similar at 0.05 significance level as they share the same letter "C." Based on Figure 3.2, the following findings were obtained:

- By comparing sample Control (control sample with 50% of LWA as a sand replacement, no precursor, and no bacteria) with all other samples; all the samples had significantly higher average compressive strength than sample Control except magnesium acetate precursor with bacteria (sample N+MgA+BP), indicating the addition of precursors without bacteria positively affected the compressive strength.
- By comparing the samples with and without bacteria (N+MgA against N+MgA+BP; N+CaL against N+CaL+BP; and N+NaL against N+NaL+BP), the addition of bacteria reduced the average compressive strength for all the precursors up to 21.8% for samples

containing magnesium acetate. Yet, this reduction was not significant for calcium lactate and sodium lactate.

• Samples N+MgA (magnesium acetate precursor only), followed by N+NaL (sodium lactate precursor only), had the highest average compressive strength compared to all other samples.

• The samples that incorporated calcium lactate showed higher compressive strength than sample Control without bacteria or precursors. As shown in Figure 3.2, the compressive strength of sample N+CaL+BP (calcium lactate precursor with bacteria) was 22% greater than sample Control. Similarly, calcium lactate precursor without bacteria (sample N+CaL) showed a 24% increase in compressive strength compared to the Control, revealing the positive impact of calcium lactate precursor on the compressive strength. This finding is consistent with the results reported in the literature (Tziviloglou et al., 2017).

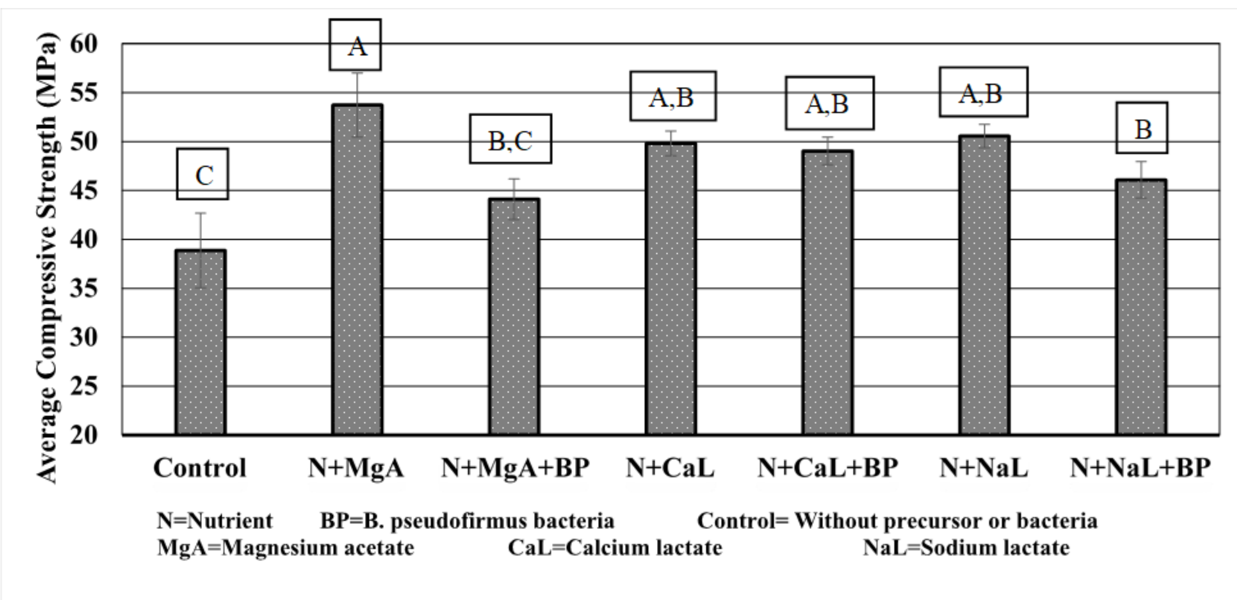


Figure 3.2. Average and standard deviation of compressive strength results for mortar samples.

3.5.2. Flexural Strength Recovery

The average flexural strength and the standard deviation results are shown in Figure 3.3 before initiating the healing phase. Figure 3.3 also shows the Tuckey's HSD Test results at a significance level of 0.05. As shown, there was no statistical difference between the flexural strength of all the samples, except between the control sample without precursor or bacteria (sample Control) and the sodium lactate precursor with bacteria (sample N+NaL+BP). This finding indicates that adding sodium lactate precursor with bacteria enhanced the flexural strength. This finding is consistent with previous studies, which indicated that lactic acid (at which sodium lactate is one of the lactic acid derivatives) effectively improves the mechanical properties of cementitious materials (Kastiukas et al., 2015; Leroux et al., 1999). This is attributed to the effect of lactic acid derivatives (such as sodium lactate) on promoting the compactness and the interlocking of the hydration products, thereby increasing the overall strength of the cementitious matrix (Singh et al., 1986).

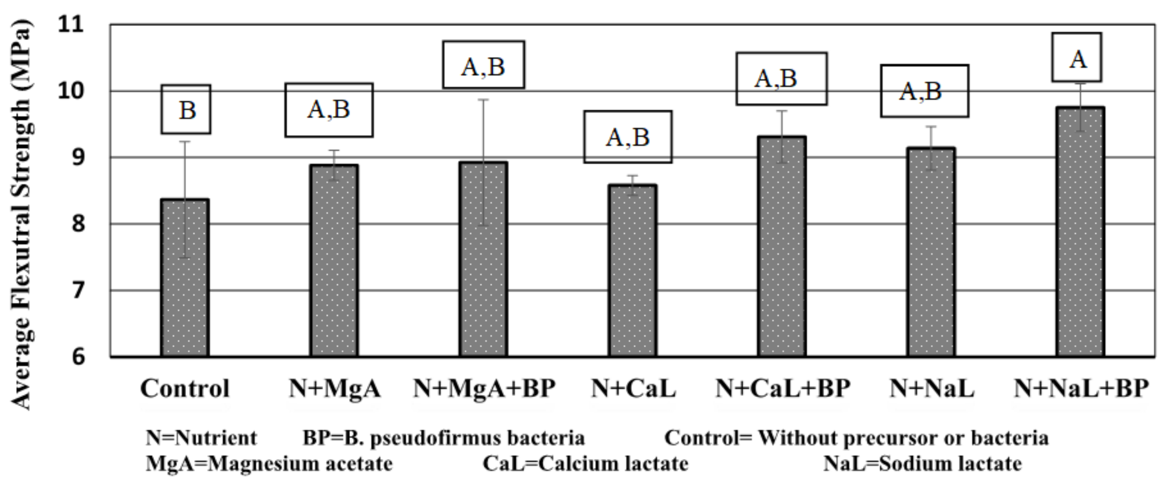


Figure 3.3. Average and standard deviation of flexural strength results for mortar samples.

Figure 3.4 presents the average values of the flexural strength recovery test along with the standard deviation and the results of the Tuckey's HSD Test at a significance level of 0.05. Results in Figure 3.4 indicated that the averages of flexural strength recovery for all the samples were not significantly different, suggesting that the addition of bacteria and precursors did not significantly affect the flexural strength recovery of the specimens. It is worth noting that the highest flexural strength recovery among the samples was for calcium lactate precursor without bacteria (sample N+CaL) with 23.6%.

