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The effect of microbiological agents on the efficiency of bio-based repair systems for concrete

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THE EFFECT OF MICROBIOLOGICAL AGENTS ON THE EFFICIENCY OF BIO-BASED REPAIR SYSTEMS FOR CONCRETE

by

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Abstract

The induction of calcium precipitation via bacterial action has been studied increasingly in past years for self-(healing/sealing) concrete applications. Several of these studies have presented promising conclusions that microbiologically induced calcite precipitation might be a useful approach for remediation and rehabilitation of shallow cracks on existing structures. Such studies have noted the necessity to encapsulate the ingredients (bacteria, nutrients and organic precursors) separately for self-healing concrete using microbiologically induced calcite precipitation. However, during mixing there is a chance that capsules or other carriers of self-healing agents may release their cargoes and affect the properties of the concrete.

Based on the above-mentioned information, the objective of this research was to evaluate whether or not shallow concrete cracks can be remediated using a bacteria-based system of repair. This research also aims to develop a new bacterial agent for use in the remediation of concrete cracks and to understand the effect of bacterial agents on the properties of cement-based mortar. The scope of this research is diverse; it requires an understanding of the factors that affect durability, water permeability, and cement properties such as initial and final setting time as well as the quality and quantity of the precipitated materials from the bacteria-based healing/sealing system.

This research is broadly divided into four stages. In spite of a number of studies on the mechanism and efficiency of bacterial self-(healing/sealing) concrete, stage one investigates the effect of bacterial self-(healing/sealing) agents on the properties of fresh and hardened concrete. This information is critical for further research and implementation of this novel material. As with any additives, this may have a negative effect on the concrete's final properties. This will be viewed with skepticism and limited uptake.

This stage included the effects of the self-(healing/sealing) agents individually and as a combined medium on the mechanical properties of fresh concrete, the hydration kinetics, and early and final setting times, as well as strength and microstructure development over time. In addition, the effects of self-(healing/sealing) agents on hardened concrete were investigated to determine whether or not capsule rupture in response to a crack would have a detrimental effect on concrete properties in the area surrounding the crack. The results showed that self-(healing/sealing) agents such as sodium citrate greatly influenced hydration kinetics when the concentration exceeded 0.05% of the cement mass. Although the self-(healing/sealing) agents at 0.5% by binder mass retarded lightly of setting time, they had little

negative effect on either 3- or 28-day strength. Calcium acetate, the dominant self-(healing/sealing) agent, acts as an accelerator while other components of the medium can have detrimental effects on the properties of fresh and hardened concrete. However, provided the quantity of self-healing/sealing agents released is below a certain threshold, it is unlikely that any detrimental effects will limit the application of bacterial self-healing/sealing concrete.

Stage two included applying the main components of the self-sealing agents (calcium lactate and yeast extract) with the ingredients of the mortar mix and as a combined medium with mortar mix (two-stage bio-concrete/mortar) to investigate the ability of *B. cohnii, B. halodurans* and *B. pseudofirmus* to induce calcite precipitation through the cracks. The results showed that the sealing materials using each one of the three bacteria with the main components of self-sealing agents were very weak and were not distributed along the crack. Moreover, the primary components of self-sealing agents in 5% bio-cement mortar of the combined medium by binder mass distributed in entire samples were unable to seal cracks. Results showed that the samples dissolved in water, meaning that with more self-healing agent (SHA-1) ingredients added to the bio-mortar, the weaker and more ineffective it was with cement.

Remediating cracks of hardening concrete with three different types of bacteria (*B. cohnii, B. halodurans*, and *B. pseudofirmus*) was performed during Stage three. Factors affecting the quantity and quality of healed materials included start and end healing time, the growth of each bacterium, and the viability of each bacterium in alkaline environment, all of which were also studied experimentally. Results showed that the three bacteria can produce calcium carbonate in a self-healing/sealing process, although *B. pseudofirmus* is the most suitable, efficient, and economical for remediating concrete cracks.

The delivery of bacteria spores inside the concrete environment has always been the most challenging task. The main objective of stage four is to study the possibility of using successful previous delivery system used to remediate concrete cracks by using mineral agent to be used as delivery systems of bacteria spores. This study investigated three different encapsulate techniques within cement mortar namely calcium alginate beads (CAB), vascular tubes, and perlite.

Results showed that CAB is very weak and very light due to low density, which cause decrease in their size, floating on the surface of mortar and poor distribution in mortar matrix.

In spite of some passive mode effect variables such as tube length and diameter, the viscosity of bacteria solutions and their agents (SHA) and the ability of the glass tube to resist internal stresses were investigated. The results showed there was not enough data to demonstrate its ability to heal cracks.

The mechanical and physical properties of uncoated and coated perlite, the ability of perlite to carry bacteria and its SHA, and the ability of bacteria and its SHA to form calcite out of perlite were investigated. The results demonstrated the ability of perlite to inoculate bacteria and its SHA, and the ability of this system to heal cracks. It is clear from the above perlite is the most suitable delivery system.

Publications

The following are publications on which the author is named:

1- Sharma, T., Alazhari, M., Cooper, R., Heath, A., Paine, K. The Requirements for autonomic microbiologically-induced calcite-precipitation in concrete. 5th International Conference on Self-Healing Materials, Durham, USA, June 2015.

2- Alazhari, M., Sharma, T., Cooper, R., Heath, A., Paine, K. Effect of bacterial self-healing agents on the early-age properties of cementitious materials. 35th Cement & Concrete Science Conference (CCSC35), Aberdeen, UK, 26-28TH August 2015.

3- Eirini Tziviloglou, Virginie Wiktor, Jianyun Wang, Kevin Paine, Mohamed Alazhari, Alan Richardson, Marielle Gueguen, Nele De Belie, Erik Schlangen and Henk Jonkers. Evaluation of experimental methodology to assess the sealing efficiency of bacteria-based self-healing mortar: Round robin test. RILEM Conference on Microorganisms-Cementitious Materials Interactions, June 2016.

4- Paine, K., Alazhari, M., Sharma, T., Cooper, R., Heath, A. Design and performance of bacteria-based self-healing concrete, 9th International Concrete Conference 2016: Environment, Efficiency and Economic Challenges for Concrete, Dundee, UK, July 2016.

5- Sharma, T., Alazhari, M., Cooper, R., Heath, A., Paine, K. Alkaliphilic *Bacillus* species show potential application in concrete crack repair by virtue of rapid spore production and germination then extracellular calcite formation. Journal of applied microbiology, February 2017.

6- Alazhari, M., Chen, X., Sharma, T., Cooper, R., Heath, A., Paine, K. Effect of microbiological growth components for bacteria-based self-healing on the properties of cement mortar.(Manuscript).

7- Alazhari, M., Sharma, T., Cooper, R., Heath, A., Paine, K. Relation between duration of healing and quantities of calcium carbonate produced by bacteria with self-healing agent.(Manuscript).

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Statement of Originality

I hereby certify that all of the work described within this thesis is the original work of the author. Any published (or unpublished) ideas and/or techniques from the work of others are fully acknowledged in accordance with the standard referencing practices.

(Mohamed S. A. Alazhari)

(February, 2017)

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List of abbreviations

SHC Self-healing concrete.

OPC Ordinary Portland cement.

ECC Engineered cementitious composites.

SAP Super absorbent polymer.

HPP Heat plasticity pipe.

SMA Shape memory alloy.

BBSHC Bacterial-based self-healing concrete.

MICP Microbially induced calcium carbonate.

MCB Mineralization controlled by biological.

MIB Mineralization induced biologically.

MICCP Microbially induced calcium carbonate precipitation.

DIC Dissolved inorganic carbon.

JCI Japan Concrete Institute.

SEM Scanning electron microscopy.

HVFA High volumes of fly ash.

ESEM Environment scanning electronic microscope.

ISAT Initial surface absorption test.

SHA Self-healing agents.

ρd Apparent density.

i Water uptake.

t Time.

SC Sorption coefficient.

SE Second electrons.

BSE Back scattered electrons.

CL Cathodoluminescence light.

CFI Colony forming unite.

GLB Glass laboratory bottle.

TSBM Two stage bio-mortar.

OD_{600} Optical density at 600.

TSRM Two stage remediation method.

LVDT Linear variable differential transformer.

CFRP Carbon fiber reinforced polymer.

CABC Calcium alginate beads concentration.

CAB Calcium alginate beads.

CT Scan computerized tomography.

ARFM Alkali resistant fiberglass mesh.

FTIR Fourier transform infrared spectroscopy.

CFU Colony forming unit.

E The amount and quality of produced healing/sealing materials.

Cp The amount of calcium carbonate healed/sealed by each bacterium.

T The range of temperature required for growth each bacterium.

Qv The quality of healing/sealing calcium carbonate.

V The viability of each bacterium in alkaline environment.

P/T The amount of calcium carbonate to time of healing/sealing.

Chapter 1

Introduction

1.1 Background

The word concrete comes from the Latin expression “concretus” which means compounded. It was used by the ancient Romans in construction of walls and roofs, and it is a heterogeneous composite material with the following constituents: cement, coarse aggregate, fine aggregate, and water.

There are two types of concrete used in the world: ordinary plain concrete which is made by mixing cement, fine aggregate and coarse aggregate with water; when steel reinforcement is added to plain concrete, it is called reinforced concrete[1]. Concrete is the most commonly used building material in the world. It is durable, available everywhere and strong and is the cheapest man-made construction material to produce and recycle[1]. Unfortunately, concrete is susceptible to many different types of damage which result in cracks. These cracks can be broadly classified as (i) structural cracks which come from design defects, construction and supervision problems; and (ii) non-structural cracks caused by ambient conditions e.g. (temperature, humidity) and/or quality of materials. Ambient conditions cause damage to concrete by freeze/thaw cycles, chemical attacks, corrosion, extreme loads and other environmental factors. Consequently, maintenance of concrete structures is recurrent and costly. Many countries in the world have spent billions of dollars every year on maintaining infrastructure such as buildings and bridges. 50% of the annual construction budget in Europe has been allocated to spend on rehabilitation and repair of the existing structure. While, 5.3 billion dollars is the average cost of bridge maintenance and repair in US[2]. Furthermore, in UK almost half of construction budget which is 80 billion pounds per a year, is estimated to be spent on repair and maintenance of existing structures[3]. These countries have also developed more durable materials and this means less frequent repairs very appealing[4].

The durability of concrete is greatly affected by the corrosion of reinforcement inside the concrete, which usually happens due to one of the aggressive agents attacks such as, when chloride penetrates concrete particularly through its cracks and breaks down the protective layer around the reinforcement. The effects of corrosion on the properties of concrete is the loss of the bond between the concrete and the steel, increasing the size of steel inside the concrete which leads to a reduce the serviceability of concrete[5].

Therefore, there have been many attempts to reduce the number and size of cracks that appear in concrete. For example, selecting high quality raw materials, having a reasonable design of mix

proportion, creating appropriate curing conditions, and having good operation structural management. However, it is impossible to prevent cracks from appearing in concrete. Over the past 25 years' researchers have attempted to develop self-healing concrete (SHC) that would alleviate costs and increase the lifespan of structures. Many researchers[6,7] have observed that some types of cracks disappear after a while, especially those that are micro sized. This type of healing is called 'intrinsic self-healing' and is common in new age concrete. However, this type of healing does not meet the durability required of concrete. Intensive researches have been done on intelligent material, which can heal cracks automatically but only once the cracks occur.

Researches on self-healing concrete were begun by Dry[8–12] in 1990; since then much work has been done to develop the first type of concrete that can heal itself. Many researchers over the last two decades have achieved huge progress in this area and have classified self-healing into two main categories: autogenous and autonomic healing[13–15]. Autonomic healing is classified into two common ways to carry the healing agent: spherical or cylindrical capsule-based self-healing and active or passive vascular-based self-healing. Once a crack appears, capsules rupture or vascular structures break and the healing agent released. The agent is transported to the crack by capillary stress or gravity.

One approach to autonomic self-healing is developing bacterial spores, nutrients, and calcium lactate to be utilized as self-healing agents for microbiologically-induced calcite-precipitation[16]. Investigations on this approach started in the late 2000's[17]. This inspired the creation of two main categories: the first achieved the bio-deposition of calcite[18,19], while the second mixed bacteria and a self-healing agent, such as calcium lactate (called calcite producer), into a cement matrix to achieve self-healing by activating bacteria and its precursors on the fracture's surface.

Researchers are developing self-healing agents (such as calcium lactate) that will be embedded in capsules with bacteria, ensuring that the agents will not be activated during the cement mixing process in order to prevent interaction before cracks appear. The main two categories of delivery are encapsulation and impregnation. Wang[20,21] demonstrates the ability of microcapsules to be used as a delivery system for bacteria and its nutrients and precursors. Instead of microcapsules, Wiktor and Jonkers[22] use light weight aggregates (LWA) as carriers of a self-healing agent and bacteria, which works as a protective. Therefore, when cracks cross through the LWA - which are placed in the weakest part of the concrete – both the bacteria and the self-healing agent produce calcite on the crack face.

Self-healing using bacteria is an innovative subject and many aspects are still under study. Jonkers et al.[23] investigate the resistance of bacteria to the harsh environment of concrete. Their results present the promising prospect of using bacteria for self-healing concrete. The maximum crack width that

could be healed by bacteria is 0.46 mm[22]. However, Bundur et al.[24] investigate the effect of cement hydration kinetics and the strength of bacterial self-healing concrete design, and the results show high influence for both kinetic hydrations and strength with a UYE medium and a concentration of bacteria.

1.2 Problem statement

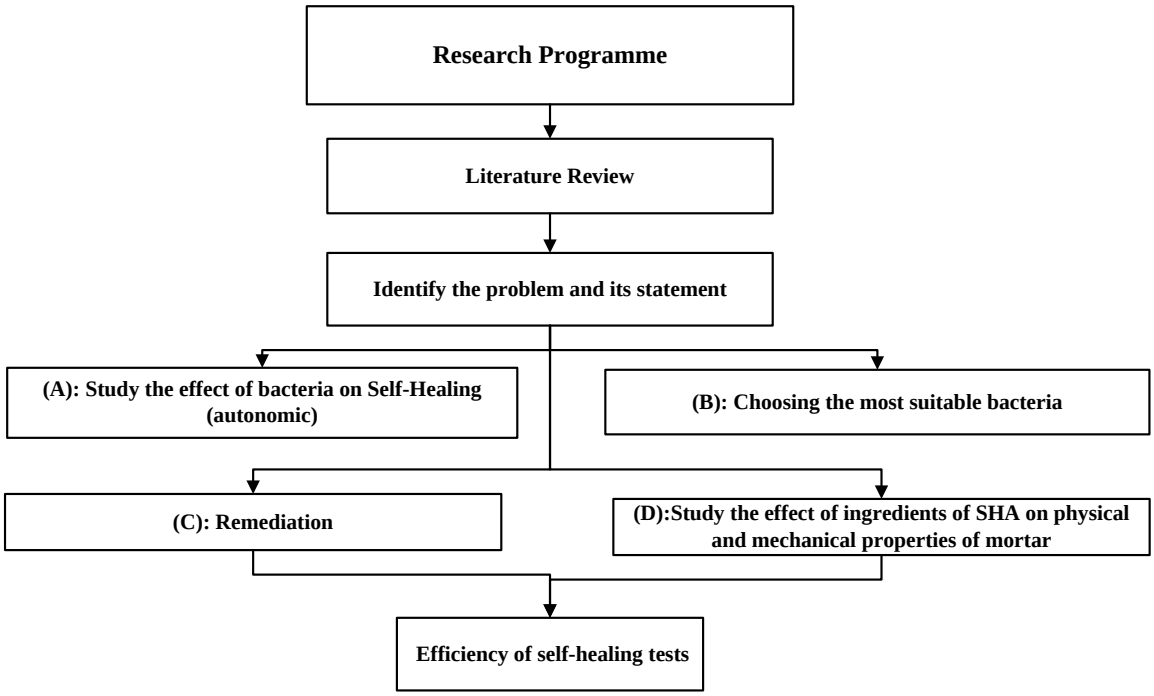
Cracks in the surface layer of concrete mainly reduce its durability, so there is a need to develop new techniques to overcome the cracks in concrete structures. There has been an attempt to address the environmental effects associated with repeated maintenance of the structures by using Portland cement. An effort in this regard is the development of self-healing concrete based on calcium carbonate precipitation by using selected bacteria. *However, more recently, materials that have the ability to adapt and respond to their environment have been developed. In concrete, at the meso-scale, research into the use of alkali-resistant spore-forming bacteria to heal cementitious materials is ongoing. This approach utilizes the metabolic activity of bacteria and mineral precursors embedded within the concrete to form inorganic inert calcium carbonate that seals cracks, prevents ingress of deleterious chemical species, and provides strength recovery. However, whilst the mechanism has been demonstrated in idealised laboratory conditions the extent of the healing/sealing that is possible and the long-term viability of the approach is still to be determined. This will be investigated in this research. The outputs of the research will lead to a resilient infrastructure that can adapt, sense and respond to damage, and which has the potential to reduce or eliminate both environmentally and economically costly repair and maintenance activities*.

1.3 Research objectives and methodology

The objective of this research is to investigate self-healing properties in cementitious materials that can be used for construction applications by using calcium carbonate precipitation built-in bacterial concrete. The research also aims to understand the behavior of bacteria and bacterial self-healing agents (SHA) were used as remediation material in concrete structural elements. The properties of this concrete will be studied, such as, survivability of three types of *bacillus* bacterial spores in concrete, the optimum amount of calcium carbonate which can be produced by these types of bacteria, the ability of three types of *bacillus* bacteria to heal/seal cracks, the durability of calcium carbonate that is produced by these types of bacteria, the effect of different concentrations of bacteria on the durability of concrete, the efficiency of bacteria when suspended in different SHA, etc.

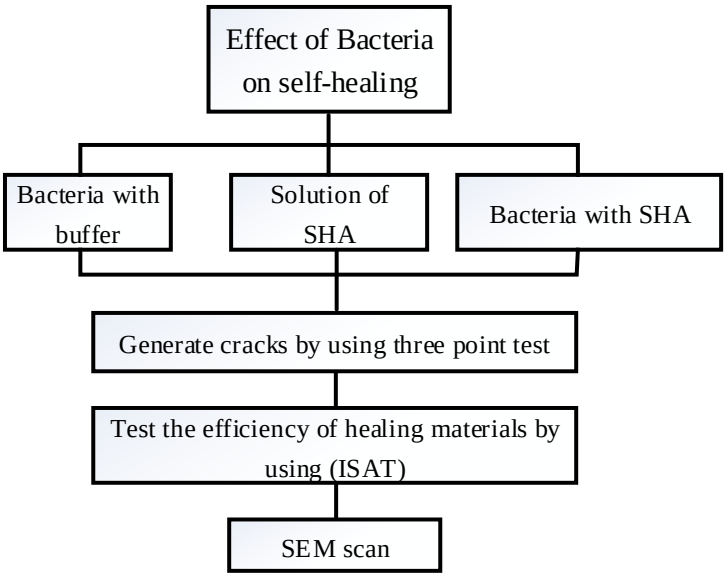
The research is broadly divided into two phases. In the first phase, primary studies are conducted on choosing the optimum number of bacterial cells which will used in remediation of concrete/mortar by producing cracks in samples and filling them with bacteria, then, the main problems will be identified.

The second phase is focusing on self-healing built-in bacterial concrete/mortar. The entire research program is illustrated in the following diagram:-



(A)- The effect of bacteria on self-sealing (autonomic) is divided into two parts:-

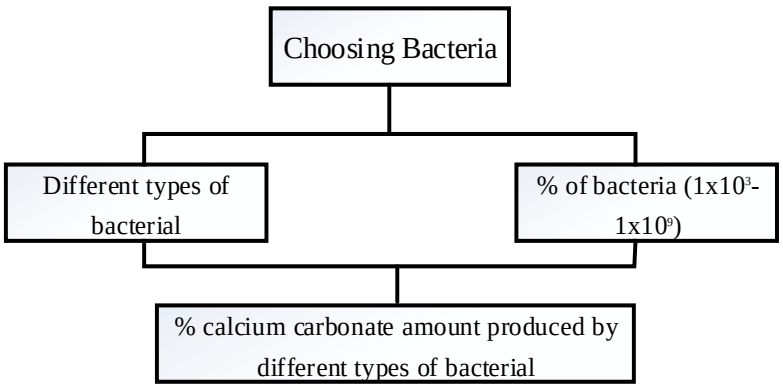
Part 1- Evaluate the effect of bacteria with buffer, solution of SHA and bacteria with SHA on concrete, as shown in the following diagram:



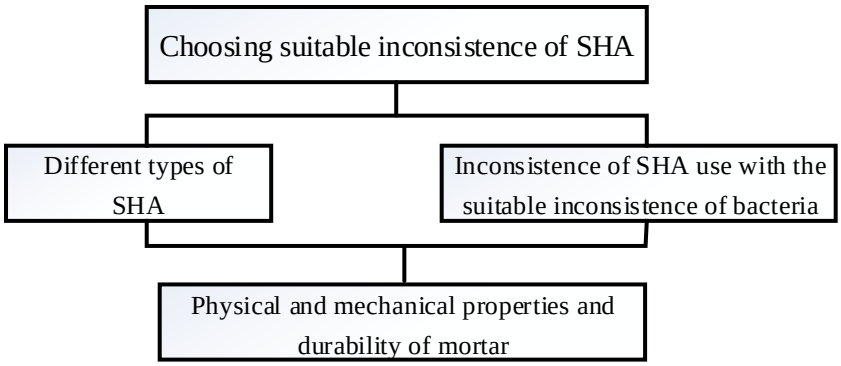
Part 2- Investigate the ability of three types of bacteria spores to survive and resist the harsh conditions inside cement paste.

(B)- Focused on choosing the most suitable bacteria to be used in this research

1- Checking the percentage of calcium carbonate precipitation and the number of cells.

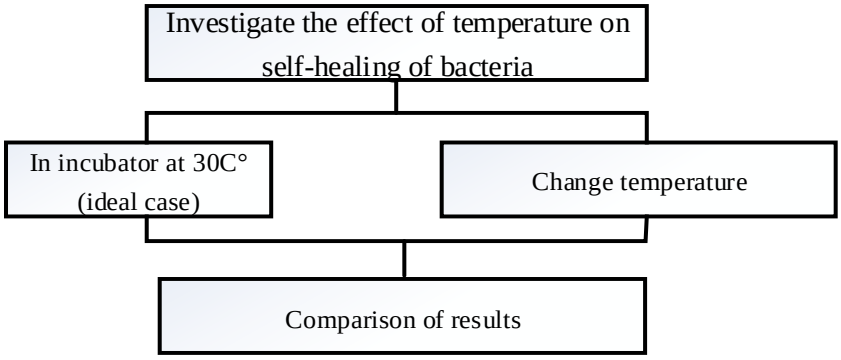


2- Choosing the most suitable percentage of SHA ingredients such as calcium nitrate or calcium chloride, …..etc. to be used as SHA of bacteria and evaluate the effect of agitation on the bacteria synthesised.



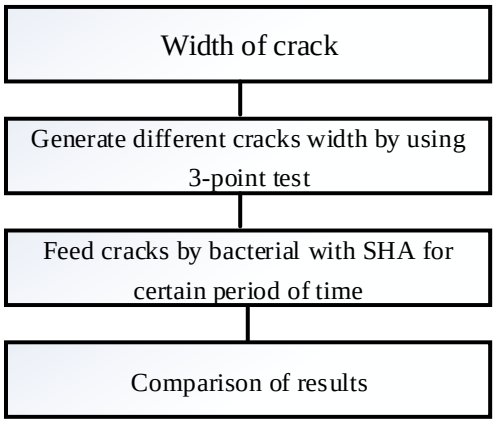
3- As stated in the above plan, the samples submerge into SHA solution for 28 days to study the effect of SHA on matrix of cement.
   i. Investigate the effect of SHA elements individual on cement matrix.
   ii. Optimism the relative quantities of SHA ingredients, which could effect on the cement matrix.
   iii. Investigate the ability of bacteria with SHA from (ii) suitability to produce calcite.

4- Evaluate the effect of temperature on the efficiency of bacteria or bacteria synthesised to induce calcium carbonate.



From the results of the four items, the most suitable bacteria will be chosen.

(C)- To determine the crack width threshold that can be healed or remediated by bacteria.



(D)- To determine the most suitable materials could be used as delivery system of bacteria

- Investigate the ability of using alginate beads, glass tube and perlite as delivery system for bacteria and SHA.
- Study various parameters of individual delivery system in order to enhance self-healing ability.
- Examine the effect of delivery system on the mechanical properties.

1.4 Outline of the thesis:

The work in this thesis is organised into nine chapters; the general introduction of this work, the research methodologies and objectives of research are presented in Chapter 1. While the summary of a review of classification and methods of self-healing in construction materials is given in Chapter 2. The review summary of classification, application and the effect of bacteria-based self-healing of concrete are presented in Chapter 3. The experimental work including used materials properties, mixtures proportion, test procedure, limits of the test results specification is described in Chapter 4. Chapter 5 explains the efficiency of bacteria and self-healing agents. The effect of *bacillus* bacteria ingredients on the properties of cement mortar and bio-cement mortar is described in Chapter 6. Chapter 7 presents sealing cracks in concrete by using *B.cohnii*, *B.halodurans* and *B.pseudofirmus*. Chapter 8 explains direct delivery systems of bacteria-based self-healing concrete, that method to protect, delivery bacterial spores, and bacterial self-healing agents into perlites as a novel. Chapter 9 presents some general conclusions from this research study, outlook, and recommendation for future studies. At the end of this thesis the lists of references and appendices are given.

Chapter 2

A review of self-healing methods in construction materials

2.1 Introduction

Mortar and concrete are the most used construction materials in the construction industry as they are easy to cast and cheap in cost. However, the major drawback of concrete is cracks, which can occur during any stage of the life of a concrete structure. These cracks can be due to the concrete material itself as in the case of not homogeneous of the material properties; due to sustained loading; due to design error or due to aggressive environmental agents. These cracks have many negative effects on the durability of concrete structures.

Nowadays, researchers are making an effort to develop concrete that can regain its performance in a short time by studying self-healing phenomena. Experimental investigations have demonstrated that cracks in concrete have the ability to seal themselves. Self-healing of cracked concrete is commonly called autogenous healing. Many researchers[25–28] have investigated the effects of crack width behavior in concrete as a function of temperature, pH and hardness of healing water and water pressure on autogenous healing. Three causes have been cited for autogenous healing to occur: physical causes, chemical causes, and mechanical causes.

2.2 Type of cracks in structural concrete

Generally, structures or elements of a structure will exhibit cracks as a result of external causes such as structural cracks (design defects and errors, construction and supervision problems, structural over stressing,….etc); or non-structural cracks caused by ambient conditions (e.g. temperature, humidity) and/or quality of materials. Ambient conditions cause damage to concrete by freeze/thaw cycles, chemical attacks, corrosion, extreme loads and other environmental factors that cannot be prevented. However, cracks should not be regarded as a structural cause (defect or failure).

The family tree shown in Figure 2.1 describes the types of structural and non-structural cracks in concrete structures that affect the durability of structural members. It highlights the simple classification of two main groups:

i. Cracks after hardening of concrete.

ii. Cracks before hardening of concrete.

The examples of the main types of non-structural cracks are depicted in Figure 2.2. They are namely plastic, early thermal contraction, early shrinkage (plastic shrinkage) due to self-desiccation and long term drying shrinkage due to low external moisture.

The Concrete Society has identified all types of non-structural cracks that could appear/happen in different parts of structural elements. Each symbol refers to one type of crack as shown in Figure 2.2[29]. The concrete society classified the location of cracks, the duration of appearance and the method of fixing them[29] Table 2.1. Plastic settlement cracks in deep sections and columns are common and it takes 10 minutes to three hours from casting time to appearance in the structure. The plastic shrinkage and restrained contraction cracks are relatively common in external slabs. Moreover, in deep beam sections and slabs, plastic shrinkage cracks are common. The time this type of crack takes to appear is 30 minutes to six hours. Restrained early thermal and drying shrinkage cracks are common in large retaining walls or columns.

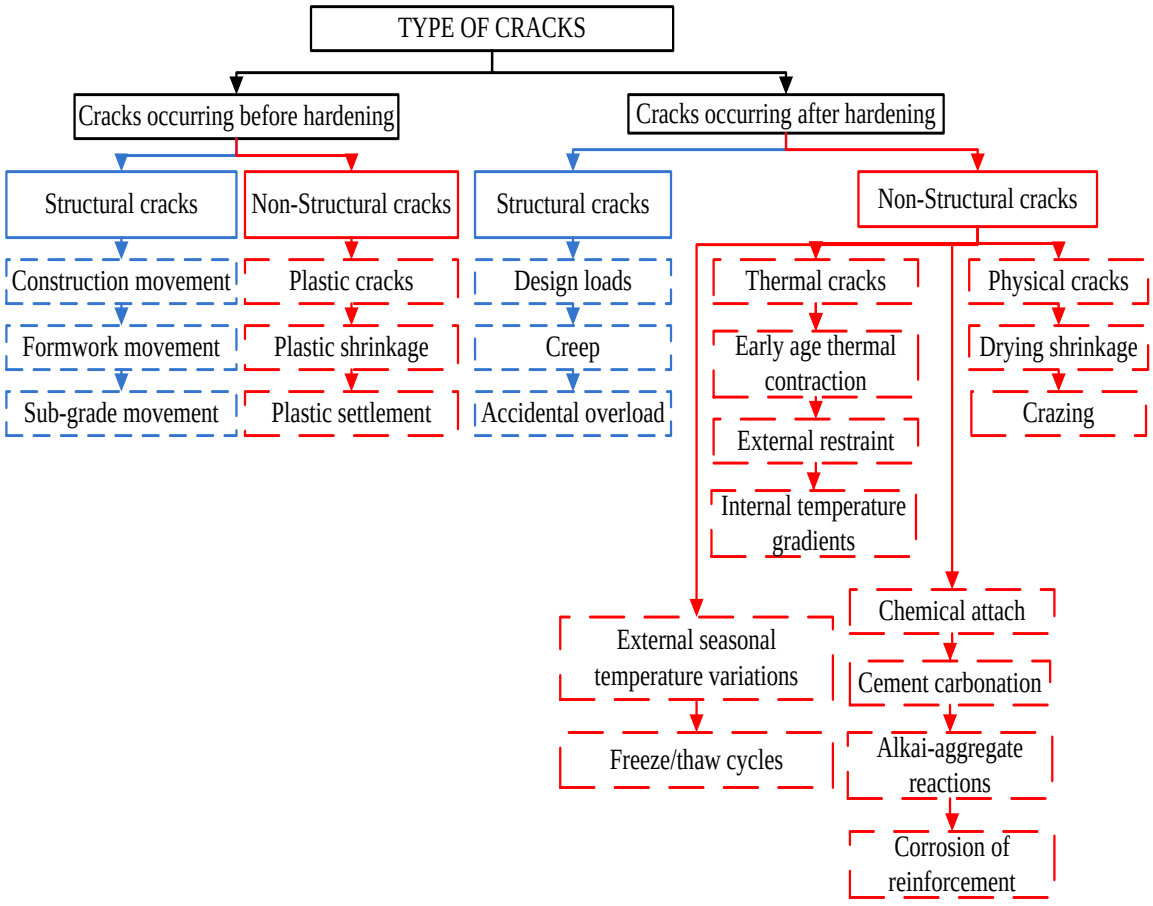


Figure 2.1: Family tree showing the main categories of cracks in concrete adapted from ref[29]

The crack width starts at the molecular level where the crack is invisible ($< nm$), to cracks on the nano scale (5 nm – 100 nm), cracks related to cement paste on the nano-micro scale (0.1 µm - 100 µm), cracks related to cement paste-sand on the micro-meso scale (0.5 mm - 5 mm), and any width

of cracks which is more than 50 μm on the macro scale[30]. However the codes and standards in the Concrete Society Technical Report Number 22[29] classify non-structural cracks in concrete as fine cracks - up to 1mm wide, wide cracks - from 1mm to 6mm wide and fractures - over 6mm wide, and cracks widths of up to 0.3 mm are aesthetically acceptable. The main objective of this review is to provide a comparison of the different healing and remediation techniques that are related to crack width.

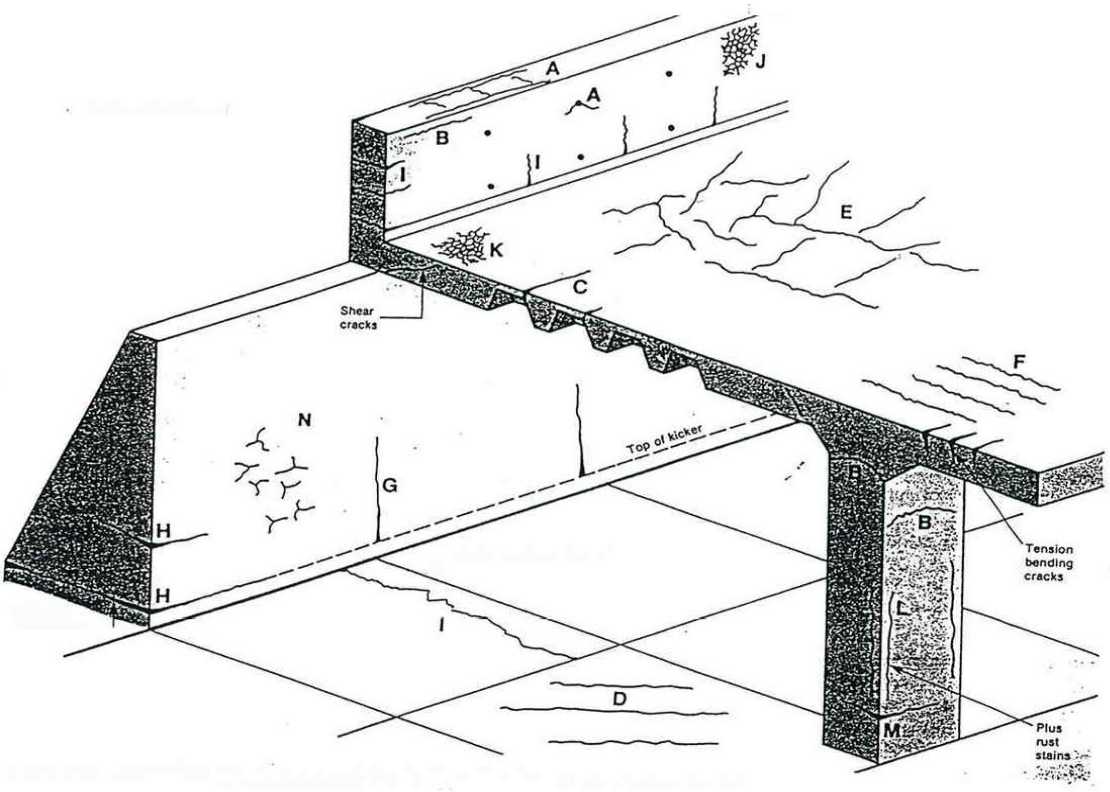


Figure 2.2: the main categories of non-structural cracks in concrete[29]

Table 2.1: gives a brief classification of non-structural cracks according to Figure 4[29].

Type of crack	Classification (see figure 4)	Most common location, form, etc.	Primary cause (excluding restraint)	Secondary cause/ factors	Remedy (assuming basic redesign is possible). In all cases reduce restraint	Time of appearance
Plastic settlement	A	Cracks over-reinforcement In deep sections	Excess bleeding	Rapid early drying conditions	Reduce bleeding (air entrainment or revibrate)	Ten minutes to three hours
	B	"Arching" cracks in columns				
	C	Cracks at change of depth in slabs/beam sections				
Plastic shrinkage	D	Diagonal cracks in roads and slabs	Rapid early drying	Low rate of bleeding	Ensure efficient early curing	Thirty minutes to six hours
	E	Random cracks in reinforced concrete slabs				
	F	Cracks over-reinforcement in concrete slabs	Rapid early drying plus steel near surface			
Early thermal contraction	G	External restraint cracks in thick walls and columns	Excess heat generation	Rapid cooling	Reduce heat and/or insulate	One day to two or three weeks
	H	Internal restraint cracks in thick slabs	Excess temperature gradients			
Early shrinkage due to self-desiccation		External restraint		Concrete with very low water/cement ratios		Hours to days
		Internal restraint				
Long term shrinkage due to external moisture loss	I	Cracking in thin slab and walls	Inefficient stress relief	High shrinkage	Reduce water content. Improve curing	Several weeks or months, up to several years
Crazing	J	Cracks "against formwork" in fair-faced concrete	Impermeable formwork	Rich mixes, poor curing	Improve curing and finishing	One to seven days sometimes much later
Crazing		Trowelled in concrete surface slabs	Power trowelled	Drying	Non	Days to months
Corrosion of reinforcement	L	Carbonation and external chlorides in columns, beams and slabs	Lack of cover/carbonation/excess salt or salt ingress	Poor-quality concrete	Eliminate causes listed	More than two years
	M	Calcium chloride in precast concrete	Excess calcium chloride			
Alkali-silica reaction	N	(Damp locations)	Reactive aggregate plus high-alkali cement		Eliminate causes listed	More than five years

2.3 Definition and Types of Self-Healing Phenomena

The self-healing phenomenon has been studied and defined by many authors[13,31–33], but in their definitions there are large differences in their interpretations, especially for the terms autogenous, autogenic and autonomic self-healing. The summary of these definitions is given hereafter: -

2.3.1 The Japan concrete institute (JCI) committee

Studies the topic of self-healing and gave a clear definition of each technical term of self-healing [13] as shown in Figure 2.3.

2.3.2 RILEM defined[14] self–healing

As "*any process by the material itself involving the recovery and hence improvement of a performance after an earlier action that had reduced the performance of the material*". RILEM divided the process of self-healing as follow:

Autogenic: "*the self-healing is autogenic when the recovery process uses material components that could otherwise also be present when not specifically designed for self-healing*".
Autonomic: "*the self-healing process is autonomic when the recovery process uses material components that would otherwise not be found in the material*".

2.3.3 Definition of self-healing given by Van Tittelboom & De Belie [15]

In their paper autogenous healing was compatible with the natural healing definition given by JCI but the autonomic healing was considered as autogenous healing and called modified autogenous healing and improved autogenous healing, depending on the material added to the original one to increase the efficiency of the healing. It is clear from these definitions that there is a difference between them, for instance, the difference between RILEM and JCI definitions to autonomic healing is that the RILEM definition is equivalent to both autonomic and activated repairing self-healing defined by JCI.

The proposed definition in this research for the healing phenomenon is divided into three areas: autogenous, autogenomic and autonomic. These are similar to the definitions given by JCI, but we introduce a new term "autogenomic", which covers the mechanism of healing done by both autogenous and autonomic together as shown in Figure 2.4. The word autogenomic comes from the word autogenic and the word autonomic (autog+nomic).

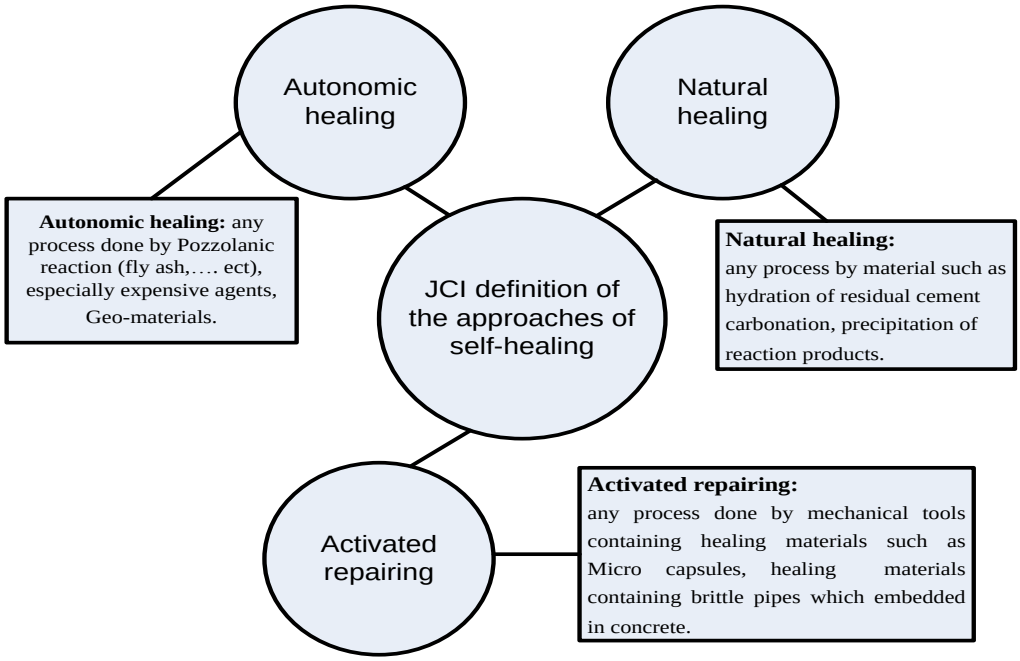


Figure 2.3: Definition of the Japan concrete institute (JCI) of self-healing phenomenon.

If **A** represents autogenous healing, **B** represents autogenomic healing, and **C** represents autonomic healing. The relation between these three can be written in terms of a Venn diagram such as:

$$B=A\cap C$$

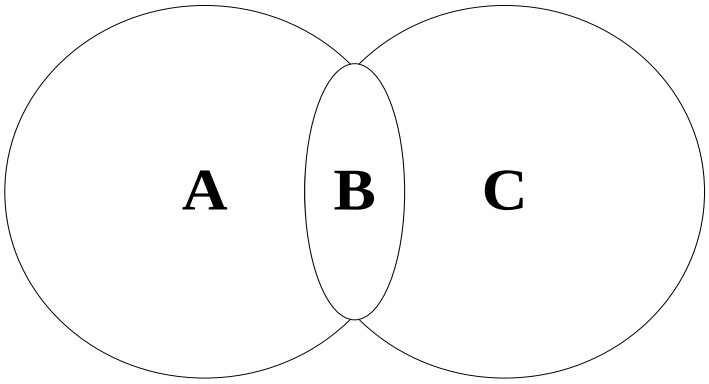


Figure 2.4: The relation between autogenous (A), autogenomic (B), and autonomic healing (C).

It is significant to know about the actual individual healing mechanism and its conditions in order to provide a systematic design for a robust system of healing abilities to apply to high performance concrete structures. In this review self-healing is divided into three types: autogenic self-healing, autogenomic self-healing and autonomic self-healing as shown in Figure 2.5.

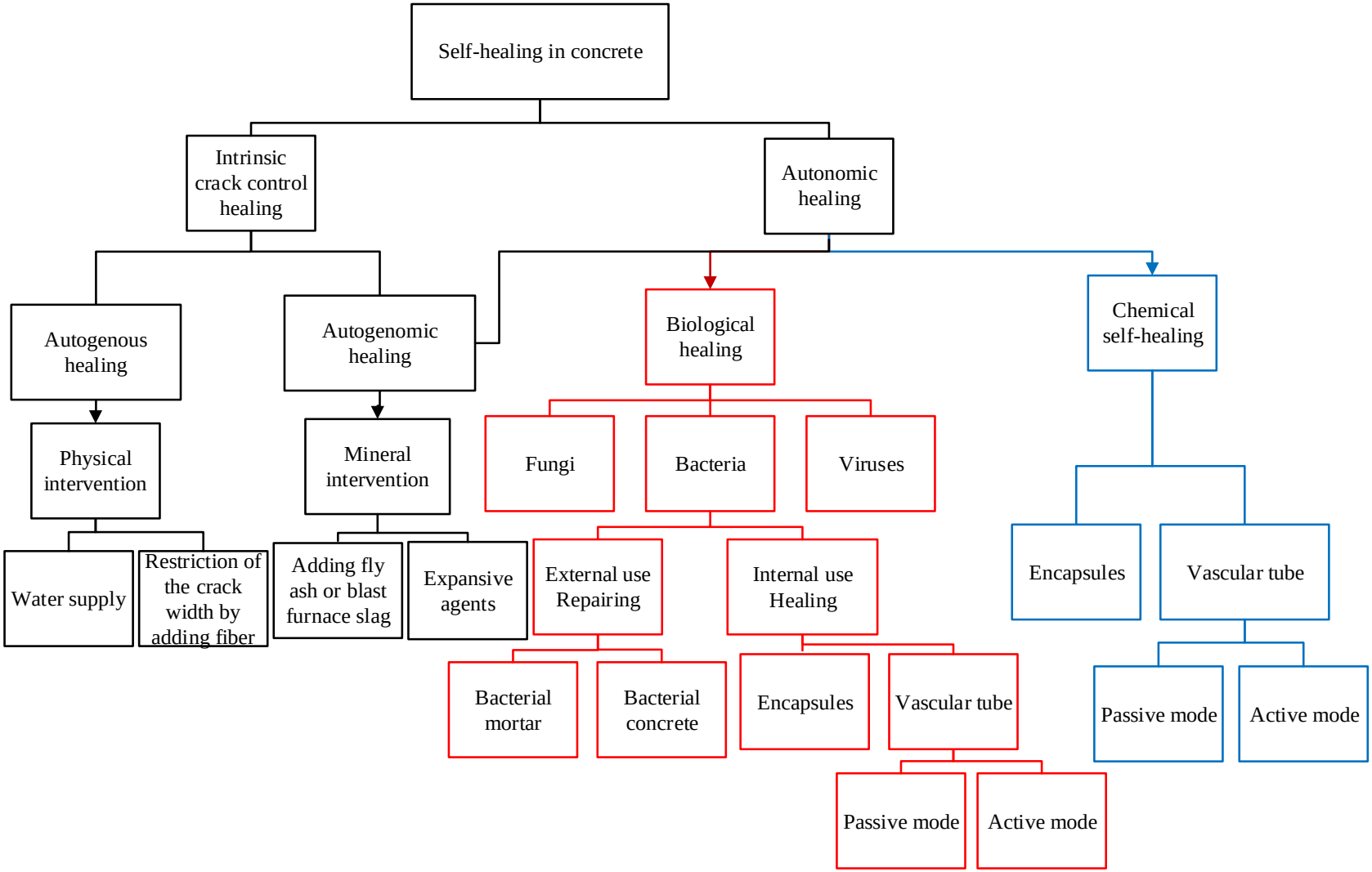


Figure 2.5: Plan shows the main categories of self-healing in concrete

2.3.4 Autogenous Self-Healing

Autogenous self-healing is the ability of cementitious material to recover itself after environmental attack due to ingress water. Autogenous phenomenon has been discussed by researchers[7,34–36] for many years. Yang et al[36] studied the autogenous healing phenomenon in engineering cementitious composites under wet-dry cycles. Their paper described the experimental investigations of resonant frequency measurements, uniaxial tensile testing and the effects of temperature during wetting-drying cycles. The analysis data confirmed reasonably robust autogenous healing of ECC in commonly encountered environments for many types of infrastructure. Ramm and Biscoping[35] investigated the autogenous healing phenomenon and reinforcement corrosion of water penetrated separation cracks in reinforced concrete. They observed that the water pressure, the degree of acid of the water and the pH value over the long term (over a 2-year period) affected reinforcement corrosion and autogenous healing of 0.1mm width cracks. No corrosion cracks were observed in any case. Corrosion started in 0.2mm width cracks depending on pH value. The conclusion of their research was that the increase in of corrosion is a function of the width of the crack, the pH value and the penetration of acid water and they found the specimens had 0.4 mm width cracks and that at pH 5.2 the highest corrosion was observed. The decrease in permeability related to a self-healing phenomenon is incorrect. This is especially common in the conclusion of the investigators, where only study and the recorded amount of inflow have been taken into consideration. What happens through the flow remains unknown as the self-healed concrete does not go through a particular permeability test. Most important is the study results in terms of the microstructure and the permeating fluid[6,37]. The main tools of autogenous healing are presented hereafter: -

Tools of autogenous self-healing

According to Hearn[6] the concrete has to create its own mechanisms to close up the cracks with time through a process called natural self-healing. These tools are shown in Figure 2.6, and each tool has its own technique to close up cracks up to 0.3mm.

- **The first tool is physical**. The technique makes the inside material swell at the two sides of the crack due to the hydration of cement paste, which will lead to the crack closing, and the more the swelling the tighter the cracks will be.
- **The second tool is chemical,** which has two types of defence:

i. When the water penetrates through the cracks inside the material, the existing inside anhydrate cement start to hydrate, and forms new products that grow into the space caused by cracks. It should be noted that continued hydration is not capable of self-closing the crack, but assuming a small crack width for cement paste + sand with size 0.05 mm - 0.1 mm on the micro/meso scale[30], and assuming simultaneous action of the physical tool (swelling) will be capable of

producing self-healing when the crack width becomes larger than 0.1 mm, the influence of hydration of cement and swelling become minor.

ii. The growth of crystals and formation of calcium carbonate on the crack face along whole its length due to some chemical reaction between carbonate ions and calcium ions from the pore water of concrete. This will lead the precipitation of calcium carbonate on the crack width. This reaction depends on many factors such as temperature, pH, width of crack and water pressure, and this process has been previously investigated[26]. It was concluded that this type of process is the most effective factor in self-healing. Whereas, the first three to five days of water exposure has the greatest autogenous healing effect

- **The third tool is mechanical,** which has two factors:

iii. Minor contribution to autogenous self-healing. Assuming there exist some fine particles that are not bonded within the hardened cement paste. They can migrate through the cracks with the help of water. In due time these particles will precipitate in the crack and partially heal it.

iv. The second is when the cracks are generated through the concrete, this will produce a fracture of small concrete particles, which will stay in the crack face, and in time these particles will partially block the cracks.

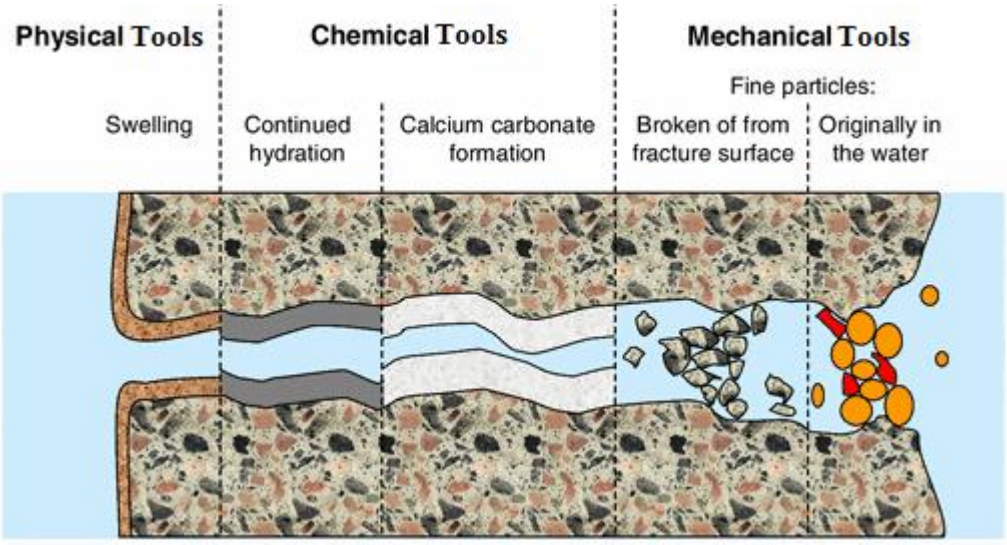


Figure 2.6: Different tools for autogenic self-healing[38]

2.3.5 Autogenomic Self-healing

Autogenomic healing is autogenous healing but the crack width is restricted by ingress of foreign new materials. It was clear from the previous discussion of autogenous self-healing, that phenomena can be used effectively when the crack width is limited to 300 μm[39]. So there should be an engineering way to introduce a fibre reinforced strain hardening to limit or control the crack width and to promote

autogenous healing to start healing the specified limited cracks in the structure or introduce agents to the material autonomically, thus promoting autogenous healing. Van Tittelboom, K. & De Belie [15] gave a good methodology for handling this process and called it modified autogenous self-healing. This review indicated that the supply of water to the material mix could be achieved by mixing super absorbent polymers (SAP) into cementitious material to provide a surcharge of water.

When cracks arise, ingress of moisture via these cracks can cause to SAP to swell again and immigrate to the cracks, causing the physical blocking of these cracks. Other autogenomic healing focuses on replacement of parts of the cement by fly-ash or blast furnace slag, which are chemically able to promote the deposition of crystals inside the cracks in the autogenous self-healing process.

2.3.6 Autonomic self-healing

Autonomic self-healing is the ability of the material to resist an environmental attack when ingress of external healing material is available. Autonomic healing is the mechanical process for repairing the damaged structures, which can be summarised in the following: -

2.3.6.1 Capsule based autonomic healing materials

Microencapsulation has been used since the 1950s and has been employed in numerous construction materials[40]. It has been used by many industries such as food, pharmaceutical, textile and chemical. A number of researchers worked to introduce autonomic self-healing in concrete, and the most common approaches are bacterial spore encapsulation[20,23,41,42] and chemical encapsulation[9,11,12,43–50]. White et al.[42] studied the polymerising agent into microencapsulation with a catalyst capable of triggering by contact with an embedded material in a structural composite matrix. The experimental results show 75% of toughness was recovered by fracture yield. Nishiwaki et al.[43] developed a smart concrete that incorporates a self-repairing system using a Self-Repairing Device (SRD), which comprises a self-diagnosis composite. This device can selectively heat the region around a crack using electricity, and uses a Heat Plasticity Pipe (HPP) to carry out repair. These pipes are embedded in the concrete and plates for smooth heat transfer between the SRD with HPP. Both thermal analysis and an experiment showed effective performance by the proposed system. The microcapsule must be sufficiently strong/stiff to resist forces that are applied to it during deformation of concrete[49], in addition to the polymerisation during the preparation of the mixture.

The microcapsule can be prepared by three methods[40]: (i) Polymerisation method, (ii) Coacervation method, and (iii) Mechanical method. The two main design parameters, diameter and thickness of the microcapsule, should be taken into consideration. The self-healing microcapsule method has the ability to resolve issues related to internal cracking and micro-cracking. When the capsules are ruptured by propagation of the concrete internal cracks, the self-healing agent is released in the

damaged region by gravitational and capillary forces. The microcapsule self-healing process (polymerisation of the healing agent) provides the following advantages[42]: low rate of shrinkage during polymerisation, long life span and low viscosity.

The capsules can take a spherical or cylindrical shape. Figure 2.7 indicates the number of researchers using each shape in the period of 1994-2012. Many researchers [29, 37, 82, 84,103-107] investigated the influence of capsule content, capsule size, the contribution of the optimal capsule size and effect of capsules on mechanical properties including the deflection capacity, the flexural strength and stiffness. Pelletier et al.[49] investigated the self-healing properties of concrete material and corrosion inhibition such as the compressive strength recovery, ductility, the toughness and the attenuation. They inserted the sodium silicate solution inside polyurethane microcapsules with sizes varying from 40-800 microns present in the concrete matrix by 2% of water volume. The results showed the samples containing capsules recovered 26% of the original value, while the controls recovered just 10% after one week. Moreover, the capsule samples showed a significant reduction in corrosion and the compressive strength was unaffected by the presence of the capsules.

Jonkers and Wiktor[51] embedded two components of biochemical agents in porous expanded clay particles and replaced part of regular concrete aggregates. Experimental results showed that this technique could heal cracks in width of up to 0.46 mm in bacterial concrete and no more than 0.18 mm wide cracks in control specimens after 100 days of submersion in water. Yang et al.[52] developed a new family of self-healing materials by designing microcapsules with an oil core as the catalyst phase in the system. The healing agents were methylmethacrylate monomer and triethylborane and silica gel shells with average diameter of 4.15 μm. The self-healing efficiency of cement mortars with microcapsules and microfibers were evaluated using permeability measurements along with a fatigue test under uniaxial compression cyclic loading. The results showed the microcapsule samples reduced the gas permeability, which might be partly attributed to the self-healing effect of microcapsules, while the fatigue test showed the inclusion of small amounts of microcapsules into carbon microfiber reinforced mortar improved the crack resistance and toughness.

Xia Hua[53] embedded three types of Engineered Cementitious Composites (ECC) with local waste materials into fine and coarse capsules and used Super Absorbent Polymers (SAP) as a water reservoir enclosed in the capsules. This technique provides water for self-healing when capsules are ruptured by propagating cracks. The results detected the recovered deflection capacity of damaged modified ECC specimens range between 65%-95% of control specimens, while rarely recovering improvement of flexural strength and stiffness. The fine capsules show more reasonable performance on mechanical properties including deflection capacity, the flexural strength, and stiffness compared to coarse capsules.

Many researchers [32, 33, 111-115] have studied the influence of cylindrical capsules or tubular capsule content, capsule size, and the contribution of the optimal capsule size and effect of capsules on mechanical properties including the deflection capacity, the flexural strength and stiffness of concrete. Van Tittelboom et al.[30] have used two types of tubular capsules to carry MEYCO MP 355 1K (BASF The Chemical Company) as a healing agent. The efficiency of glass tubular capsules and ceramic tubular capsules were compared. The results concluded that the glass and ceramic tubes have similar performance and the chemical agent was a very appropriate healing agent for obtaining self-healing properties in cementitious materials. Furthermore, Van Tittelboom et al. [27] used the same system to deliver bacteria to concrete by using glass tubular capsules, and protected the bacteria from the high pH inside the concrete by using silica gel or polyurethane as the carrier. The results, according to the thermogravimetric analysis of the $CaCO_3$ precipitated by bacteria in silica gel was 25% by mass while in polyurethane it was only 11% by mass. However, the strength regain was 60% for the cracked mortar healed by polyurethane immobilized bacteria while it was just 5% for mortar healed by silica gel immobilised bacteria.

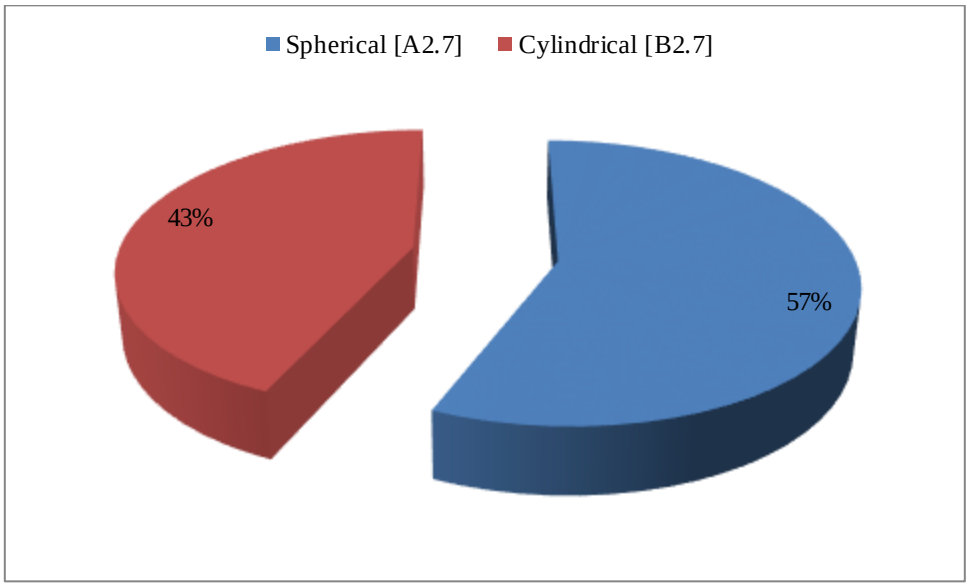


Figure 2.7: Percentage use of cylindrical shape capsules and spherical shape capsules for self-healing in previous literature (Ref. No 23) (All the references for corresponding graphs of Figures 2.5,2.6,2.8-2.11 and 2.14 are summarized in Appendix I and their codes are A2.5, B2.5, etc).

2.3.6.2 Vascular autonomic self-healing

Vascular autonomic self-healing materials consists of embedding repair (healing) material in hollow fibers or tubes which connect the interior and the exterior of the structure with the repair matrix before it is subjected to damage. This system can be a one channel vascular system, which can carry one component of the healing agent, or a multi-channel system, which uses a combination of multi-

component healing agents. Therefore, when cracking occurs, this healing material is released from inside the tube or fibers and enters into the structural matrix.

These released materials will penetrate into cracks and re-bond to the original structural material of the structure. The self-healing/repair concept has been used by many authors starting in 1990 by Dry, who used this approach for passive smart self-repair in different types of structures such as beams, frames and bridges and also developed an anti-corrosion system by using calcium nitrite inside porous fibers and coated the samples with a salt-sanative substance to control the response. The results showed that the system delayed the onset of corrosion by at least three weeks in the laboratory specimens and the total amount of corrosion was reduced by more than half[10,54–56].

Other authors[2,57–59] have investigated this type of approach of using different healing agents for decades. Sangadji and Schlangen[60] used a different technique, which tried to imitate the process of bone healing. Their experimental procedure was to create a porous network to simulate the 'spongious bone' by putting porous concrete inside the concrete structure. They used cylindrical and beam samples for their experiment and injected healing agents manually after creating cracks on the specimens. The results showed that macro-cracks were sealed and also the strength of the concrete was regained. Nishiwaki et al.[61] developed a self-healing system for concrete by using a self-diagnosis composite as a healing device. This heating device and pipe containing a repair agent made of heat-plasticity organic film were embedded in the concrete, followed by heating around the crack by the heating device and pipes and they found that the repair agent filled up the crack and the repair agent in the crack hardened. They also confirmed their experiment results by comparison with three-dimensional analysis.

Sun et al. [46] studied the efficiency of hollow glass fibres on the self-healing performance of micro cracks in a concrete bridge using a repair agent. Moreover, Joseph et al. [47] studied the effect of the diameter and longitude of glass reservoirs on self-healing and the level of reinforcement and loading rate on the amount of self-healing. The conclusion of their experiments was that during the first and second loading cycles healing occurs. Dry [9] studied the comparison between two modes of repair: resin injected manually after cracking, and self-repair by presenting the adhesive for repair in the matrix at the time of cracking occurs. The conclusion showed the manual resin injection technique controlled variables such as the quantity and location of the resin injection, while the tubular capillary had less or no control. Kuang et al. [116] investigated the recycling static loading on concrete beams with shape memory alloy (SMA) wire as reinforcement and brittle fibre containing adhesives. The results showed this technique improved self-restoration capacity of concrete beams. Figure 2.8 shows a comparison between the number of papers using vascular self-repair and using encapsulates healing over the same period (1994-2012). It is clear from Figure 2.8 that the number of research papers using capsules for self-healing is approximately three times that of self-healing using the vascular system.

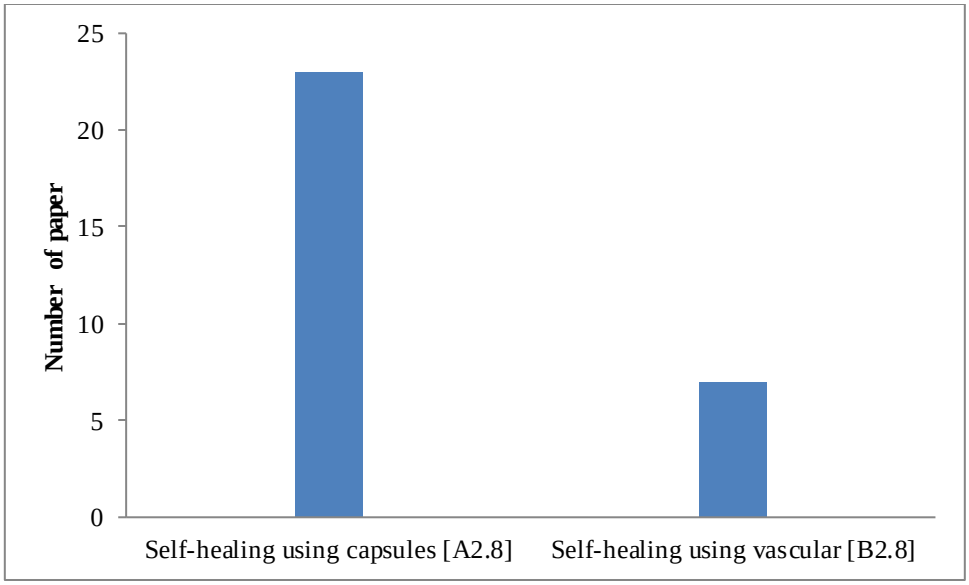


Figure 2.8: Number of papers using chemical capsule agents and direct vascular feeding.

2.3.6.3 Bacteria-based self-healing concrete (BBSHC)

Synthetic materials like epoxies are used for remediation of concrete cracks but repair using these materials has a number of disadvantageous aspects, such as different thermal expansion coefficients with respect to the concrete and environmental and health hazards. BBSHC concrete induced calcium carbonate precipitation is considered to be an alternative and an environmental friendly crack repair technique. BBSHC concrete is considered a promising solution to cracked concrete to reduce the high repair cost. The definition of BBSHC concrete is especially concrete made from bio-chemical agents to increase the lifespan or the durability of concrete structures by sealing the cracks. BBSHC concrete is also called bacterial concrete or self-healing concrete or bio-concrete or Bioengineered concrete. Besides the external application of bacteria in the case of crack repair, microorganisms have also been applied in the concrete mixture, and strength and permeability have been the dominant criteria for evaluating the self-healing efficiency. However, types of bacterial materials, classification and application of bacteria in natural and artificial will be discussed in chapter 3.

Figure 2.9 suggests that the most promising research area is the autonomic healing and the least is autogenous, which was covered period since 1985 until 2014 carried out to study the self-healing phenomenon.

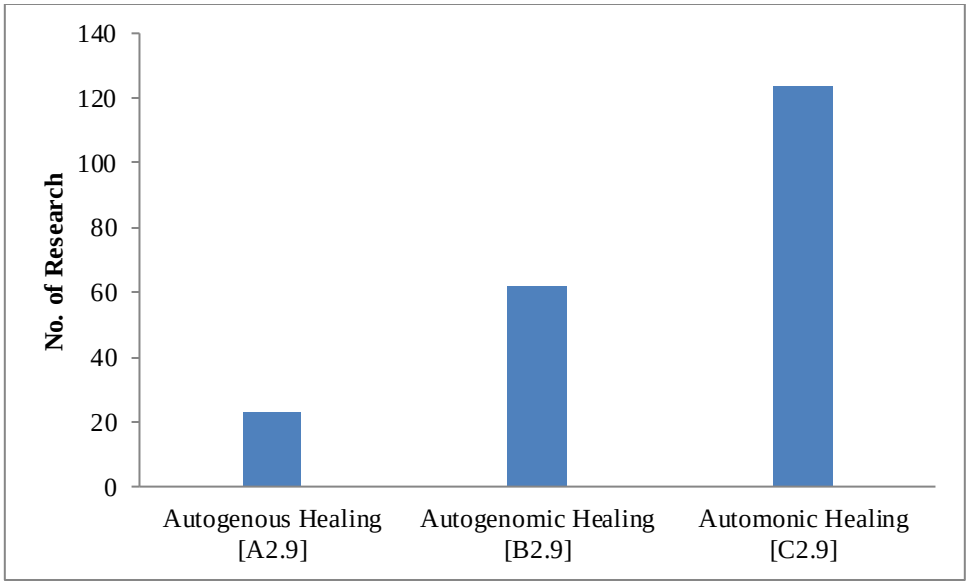


Figure 2.9: Number of previous research papers over the period 1985-2014 carried out to study the healing phenomenon.

The relation between the number of publications using both mineral and chemical agents and using bacteria is illustrated in Figure 2.10.

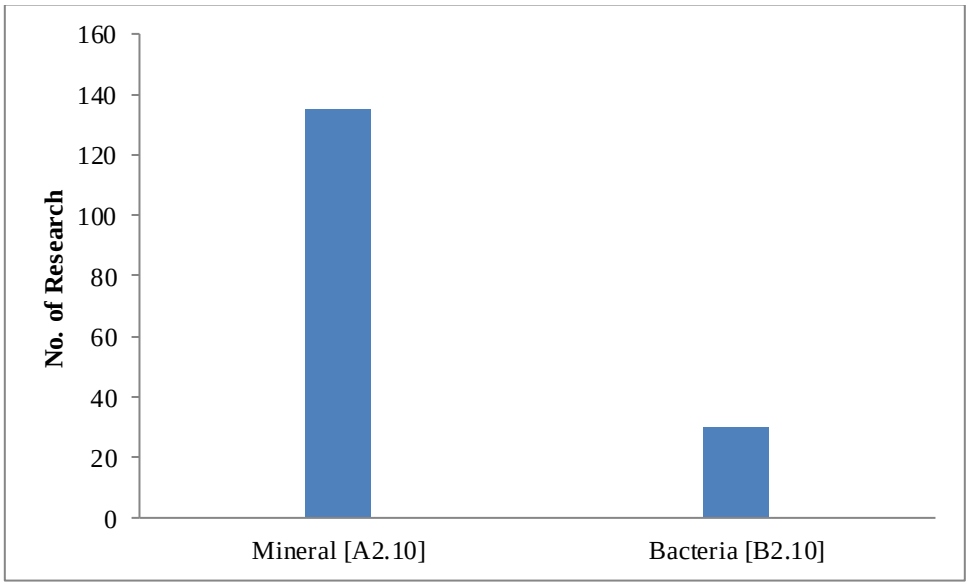


Figure 2.10: Number of research papers using mineral and chemical agents, and bacteria over the period 1985-2014.

2.4 Tests of self-healing capabilities

In the last decade several approaches have aimed at making protecting concrete against environmental effects by using self-healing techniques. The common testing procedure for different materials classification is one of the challenges, because there are no unified criteria to assess self-healing test

results. One of the major goals of the researchers[62] is to develop a universal standard procedure to evaluate the efficiency of self-healing capabilities.

In general, different techniques and tests are used to evaluate the healing capabilities and performance such as stress tests, the permeability test and scanning electron microscopy (SEM). These tests are carried out to find the efficiency of regaining: mechanical properties, sealing the cracks and to analyse the chemical components respectively as illustrated in Figure 2.11.

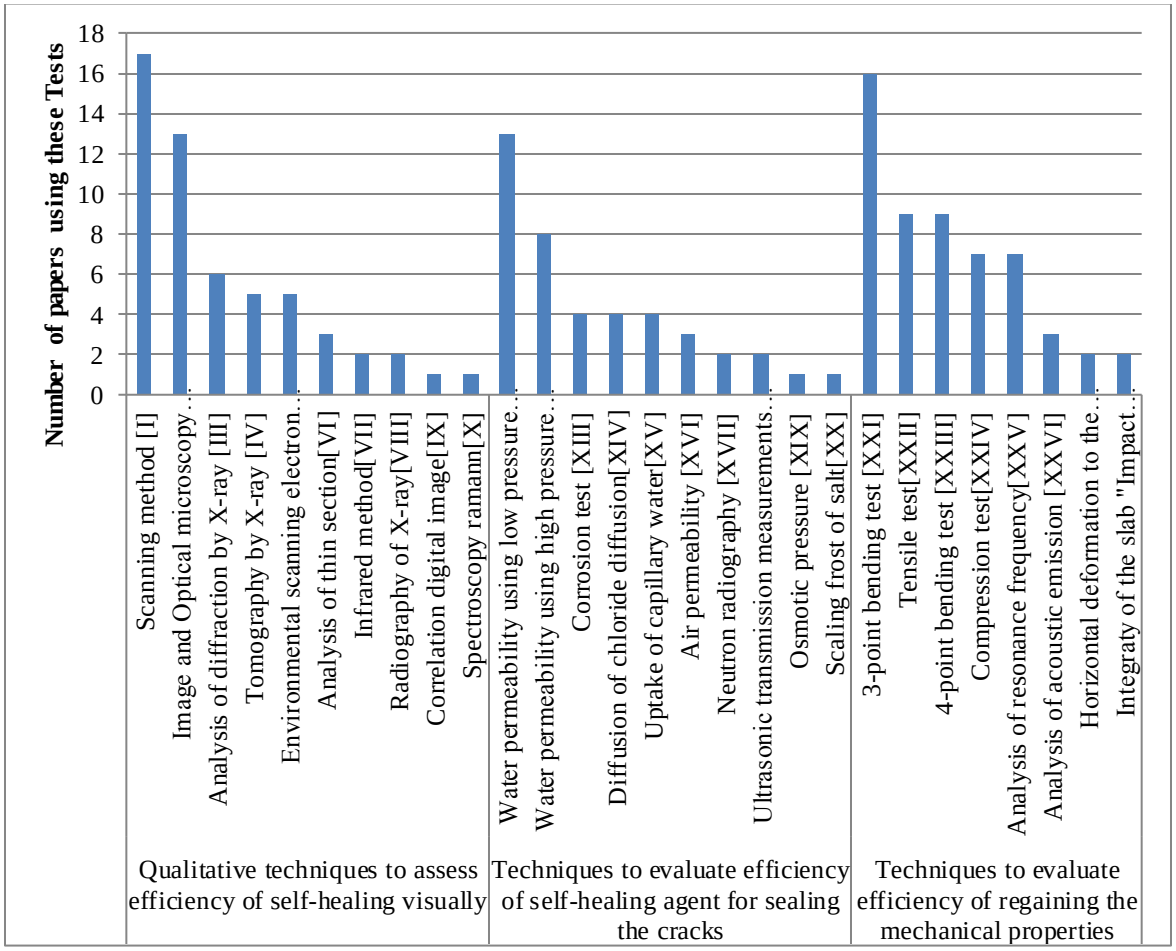


Figure 2.11: Tests used to evaluate the efficiency of self-healing according to the literature.

2.5 Summary and discussion

The goal of this view is to provide an overview of different methodologies, allowing for quantitative evaluation of different self-healing approaches, such as intrinsic healing with crack control width, bacterial encapsulation and crack repair, chemical encapsulation, chemical in glass tubing, and mineral admixture.

2.5.1 Intrinsic (autogenous and autogenomic) healing

The intrinsic crack healing in concrete matrix is the most widely studied[7,35,36],[63–65]. This type of phenomenon can be classified as being autogenous and autogenomic healing as explained in sections 2.3.4 & 2.3.5. This phenomenon can heal cracks up to 300 μm[39], since the intrinsic mechanisms of continuing hydration of pozzolanic reaction and carbonation in cementitious materials are known to have a long life span. Also this type of healing will continue to occur as long as the pozzolanic material and environment conditions are adequate.

Advantages

- Friendly to the environment.
- Long life span and costs less.
- Healing material distributed everywhere in the structure.
- After healing, the healed material gains tightness and strength.
- Demonstrated ability of gaining strength for the healing material exposed to more than one cycle of loading and under different environments.

Disadvantages

- Healing limited to small crack width.
- Highly alkaline and chloride environments.
- There is not enough data for proper assessment.

2.5.2 Chemical encapsulation

A variety of chemical agents contained in microcapsules such as epoxy[42], $Ca(OH)_2$[42], Na_2SIO_3[49], retarder agent[66] and also a variety of shell material, gelatin[44], wax[66], ceramics[45], and glass[37] have been used to facilitate the chemical self-healing of concrete.

Advantages

- Long serviceability life depends on the healing agent concentration in capsule.
- Healing more than one defective place at the same time.
- Capsules can be distributed almost uniformly in concrete.
- Storing healing agent for future use.
- Regain mechanical strength when it is healed.

Disadvantages

- The bond between capsules and boundary is not well known.
- It is not reliable due to inadequate data for proper assessment.
- The more capsules used the less stiff the cross section will be.
- No previous study has been done on the healing behaviour for different climate.
- The previous tests were done for one healing cycle (the test was not repeated for more than one sample).

2.5.3 Mineral admixtures

Mineral admixtures have been used as a tool for the self-healing of concrete cracks by promoting the deposition of crystals inside the crack. Kashi[67] used expansive agents (C_4A_3S, $CaSO_4$ and CaO) and some researchers[68–71] replaced part of cement with fly ash or blast furnace slag and found that the healing is effective for cracks up 0.22mm. Sahmaran et al.[68] studied replacement high volumes of fly ash (HVFA) ratio (0%, 35%, and 55%) with cement and water. The cement ratio was 0.35 and their observation indicated that HVFA lost 27% of their compressive strength when the initial loaded up to 90% of their ultimate strength, while after 28 days curing in water that reduction was only 7% which indicating essential healing. Termkhajornkit et al.[71] investigated the ability of fly ash cement systems to self-heal after shrinkage of cracks occurred by considering mechanical properties and hydration reactions and products. The results showed the mechanical properties such as compressive strength and hydration reactions and products increased with an increase of the replacement ratio of fly ash. By replacing 10% of cement with a combination of chemical, geo-materials agents and expansive agents, crack healing can be reached[72],[73].

Advantages

- Efficient healing.
- There is compatibility between cement matrix and the healing product.
- Life span is a function of reactivity of the additives and unhydrated materials.
- It is suitable to be used for underground structure.

Disadvantages

- Needs good treatment to avoid undesirable expansion.
- There is no guarantee of obtaining the required healing.
- There is not enough data to be used as guide for proper assessment.
- No study has been done for different climate conditions.
- Uniformly distributed in mix.

2.5.4 Chemical in glass/fiber vascular tubing

The glass vascular tubing system consists of a network of hollow tubes connecting the internal and the external cracks of the structure. There are two types of vascular tubing system, namely one-channel vascular tubing which has been studied by Joseph et al.[17],[47],[83],[84] and the other system called multi-channel tubing system which has been studied by Mihashi et al.[76] and Dry and McMillan[12].

These types of systems consist of two different modes namely active mode, where the crack is healed from the embedded tube in the concrete. The other is passive mode; the self-healing agent is discharged from outside of the structure container through the glass tube. The results showed that the active mode can improve the tightness of the cracks, and the passive mode can improve the flexural toughening.

Advantages

- The healing agent is adjustable and released upon request.
- Can be used effectively under multi damages.
- The life span is partially long and depends on the healing agent used.
- Gaining strength and tightness is acceptable but not 100% efficiency.
- This system can carry any type of healing agents.
- The external environmental has no effect on the healing mechanism.

Disadvantages

- Difficulty in orientation of the glass fibers and casting.
- The greater the number of tubes used the less effective the structural cross section and mechanical properties will be.
- There is not enough data from the previous study to be used for proper assessment.
- When the glass tubes are used, this type of system cannot be reused to heal the healed cracks due to the brittleness of the glass.
- If the glass tubes are used, this glass might affect the cement matrix.

2.5.5 Summary of advantages and disadvantages of using bacteria in concrete

Advantages

- It can be used for remediation of external cracks by filling the cracks with bacteria, nutrients and suitable sand; this will lead to improvements in strength and higher stiffness.

- Formation of biological binders and surface treatment to the stones, by using the same bacteria and procedure for crack repair.
- Improvement in compressive strength and durability of concrete when used as remediation (external self-healing) or as bio-concrete.
- Regain tightness, which leads to reduction in corrosion of reinforced concrete.
- The precipitation material produced by bacteria is compatible with the original material.

Disadvantages

- The primary cost of biological concrete (bio-concrete and use of bacteria in external self-healing as remediation and/or repairing) is about double that of traditional concrete[77], but this cost is reduced due to the reduction of maintenance cost.
- Inserting bacteria in capsules and mixing them with the concrete leads to a decrease in compressive strength due to a large amount of capsules inside concrete.
- Different bacteria need different nutrients and different atmospheres.
- Cost precaution: need to protect bacteria from the effect of high pH in concrete.
- There is no standard or code that specifies the bacterial concrete mix design.

2.6 Concluding remarks and future studies

In this review the five self-healing approaches, namely: bacteria-based self-healing of concrete, mineral admixture, chemical encapsulation, chemical in glass tubing encapsulation, and intrinsic healing with restricted crack width have been studied, and found to be effective to some extent. It is not possible to predict in detail which approach would be better than the other. In spite of the fact that the autogenous healing is always restricted to healing of small cracks and also its reliability of healing is lower, in the past, a lot of research was concentrated on autogenous and autogenomic healing. The self-healing approaches reviewed in this chapter are a relevant research topic involving microbiology, chemistry, civil engineering, and materials science. It is recommended to investigate a combination of the presented five different approaches to come up with a new self-healing approach.

Without in situ application of these five self-healing approaches, the benefit of reducing the cost and extended the life span of the structure will not be reached. Concrete mix design using the criteria bounded by each approach and the unified method of testing of the healed material and development the international standard is highly recommended. Intensive research is needed on the healed material to find its reliability in case of creep, stress and mechanical properties, of the healed sections.

2.7 Some factors influencing the selection of the proposed research

Referring to the literature review specifically, summary and discussion, it is clear that in order to develop self-repairing and environmentally friendly concrete one has to choose bio-deposition self-healing approaches. After surveying the University of Bath laboratory equipment facilities, the most suitable subjects to be studied are: -

a. Bio-mortar.
b. Remediation of cracks in mortar/concrete by MICP.
c. Bacteria-based self-healing concrete (BBSHC)

Due to the following: -

1. The literature review demonstrated the healing mechanism is dependent on the external environment, which will lead to low versatility.
2. The literature review indicated no tests have been done with more than one cycle of tests, which will lead to less reliability of the test results.
3. The specified optimum bio-concrete mix is still under research.
4. The previous study used one type of bacterium for studying the healing efficiency, and did not demonstrate a comparison study between the healing efficiency of these bacteria.
5. There is a lack of information in the literature about the amount of healing efficiency if two or three types of bacteria are used at the same time for healing the concrete.

The research work of this thesis will provide answers to investigate the ability of healing for more than one cycle, demonstrate and present comparison between three types of bacterium, and account the amount of calcite produced by the three types of bacterium to seal/heal the crack.

Figure 2.11 given in the literature review indicated that the most tests have used by researchers to evaluate the self-healing efficiency are: -

1- Qualitative assessment of the efficiency of self-healing visually.
   - Scanning by using electron microscopy.
2- The efficiency of self-healing agents for sealing the cracks.
   - Water permeability using low pressure.
   - Water permeability using high pressure.
3- The efficiency of regaining the mechanical properties.
   - 3-point bending test.
   - Tensile test (pull out test).
   - Compression test.

Chapter 3

A review of classification, application, and the effect of bacteria-based self-healing of concrete

3.1 Introduction

The factors effect on the durability of concrete are well-known and the main factor of contaminants concrete is cracks. For many years researchers attempted to develop a smart concrete that can heal itself and they invented many types of autonomic self-healing approaches. For example, microbiologically-induced calcite-precipitation by bacteria is utilization in concrete. Both bacteria and its precursors embedded within the concrete to produce mineral, usually calcite to heal the cracks. However, this approach creates a number of challenges for engineering and scientific specially for BBSHC aspects. This chapter provides a review of classification of bacteria types, previous and ongoing application of most utilized bacteria on construction materials, and the effects of procures materials and bacteria spores/cells on physical and mechanical properties of fresh and hardened concrete.

3.2 Classification of bacteria

3.2.1 Bacteria are generally classified with respect to:

- Shapes, which can be divided into

  i. Rod-shaped bacteria (Bacilli).
  ii. Sphere-shaped bacteria (Cocci).
  iii. Spiral-shaped bacteria (Spirilla).

- Classification related to Gram strain

  The Gram straining method is the ability of an agent used to bind the cell wall of the bacteria, which can be Gram positive or Gram-negative. Gram positive stains crystal violet blue while Gram negative stains a safranin/basic fuchsine pink colour. The gram staining depends on the presence of teichoic acid in the cell wall of bacteria, where teichoic acid is only present in gram positive bacteria.

- Classification related to oxygen requirement

  The microorganisms are classified according to the requirement for oxygen into four groups: obligate aerobic or strict, which grow in the presence of oxygen. Facultative anaerobic bacteria grow either with or without presence of free oxygen. Some types of bacteria are microaerophilic that have best grow when the concentration of oxygen molecular is low[294].

3.2.2 Classification of carbonate precipitation from microorganism's activities

The main source of carbon is present in carbonates in the form of limestone and dolomite, which cover around 41.9% of the total carbon on Earth[285]. Natural processes (biogenic origin) at the Earth's surface strata occurring due to microorganism such as bacteria, fungi, algae, and metazoa produce insoluble carbonate[286]. It is significant to know about the types of carbonate precipitation due to microbial activities. In this review the carbonate precipitate is divided into biologically controlled mineralisation, microbially induced calcium carbonate precipitation (MICP), and biologically induced mineralisation as shown in Figure 3.1.

- **Mineralization controlled biologically (MCB)**

Mineralization controlled by biological (MCB) processes could be one of the following types:

i. **Extra-cellular MCB**, where the production outside the cell is a macromolecular matrix in an area considered a site of mineralisation. This type of process is more popular in the natural environment precipitation and as biotechnological application in construction fields. Microorganisms produce calcium carbonate extracellularly in the presence of a calcium source through one of two metabolic pathways, namely the autotrophic pathway and the heterotrophic pathway.

ii. **Inter-cellular MCB** is a process that generally occurs in single-celled organisms, wherein the epithelium substrate governs the nucleation and growth of specific phases of bio-minerals in large surface areas of the cells. This kind of mineralization can be seen in calcareous algae, in which calcite nucleates and grows perpendicular to the cell surface[287,288].

iii. **Intra-cellular MCB** is a crystallization process due to compartmentalisation that occurs in direct nucleation specialised vesicles in cell bio-minerals[289]. This type of process is carried out by many sorts of eukaryotes and prevalently tissue-forming multicellular ones[290,291].In MCB the process is controlled by the organism, and the organism synthesises minerals independent of environmental conditions.

- **Mineralisation induced by biological processes**

According to Frankel and Bazylinski[292] mineralisation induced biologically (MIB) is that the metabolic secretions of microorganisms react with the presence of ions or compounds in the environment, which subsequently precipitate into mineral particles. The formed bio- minerals are due to nucleation and grow extracellularly, chiefly as calcite precipitate in the MIB process.

These types of bacteria are used by researchers in different building materials, as shown in Table 3.1.

3.2.3 Calcium carbonate precipitation by microorganisms

Microbially induced calcium carbonate precipitation (MICP) is a bio-mineralisation process, and occurs by either: MCB or MIB[290]. Production of calcium carbonate could be due to natural or artificial methods by microbial activities.

MICP is a biochemical process, and this process has four key factors to complete the process by the microorganisms; (a) The concentration of calcium to produce calcium carbonate, (b) the dissolved carbon concentration for calcite precipitation, (c) the pH concentration and the availability of nucleation sites, and (d) the microorganism's ability to nucleate. $CaCO_3$ precipitation depends on the solubility constant (k_s) and the ion activity product (k_{iap}). If $k_{iap} > k_s$ the system is oversaturated and precipitation is likely[293].

Theoretically, calcium carbonate precipitation occurs in nature following several processes:

i. Chemical precipitation due to evaporation from saturated solution by increasing the temperature and/or decreasing the pressure.
ii. Production of external and internal skeleton by eukaryotes.
iii. CO_2 pressure derivation under the effect of autotrophic processes.
iv. Fungal mediation.
v. Bacterial mediation.

There are six main groups of microorganism that can induce $CaCO_3$ formation in nature and artificially in microorganism[133] as shown in Figure 3.1. Zhu and Dittrich[294] compared six types of metabolisms, namely; photosynthesis, ureolysis, denitrification, ammonification, sulfate reduction and methane oxidation, and their results showed that the most published studies in the natural field were on photosynthesis at around 1128 articles followed by 120 articles on ureolysis. In contrast, the artificial field showed that ureolysis was the dominant developed technology, by 19 articles, followed by 13 articles on photosynthesis. Moreover, sulfate reduction is studied widely, but it is taken less advantage of in engineering projects.

a- Photosynthesis

Photosynthesis is the process used by some kinds of organisms such as *cyanobacteria* and *algae* to synthesize $CaCO_3$ from carbon dioxide and water in the presence of calcium ions, using sunlight. Photosynthetic bacteria are classified into two groups; oxygenic and anoxygenic bacteria. The difference between them is in the utilisation of the electron donor type to produce methanol. In oxygenic bacteria water acts as an electron donor, while in anoxygenic bacteria hydrogen sulphide (H_2S) acts as an electron donor[295].

A result of photosynthetic activities in microorganisms is an increase in pH due and subsequent increase in carbonate concentration, which means this process possibly occurs only in the presence of carbon dioxide in the surrounding environment. This gives an indication that the photosynthetic pathway would be useful for a concrete construction area, which is exposed to both carbon dioxide and light.

b- Ureolysis

The ureolysis process is similar to the photosynthesis process, which has an effect on the concentration of the dissolved inorganic carbon (DIC) and the pH of the surrounding environment during urea hydrolysis. The pathway involving enzymatic hydrolysis of urea involves the use of bacteria that are capable of converting urea to ammonia and carbon dioxide[97,296]. An example of ureolytic bacteria is *Sporosaricina pasteurii*[297]. In the reaction, one mole of urea is converted to one mole of carbonate and two moles of ammonium ions. This reaction increases the pH from neutral to alkaline conditions resulting in the formation of carbonate ions that precipitate to form calcium carbonate, most usually in the form of calcite. Urea is considered nitrogen source for many kinds of microorganisms such as fungi (*Ustilago sp.*,*Neurospora sp.*, and *Aspergillus sp.*), *bacillus* (*B. pasteurii, B. subtilis, B. lentus,* and *B. sphaericus*), *lactobacillus, purple sulfur and non-sulfur bacteria*, etc[298].

In the artificial field the most common ureolytic bacterium is *genus bacillus*. The property of this type of *genus bacillus* is that it is aerobic, is rod-shaped, Gram-positive, and the cell size is between 1 to 10 μm[298].

c- Denitrification

Denitrification is extraction or loss of nitrogen or its compounds, and this process is performed commonly by bacteria living in soil, which usually results in reduced amounts of nitrates by escape into the air. This process is generally done under anaerobic conditions, and the resource of energy and cell growth used by microorganisms is nitrate[299]. According to Karatas, many kinds of bacteria have the capability of reducing nitrate, including *pseudomonas, bacillus, micrococcus,* and *thiobacillus*[300]. This process increases the pH from neutral to alkaline conditions in the surrounding medium resulting in the formation of carbonate ions that precipitate to form calcium carbonate. A few studies have been published on this type of process by observing crystals of calcium carbonate formed around the two isolated bacteria cells namely *Pseudomonas aeruginosa* and *Diaphorobacter nitroreducens* [301].

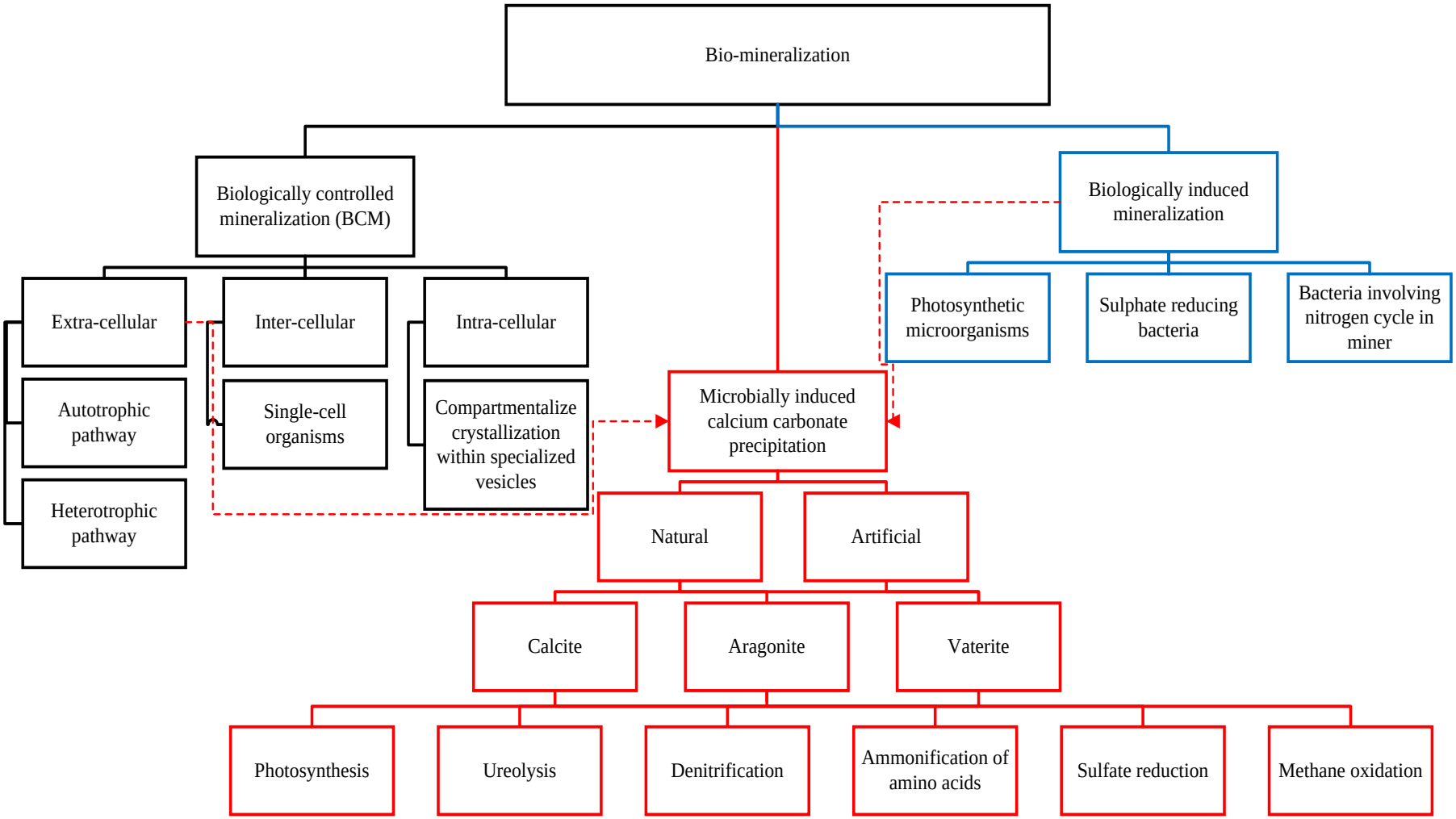


Figure 3.1: Plan shows the main categories of Bio-mineralization.

d- Ammonification of amino acid

Ammonification of amino acids is the process of bacteria or fungi converting organic nitrogen into ammonium (NH_4^+). This process is also called mineralisation. The main characteristics are aerobic conditions, Gram-negative, rod-shape cell, heterotrophic, non-pathogenic and the resource of energy and bacterial cell growth is amino acids[302]. Some species of myxobacteria such as *Myxococuss xanthus* can precipitate carbonate[303].

e- Sulfate reduction

The main characteristics of sulfate reduction bacteria (SRB) are that they are anaerobic, ubiquitous, prokaryotic, Gram-positive, highly diverse phylogenetically and morphologically, and members of a heterogeneous group of eubacteria and archaebacteria[292]. SRB convert sulfates to sulfides while oxidizing organic carbon to bicarbonate, and during this reaction both saturation state and pH increase [304]. Some species of bacteria such as *cyanobacteril* can precipitate carbonate underneath the surface layers[305]. On the other hand, the *Desulfovibrio* bacterial family is used widely in biotechnology and is applied to remove sulphates from gypsum[306].In addition the *D. desulfuricans* strain D20 was used by Bosak and Newman to precipitate calcium carbonate by providing heterogeneous nucleation sites[307].

f- Methane oxidation

Methane oxidation is an anaerobic microbial process occurring in freshwater sediments and anoxic marine areas to precipitate calcium carbonate, whilst the aerobic methane oxidation process dissolves carbonates by increasing acidity[308]. According to Boetius et al., a large portion of methane in marine sediments is converted to CO_2 through anaerobic oxidation[309].

g- Carbonate precipitation due to microbes in natural environments

The native bacterial community always influences environmental conditions, thus will change the environment due to its metabolic activities[310]. The precipitation of carbonate minerals by microbes occurs in a wide range of environments, such as caves, marine and fresh water, hot springs, and soil[311]. The overview of microbial induced carbonate formation in natural environments is shown in Table 3.1.

Table 3.1: overview of microbial induced carbonate formation in natural environments

Embedment in natural	Mechanism of precipitation	microorganism	Type of precipitation	Ref.
Caves	photosynthetic and ureoly	Cyanabacteria,	Stalactites and stalagmites	[312–314]
fresh water	Photosynthetic, denitrification, sulfate reduction, methane oxidation and ureolysis	Picocyanobacteria and cyanobacterial	Oligotrophic, hypereutrophic, europhic, and mesotrophic	[315–317]
Marine water	Photosynthetic, sulfate reduction, methane oxidation, denitrification, and ureolysis.	Cyanabacteria, and non-phototrophic bacteria	Carbonated sediments, thrombolites, and stromatolites.	[314,318,319]
hot springs	Photosynthetic.	Photoautotroph, heterotrophs, and Cyanabacteria such as *Thermosynechococcus elongatus*.	Travertine, aragonite, and dendritic pool.	[320–323]
Soil	Photosynthetic, denitrification, sulfate reduction, and ureolysis	Ureolytic bacteria, such as *Bacillus sp.,* S. *pasteurii, Pseudomonas calcis and Pseudomonas denitrificans,myxococcus*.	Calcite, polymorphs of calcite and calcium carbonate.	[324–327]

3.3 Viability of bacteria in concrete

Bacterial surfaces play an important role in calcium precipitation[92], due to the presence of negatively charged groups at high pH, positively charged metal ion could be found on bacteria surfaces. Commonly, carbonate precipitates develop on the external surface of bacterial cells by successive stratification and bacteria can be embedded in growing carbonate crystals[328]. The key roles of pH and calcium metabolism in microbial carbonate precipitation are described by Hammes[329]. The bacteria can be used externally to remedy the external cracks in concrete cracks or to remedy stones as illustrated in Figure 3.2 or can be used inside concrete mix as spore self-sufficient self-healing or can be used in capsular state inside tubers as illustrated in Figure 3.3.

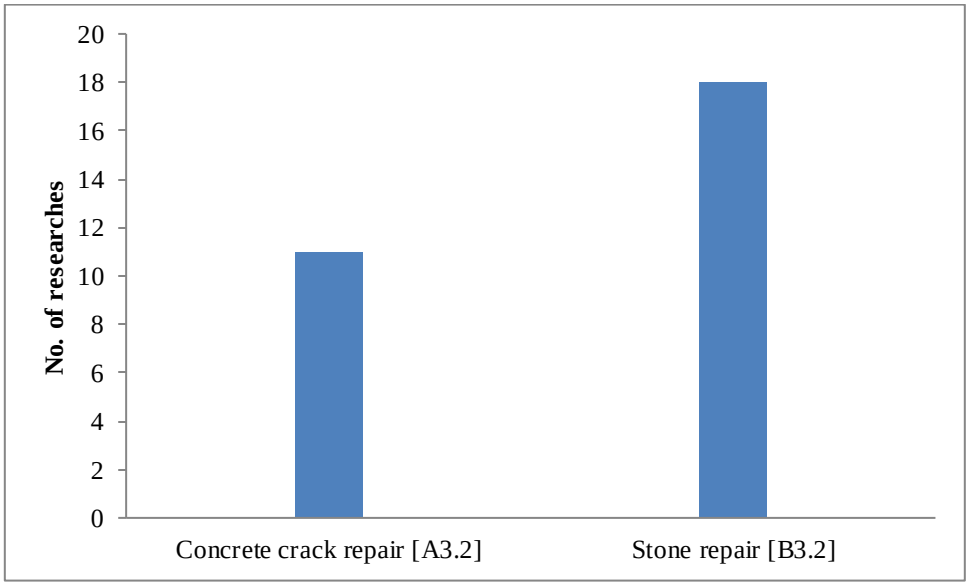


Figure 3.2: Number of researches for repairing both concrete and stone in period (1990-2012).

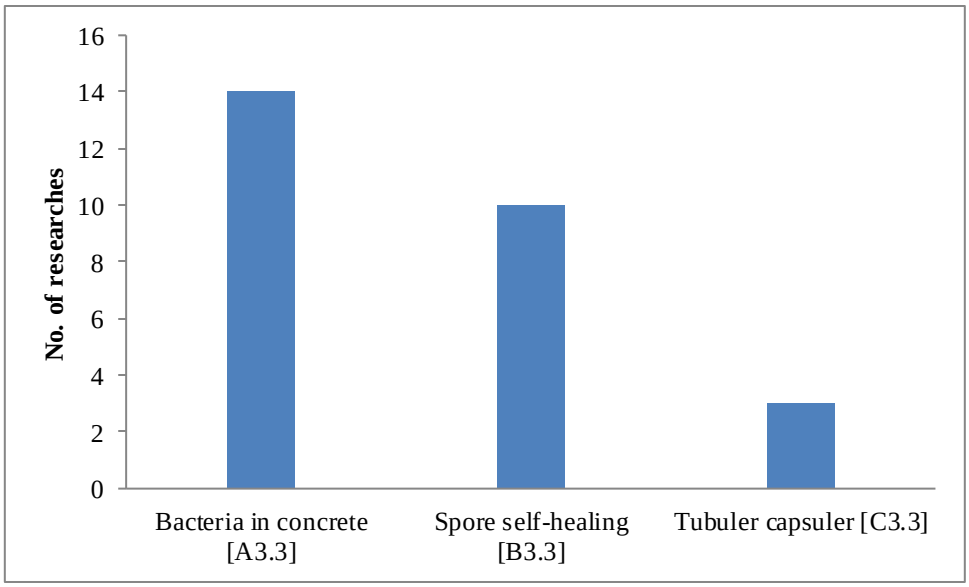


Figure 3.3: Number of published papers using different technique of bioengineering over the period. 2005-2013.

3.4 Types of bacteria used in construction materials

In construction there are various types of bacteria used, as shown in Table 3.2.

Table 3.2: Different bacteria used in building materials in the literature.

Application	Type of bacteria	References
Stone	*Calcinogenic bacteria* *Myxococcus Xanthus* *Bacillus Sphaericus*	[102] [103] [17,104,105,330]
Bacterial mortar	*Bacillus Cereus*	[106]
Repair of cracks in concrete	*Bacillus Pasteurii (Sporosarcina pasteurii)*	[224]
Biological concrete	*Bacillus Sphaericus* *Shewanella* *Bacillus Pasteurii*	[115] [116] [114]
Self-healing concrete	*Bacillus Pseudofimus* and *Cohnii*	[127,118,130]

3.5 Different applications of Bacteria in construction

A variety applications of calcium carbonates have been used commercially in the last two decades, and still a wide range of potential areas is under development. For example, oil recovery, soil, stone, and bio-cementation. The cementitious property for biotechnology makes calcite suitable for CO_2 absorption and restoration of construction materials. The induction of calcium carbonate precipitation by ureolysis, photosynthesis, denitrification, and ammonification has been used in this biotechnology[18,301,331,332].

3.5.1 Oil well

Crude oil is one of the main sources of energy in the world, thus crude oil affects the economy, politics and technology. Biotechnology is used in the development of extraction of oil wells and plugging fractures in areas of water production to avoid excessive production of water for oil recovery.

Conventional methods of recovery of oil are conducted in two phases: (1) in the primary phase between 10-15% of the original oil in place is recovery and (2) in the secondary phase around 15% of the original oil in place is recovery by water flooding[333]. Subsequently, nearly 65% of oil is left in the reservoir by the end of conventional methods. However, while there are many attempts to increase the percentage of extraction of oil by chemical flooding or thermal processes, these methods are costly, environmentally hazardous, and leave

undesirable residues which are difficult to remove[333]. In contrast, biotechnology presents an eco-friendly and cost-effective strategy. The most common application of biotechnology is to produce biopolymers, bio-surfactants, gases, acids, and solvents to reduce interfacial tension between oil and water, oil and rock interfaces[334]. On the other hand, a variety of *bacillus* strains such as *Bacillus pasteurii* have been used to plug high permeability areas, and leads to prevention or reduction of water flooding during oil recovery and improves the yield from oil wells[93]. Zhong and Islam investigated the microbial mineral plugging process to repair fractures in oil wells and to study the factors affecting that process[93]. The details of their study have been shown in Table 3.1.

3.5.2 Soil

The soil mechanical properties (friction, permeability, cohesion, and stiffness) are important for engineering construction, which serves as the basis for railways, roads, dams, slopes and dunes [335]. Therefore, the stability of soil is required for desired land uses for construction purpose, especially for those whose mechanical properties are insufficient. One of the strengthening techniques for soil is chemical grouting, but it is costly and to treat large volumes requires many injection wells[336]. The most suitable solution for soil stability is bio-grout or/and bio-cementation, which are eco-friendly and cost-effective strategies. The most biotechnological studies in sand were based on hydrolysis of urea to precipitate $CaCO_3$[335].

The first demonstration in vitro of soil bacterial ability to precipitate calcium carbonate was in 1973 by Boquet et al[337].The bio-cementation process was intensively studied by most research groups on sand[115,338,339]. The demonstration of their experiment was by filling a tube with mixed sand with bacterial culture or filling it with sand and dripping bacterial cultural solution through the sand column. In a few days the sand formed a solid column in the tube. Achal et al.[115] estimated the weight of $CaCO_3$ formed in sand samples plugged by *Bacillus pasteurii*, which was 24% and later they used a mutant of *Bacillus pasteurii* to increase $CaCO_3$ weight by 33%[115]. Moreover, Dejong et al.[340] demonstrated that the strength of soil increased significantly due to bio-mineralization of *Bacillus pasteurii*, while Rong et al. found the strength of the column of bio-sand can reach up to 6.1 MPa[339]. The precipitation method of calcium carbonate was applied in large column scale soil by using *Sporosarcina pasteurii* (DSMZ 33) for an improvement project[341]. The mechanical soil properties such as stiffness, permeability, volumetric response, and shear strength were significantly improved[19,342]. The improvement that happened in the soil reduces the impact and the cost on the environment[19].

3.5.3 Stone

The main factor in deterioration of ornamental stone, historical building and monumental stones, and urban stone heritage is weather. Many maintenance treatments to protect these historical objects have been introduced to enhance stones before untreatable damage occurs. The first applied biotechnology to protect ornamental stone was in 1990 by Adolphe et al.[343]. In 1993 the first application in situ was carried out in Thouars on Saint Médard Church tower. An area of 50 m^2 of Tuffeau limestone was treated[106]. The evolution of treatment was evaluated twice, after 6 months and again after one year in 20 points over the treatment surface. The measurements confirmed the consistency and good quality of organic carbonate. Similar procedures followed on two types of limestone statues (Tuffeau, and Saint-Maximim), placed in different climatic environments. The experiments revealed that two types of limestone had good protection due to surficial bio-mineralization by carbonatogenic bacteria[106]. Additionally, it was reported that the bio-treatment using microbial carbonate precipitation on the Santa Maria Church in Italy did not impact the aesthetic appearance[344]. Dick et al.[18] investigated the ability of *B. sphaericus* to deposit a layer of carbonate on the stone surface by testing the water absorption rate of treated and untreated Euville limestone. They proposed immersing limestone cubes in liquid medium for 2 weeks with 1% of the different strains, and during this period the samples were rewetted every 2 h by shaking for 5 min. Then, calcium chloride was added to the medium to precipitate calcium carbonate. Then, for a week, the samples were suspended in fresh medium for the second phase, and calcium chloride added in the fourth week in sterile conditions at 28°C. Their results showed that the treated samples decreased water absorption rate by about 50% compared to untreated samples. Richardson et al.[345] proposed using *sporosarcina pasteurii* to produce calcite as a binding base and investigated the impact of MICP as a natural binding source for the treatment of historic natural stone (sandstone and limestone). The results confirmed that possibility of using bacterial precipitation as stone particle cementation.

In 1991 Heselmeyer et al.[346] used *Desulfovibrio vulgaris* to obtain complete removal of gypsum crusts in laboratory conditions from marble samples. Furthermore, Ranalli et al.[347] optimised the Heselmeyer procedure, where they used sepiolite as a carrier system for *D. vulgris* and *Desulfovibrio desulfuricans*. This system provided anaerobic conditions, shortened time for treatment, and humidity for bacteria. Cappitelli et al.[348,349] used Carbogel

as a delivery system for bacteria. The results presented high retention allowance for the delivery system and significantly decreased the entrapment time needed for microorganisms.

Additionally, Castanier et al.[350] applied the ammonification of amino acids isolated from soil (*B. cereus*) to decayed limestone, which was among the first attempts to explore this method to be used in preserving a historic property. *M. xanthus* was found capable of producing cohesive carbonate, which was remediated limestone[331]. The biological materials composites by bacteria can produce new crystals more resistant to stress than those in the original stone. Biological crystals depend on the composition of the culture medium.

3.5.4 Concrete

Besides the application of biotechnology in the petroleum field, geotechnical engineering field, and building materials, biotechnology has also been used potentially for binder-based materials generation such as self-healing of binder material in mortar/concrete, improving development strength, and remediation cracks in concrete and building materials.

3.5.4.1 Biological mortar/ bio-groud

The knowledge due to bio-concepts has been used for more than two decades in the development of biological mortar for the purpose of repair of small cracks in concrete and cavities on stone surfaces. Referring to De Muynck et al.[120] biological mortar consists of bacteria, nutrients containing calcium salt, and limestone powder. The three main components of biological mortar dosage were optimised to obtain sufficient mortar, which resists surface tension in the presence of micro cracks and towards fracturing. During the in vitro experiments to evaluate different parameters of biological mortar, the best proportion that was obtained was 25% of bacterial paste (containing 10^9 cells mL^{-1}), 25% of nutritional medium, and 50% of limestone powder with particles of stone between 40 and 60 μm. The mortar was applied and tested in small scale on the Amiens Cathedral sculptures and on the church portal of Argenton-Château in France. The observation after two years of treatment showed a satisfying appearance in the repaired areas[106].

3.5.4.2 Crack remediation in concrete

Hair cracks are quite common in concrete due to chemical attachment, freeze-thaw cycles, …..etc. A large number of maintenance materials are commercially available to repair these

kind of cracks. However, these maintenance materials have a number of disadvantages, such as the difference in thermal expansion between concrete and these materials, strength, and degradation over time.

Nowadays, many researchers propose using biotechnology in concrete constructions, and in 2001 Ramachandran et al.[224] studied the remediation of concrete by using *B. pasteurii*, nutrients and sand as filling material. The results obtained from samples with microbiological treatment significantly increase mechanical properties such as compressive strength and stiffness values compared to those without microbiological treatment. Moreover, Day et al.[96] considered different types of filling materials with microbiological methods to seal cracks and their effect on the efficiency of repairing cracks. The results presented high stiffness improvement in beams treatment by using bacteria and polyurethane compared to sand, lime, fly ash, and silica as filling materials. De Belie and De Muynck[99] investigated the ability of *B. sphaericus* with ceramic as a filling material to seal concrete cracks. The authors protected *B. sphaericus* from the high pH in concrete by immobilising it in a silica solution, which presented an additional salt. Their observations showed that samples repaired by bacteria with ceramic were similar to samples treated by epoxy injection, which had decreased the water permeability.

Many researchers[17,99,351] have got positive results, such as an improvement in strength of concrete, increased and improved durability, and a decrease in both permeability and surface water absorption in their laboratory investigations. The compressive strength is significantly improved by applying microbiological processes[20,224,225,352]. Furthermore, the surface of concrete improved due to microbiological treatment, in which the water absorption rate was decreased[104,353]. Richardson et al.[354,355] investigated the ability of bacteria and its nutrients and precursor on surface finish as an environmentally friendly way to repair concrete cracks instead of traditional methods. The results demonstrated that *S. pasteurii* has the potential and subsequently MICP holds in enhancing the integrity of completion to concrete, without using chemical based sealants. Further, they found *S. pasteurii* can seal micro-cracks to a depth of about 20 mm.

3.5.4.3 Bacteria-based self-healing of concrete

Concrete is made from biochemical agents, and microorganisms and agents are applied in the mixture of concrete to increase the lifespan or the durability of concrete structures by sealing

the cracks. Still there is a gap of knowledge on the consequences of supplying these materials directly to the mix, such as the mechanical properties of the concrete (strength, creep, modulus of elasticity), durability (both of which depend on concrete microstructure), and the interactions that could happen between the biomass and the matrix of the cement.

The first investigation on BBSHC concrete was in 1998 to improve the compressive strength of cube mortar samples, by Ramakrishnan et al.[351]. In 2001 Ramakrishnan et al.[224] studied in detail the BBSHC concrete. The authors cast mortar samples with dimensions 50.8x50.8x50.8 mm, which contained 660g sand, 240g cement, and 16.4 ml of *B. pasteurii* suspended in phosphate buffer. All samples were demoulded after 24 hours and cured in a medium of urea-$CaCl_2$ for 7 and 28 days. The results demonstrated a significant increase in compressive strength, specially for the 28 day samples, which was confirmed by Achal et al.[356] In 2005 Ghosh et al.[116] investigated the effect of adding *Shewanella* on the compressive strength of mortar samples. The experimental procedure was to cure the samples in air instead of a medium containing nutrients. The water-cement ratio was 0.4 for all samples, and the concentration of bacterial cells was 10^5 cell mL^{-1}. The results showed an increase in compressive strength of about 25% compared to control samples after 28 days.

3.5.4.4 Self-healing concrete

Self-healing concrete has both bacteria and nutrients inserted in the concrete matrix through microcapsules, vascular network, and/or combined between microcapsules and vascular methods. The first application of self-healing concrete as autonomous healing was in 2007 by Jonker and Schlangen[127]. The authors found the most suitable bacteria that might be able to survive for a long period in concrete were *Bacillus pseudofirmus* DSM 8715 and *Bacillus cohnii* DSM 6307. In the first stage of their research, bacteria were added as spores directly to the concrete mix, as bacterial spores can endure extreme chemical and mechanical stresses, and calcium lactate was added as a nutrient for bacteria. Their results by ESEM scan showed the presence of massive amounts of $CaCO_3$ precipitates in the seven day samples.

Bacteria and their nutrients are encapsulated in the form of spores and protected against forces during mixing by clay particles or by mixing suitable sand, and are used for crack repair. The studies by[22,128,129] using this type of technique indicated that crack healing improved. Wiktor and Jonkers[51] found that the maximum crack width that can be healed by this type of approach is 0.46 mm, and the spores only remained viable for a limited period.

Jonkers[129] showed the encapsulated spores are viable for at least six months. Moreover, Wang et al.[20] investigated the ability of silica gel and/or polyurethane to protect bacteria through concrete mixing and casting. The authors' experimental results showed the ability of silica gel to protect and its ability to precipitate $CaCO_3$ was higher compared to polyurethane, (25% by mass in silica gel while just 11% by mass in polyurethane) according to thermogravimetric analysis. Therefore, Ersan et al.[357] addressed the shortcomings of immobilisation by encapsulation of bacteria in silica gel, hydrogel, expanded clay, metakaolin, zeolite and granular activated carbon. The survivability of embedded bacteria in silica gel under harsh conditions was investigated and hydrogel was applied to immobilisation of bacteria[358,359]. It was observed that bacteria in hydrogel have the ability to fill crack widths up to 0.5 mm by $CaCO_3$ precipitation, due to the extra water provided by the swollen hydrogel. The bacterial spores approach works efficiently in a wetted environment like underground or water-containing structures. Table 3.3 gives a summary for all bio-deposition self-healing approaches.