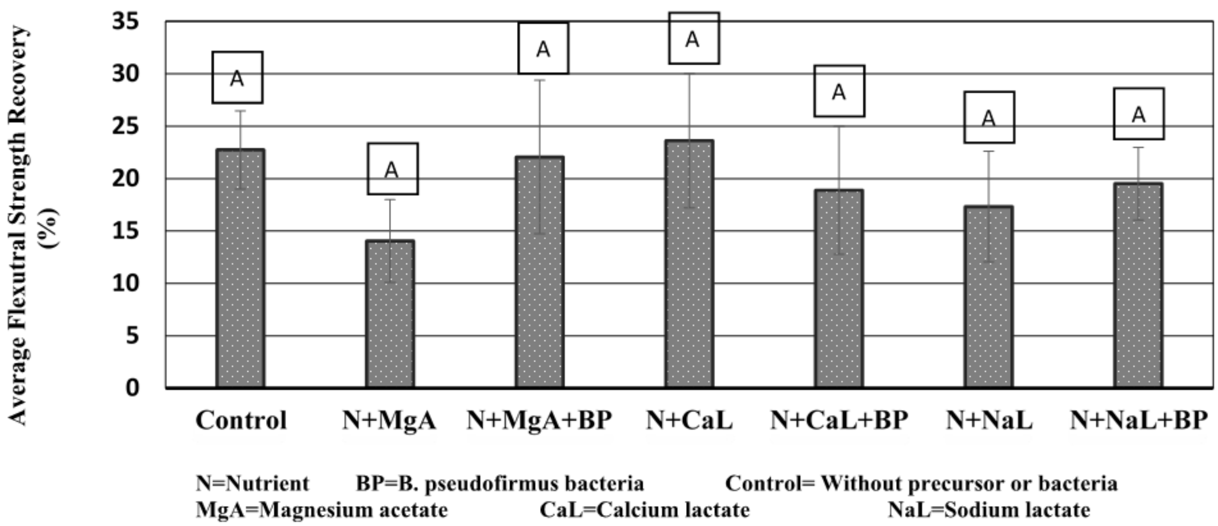


Figure 3.4. Average and standard deviation of flexural strength recovery (%) results for mortar samples.

3.5.3. Crack-Sealing Efficiency

Light microscopy was used to monitor the healing efficiency of the specimens during the healing period of the wet-dry cycles. Images were acquired at specific time intervals at 3,7,14, and 28 days during the healing phase. The crack was initiated at the bottom of the specimen during the three-point bending test and extended to the side faces. PVA fibers were utilized in the mixture

design to provide ductility and prevent sudden failure of the specimen. Although the three cracked faces of the beams were captured in the images, the analysis for crack measurement was restricted to only the two side faces. Considering that the side cracks covered a wide range of crack widths due to crack propagation from the bottom to the sides of the beam (Milla et al., 2019). The crack healing efficiency at different time intervals (3,7,14, and 28 days) was calculated by using Equation 4:

$$Healing\ Efficiency\ (\%) = \left(\frac{CW_i - CW_t}{CW_i}\right) \times 100 \qquad (2)$$

where,

CW_i =Initial crack width on day zero,

CW_t =Crack width at a particular time interval.

The healing efficiency % of the side cracks and the Tuckey's HSD Test results are shown in Figure 3.5. Based on the results shown in Figure 3.5, the following findings were drawn:

• Sodium lactate precursor with bacteria (sample N+NaL+BP) exhibited the highest self-healing efficiency, as high as 75% after 28 days of the healing phase, as shown in Figure 3.5. The main reason is that lactic acid and its derivatives, such as calcium and sodium lactate, play an essential role in microbial-induced minerals precipitation. They produced carbon dioxide by metabolic conversion of the precursors by utilizing heterotrophic bacteria (Mors & Jonkers, 2017).

• The significance of the differences between the healing efficiencies of the samples was examined by conducting an ANOVA test. The p-value of 0.013 was less than 0.05 (significance level=0.05), which indicates a significant difference between the averages of the samples. Tuckey's HSD Test was conducted to determine the differences between

each pair of samples, as shown in Figure 3.5. The only pair with a significant difference were sodium lactate with bacteria (sample N+NaL+BP) and sample Control. This finding led to the positive impact of sodium lactate precursor with bacteria on the self-healing efficiency of mortar samples when they were impregnated into lightweight aggregate.

• Sand and fine lightweight (sample Control) had the lowest healing efficiency averaging 44% after 28 days. The healing in the Control sample occurred due to the autogenous healing capability of concrete. The autogenous healing occurred because of the hydration of cement and calcite formation by the reaction of water and carbon dioxide (Hearn, 1998).

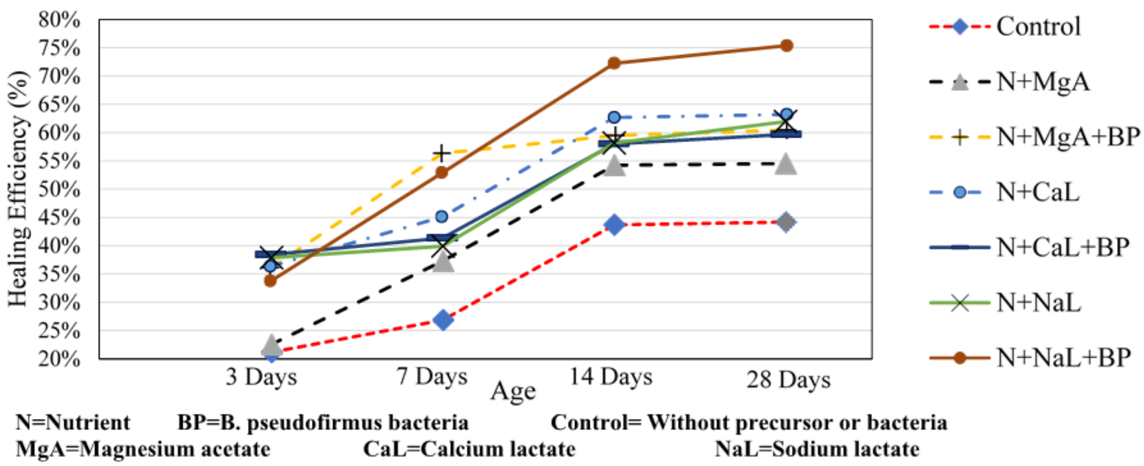


Figure 3.5. Healing Efficiency (%) of Side cracks and Tuckey's HSD Test results for mortar samples.

Table 3.3. Statistical Analysis Results of Self-healing Efficiency After 28 Days of a Healing Regime

Sample ID	Average (after 28 days of healing regime)	Tuckey's HSD Test	Variance (after 28 days of healing regime)
Control	44.2%	A	0.078
N+MgA	54.5%	A,B	0.0082
N+MgA+BP	60.5%	A,B	0.0159
N+CaL	63.2%	A,B	0.0612
N+CaL+BP	59.7%	A,B	0.0152
N+NaL	62%	A,B	0.0064
N+NaL+BP	75.4%	B	0.0052

Furthermore, Figure 3.6 represents the reduction in the crack size at the end of the self-healing regime of wet-dry cycles for one of the bacteria-containing samples (i.e, Magnesium acetate with bacteria (N+MgA+BP).

(a) (b)

Figure 3.6. Sample magnesium acetate with bacteria (N+MgA+BP) under the light microscope: (a) Before starting the wet-dry cycles; (b) After 28 wet-dry cycles showing part of the calcite precipitations

3.5.4. Best-Performing Sample

Based on the previous analysis, it was concluded that sodium lactate precursor with bacteria (sample N+NaL+BP) achieved the best results, as supported by the following key findings:

- The compressive strength of sodium lactate precursor with bacteria (sample N+NaL+BP) was significantly higher than the sample Control.

- The flexural strength recovery of sodium lactate precursor with bacteria (sample N+NaL+BP) was not statistically different from the sample Control, which means that the addition of sodium lactate with bacteria did not have a significant negative impact on the flexural strength recovery.

- The flexural strength of sodium lactate precursor with bacteria (sample N+NaL+BP) was the highest among all the samples and was significantly different from the sample Control.

- The crack healing efficiency of sodium lactate precursor with bacteria (sample N+NaL+BP) was the highest among all the samples, which is consistent with the results of previous studies (Buller et al., 2019).

3.5.5. Healing Product Characterization

The secondary electron (SE) images of the healing products produced in the cracks were obtained for all the samples, see Figure 3.6. As shown in Figure 3.6 (a to c), all the bacteria-containing specimens showed calcite crystals, indicating a successful self-healing process. In addition, Figure 3.6 (d) showed that the control sample (without bacteria or precursors) revealed fewer calcite crystals, which resulted from the autogenous healing produced by the moisture continuously present during the wet/dry cycles (J. Y. Wang, Snoeck, et al., 2014).

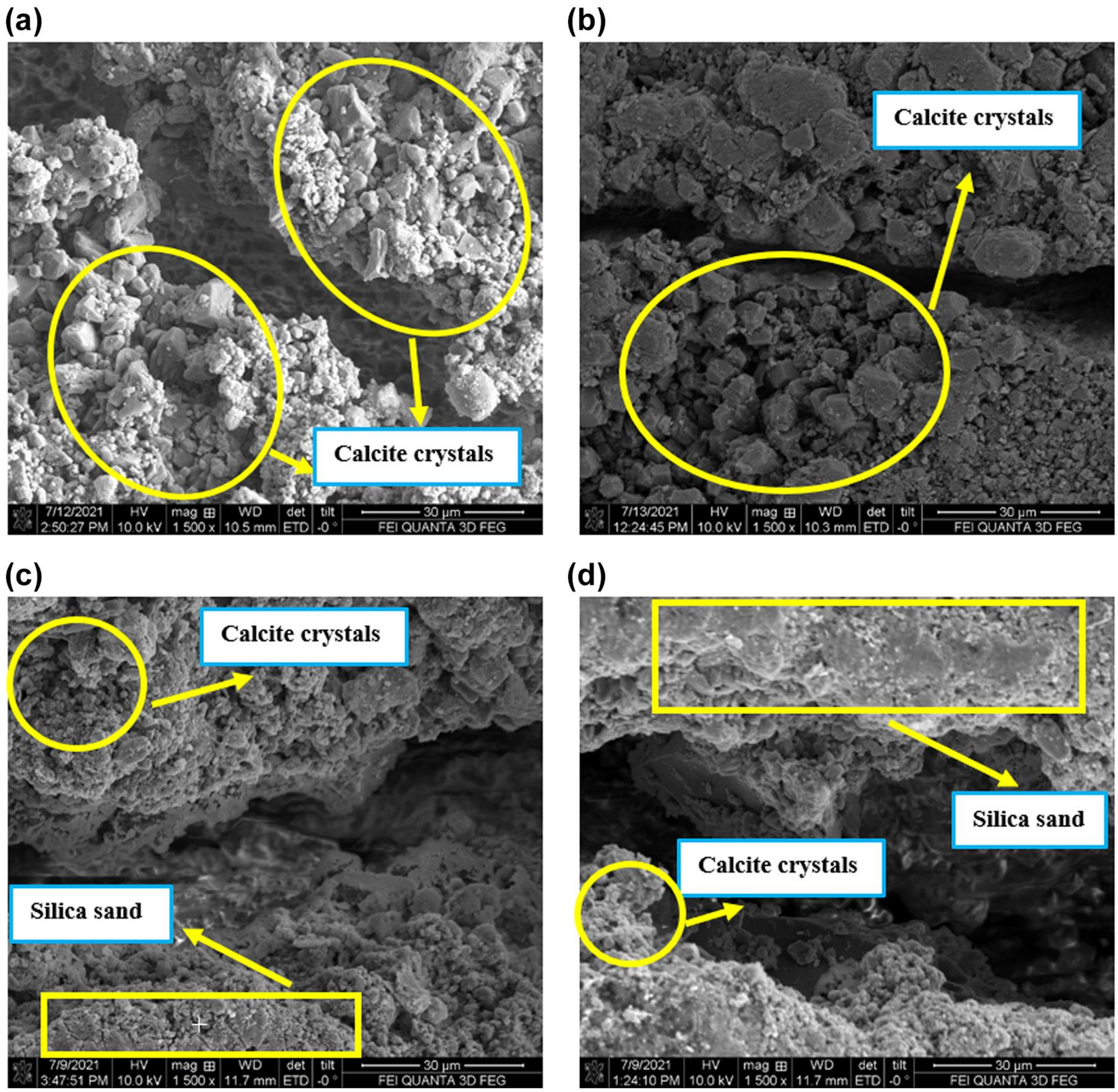


Figure 3.7. SEM images of healing products in mortar specimen crack for (a) Magnesium acetate precursor with bacteria (N+MgA+BP); (b) Calcium lactate precursor with bacteria (N+CaL+BP); (c) Sodium lactate precursor with bacteria (N+NaL+BP); (d) Control sample without precursor or bacteria (Control).

EDS using point analysis was conducted on one beam per sample to validate the existence of healing products in the samples. EDS spectrums were gathered on the spots that showed visible healing products throughout the cracks. A total of 30 data points were selected on each beam per 7 samples (overall 210 points), and the data were utilized in plotting the atomic ratio plot shown

in Figure 3.7. The C-S-H region comprehended between 0.45 to 0.55 Si/Ca and 0.04 to 0.08 Al/Ca for pure cement pastes (Winter, 2012). As shown in Figure 3.7, most of the points were in the calcium-rich crystal region, indicating the availability of calcium-rich crystal healing products such as calcite (CaCO3) or calcium hydroxide (CH). In addition, a significant number of the points were located in the interaction area (outside the C-S-H region and the calcium-rich crystal region). The interaction region can be attributed to the effect of scattering skirt electrons that produce X-rays at points hundreds of microns away from the targeted location, thus showing the characteristics of more than one phase at a time, including both C-S-H and calcium-rich crystals regions (Winter, 2012). By comparing the number of points in the calcium-rich crystals region, the bacteria containing samples, namely; samples N+MgA+BP, N+CaL+BP, and N+NaL+BP, had 40% of their points in this region, against 21% for the control samples, namely; samples Control, N+MgA, N+CaL, and N+NaL. This indicates more self-healing products in the bacteria-containing samples than in the control samples. Table 3.4 shows the results of the averages and standard deviation of the atomic weight percentage (atomic wt %) per sample, where the atomic weight percentage is the percentage of atoms of the element to the total atoms in the sample. In addition, the number of points in the calcite-rich crystals region is shown for each sample.

Table 3.4. Statistical Analysis Results of EDS Points After 28 days of Healing Regime

Sample ID	Number of Points	Number of Points in Calcite Rich Crystals region	Average		Standard Deviation	
			Al/Ca (Atomic wt %)	Si/Ca (Atomic wt %)	Al/Ca (Atomic wt %)	Si/Ca (Atomic wt %)
Control	30	5	0.376	0.685	0.712	1.081
N+MgA	30	6	0.016	0.035	0.016	0.026
N+MgA+BP	30	5	0.069	0.302	0.080	0.442
N+CaL	30	8	0.979	3.258	1.280	3.431

table cont’d

Sample ID	Number of Points	Number of Points in Calcite Rich Crystals region	Average		Standard Deviation	
			Al/Ca (Atomic wt %)	Si/Ca (Atomic wt %)	Al/Ca (Atomic wt %)	Si/Ca (Atomic wt %)
N+CaL+BP	30	25	0.011	0.544	0.021	2.765
N+NaL	30	6	0.216	1.218	0.378	2.27
N+NaL+BP	30	6	0.101	0.24	0.11	0.217