3.6 Effects of hostile environments of concrete on bacteria

Since its invention, bacteria-based self-healing of concrete has created a number of challenges[360]. Many of these challenges are linked to the ability of bacteria to germinate, survive, and grow in the myriad hostile environments present in concrete[23,315,361–364].

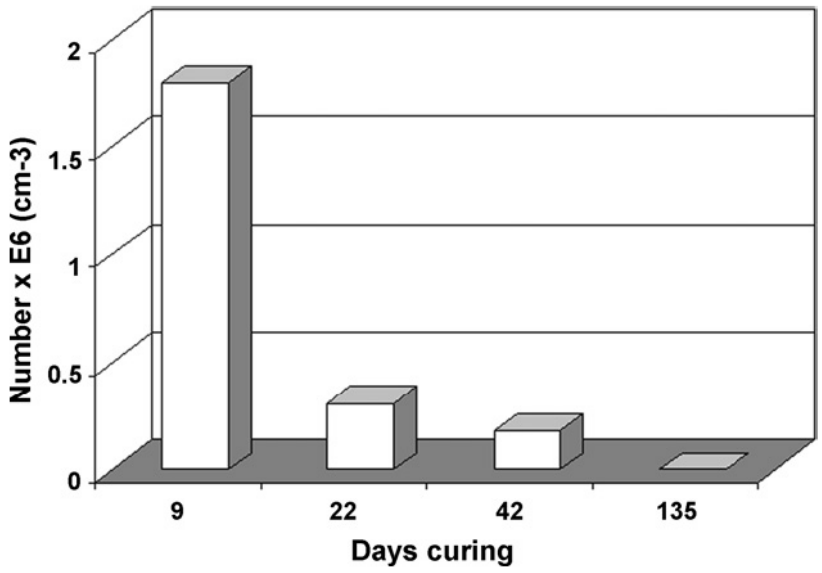


Figure 3.4: Estimated number of viable bacterial spores (*B. cohnii*) inside cement paste at different ages[23]

The ability of bacterial live cells and their spores to survive in concrete has been investigated by a number of researchers. Jonkers et al.[23] added *bacillus cohnii* spores directly to cement paste to identify the time these spores remain viable in high alkaline environment and showed that bacteria spores can survive up to four months (Figure 3.4).

However, they proposed that bacterial live cells have a few limited to stay alive in same state of spores because of cement hydration process and early strength stresses, which crush the micro size cells. Soltmann et al.[361] added non-alkaliphile *rlodecoccus ruber* live cells directly to a magnesium phosphate cement (MPC), demonstrating that pH has no effect on viability and that the main factor is the hydration of the cement. The results showed that viability duration of *R.ruber* was 19 days, even as the pH of cement was close to neutral. Moreover, Achal et al.[362] mixed *bacillus megatarium* ATCC 14581 with Portland cement (PC) paste and (PC with different percentage of fly ash) paste. The results demonstrated that the number of surviving cells increased with increasing levels of fly ash in the paste. Zhu et al.[363] investigated cyanobacteria *Synechococcus* PCC8806 cell viability in the cement solution and their results confirmed that *Synechococcus* PCC8806 can survive without protection at pH 11.7. Furthermore, a number of researchers[315,364] found that *Synechococcus* PCC8806 can survive in a wide range of hostile environments such as concrete, desert settings, and ologotrophic lakes. However, the viability for long-term survival still needs to be studied.

Table 3.3: Summary of self-healing bio-deposition approaches.

	Biological mortar	Remediation of cracks in concrete using bacteria	Bacterial concrete	Bacteria in tubular capsules	Bacteria encapsulation (Spores)
Function	Binder-based materials	Remediation of cracks externally	To be use in concrete mix for self-healing	For repairing the cracks at specified internal locations	Healing the concrete for internal cracks at any location
Component mix giving the best results	1:1:2 Bacteria/nutritional /limestone	10% (*S. pasteuri* + silica fume) and 90% sand maximum crack width 0.9 mm and depth 3 mm when *bacillus* + *sphaericus* in silica gel+ calcium source	No specified optimum concrete mix, and still under research	Different tubes filled with different agent and bacteria	Spores consists from specified bacterial and its nutrition
Versatility	Demonstrated healing mechanism dependent on external environment. Low versatility	Demonstrated healing mechanism dependent on external environment. Low versatility	Demonstrated healing mechanism dependent on external environment. Low versatility	The healing mechanism is functioning inside the specimen and independent on external environment. Low versatility	Needs continuous water exposure. Low versatility
Reliability	No tests have been done with more than one loading test	No tests have been done with more than one loading test	No tests have been done with more than one loading test	No tests have been done with more than one loading test	No tests have been done to test the ability of spores to heal the renewable cracks for another cycle of loading
Quality insurance	After the treatment indicated a satisfactory appearance of the repaired zones	Regain strength and tightness	Strength, durability and tightness	Regain strength + permeability	Regain strength and tightness
Pervasiveness	Bacteria uniformly distributed in the mortar, but the mortar is applied in one place for repair	Bacteria uniformly distributed in the mortar, but the mortar is applied in one place for repair	Bacteria uniformly distributed in the mortar, but the mortar is applied in one place for repair	The tubes have to be placed in specified locations	Distributed uniformly in the concrete
Reliable data to be used for assessment	No	No	No	No	No
References	92,106,93,341,365	224,93–100,366	114–116,117	97,132,20,130	126,77,127,23

3.7 Effects of bacteria and bacterial self-healing agents on concrete

3.7.1 Effects of vegetative cells of bacteria

According to previous studies[23,361,362], bacteria cells and spores remain viable for a short time in the early-age properties of concrete and have a limited effect. Indeed, extensive research was conducted with different bacteria species to improve the durability and strength of concrete[17,20,104,116,117,121,127]. Further, some researchers[116,224,367] investigated the relation between the proportion of bacteria and compressive strength. Their observations showed that compressive strength improved with an increased proportion of bacteria, as a result of the precipitate $CaCO_3$, due to their nucleation for reactions, or filling the cement mortar pores with filler material. The strength was improved by about 25%[116] to 33%[367] as a result of microbiologically-induced mineral precipitation. The compressive strength of mortar specimens was significantly improved (around 18%) by the introduction of aerobic microorganisms (*B. pasteurii* & *Pseudominas aeruginosa*)[224,351]. Jonkers and Schlangen[127] studied the influence of higher levels of bacteria (10^9 cell/ml) in concrete and found that bacteria has the potential to produce immobilizing material for self-healing, but this has no effect the strength. Bundur et al.[24] investigated the impact of adding vegetative bacterial (*S. pasteuri*) cells in a cement paste on early-age cement mortar properties. The results showed that the hydration kinetics of cement paste mixed with vegetative cells were greatly influenced and early-age strength was low. The hydration and strength after 28 days was also affected. Further, they added dead bacterial cells to cement paste and observed that its strength gain was less than live cell samples (Figure 3.5). This means that both live and dead cells delay hydration kinetics, but the metabolic activity of live cells during the hydration process might partially reduce the negative effect on hydration kinetics. On the other hand, growth medium added to cement mortar without bacterial cells had no significant effect in strength[356].

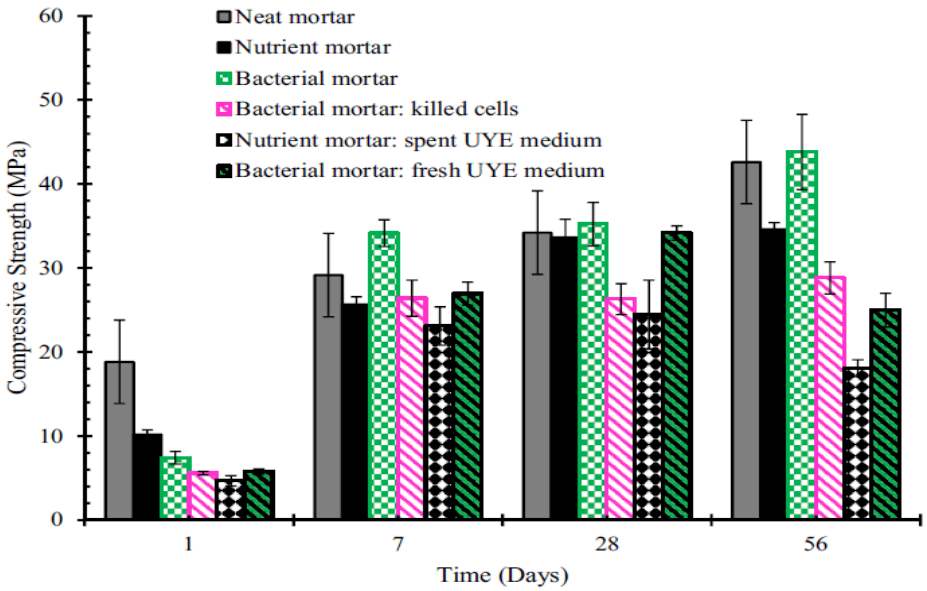


Figure 3.5: Influence of bacterial live and dead cells, and self-healing agents on the compressive strength of mortar[24]

De Muynck et al.[17] widely studied and reported the effects of calcite precipitation produced by bacteria on the durability of mortar samples, and results demonstrated that the surface of calcite crystal deposition reduced the absorption of samples by 65 to 90% depending upon filling material capacity. However, the carbonation rate was reduced by 25 to 30% and chloride migration decreased from 10 to 40%. Furthermore, calcite crystal morphology was impacted both by the bacterial culture type and the composition of the medium. Further, Ghosh et al.[368] observed that microbial mineral precipitation material showed a better crack repairing performance and durability than autogenous healing due to the materials of normal concrete.

3.7.2 Effects of bacterial self-healing agent

Most research regarding the bacteria-based self-healing of concrete has intensively concentrated on demonstration of calcium carbonate formation (calcite) as the healing compound. Many types of bacteria have urea hydrolysis pathways to produce calcium carbonate, but the source of Ca ions of this pathway for most research was calcium chloride. Recently, many researchers have replaced calcium chloride with calcium nitrate[21,359,369,370]. To avoid introducing chloride ions into the fresh concrete, the chloride ion (Cl^-) content permitted in fresh reinforced concrete according to BS 1881: part 124[371] is usually limited to not exceed by 0.3% of cement mass to prevent reinforcement inside concrete from initiate

corrosion and loss bond between steel and concrete. On the other hand, calcium nitrate has been used for a long time as an anti-freeze, to accelerate hydration, to increase the degree of hydration and as a setting accelerator, and has good defence against chloride to induce corrosion of steel in concrete, which is effective as an anodic inhibitor[21,372]. The disadvantage of this healing process is the formation of the ammonium particles that cause extreme ecological nitrogen loading[23]. It has additionally been recommended that the nearness of nitrifying microorganisms inside concrete could change alkali to nitric, leading to corrosion as a result of the forceful assault on concrete[373]. However, this has never been noticed in self-healing concrete. Wang et al.[21] have proposed that urea [4% by mass of cement] causes a delay in hydration. Furthermore, isothermal conduction calorimetry tests embraced by Bundur et al.[24] have demonstrated that urea has almost no impact at 0.5% by mass of concrete.

For metabolic pathways, various organic calcium precursors can be used for microbiology, of which the most prevalent has been calcium acetate. Testing by Jonkers et al.[23] hypothesized that the main calcium precursor that might be added specifically to the concrete without a loss of strength is calcium lactate. Cunniffe[374] demonstrated that calcium lactate has no effect on the strength of concrete. Further results showed that calcium lactate might increases the compressive strength when used at an optimum rate of around 1 to 2% by cement mass (Figure 3.6).

A conceivable purpose behind the change in execution is that adversely charged lactate particles may adsorb onto the surface of cement grains, giving a plasticizing impact and making more cement surfaces accessible for hydration. Furthermore, Cunniffe[374] demonstrated that calcium lactate could expand the initial setting time, but delay the final setting time. However, the organic calcium precursor should be epitomized before the expansion of concrete, as is currently the best practice for this pathway, then the impact of the precursor on early-age properties could be inconsequential unless there was a mass failure of capsules during the mix or cast.

Luo and Qian[375] studied the influences of bacteria-based self-healing agents on cementitious materials hydration kinetics, rheology, and compressive strength. Their study used three types of bacteria-based self-healing agents, namely RB agent consisting of calcium lactate and bacteria spores powder; JB agent consisting of calcium formate and bacteria spores powder; and NB agent consisting of calcium nitrate and bacteria spores powder. The ratio of self-

healing agents to ordinary Portland cement was 0%, 1%, 2%, and 3%, respectively. The results showed that the addition of bacteria-based self-healing agents greatly influences cement paste hydration kinetics. The rheology of different dosages of cement mortar with bacteria-based self-healing agents RB, JB, and NB were verified. Moreover, their result for the RB agent decreased early compressive strength, but this increased at day 28 compared to the control (Figure 3.7).

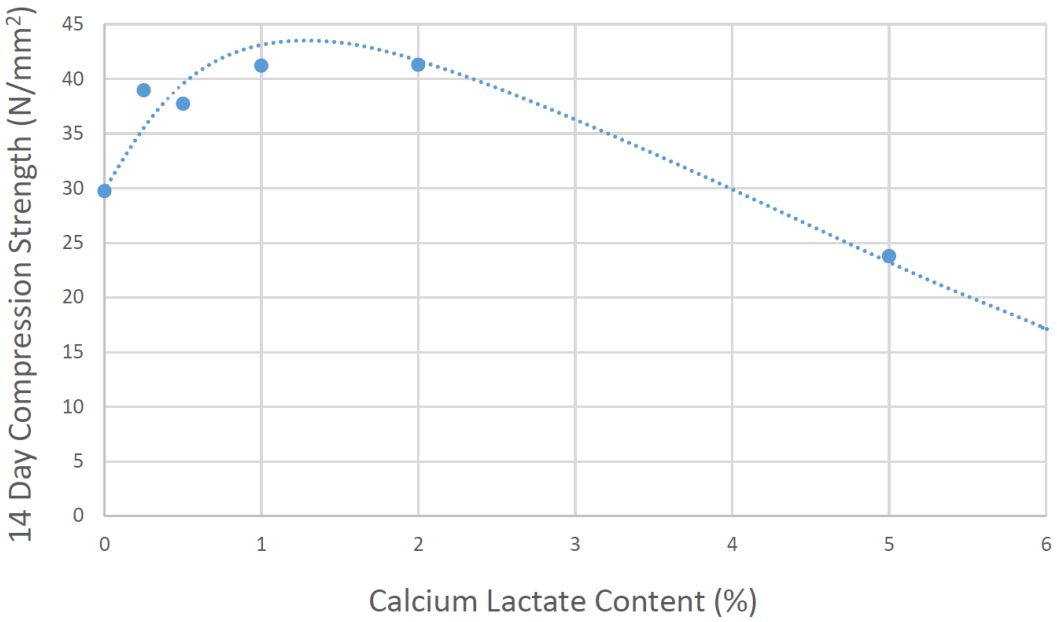


Figure 3.6: Effect of calcium lactate percentage by cement mass on compressive strength of mortar[374]

Furthermore, Thiyagarajan et al.[376] presented a study of the influence of *bacillus licheniformis* (10^6 cells/ml and 10^8 cells/ml) activity on rheology and compressive strength of cement mortar in different curing media, namely normal water (NW), Luria Bertania broth (LBC), and wastewater curing (WWC). They found that the amount of water required for bacterial culture samples is less than the control for obtaining the standard consistency and setting time values for both concentrations of bacteria when the medium were the same. Further, they observed that the strength gain over the duration of the test was non-uniform, indicating that the activity of bacteria was highly dependent on the type of medium as well as the curing period.

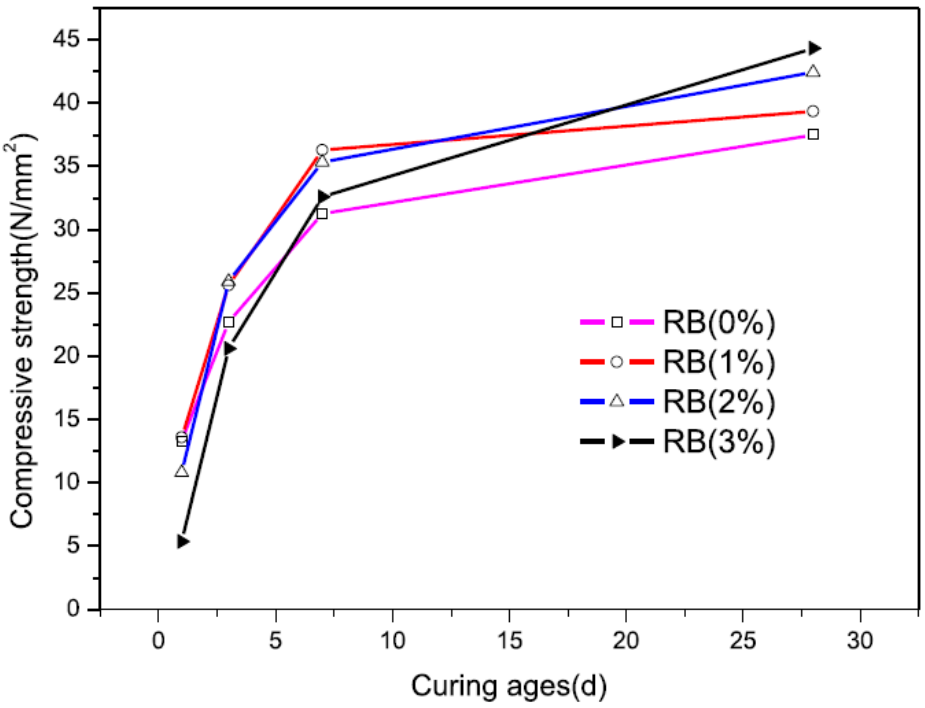


Figure 3.7: Effect of RB agent percentage by cement mass on compressive strength of mortar[375]

Yeast extract (YE) is usually used as a wellspring of carbon for the urea hydrolysis pathway and as a wellspring of nitrogen for the metabolic pathway. It additionally contains other key minerals such as water soluble vitamins, amino acids, peptides, and carbohydrates. Research demonstrates that YE added directly to concrete delays the setting of the cement and hardening of the concrete. For instance, Bundur et al.[24] showed that YE has a huge impact in retarding the hydration of cement. Inside a supplement mix comprised of tris, urea, and YE (1% by mass of concrete), the compound of YE had the best impact on the energy of hydration as shown by isothermal conduction calorimetry (Figure 3.8). Wang et al.[21] reported that isothermal conduction calorimetry of added YE directly to cement paste was 0.8 by cement mass.

Jonkers et al.[23] demonstrated that the expansion of YE (1% by mass of binder) prompts a diminishment in the strength of cement. However, Cunniffe[374] proposed that, when used underneath 0.5% by mass of binder, YE has no impact on mortar quality (Figure 3.9).

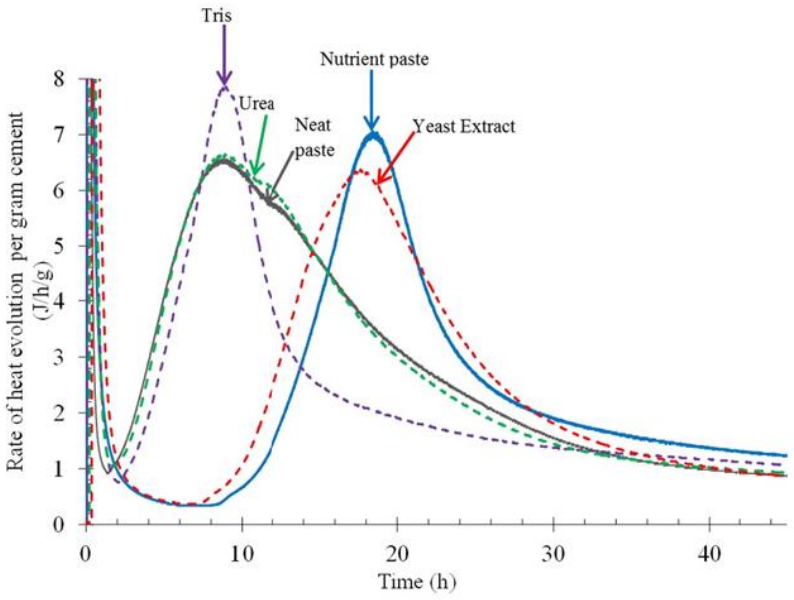


Figure 3.8: Effect of self-healing agent components on hydration of cement paste[24]

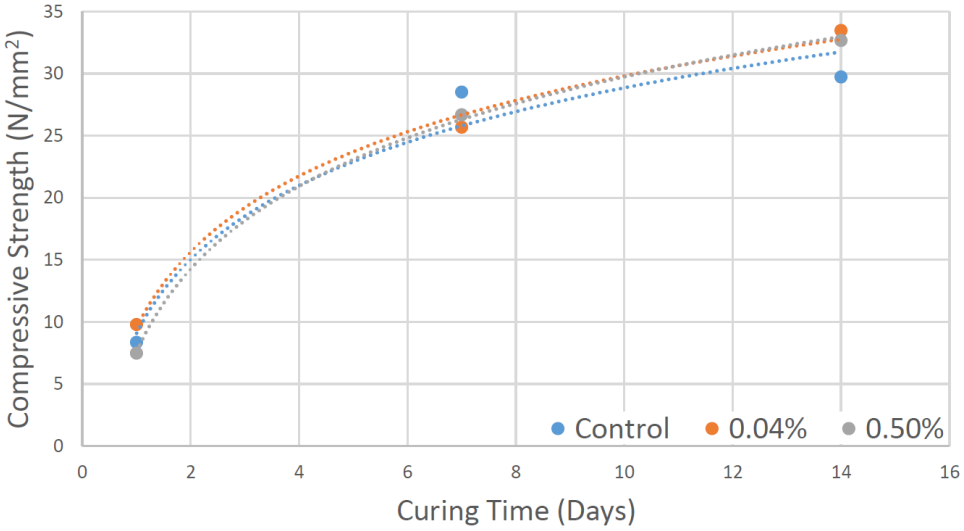


Figure 3.9: Effect of calcium lactate percentage by cement mass on compressive strength of mortar[374]

The use of sugar can be classified into three categories: effective retarders, good retarders, and non-retarders. For example, glucose and dextrose are known as set and hardening retarders of concrete essentially through their capacity to restrict the development of calcium silicate hydrate (C–S–H)[377] by adsorption onto calcium hydroxide and calcium silicates[378]. In the early 1990s, molasses was used as a retarder in channel construction between England and France to prevent residual concrete from setting because washing it out underground was impossible[5]. However, the impeding impact of sugars increments with sugar substance and it

has been recommended that a sugar substance of 0.1% by mass of binder can prompt an uncertain postponement of hardening[5]. Further, Abalaka[379] studied the effects of different concentrations of sugar by cement mass on physical properties of OPC past and concrete. Results showed that a long period of initial set time occurred at 0.06% of sugar concentration and 0.35 mm soundness value (Figure 3.10), and strength of concrete was improved by 3.62% at 28 days, though the compressive strength started to decrease significantly from 0.1% to 1% (Figure 3.11). In any case, there have been a few recommendations that past a sugar substance of around 0.15%, the impeding impact decreases and that for sugar substance in overabundance of 0.3% by mass of cement, nucleation impacts rise and the sugar acts as accelerator[377].

Sugars additionally have a more noteworthy retarding impact when they are added after few minutes of mixing water and cement[377].

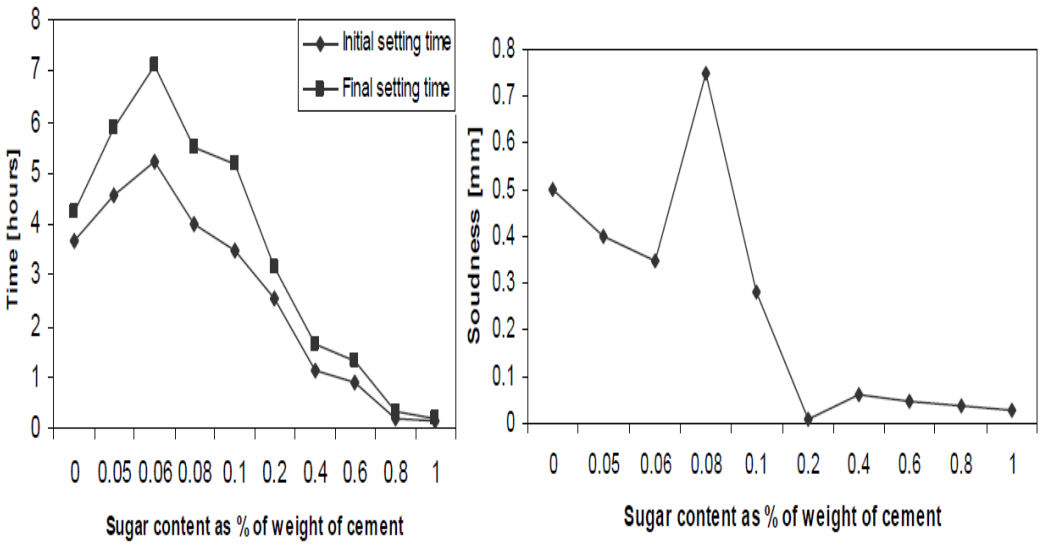


Figure 3.10: Effect of different percentage of sugar by cement mass on setting time and soundness value[379]

For metabolic pathways, numerous researchers have used a standard growth media consolidated with an organic precursor[380–382] like B4 or modified B4 medium comprising around 0.4-0.5% yeast extract, 0.5% glucose or dextrose, and 0.25%-1.5% calcium acetate. A Luria-Bertani broth supplemented with calcium acetate has also been used, consisting of, for instance, 1% tryptone, 0.5% yeast extract, 0.05% calcium chloride, and 1% calcium acetate[381].

Thus, the research investigation at the University of Bath will use calcium acetate as a less expensive and more accessible option to calcium lactate. Investigate the effect of the self-healing agents individually and as a combined medium on the mechanical and physical properties of fresh mortar, hydration kinetics, and early microstructure, as well as on the strength and microstructure development over time.

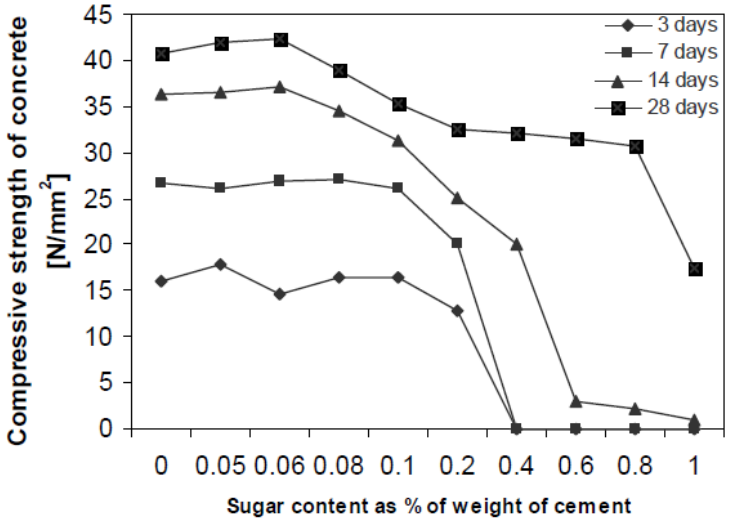


Figure 3.11: Effect of different percentage of sugar by cement mass on compressive strength of concrete[379]

3.7.3 Temperature

In connection to their capacity to be used as a part of concrete presented to various introduction the decision of microorganisms used additionally relies on upon their capacity to adapt to hot temperatures (thermophilic), frosty temperatures (psychrophilic), and salt-and fresh water situations. A significant number of these characteristics have not been considered in any suitable element in self-healing concrete and research trials to date have considered the germination of spores and precipitation of calcite at conditions near ideal for the bacterial species used, which is generally somewhere around 25 and 45°C for most alkaliphilic microbes[383]; the revelation of a calcite precipitating alkaliphile with comparative growth attributes would have a critical impact on the improvement of microorganisms based self-healing concrete. However, some alkaliphiles do demonstrate psychrophilic conduct. For instance, strain 207 is an aerobic coccus with a diameter of 0.8-1.2 mm that can grow at temperatures between -5 and 39°C and pH 8.5, and the optimum growth pH value decreased with increasing temperature[384]. Further, Kimuka and Horikoshi[384] studied the preliminary

characterization for three isolated bacteria, namely strain 207, strain 235 and strain 602, able to grow between 0 and 40°C and pH between 7.4 and 11.5. The results demonstrated that strain 235 and strain 602 could growth at pH between 6 and 12, with an optimum of 10.0 for strain 235 and the optimum pH for strain 602 was 9.5 to 10.0 at 10°C and 20°C, respectively (Figure 3.12).

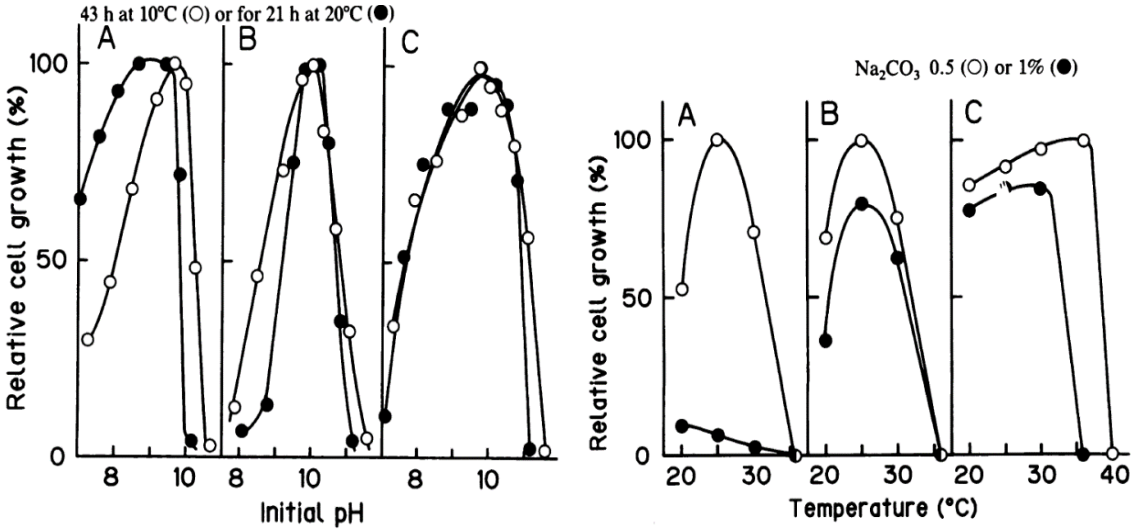


Figure 3.12: effects of pH and temperature on the growth isolated bacteria: (A) strain 207, (B) strain 235 and (C) strain 602[384]

A few strains of B. sphaericus, B. megaterium, and B. firmus, have appeared to preciptate calcite in B4 medium even at temperatures as low as 4°C[385]. While the precipitation was slower than that at higher temperatures, this provides proof that self-healing at lower temperatures using bacillusis is conceivable. Cacchio et al.[385] monitored the formation of calcium carbonate using bacteria from the arthrobacter species. These are oligotrophic microscopic organisms that can develop in exceptionally dry conditions, at low temperatures, and with constrained nutrient. However, most bacteria of this variety are not spore-producing, which generally makes them an unsatisfactory possibility for self-healing concrete.

Ghosh et al.[116] performed examinations to enhance the compressive strength of mortars using bacteria of the Shewanella genus, a thermophilic anaerobic microorganism. They did not examine its ability to grow in concrete at various temperatures, though they did watch the bacteria grow and precipitate minerals up to a pH of 11[386].

3.7.4 Crack size

For the elements of structural concrete to be durable and serviceable, the appearance of cracks must be controlled because they allow the entrance of water and different polluting influences like chloride and sulfate particles into the matrix of concrete, prompting untimely network corruption. Cracks in concrete must be minimized to enhance the life span and reduce maintenance. A number of studies on this theme have been done, mainly focusing on the impact of crack self-healing[22,65,133,387,388].

The main principle of using microbials is induced $CaCO_3$ precipitation to close micro cracks by adding bacterial cells directly to cracks or embedded bacteria spores inside concrete, which might grow in the presence of oxygen, water, or moisture followed by nutrients and a precursor to produce calcite that heal and seal cracks. Therefore, bacterial healing has been demonstrated to be valuable at recovering the low permeability of concrete when subjected to micro scale estimated (or smaller) cracks. Previous results demonstrated that cracks could be healed up to one mm within 3 to 14 weeks depending on many factors like the type of bacteria; amount and type of biochemical agents; processes of biochemicals; and crack width[20–22]. Nuguroho et al.[389] sealed cracks in cement mortar using bacteria as a self-healing agent and results showed that the bacteria self-healing agent could effectively seal cracks and prevent water flow up to 0.25 mm (Figure 3.13). Qian et al.[390] investigated the ability of *bacillus mucilaginous L3* to heal samples of 40 mm x 40 mm x 160 mm with w/c 0.4 cracks.

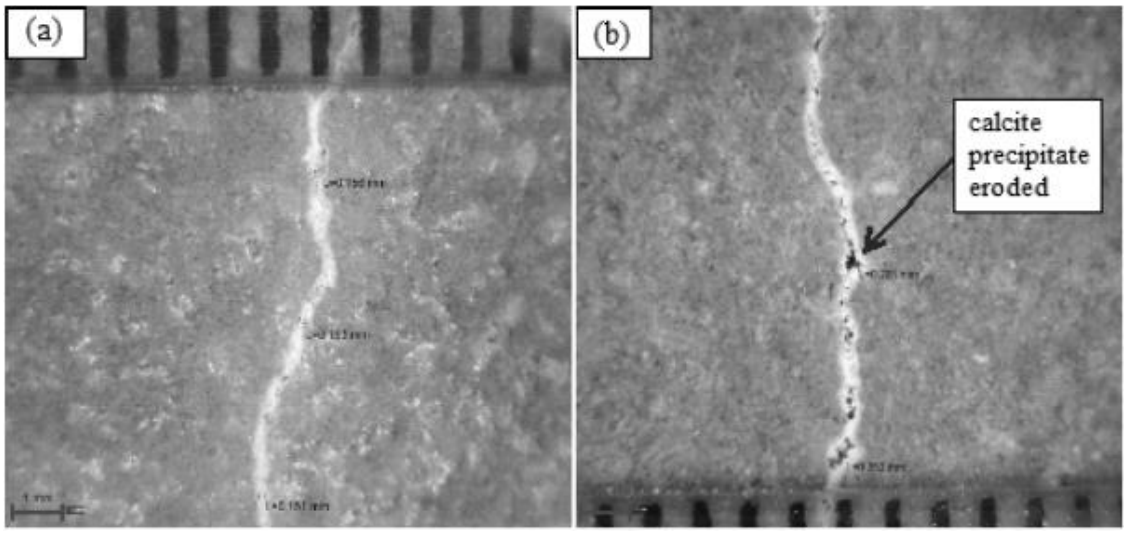


Figure 3.13: Crack healing/sealing by bacteria self-healing agent after permeability test: (A) crack width 0.15 mm, (B) crack width 0.25 mm[389]

The results demonstrated that this technique can completely heal 50-100 μm cracks. Erşan et al.[391] found that the most noteworthy crack width that could healed by the bacteria was 370 ± 20 μm in 28 days and 480 ± 16 μm in 56 days. Water tightness recapture up to 85% was accomplished toward the end of 56 days for a crack width of 465 ± 21 μm. Essentially by including the preserved bacterial spores and nutrient added substances into mortar samples, cracks up to 460 μm wide were completely sealed in 100 days[22]. Further, microbial precipitation calcite following ureolysis pathways induced cracks up to 970 μm to be significantly sealed after 56 days of inundation under water[21].

On the other hand, a new technique to control crack width developed at Cardiff University generated internal pre-stressing to close existing cracks or make them very narrow, so that they might be healed by a bacteria-based self-healing microcapsules system[392]. The efficiency of bioremediation in cementitious samples to heal cracks presented by various researchers is summarized in Table 3.4.

Table 3.4: The efficiency of bio-remediation in cementitious samples to heal cracks on MICP technology[393]

Samples type	Sample dimension (mm)	Bacteria type	Crack width (mm)	Efficiency evaluation	Ref.
Concrete samples	160 x 160 x 70	*B. sphaericus*	0.3	Decrease water permeability	[99]
Reinforced mortar	40 x 40 x 160	*B. alkalinitrilicus*	0.05-1.00	Oxygen diffusion barrier, self-healing	[22]
Cement mortar	50.8 x 50.8 x 50.8	*S. pasteurii*	3.175	Improve compressive strength by 61%	[224]
Cement mortar	50.8 x 50.8 x 50.8	*S. pasteurii (encapsulated)*	3.18	Improve compressive strength by 12%	[97]
Cement mortar	40 x 40 x 40	*B. pseudofirmus B. cohnii*	----	Prevent crack against water ingress	[394]
Concrete sample	160 x 160 x 70	*B. sphaericus*	0.3	Decrease water permeability and crack visually sealed	[100]
Reinforced sample	40 x 40 x 360	*B. sphaericus*	0.35-0.50	Decrease water permeability and regain strength	[20]
Reinforced sample	30 x 30 x 360	*B. sphaericus*	0.20-0.22	Decrease water permeability and healed crack by 48-80%	[21]

It is clear from the previous studies that microorganisms could heal cracks ranging from 50 μm to 3.20 mm thick. However, cracks could be healed by bacteria without filling materials only up to 0.45 mm wide. Thus, the research at the University of Bath will demonstrate the size of crack that can be healed or sealed by bacteria and the ability of bacteria to re-heal or re-seal the crack as a second cycle of healing.

3.8 Concluding remarks and factors influencing the selection of the proposed research

This review considered the effect of concrete properties on bacteria; the impact of both bacteria and self-healing agent on the physical and mechanical properties of concrete; the effect of variation temperature on the ability of bacteria to produce and precipitate calcite; and the efficiency of sealing and healing material corresponding to crack width and with restricted crack width and were each found to be effective to some extent. It is clear that developing an environmentally-friendly concrete system that can heal or repair itself requires the study of the following aspects:

1. Three types of *bacillus* metabolic pathways that grow and precipitate calcite at hot temperature (thermophilic), namely *b.cohnii, b.halodurans* and *b.pseudofirmus*, are still under research, but the relation between number of bacteria and the amount of calcium carbonate produced has not been studied yet.
2. The literature review indicated that no studies have tested the ability of bacteria spores to stay viable in concrete over the long term in concrete.
3. There is a lack information in the literature about the negative effects of self-healing agent (precursor and nutrients) on early-age and the fresh properties of concrete and the limitation of self-healing agent, which does not affect the physical and mechanical properties of concrete.
4. Previous studies have demonstrated the ability of these bacterial species to germinate spores and precipitate calcite at conditions near ideal, which is often somewhere between 25 and 35°C for alkaliphilic microorganisms. However, the ability of bacterial species to germinate and produce calcite at various ranges of temperature still needs further study.
5. The literature review presented a wide range of crack widths (50 μm – up to 3.18 mm) that could be healed or sealed by bacteria-based self-healing. However, there is

still a lack of information in the literature about the crack width that could be healed by bacteria with its self-healing agent without filling material such as sand.

The research work of this thesis displays the consequences of an extensive examination program that gives answers to these inquiries and contributes to the principal understanding of the previous questions.

Chapter 4
Methodology

4.1 Introduction

The materials used in this thesis are described in detail in this chapter, including the mix proportions of mortar specimens, casting and manufacture of fresh mortar, and demoulded and curing of samples. In the second part of this chapter is the evaluation of fresh properties of cement such as initial and final setting time, hydration kinetic of cement, and the mechanical and physical properties such as flexural, compressive strength of the mortar, unit weight, apparent density, sieve analysis, absorption and surface moisture. The efficiency of self-healing agents for sealing/healing the cracks using the permeability test (initial surface absorption test ISAT and capillary water absorption). Qualitative assessment of the efficiency of self-healing visually using the scanning electron microscope (SEM) results (to evaluate the durability of concrete) are also given, as well as microorganism selection, growth medium stock, and biology tests such as cultivation of bacteria by centrifugation and washing it, serial dilution-tube method, spectrophotometry, and alkaline agar plates, are also given.

4.2 Materials

4.2.1 Preparation of test specimens

The materials used for making mortar specimens was designed according to EN 196-1:2005[395], by taking W/C = 0.5, fine aggregate was used standard CEN sand for A/C = 3, and fly ash cement type CEM II/B-V 32.5R. The size of the samples was 40x40x40mm for compressive strength, and 40x40x160mm for flexure strength and a suitable percentages of alkali resistant fiberglass mesh was added to each mould to avoid penetrating throughout the depth, the mesh placed around 15 mm from the base.

4.2.2 Casting and manufacture of fresh mortar

The cement CEM II/B-V 32.5R was used and mixed with water in a CONTROLS **65-L0006/AM** AUTOMIX mixer for about 30 seconds at the low speed. The standard sand was added steadily to the cement paste and mixing continued during the next 30 seconds. The mixer was stopped for 90 seconds. The first 30 seconds was used to scrape any of the mix

adhering to the walls of the bowl and put it in the centre by hand, and then the mix was left to rest for 60 seconds. The mixer was switched on again at high speed and continued mixing for an additional 60 seconds, the procedure of mixing followed by BS EN 196-1[395]. Immediately after mixing was finished the fresh mortar was cast into moulds and compacted for 60 seconds by using a vibration table to remove air trapped in the cement mortar, then finishing the surface level of the mortar by using spatula.

4.2.3 Demoulding and curing of specimens

After casting, the specimens were cured in a curing room for 24h at 20℃ ±2℃ and a relative humidity of 95%. After that, the specimens were demoulded, using a hand drill to remove the screws, which provided the restraint for the mould. Once the restraint was removed the specimen was taken out from the mould, the specimens were immersed in water at 20℃ until the day of the test.

4.3 The procedure of tests used

In this study, setting time, hydration kinetic, compressive strength, flexural test, permeability tests, physical properties of aggregates (perlite), and SEM analysis were used. These test methods are briefly summarised below.

4.3.1 Setting time test

Setting time tests (Vicat Needle tests) were conducted according to BS EN 196-3:2005[396] to determine the initial and final setting time of the cement pastes. Methods of testing cement-part 3: determination of setting time and soundness.

4.3.2 Calorimeter analysis

The isothermal conduction calorimeter test monitors a heat method of hydration over time. A calmetrix I-Cal 4000 was used to determine the rate of heat evolution of cement paste hydration kinetics using isothermal conduction calorimetry at 20°C for 72 hours as shown in Figure 4.1.

4.3.2.1 Procedure

All the cement paste with SHA-1 was used as an input and the outputs were the rate of heat in w/g and time in hours. The first experiment was done without any bacterial self-healing

agents (SHA-1), only cement paste, this mix was called the control mix or reference mix. The other three mixes were prepared by using 0.1, 0.3 and 0.5% of SHA-1 from the mass of cement to find the optimum percentage of agent, which had no or had minor effects on cement hydration.

All samples were prepared by mixing 20 g of water, different proportion of SHA-1 and 40 g of cement by hand for one minute. Forty grams of cement paste was used for all isothermal calorimeter analysis samples. However, another sets were prepared to investigate the impact of individual components of SHA-1 on the hydration kinetics.

The temperature was maintained at 20°C throughout the testing for all samples and F/P-Cal Logger software was used to gather and analyse the experimental data. The investigated shape of the hydration curve is presented according to qualitative analysis.

Figure 4.1: Isothermal conduction calorimetry machine.

4.3.3 The efficiency of regaining the mechanical properties

4.3.3.1 Specimen preparation

The specimens were prepared from the same batch and therefore the procedure of mixing was the same for all tests. For each mix proportion batch fill three standard 40 mm x 40 mm x 160 mm prismatic moulds were made, based on BS EN 196-1: 7.2.[395].

4.3.3.2 Compressive strength

The prepared samples of fresh concrete/mortar were tested for compressive strength by a compressive machine based on BS 1881-116:1983[397] (testing concrete) as shown in Figure 4.2. Each sample is centered between the two parallel discs. According to BS the reference rate of load is increased by 0.30 ± 0.05 N/(mm^2.s) until no greater force can be sustained and for the reference specimen, the load is investigated up to failure. After the duration time of healing has passed, each sample is retested to failure.

Figure 4.2: Compressive strength machine.

4.3.3.3 Flexural strength test

The experimental procedure to determine the flexural strength was adapted from BS EN 196-1:2005 [395]. The samples were tested before and after healing by using a three-point bend test (flexural strength) as shown in Figure 4.3. The specimen is supported by two parallel beams and compressed by one central beam. The load is increased steadily by 0.04-0.06 N/mm^2, then the load is stopped after the specimen has reached the maximum load.

Figure 4.3: 3-point bending test machine.

4.3.4 Physical properties of aggregates (perlite)

The percentage of aggregate in concrete to provide savings in the cost of concrete ranges between 70% to 75%. Therefore, there are some of the properties/characteristics of aggregate that influence the properties of the concrete mix as follow.

1. Unit weight according to EN 1097-3[398] is the weight of the aggregate required to fill a container of a specified unit volume.
2. Apparent density (*p*d) is the ratio between the apparent volume and dry specimen mass of a perlite sample.
3. Sieve analysis according to BS EN 933- Part-1[399] is a division method of material into fragment size by passing it through a sieve with decreasing size of slots.
4. Absorption and surface moisture according to EN 1097-6[400] is the difference between very dry aggregate weight and the weight of saturated surface dry aggregate conditions. It depends on the amount of moisture content in aggregates such as over-dry aggregate, dry aggregate, saturated surface dry aggregate and moist aggregate as shown in Figure 4.4.

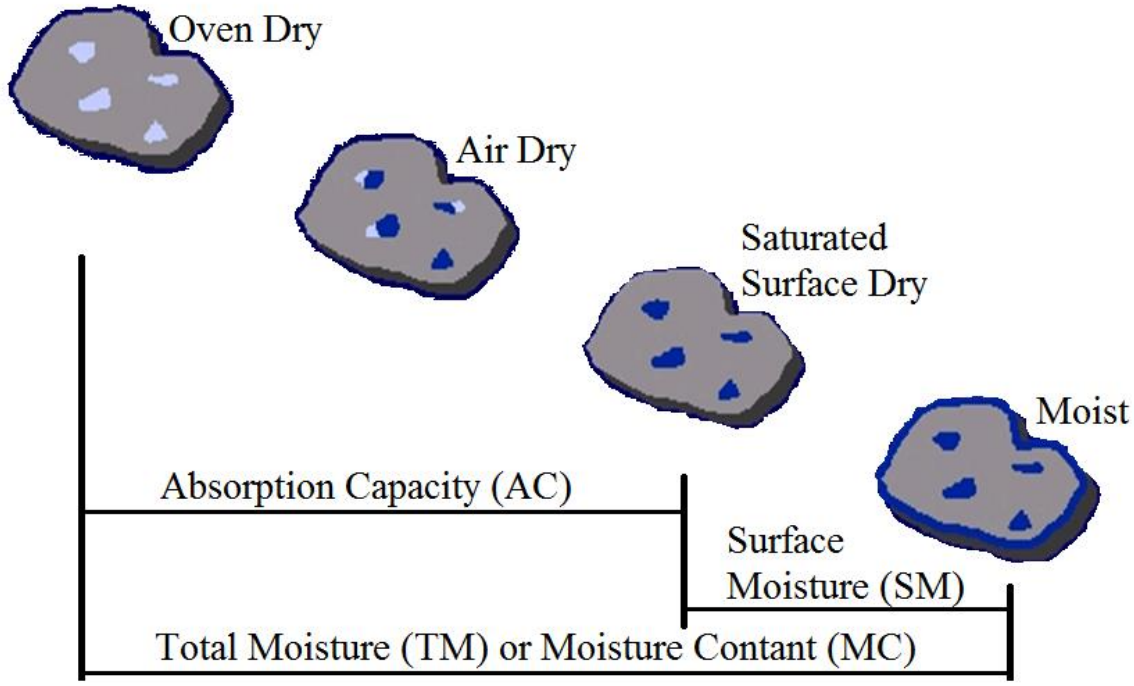


Figure 4.4: Plan of the amount of moisture content in aggregates.

4.3.5 The efficiency of self-healing agents for sealing/ healing the cracks

4.3.5.1 Initial surface absorption test (ISAT)

The water permeability test is a convenient, reliable and non-destructive in the laboratory for assessing mortar durability at the mix design stage. Moreover, this test system plays complementary rates to existing methods assessing durability such as carbonation test and rapid chloride permeability test (BS 1881-5:1970[401]). Methods of testing hardened concrete for other than strength are shown in Figure 4.5. The tested samples dimensions were 40x40x160 mm, and the mortar samples remained in the laboratory temperature at 20±2℃ for a minimum period of 48 h before starting the absorption procedure.

4.3.5.1.1 Principle

The flow water rate passes into the surface of the concrete sample per unit area under its own weight after constant applied head and temperature in water reservoir.

4.3.5.1.2 Procedure

- The procedure started by fixing the cap on the specimen with foam or silicone to prevent any loss of water from the cap.
- The position of the reservoir was set above the cap, and the head of water was taken 200±20 mm above the surface of the sample and connected to the inlet of the cap with fixable tube with tap on it.
- The temperature of the water used in the experiment remained at 20℃ and should not exceed 22℃.
- The test was started by closing the fixable tube with the tap and filling the reservoir with water, and then the tap was opened to allow the water to run into the cap and come out from the outlet tube until no more air existed inside the system. The time should be recorded when the test is started. During the test, care should be taken to prevent the air coming into the system by letting the reservoir empty itself.
- The readings were taken after 10 min, 30 min, 1 hour and 2 hours from the start of the test. At each period, the tap was closed and when water started to flow in the capillary tube in the opposite direction the stopwatch was started to record the time, referring to Table 4.1, the period during which movement is noted was determined.

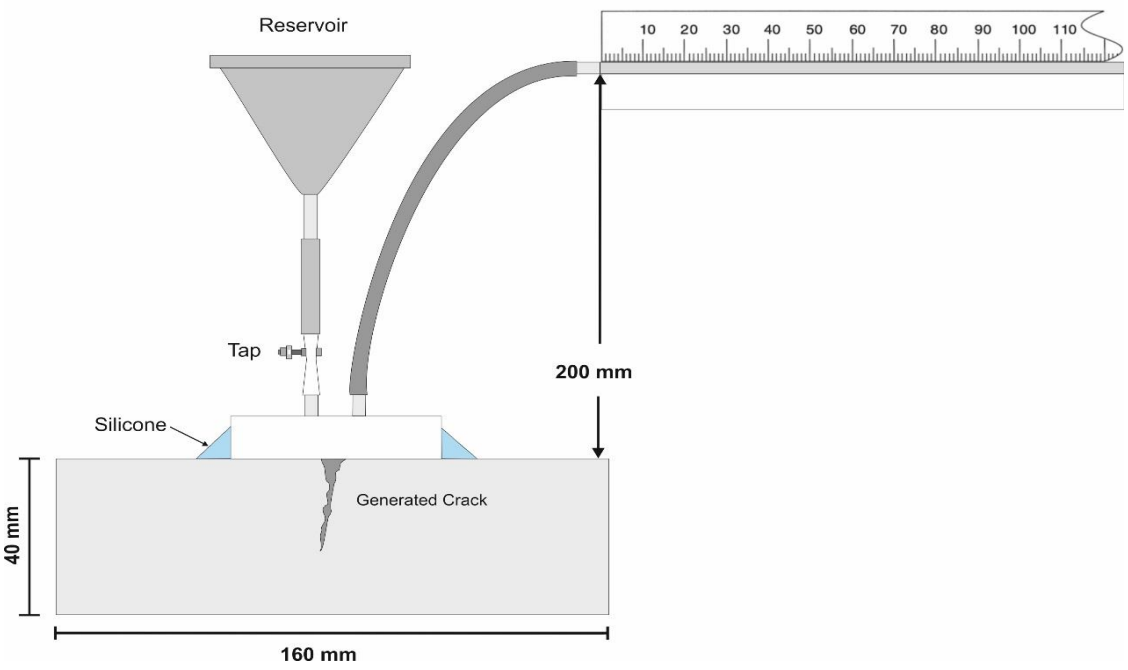


Figure 4.5: Apparatus for surface absorption of concrete test [401].

Table 4.1: Determination of period of movement[401].

Number of scale units moved in 5 s	Period during which movement is noted
Less than 3	2 minutes
3-9	1 minute
10-30	30s
More than 30	Record initial surface absorption as more than 360 ml/m^2 per second

4.3.5.2 Permeability test via capillary water absorption

The water permeability test via water absorption is non-destructive test in the laboratory for assessing mortar durability at certain age. Moreover, this test system plays complementary rates to existing methods assessing durability via determination of resistance of capillary absorption EN 13057:2002[402]. The tested samples dimensions were 40x40x160 mm, and the mortar samples remained in the oven temperature at 40±2℃ for a minimum period of 7 days, until the change of weigh became constant. Then the samples kept at 20±2℃ with relative humidity 60±10 % for 24 hours before starting the absorption procedure.

4.3.5.2.1 Procedure

- All samples covered partially by waterproof before start the test by using one of waterproof coating (e.g. aluminum adhesive foil or any kind of epoxy suitable for concrete). The minimum height at both side was 20 mm from the bottom surface and all bottom surface except a section of 20 mm x 40 mm centered on the cracked section as shown in Figure 4.6.
- The samples were weighed before start the test referring as (W_1), the crack width was measured before the test.
- The level of water when the samples surface exposed to water in the container shall not exceed more than (2.0±1.0) mm.
- The samples were weighed after starting the test for a period of 8 hours frequently (after 0.25 hour, 0.5 hour, 1 hour, 2 hours, 4 hours, 6 hours, and 8 hours), then removed the excess of water on their surface before each weigh.

- The data got it from water absorption test used to plot the relation between water uptake with the square root of time. The following formula was utilized to calculate the sorption coefficient (SC) that adopted from Hall[403] for materials with little suction as following equation:

$$i = A + SCt^{\frac{1}{2}} - Bt$$

Where:

i: the water uptake in g.

A and B: constant values.

t: time in hours.

SC: the sorption coefficient in $gh^{-1/2}$.

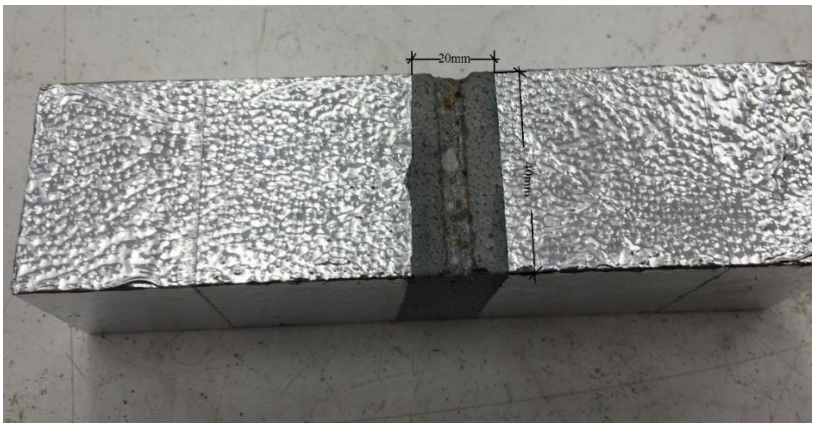


Figure 4.6: Apparatus for surface absorption of concrete test

4.3.6 Qualitative assessment of the efficiency of self-healing visually

4.3.6.1 SEM analysis

SEM is a type of microscope that focuses a high energy electron beam on a very fine scale of sample to produce images of this part of the sample by scanning it.

4.3.6.1.1 Principle

The electron beam focuses on the sample and comes into contact with atoms of the sample particles, then the sample's surface topography, morphology (the micro particles composing of the object size and shape), crystallographic data (the structural atoms of the object), and composition (elements and compounds included in the object and the relative amounts of

them)[404] might be measured and detected by producing various signals. These signals contain information about the sample. The signal type produced by a SEM are secondary electrons (SE), which is standard in all SEMs, back scattered electrons (BSE), characteristic X-ray, light cathodoluminescence (CL) and transmitted electrons. The basic details of SEM scan are briefly described as follows and in Figure 4.7[405].

4.3.6.1.2 Procedure

- Typically, the first step is to take pieces from the cracks where the bacteria made their precipitation of calcium carbonate inside these cracks. Then these pieces were crushed until they became a powder.

- **FIXATION**

  The powder was fixed on small cylinders by special glue as shown in Figure 4.8, and fixed in the cylindrical disc.

- **DEHYDRATION AND DRYING**

  Removal of the water from the samples by leaving them in the vacuum chamber for 24 hours in drying air.

- **COATING**

  The sample powder was electrically non-conductive. This property complicates SEM observation. The way to overcome charging problems was to deposit a very thin (nm) conductive material layer, such as gold, on the sample surface. The coating instrument was shown in Figure 4.9.