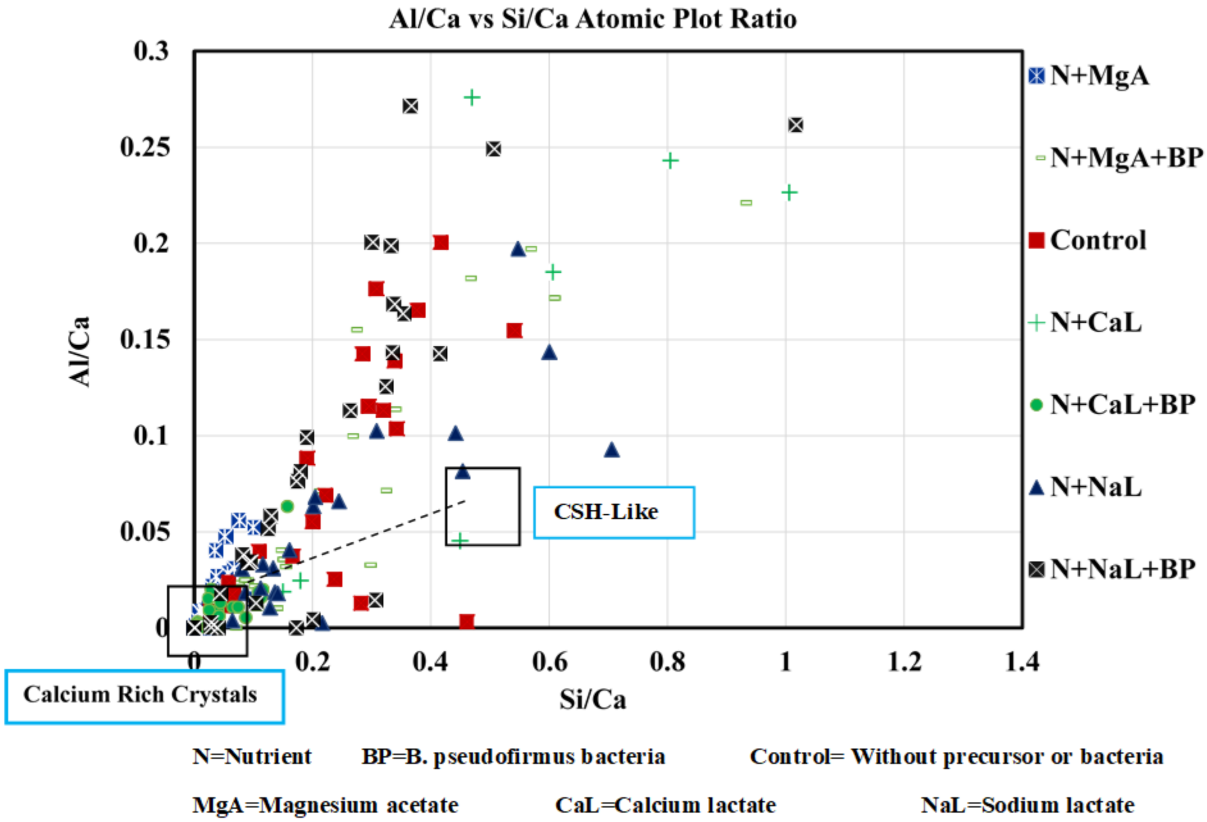
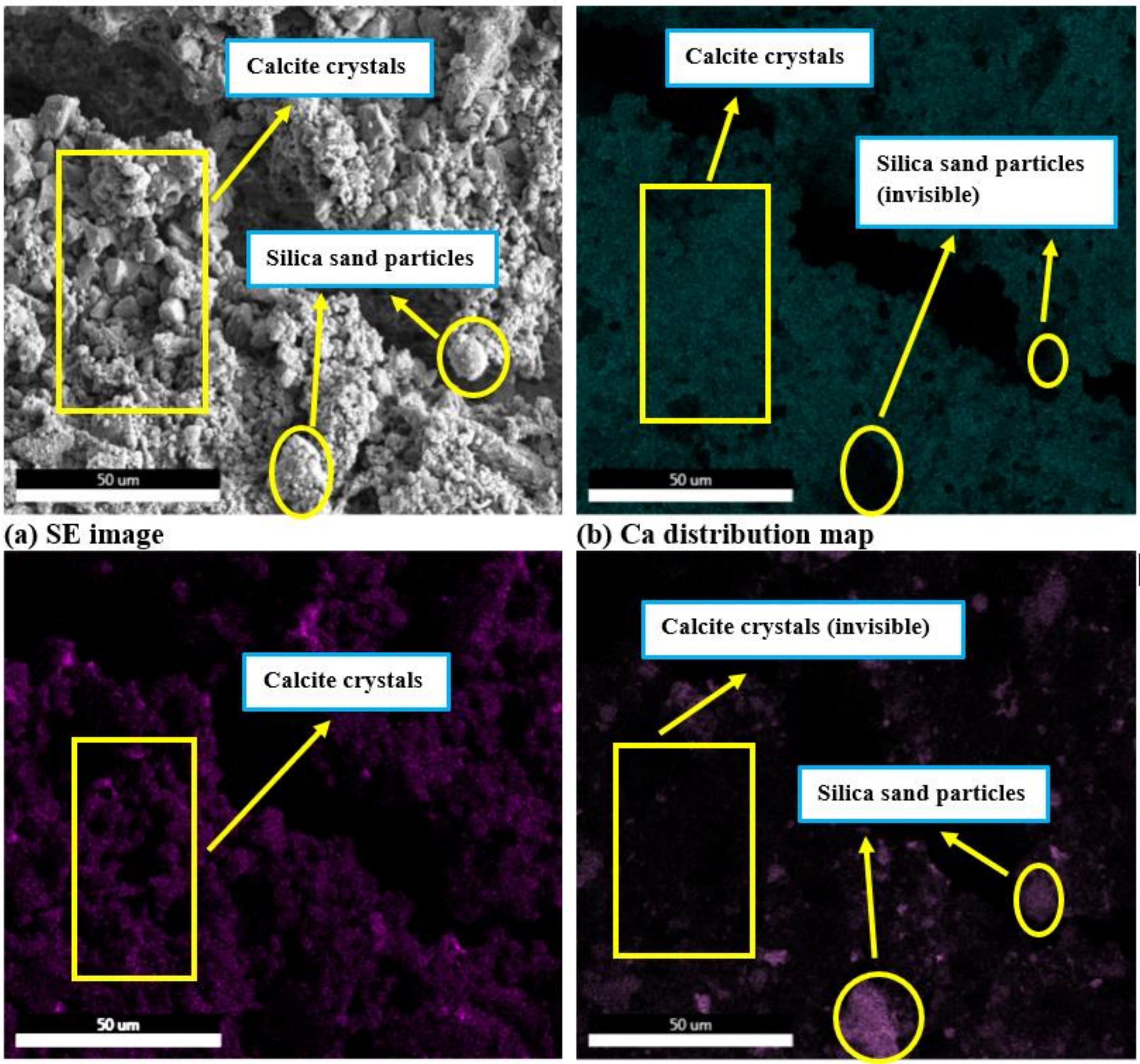


Figure 3.8. Atomic ratio plot products displayed throughout the cracks

Besides, EDS maps were generated to validate the previous findings regarding the chemical composition and morphology of the healing products. Figure 3.8 presents four different maps of the same spot shown in Figure 3.6 (a) for sample N+MgA+BP as an example of the bacteria-containing samples. The four maps show the specimen's SE image, calcium distribution, carbon distribution, and silica distribution, respectively. By evaluating Figure 3.8, the calcite crystals region can be observed in Figures 3.8(b) and 3.8(c) of the main components of calcite; calcium

and carbon distributions, respectively. Meanwhile, the calcium-rich crystals region was vacant in Figure 3.8(d) of silica distribution. The figure shows the correlation between the carbon and calcium contents as the healing products' main components. Moreover, some silica sand particles were detected in these maps due to the high concentrations of silicon bulks in those regions. This is similar to the EDS mapping findings reported in a previous study (Arce et al., 2017).



(a) SE image (b) Ca distribution map

(c) C distribution map (d) Si distribution map

Figure 3.9. SE image of crack after healing with their respective Ca, Si, and C distribution maps of magnesium acetate precursor with bacteria (N+MgA+BP): (a) SE image; (b) Ca distribution map; (c) C distribution map; and (d) Si distribution map.

Note: N = nutrient; MgA = magnesium acetate; BP = B. pseudofirmus bacteria; CaL = calcium lactate; NaL = sodium lactate; SE = secondary electron.