- **SEM scan images**

  Fixed the cylindrical disc on the stage of the SEM scanner. The electron gun forms a fine probe of electrons under high vacuum conditions. This current was accelerated towards the specimen while limited and focused using the magnetic metal lens and openings in a concentrated, thin, monochromatic beam. The beam irradiated the sample and due to the interactions inside the irradiated samples, the electron beam was affected. All these interactions and effects were detected and transformed into images or other analysis methods.

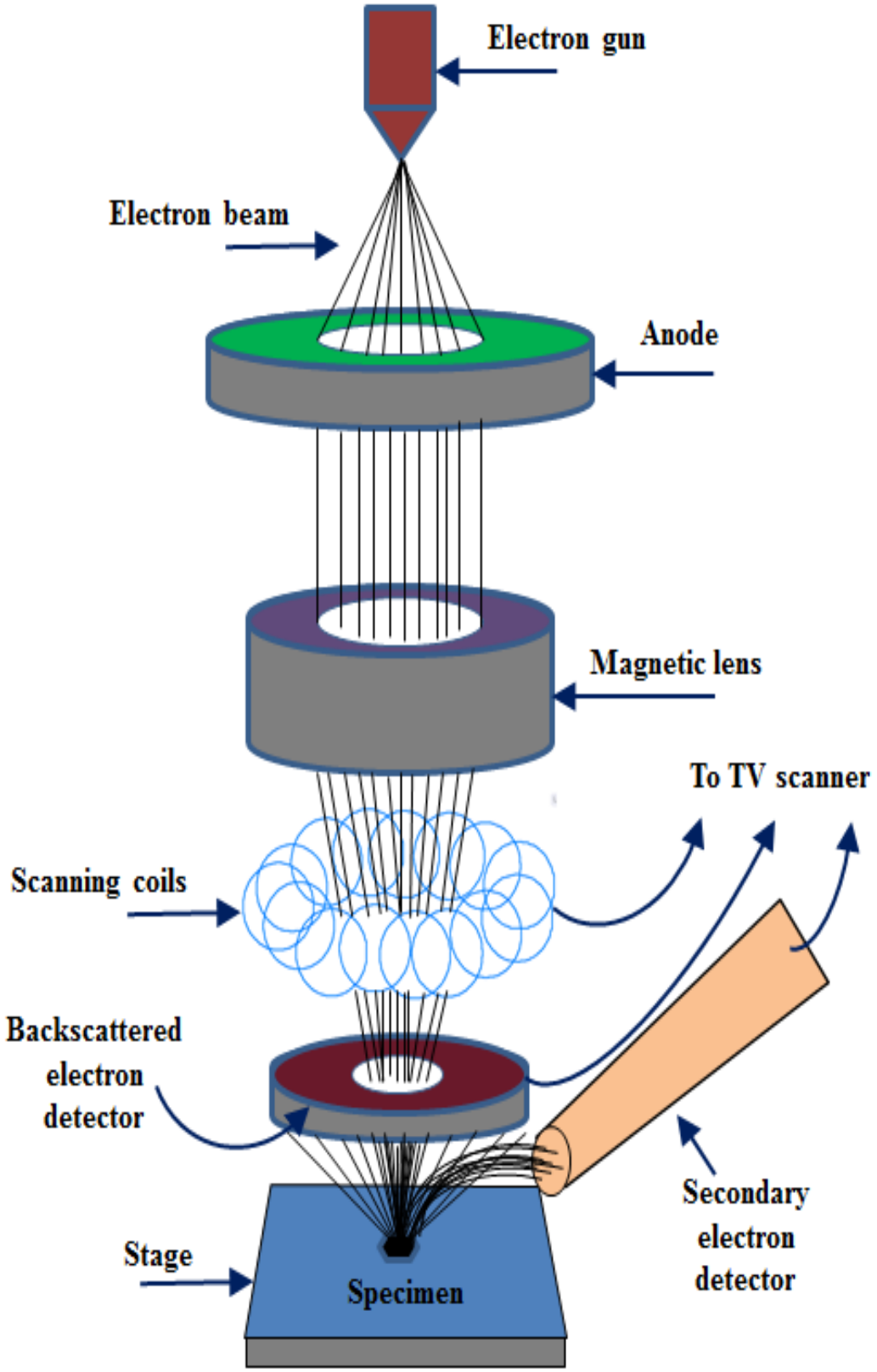


Figure 4.7: The mechanism of SEM scan adapted from Stefanaki[405].

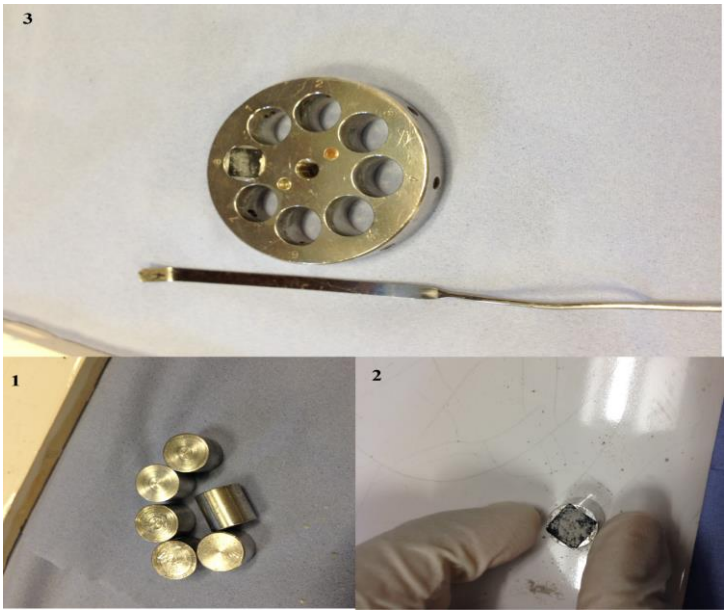


Figure 4.8: Steps of fixing cement powder on the sample holder (stub).

Figure 4.9: Sputter coating.

4.4 Microorganism selection, growth medium stock and biology tests

4.4.1 Cultivation of bacteria

Three types of bacterial strains form the German collection of microorganisms cell cultures (DSMZ) were used in this research, based on previous studies that have used these microorganisms in their studies, such as Jonkers[23,77,127,179,271]: *Bacillus pseudofirmus* type strain DSM 6950, *Bacillus conhii* type strain DSM 6307 and *Bacillus halodurans* type strain DSM 497.

4.4.2 Growth Medium stock

The following proportions of solutions for bacteria with buffer, bacterial self-healing agents (SHA) and bacteria with SHA were used. These solutions of compounds were used for remediation after 7 and 28 days by injecting them into cracks that were generated by a three point bending test.

4.4.2.1 Bacteria with buffer:

- 50ml in a 10:1 ratio of LB medium: sesquicarbonate.
- Inoculated with three types of *Bacillus* family (*pseudofirmus*, *conhii*, *halodurans*).

Where LB: Luria broth as the growth medium (medium composition: 10 g of Tryptone, 10 g of NaCl, 5 g yeast extract, 0.42 g $NaHCO_3$, and 0.53 g Na_2CO_3 per litre distilled water; pH 9.5).
Sesquicarbonate: (medium composition: 4.2 g $NaHCO_3$, and 5.3 g Na_2CO_3 per 100 ml distilled water)

4.4.2.2 Calcite production bacterial self-healing agents (SHA-1):

- Sodium glutamate 0.4 g/L.
- Alanine 0.2 g/L.
- Inosine 0.2 g/L.
- Yeast extract 2.2 g/L.
- Calcium acetate 7.1 g/L.
- Sodium citrate 2.0 g/L.
- Magnesium chloride 0.2 g/L.
- Monobasic potassium phosphate 0.1 g/L.
- Sodium chloride 0.2 g/L.
- Manganese (II) sulfate 0.1253 g/L.
- SL12B (trace element solution) – 1ml added post-autoclave
- Distilled water 1000ml.

SHA-1 was fortified post-autoclave with:

- 100mM tri-sodium citrate (5.16g/L).
- 50mM $NaHCO_3$ (4.2g/L).

- 25mM $Na2CO_3$ (5.3g/L).

It contains ten ingredients, namely calcium acetate, which is very important as an inducer for bacteria to produce calcite; sodium citrate as a sole source of carbon and energy; yeast extract is complex source of nitrogen, vitamins, amino acids and carbon for bacteria to grow; alanine and inosine are two germination triggers that help spores to form vegetative cell; sodium glutamate is an important molecule for all living organ-isms; magnesium chloride is mainly found inside the cells and is necessary for the metabolism of carbohydrates; sodium chloride, which controls the water activity that is required for the grown of vegetative cells and endospores; manganese sulphate monohydrate, which is required for sporulation vegetative cells from spores, and monobasic potassium phosphate, which used as a buffering agent.

4.4.2.3 Alkaline B4 modified bacterial self-healing agents (SHA-2):

- Yeast extract – 1.0g/L.
- Dextrose – 1.0g/L.
- Calcium acetate (Ca-acetate) – 5.0g/L.

SHA-2 was made alkaline post autoclave by the addition of:

- 100mM tri-sodium citrate (5.16g/L).
- 50mM $NaHCO_3$ (4.2g/L).

4.4.2.4 Bacterial self-healing agents (SHA-3):

- Yeast extract – 4.0g/L.
- Dextrose – 4.0g/L.
- Calcium acetate (Ca-acetate) – 100.0g/L.

SHA-3 was made alkaline post autoclave by the addition of:

- 100mM tri-sodium citrate (5.16g/L).
- 50mM $NaHCO_3$ (4.2g/L).

The summary of both types of medium as solutions and as powder for three types of SHA is presented in Table 4.2.

Table 4.2: The quantity of used an ingredients of bacterial medium SHA.

Ingredients	SHA-1		SHA-2		SHA-3	
	Powder (%)	Gram/litre	Powder (%)	Gram/litre	Powder (%)	Gram/litre
Calcium acetate	55.94	6	71.43	5.0	92.60	100.0
Sodium citrate	18.65	2				
Yeast extract	12.12	1.3	14.29	1.0	3.70	4.0
Dextrose	---	---	14.29	1.0	3.70	4.0
Na-glutamate	3.73	0.4	---	---	---	---
Alanine	1.86	0.2	---	---	---	---
Inosine	1.86	0.2	---	---	---	---
$MgCl_2$	1.86	0.2	---	---	---	---
NaCl	1.86	0.2	---	---	---	---
$MnSO_4$	1.17	0.1253	---	---	---	---
Kh_2PO_4	0.93	0.1	---	---	---	---
100mM $Na_3C_6H_5O_7$	Not required	5.16	Not required	5.16	Not required	5.16
50mM $NaHCO_3$	Not required	4.20	Not required	4.20	Not required	4.20
25mM Na_2CO_3	Not required	5.30	Not required	---	Not required	---
Total	100	25.39	100	16.36	100	117.36

4.4.3 Centrifugation and washing of bacteria

The instrument used to separate ingredients of a complex mixture using a process called centrifugation, by spinning complex mixtures at very high speeds (5000 rpm, for 10 minutes at 4°C). The components of a given mixture are subjected to centrifugal force, making the more dense particles migrate away from the axis of rotation and the lighter particles move toward it. These particles can deposit in the bottom of the tube in what is known as a pellet, and the isolated specimen, or the remaining solution, the supernatant may be treated or analysed by taking it out of the tube as shown in Figure 4.10. Then the deposited pellets can be washed with sterile water many times and finally bacterial self-healing agents added to it and mixed - they will now be ready to use.

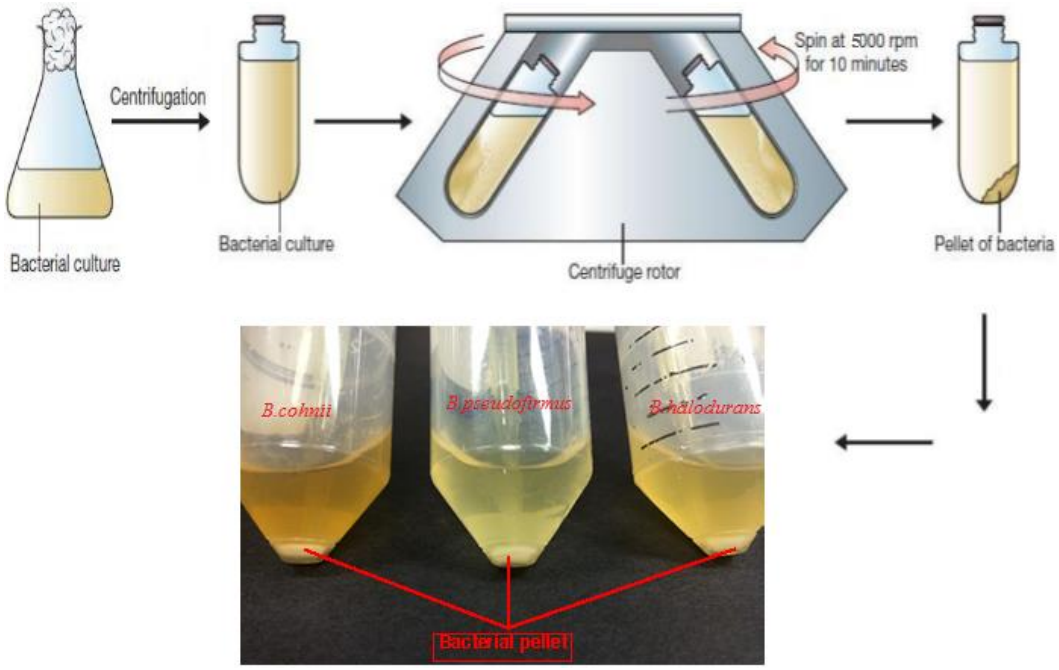


Figure 4.10: Plan of centrifugation process.

4.4.4 SERIAL DILUTIONS – TUBE METHOD

The serial dilutions method was used to estimate the number of bacterial cells in the solution of bacteria with buffer or bacteria with medium or bacterial spores by measuring one gram from that material and mixing it with 9 ml of specific solution to count the number of cells.

4.4.4.1 Principle

Serial dilution is a common technique that uses a small amount of serum or solute and one of the most common series doubles the dilution factor with each transfer (1:2, 1:4, 1:8....). These dilutions can be done in micro titer plates or test tubes depending on the volumes of sample and diluents used.

4.4.4.2 Materials

Plastic test tubes, LB, Sesqui, alkaline nutrient agar, distilled water.

4.4.4.3 Procedure

- Serial dilutions were made using LB:Sesqui of 10:1.
- 1ml of suspension to 9ml of LB:Sesqui was used to make the ×10 dilutions of each step.
- For example:
  1ml of stock +9ml of LB:Sesqui ⇒ 1/10 dilution.
  1ml of the 1/10 dilution + 9ml of LB:Sesqui ⇒ 1/100 dilution.
- This was repeated until $1/10^8$ dilution was achieved.
- 100ml of 10^{-5}, 10^{-6}, 10^{-7} and 10^{-8} was plated onto alkaline nutrient agar and incubated overnight at 30°C.
- 10^{-5} was used to count colonies; at $1/10^{-5}$ there were 421 colonies.
- 421 cfu in 100ml of 10^{-5} dilution = 4210 cfu ml^{-1} at 10^{-5}.
- 4210*100000 = no. cfu ml^{-1} of stock culture = $4.21*10^8$ cfu ml^{-1}.
- The solution applied to the concrete is a 1/10 dilution so therefore has a concentration of $4.21*10^7$ cfu ml^{-1}.
  Where cfu is colony forming units.

4.4.5 Spectrophotometry

Spectrophotometry is a method used to measure the intensity value of a light beam passing through the sample solution. It measured the absorbance of light of the solution.

4.4.5.1 Principle

The principle of spectrophotometry is that; each solution compound absorbs or transmits light over a particular range of the wavelength spectrum as shown in Figure 4.11. This principle can be used to determine the amount of a known solution substance by measuring its density.

4.4.5.2 Procedure

The bacterial self-healing agents were prepared, then mix bacteria with bacterial self-healing agent solutions, took 50 mL of it using a pipette and follow the instructions below, then put the rest of the bacteria with bacterial self-healing agent solution in a shaking incubator at 30°C.

- Prepare a labelled cuvette containing 50 mL distilled water as a reference cuvette and the other three labelled cuvettes containing 50 mL bacteria with bacterial self-healing agents.
- The spectrophotometer Prepared to be ready to read the OD_{600} on the screen and the reference cuvettes containing water used to 'zero' the spectrophotometer until the screen display OD_{600} is zero.
- Each of the cuvettes containing bacteria with bacterial self-healing agents solution were placed and read the absorbance of the solution through the digital display.
- All the above steps were repeated for each individual reading of the samples.

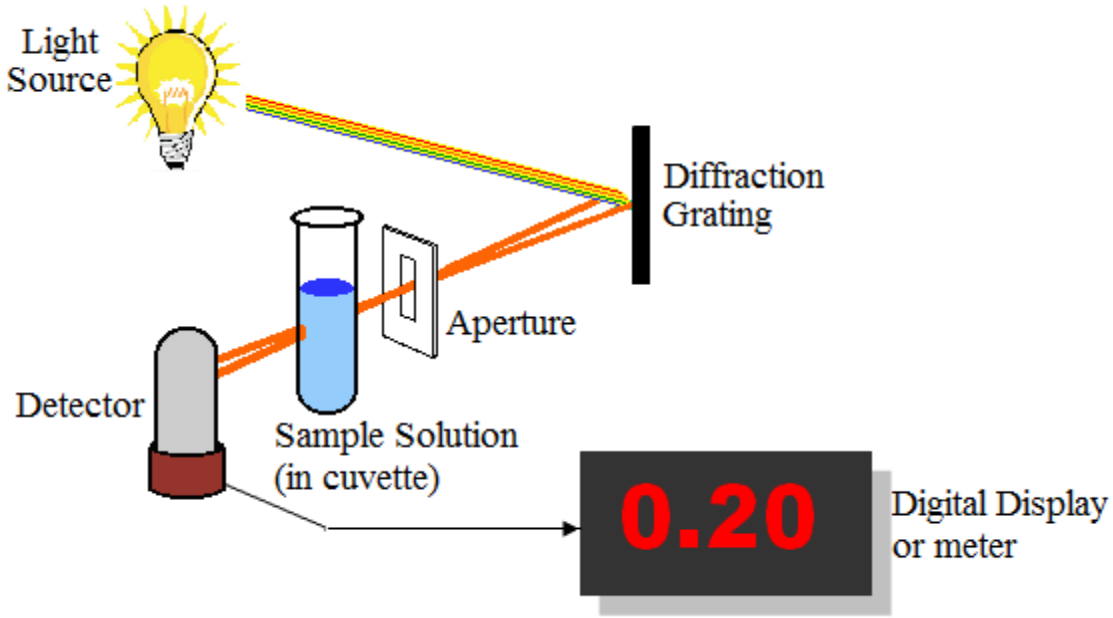


Figure 4.11: Plan of the basic structure of spectrophotometry.

4.4.6 Preparation of Alkaline Agar plates

4.4.6.1 Introduction

Agar plate contains a growth medium (Agar with nutrients) used to microorganisms culture. This method used to place microorganisms on plates for growing into individual colonies, which is ideal temperature inside incubator at 30°C. Each colony referring to individual

organism, which could be visible in about 24 hours. Agar plates used to estimate the concentration of microorganisms in the culture.

4.4.6.2 Materials

The following materials were used to prepare alkaline agar plates:

Agar, nutrients containing on (tryptone, yeast extract, sodium chloride), distilled water, glass laboratory bottle (GLB) and sterile Petri dishes.

4.4.6.3 Procedure

- The ingredients of alkaline agar were weighed out 16g agar, 10g tryptone, 5g yeast extract and 5g sodium chloride.
- Both yeast extract and sodium chloride were added to 1000ml of sterile water in GLB on a hotplate-stirrer with gentle stirring until both components completely dissolved in sterile water.
- Added slowly the agar to 1000ml of nutrients solution in GLB on a hotplate-stirrer with gentle stirring by magnetic stirrer until the solution became homogenous.
- The agar solution was kept in autoclaved for sterilize at 150°C.
- The magnetic stirrer was removed from GLB, closed the lid of bottle.
- Agar solution allowed to settle down until 55-50°C.
- Finally, poured the agar solution into sterile Petri dishes to cover about quarter of the plate in sterile condition (airflow desk).

Chapter 5

The efficiency of bacteria and self-healing agents

5.1 Introduction

People have used microorganisms for centuries for purposes going from the generation of liquor by yeasts to the production of different type of proteins, for example, insulin, by genetically engineered modified of bacteria. Recently, microorganisms have been used particularly to attempt and decrease the effect of people on nature and illustrations incorporate the utilization of anaerobic microorganisms in water treatment and in biogas generation. Microorganisms specifically can possibly lessen the environmental effect of one industry; this is construction industry. The aim of the research presented in this chapter was to present the basic experiments on ability of bacteria spores to survive on cement paste presented as stage one. In stage two ability of bacteria to growth in different types of SHA. In stage three capability of bacteria to produce calcite.

In stage four part one a comparison between the efficiency of three types of SHA to precipitate calcium carbonate with three different types of bacteria is given and the most suitable bacteria for the further studies is discussed. Information about SHA-1, SHA-2, and SHA-3 is given in sections 4.4.2.2, 4.4.2.3, and 4.4.2.4 respectively. In stage four part two and three study the relation between number of bacteria cells or spores and calcite amount produced, and in stage five the effect of variation of temperature on the ability of bacteria to growth. The *bacillus* bacteria have chosen based on produce long-lived endospores.

5.2 Experimental programme

5.2.1 Cultivation of bacteria and viability of bacterial spores in cement paste (stage one)

All types of bacterial strains were selected for this research and were formed from a German collection of Microorganisms and cell cultures (DSMZ), their properties and specification were explained in Chapter 4.

The spores were prepared as follows: an overgrown cell culture, 1ml, was added to 100ml of the spore producing medium[127] and was incubated at 30℃ on an orbitary shaker for 72 hours. After confirming spore formation by Fulton-Schaffer spore staining method, the spores were centrifuged at 10,000 rpm for 15 minutes. The spore pellet was washed three times with a

chilled 10mM Tris HCl buffer, pH 9.5. The spore pellet was then freeze dried to obtain a spore powder, which was then stored in desiccator. Then these spores were added to the cement to make cement paste. The viability of spores (ability to germinate) of *B.pseudofirmus, B.cohnii,* and *B.halodurans* were studied. The experiment was conducted under sterile/aseptic conditions by using small plates of cement paste (46 x 46 x 8 mm with 0.5 w/c ratio) and spores have been added to them. Three cement paste specimens crushed for each of the species of bacteria at 1, 3, 7, 14, 21, 28, 56, 73 and 93 days and one gram was taken from each sample. One gram of sample with bacteria spores was obtained each time period and serially diluted (10^{-1}-10^{-9}) in test tubes. Then they vortexed for two minutes to provide homogeneity and the sterile 15 ml falcon tubes were used to make the solution by adding 1 ml of buffer with cement powder with 9ml of buffer, then odd numbers of 1/10 to $1/10^{9}$ dilutions were chosen to plate onto alkaline agar plates. Sterile, packaged plastic spreaders were individually used to spread the suspension onto alkaline agar plates, and finally all alkaline agar plates were incubated overnight at 30℃. If colony numbers were not obtained (between 20-500) at these dilutions, then additional plates of dilutions were made. Then the ability of growth and precipitation of calcium carbonite in the flask via the bacteria was studied.

5.2.2 Selection of an appropriate SHA for bacteria growth (stage two)

This research has three types of *Bacillus* bacteria and investigated their ability to seal and heal the micro-cracks in mortar. In this study, *B.pseudofirmus* was chosen to investigate the growth curve for SHA-1, SHA-2, and SHA-3 mediums. To obtain germination of *B.pseudofirmus* cells, 1ml of its culture stock was inoculated to 50 ml of a Luria Bertani (LB) medium at 30℃ and 150 rpm on an orbitary shaker overnight. In addition, inoculated 1.2ml of *B.pseudofirmus* cells grew aerobically in 120 ml of medium until the stationary reached the growth period. This procedure was repeated each time for each type of SHA. When both the growth of microorganisms and the growth rate dropped down, this is called the starting of the stationary growth period. one ml of sample solution was obtained at zero hours, 2 hours, 4 hours, 6 hours…etc, until the reading of the growth curve was stable and the serial was diluted (10^{0}-10^{-4}) in test tubes, by using OD_{600} (optical density measurement at 600 nm), one could take measurements by a Thermo Spectronic. The growth curve of *B.pseudofirmus* with three types of SHA was generated by correlating absorbance of solution at OD_{600} at certain time.

5.2.3 Growth and quantification of calcite precipitation by bacteria (stage three)

To identify the ability of three bacillus bacteria to precipitate calcite. 48 well titre plates were used and 24 wells were divided to three sets namely as follow:

- Set 3-1, the isolates of *Bacillus pseudofirmus* (four inoculated wells).
- Set 3-2, SHA-2 control wells (four inoculated wells).
- Set 3-3, the isolates of *Bacillus cohnii*.
- Set 3-4, SHA-2 control wells.
- Set 3-5, the isolates of *Bacillus halodurans*.
- Set 3-6, SHA-2 control wells.

All sets were kept overnight at 30℃.

5.2.4 Optimising calcium carbonate precipitation by three types of medium (SHA-1, SHA-2, and SHA-3) with *B.pseudofirmus*, *B.halodurans*, and *B.cohnii* (stage four part one)

To determine the influence of all three types of SHA on the morphology of the calcium carbonate precipitated, the spores of bacteria were centrifuged at 10,000 rpm for 15 minutes. The spore pellet was washed three time with chilled 10mM Tris HCl buffer, pH 9.5. The spore pellet was then freeze dried to obtain a spore powder, then stored in desiccator. These spores were grown at 30℃, aerobically, and in 150 rpm shaking conditions for 24 hours. From the cultures of the three types of bacteria, a 1ml portion was sampled in serially diluted (10^{-1}-10^{-9}) concentrations in test tubes to obtain the total cell number of each bacteria before they were added into flasks. Three types of SHA were prepared as stated in sections 4.4.2.2 – 4.4.2.4. Each type of SHA was divided into 12 samples as shown in Figure 5.1 (working volume of each sample, 20 ml) as follow:

- Set 4-11, three control samples of SHA-1 were taken as a reference.
- Set 4-12, three samples of SHA-1 were mixed with 1ml (3.19×10^{11} spores) of the *B.pseudofirmus* culture.
- Set 4-13, three samples of SHA-1 were mixed with 1ml (2.05×10^{11} spores) of the *B.cohnii* culture.

- Set 4-14, three samples of SHA-1 were mixed with 1ml (1.18 x10^{11} spores) of the *B.halodurans* culture.
- Set 4-21, three control samples of SHA-2 were taken as a reference.
- Set 4-22, three samples of SHA-2 were mixed with 1ml (3.19 x 10^{11} spores) of the *B.pseudofirmus* culture.
- Set 4-23, three samples of SHA-2 were mixed with 1ml (2.05 x 10^{11} spores) of the *B.cohnii* culture.
- Set 4-24, three samples of SHA-2 were mixed with 1ml (1.18 x10^{11} spores) of the *B.halodurans* culture.
- Set 4-31, three control samples of SHA-3 were taken as a reference.
- Set 4-32, three samples of SHA-3 were mixed with 1ml (3.19 x 10^{11} spores) of the *B.pseudofirmus* culture.
- Set 4-33, three samples of SHA-3 were mixed with 1ml (2.05 x 10^{11} spores) of the *B.cohnii* culture.
- Set 4-34, three samples of SHA-3 were mixed with 1ml (1.18 x10^{11} spores) of the *B.halodurans* culture.

Then, all sets were incubated on a shaker at 30℃ and at 150 rpm for eight days, after that they were continually observed to detect which sample or samples had performed well. All liquid solution was taken at the end of incubation time from each flask for calcium carbonate analysis. However, the variation of the number of spores between the three types of bacteria due to cell/spore size of each type of them. The colonies of *hardorans* are white circular with a marginally filamentous edge, which producing spores are ellipsoidal (0.5 to 0.6 x 0.8 to 1.2 µm). While, the colonies of *pseudiformus* are yellow round with irregular margins, which producing spores are oval (0.5 to 0.7 x 0.5 to 1.2 µm)[406].

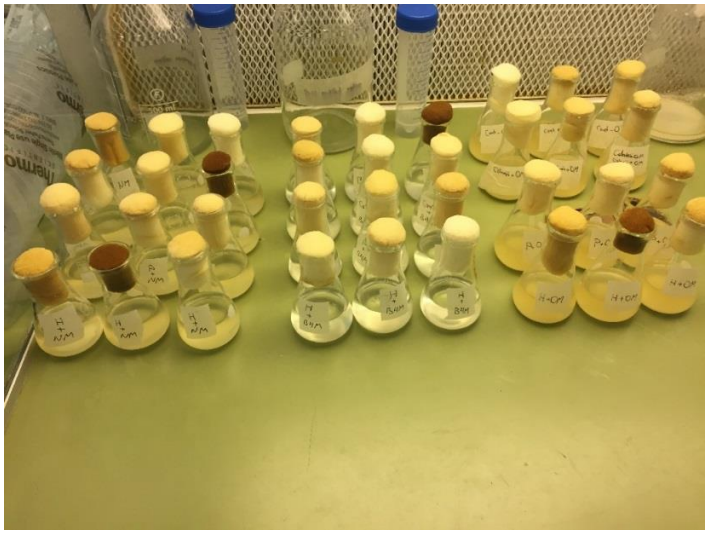


Figure 5.1: Cultures of three types of bacteria preparations with different SHA.

5.2.5 Relation between number of bacteria and quantification of calcite precipitation *in vitro* (stage four part two)

Different volumes of pre-grown suspension of *B. pseudofirmus* culture, 7.4 x 10^9, 1.85 x 10^{10}, 3.7 x 10^{10}, 5.55 x 10^{10}, 7.4 x 10^{10} CFU was added to three calcite precipitating medium, SHA-1, SHA-2 medium and SHA-3 medium respectively. The reaction mixtures were incubated at 30°C, 150 rpm for 8 days. The samples were withdrawn, centrifuged at 10,000 rpm for 10 minutes. After decanting the supernatant, 2 ml of sterile water was added to the pellet and mixed until homogenous suspension was attained. This homogeneous suspension was subjected to filtration using 3 µ nitrocellulose membrane and calcium carbonate crystals were collected from the membrane and they had been left to dry for 72 hours at 50±2℃ and weighed. The relation between amount of bacteria CFU and ratio between calcium carbonate by *B.pseudofirmus* and the amount of calcium acetate were investigated.

5.2.6 Calcite amount precipitated by SHA-3 with *B.pseudofirmus* (stage four part three)

A containing (cell number) of was inoculated to 10 mL calcite medium SHA-3 (Calcium acetate 100 grams, 4 grams of Yeast Extract and 4 grams of dextrose per litre). The reaction mixtures were incubated at 30°C, 150 rpm for 3, 6, 8, 13 and 15 days. The samples were withdrawn, centrifuged at 10,000 rpm for 10 minutes. After decanting the supernatant, 2 ml of sterile water was added to the pellet and mixed until homogenous suspension was attained. This homogeneous suspension was subjected to filtration using 3 µ nitrocellulose membrane

and calcium carbonate crystals were collected from the membrane and they had been left to dry for 72 hours at 50±2℃ and weighed.

5.2.7 Effect of temperature on the growth of bacteria (stage five)

The climates across the world have huge variation. Therefore, it is important to investigate the ability of the three *Bacillus* species strain on dynamic growth with potential for incorporation into self-healing concrete. The assessment of dynamic growth of *B.cohnii, B.pseudofirmus,* and *B.halodurans* were measured turbidity over time at a particular temperature. Temperatures ranges that effect on bacteria growth were investigated. The range of temperature of these types of bacteria from 25℃ to 40℃ grow in ranged between 20℃ to 55℃[407]. Temperature is an issue in UK, which average temperature in UK is ranged from ≈3℃ to ≈22℃. However, in this stage is performing proof of principle. The *bacillus* isolates that grow 20℃ to 55℃ range would still be of value in warmer regions[408].

5.3 Results and discussion

5.3.1 Survivability of bacteria spores inside the cement paste (stage one)

The viability of spores in terms of CFU were monitored and the results indicated that the number of viable spores decreased steadily during the period of 93 days as illustrated in Figure 5.2. The number of each type of bacteria decreased by about 0.1% of the initial number. The processes of cement hydration had minor effects on the survivability of bacterial spores, also it was observed that the mechanical stresses due to mixing and hardness of cement paste and the effect of a high alkalinity environment had no effect on the survivability of the number of bacterial spores. The fluctuated results of survivability of spores was due to the method of extraction of spores from the cement paste, which affected the CFU and the number of spores that were extracted was much less than the number of spores added to the cement paste. It was of interest to see whether these spores survived the process of mixing, a high pH, setting and hardening of cement paste for a period of more than three months, which gave an indication that these spores can be used in bio-concrete/bio-mortar.

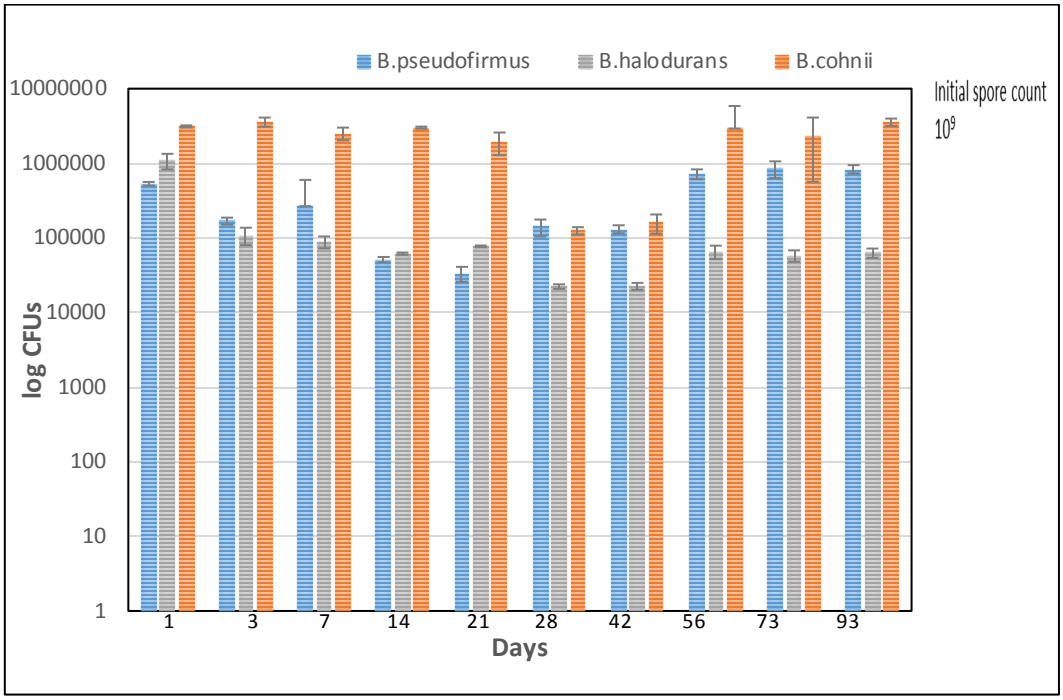


Figure 5.2: Relation between time and CFU/gram.

5.3.2 Determination of growth of bacteria in a calcite medium (stage two)

The results of studying the growth curve of *B.pseudofirmus* in three different calcite precipitating mediums (SHA-1, SHA-2 and SHA-3) are presented in Figure 5.3. It was found that based on optical density (OD_{600}), growth conditions in SHA-3 were more favourable than SHA-1 and SHA-2. However, growth conditions in SHA-1 was faster during first eight hours, which could be due to the fact that it has ten ingredients including inosine and alanine, and some of their properties are germination triggers, specific to *Bacillus*, which aid the formation of vegetative cells from spores. Overnight growth conditions in SHA-3 was dominant until the end of the test, which might due to the mount of calcium acetate and amount of energy sources (yeast extract and dextrose) compared to SHA-2 and SHA-1. Moreover, the stationary phase in both SHA-2 and SHA-3 occurred before SHA-1 due to the presence of dextrose in them.

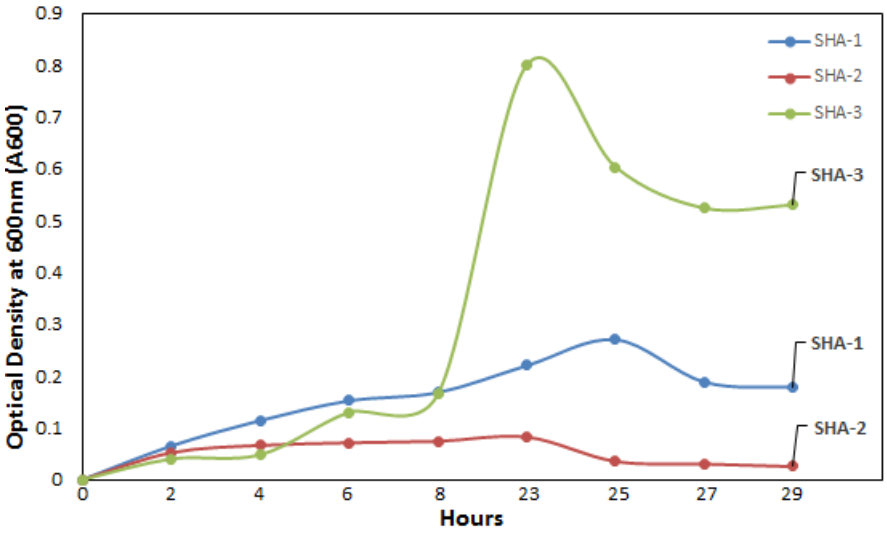


Figure 5.3: Growth of *B.pseudofirmus* at 30℃ in SHA-1, SHA-2 and SHA-3 mediums.

5.3.3 Capability of three isolates of alkaliphilic bacteria to precipitate calcite (stage three)

The ability of the three types of isolates of bacteria for kinetics of the precipitate in SHA-2 medium was studied. The aim of this experiment was to investigate the high throughput screen of these isolated bacteria for incorporation into the application of self-healing concrete. After 18 hours all plates were assessed and the results indicated that all of them already presented bacterial growth, and clearly showed the presence of a precipitate. The crystals of precipitate from the isolates of *Bacillus* bacteria might clearly be seen under a light microscope oil immersion with a x100 lens, as shown in Figure 5.4. However, the crystals presented in this experiment might not confirm them as calcite and the method of confirming the presence of calcite is presented in Chapter 6 & 7.

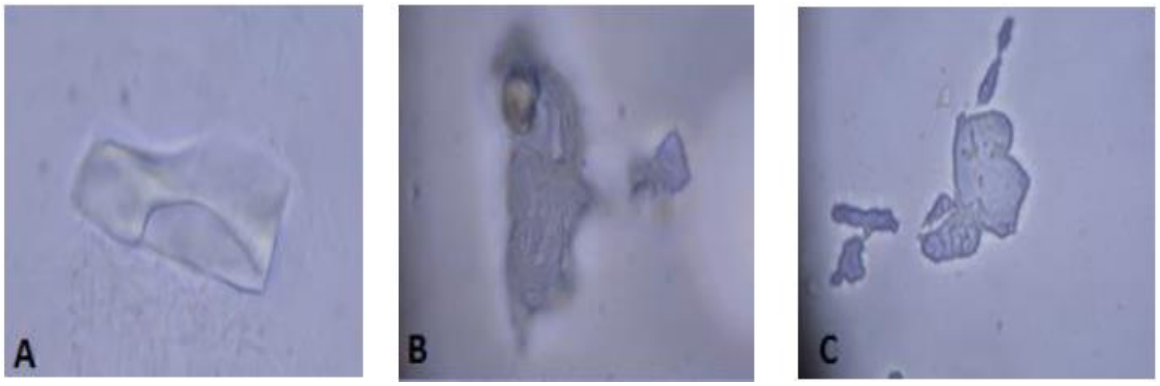


Figure 5.4: crystals under a light microscope. A is from *B.cohnii,* B is from *B.halodurans*, and C is from *B.pseudofirmus*.

5.3.4 Amount of calcium carbonate precipitated (stage four part one)

The increasing densities of all culture samples with SHA was observed and compared to SHA cultures. There were precipitated into sets (4-2, 4-3, 4-4), as shown in Figure 5.5. All liquid solutions were centrifuged at high-speed (5,000 rpm for 10 minutes) at 4℃ to separate the materials (calcium carbonate, organic materials) from the culture solution.

Figure 5.5: Culture of *B.pseudofirmus* with SHA-3 (set 1-2) and SHA-3 (set 1-1) after eight days of incubation.

Weighing of the precipitate materials inside falcon tube was conducted on all sets after all samples of sets were dried on an oven at 50±2℃ for a minimum of five days. The calcium carbonate amount from sets [(4-12 to 4-14), (4-22 to 4-24), (4-32 to 4-34)] is shown in Figures 5.6-5.9. The amount of calcium carbonate (mg/mL) from the three types of bacillus in SHA-1 for a certain time (eight days) is illustrated in Figure 5.6. It is clear that set 4-12 produced the highest amount of calcium carbonate, 12% higher than the amount produced by set 4-13 and 65% higher than calcite produced by set 4-14.

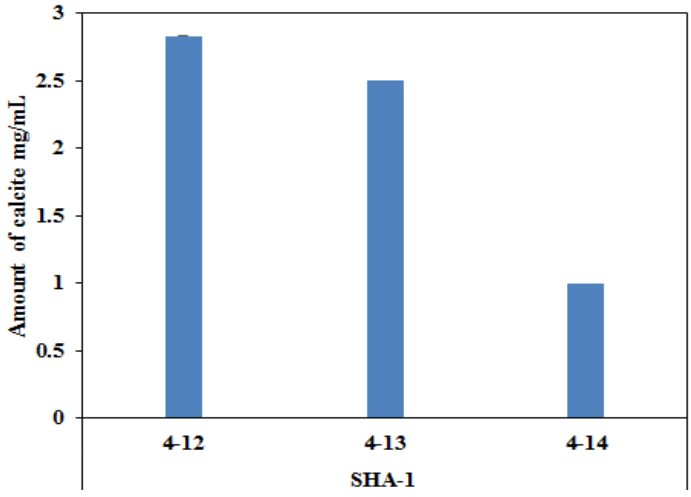


Figure 5.6: Amount of calcium carbonate produced by sets (4-12 to 4-14).

In contrast, the amount of calcite produced by set 4-22 was the lowest compared to the other two sets, as shown in Figure 5.7. In terms of its quantity, it represents 51% of the quantity produced by set 4-23 with SHA-2, while it is presents 60% of the quantity produced by set 4-24.

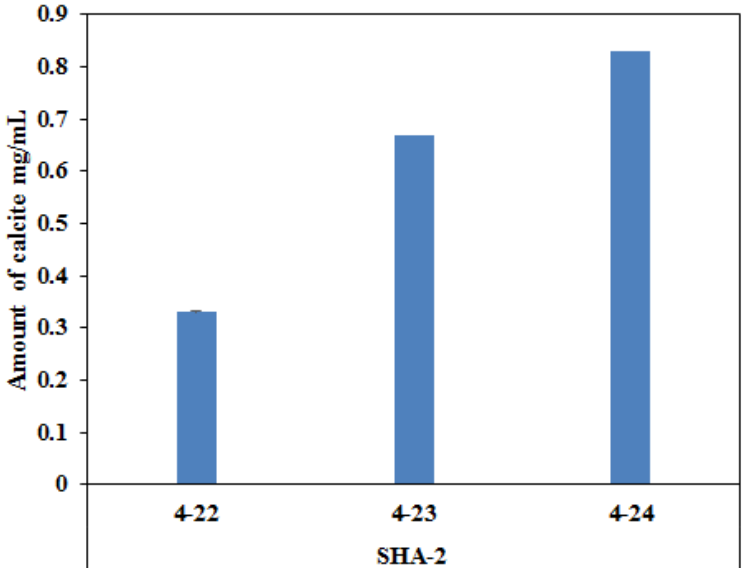


Figure 5.7: Amount of calcium carbonate produced by sets (4-22 to 4-24).

The amount of calcium carbonate produced by set 4-32 was dominant and the least amount of calcium carbonate was produced by set 4-33, as presented in Figure 5.8. The amount of calcium carbonate produced by set 4-32 was higher than the amount produced by set 4-33, which was around 73% and approximately 46% more than the calcite produced by set 4-34.

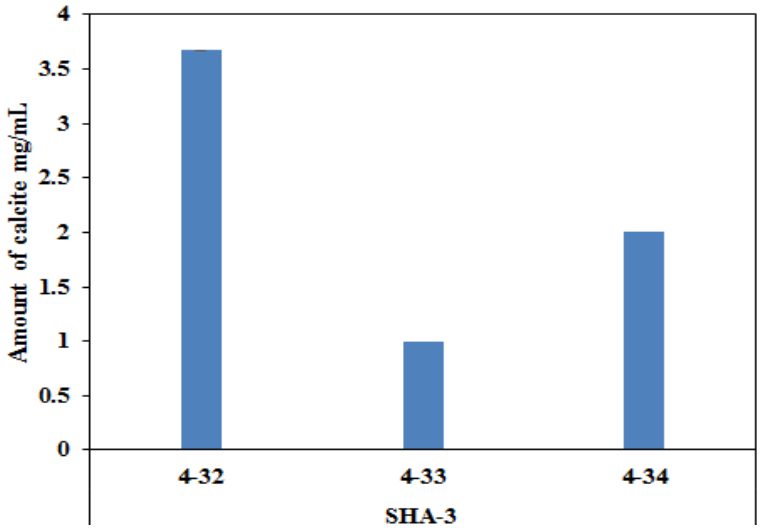


Figure 5.8: Amount of calcium carbonate produced by three sets (4-32 to 4-34).

It was observed that the amount of calcium carbonate produced by set 4-12 and set 4-32 was the highest compared to the amount produced by set 4-23 and set 4-34 as shown in Figure 5.9. However, the amount of calcium carbonate from set 4-32 was higher than set 4-12 (around 23% by weight). The calcite produced by set 4-34 was higher than that produced by set 4-14 (around 50% by weight).

The amount of calcium acetate in SHA-3 was higher than that in SHA-2 (around 20 times by weight) and energy resources was higher in SHA-3 around four times by weigh than that in SHA-2. It was observed that the amount of calcite produced by set 4-22 was less than that amount produced by set 4-24, while it was higher in set 4-32, that could be due to not filtered and calcite is not separated from the cells of bacteria. Whereas, *Halodurans* forms long chains compare to *pseudofirmus* chains. Or could be due to the amount of energy resources was not enough to germinate all spores to cells or to divisions the cells and produce the energy inside the cell to produce calcite. Or could be due to some component of SHA-2 is triggering more calcite, which means that SHA-2 is an optimum medium for calcite precipitation in case of *halodurans* but not *pseudofirmus*.

Though they belong to same genus, their needs are different. *Halodurans* is halophilic (salt tolerant) whereas *pseudofirmus* is not. Hence the difference in the yield.

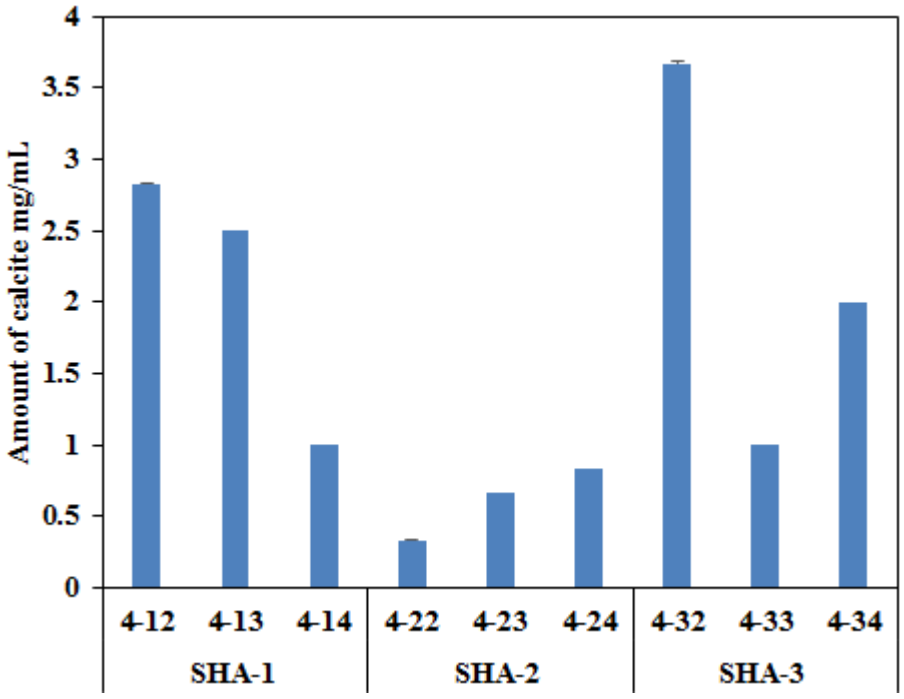


Figure 5.9: Amount of calcium carbonate produced by sets [(4-12 to 4-14), (4-22 to 4-24), (4-32 to 4-34)].

5.3.5 Calcite amount precipitated by SHA-3 with *B.pseudofirmus* (stage four part two)

The calcium carbonate amount from set 4-32 was studied. The amount of calcium carbonate (mg/ml) from set 4-32 for period of time is illustrated in Figure 5.10. It is clear that curve increased with time until eight day. Then, curve was reached stationary phase after eight day, which the amount of calcite at eight day was 3.87 mg/ml and this amount increased by 1.8%.

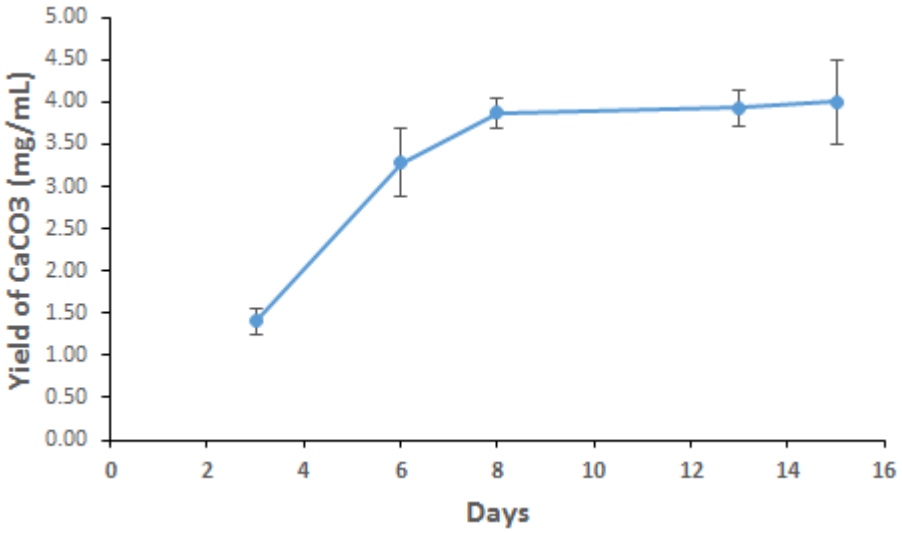


Figure 5.10: Amount of calcium carbonate due to set 1-2 with SHA-3.

5.3.6 Effect of inoculum volumes on calcium carbonate precipitation in various calcite screening medium (stage four part three)

The relationship between amount of bacteria CFU and ratio between calcium carbonate by set 4-32 and the amount of calcium acetate were analyzed as shown in Figure 5.11. The results presented that calcium carbonate/calcium acetate ratio for three types of medium increased with CFU of bacteria. High calcite/calcium acetate ratio for SHA-1 was 0.62 when calcium acetate/amount of bacteria ratio was 0.014, then the ratio decreased steadily with increasing the ratio of calcium acetate/amount of bacteria. High ratio for SHA-2 was 0.55 when the number of bacteria was 7.4 x 10^{10}, then the cure decreased significantly with increasing the ratio of calcium acetate/amount of bacteria.

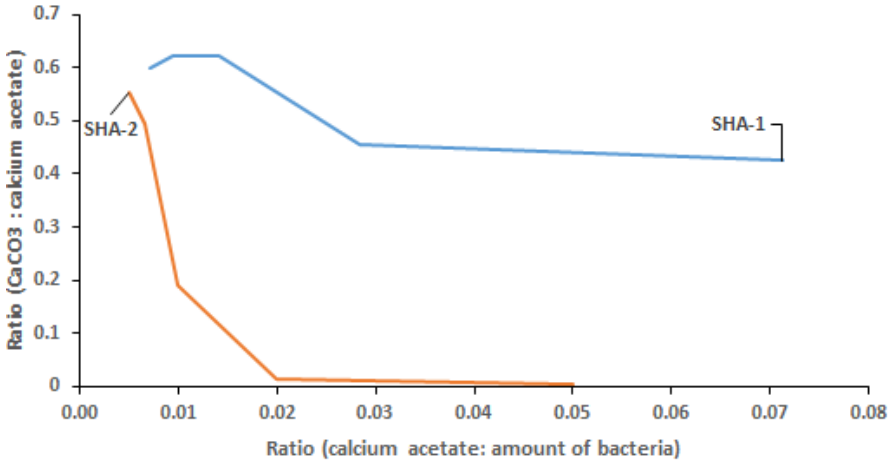


Figure 5.11: The ratio of calcite/calcium acetate with various inoculum volumes of bacteria.

However, the results presented in Figure 5.12 showed that ratio of calcium carbonate/calcium acetate decreased with ratio of calcium acetate/amount of bacteria until ratio of calcium acetate/amount of bacteria reached 0.4, and then it increased steadily due to huge amount of calcium acetate in SHA-3 compared to SHA-1 and SHA-2. Whereas, the percentage of calcium acetate in SHA-1 and SHA-2 compared to SHA-3 is 7.1% and 5% respectively, which mean the amount of calcium carbonate produced by the volume of bacteria from calcium acetate is small compared to that produced in SHA-1 and SHA-2. Thus, the volume of bacteria have been increased significantly to guarantee convert the whole amount of calcium acetate in SHA-3 to calcium carbonate.

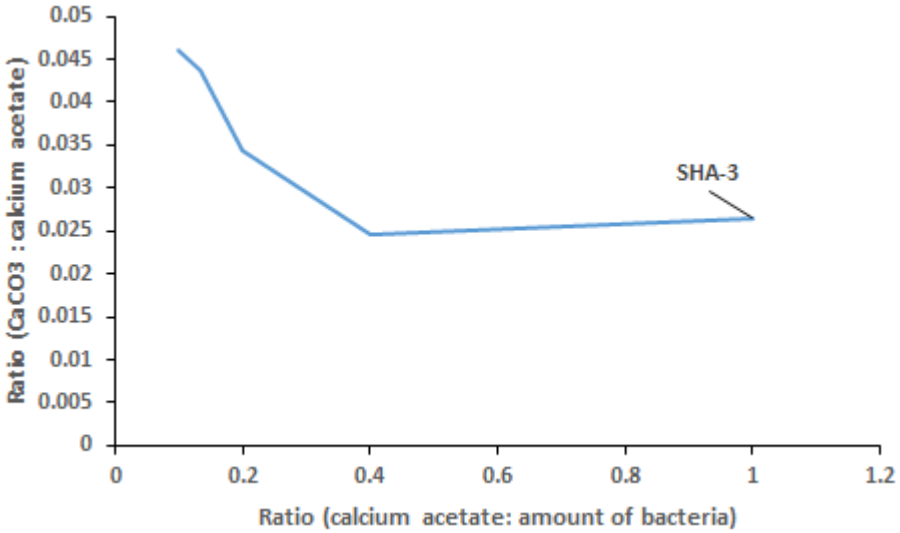


Figure 5.12: The ratio of calcite/calcium acetate with various inoculum volumes of bacteria.

5.3.7 The effect of temperatures on the ability of three *Bacillus* species strain on growth dynamically (stage five)

The results presented in Figure 5.13 showed that both *B.cohnii* and *B.pseudofirmus* have a stationary pattern at 25℃, at which they greatly grew. In contrast, *B.halodurans*' growth significantly reduced. Moreover, for both *B.cohnii* and *B.pseudofirmus*, their growth was reduced by increasing the temperature, if the data at 30℃ for *B.cohnii* is ignored. *B.halodurans* has a stationary pattern at 30℃ and 40℃ and the lowest growth was recorded at 25℃, while its growth at 35℃ dropped below 40℃ after around 25℃. Overall, three types of bacteria showed significant growth difference between 25℃ and 40℃, accepting the result of *B.halodurans* at 25℃, which gives the indication that the range of growth of bacteria is between 25℃ and 40℃. However, the results of the three strains of *Bacillus* that were tested in these experiments demonstrated their ability to produce calcite as self-healing agents of concrete at different temperatures.

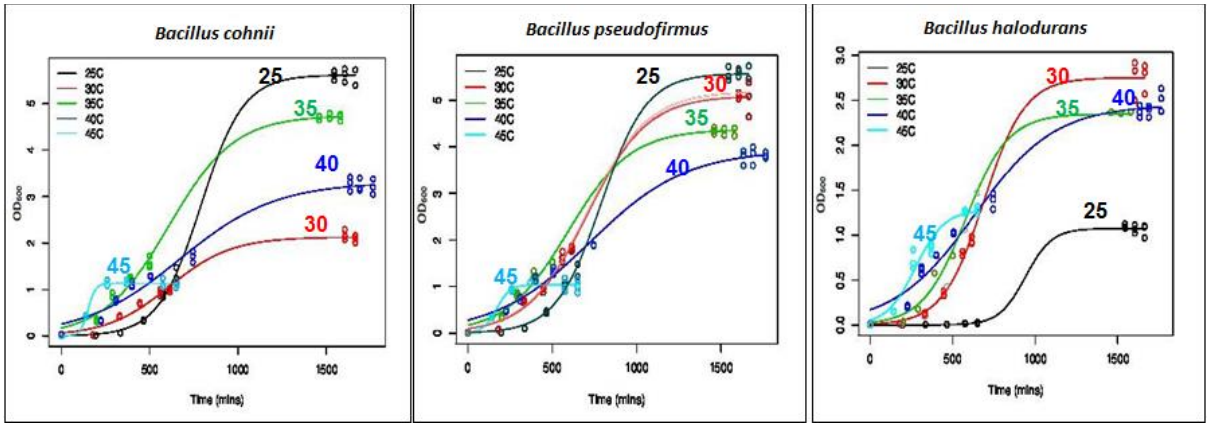


Figure 5.13: The growth of three *Bacillus* species at different temperature.

5.4 Conclusion

This section gathers the conclusions drawn from the experimental work reported in this chapter. The specific objectives of this work were described in the introduction of this chapter, and the experimental stages were designed to satisfy these objectives, which has led to the specific conclusion. These conclusions are drawn from the experimental results presented in the previous sections:

a. The processes of cement hydration, the mechanical stresses due to mixing and hardness of cement paste and the effect of a high alkalinity environment had minor effects on the survivability of bacterial spores, conformed that spores for all three types can stay inside mortar/concrete for more than three months. Thus, can use them in self-healing concrete.
b. According to optical density (OD600), growth conditions in SHA-3 were more favourable than SHA-1 and SHA-2 because the percentage of calcium acetate in SHA-1 and SHA-2 compared to SHA-3 is 7.1% and 5% respectively. However, growth conditions in SHA-1 was faster during first eight hours due to it has ten ingredients which make microorganisms grow faster as described in Chapter 4.
c. Study the capability of three isolates of alkaliphilic bacteria to precipitate calcite clearly showed the presence of the crystals of precipitate from the isolates of Bacillus bacteria. The screening of all three types of bacteria unified that all of them can produce calcite, which mean they can apply them as self-healing agent to heal/seal cracks in mortar/concrete.
d. It was found that the three studied bacteria, *B.pseudofirmus*, *B.halodurans*, and *B.cohnii*, have the ability to generate calcium carbonate as a sealing material in the healing process.
e. *B.pseudofirmus* has a lower precipitation time ratio (defined as the ratio between the amount of calcium carbonate precipitation to amount of SHA) over the other two bacteria (*B.cohnii*, and *B.halodurans*).
f. Both *B.pseudofirmus* and *B.cohnii* can grow in temperatures that range between 25℃ and 30℃. *B.halodurans* can grow in temperatures up to 40℃, which can be used to remediate concrete cracks built in hot climate regions.

Chapter 6
The effect of *Bacillus* bacteria and SHA on the properties of bio-cement mortar

6.1 Introduction

Cracks in concrete leads to an increase in its permeability, which can result in a loss of its durability. Self-healing concrete is a method by which concrete can recover its permeability autonomically without the need for human intervention. Microbiologically-induced calcite-precipitation is a self-healing concrete that has been studied in recent years. It is now known that the necessity to encapsulate all the ingredients (bacteria, nutrients and organic precursors) prior to mixing. However, during mixing there is a chance that capsules or other carriers of the self-healing agents may release their cargo. A key question is whether the released materials effect the cement mortar properties. The aim of the research presented in this chapter was to investigate the effect of the spilt medium and Precursor from some microcapsule ruptures during the mixing process of concrete components in the early-age properties of cementitious materials. For example, setting time, hydration kinetics, compressive, flexural strength, and microstructure of cement were studied. This phenomenon is simulated by adding the different self-healing agent (SHA) ingredients with different percentages directly to the mortar. This is then studied to determine their effect on mortar matrix properties. Subsequently, the study has been reported in Mohamed et al.[409,410]

This chapter is divided into three stages, **the first stage** covers the remediation of mortar cracks using two stage bio-mortar (TSBM) (the first part involves making samples from mortar mixed with bacterial agent ingredients, such as yeast extract and calcium lactate. The second part begins after the samples hardened. A crack is generated by using a three-point bending test and is injected with suitable bacteria. This concludes the second part).

In the second stage, the effect of SHA-1 solution on compressive strength for development mortar and long-term properties of cementitious materials such as compressive strength for hardened mortar were investigated.

Finally, **in the third stage**, the effect of SHA-1 ingredients on the fresh properties of bio-cement mortar and microstructure of cement paste were presented and discussed.

6.2 Experimental programme

6.2.1 Growth Medium stock

Growth medium was divided into two main parts: nutrients used as an energy resource for bacteria to germinate and grow from spore to cell and a precursor, which is required to produce calcium carbonate as shown in the equation below.

$$CaC_4H_6O_4 + 4O_2 \rightarrow CaCO_3 + 3CO_2 + 3H_2O$$

The SHA used in this work was SHA-1 (see Chapter 4).

Table 6.1 presents the percentage of each component of SHA-1 and the percentage of each component of modified SHA-1, which was studied as part of their effect on compressive strength for development mortar and long term properties of cementitious materials such as compressive strength for hardened mortar (stage two) and the early-age properties of cementitious materials e.g. cement hydration kinetics, setting time (stage three).

Table 6.1: The quantity of used ingredients of bacterial medium SHA-1 and modified SHA-1 as % of cement

Ingredients	% of each proportion of SHA-1	% of each proportion of modified SHA-1
Calcium acetate	55.94	65.20
Sodium citrate	18.65	3.11
Yeast extract	12.12	18.40
Na-glutamate	3.73	3.73
Alanine	1.86	1.86
Inosine	1.86	1.86
$MgCl_2$	1.86	1.86
NaCl	1.86	1.86
$MnSO_4$	1.17	1.17
Kh_4PO_4	0.93	0.93
Total	100.00	100.00

6.2.2 Preparation of samples

6.2.2.1 Mix properties

The materials used for making mortar specimens are divided into two parts. Firstly, normal mortar (series A). Secondly, bio-mortar is divided into three series B, C, and D as given in Table 6.2. The design of normal mortar was according to EN 196-1:2005[395], by taking W/C = 0.5, A/C = 3, and fly ash cement type CEM II/B-V 32.5R. The size of the samples was

40x40x40mm for compressive strength, and 40x40x160mm for flexure strength and a suitable percentages of alkali resistant fiber glass mesh was added to each mould. While bio-mortar (has same proportion of normal mortar with directly adding SHA powder during mix) is based on a previous study carried out at the University of Bath 'The Effects of Calcium Lactate on Early Age Concrete' by Cunniff[374], and all mix proportions for stage one are given in Table 6.2 and Table 6.3.

Table 6.2: Mix proportions of test specimens.

Design mortar according to EN 196-1:2005[395](40x40x160)								
Series	W/C	A/C	Cement	Standard sand	Water	Calcium lactate	Yeast extract	Calcium lactate with Yeast extract
A (control)	0.5	3.0	450g	1350g	225g	-----	-----	-----
B	0.5	3.0	450g	1327.5g	225g	22.5g (5%)	-----	-----
C	0.5	3.0	450g	1347.75g	225g	-----	2.25g (0.5%)	-----
D	0.5	3.0	450g	1325.25	225g	-----	-----	24.75

The quantity of each group of samples prepared for development of mortar compressive strength and long term properties of cementitious materials such as compressive strength for hardened mortar for stage three are given in Table 6.3.

Table 6.3: Mix Proportion of Samples Based on Self-Healing Agents SHA-1.

Group	Component/Cement Mass Ratio	SHA-1 Component/g	Cement/g (CEM II/B-V)	Water/g	Standard Sand/g
Control	--	--	450	225	1350
Complete SHA-1	0.5%	2.25	450	225	1347.75
Complete SHA-1	1.4%	6.30	450	225	1345.70
Complete SHA-1	2.2%	9.90	450	225	1340.10

6.2.2.2 Generating cracks

A three-point bending test was applied to each prism to create a crack at the middle of the samples. For all samples, the crack width was measured by using microscope with x5 magnification. To achieve the same crack width for all samples was challenging since the cracks openings simulate real cracks, which occur rapidly in concrete with possibility of controlling it. Therefore, the crack widths ranged between 0.24 and 0.53 mm.

6.2.3 Stage one:

In this section, the idea of remediation of mortar cracks presented in this stage was to use a two stage bio-mortar remediation method, which can also be called 'two stage remediation method (TSRM)'. In this method, the bio-mix is created first by mixing mortar components with bacterial agent ingredients, and then after the mortar gets harder, the crack is generated and then the specified type of bacteria is injected in the specified crack. The primary study was done according to the plan shown in Figure.6.1 with three categories. The code of each set was presented in Table 6.4

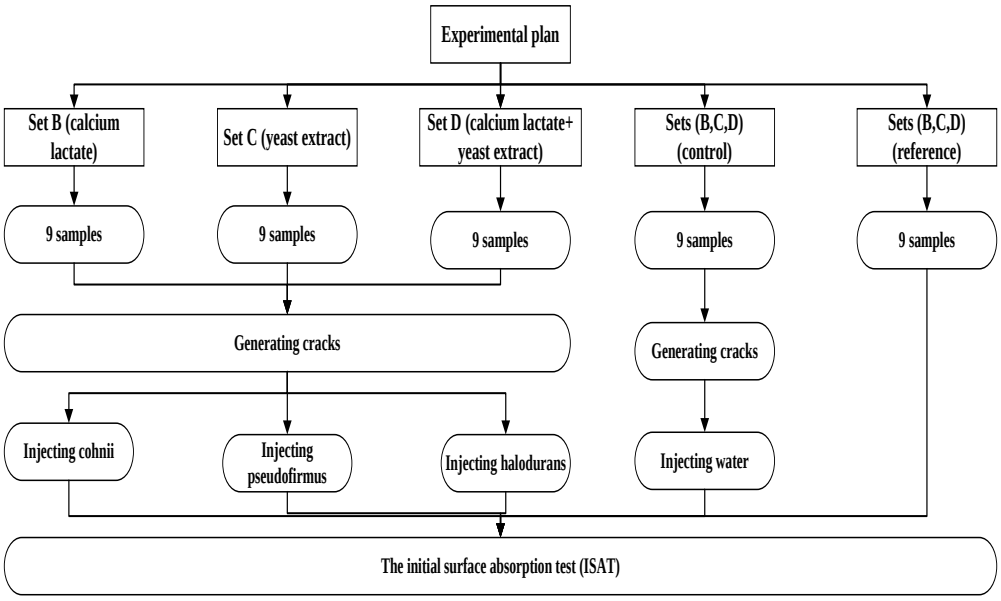


Figure 6.1: Plan of bio-mortar experiment.

Table 6.4: Codes number for all samples in stage one part1

	0	1	2	3	4
A	Normal mortar	---	---	---	---
B	$C_6H_{10}CaO_6$ mortar	$C_6H_{10}CaO_6$ mortar	$C_6H_{10}CaO_6$ mortar	$C_6H_{10}CaO_6$ mortar	$C_6H_{10}CaO_6$ mortar
C	Yeast extract mortar	Yeast extract mortar	Yeast extract mortar	Yeast extract mortar	Yeast extract mortar
D	($C_6H_{10}CaO_6$ + yeast extract) mortar	($C_6H_{10}CaO_6$ + yeast extract) mortar	($C_6H_{10}CaO_6$ + yeast extract) mortar	($C_6H_{10}CaO_6$ + yeast extract) mortar	($C_6H_{10}CaO_6$ + yeast extract) mortar
	Reference set	Control set (water only)	*B.pseudofirmus* set	*B.cohnii* set	*B.halodurans* set

6.2.3.1 Treatment of samples and monitoring the cracks

Prisms of bio-deposition were placed in water to about half of their depth to minimise the immigration of treatments laterally out of the crack. The duration was 28 days and on each day the specimens were fed twice in 24 hours by sterile 3ml syringes tipped with non-pyrogenic needles, which were used to accurately dispense the treatment directly into the cracks. Each day the crack was observed by using a crack detection microscope supplied from C&D (Microservies) Ltd. The mortar blocks were then stored in an incubator overnight at 30°C. Then after healing, the cracks were measured again by using microscope and the efficiency of the crack-sealing materials are studied.

6.2.3.2 Initial surface absorption test (ISAT)

In the literature review, different techniques and tests were used to evaluate the healing capabilities and performance. For example, qualitative techniques to assess the efficiency of self-healing visually, such as scanning methods. This technique was used in all sections, which gave us an indication about the type and condition of the healing material visually. In order to evaluate the efficiency of the sealing materials, the permeability using low pressure or initial surface absorption test (ISAT) was done to all samples according to BS1881-1970[401]. The target of this test was to compare the efficiency of crack sealing material before and after the bio-treatment.

6.2.4 Stage two:

This stage was divided to two parts as follow:

First part has four groups of mortar prisms were prepared in accordance with EN 196-1[395] with the addition of 0, 0.5, 1.4 and 2.2% of SHA-1 by mass of cement. They were cured in a curing room at 20°C and the relative humidity was 80% as shown in Table 6.3. The prisms were tested at 3, 7, 14, 21 and 28 days to investigate the effect of SHA-1 on develop strength of mortar.

Second part mortar prisms prepared according to BS and the proportion was presented in **section 6.2.2**. The specimens were cured as previous part for 24 h, then cured in water for 359 days. They were then kept in SHA-1 solution (10.7 g/l) (SHA-1 was summarised in Section 4.4.2.2), whilst control prisms were maintained in water. Compressive strength tests were

carried out on samples at 360+ 3, 7, 14, 21 and 28 days to investigate the effect of SHA-1 solution on hardened mortar strength.

6.2.5 Stage three:

This stage was divided to three parts as follow:

6.2.5.1 Setting time test

Setting time tests (Vicat Tests) were conducted according to BS EN 196-3:2005[396] to determine the initial and final setting time of the cement pastes. Methods of testing cement-part 3: determination of setting time and soundness. Fly ash cement type CEM II/B-V 32.5R produced by Lafarge cement UK Ltd was used for the experiment with the consistent use of W/C used throughout this study (0.33) and each set had three samples. Five hundred grams of cement was mixed with 165g of water for control mixes paste. Similarly, the procedure was done with the rest of sets as presented in Table 6.5.

Table 6.5: Effects of self-healing agents SHA-1 components on setting time of cement.

Group	Component/Cement Mass Ratio	Self-Healing Agents, g	Cement, g
Control	0	0	500
SHA-1	0.5%	2.50	500
Ca-acetate	0.28%	1.40	500
Na-citrate	0.09%	0.45	500
YE	0.06%	0.30	500

6.2.5.2 Preparation of paste samples for generating and analysing experimental data by Isothermal calorimeter analysis

The effects of SHA-1 on concrete properties was studied by investigating the effect of SHA-1 on cement hydration as a function of time, and by using an advanced laboratory isothermal conduction calorimetry machine, Calmetrix I-Cal 4000. In this machine, all the cement paste with SHA-1 was used as an input and the outputs were the rate of heat in w/g and time in hours. The temperature was maintained at 20°C throughout the testing for all samples and F/P-Cal Logger software was used to gather and analyse the experimental data. The first experiment was done without any bacterial self-healing agents (SHA-1), only cement paste, this mix was called the control mix or reference mix. The other three mixes were prepared by using 0.1, 0.3 and 0.5% of SHA-1 from the mass of cement to find the optimum percentage of

agent, which had no or had minor effects on cement hydration. It was found in **section 6.3.3.1** that 0.5% of SHA-1 had a minor effect on the strength of hard mortar. Therefore, in this section the study concentrated on the effect of SHA-1 on cement hydration. Three percentage were chosen (0.1, 0.3, and 0.5%) to be studied. In order to reach the optimum percentage of SHA-1 to be used in bio-concrete mix, the water/cement ratio was taken at 0.5 for all cement paste and cement weigh was taken as 40 g for all paste samples. The investigated shape of the hydration curve is presented according to qualitative analysis, as shown in Figure 6.2

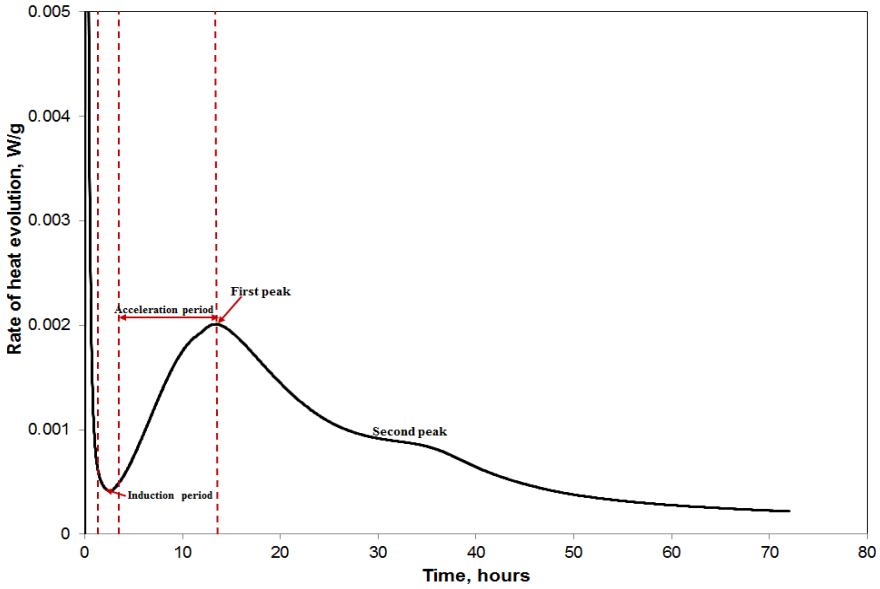


Figure 6.2: A typical hydration curve for cement paste.

Since the SHA-1 is composed of ten components, then the second phase of this investigation was to elucidate which component of these ten ingredient (calcium acetate, yeast extract, sodium citrate, sodium glutamate, alanine, inosine, magnesium chloride, sodium chloride, manganese sulfate, and monopotassium phosphate) affects the properties of concrete at an early stage. Therefore, the method of elimination was implemented as follow:

i. Study the effect of each element in the SHA-1 on the properties of mortar.
ii. From the investigation presented in 'a' of this section, one can find that which ingredient was the most effective of the SHA-1 on concrete properties. Then, investigated the element in order to reach the optimum percent so that has no effect on concrete.
iii. The optimum percent of element was found in 'b', which was used with all other ingredient values (0.1, 0.3, and 0.5%) and it presented as SHA-1 modified.

6.2.5.3 Microstructure of cement paste

The cement used in this investigation was Portland-fly ash cement CEM II/B-V 32.5R produced by Lafarge cement company UK Ltd. Two sets of cement paste were prepared by mixing 0.5% of SHA-1 by cement mass with 0.5 water cement ratio. The other two sets were prepared without any calcium source. 20 g of water, 0.5% SHA-1, and 40 g of cement were mixed to prepare all samples, which were prepared by hand for one minute. After this, all samples were cured in a curing room at 20°C with a relative humidity of 80% until they were tested by SEM.

6.3 Results and discussion

6.3.1 Stage one part 1: Ability of bacteria to remediate of mortar cracks using bio-mortar

In this stage some experimental works were carried out on injected bacteria with buffer into crack of bio-mortar to explore their growth ability to precipitate calcite as well as their ability to remediate a crack was also investigated.

The first set was calcium lactate specimens. The set B-1 is the control set and the crack width was 0.42 mm and was injected with water only for 28 days, as shown in Figure 6.3A. From Figure 6.3B, one can observe that the crack was healed by a white material and it was healed by an autogenous healing method.

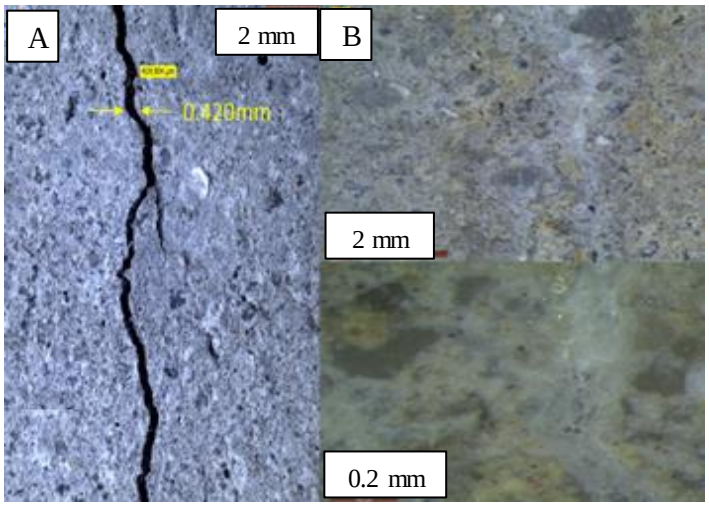


Figure 6.3: Set B-1 injected by water only at tension zone. A: before; B: after.

In the set B-2, the crack width was 0.36 mm and it was treated by injecting *B.pseudofirmus* with a buffer as shown in Figure 6.4A. One can observe from Figure 6.4B that the crack was healed by a poor yellow-like material and the healing was scattered at some places along the crack length.

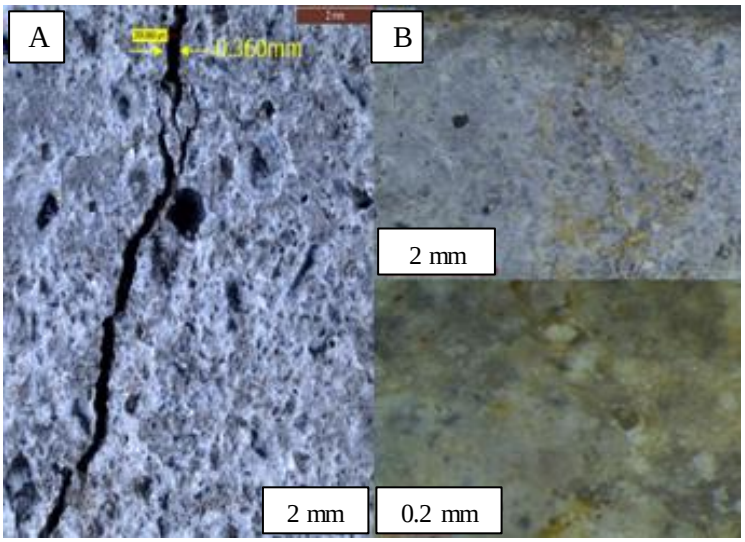


Figure 6.4: Set B-2 injected *B.pseudofirmus* with buffer at tension zone. A: before; B: after.

The set B-3 was injected for 28 days as shown in Figure 6.5A. The width of the crack was 0.32 mm, which was measured using a microscope before the healing process. The set B-3 produced a poor white-like sealing material with yellow-like points across the crack, as shown in Figure 6.5B.

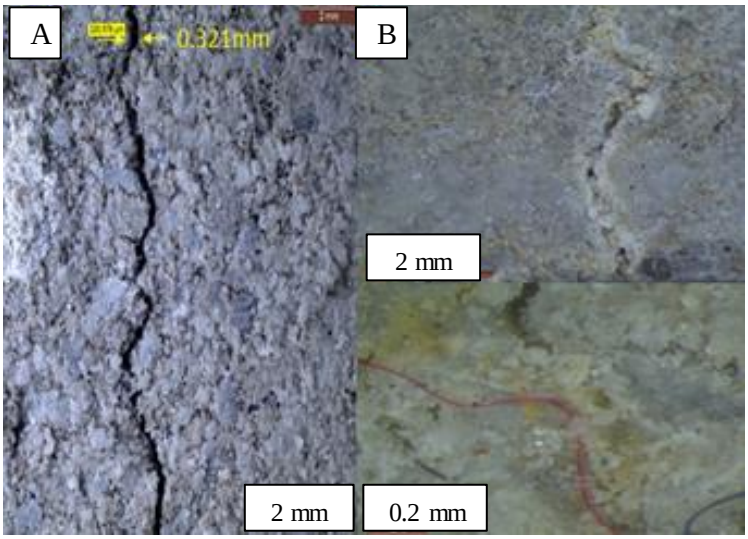


Figure 6.5: Set B-3 injected by *B.cohnii* with buffer at tension zone. A: before; B: after.

In the set B-4 the crack width was measured as 0.30 mm and it was injected for 28 days as shown in Figure 6.6A. It was clear from Figure 6.6B the set B-4 produced sealing materials have a yellow-like colour and the sealing/healing was limited to some places along the crack.

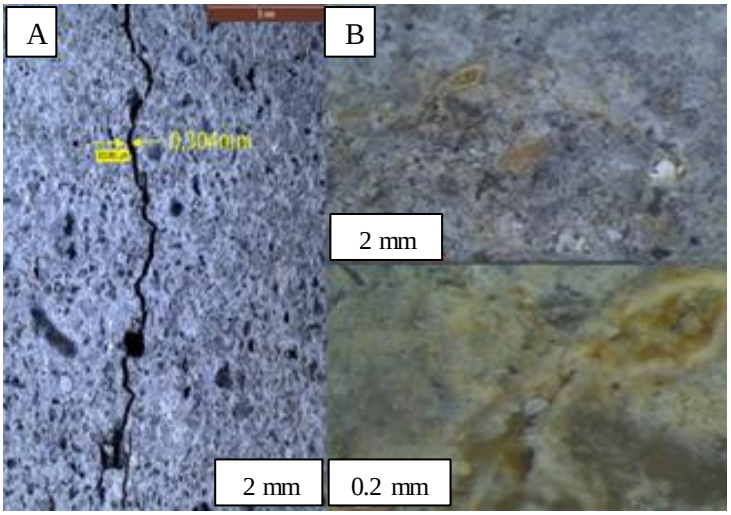


Figure 6.6: Set B-4 injected by *B.halodurans* with buffer at tension zone. A: before; B: after.

The second set was the yeast extract specimens. The set C-1 was the control samples. The crack width was 0.37 mm and it was injected with water only as shown in Figure 6.7. From Figure 6.7A, one can observe that the crack was healed by white-like material and Figure 6.7B and Figure 6.7C show this material healed the crack was poor and it was healed by an autogenous healing method.

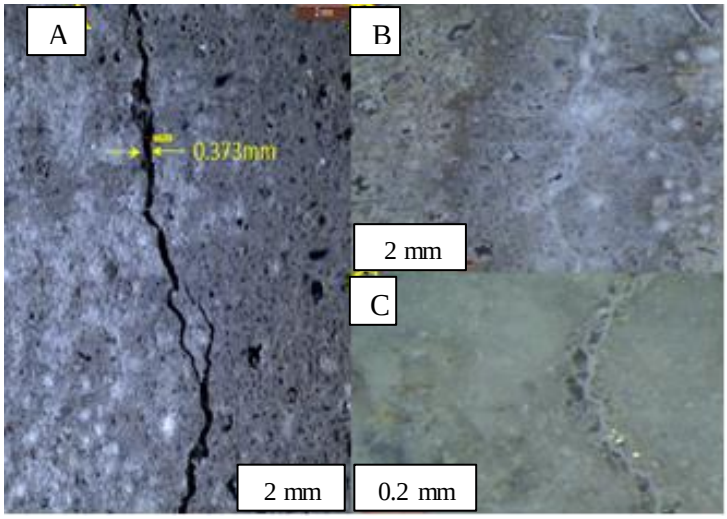


Figure 6.7: Set C-1 injected by water only at tension zone. A: before; B-C: after.

In the set C-2, a microscope was used to measure the width of the crack before healing. It was found to be 0.35 mm and then it was treated, as shown in Figure 6.8A. The duration of treatment was 28 days and set C-2 produced sealing materials that had a brown-like colour as shown in Figure 6.8B. The crack was sealed by thin a layer as shown in Figure 6.8C.

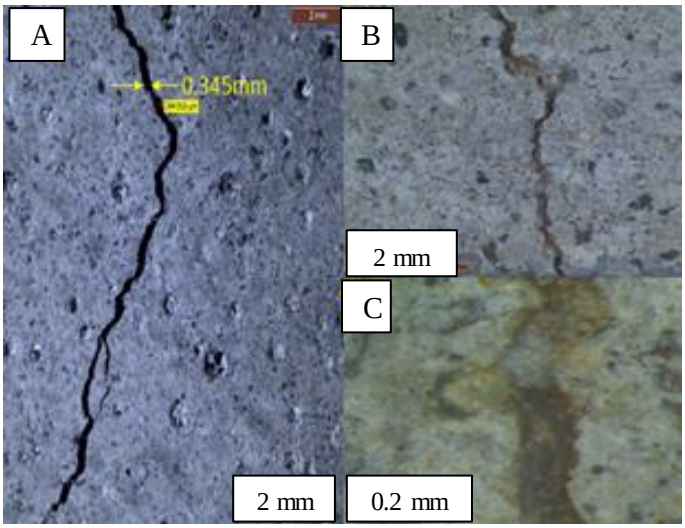


Figure 6.8: Set C-2 injecting by *B.pseudofirmus* with buffer at tension zone. A: before; B-C: after.

The set C-3 was treated by *B.cohnii* with buffer as shown in Figure 6.9. The width of the crack was considered as 0.35 mm before healing. It was injected for 28 days as shown in Figure 6.9A. The set C-3 produced a poor white-like/yellow-like material across the crack as shown in Figure 6.9B.

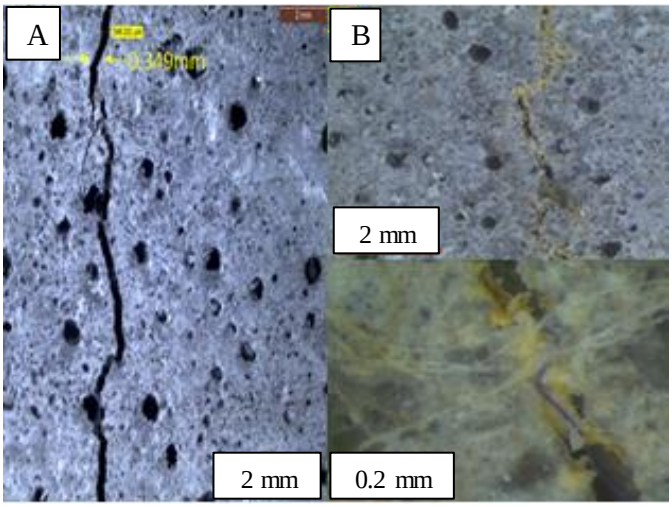


Figure 6.9: Set C-3 injected by *B.cohnii* with buffer at tension zone. A: before; B: after.

The fourth set was C-4. A microscope was used to measure width of the cracks before healing with *B.halodurans*. The crack width was 0.39 mm as shown in Figure 6.10A. Figure 6.10B shows sealing of the crack by a white material due to it having been injected for 28 days of treatment. From Figure 6.10C one can see the white-like material that sealed the crack is of poor quality and resembled a crystal material.

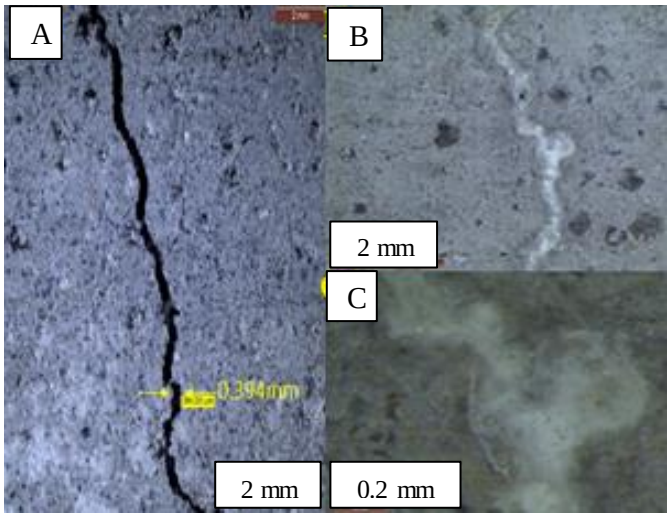


Figure 6.10: Set C-4 injected by *B.halodurans* with buffer at tension zone. A: before; B-C: after.

The third set was 'D' (calcium lactate with yeast extract). The set D-1 was the control samples. The crack width was 0.27 mm and it was injected with water only, as shown in Figure 6.11. From Figures 6.11A and B one can observe that the crack was healed by a white, thin, poor quality material layer, which indicates that the healing took place by an autogenous healing method.

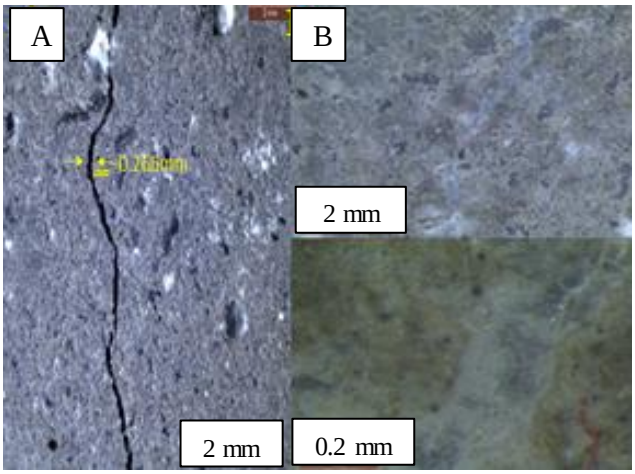


Figure 6.11: Set D-1 injected by water at tension zone. A: before; B: after.

In the set D-2, the crack width was 0.38 mm and it was injected with *B.pseudofirmus* with a buffer, as shown in Figure 6.12A. set D-2 produced sealing materials that had a white-like colour and there were some yellowish points across the crack, as shown in Figure 6.12B. The sealing material was of a poor quality and the sealing material sealed the crack by a thin layer, as presented in Figure 6.12C.

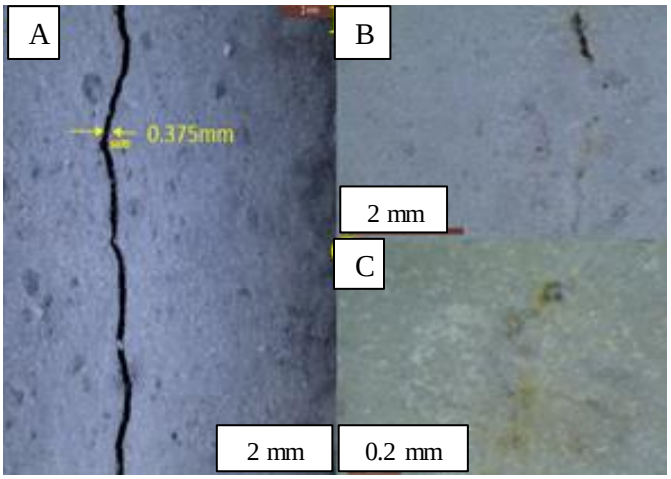


Figure 6.12: Set D-2 injected by *B.pseudofirmus* with buffer at tension zone. A: before; B-C: after.

In the set D-3 the crack width was 0.40 mm. Three samples were injected with *B.cohnii* + buffer after the crack was generated, as shown in Figure 6.13A. The set D-3 produced sealing materials, which sealed the opening of the cracks slowly at both crack faces. The crack by the end of sealing time disappeared, as shown in Figure 6.13B. The sealing material shows a thin yellow-like, poor quality layer. This is illustrated in Figure 6.13C.

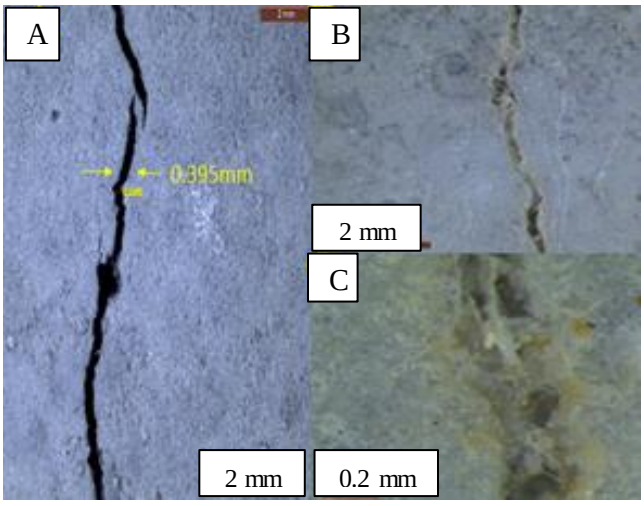


Figure 6.13: Set D-3 injected by *B.cohnii* with buffer at tension zone. A: before; B-C: after.

In the set D-4 the microscope was used to measure the width of cracks before healing. The crack width was 0.40 mm as shown in Figure 6.14A. Figure 6.14B shows the sealing of the crack by a white material due to injecting for 28 days of treatment. It is clear from Figure 6.14C that the material that sealed the crack was of poor quality and it was a thin layer of white-like/yellow-like material across the crack.

The fifth sets were the reference samples set, which were kept without generating cracks and they remained at the same conditions as the fourth set's conditions until the ISAT test.

The summary of experimental data and observed results of the four categories is given in Table 6.6.

Table 6.6: Summary of experimental data and observation results for four categories.

Action taken and observed	**Control samples (water only) no bacteira**			***B.pseudofirmus***			***B.cohnii***			***B.halodurans***		
Type of sample	B-1	C-1	D-1	B-2	C-2	D-2	B-3	C-3	D-4	B-4	C-4	D-4
Crack width	0.37	0.37	0.47	0.36	0.36	0.36	0.32	0.34	0.40	0.30	0.35	0.40
pH value	10	10	10	10	10	10	10	10	10	10	10	10
Temperature	$22C^{o}$	$22C^{o}$	$22C^{o}$	$30C^{o}$	$30C^{o}$	$30C^{o}$	$30C^{o}$	$30C^{o}$	$30C^{o}$	$30C^{o}$	$30C^{o}$	$30C^{o}$
Start of healing days	12	13	14	13	15	12	9	9	9	12	13	10
End of healing days	21	22	20	23	23	21	23	24	22	24	25	23
Finishing experiment	28	28	28	28	28	28	28	28	28	28	28	28
Type of healing	Autogenous			Sealing cracks			Sealing cracks			Sealing cracks		
Remark				Poor yellow-like material and the healing was limited to some places along the crack			Poor white-like material and there were some yellow-like points across the crack			Poor yellow-like material and the healing was limited to some places along the crack		

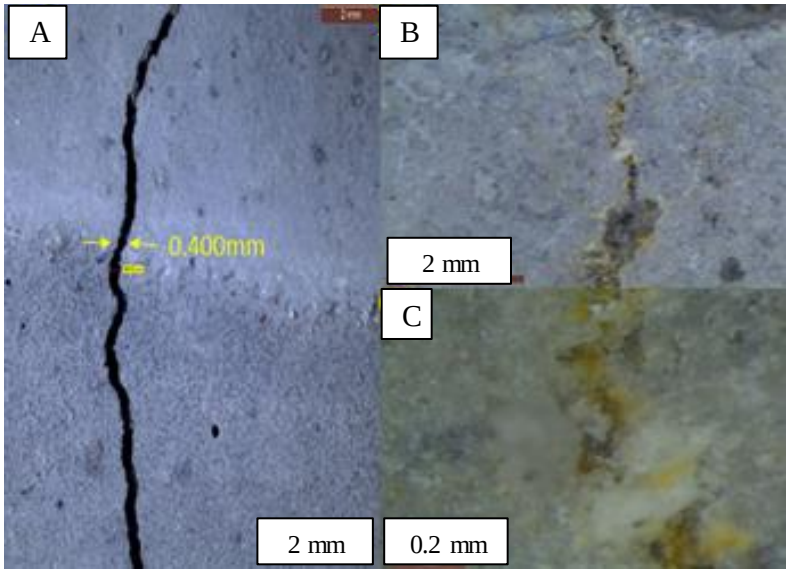


Figure 6.14: Set D-4 injected by *B.halodurans* with buffer at tension zone. A: before; B-C: after.

6.3.1.1 Testing the efficiency of the produced self-healing material by using initial surface absorption procedure (ISAT)

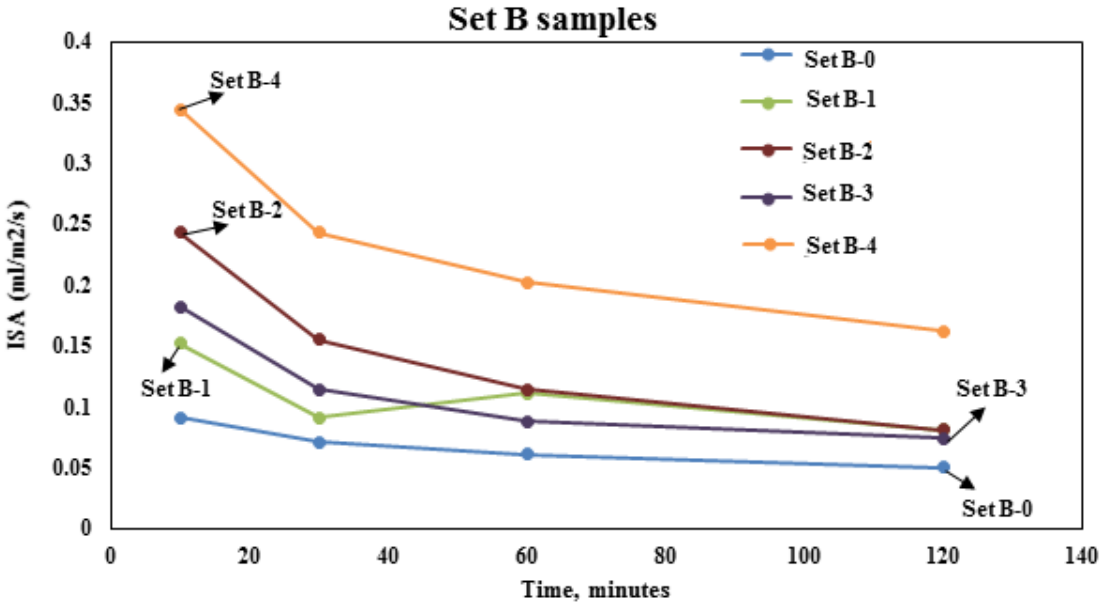


Figure 6.15: Initial Surface Water Absorption Rate for set B and uncracked samples.

The test was carried out for all sets (A, B, C, D) with or without adding bacterial agent ingredients, plus the control samples (B-1, C-1, D-1) as well as the reference samples (A-0, B-0, C-0, D-0). The results are illustrated in Figures 6.15-6.21. From the results shown in

these graphs, one can note that all the graphs have the same pattern, which indicated that the rate of ISAT results gradually decreased with time as the prisms became saturated with water.

The effect of the three bacterial ingredients (calcium lactate, yeast extract and calcium lactate + yeast extract) on the healing of the three bacteria (*B.cohnii, B.pseudofirmus,* and *B.halodurans*) are shown in these graphs. They give fluctuating results, which indicates that the healed material was poor in quality.

The healing material from set B-4 was the worst with respect to the other two bacteria when calcium lactate is used as an ingredient, as shown in Figure 6.15. set C-2 gave the worst healing material when yeast extract was used as an ingredient as shown in Figure 6.16.

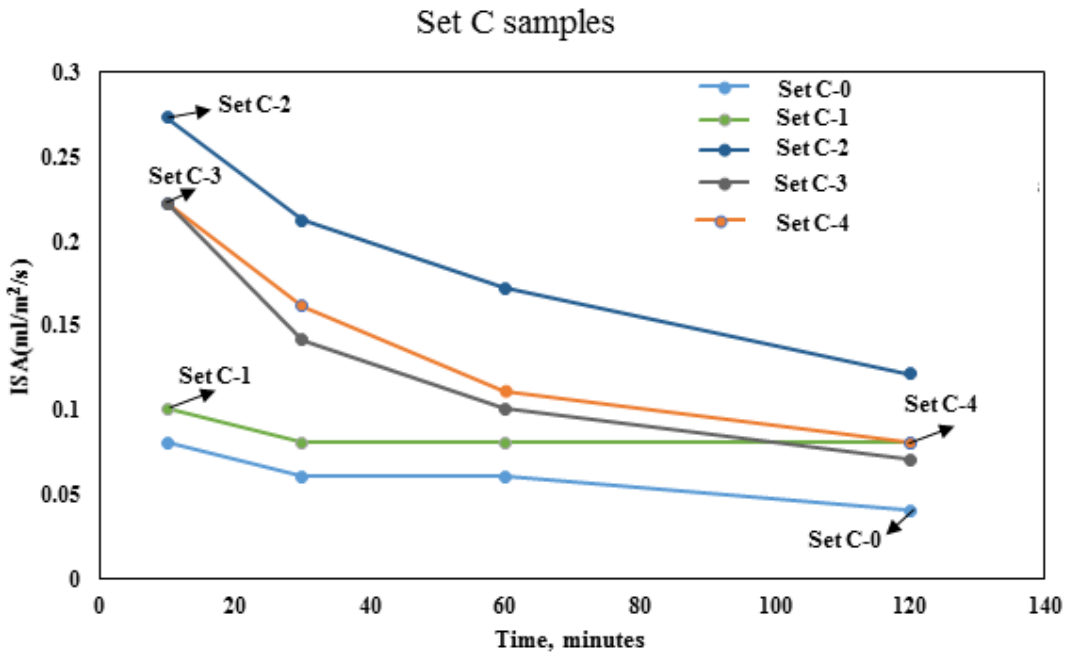


Figure 6.16: Initial Surface Water Absorption Rate for set C and uncracked samples.

The worst value recorded by ISAT in this study was given when both calcium lactate and yeast extract were used as an ingredient for each type of studied bacteria as shown in Figure 6.17. The yeast extract ingredient gave the worst results when it was used with both set C-2 and set B-2, as shown in Figures 6.18 and 6.19. It may be noted from Figure 6.20 that sets (D-2, D-3, and D-4) gave the highest value of the permeability coefficient for the three studied ingredients.

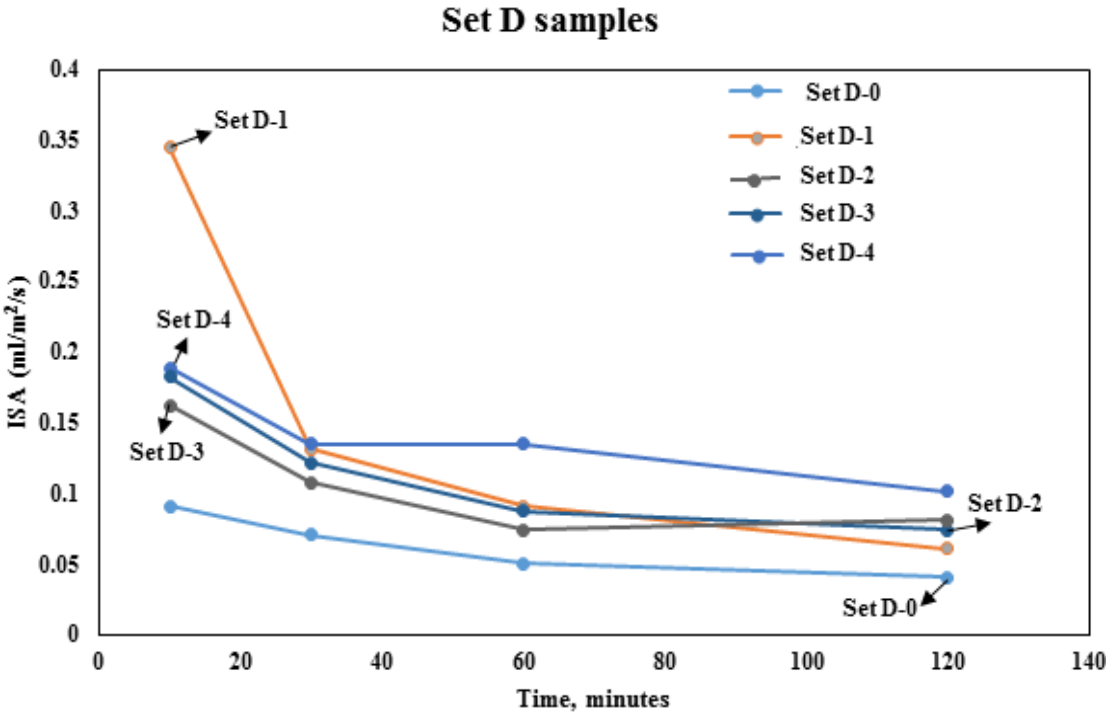


Figure 6.17: Initial Surface Water Absorption Rate for set D and uncracked samples.

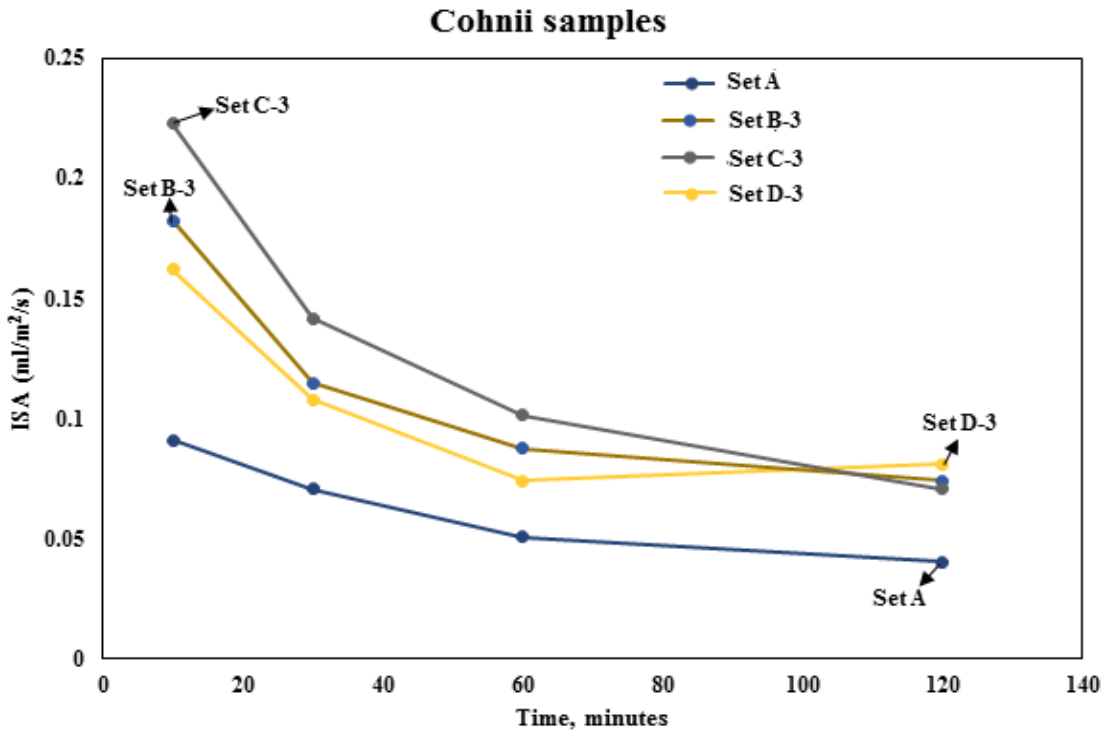


Figure 6.18: Initial Surface Water Absorption Rate for sets injected by *cohnii* bacteria and Sets A.

The autogenous healing sample (control) gave better results than the two stage bio-mortar results but the highest value of permeability was recorded in set D in the first 30 minutes.

However, the lowest value of permeability for set D was recorded for three sets (D-2, D-3, and D-4) after 30 minutes until the end of the test. Compression between the permeability results obtained from the reference sample (bio-cement mortar without cracks) and permeability results obtained from the normal cement mortar, as shown in Figures 6.21, gave us another indication that the cement mortar samples were extremely effected by the presence of bacterial agent ingredients in bio-cement mortar, which may be due to the interaction of the ingredient components with cement components. The poor performance of the two-stage bio-cement mortar may be due to the following:

- Poor distribution of the ingredients in the cement mortar matrix may lead to a shortage of bacterial food in the crack and its adjacent areas, which could result in a deficiency in healing materials.
- The number of ingredients used in bio-cement mortar was not enough to feed the bacteria; this lead to a deficiency in quality and amount of healing material, which will not be enough to seal the crack.
- The permeability of bio-cement mortar is not a simple function of its porosity, but depends on many factors such size, distribution, and continuity of the pores.

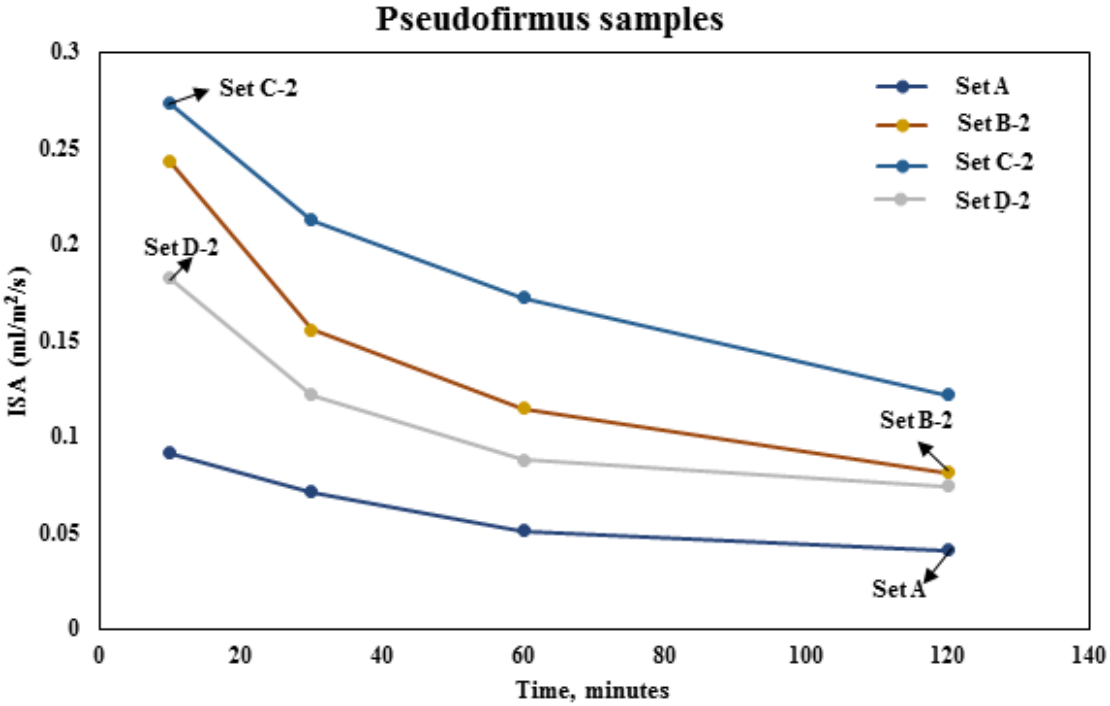


Figure 6.19: Initial Surface Water Absorption Rate for sets injected by *Pseudofirmus* bacteria and set A.

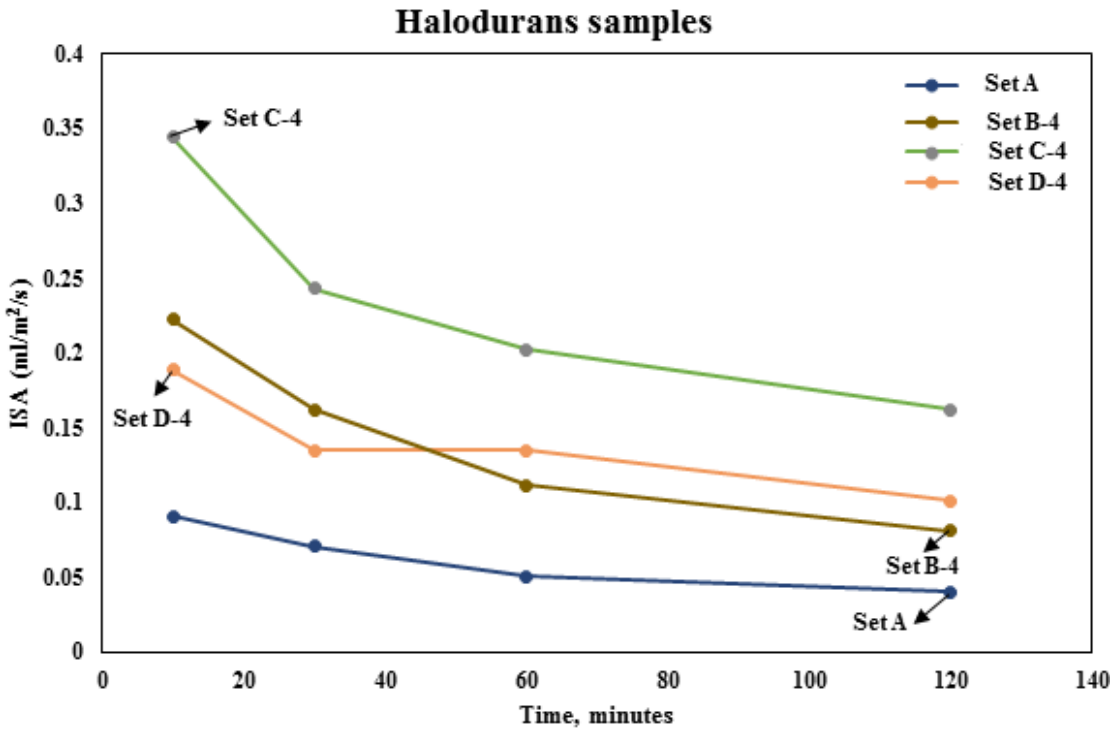


Figure 6.20: Initial Surface Water Absorption Rate for series injected by *halodourans* bacteria and series A.