3.6. Summary and Conclusions

The objective of this study was to optimize the crack healing efficiency of bacterial concrete in subtropical climates through the vacuum impregnation of bacteria into a lightweight aggregate (LWA). To achieve this objective, three sets of mortar specimens were prepared, including three precursor types (magnesium acetate, calcium lactate, and sodium lactate) with bacteria. In addition, three mortar samples were prepared with the three precursors but without bacteria, and one more control sample was prepared without bacteria or precursor for comparative purposes. Compressive strength, flexural strength, crack width measurements, and scanning electron microscopy with energy x-ray dispersive spectroscopy (SEM/EDS) were conducted for all the mortar specimens prepared in this study. The main conclusions of this study were as follows:

- The compressive strength of sodium lactate precursor with bacteria (sample N+NaL+BP) was significantly higher than the sample Control.
- The flexural strength recovery of sodium lactate precursor with bacteria (sample N+NaL+BP) was not significantly different from the sample Control, which indicates that the addition of sodium lactate with bacteria did not significantly affect the flexural strength recovery.
- The crack healing efficiency of sodium lactate precursor with bacteria (sample N+NaL+BP) was the highest among all the samples at 75%. The control sample without precursor or bacteria (sample Control) exhibited the lowest self-healing efficiency at 44%.

- Sodium lactate precursor with bacteria (sample N+NaL+BP) achieved the best results in crack healing efficiency without negatively affecting the compressive strength and the flexural strength recovery of the mortar samples.
- Calcite crystals healing products were identified in the samples that achieved the most self-healing efficiency during the scanning electron microscopy (SEM) coupled with energy dispersive spectroscopy (EDS) and in the EDS maps.

In light of the obtained results, future work should focus on scaling up the mortar samples to concrete to study the effects on the evaluation of self-healing efficiency, mechanical properties, and permeability. The flexural strength and strength recovery should also be evaluated using a four-point bending test on the concrete and mortar samples because it indicates the material's flexural strength better than the three-point bending test. In addition, this study considered fixed fiber content in all the samples even though the flexural strength increases by increasing the PVA fibers (Zhang et al., 2019). As such, different fiber contents should be considered as a variable in future work while comparing different sodium lactate with bacteria samples (the best-performing sample in this study).

3.7. Acknowledgments

The authors would like to acknowledge the financial support of Tran-SET through the grant 18CLSU01 and of the Louisiana Transportation Research Center (LTRC).

3.8. Author Contributions

The authors confirm their contribution to the paper as follows: study conception and design: O. Omar, M. Mousa, M.Hassan, G.Arce, J.Milla; data collection: O.Omar, R.Hungria, A.Gavilanes; analysis and interpretation of results: O.Omar, M.Mousa, G.Arce; draft manuscript preparation:

O.Omar, M.Mousa, M.Hassan, G.Arce, J.Milla, T.Rupnow. All authors reviewed the results and approved the final version of the manuscript.

4. SUMMARY AND CONCLUSIONS

Concrete is the most broadly used construction material due to its remarkable mechanical properties and cost-effectiveness. But it is susceptible to high tensile stresses, which causes cracking. The cracks jeopardize the concrete by increasing the permeability and ingressing harmful substances, which deteriorate the durability of concrete. On the other hand, developing sustainable concrete can reduce greenhouse gas (GHG) emissions from the concrete industry. Thus, implementing self-healing concrete technologies is a promising approach to enhance the durability of the transportation infrastructure and reduce the usage of concrete for rebuilding. Among these technologies, bacterial concrete, as an autonomic self-healing technique, showed promising results in sealing microcracks.

This study accommodated the crack healing efficiency of bacterial concrete in subtropical climates through the vacuum impregnation of bacteria into a lightweight aggregate (LWA). Three sets of mortar specimens were prepared to achieve this objective, including three precursor types (magnesium acetate, calcium lactate, and sodium lactate) with bacteria. In addition, three mortar samples were prepared with the three precursors but without bacteria, and one more control sample was prepared without bacteria or precursor for comparative purposes. Compressive strength, flexural strength, crack width measurements, and scanning electron microscopy with energy x-ray dispersive spectroscopy (SEM/EDS) were conducted for all the mortar specimens prepared in this study.

This study was the first to evaluate sodium lactate as a precursor using vacuum impregnation into lightweight aggregate technique. To evaluate its efficiency as a precursor for converting organic salts metabolic pathway. Simultaneously, the study was among a few studies that compared three different precursors: magnesium acetate, calcium lactate, and sodium lactate, along with bacteria, in terms of self-healing efficiency. The comparison was conducted by 50% LWA as sand replacement samples. The followings are the main findings of the study:

- The addition of sodium lactate and bacteria significantly increased the compressive strength compared to the sample Control.
- However, the addition of sodium lactate and bacteria did not significantly affect the flexural strength recovery compared to the sample Control, indicating that adding sodium lactate with bacteria did not significantly affect the flexural strength recovery.
- The crack healing efficiency of sodium lactate precursor with bacteria was the highest among all the samples at 75%. The control sample without precursor or bacteria exhibited the lowest self-healing efficiency at 44%.
- Sodium lactate precursor with bacteria achieved the best results in crack healing efficiency without negatively affecting the compressive strength and the flexural strength recovery of the mortar samples.
- On the other hand, the samples incorporating calcium lactate showed higher compressive strength than the sample Control without bacteria or precursors. The compressive strength of the sample calcium lactate precursor with bacteria was 22% greater than sample Control. Similarly, calcium lactate precursor without bacteria showed a 24% increase in compressive strength compared to the Control, revealing the positive impact of calcium lactate precursor on compressive strength.

- Calcite crystals healing products were identified in the bacteria-containing samples during the scanning electron microscopy (SEM) coupled with energy dispersive spectroscopy (EDS) and the EDS maps.

In conclusion, sodium lactate precursor with bacteria (sample N+NaL+BP) achieved the best results according to the following key factors regarding the mechanical properties; compressive strength; which was significantly higher than the control sample, and the flexural strength of it was the highest among all the samples. Most importantly, the self-healing efficiency was the highest among the samples at 75%.

5. FUTURE WORK

In light of the obtained results, future work should be focused on the following:

- The mortar samples should be scaled up to concrete samples to study the effect on the concrete matrix.
- The durability and water permeability should be evaluated in addition to the mechanical properties and self-healing efficiency.
- The flexural strength and strength recovery should also be evaluated using a four-point bending test on the concrete and mortar samples because it better indicates the material's flexural strength than the three-point bending test.
- This study considered fixed fiber content in all the samples even though the flexural strength increases by increasing the PVA fibers. Different fiber contents should be regarded as a variable in future work while comparing different sodium lactate with bacteria samples (the best-performing sample in this study).
- Further analysis and quantification for the self-healing products should be conducted using more investigation equipment, such as Raman spectroscopy and XRD analysis.

APPENDIX.

COPYRIGHT PERMISSION

Optimization of the Self-Healing Efficiency of Bacterial Concrete Using Impregnation of Three Different Precursors into Lightweight Aggregate

Author: Omar Omar, Momen R. Mousa, Marwa Hassan, Ricardo Hungria, et al.

Publication: Transportation Research Record

Publisher: SAGE Publications

Date: 2023-03-01

Gratis Reuse

If you are a SAGE journal author requesting permission to reuse material from your journal article, please note you may be able to reuse your content without requiring permission from SAGE. Please review SAGE's author re-use and archiving policies at https://us.sagepub.com/en-us/nam/journal-author-archiving-policies-and-re-use for more information.

If your request does not fall within SAGE's reuse guidelines, please proceed with submitting your request by selecting one of the other reuse categories that describes your use. Please note, a fee may be charged for reuse of content requiring permission. Please submit a ticket through the SAGE Permissions Portal if you have questions.