- The chemical inter reaction of bacterial agent ingredients with the cement greatly affected the productivity and quality of the healing material, some ingredients may have given adverse reactions with cement.

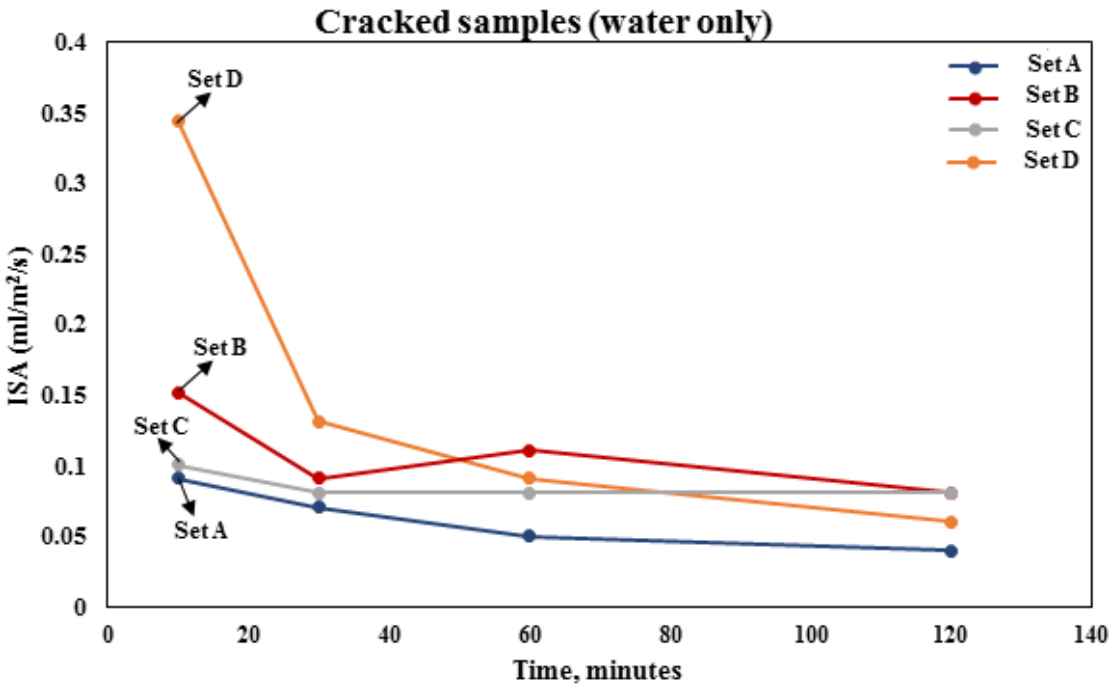


Figure 6.21: Initial Surface Water Absorption Rate for the normal cement mortar set and reference set.

All or some of the above reasons may affect the performance of two stage-bio mortar and as such, the conclusion of this section is that more experimental studies are needed in order to find the actual effect of each bacterial ingredient on the rate of hydration of the fresh bio-mortar.

6.3.2 Stage one part 2: Influence of increasing the ingredients of SHA on healing cracks in bio-mortar

In the previous section, it has been shown that sealing materials produced by all three *Bacillus* bacteria was not enough to seal the cracks as a result of one or all reasons mentioned above. Moreover, in section 7.3.1, SHA-1 (calcite producing bacterial self-healing agents) with *B.pseudofirmus* have demonstrated that calcium carbonate was precipitated in the repairing stage. In this stage, an investigation into the ability of the ingredients of SHA-1, given in Table 6.1, with the all three *Bacillus* bacteria to precipitate calcium carbonate as bio-mortar is presented. However, the number of ingredients in the previous section and also in SHA-1 are of a small amount if they are to be added to mortar directly and would be insufficient for bacterial growth.

The experimental set up and methodology of this set followed the same procedures of the previous experimental sets. This set was divided into two sets. The first set had 15 samples and SHA-1 was used in the proportion of 5% by mass of cement distributed in the whole samples. The second set used the same proportion but was applied into the cover layer of the sample only. The specimens were cured at 80% humidity and a temperature of 20℃ in a conditioning room for 24 hours, then immersed into water at 20℃ for 27 days. After 28 days of curing, all samples were taken out for flexural tests but the bio-mortar in the first set and the bio-layer mortar in the second set were dissolved in water.

6.3.3 Stage two: the effect of self-healing agent on mechanical properties for development and hardened mortar

6.3.3.1 Effect of SHA-1 on development of strength of mortar

The results showed that 2.2% of the medium group significantly reduced the strength development and the entire test results were close to zero as shown in Figure 6.22. 1.4% of the medium increased steadily until 14 days, then it fluctuated for a two-week period, but the results are still very low compared to control sample results.

On the other hand, both groups (control and 0.5% medium) had close results but by increasing the time, the 0.5% medium started lowering its compressive strength results.

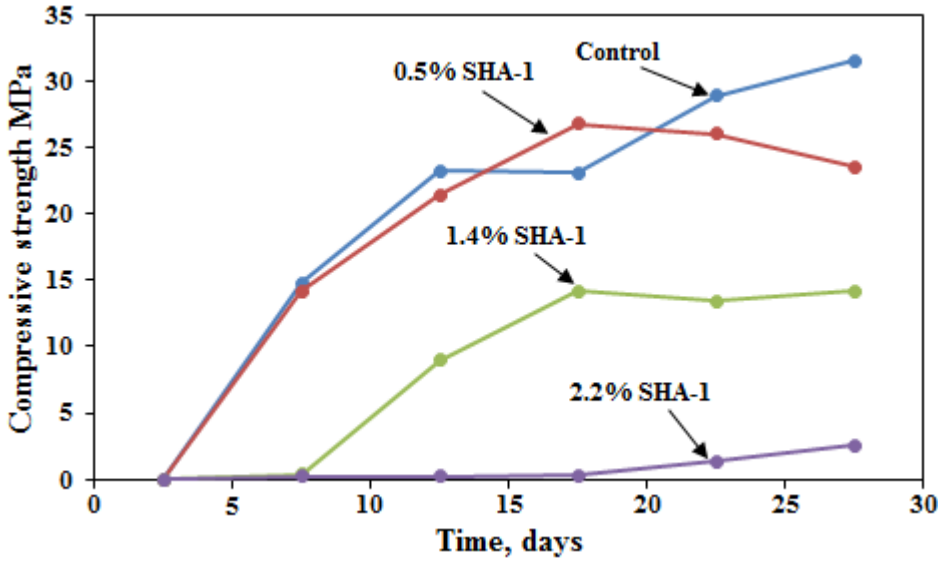


Figure 6.22: Effect of medium on compressive strength development.

6.3.3.2 Effect of medium on hardened mortar

Figure 6.23 shows the results of compressive strength tests for hardened mortar. It was concluded that compressive strength of mortar immersed in a medium decreased by around 25% to 30% compared to the control prisms. The decrease in compressive strength could be due to changes of cement matrix as a result of its reacting with one of the SHA-1 ingredients.

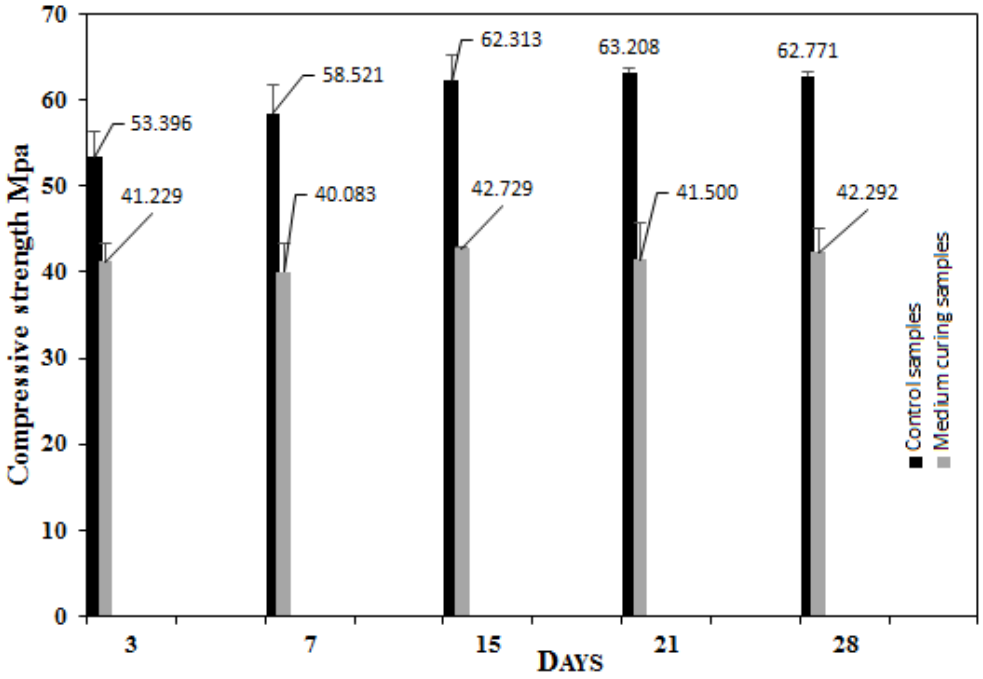


Figure 6.23: Effect of medium on compressive strength of mortar.

The impact of the chemical reaction between the cement matrix and the ingredients of SHA-1 can be understood by investigating the component of each ingredient, as presented in Table 6.1, or this decrease in compressive strength of mortar could have happened as a result of high concentration of some components such as yeast extract (12.12%), sodium citrate (18.65%) and calcium acetate (55.94%) inside the medium.

6.3.4 Stage three: the effect of bacterial self-healing agents on setting time, hydration and microstructures of cement matrix

From the result of the stage one and two, in which the bacterial self-healing agent ingredients effected the bio-cement mortar properties extremely due to unknown chemical reactions between the ingredients and mortar matrix and some other factors that also had minor effects. Therefore, the goal of this stage was to study the effect of each bacterial self-healing ingredient component on the properties of bio-cement mortar.

6.3.4.1 Effects of self-healing agents on setting of cement paste

Test results of the initial and final setting times are shown in Figure 6.24. The control paste had an initial setting time of approximately 250 minutes and a final setting time of round 350 minutes.

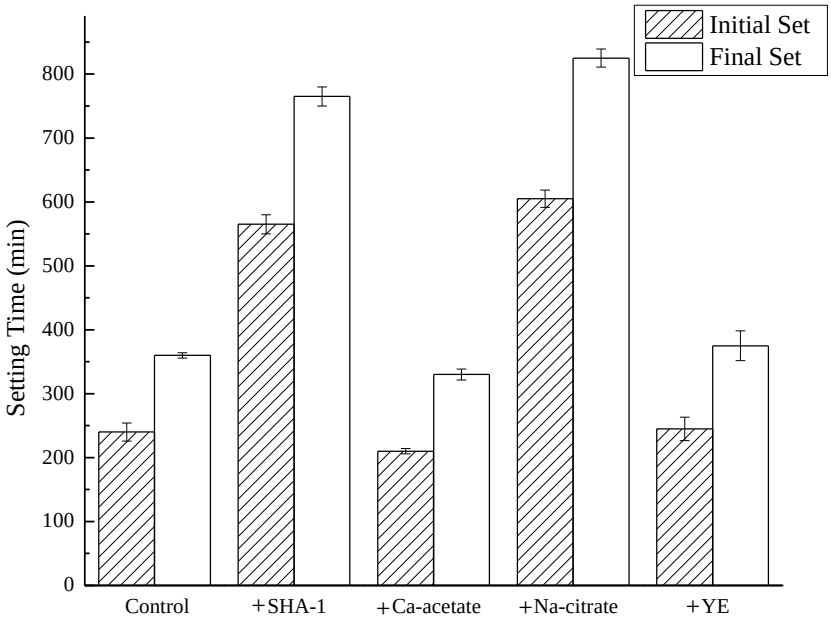


Figure 6.24: Effects of self-healing agents on setting of cement paste.

The addition of SHA-1 led to a significant retardation of setting – delaying the initial set by 300 minutes and the final setting by 400 minutes. Of the three major components, it can be seen that sodium citrate played the dominant role in slowing the setting of the cement. The retarding effect of sodium citrate was most probably due to the adsorption of citrate ions on cement particles, in a similar manner to how citric acid affects the hydration of concrete[411]. Neither, yeast extract nor calcium acetate had any significant effect on cement setting.

6.3.4.2 Hydration kinetics

The weigh of each ingredient of SHA-1 is presented in Table 6.1 and the results are given in Figure 6.25. It is clear from the curve that the induction period of the control paste (cement paste only) was considerably extended when SHA-1 was added to the cement by mass weigh. However, by increasing the SHA-1, a delay was observed. SHA-1 was greatly effecting the property of fresh cement paste by delaying the setting time for more than 20 hours and 30 hours when the SHA-1 made up 0.3% and 0.5% of the cement respectively. However, when 0.1% of the cement paste was the bacterial self-healing agent, cement hydration was slightly slowed.

It should also be noted that for all percentages of SHA-1, the rate of hydrations was lower up to its peak and then the rate of hydration increased drastically, except for 0.1% of SHA-1,

which gave a higher rate of hydration starting at the peak up to the initial setting time of the cement. The conclusion of these experimental results was that cement pastes with 0.3% and 0.5% SHA-1 cannot be used in bio-concrete/bio-mortar but percentages of 0.1% can be used but it will not provide enough food (bacterial self-healing agents) for the bacteria to generate self-healing precipitation.

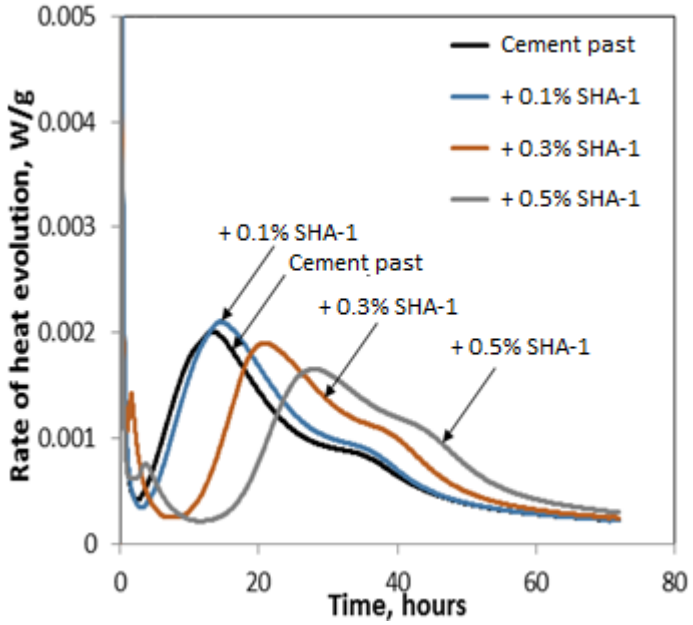


Figure 6.25: The effect of medium on rate of heat evolution.

a- The results are presented in Figures (6.26 to 6.31). From these Figures, one can classify the results into four groups: **Group one** includes yeast extract, calcium acetate, alanine, sodium glutamate, magnesium chloride. Their results are presented in Figure 6.26. It is clear this group increased the rate of hydration of cement and caused no significant retardation. However, yeast extract contained carbohydrates and sugar, which are known as good retarders, especially the induction period. The hydration kinetics was greatly influenced when the concentration of yeast extract exceeded 1% of the cement mass[409]. In this study, the percentage of yeast extract used was 0.036% to avoid the effect on hydration kinetics but at the same time, it was enough to germinate bacteria spores.

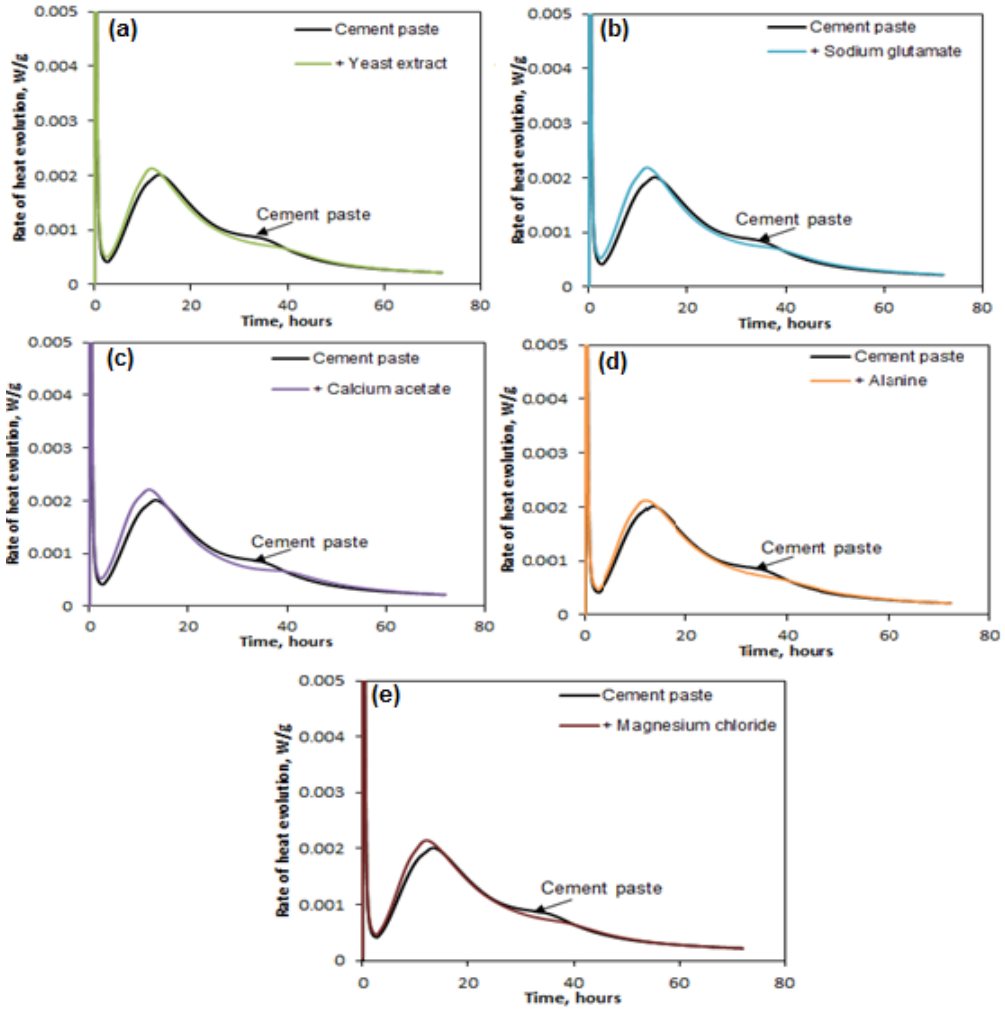


Figure 6.26: The effect (a) yeast extract, (b) sodium glutamate, (c) calcium acetate, (d) alanine, and (e) magnesium chloride on rate of heat evolution.

Group two included manganese sulfate monohydrate. The results of this group are presented in Figure 6.27; one can observe from this result that this group showed no change in the induction period, while the peak rate of hydration in dormant period compared to the reference/control paste sample was reduced. However, it caused no significant retardation. According to Nocuń-Wczelik et al.[412] hydration kinetics are greatly influenced when the concentration of manganese sulfate monohydrate exceeds 1% of cement mass. In this present study, the percentage of manganese sulfate monohydrate was 0.03%.

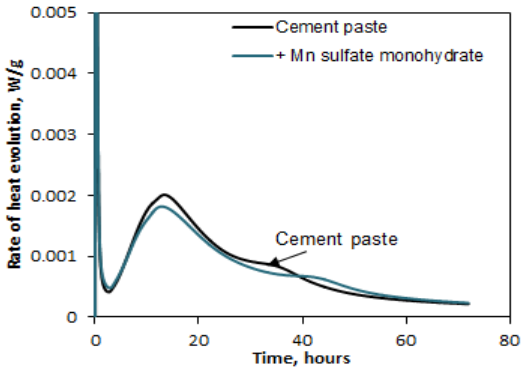


Figure 6.27: The effect of manganese sulfate monohydrate rate of heat evolution.

Group three included sodium chloride, inosine, and potassium hypophosphite and the results are presented in Figure 6.28. This group showed no effect on the rate of hydration during the hydration process.

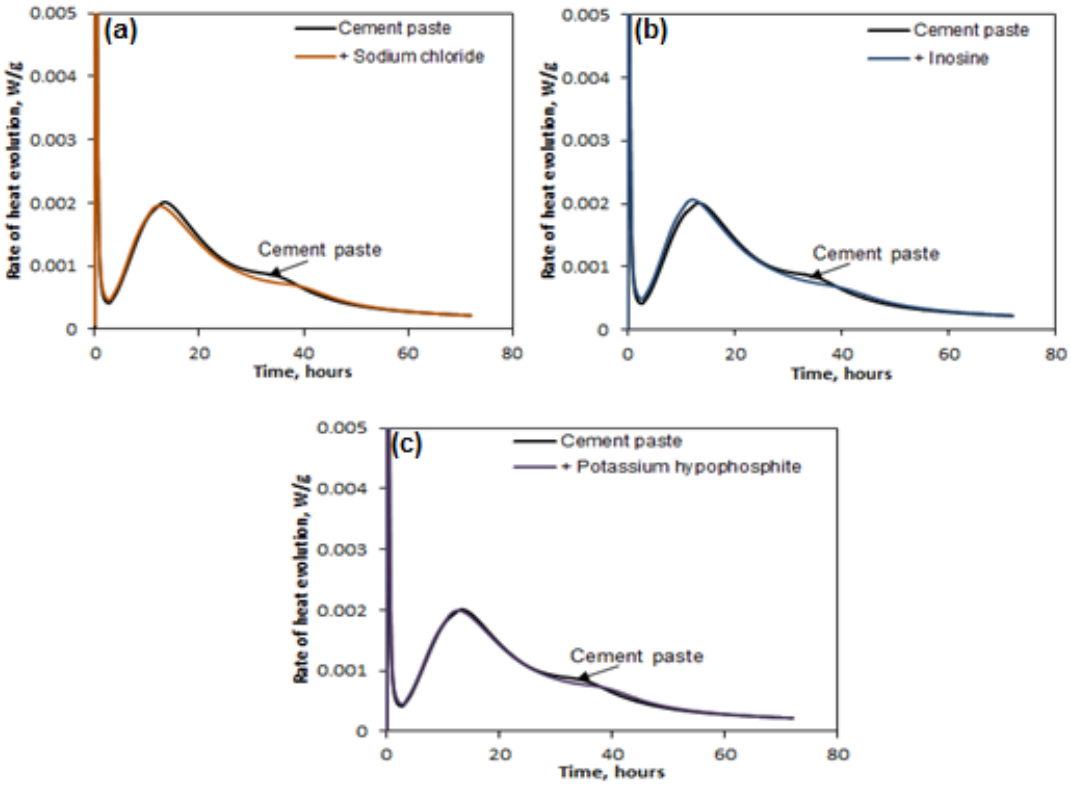


Figure 6.28: The effect of (a) sodium chloride, (b) inosine, and (c) potassium hypophosphite on rate of heat evolution.

Group four included sodium citrate and the results are presented in Figure 6.29. This group extremely effected the rate of hydration by delaying its chemical reactions for more than three, four or five times the initial and final setting time. It may be concluded that

sodium citrate was the component of most single significance but that it's use may also have led to a series of additional complex equations, resulting in far greater retardation than when used alone. As observed earlier, the retarding effect of sodium citrate was mostly due to the adsorption of citrate ions on the cement particles, which prevented access of water.

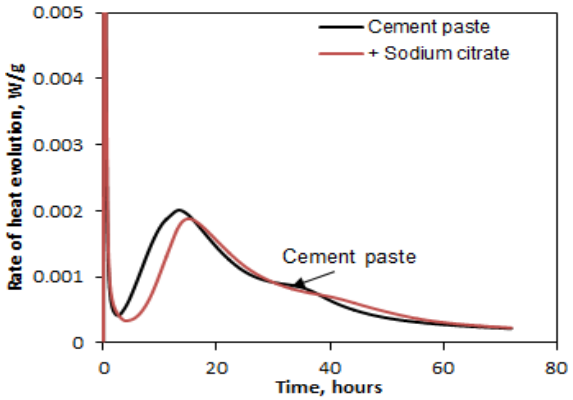


Figure 6.29: The effect of sodium citrate on rate of heat evolution.

b- From the investigation presented in 'a' of this section, one can conclude that the sodium citrate was the most effective item of the SHA-1 ingredients on concrete properties. In this section, the sodium citrate was investigated in order to reach the optimum percent of sodium citrate so that it has no effect on mortar/concrete. Decreasing each time, the sodium citrate percentages were taken from 0.3 up to 0.05 by 17%. The cement components are presented in Table 6.1. All samples were prepared, curved, and listed according to ASTM C1702[413]. The results are presented in Figure 6.30. It is clear from this graph that the optimum sodium citrate percentage, which had no effect to the rate of hydration of cement, was 0.05%.

c- The optimum percent of sodium citrate was 0.05%, which was used with all other ingredient values (0.1, 0.3, and 0.5%), as given in Table 6.1. The samples were prepared and the results are presented in Figure 6.31. From these results, one can conclude these modified SHA-1 ingredients with sodium citrate at the optimum percentage (0.05%) had no effect on concrete properties.

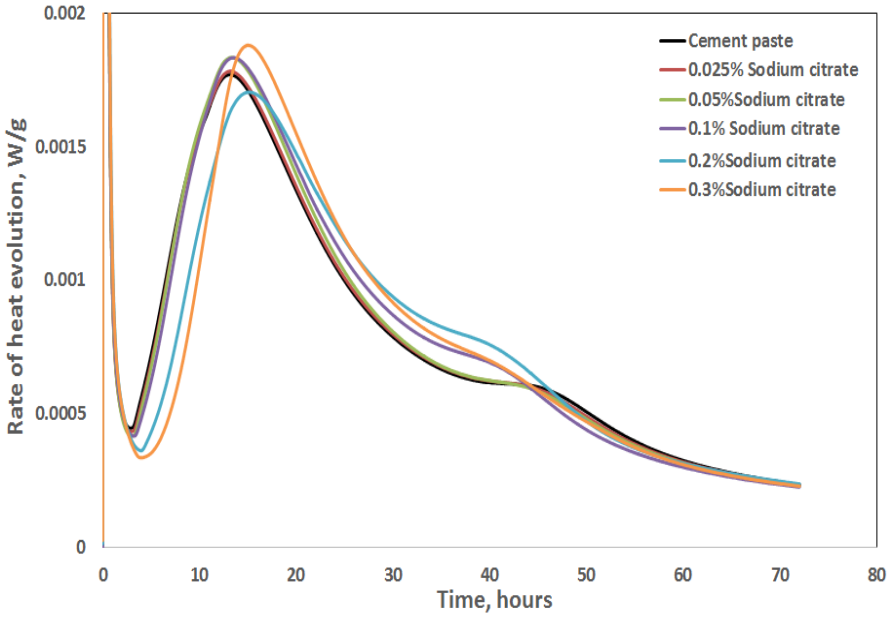


Figure 6.30: The effect of sodium citrate percentage on the rate of heat evolution.

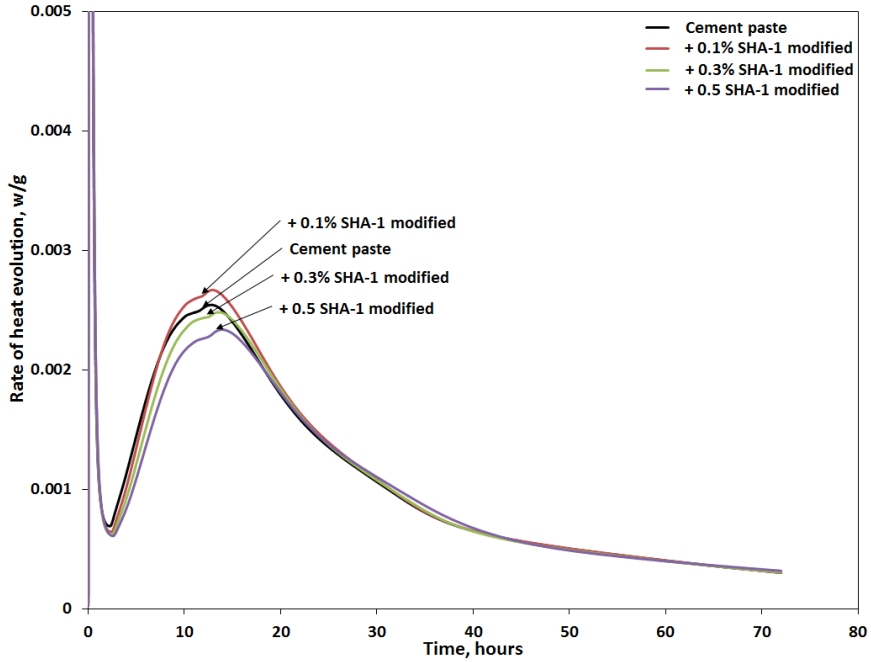


Figure 6.31: The effect of 0.05% sodium citrate with whole SHA-1 on the rate of heat evolution.

6.3.4.3 Microstructure of cement paste

The microstructure of the control mortar and that containing SHA-1 were observed at seven days (Figure 6.32) and 28 days (Figure 6.33). Each figure shows the microstructure at a magnification of x2000 and x5000 respectively. From the scan pictures, the similarity in

compressive strength of pure cement paste and cement paste with 0.5% SHA-1 was further confirmed as they had similar microtopographies, although there were some unknown crystals separated from the C-S-H structure (7d, 0.5%, ×2,000; 28d, 0.5%, ×5,000). The needles-like habit of ettringite was showed clearly in Figure 6.32 for the control paste sample (×2,000), while the needles became clear for cement paste with 0.5% SHA when the magnification increased to ×5,000. The number of ettringite needles decreased with the increased age of cement paste, as shown in Figure 6.33. However, the number of needles and shape in cement paste with 0.5% SHA remained the same. Therefore, the chemical compositions of the unknown materials require further investigation, but it is likely to be the precipitates of the soluble self-healing agents formed during drying.

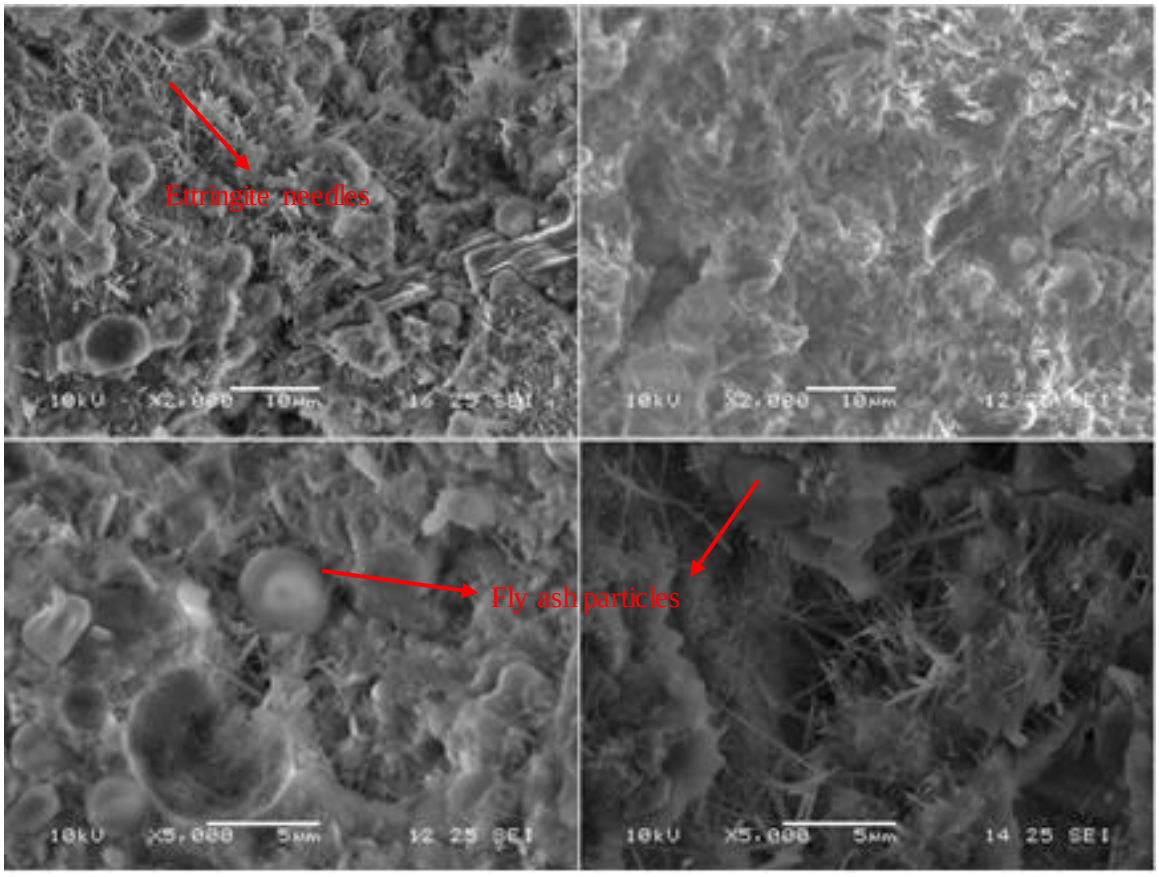


Figure 6.32: Effects of self-healing agents on seven day cement paste - microtopography at different magnifications (the left: pure cement paste; the right: cement paste with 0.5% SHA-1).

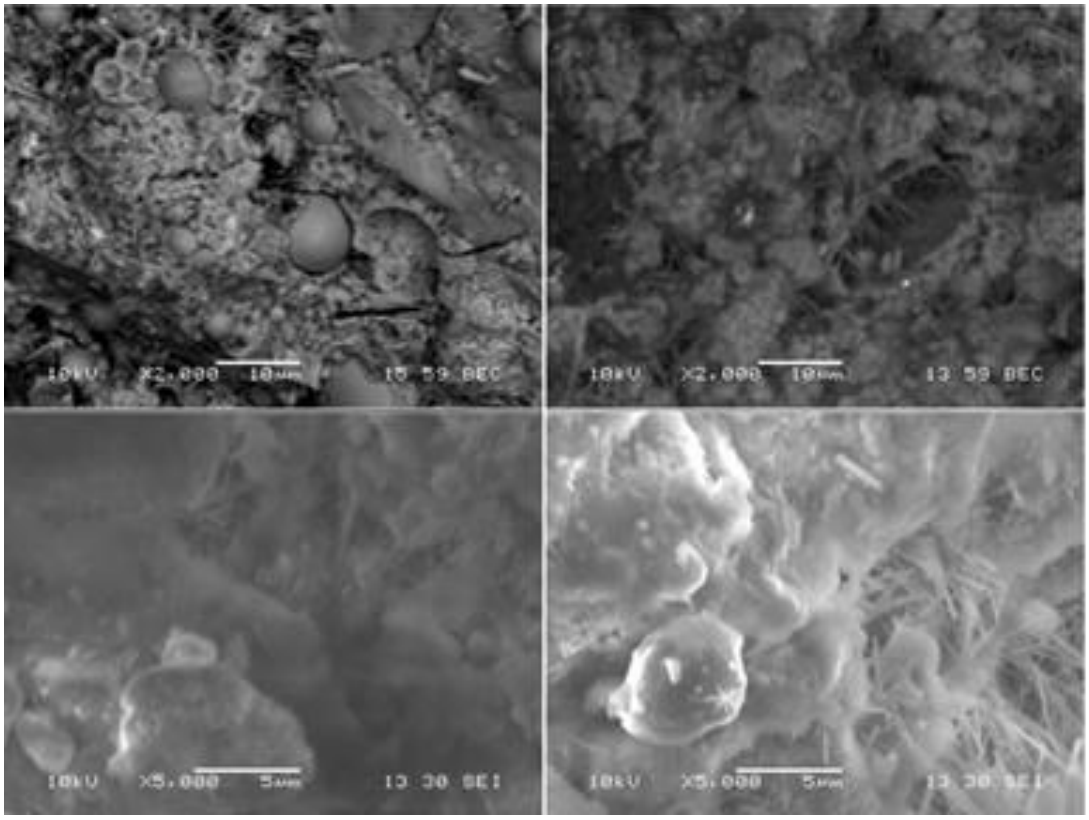


Figure 6.33: Effects of self-healing agents on 28 day cement paste - microtopography at different magnifications (the left: pure cement paste; the right: cement paste with 0.5% SHA-1).

6.4 Conclusion

The following main conclusions are derived from this chapter:

1- The results indicated that number and poor distribution of the ingredients used in the cement mortar matrix may lead to a shortage of bacterial food in the crack and its adjacent areas; this lead to a deficiency in quality and amount of healing material, which will not be enough to seal the crack.

2- The permeability of bio-cement mortar is not a simple function of its porosity, but depends on many factors such as size, distribution, and continuity of the pores. The chemical inter reaction of bacterial agent ingredients with the cement greatly affected the productivity and quality of the healing material, some ingredients may have given adverse reactions with cement.

3- The result of mixing SHA-1 in the proportion of 5% by mass of cement distributed in the whole samples. The second set used the same proportion but was applied into the cover

layer of the sample only. After 28 days of curing, all samples were taken out for testing but the bio-mortar in the first set and the bio-layer mortar in the second set were dissolved in water due to react some SHA-1 ingredients with cement matrix, that effect on hardened of mortar and a mechanical properties of cement.

4- The impact of ingredients of self-healing agents SHA-1 on the early-age properties of cement pastes and mortars were investigated.

   a. It was observed that the use of sodium citrate (when concentration exceeded 0.05% of cement mass) causes a retardation of the cement that is also likely responsible for reductions in compressive strength development. This ingredient should therefore not be used in self-healing medium.

   b. It was shown that the release of complex self-healing agents into early age concrete (at 0.5% by mass of cement) has no significant effect on setting, strength development, and microstructure of the concrete provided sodium citrate is not used.

Chapter 7
Sealing cracks in concrete by using *Bacillus* bacteria with SHA

7.1 Introduction

This chapter describes work to investigate the most efficient self-sealing technique produced by the three types of bacteria. This chapter covers six stages of investigation:

- Stage one; the efficiency of *B.pseudofirmus* with SHA-1 for sealing cracks is given.
- Stage two; the efficiency of each type of the three bacteria with SHA-2 for sealing cracks is presented, as is a comparison between the three types of bacteria, with respect to the sealing time and quantity of bacteria needed with SHA-2 in order to generate sealing.
- Stage three; the efficiency of *B.pseudofirmus* with SHA-3 for the sealing of cracks is studied.
- Stage four; investigate the ability of *bacillus Pseudofirmus* with SHA-3 for re-healing cracks in concrete.
- Stage five; study relation between duration of healing and quantities of calcite produced by *Pseudofirmus* with SHA-3.
- Stage six; the ability of the three types of bacteria to grow dynamically in the varying temperatures, the maximum crack width that could be sealed by these bacteria and efficiency of the three types of bacteria to seal cracks at different temperatures are given.

7.2 The experimental outline

The ingredients of cement mortar were mixed and a suitable percentage of alkali resistant fiber glass mesh was added to each mould as described in Chapter 4. The cracks were generated by exposing each mould to three-point bending test.

7.2.1 Stage one

This research was conducted to find the effect of *B.pseudofirmus* bacteria with a buffer and *B.pseudofirmus* with medium on the sealing of cracks in concrete. Four experimental sets with 12 samples were prepared. The mortar prisms were kept at 95% humidity and 20℃ in a conditioning room for 24 hours followed by six-day immersion in water at 20℃. The cracks were generated by using a flexural strength testing machine, after a seven-day curing period,

to carry out the three point bending test. *Bacillus pseudofirmus* is capable of precipitating a suitable amount of calcite, as shown by Sharma[414], and it was chosen for this set of experiments, which were in vitro experiments. The specimens were divided into four experimental sets as follow:

- Set 1-1 three control cracked specimens were injected with water only.
- Set 1-2 three cracked specimens were injected with SHA-1 (medium control).
- In addition, three cracked specimens were injected with bacteria in a buffer is presented as set 1-3.
- Three cracked specimens were injected with bacteria + calcite producing bacterial self-healing agents (SHA-1) is known as set 1-4, as presented in Chapter 4.

To reduce the movement of liquid out of the crack and into the body of the concrete through pores via capillary action, during the treatment period, the mortar prisms were placed to approximately half their depth in water in a tray. The quantity of solution injected into each specimen during the healing process of the specimens was taken at around 10 days. Each day the specimens were fed twice in the morning and afternoon. Sterile 3ml syringes (non-pyrogenic tipped needles) were used to accurately dispense the treatment directly into the cracks. Then, the mortar prisms were stored in an incubator at 30°C. The cracks' widths were measured by using a Leica microscope before and after healing, while the calcite within the cracks was observed and measured by SEM imaging. The crack widths of these set ranged between 0.33 mm and 0.40 mm.

7.2.2 Stage two

In this stage, five experimental sets, with 15 samples in total. The procedures of design and casting the mortars samples were followed the same procedures in stage one. The specimens were cured at 95% humidity and at a temperature of 20℃ in a conditioning room for 24 hours, then immersed into water at 20℃ for 13 days, then kept in a carbonation chamber for another 14 days. A three-point bending test was applied to each prism to create a crack at the middle of the samples. For all samples, the crack width was measured by using microscope with x5 magnification. The crack widths ranged between 0.3 and 0.58 mm. Following this, they were prepared as follows:

- Set 2-0 three reference specimens were placed to about half their depth in water for the duration of the experiment with no other treatment.
- Set 2-1 three control cracked specimens were injected with water only.
- Set 2-2 three cracked specimens were injected with SHA-2 (medium control).
- Set 2-3 three cracked specimens were injected with *B.pseudofirmus* and (SHA-2).
- Set 2-4 three cracked specimens were injected with *B.cohnii* and (SHA-2).
- Set 2-5 three cracked specimens were injected with *B.halodurans* and (SHA-2).

Prisms were placed in water at about half of their depth to minimise the movement of treatments laterally out of the crack by virtue of capillaries through the mortar matrix. The duration was 28 days and on each day the specimens were fed twice in 24 hours by sterile 3ml syringes that had tipped non-pyrogenic needles. These were used to accurately dispense the treatment directly into the cracks. The mortar blocks were then stored in an incubator at 30°C. Then after healing, the cracks were measured again by microscope. After 28 days of treatment the healing of these sets was examined by way of permeability tests, microscope SEM images and EDX analysis.

7.2.3 Stage three, and stage four

The third stage of this research was conducted to find the effect of the *B.pseudofirmus* bacteria with SHA-3 on accelerating the healing process.

Nine mortar specimens with a size of 40x40x160mm were prepared as previous stages. The mortar prisms were kept at 95% humidity and at 20℃ in a conditioning room for 24 hours, followed by 27 days of immersion in water at 20℃. A three-point bending test was applied to each prism to generate a crack at the middle of the samples. The samples were tested until failure according to the procedure described in BS EN 196-1[395]. The load during the test was applied to a face cast against alkali resistant fiber glass mesh face of the mould. For all samples, the crack width was measured using a linear variable differential transformer (LVDT) sensor as shown in Figure 7.1 and the crack width was increased by a speed of 0.025 mm/s until a crack width of 0.35 mm was reached. This can be seen in Table 7.1. The cracks were created in samples by subjecting them to a flexural strength testing machine after a 28-day curing period in order to create a single crack at the centre of the sample, after generating a 2 mm notch on their bottom side. The specimens were divided into four experimental sets as follow:

- Set 3-0 three reference specimens were placed to about half their depth in water for the duration of the experiment with no other treatment.
- Set 3-1 three control cracked specimens were injected with water only.
- Set 3-2 three cracked specimens were injected with SHA-3 (medium control).
- Set 3-3 three cracked specimens were injected with bacteria + SHA-3 (testing specimens).

Prisms were placed in water to about half of their depth to reduce the movement of liquid out of the crack and into the body of the concrete through pores via capillary action during the treatment period. Additionally, the mortar prisms were placed to approximately half their depth in water on plastic support trays. The duration was seven days and the specimens were fed once in 24 hours, directly into the cracks, by sterile 3ml syringes that had non-pyrogenic needles. The mortar blocks were then stored in an incubator at 30°C.

Table 7.1: LVDT measurement of crack width

Damage introduction	(t= 28 days)	temperature =19.4°C	humidity=40%
Specimen	Peak load (kN)	W1 (μm)	W2 (μm)
$[Set\ 3\text{-}1]_1$	2.48	356	140
$[Set\ 3\text{-}1]_2$	2.54	353	131
$[Set\ 3\text{-}1]_3$	2.15	360	126
$[Set\ 3\text{-}2]_1$	2.35	352	142
$[Set\ 3\text{-}2]_2$	2.68	354	131
$[Set\ 3\text{-}2]_3$	2.25	350	134
$[Set\ 3\text{-}3]_1$	2.38	350	140
$[Set\ 3\text{-}3]_2$	2.54	352	130
$[Set\ 3\text{-}3]_3$	2.58	350	131

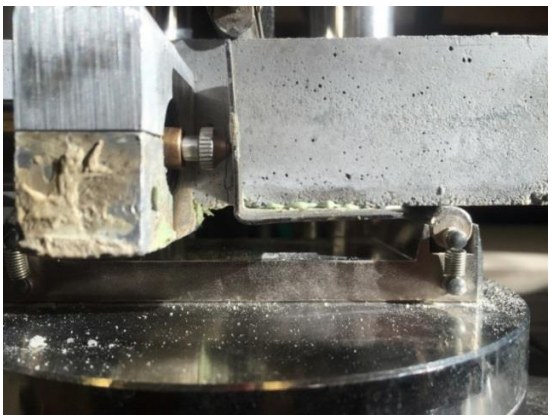


Figure 7.1: 3-point-bending test with LVDT sensor.

These sets were examined after healing period by using permeability tests (ISAT, capillary water absorption), microscope SEM images and EDX analysis. The same procedures were followed in fourth stage. Subsequently, the study has been reported in Sharma et al.[415].

7.2.4 Stage five

A 12 mortar specimens were prepared, casted, cured, a three point bending test, and treated same as stage three. For all samples, the crack width was measured using a linear variable differential transformer (LVDT) sensor until a crack width of 0.35 mm was reached. This can be seen in Table 7.2. The specimens were divided into five experimental sets as follow:

- Set 5-0, three reference specimens were placed to about half their depth in water for the duration of the experiment with no other treatment.
- Set 5-1, three control specimens were injected with water only.
- Set 5-2, three cracked specimens were injected with bacteria + SHA-3 once.
- Set 5-3, three cracked specimens were injected with bacteria + SHA-3 on first, third, fifth and seventh days.
- Set 5-4, three cracked specimens were injected with bacteria + SHA-3 (testing specimens) for seven days.

Then after healing, the cracks were measured again by microscope.

Table 7.2: LVDT measurement of crack width

Damage introduction	(t= 28 days)	temperature =19.4°C	humidity=40%
Specimen	Peak load (kN)	W1 (μm)	W2 (μm)
$[Set\ 5\text{-}1]_1$	3.95	425	284
$[Set\ 5\text{-}1]_2$	3.25	355	187
$[Set\ 5\text{-}1]_3$	2.97	398	191
$[Set\ 5\text{-}2]_1$	3.22	354	179
$[Set\ 5\text{-}2]_2$	3.19	350	181
$[Set\ 5\text{-}2]_3$	3.25	345	184
$[Set\ 5\text{-}3]_1$	3.40	349	151
$[Set\ 5\text{-}3]_2$	2.99	349	144
$[Set\ 5\text{-}3]_3$	2.90	349	156
$[Set\ 5\text{-}4]_1$	3.07	349	145
$[Set\ 5\text{-}4]_2$	2.98	353	171
$[Set\ 5\text{-}4]_3$	3.06	350	136

7.2.5 Stage six

The sixth stage was divided to two groups. Each one had five experimental sets, with 15 samples in total (treated for 360) days. The immersing of samples into water for long periods of time cause corrosion in alkali resistant fiber glass mesh. Therefore, Carbon Fiber Reinforced Polymer (CFRP) was applied to all samples at the tension zone, and a three-point

bending test was applied to each prism to create a crack at the middle of the samples, as shown in Figure 7.2. For all samples the crack width was controlled by using plastic spacers with 0.4 mm thickness and the minimum crack widths was 0.4 mm at the edges of each sample. Each group of mortar blocks was divided into five experimental sets plus reference set as follows:

- Set 6-0, three reference specimens were placed to about half their depth in water for the duration of the experiment with no other treatment.
- Set 6-1, three cracked specimens as control were injected with water only.
- Set 6-2, three cracked specimens were injected with SHA-2 (medium control set).
- Set 6-3, three cracked specimens were injected with *B.pseudofirmus* + SHA-2.
- Set 6-4, three cracked specimens were injected with *B.cohnii* + SHA-2.
- Set 6-5, three cracked specimens were injected with *B. halodurans* + SHA-2.

After 28 days of treatment the healing of these sets was observed by using microscope with x5 magnification.

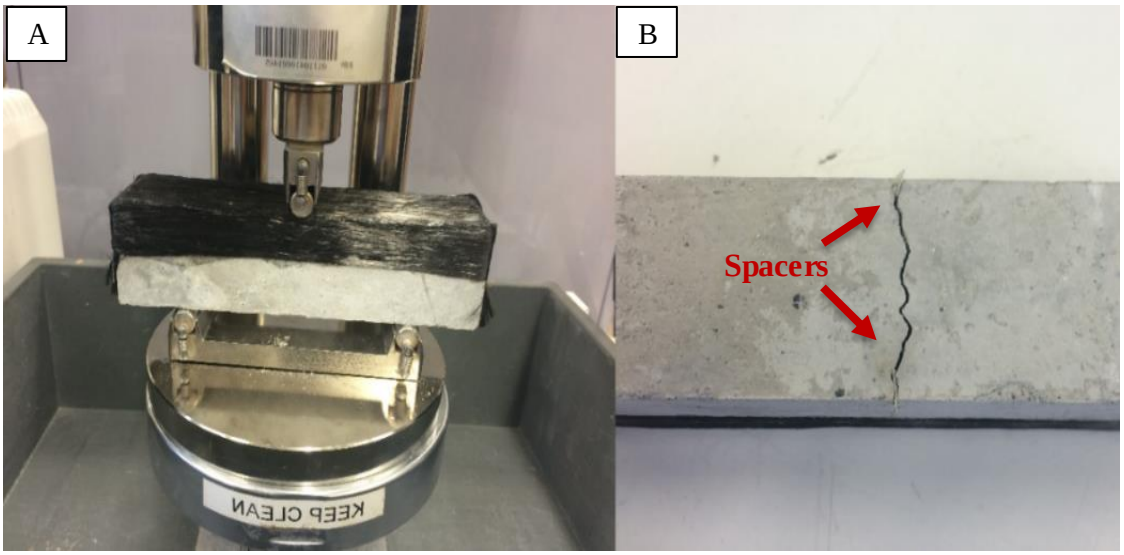


Figure 7.2: (A) Flexural strength test, (B) position of spacers into crack.

Prisms were placed in water to about half of their depth to minimise the movement of treatments laterally out of the crack by virtue of capillaries through the mortar matrix. The duration was 28 days and each the specimen was fed twice in 24 hours by sterile 3ml syringes that were tipped with non-pyrogenic needles. These were used to accurately dispense the treatment directly into the cracks. The first group of mortar blocks was then stored in an

incubator at 40°C and the second group of mortar blocks were then stored in a curing room at 20°C and 95% humidity. Then after healing, the cracks were measured again by using microscope.

7.3 Results and discussion

7.3.1 Study the ability of *Bacillus pseudofirmus* for healing cracks in concrete (stage one)

Figures 7.3 and 7.4 present the results of the set 1-1. Figure 7.3A shows the cracks at tension zone for the control samples. The width of the crack was 0.32 mm before the healing process, and the healing across the crack was autogenous healing, and the crack was not completely healed, as shown in Figure 7.3B. The healing in crack occurred due to the autogenous process that as results of the progress of incomplete hydration in cement in mortar samples. However, the amount of anhydrate particles in the cement mortar was not enough for this type of healing in this experiment. According to BS8007 'the design of concrete structures for retaining aqueous liquids', it is specified that a crack width of up to 0.2 mm will sealed by autogenous methods within 28 days, and up to 0.1 mm will be sealed within 14 days. The SEM scan analysis of the material in the sealed crack showed that the formation of the main shape of the set 1-1 sample surface structure was mainly hexagonal flakes, as shown in Figure 7.4. The crystallisation of the hexagon is the main product of hydration in almost all types of cement[416].

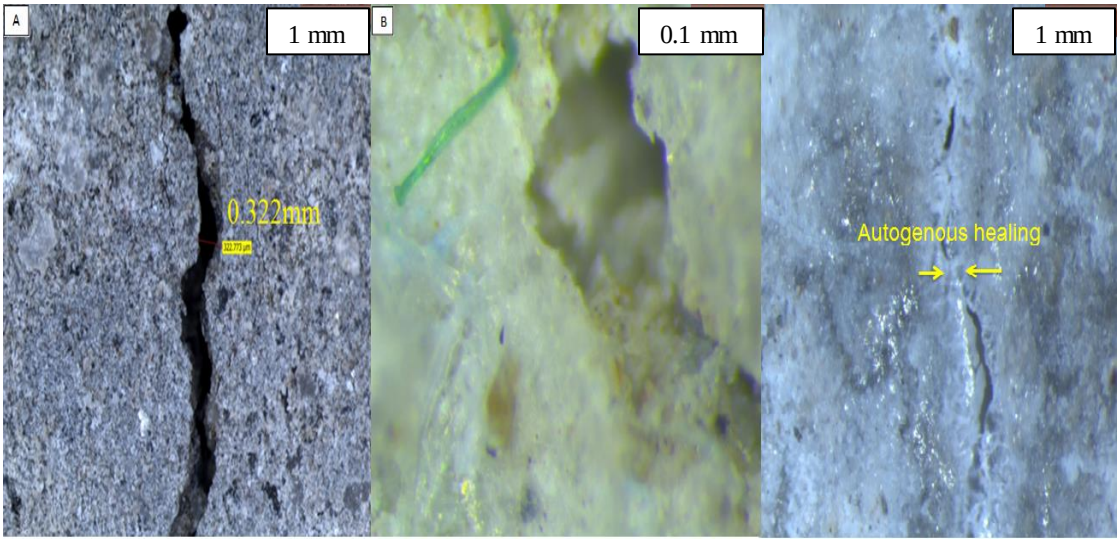


Figure 7.3: Set 1-1 at tension zone. A: before; B: after.

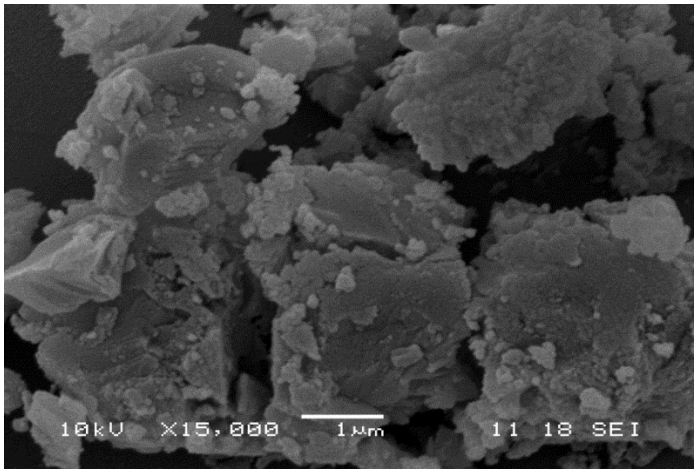


Figure 7.4: SEM image of set 1-1.

Figures 7.5 and 7.6 present the results of set 1-2. Figure 7.5A shows the cracks at tension zone for the SHA-1 samples. The crack width was measured by using a Leica microscope before healing and found 0.38mm. Figure 7.5B shows the sealing of the crack by low quality materials, which could be due to inorganic components in mineral SHA-1, or could be due to one of its components that may react with the cement matrix, or could be a combination of inorganic components precipitation and autogenous healing in the sample. Figure 7.6 demonstrates a small amount of calcium carbonate owing to inorganic components in SHA-1 (the mineral medium). It is clear a small amount of crystals of calcium carbonate was obtained in the presence of autogenous process and the rest was inorganic materials, which came about as a result of precipitation component materials from the injected SHA-1.

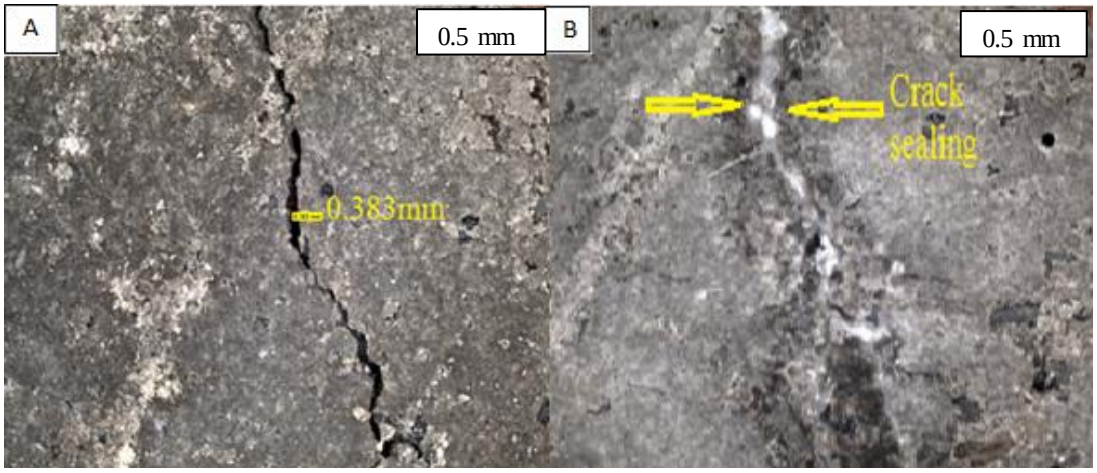


Figure 7.5: Set 1-2 at tension zone. A: before; B: after.