BACK

CLOSE WINDOW

REFERENCES

Alderete, N. M., Villagrán Zaccardi, Y. A., & De Belie, N. (2019). Physical evidence of swelling as the cause of anomalous capillary water uptake by cementitious materials. *Cement and Concrete Research*, *120*, 256–266. https://doi.org/10.1016/j.cemconres.2019.04.001

Alghamri, R., Kanellopoulos, A., & Al-Tabbaa, A. (2016). Impregnation and encapsulation of lightweight aggregates for self-healing concrete. *Construction and Building Materials*, *124*, 910–921. https://doi.org/10.1016/j.conbuildmat.2016.07.143

Aliko-Benítez, A., Doblaré, M., & Sanz-Herrera, J. A. (2015). Chemical-diffusive modeling of the self-healing behavior in concrete. *International Journal of Solids and Structures*, *69–70*, 392–402. https://doi.org/10.1016/j.ijsolstr.2015.05.011

Andalib, R., Abd Majid, M. Z., Hussin, M. W., Ponraj, M., Keyvanfar, A., Mirza, J., & Lee, H. S. (2016). Optimum concentration of Bacillus megaterium for strengthening structural concrete. *Construction and Building Materials*, *118*, 180–193. https://doi.org/10.1016/j.conbuildmat.2016.04.142

Arce, G. A., Hassan, M. M., Mohammad, L. N., & Rupnow, T. (2017). Characterization of Self-Healing Processes Induced by Calcium Nitrate Microcapsules in Cement Mortar. *Journal of Materials in Civil Engineering*, *29*(1), 04016189. https://doi.org/10.1061/(asce)mt.1943-5533.0001717

Arce, G., Noorvand, H., Hassan, M., Rupnow, T., & Hungria, R. (2019). Cost-Effective ECC with Low Fiber Content for Pavement Application. *MATEC Web of Conferences*, *271*, 07001. https://doi.org/10.1051/matecconf/201927107001

Bang, S. S., Galinat, J. K., & Ramakrishnan, V. (2001). Calcite precipitation induced by polyurethane-immobilized Bacillus pasteurii. *Enzyme and Microbial Technology*, *28*(4), 404–409. https://doi.org/https://doi.org/10.1016/S0141-0229(00)00348-3

Basheer, L., Kropp, J., & Cleland, D. J. (2001). Assessment of the durability of concrete from its permeation properties: A review. *Construction and Building Materials*, *15*(2–3), 93–103. https://doi.org/10.1016/S0950-0618(00)00058-1

Bashir, J. (2016). Bio Concrete- The Self-Healing Concrete. *Indian Journal of Science and Technology*, *9*(1), 1–5. https://doi.org/10.17485/ijst/2016/v9i47/105252

Bhavya, K., & Sanjeev, Dr. N. (2017). Effect of Different Types of Coarse Aggregates on Physical Properties of Mostly Used Grades M20, M25, M30 of Concrete. *IOSR Journal of Mechanical and Civil Engineering*, *14*(01), 46–51. https://doi.org/10.9790/1684-1401024651

Brinkman, L., & Miller, S. A. (2021). Environmental impacts and environmental justice implications of supplementary cementitious materials for use in concrete. *Environmental*

Research: Infrastructure and Sustainability, *1*(2), 025003. https://doi.org/10.1088/2634-4505/ac0e86

Buller, A. S., Abro, F. U. R., Lee, K. M., & Jang, S. Y. (2019). Mechanical recovery of cracked fiber-reinforced mortar incorporating crystalline admixture, expansive agent, and geomaterial. *Advances in Materials Science and Engineering*, *2019*. https://doi.org/10.1155/2019/3420349

Busch, P., Kendall, A., Murphy, C. W., & Miller, S. A. (2022). Literature review on policies to mitigate GHG emissions for cement and concrete. *Resources, Conservation and Recycling*, *182*(February), 106278. https://doi.org/10.1016/j.resconrec.2022.106278

De Belie, N. (2016). Application of bacteria in concrete: a critical evaluation of the current status. *RILEM Technical Letters*, *1*, 56. https://doi.org/10.21809/rilemtechlett.2016.14

De Belie, N., Gruyaert, E., Al-Tabbaa, A., Antonaci, P., Baera, C., Bajare, D., Darquennes, A., Davies, R., Ferrara, L., Jefferson, T., Litina, C., Miljevic, B., Otlewska, A., Ranogajec, J., Roig-Flores, M., Paine, K., Lukowski, P., Serna, P., Tulliani, J. M., … Jonkers, H. M. (2018). A Review of Self-Healing Concrete for Damage Management of Structures. In *Advanced Materials Interfaces* (Vol. 5, Issue 17). Wiley-VCH Verlag. https://doi.org/10.1002/admi.201800074

Edvardsen, C. (1999). Water permeability and autogenous healing of cracks in concrete. *ACI Materials Journal*, *96*(4). https://doi.org/10.14359/645

Erşan, Y. Ç., Hernandez-Sanabria, E., Boon, N., & De Belie, N. (2016). Enhanced crack closure performance of microbial mortar through nitrate reduction. *Cement and Concrete Composites*, *70*, 159–170. https://doi.org/10.1016/j.cemconcomp.2016.04.001

Fahimizadeh, M., Abeyratne, A. D., Mae, L. S., Raman Singh, R. K., & Pasbakhsh, P. (2020). Biological self-healing of cement paste and mortar by non-ureolytic bacteria encapsulated in alginate hydrogel capsules. *Materials*, *13*(17). https://doi.org/10.3390/MA13173711

Gardner, D., Jefferson, A., Hoffman, A., & Lark, R. (2014). Simulation of the capillary flow of an autonomic healing agent in discrete cracks in cementitious materials. *Cement and Concrete Research*, *58*, 35–44. https://doi.org/10.1016/j.cemconres.2014.01.005

Glatz, B. A., & Anderson, K. I. (1988). Isolation and Characterization of Mutants of Propionibacterium Strains. *Journal of Dairy Science*, *71*(7), 1769–1776. https://doi.org/10.3168/jds.S0022-0302(88)79744-1

Hassan, M., Milla, J., Rupnow, T., & Soysal, A. (2019). *Self-Healing Concrete using Encapsulated Bacterial Spores in a Simulated Hot Subtropical Climate*. https://orcid.org/0000-0001-9144-8545

Hearn, N. (1998). Self-sealing, autogenous healing and continued hydration: What is the difference? In *Materials and Structures/Mat~riaux et Constructions* (Vol. 31).

Hilloulin, B., Van Tittelboom, K., Gruyaert, E., De Belie, N., & Loukili, A. (2015). *Design of polymeric capsules for self-healing concrete Design of polymeric capsules for self-healing concrete 1 2*. *55*, 298–307. https://doi.org/10.1016/j.cemconcomp.2014.09.022ï

Hizami Abdullah, M. A., Harmiza Abdullah, N. A., & Tompang, M. F. (2018). Development and Performance of Bacterial Self-healing Concrete - A Review. *IOP Conference Series: Materials Science and Engineering*, *431*(6). https://doi.org/10.1088/1757-899X/431/6/062003

Hong, G., Song, C., & Choi, S. (2020). Autogenous healing of early-age cracks in cementitious materials by superabsorbent polymers. *Materials*, *13*(3). https://doi.org/10.3390/ma13030690

Houtsma, P. C., Kant-Muermans, M. L., Rombouts, F. M., & Zwietering, M. H. (1996). Model for the combined effects of temperature, pH, and sodium lactate on growth rates of Listeria innocua in broth and bologna-type sausages. *Applied and Environmental Microbiology*, *62*(5), 1616–1622. https://doi.org/10.1128/aem.62.5.1616-1622.1996

Hungria, R., Hassan, M. M., Arce, G., Mousa, M., King, G., Milla, J., & Rupnow, T. (2023). Optimizing the Self-Healing Efficiency of Hydrogel-Encapsulated Bacteria in Concrete. *Journal of Materials in Civil Engineering*, *35*(4). https://doi.org/10.1061/(ASCE)MT.1943-5533.0004709

Hungria, R., Mousa, M., Hassan, M., Omar, O., Gavilanes, A., Arce, G., Milla, J., & King, G. (2021). Effect of Using Magnesium Acetate on the Self-Healing Efficiency of Hydrogel-Encapsulated Bacteria in Concrete. *Tran-SET 2021 - Proceedings of the Tran-SET Conference 2021*, 163–175. https://doi.org/10.1061/9780784483787.018

Irwan, J. M., Anneza, L. H., Othman, N., Faisal Alshalif, A., Zamer, M. M., & Teddy, T. (2016). Calcium Lactate addition in Bioconcrete: Effect on Compressive strength and Water penetration. *MATEC Web of Conferences*, *78*, 0–5. https://doi.org/10.1051/matecconf/20167801027

Italia, H., Patel, I., & Shah, J. (2016). Experimental Study of Bacterial Self-healing Effect on Concrete: A Review. In *Journal of Civil Engineering and Environmental Technology* (Vol. 3, Issue 1). http://www.krishisanskriti.org/Publication.html

Jonkers, H. (2011). *Bacteria-based self-healing concrete* (Vol. 56, Issue 1).

Jonkers, H. M., Thijssen, A., Muyzer, G., Copuroglu, O., & Schlangen, E. (2010). Application of bacteria as self-healing agent for the development of sustainable concrete. *Ecological Engineering*, *36*(2), 230–235. https://doi.org/10.1016/j.ecoleng.2008.12.036

Jose, M., Alonso, C., Eloir, C., Ortiz, L., Omar, S., Perez, G., Narayanasamy, R., Fajardo, J., & Miguel, S. (2018). *Improved strength and durability of concrete through metabolic activity of ureolytic bacteria*. *25*(22), 21451–21458. https://doi.org/10.1007/s11356-017-9347-0

Joshi, S., Goyal, S., Mukherjee, A., & Reddy, M. S. (2017). Microbial healing of cracks in concrete: a review. *Journal of Industrial Microbiology and Biotechnology*, *44*(11), 1511–1525. https://doi.org/10.1007/s10295-017-1978-0

Juliafad, E., Rani, I. G., Rifwan, F., & Yose Fajar, P. (2019). Concreting workmanship in Indonesia study case: Padang City, West Sumatra, Indonesia. *International Journal on Advanced Science, Engineering and Information Technology*, *9*(1), 300–306. https://doi.org/10.18517/ijaseit.9.1.7201

Kastiukas, G., Zhou, X., Castro-Gomes, J., Huang, S., & Saafi, M. (2015). Effects of lactic and citric acid on early-age engineering properties of Portland/calcium aluminate blended cements. *Construction and Building Materials*, *101*(December), 389–395. https://doi.org/10.1016/j.conbuildmat.2015.10.054

Kim, H. K., Park, S. J., Han, J. I., & Lee, H. K. (2013). Microbially mediated calcium carbonate precipitation on normal and lightweight concrete. *Construction and Building Materials*, *38*, 1073–1082. https://doi.org/10.1016/j.conbuildmat.2012.07.040

Krishnapriya, S., Venkatesh Babu, D. L., & G., P. A. (2015). Isolation and identification of bacteria to improve the strength of concrete. *Microbiological Research*, *174*, 48–55. https://doi.org/10.1016/j.micres.2015.03.009

Kumari, C., Das, B., Jayabalan, R., Davis, R., & Sarkar, P. (2016). *Effect of Nonureolytic Bacteria on Engineering Properties of Cement Mortar Effect of Nonureolytic Bacteria on Engineering Properties of Cement Mortar*. *October 2017*. https://doi.org/10.1061/(ASCE)MT.1943-5533.0001828

Leroux, L., Hatim, Z., Frèche, M., & Lacout, J. L. (1999). Effects of various adjuvants (lactic acid, glycerol, and chitosan) on the injectability of a calcium phosphate cement. *Bone*, *25*(2 SUPPL. 1), 31–34. https://doi.org/10.1016/S8756-3282(99)00130-1

Li, V. C., & Herbert, E. (2012). Robust self-healing concrete for sustainable infrastructure. *Journal of Advanced Concrete Technology*, *10*(6), 207–218. https://doi.org/10.3151/jact.10.207

Li, V. C., & Yang, E.-H. (2007). Self Healing in Concrete Materials. In S. van der Zwaag (Ed.), *Self Healing Materials: An Alternative Approach to 20 Centuries of Materials Science* (pp. 161–193). Springer Netherlands. https://doi.org/10.1007/978-1-4020-6250-6_8

Mihashi, H., & Nishiwaki, T. (2012). Development of engineered self-healing and self-repairing concrete-state-of-the-art report. In *Journal of Advanced Concrete Technology* (Vol. 10, Issue 5, pp. 170–184). https://doi.org/10.3151/jact.10.170

Milla, J., Hassan, M. M., Rupnow, T., & Daly, W. H. (2019). Measuring the crack-repair efficiency of steel fiber reinforced concrete beams with microencapsulated calcium nitrate. *Construction and Building Materials*, *201*, 526–538. https://doi.org/10.1016/j.conbuildmat.2018.12.193

Mohammad, I., Khattab, A., Shekha, H., & Abdi, M. A. (2019). *Study on Self-healing Concrete types-A review*. *2*(1), 76–87. https://doi.org/10.26392/SSM.2019.02.01.076

Mors, R. M., & Jonkers, H. M. (2017). Feasibility of lactate derivative based agent as additive for concrete for regain of crack water tightness by bacterial metabolism. *Industrial Crops and Products*, *106*, 97–104. https://doi.org/10.1016/j.indcrop.2016.10.037

Mote, S., & Ghodke, P. (2018). THE SELF-HEALING CONCRETE-A REVIEW Anaerobic Digestion of MSW leachate View project THE SELF-HEALING CONCRETE-A REVIEW. In *International Journal of Advances in Engineering & Technology* (Vol. 11). https://www.researchgate.net/publication/328094234

Muynck, W. De, & Verstraete, W. (2008). *Bacterial carbonate precipitation as an alternative surface treatment for concrete*. *22*, 875–877. https://doi.org/10.1016/j.conbuildmat.2006.12.011

Neville, A. (2002). Autogenous healing: a concrete miracle? *Concrete International*.

Omar, O., Mousa, M., Hassan, M., Hungria, R., Gavilanes, A., Arce, G., Milla, J., & Rupnow, T. (2021). Vacuum impregnation of bacillus pseudofirmus into fine lightweight aggregate. *Tran-SET 2021 - Proceedings of the Tran-SET Conference 2021*, 365–372. https://doi.org/10.1061/9780784483787.036

Omar, O., Mousa, M. R., Hassan, M., Hungria, R., Gavilanes, A., Arce, G., Milla, J., & Rupnow, T. (2022). Optimization of the Self-Healing Efficiency of Bacterial Concrete Using Impregnation of Three Different Precursors into Lightweight Aggregate. *Transportation Research Record: Journal of the Transportation Research Board*, 036119812211265. https://doi.org/10.1177/03611981221126513

Omar, O., Noorvand, H., Hassan, M., Asce, M., Subedi, S., Milla, J., Rupnow, T., Asce 6 1 Graduate, M., Assistant, R., & Turner, B. S. (2022). *Effect of Using Calcium Lactate with Bacillus pseudofirmus Bacteria on Self-Healing Efficiency of Bacterial Concrete*.

Palin, D., Wiktor, V., & Jonkers, H. M. (2016). A bacteria-based bead for possible self-healing marine concrete applications. *Smart Materials and Structures*, *25*(8). https://doi.org/10.1088/0964-1726/25/8/084008

PCA. (2002). Types and Causes of Concrete Deterioration. *Portland Cement Association, PCA R & D Se*, 1–16. https://www.cement.org/docs/default-source/fc_concrete_technology/durability/is536-types-and-causes-of-concrete-deterioration.pdf?sfvrsn=4&sfvrsn=4

Rajczakowska, M., Habermehl-Cwirzen, K., Hedlund, H., & Cwirzen, A. (2019). The Effect of Exposure on the Autogenous Self-Healing of Ordinary Portland Cement Mortars. *Materials*, *12*(23), 3926. https://doi.org/10.3390/ma12233926

Ramachandran, S. K., Ramakrishnan, V., & Bang, S. S. (2001). Remediation of Concrete Using Microorganisms. *ACI Materials Journal*, *98*, 3–9. https://doi.org/10.14359/10154

Rooij, D., R., M., & Schlange, E. (2011). *"Self-healing phenomena in cement-based materials." Draft of State-of-the-Art Report of RILEM Technical Committee 221-SHC.*