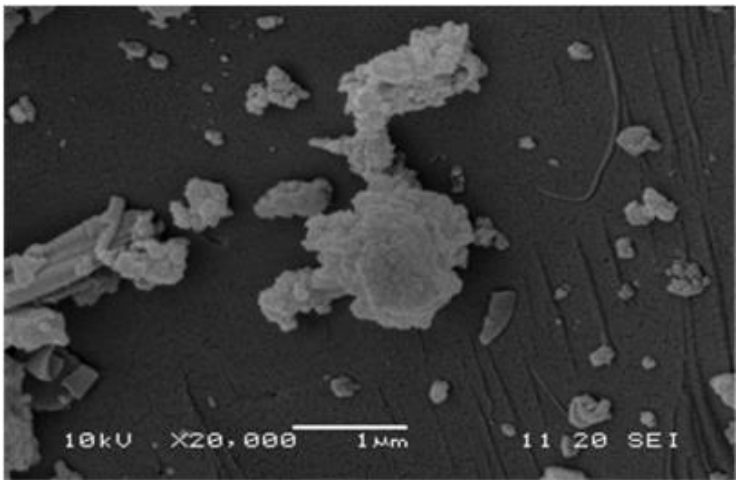


Figure 7.6: SEM image of set 1-2.

Figures 7.7 and 7.8 present the results of set 1-3. Figure 7.7A shows the cracks at tension zone for bacterial samples and the width of the crack was 0.38mm before the healing process There is no healing shown in the crack by using bacteria with a buffer, just small places, as shown in Figure 7.7B. The small places healed could have occurred due to the autogenous processes that happen as results of the progress of incomplete hydration of the cement in mortar samples, or could have happened due to the complex structure of the adhering of colonies or vegetative cells of bacteria. The SEM scan shows the material that sealed the crack was bio-film in some places in crack, which could be as result of bacterial cells sticking to each other on a surface to build-up a network between each other, together with small particulars of calcium carbonate obtained in the presence of the autogenous process with biofilm, as shown in Figure 7.8.

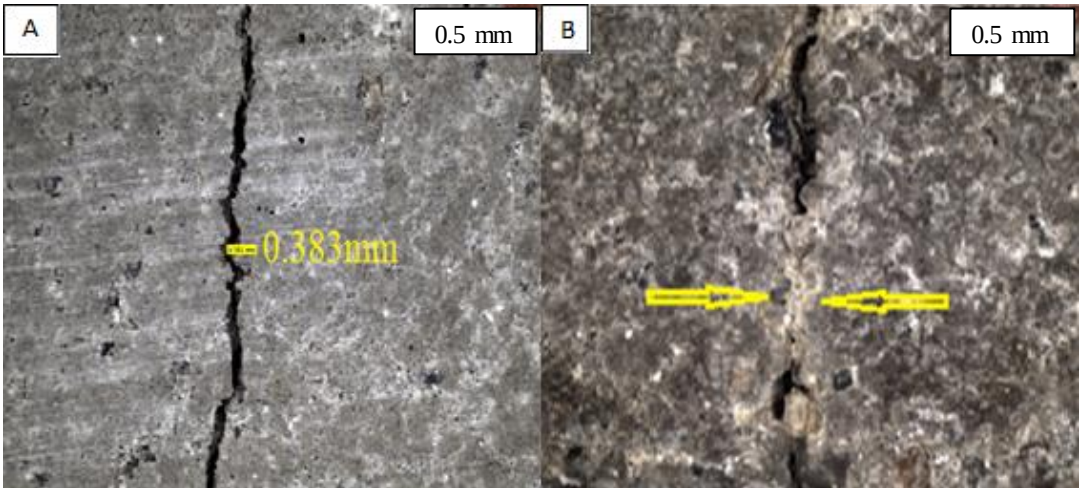


Figure 7.7: Set 1-3 at tension zone. A: before; B: after.

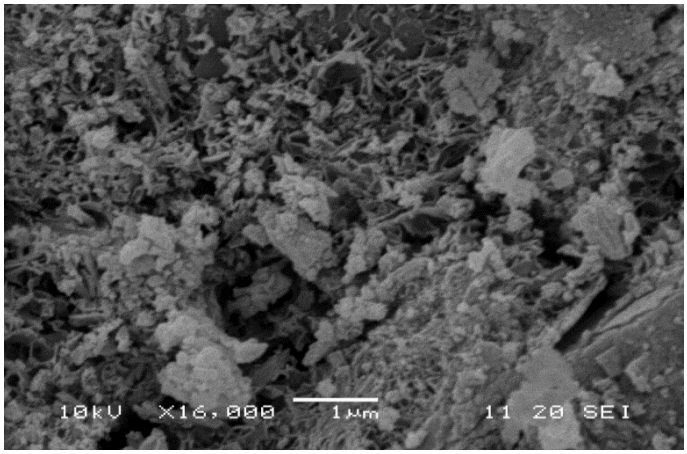


Figure 7.8: SEM image of set 1-3.

Figures 7.9 and 7.10 present the results of set 1-4. Figure 7.9A shows the cracks at the tension zone after the samples had been cured in water for seven days, and also it shows the width of the crack (0.33 mm) before the healing process, which was measured by the microscope. Figure 7.9B shows the healing of the crack of the samples injected with a suspension of SHA-1 inoculated with *B.pseudofirmus*. Figure 7.10 presented the presence of a crystal layer through the entire crack. The SEM images showed crystalline $CaCO_3$ in two main shapes. One of these shapes is needle-like and the other is lamellar rhombohedra[416].

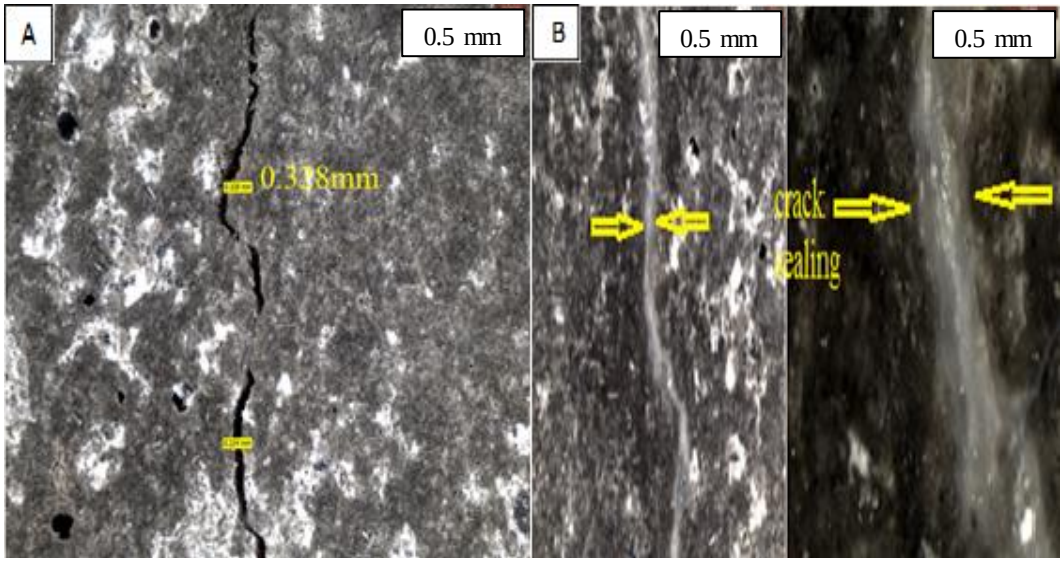


Figure 7.9: Set 1-4 at tension zone. A: before; B: after.

The observation of $CaCO_3$ precipitation based on bacteria was only on crack surfaces of samples, which means its formation was related to bacterial activity.

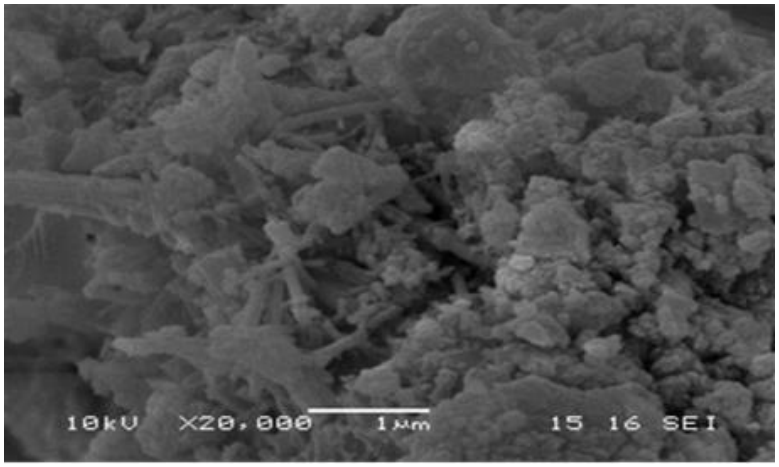


Figure 7.10: SEM image of set 1-4.

7.3.1.1 Summary

To summarize:

1- *B.pseudofirmus* with SHA-1 has potential for sealing cracks in concrete by producing calcite.
2- The precipitation from SHA-1 is an inorganic material due to the depositing of some of its ingredients within the crack.
3- *B.pseudofirmus* with a buffer could not heal cracks by calcium carbonate due to the lack of resources, as explicated in the following equation:

$$CaC_4H_6O_4 + 4O_2 \rightarrow CaCO_3 + 3CO_2 + 3H_2O$$

4- The sealing in control samples happened due to unhydrate cement particles during mixing time. This process is called autogenous healing, and this type of healing could heal crack widths up to 0.2 mm.

One can conclude from this study that the bacteria type *B.pseudofirmus* has the ability to produce enough calcium carbonate if it gets enough SHA. Therefore, it can be used to seal the cracks in mortar and concrete. Further studies in the next sections will demonstration the ability of three types of *bacillus* to seal cracks in mortar samples.

7.3.2 Comparison between the efficiency of three types of bacteria for healing cracks (stage two)

7.3.2.1 The ability of bacteria on sealing cracks in mortar

The specimens were divided into five experimental sets, set 2-1 was the control samples, the crack width was 0.32 mm and they were injected with water only as shown in Figure 7.11. From Figure 7.11A one can observe that the crack was healed by white material, while the Figure 7.11B and Figure 7.11C were clearly healed and it was clearly healed by one of the autogenous healing methods.

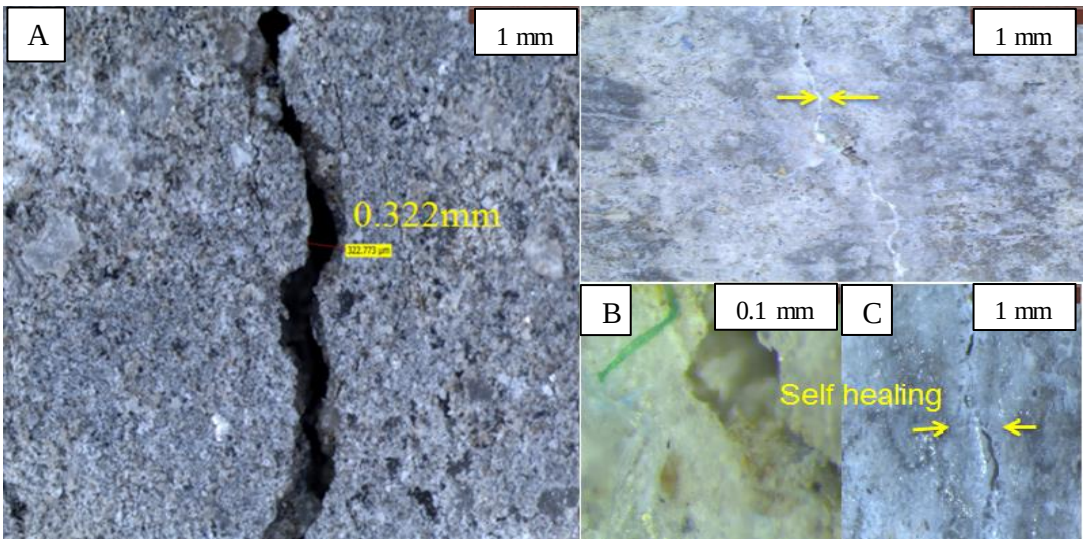


Figure 7.11: Set 2-1 at tension zone. A: before; B-C: after.

The set 2-2 was the SHA-2 control medium. The crack width was 0.53mm and they were injected with SHA-2 only, as shown in Figure 7.12A The crack was completely sealed with SHA-2, as shown in Figure 7.12B, with low quality material as shown in Figure 7.12C, which could be due to inorganic components in SHA-2 (mineral medium) or could be due to one of its components reacting with the cement matrix.

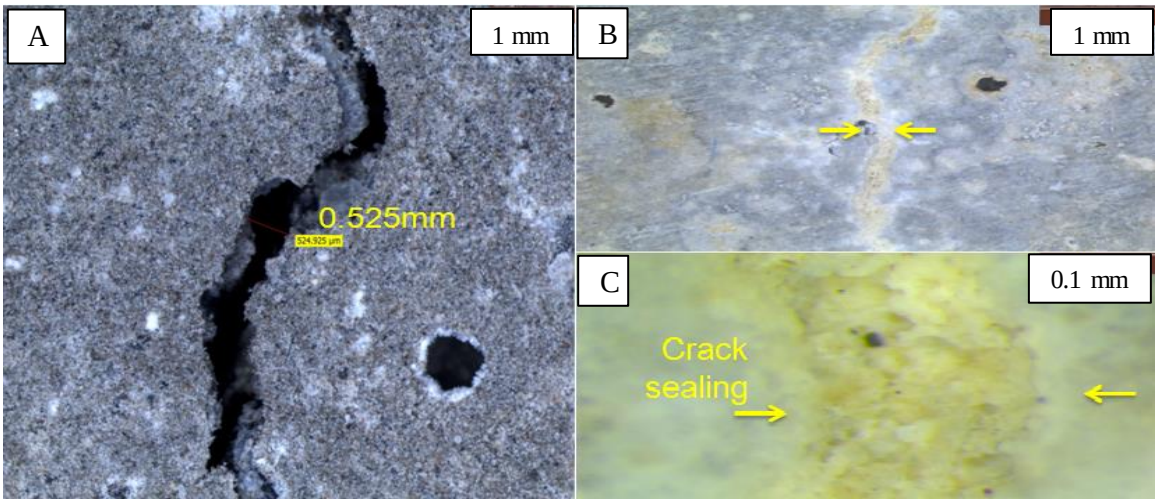


Figure 7.12: Set 2-2 2 at tension zone. A: before; B-C: after.

The set 2-3 was injected with *B.pseudofirmus* + SHA-2 (testing samples), as shown in Figure 7.13. A microscope was used to measure the width of the crack before healing and was found to be 0.50 mm, as shown in Figure 7.13A. The duration of treatment was 28 days and *B.pseudofirmus* with SHA-2 produced sealing materials have an orange-like colour, which sealed the opening of cracks slowly, at both crack faces. By the end of sealing time the crack disappeared, as shown in Figure 7.13B. The healed sealing material generated by *B.pseudofirmus* shows a significant difference between the amount of sealing material produced, compared to the sealed materials produced by SHA-2 (medium control set) or autogenous healing (control set). Microscopic examination showed the orange-like coloured material healed by the *B.pseudofirmus* set, compared to the other sets. The orange-like coloured material sealed the whole crack by 'crystal-resembling' materials, as shown in Figure 7.13C.

The set 2-4 was *B.cohnii* + SHA-2 and the crack width was 0.37 mm, as shown in Figure 7.14A. Three samples were injected with *B.cohnii* + SHA-2. The duration of treatment was 28 days and on each day the specimens were fed twice - in the morning and afternoon. The bacteria with SHA-2 produced sealing materials, which sealed the opening cracks slowly, at both crack faces, and the crack, by the end of sealing time, disappeared. This is shown in Figures 7.14B.

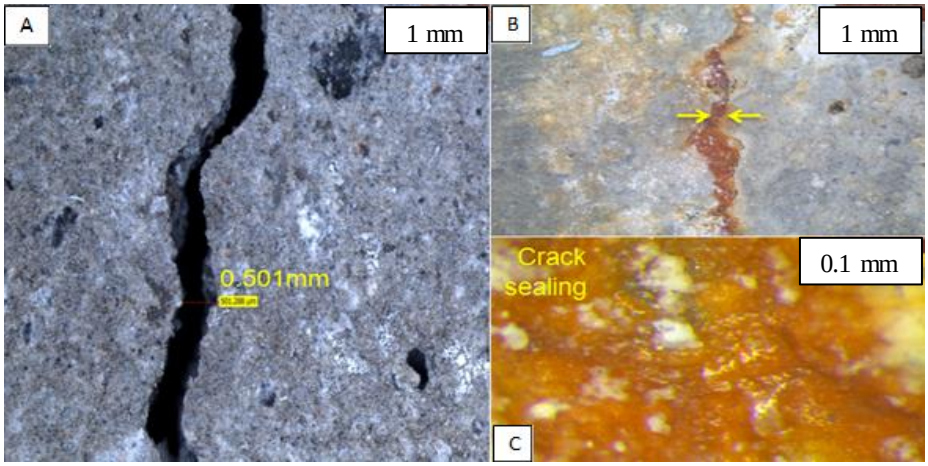


Figure 7.13: Set 2-3 at tension zone. A: before; B-C: after.

The sealing material shows a significant difference between the amounts of sealing material produced compared to those sealed materials induced by SHA-2 or autogenous healing, which happened to the set 2-1. Microscopic examination showed high quality materials sealing the cracks for set 2-4, compared to the set 2-1 and set 2-2, as shown in Figure 7.14C.

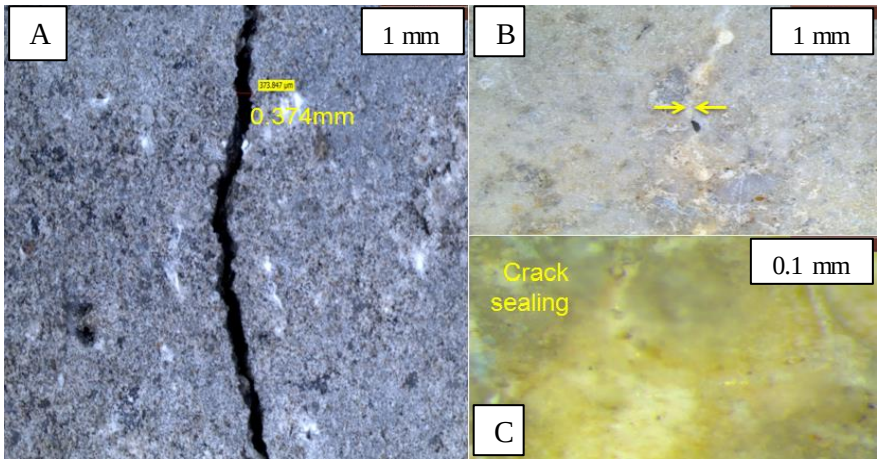


Figure 7.14: Set 2-4 at tension zone. A: before; B-C: after.

The set 2-5 was *B.halodurans*+ SHA-2. The width of cracks before healing was 0.53 mm wide, as shown in Figure 7.15A. Figure 7.15B shows the sealing of the crack by a dark red-yellow material. This was due to the injection of a suspension of SHA-2 inoculated with *B.halodurans* after 28 days of treatment. It is clear from Figure 7.15C that the dark red-yellow material that sealed the crack is a crystal material, compared to both the set 2-1 and set 2-2.

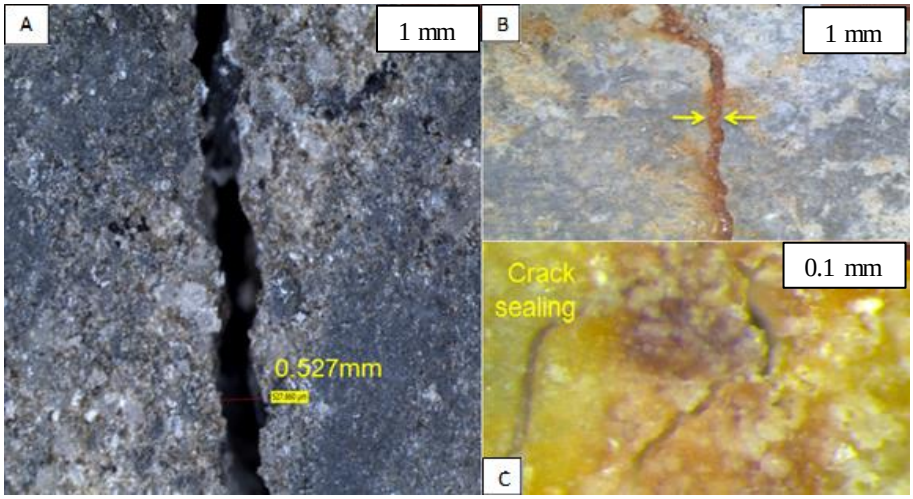


Figure 7.15: Set 2-5 at tension zone. A: before; B-C: after.

The summary of experimental data and observed results of the five sets is given in Table 7.3.

Table 7.3: The summary of the experimental data and observed results of stage two

Type of sample / Action taken and observed	Set 2-1	Set 2-2	Set 2-3	Set 2-4	Set 2-5
Crack width	0.32mm	0.53mm	0.53 mm	0.37mm	0.50mm
pH value	10	10	10	10	10
Temperature	22°C	30°C	30°C	30°C	30°C
Start of healing	After 3 days	After 19 days	After 4 days	After 13 days	After 19 days
Finishing of healing	After 9 days	After 27 days	After 9 days	After 19 days	After 25 days
Finishing of experiment	After 28 days	After 28 days	After 28 days	After 28 days	After 28 days
Type of healing	Autogenous	Sealing cracks	Sealing cracks	Sealing cracks	Sealing cracks
Remark	Autogenous healing materials.	Precipitation of an inorganic component or its components reacted with the cement matrix.	Precipitate had crystal shape and was of non-permeable materials.	Precipitate had crystal shape and was of non-permeable materials.	Precipitate had crystal shape and was of non-permeable materials.

7.3.2.2 Initial surface absorption test (ISAT)

The initial surface absorption test (ISAT) procedure was carried out in accordance with BS 1881-5:1970[401]. The aim of this test was to compare the efficiency of crack filling material before and after treatment for mortar with 0.5w/c, as shown in Figure 7.16. The graph of ISAT test indicates that the effecting of set 2-3 at 10 minutes was best than set 2-0. This means that the set 2-3 had the ability to increase durability of mortar by reducing the pores size in samples to around 25% of the set 2-0. Further, the set 2-4 reduced the absorption by around 8% compared to the set 2-0. While the set 2-5 was close to the set 2-0, it exceeded the set 2-0 about 8% only. However, from 30 minutes until the end of the test both sets (2-3 and 2-4) effectively reduced the ISAT and became close to the set 2-0, while the set 2-1 after it had been cracked was out of range. The graph also shows that the ISAT rate for all sets decreased with time. The rate of ISAT gradually decreased with time as prism became saturated with water. It is also clear from Figure 7.16 that the efficiency of the sealing material that was produced by the three types of bacteria are of a high quality and these materials have the lowest ISAT rate, compared with the set 2-1 and the set 2-2.

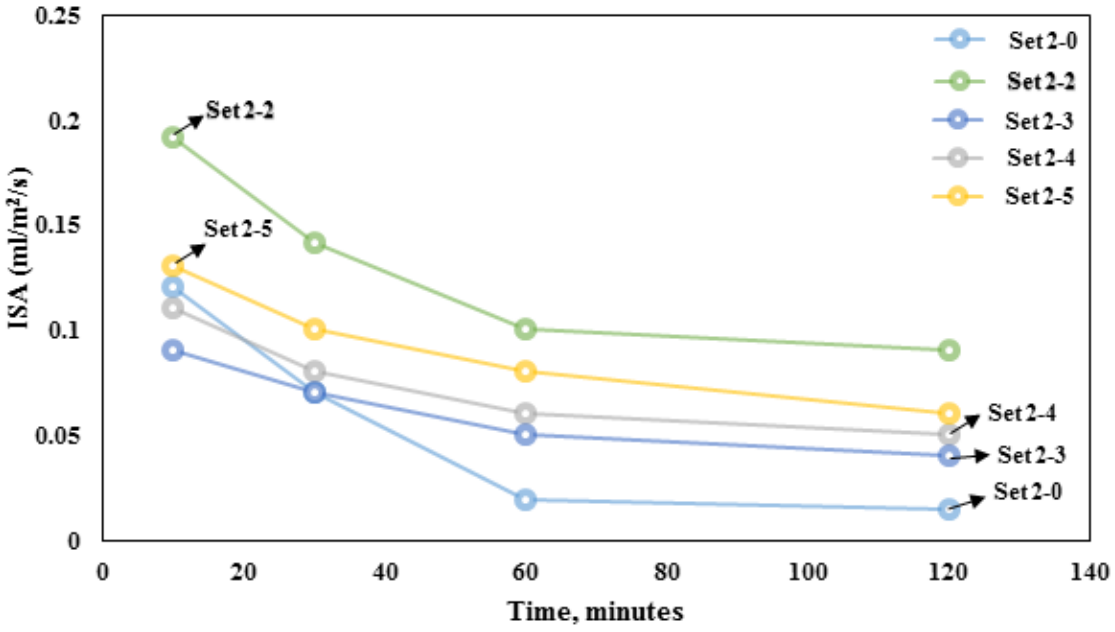


Figure 7.16: Initial Surface Water Absorption Rate for sets (2-2 to 2-5) and set 2-0.

Meanwhile the material sealed by the set 2-2 gave poor quality materials and its ISAT rate was the highest compared to the ISAT rate of the set 2-0. Figures 7.17 to 7.20 show set 2-3, set 2-4 and set 2-5 compared with the set 2-0. One can see the most efficient set was the set 2-

3, which reduced absorption during the first 30 minutes by between 0 to 25% compared the reference set.

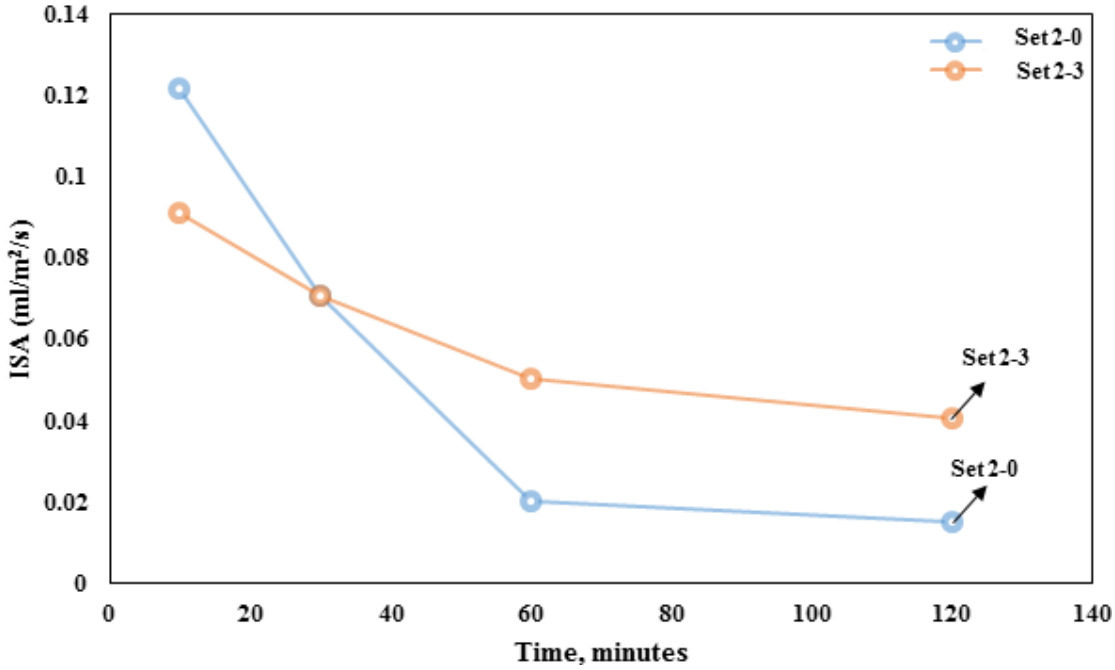


Figure 7.17: Initial Surface Water Absorption Rate for set 2-3 and set 2-0.

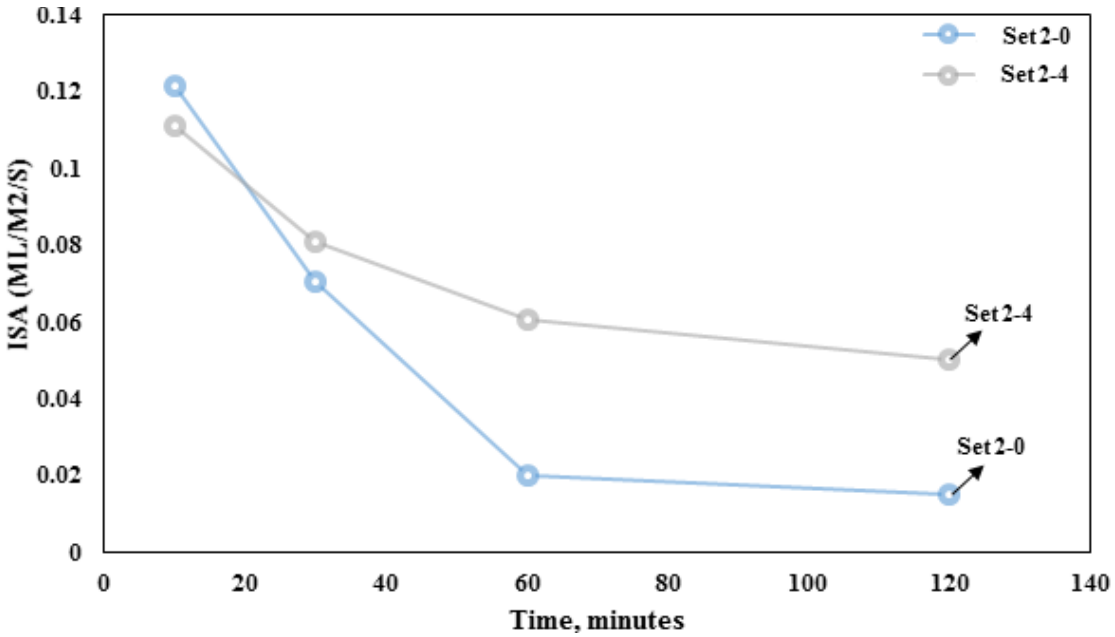


Figure 7.18: Initial Surface Water Absorption Rate for the set 2-4 and the set 2-0.

This is followed by the set 2-4, as shown in Figure 7.18, which reduced absorption around by 8% until 10 minutes, at which point it exceeded by 14% at 30 minutes compared to the set 2-0. Figure 7.19 shows the relation between the set 2-0 and the set 2-5, which ISAT values for

set 2-5 exceeded by about 8% and 43% at 10 and 30 minutes respectively compared to set 2-0. The relation between the set 2-2 and the set 2-0 is illustrated in Figure 7.20. The results presented are the average absorption by the set 2-2 samples at 10 minutes, which shows it was nearly 60% more than the average absorption of the set 2-0 and around 100% at 30 minutes. One can see from the presented results that the absorption from the set 2-0 samples were more than ten times that of the closed bacteria set (set 2-5) at 10 minutes, while around two and half at 30 minutes.

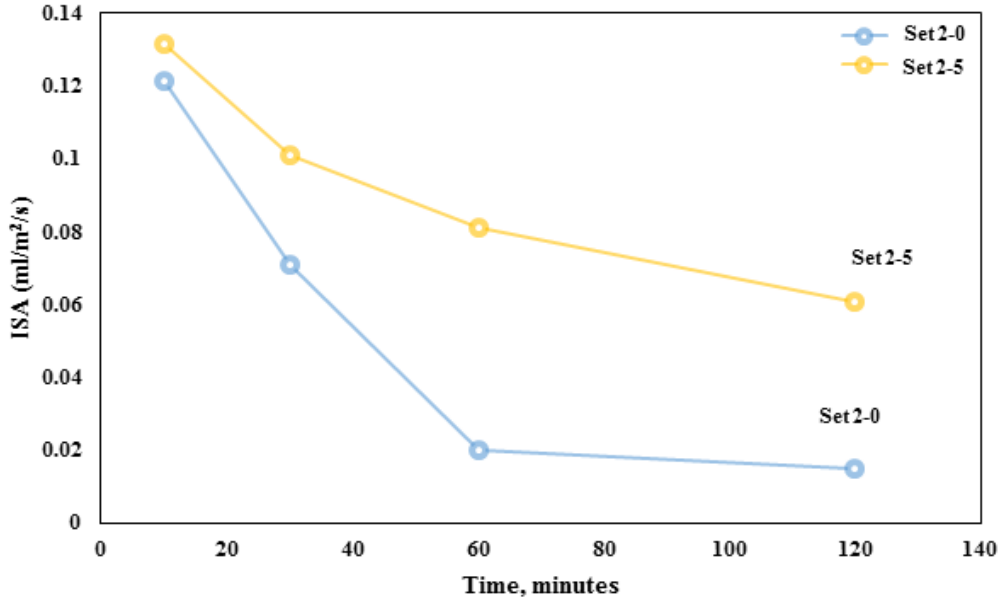


Figure 7.19: Initial Surface Water Absorption Rate for the set 2-5 and the set 2-0.

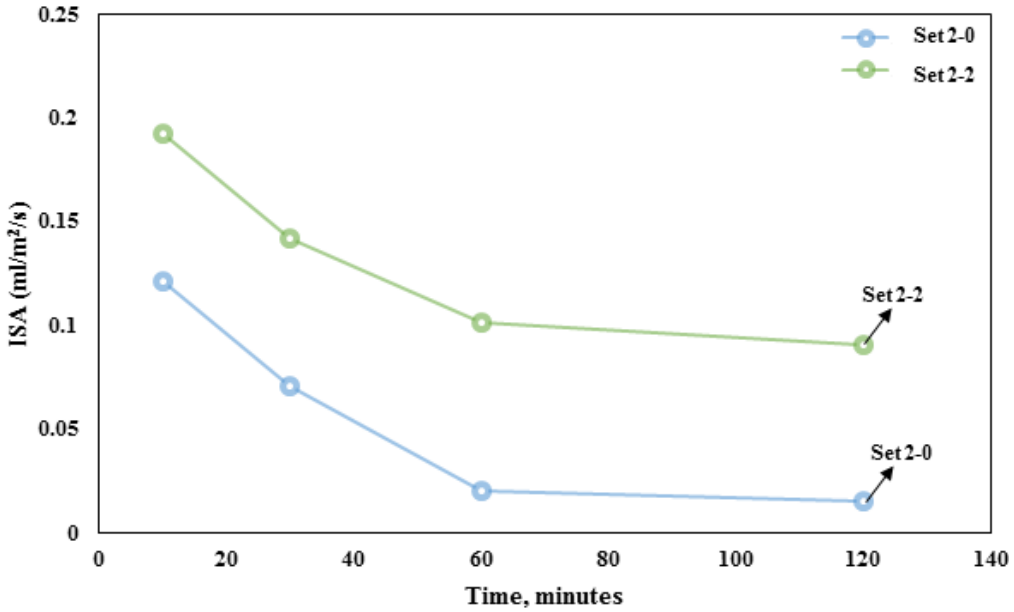


Figure 7.20: Initial Surface Water Absorption Rate for set 2-2 and set 2-0.

7.3.2.3 Visualisation tests of the healing cracks

All samples were subjected to ISAT before a SEM scan was taken. The scanning electron microscope images (SEM) of all samples indicated that sealing cracks were observed in all tested samples, including the control samples. SEM images of untreated surfaces of mortar samples are shown in Figure 7.21.

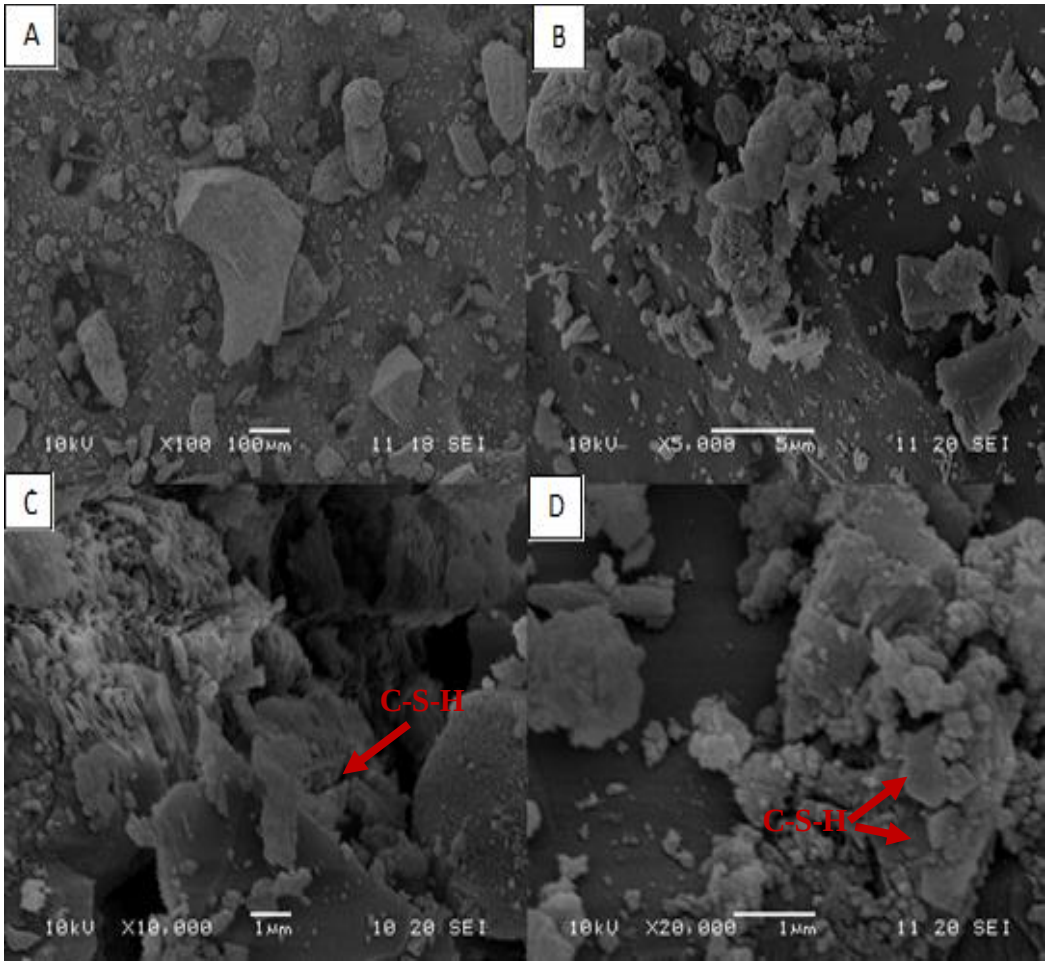


Figure 7.21: SEM images show an untreated mortar samples of set 2-1 after autogenous healing.

The SEM scan used a magnification range of 100 to 20,000 times the images of control samples mixture and according to the observations of SEM analysis, it was found that the main shape of the control sample surface structure was mainly hexagonal flakes, as shown in Figures 7.21C and D. The major elements shown according to EDX analysis (Figure 7.22) are Ca, Si, and O. These elements refer to the previous study[416] and are a colloid C-A-S-H. The key factors in cementitious materials to produce strength are C-S-H and C-A-S-H.

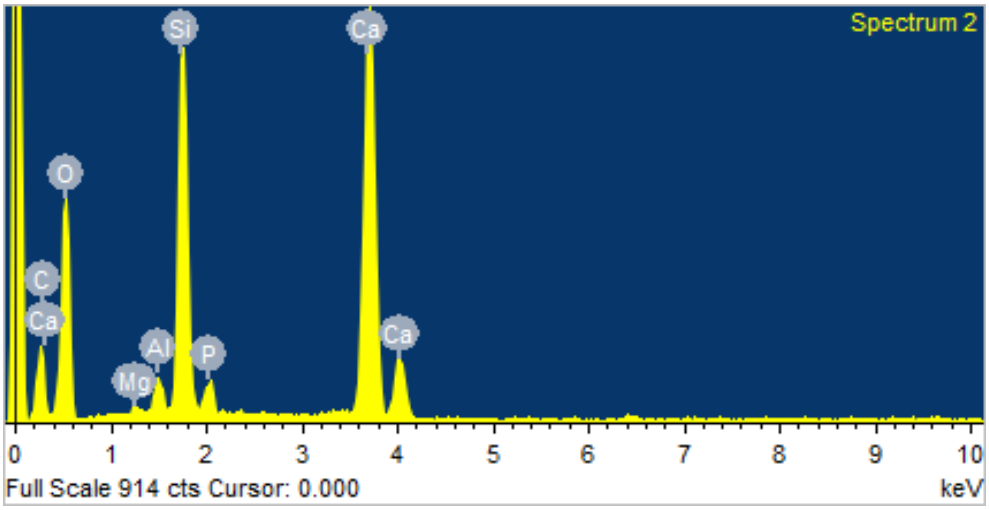


Figure 7.22: EDX diffraction of untreated mortar samples of set 2-1 after autogenous healing.

The observations of set 2-2 by SEM scan are presented in Figure 7.23. The SEM scan magnification was taken at 10,000 and 15,000 times the images for the set 2-2 mixtures. According to SEM analysis, the whitish precipitates of organic materials could be clearly observed through the mixture on the surface of the samples, as shown in Figure 7.23. This indicates that the precipitation of SHA-2 was rich by organic carbon and possibly organic compounds dervied from present precipitation yeast extract and/or calcium acetate and/or dextrose.

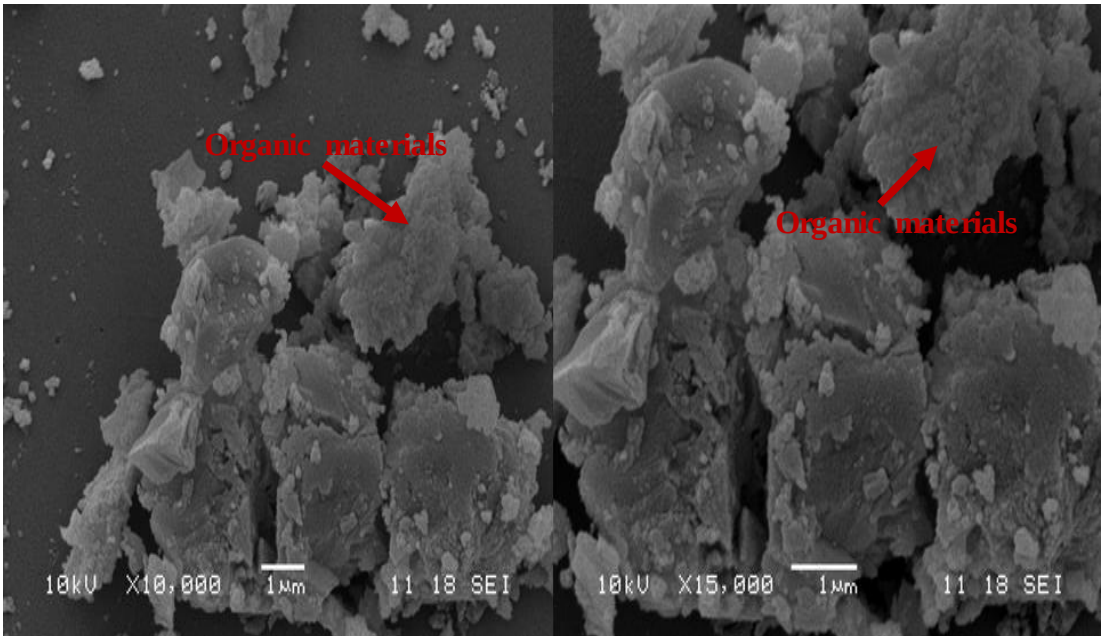


Figure 7.23: SEM images show set 2-1 after 28 days of treatment.

Mortar set 2-3 with 40x40x160mm cracks that were treated with *B.pseudofirmus* and SHA-2 (calcium source) for 28 days performed at a high quality and produced a healing material into the cracks, as presented in Figures 7.24A and B. The SEM scan magnification was taken between 50 to 1,500 times for the images for the set 2-3 samples mixture.

Figures 7.24C and D show the samples treated with *B.pseudofirmus*+SHA-2 and the carbonate crystals present on the surface, which resulted out of bio-deposition treatment.

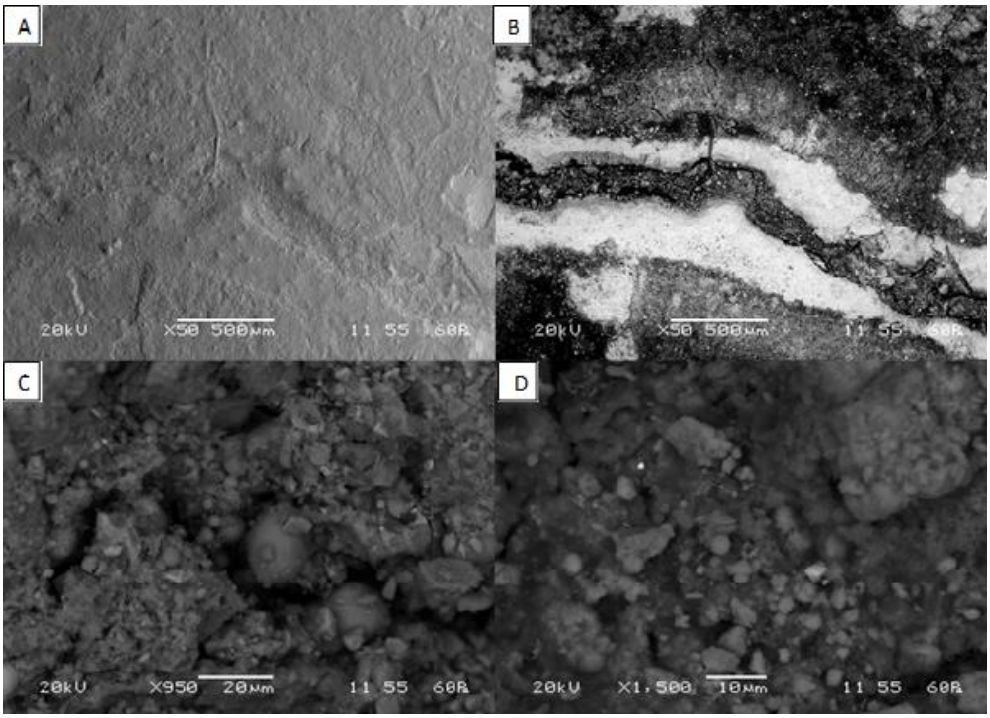


Figure 7.24: SEM images show set 2-3 after 28 days of treatment.

SEM analysis revealed large variation in crystals size in the surface covered layer of cracks. They have a range of 5-20μm through sample surface. The presence of this type of crystal gives evidence of the carbonate precipitation by bacterial mediation. Essentially, there were three different morphologies of crystals, rhombic crystals, polygonal plate like crystals, and cubic crystals. Many researchers are in agreement with the result presented in Jonkers and de Niele[99,118], which showed that *B.cohnii* and *B.pseudofirmus* helped in calcite precipitation in mortar samples. Figure 7.25 shows the EDX analysis for samples treated by *B.pseudofirmus*+SHA-2 to determine the calcium carbonate induced. The healing samples were left to dry for 24 hours at 45°C, then the calcium carbonate precipitation was collected

by crushing around the healing area by using pestle and a mortar. The aluminum holder was used to pack the powdered sample and analysed by using EDX and SEM. The major elements revealed by EDX analysis for set 2-3 were Ca, Si, O with (Ti and Fe), C, and Al. Consequently, both *B.pseudofirmus* and *B.cohnii* produced enough calcium carbonate compared to the amount of calcium carbonate produced by *B.halodurans*.

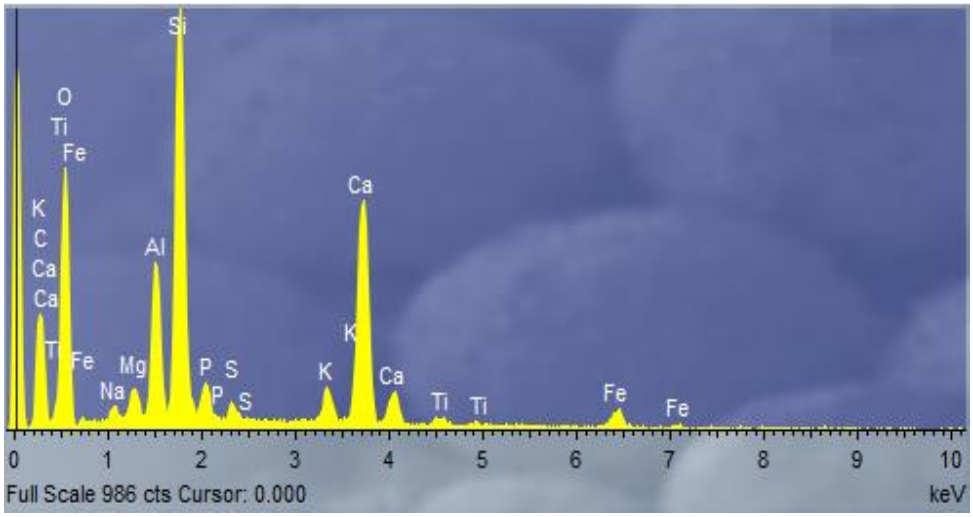


Figure 7.25: EDX analysis of set 2-3 after 28 days of treatment.

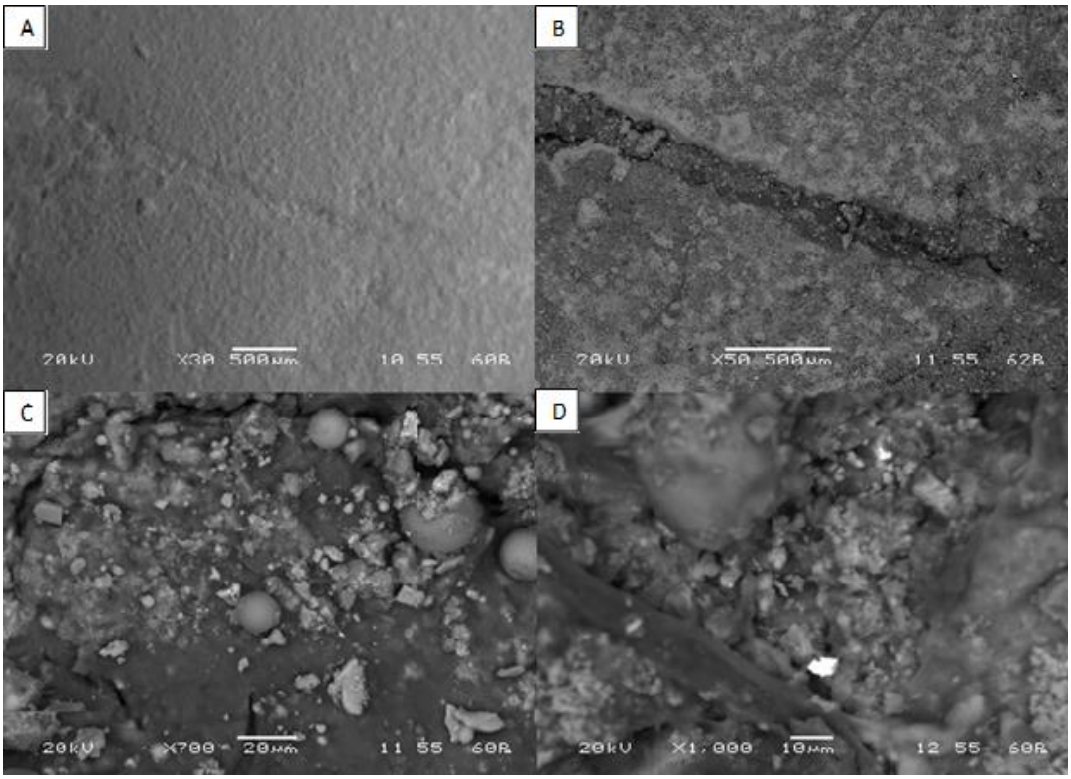


Figure 7.26: SEM images show set 2-4 after 28 days of treatment.

Treatment of set 2-4 with 40x40x160mm cracks with *B.cohnii* +SHA-2 (calcium source) produced a high quality healing material in the cracks, as presented in Figures 7.26A and B. The SEM scan magnification was taken between 500 and 1,500 times the images for the *B.cohnii* +SHA-2 samples mixtures. Figures 7.26C and D shows the samples treated with *B.cohnii* +SHA-2 and the carbonate crystals present on the surface, which resulted from the bio-deposition treatment.

An aluminium holder was used to pack the powdered sample, which was analysed using EDX and SEM at the Physical department after the healing samples had been left to dry for 24 hours at 45℃. The major elements revealed by EDX analysis for set 2-4 were Ca, Si, Al, C, and O with Fe, as shown in Figure 7.27.

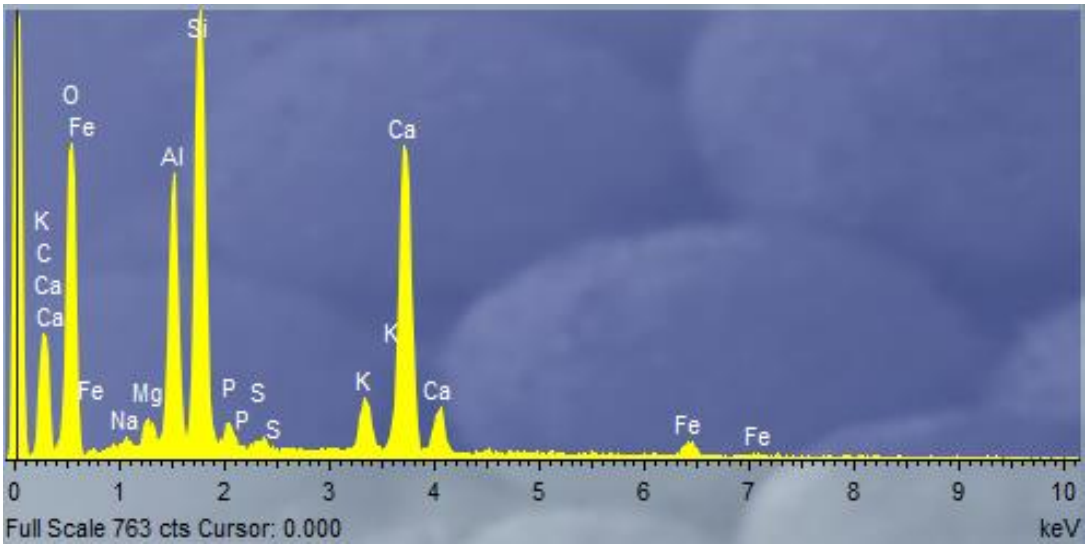


Figure 7.27: EDX analysis of set 2-4 after 28 days of treatment.

The set 2-5 were used to treat crack widths of 0.53 mm and the SEM images show they performed well and produced a high quality healing material that sealed the crack, as presented in Figure 7.28A and B. The level of magnification was ranged 50 x to 1,500 x the magnification of the images for set 2-5. Figure 7.28C and D show the efficiency of the healing materials produced by set 2-5 that sealed the crack. According to SEM analysis the healing material was crystals of carbonate present on the surface due to bio-deposition treatment by *B.halodurans*.

Figure 7.29 shows the EDX analysis for set 2-5 to determine calcium carbonate produced by three types of bacteria. The healing samples were left to dry for 24 hours at 45℃, then the

calcium carbonate precipitation was collected by crushing around the healing area by using a pestle and a mortar. The aluminum holder was used to pack the powdered sample. The EDX analysis for the samples processed by set 2-5 present that the major elements are O, Al, Si, C, and Ca with K, as shown in Figure 7.29.