Schreiberová, H., Bílý, P., Fládr, J., Šeps, K., Chylík, R., & Trtík, T. (2019). Impact of the self-healing agent composition on material characteristics of bio-based self-healing concrete. *Case Studies in Construction Materials*, *11*. https://doi.org/10.1016/j.cscm.2019.e00250

Seifan, M., Samani, A. K., & Berenjian, A. (2016). Bioconcrete: next generation of self-healing concrete. In *Applied Microbiology and Biotechnology* (Vol. 100, Issue 6, pp. 2591–2602). Springer Verlag. https://doi.org/10.1007/s00253-016-7316-z

Shaikh, A., & John, R. (2017). *Self-Healing Concrete By Bacterial And Chemical Admixtures*. http://www.ijser.org

Sharma, T. K., Alazhari, M., Heath, A., Paine, K., & Cooper, R. M. (2017). *Alkaliphilic Bacillus species show potential application in concrete crack repair by virtue of rapid spore production and germination then extracellular calcite formation*. https://doi.org/10.1111/jam.13421

Silva, D. M. G. da, Cappellesso, V. G., Garcia, M. G. L., Masuero, A. B., & Molin, D. C. C. D. (2021). Calcium hydroxide influence in autogenous self-healing of cement-based materials in various environmental conditions. *Ambiente Construído*, *21*(2), 209–224. https://doi.org/10.1590/s1678-86212021000200522

Singh, N. B., Singh, S. P., & Singh, A. K. (1986). EFFECT OF LACTIC ACID ON THE HYDRATION OF PORTLAND CEMENT. In *CEMENT and CONCRETE RESEARCH* (Vol. 16).

Sisomphon, K., Copuroglu, O., & Fraaij, A. (2011). Application of encapsulated lightweight aggregate impregnated with sodium monofluorophosphate as a self-healing agent in blast furnace slag mortar. *Heron*, *56*(1–2), 17–36.

Souradeep, G., & Kua, H. W. (2016). *Encapsulation Technology and Techniques in Self-Healing Concrete*. https://doi.org/10.1061/(ASCE)MT.1943-5533

Soysal, A., Milla, J., King, G. M., Hassan, M., & Rupnow, T. (2020). Evaluating the Self-Healing Efficiency of Hydrogel-Encapsulated Bacteria in Concrete. *Transportation Research Record*, *2674*(6), 113–123. https://doi.org/10.1177/0361198120917973

Susanto, S. A., Hardjito, D., & Antoni, A. (2021). Review of autonomous self-healing cementitious material. *IOP Conference Series: Earth and Environmental Science, 907*(1). https://doi.org/10.1088/1755-1315/907/1/012006

Tziviloglou, E., Wiktor, V., Jonkers, H. M., & Schlangen, E. (2016). Bacteria-based self-healing concrete to increase liquid tightness of cracks. *Construction and Building Materials, 122,* 118–125. https://doi.org/10.1016/j.conbuildmat.2016.06.080

Tziviloglou, E., Wiktor, V., Jonkers, H. M., & Schlangen, E. (2017). Selection of nutrient used in biogenic healing agent for cementitious materials. *Frontiers in Materials*, *4*(June), 1–7. https://doi.org/10.3389/fmats.2017.00015

Van Tittelboom, K., & De Belie, N. (2013). Self-healing in cementitious materials-a review. *Materials*, *6*(6), 2182–2217. https://doi.org/10.3390/ma6062182

Vijay, K., & Murmu, M. (2019). Effect of calcium lactate on compressive strength and self-healing of cracks in microbial concrete. *Frontiers of Structural and Civil Engineering*, *13*(3), 515–525. https://doi.org/10.1007/s11709-018-0494-2

Vijay, K., Murmu, M., & Deo, S. V. (2017). Bacteria based self healing concrete – A review. In *Construction and Building Materials* (Vol. 152, pp. 1008–1014). Elsevier Ltd. https://doi.org/10.1016/j.conbuildmat.2017.07.040

Wahyudin, U., Hariri, R. El, & Darmawan, D. (2013). Developing Image of Higher Education Performance. *International Journal of Communications, Network and System Sciences*, *06*(09), 407–413. https://doi.org/10.4236/ijcns.2013.69044

Wang, J., Dewanckele, J., Cnudde, V., Van Vlierberghe, S., Verstraete, W., & De Belie, N. (2014). X-ray computed tomography proof of bacterial-based self-healing in concrete. *Cement and Concrete Composites*, *53*, 289–304. https://doi.org/10.1016/j.cemconcomp.2014.07.014

Wang, J., Mignon, A., Snoeck, D., Wiktor, V., Van Vliergerghe, S., Boon, N., & De Belie, N. (2015). Application of modified-alginate encapsulated carbonate producing bacteria in concrete: A promising strategy for crack self-healing. *Frontiers in Microbiology*, *6*(OCT), 1–14. https://doi.org/10.3389/fmicb.2015.01088

Wang, J., Van Tittelboom, K., De Belie, N., & Verstraete, W. (2012). Use of silica gel or polyurethane immobilized bacteria for self-healing concrete. *Construction and Building Materials*, *26*(1), 532–540. https://doi.org/10.1016/j.conbuildmat.2011.06.054

Wang, J. Y., De Belie, N., & Verstraete, W. (2012). Diatomaceous earth as a protective vehicle for bacteria applied for self-healing concrete. *Journal of Industrial Microbiology and Biotechnology*, *39*(4), 567–577. https://doi.org/10.1007/s10295-011-1037-1

Wang, J. Y., Snoeck, D., Van Vlierberghe, S., Verstraete, W., & De Belie, N. (2014). Application of hydrogel encapsulated carbonate precipitating bacteria for approaching a realistic self-healing in concrete. *Construction and Building Materials*, *68*, 110–119. https://doi.org/10.1016/j.conbuildmat.2014.06.018

Wang, J. Y., Soens, H., Verstraete, W., & De Belie, N. (2014). Self-healing concrete by use of microencapsulated bacterial spores. *Cement and Concrete Research*, *56*, 139–152. https://doi.org/10.1016/j.cemconres.2013.11.009

Wiktor, V., & Jonkers, H. M. (2011). Quantification of crack-healing in novel bacteria-based self-healing concrete. *Cement and Concrete Composites*, *33*(7), 763–770. https://doi.org/10.1016/j.cemconcomp.2011.03.012

Wiktor, V., & Jonkers, H. M. (2016). Bacteria-based concrete: From concept to market. *Smart Materials and Structures*, *25*(8). https://doi.org/10.1088/0964-1726/25/8/084006

Winter, N. B. (2012). *Scanning electron microscopy of cement and concrete*. Woodbridge : WHD Microanalysis Consultants Ltd. http://lib.ugent.be/catalog/rug01:002084226

Wu, M., Johannesson, B., & Geiker, M. (2012). A review: Self-healing in cementitious materials and engineered cementitious composite as a self-healing material. *Construction and Building Materials*, *28*(1), 571–583. https://doi.org/10.1016/j.conbuildmat.2011.08.086

Zhang, P., Ling, Y., Wang, J., & Shi, Y. (2019). Bending resistance of PVA fiber reinforced cementitious composites containing nano-SiO2. *Nanotechnology Reviews*, *8*(1), 690–698. https://doi.org/10.1515/ntrev-2019-0060

Zhang, W., Zheng, Q., Ashour, A., & Han, B. (2020). Self-healing cement concrete composites for resilient infrastructures: A review. *Composites Part B: Engineering*, *189*, 107892. https://doi.org/10.1016/J.COMPOSITESB.2020.107892

VITA

Omar K. Omar was born in 1991 in Cairo, Egypt. In 2014, he finished his Bachelor of Science in Civil Engineering from Cairo University, Egypt. From 2014 to 2019, he worked on highly demanding mega projects in Egypt. In 2020, he pursued his Ph.D. in Engineering Science at Louisiana State University. Meanwhile, he plans to receive his Master of Engineering Science in August,2023. His research interest includes sustainable construction materials, concrete materials, material characterization, novel materials, concrete pavement, and self-healing concrete.