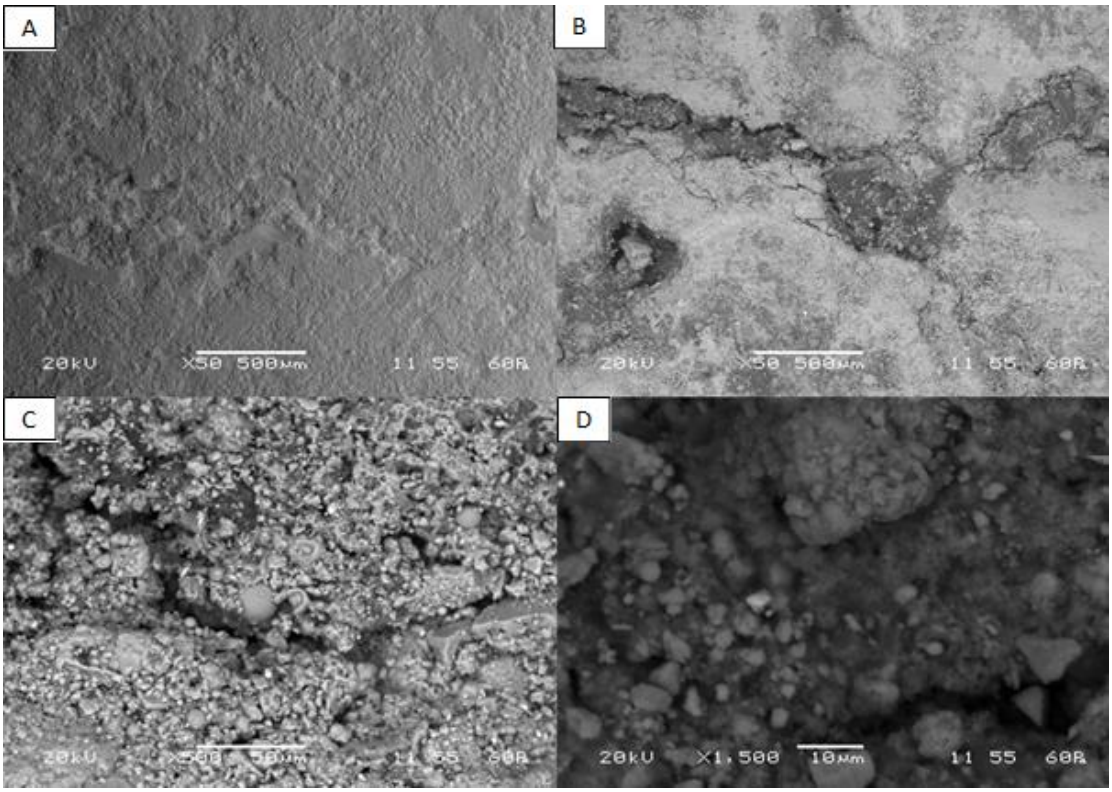


Figure 7.28: SEM images show set 2-5 after 28 days of treatment.

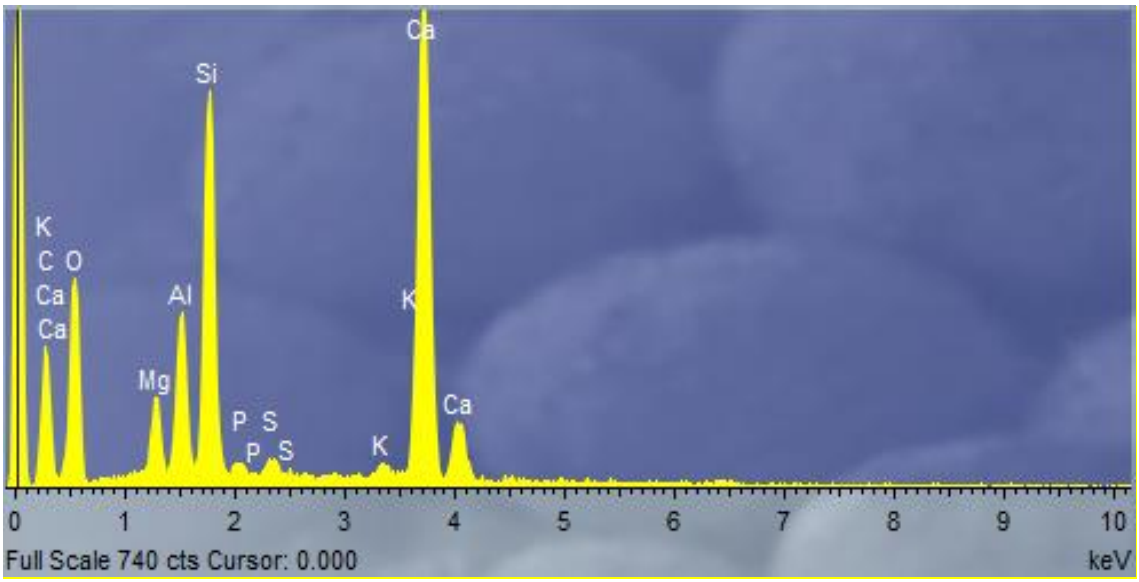


Figure 7.29: EDX analysis of set 2-5 after 28 days of treatment.

7.3.2.4 Summary

To summarize:

1- The initial absorption surface test (IAST) analysis presented the efficiency of materials produced by *B.pseudofirmus* as better than the reference set. It showed that the efficiency of materials produced by *B.cohnii* and *B.halodurans* are close to the reference set. However, the IAST analysis of the control and the SHA-2 medium control sets showed poor efficiency of the materials that sealed the cracks.
2- The control set and the SHA-2 medium control was observed during the duration of the treatment and both of them sealed the cracks. However, the control set showed autogenous healing of the crack, while the SHA-2 medium control crack was sealed by inorganic materials due to the ingredients of the precipitation, according to SEM images and EDX analysis.
3- The materials produced by the three types of bacteria according to SEM images and EDX analysis was confirmed as calcite.
4- *B.pseudofirmus* and *B.cohnii* with SHA-2 produced enough calcium carbonate compared to the amount of calcium carbonate produced by *B.halodurans* with SHA-2.

7.3.3 Ability of Bacillus *pseudofirmus* with SHA-3 for healing cracks in mortar (stage three)

7.3.3.1 The ability of bacteria on sealing in mortar

The specimens were divided into four experimental sets. The set 3-0 was reference and consisted of three samples without cracks, the set 3-1 a control and consisted of three samples. The crack width was taken between 0.13 and 0.14 mm and was injected with water only as shown in Figure 7.30A. From Figure 7.30A one can observe that the crack was healed by a crystal coloured material across both edges of the crack, while Figure 7.30B shows the material that healed the crack was from autogenous healing methods.

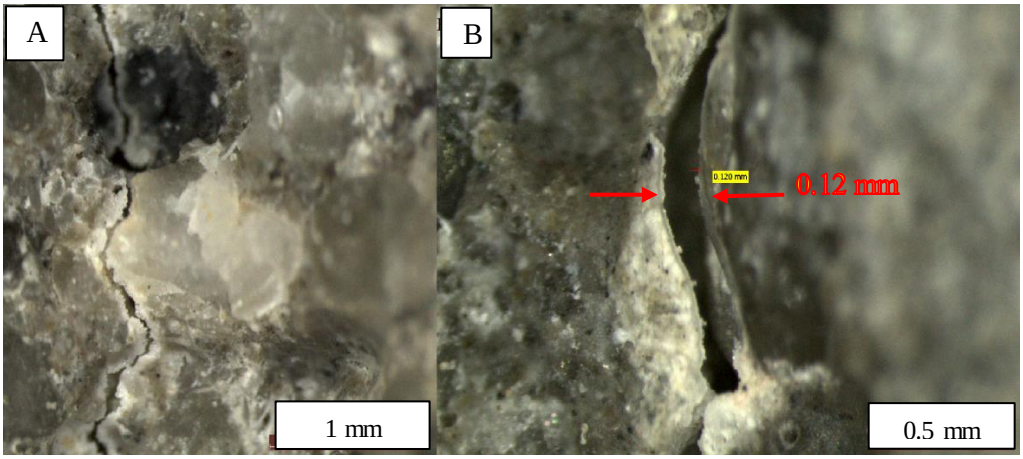


Figure 7.30: Crack before and after healing by injecting water for set 3-1 at tension zone.

The set 3-2 was the SHA-3 control medium; the crack widths were between 0.13 and 0.14 mm and they were injected with SHA-3 only as shown in Figure 7.31A. The crack was sealed with SHA-3 in some places across the crack as shown in Figure 7.31B, and it is clear that the material is a permeable.

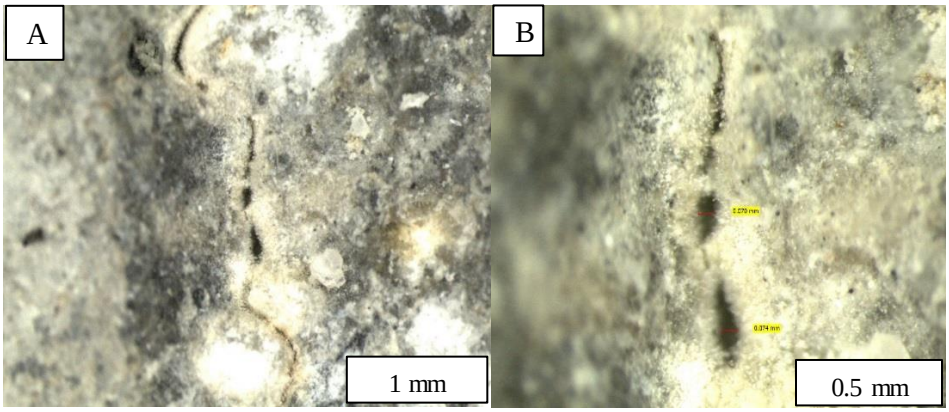


Figure 7.31: Crack after healing by injecting SHA-3 for set 3-2 at tension zone.

The materials that sealed the crack could be due to inorganic components in SHA-3 or could be due to one of its components reacting with the cement matrix. Hence, all samples injected with SHA-3 were washed with water and small plastic brush to remove any organic materials that had stuck across the crack as shown in Figure 7.32A and 7.32B. One can see that all the organic components were dissolved by water and disappeared.

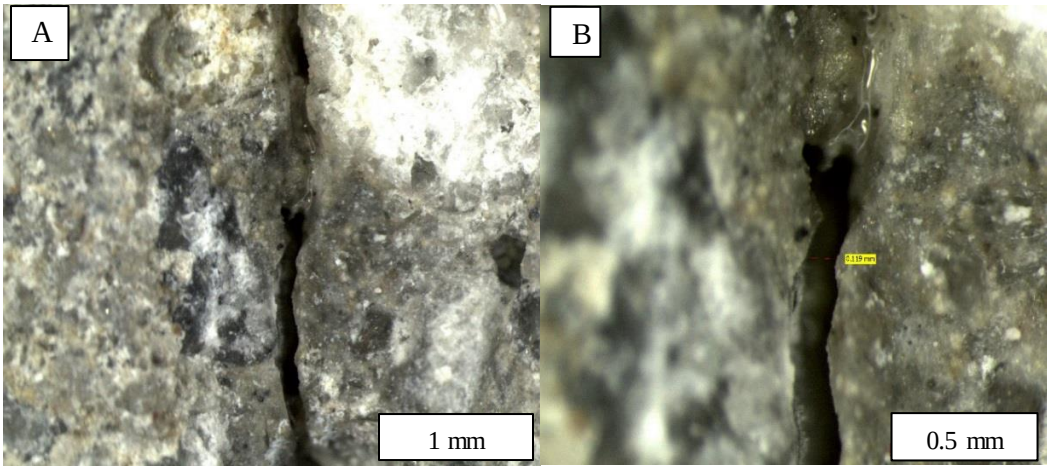


Figure 7.32: Crack after healing by injecting SHA-3 for set 3-2 and after washing at tension zone.

The set 3-3 is *B.pseudofirmus* + SHA-3. The crack width domain was between 0.13 and 0.14 mm as shown in Figure 7.33A. The set had three samples, which were injected with *B.pseudofirmus* + SHA-3. For the whole healing duration of the samples (seven days), they were fed each day, once, with one ml of *B.pseudofirmus* + SHA-3.

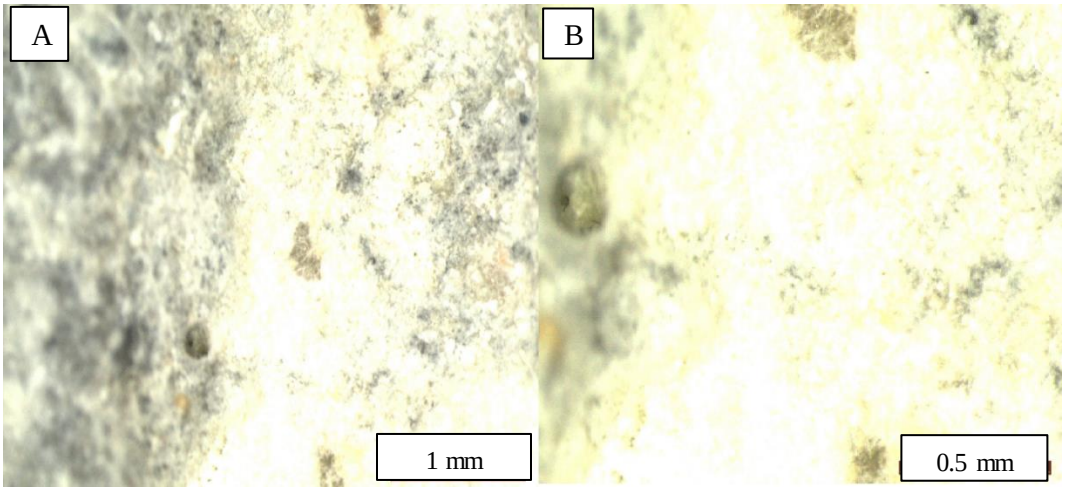


Figure 7.33: Crack after healing for set 3-3 at tension zone.

The set 3-3 produced sealing materials, which sealed the openings of the cracks slowly at both crack faces, and the crack by the end of sealing time disappeared, as shown in Figures 7.33B. The sealing material shows a significant difference between the produced amounts of sealing material compared to those sealed materials induced by set 3-2 or set 3-1, which happened to the set 3-2 after washing samples with water and small plastic brush.

Microscopic examination showed non-permeable materials sealing the cracks for the set 3-3, as shown in Figure 7.34, compared to the set 3-1 and the set 3-2, which were dissolved in water.

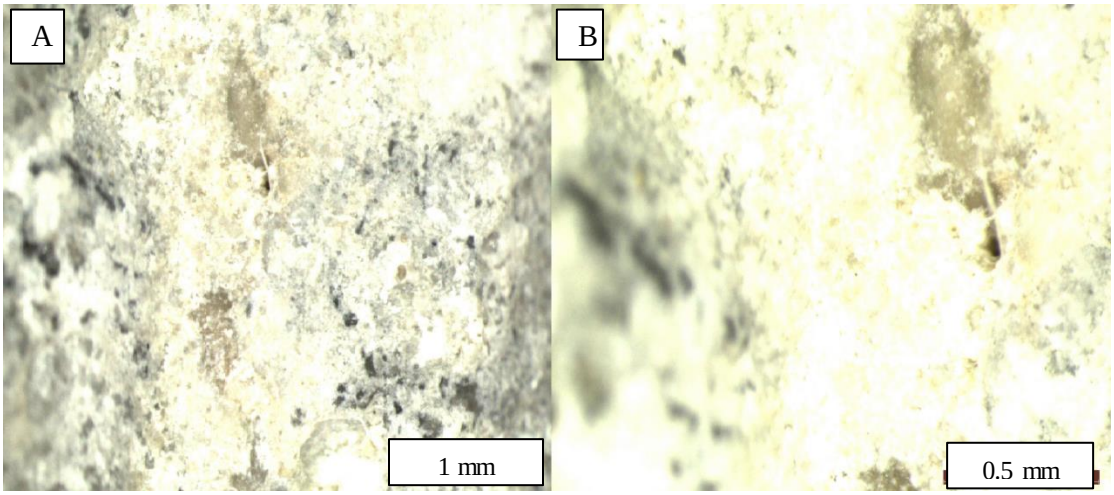


Figure 7.34: Crack after healing by injecting *B.pseudofirmus* in SHA-3 for set 3-3 at tension zone after washing.

7.3.3.2 Initial surface absorption test

The aim of this test was to compare the results of filling material after treatment for the three sets with the reference set (three samples without generated cracks), as shown in Figure 7.35. This graph indicated that the average of the set 3-3 at 10 and 30 minutes was better than the set 3-0. However, from 60 minutes to the end of the test, the ISAT of the testing set was effectively reduced and became close to the set 3-0, while the set 3-1 injected with water after it had been cracked was out of range. The graph also shows that the ISAT rate for all sets decreased with time. The rate of ISAT gradually decreased with time as the prism became saturated with water. In addition, Figure 7.35 confirmed that the efficiency of the sealing material that was produced by bacteria is of a non-permeable and these materials have the lowest ISAT rate compared to the set 3-1 and the set 3-2. Meanwhile the material sealed by the set 3-2 gave poor quality materials and its ISAT rate was the highest compared to the ISAT rate of the set 3-0.

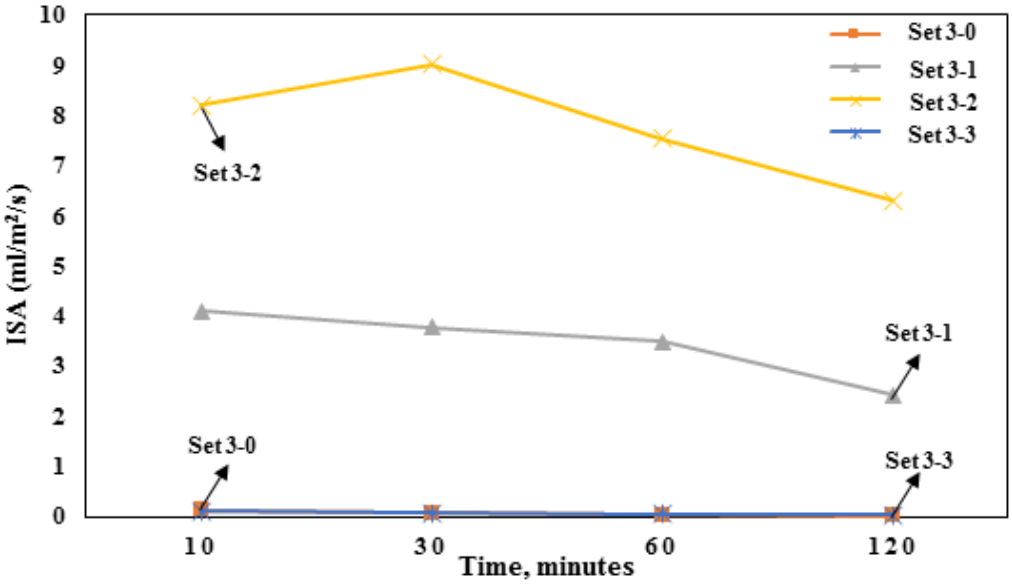


Figure 7.35: Initial Surface Water Absorption Rate for three sets and set 3-0.

Figures 7.36 indicated that the average of the set 3-3 in the first 30 minutes was better than the set 3-0, which means the set 3-3 has the ability to increase durability of mortar by reducing the pore sizes in samples by around 20% of the set 3-0. Further, at 30 minutes, the average of the set 3-3 reduced the absorption to around 9.1% compared to the set 3-0. However, for 60 and 120 minutes the set 3-3 was effectively reduced in terms of the ISAT rate and became close to the set 3-0. It also exceeded by 7.7% and 10.33% respectively compared to the set 3-0.

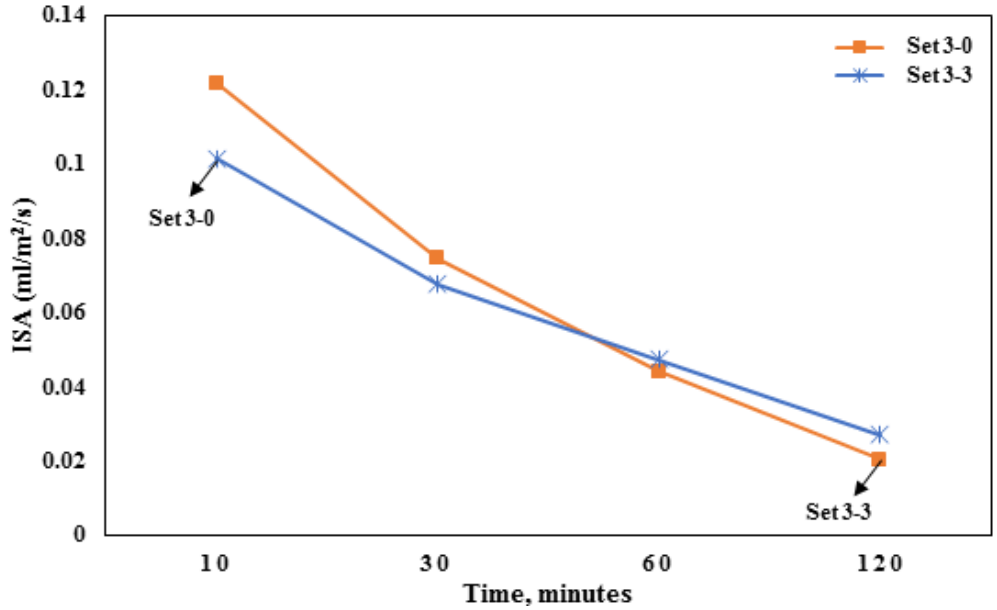


Figure 7.36: Initial Surface Water Absorption Rate for set 3-3 and set 3-0.

Figure 7.37 shows the relation between the set 3-0 and the set 3-2. This relation showed the percentage of absorption of the set 3-2 compared to the set 3-0. The results presented show that the average absorption by the set 3-2 samples was around 70 times higher compared to the close point of the average absorption from set 3-0. Hence, the material in the set 3-2 was organic, which dissolved in water and produced poor quality materials. Its ISAT rate was the highest compared to the ISAT rate of the reference set.

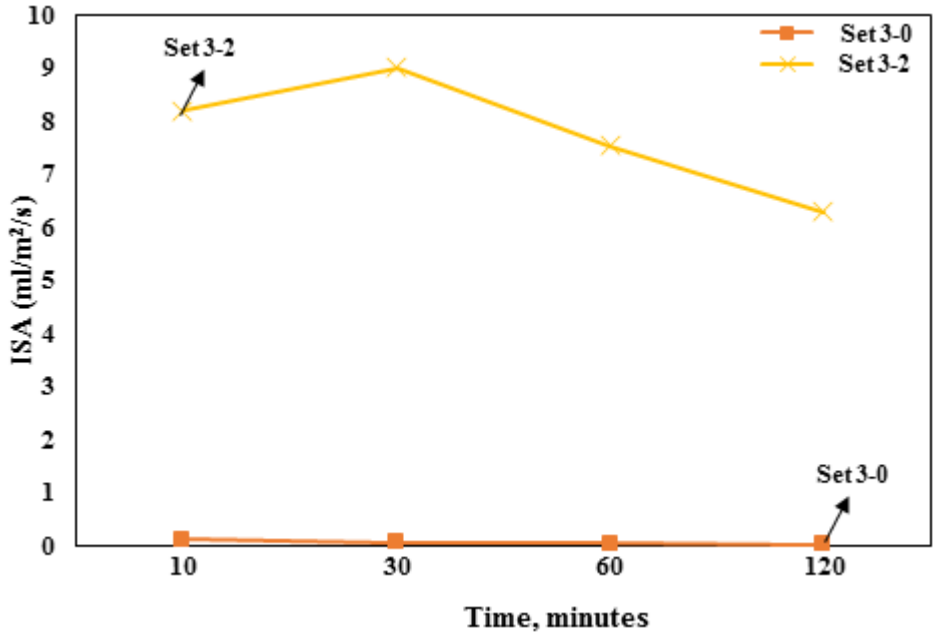


Figure 7.37: Initial Surface Water Absorption Rate for the set 3-2 and the set 3-0.

7.3.3.3 Permeability via water absorption

The procedure of the permeability test via capillary water absorption followed the HEALCON document[401]. Figure 7.38 shows the average sorption coefficient (SC) of each set calculated - each set has three samples. The results revealed that the average of the set 3-1 SC were the most absorbent, while the average of the set 3-3 SC were the least absorbent. The set 3-3 had the ability to increase the durability of mortar by reducing the pore size in samples, whereas the average SC of its set was less than the average SC of the set 3-0 by around 52%. Further, the average of the set 3-3 reduced the absorption by around 40% compared to the average of the set 3-2.

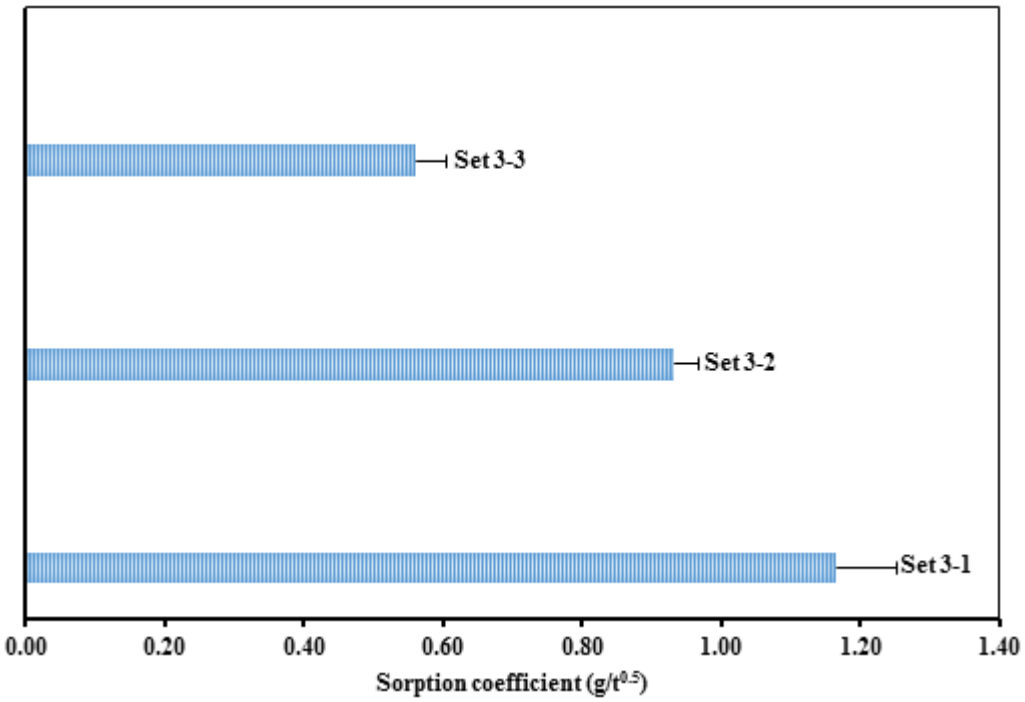


Figure 7.38: Average sorption coefficient calculated from permeability test via water absorption.

7.3.3.4 Visualisation tests on the healing cracks (SEM/EDX)

All samples were subjected to ISAT before a SEM scan was taken. The scanning electron microscope (SEM) images for all samples indicated that sealing of cracks were observed in the set 3-3, while set 3-1 and set 3-2 were not sealing. SEM images of particles of untreated mortar surface samples is shown in Figure 7.39.

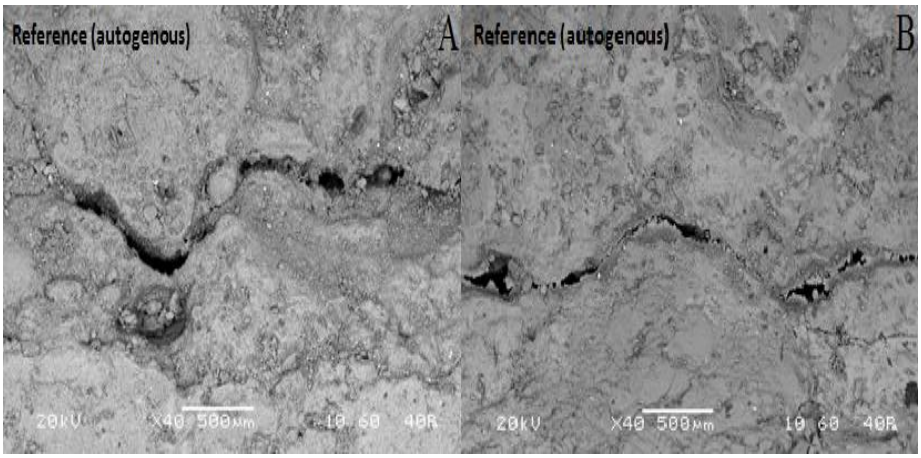


Figure 7.39: SEM images show set 3-1 after autogenous healing.

The SEM scan used magnified the images of set 3-1 by 40 times and according to the observations of the SEM analysis, it was found that the crack was not healing except in small places due to autogenous healing, as presented in Figure 6.39A and B. The major elements, according to EDX analysis - in Figure 7.40, were Ca, Si, O and Fe. These elements refer to the previous study[416] and are a colloid C-A-S-H. The key factors in cementitious materials to produce strength are C-S-H and C-A-S-H.

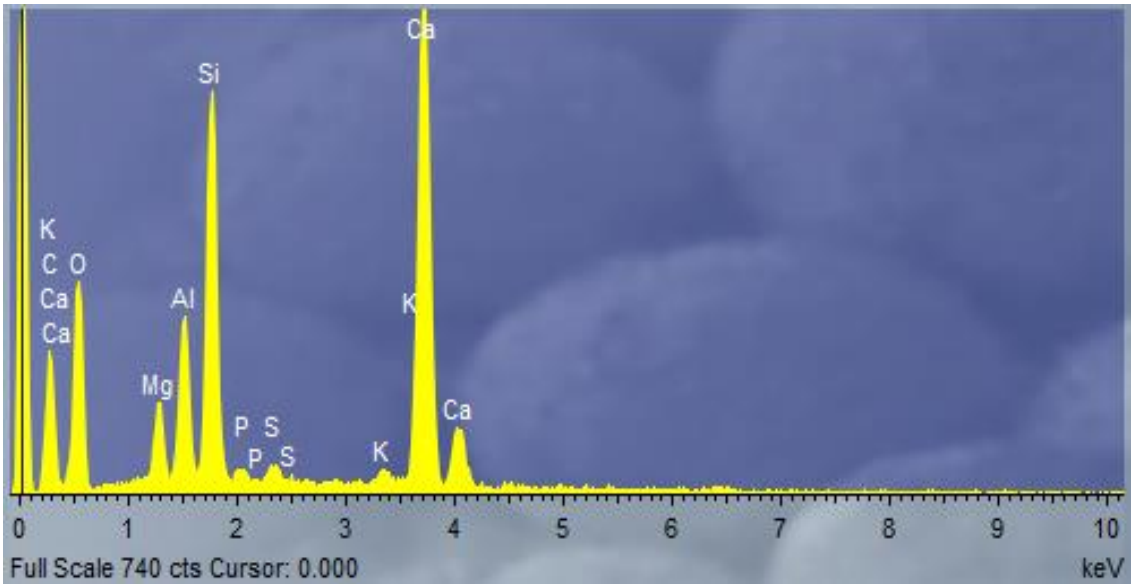


Figure 7.40: EDX diffraction of set 3-1 after autogenous healing.

The observations from the set 3-2 by the SEM scan are presented in Figure 7.41. The SEM scan magnification was taken same as set 3-1 magnification. According to the SEM analysis, the crack still existed and there were some places through the crack that closed by way of deposits of calcite (calcium carbonate) as shown in Figure 7.41. That indicates that the precipitation of SHA-3 was rich by organic carbon and possibly organic compounds derived due to any present precipitation from yeast extracts and/or calcium acetate and/or dextrose, which was dissolved due to washing them with water.

All samples were cut to be suitable for the aluminum holder, which were analysed using EDX and SEM at the physical department after the healing samples had been left to dry for 72 hours at 45℃. The major elements revealed by EDX analysis for the set 3-2 were Ca, Si, Sn, W, and O with Fe, as shown in Figure 7.42.

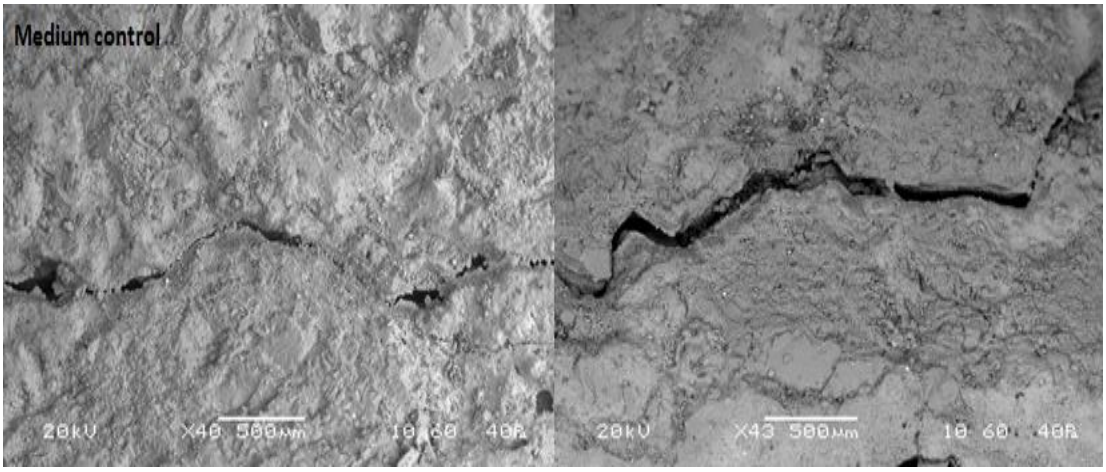


Figure 7.41: SEM images show set 3-2 after 28 days of treatment.

Treatment of set 3-3 with 40x40x160mm cracks with *B.pseudofirmus* and SHA-3 for 28 days produced non-permeable materials into the cracks, as presented in Figures 7.43. The SEM scan magnification was x500 for set 3-3.

Figures 7.43 shows the samples treated with *B.pseudofirmus*+SHA-3 (set 3-3) and the carbonate crystals present on the surface, which resulted from bio-deposition treatment.

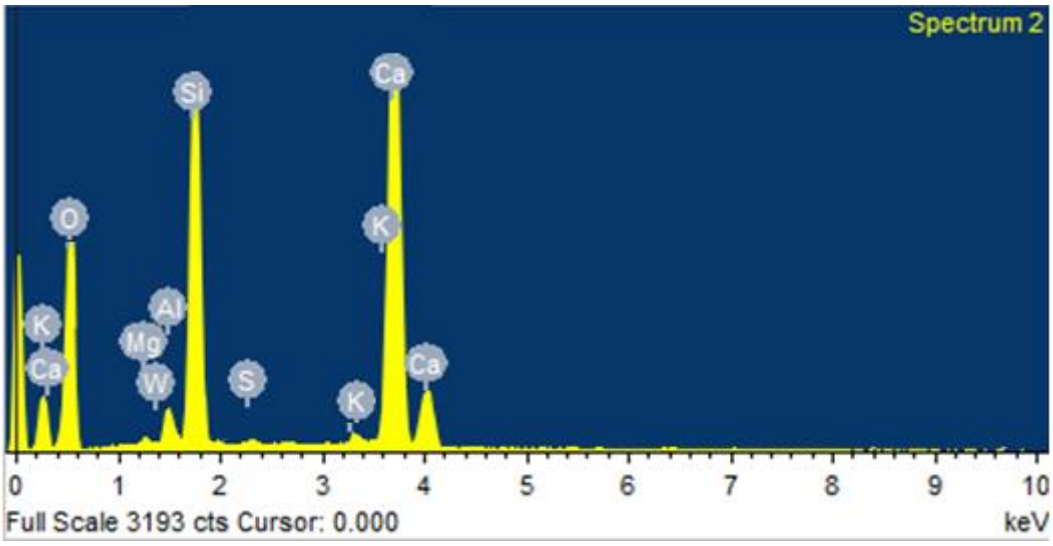


Figure 7.42: EDX analysis of set 3-2 after 28 days of treatment.

SEM analysis revealed large variation in crystals size on the surface covering the layer of cracks, which had a range of 5-20μm through the sample surface. The presence of this type of crystal gives evidence of the carbonate precipitation by bacterial mediation. Essentially, there were three different morphologies of crystals, rhombic crystals, polygonal plate like crystals,

and cubic crystals. Many researchers are in agreement with the result presented in Jonkers and De Niele[99,118], which showed that *B.pseudofirmus* helped in calcite precipitation in mortar samples.

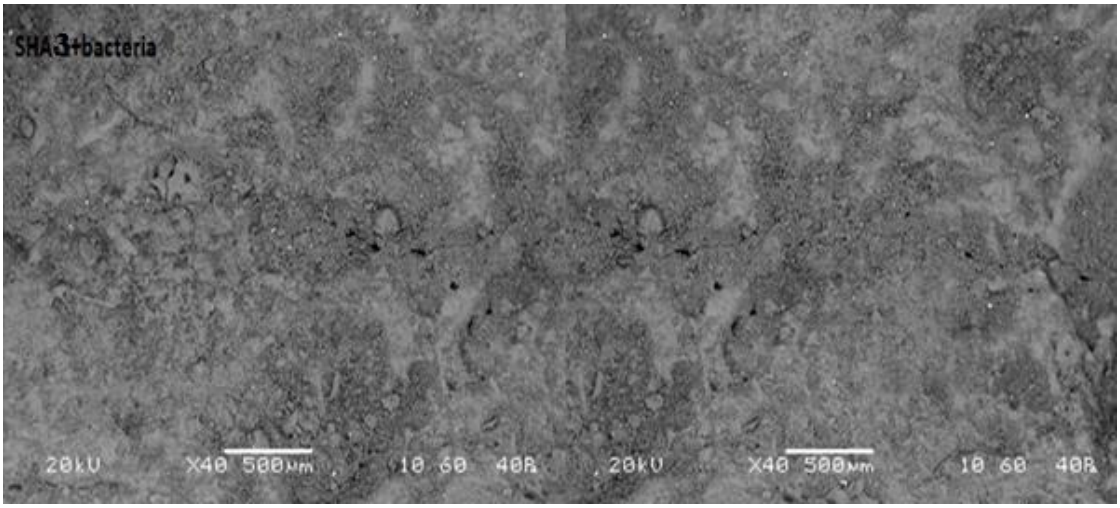


Figure 7.43: SEM images show set 3-3 after 28 days of treatment.

Figure 7.44 shows the EDX analysis for set 3-3 treated with *B.pseudofirmus* with SHA-3 to determine the calcium carbonate induced. The major elements revealed by EDX analysis for samples treated with *B.pseudofirmus* with SHA-3 were Ca, Si, O, and C. Therefore, set 3-3 produced enough calcium carbonate through cross section of crack.

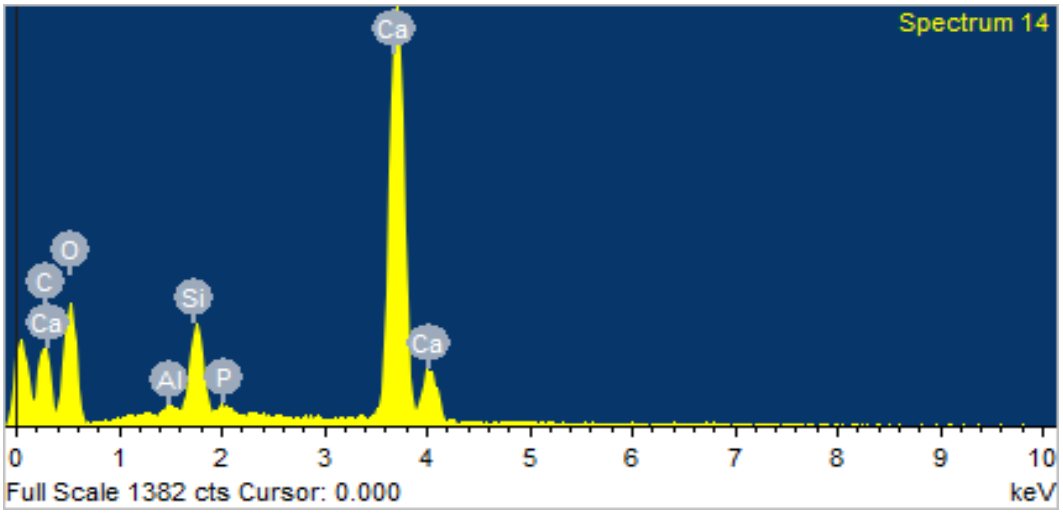


Figure 7.44: EDX analysis of set 3-3 after 28 days of treatment.

The summary of this stage and the next two stages were summarized in the end of stages four and five.

7.3.4 Ability of Bacillus *pseudofirmus* with SHA-3 for re-healing cracks in concrete (second cycle) (stage four)

Three samples of set 3-3 have re-generated cracks using flexural test and cracks width were measured by LVDT sensor. The samples crack size were ranged between 130 µm and 240 µm. For the whole healing duration of the samples (seven days), they were fed each day, once, with 1 ml of *B.pseudofirmus* + SHA-3. The procedures of this stage were taken same as stage three and the set 3-3 has renamed as set 4-1.

7.3.4.1 The ability of bacteria to re-heal mortar samples

Set 4-1 re-produced sealing materials, which re-sealed the openings of the cracks slowly at both crack faces, and the crack by the end of sealing time disappeared, as shown in Figures 7.45.

Microscopic examination showed non-permeable materials sealing the cracks for the set 4-1, as shown in Figure 7.45B.

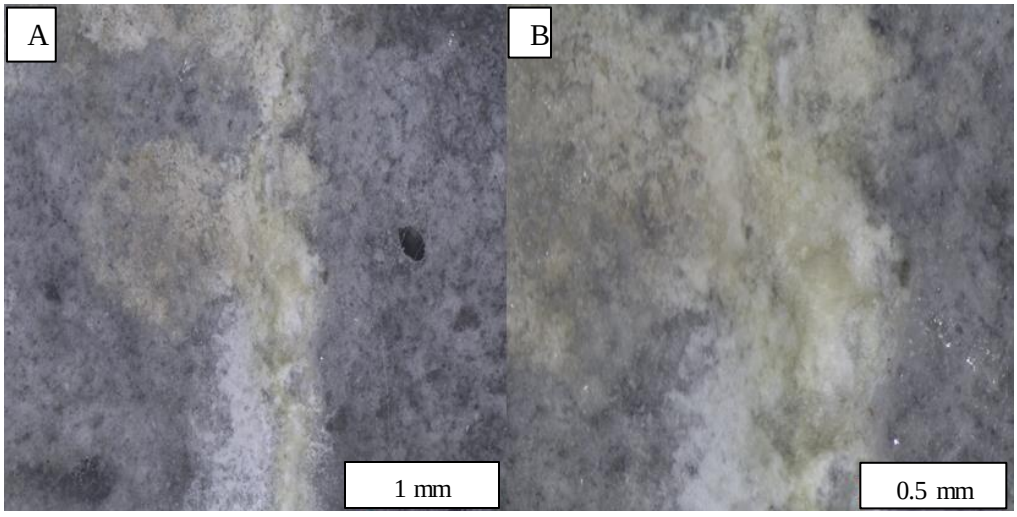


Figure 7.45: Crack after healing by injecting *B.pseudofirmus* in SHA-3 for set 4-1 at tension zone after washing.

7.3.4.2 Initial surface absorption test (ISAT)

Figures 7.46 indicated that the set 4-1 in the first 10 minutes was better than the reference set (set 3-0) without a crack, which means the set 4-1 has the ability to increase durability of mortar by reducing the pore sizes in samples by around 8% of the set 3-0 even after

regenerated crack. However, from 30 and 120 minutes the testing set was effectively reduced in terms of the ISAT rate and became close to the reference set without a crack.

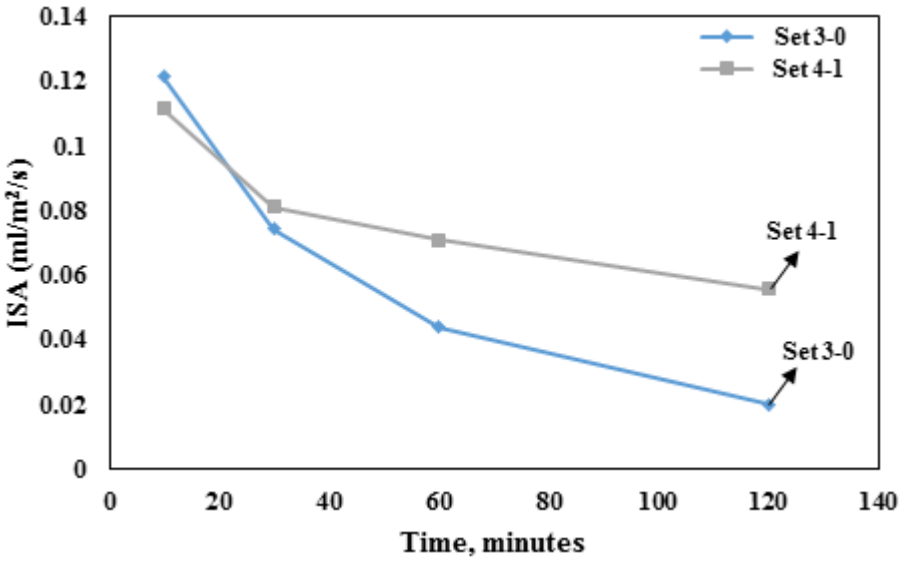


Figure 7.46: Initial Surface Water Absorption Rate for set 4-1 and set 3-0 (second cycle).

Figure 7.47 shows that the ISAT of the set 4-1 is to close to set 3-3 and set 3-0. It is clear the *Pseudofirms* bacteria sets (set 3-3 and set 4-1) have the ability to heal crack for second cycle and the efficiency of healing material that produced by it is high quality.

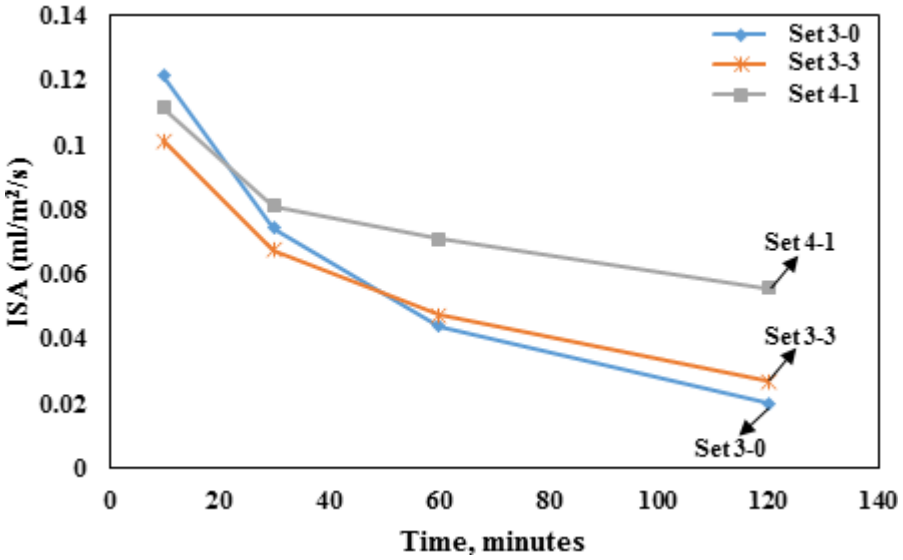


Figure 7.47: Initial Surface Water Absorption Rate for set 3-3, set 4-1 and set 3-0.

7.3.4.3 Permeability via water absorption

Figure 7.48 shows the average sorption coefficient (SC) of each set calculated - each set has three samples. The results revealed that the average of set 3-3 SC were the most absorbent, while the average of set 4-1 SC were the least absorbent. The sets in both cycle had the ability to increase the durability of mortar by reducing the pore size in samples.

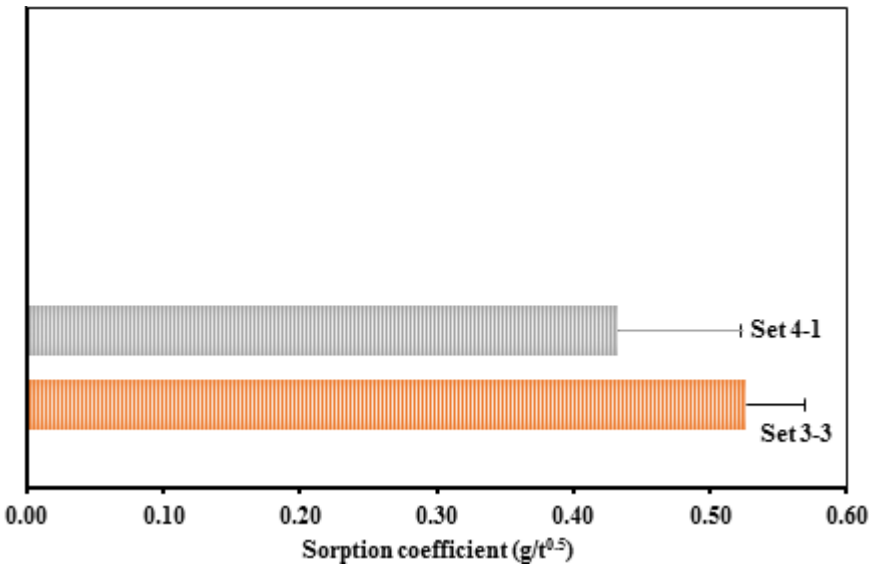


Figure 7.48: Average sorption coefficient calculated from permeability test via water absorption.

7.3.4.4 Summary

The ability of *Bacillus pseudofirmus* to re-heal cracks for a second cycle was investigated in this section. Based on the results one can conclude that, bacteria with SHA-3 can re-healed crack for second cycle, the crack width which can bacteria re-healed was ranged between 125 µm to 240 µm. The ISAT analysis and capillary water absorption presented the efficiency of materials produced by *B.pseudofirmus* with SHA-3 (set 3-3 and set 4-1) for both cycles as better than the set 3-0.

7.3.5 Ability of Bacillus *pseudofirmus* with SHA-3 for healing cracks in concrete (stage five)

7.3.5.1 The ability of bacteria on sealing in mortar as function of time

The specimens were divided into five experimental sets. The set 5-0 was reference and consisted of three samples without cracks, the set 5-1 a control and consisted of three

samples. The crack width was taken between 0.19 and 0.28 mm and was injected with water only as shown in Figure 7.49A. From Figure 7.49A one can observe that the crack was healed by a crystal coloured material across both edges of the crack, while Figure 7.49B shows the material that healed the crack was from autogenous healing methods.

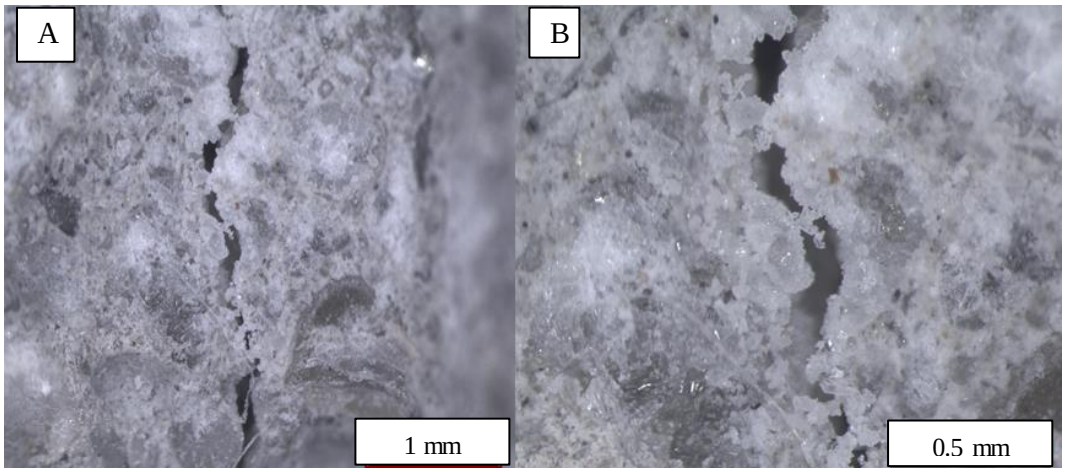


Figure 7.49: Crack before and after healing by injecting water for set 5-1 at tension zone.

The set was the set 5-2; the crack widths were between 0.179 and 0.184 mm and they were injected with bacteria + SHA-3 once only as shown in Figure 7.50A. The crack was sealed in some places across the crack as shown in Figure 7.50B, and it is clear that the material is of low quality and it is mix between autogenous and autonomic healing.

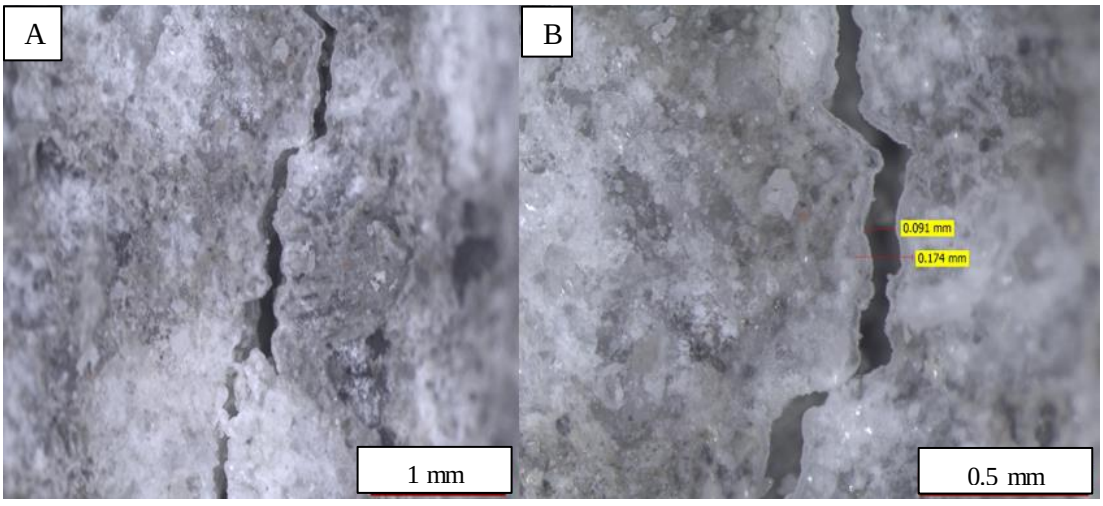


Figure 7.50: Crack after healing by injecting *pseudofirmus* with SHA-3 once for set 5-2 at tension zone.

The crack width domain was between 0.144 and 0.156 mm in set 5-3 as shown in Figure 7.51A. The set had three samples, which were injected with *B.pseudofirmus* + SHA-3. For the whole healing duration of the samples (seven days), they were fed for (first, third, fifth and seventh) days, once, with 1 ml of *B.pseudofirmus* + SHA-3.

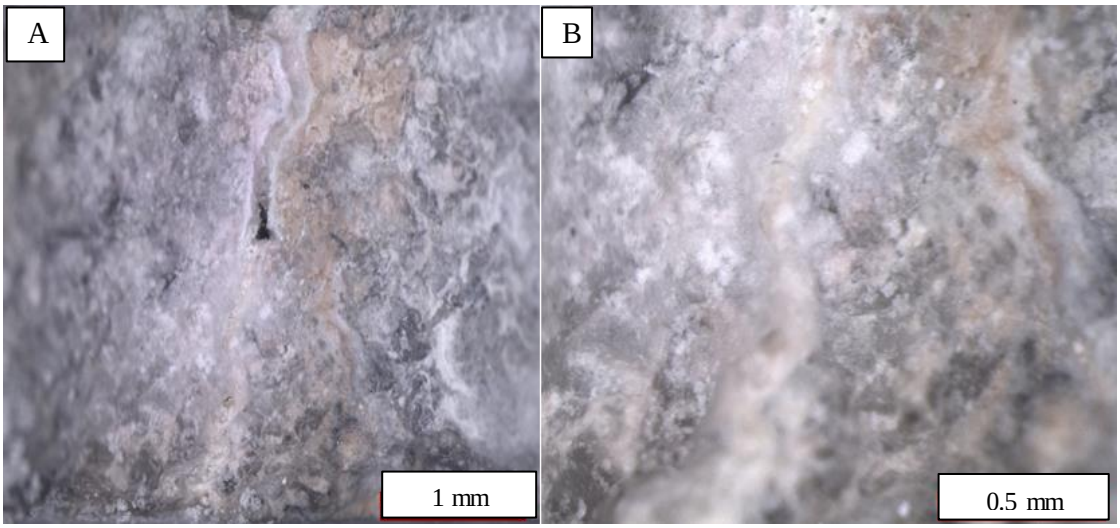


Figure 7.51: Crack after healing by injecting *B.pseudofirmus* with SHA-3 on (first, third, fifth, and seventh) days at tension zone.

The bacteria with SHA-3 produced sealing materials, which sealed the openings of the cracks slowly at both crack faces, as shown in Figures 7.51B. The sealing material shows a significant reduced the crack width compared to those sealed materials induced by set 5-1 or set 5-2. However, the time of complete the process was not enough because still some places through the crack not healing. Microscopic examination showed non-permeable materials sealing the cracks for the set 5-3, as shown in Figure 7.51B.

The crack width domain was between 0.136 and 0.171 mm for set 5-4 as shown in Figure 7.52A. The set had three samples, which were injected with *B.pseudofirmus* + SHA-3. For the whole healing duration of the samples (seven days), they were fed each day, once, with 1 ml of *B.pseudofirmus* + SHA-3.

The bacteria with SHA-3 produced sealing materials, which sealed the openings of the cracks slowly at both crack faces, and the crack by the end of sealing time disappeared, as shown in Figures 7.52B. The sealing material shows a significant difference between the produced amounts of sealing material with time of healing compared to those sealed materials induced

by set 5-3 or set 5-2 or set 5-1, which occurred to the set 5-1 after washing samples with water and small plastic brush.

Microscopic examination showed non-permeable materials sealing the cracks for the sets (5-3 and 5-4), as shown in Figures 7.51 and 7.52.

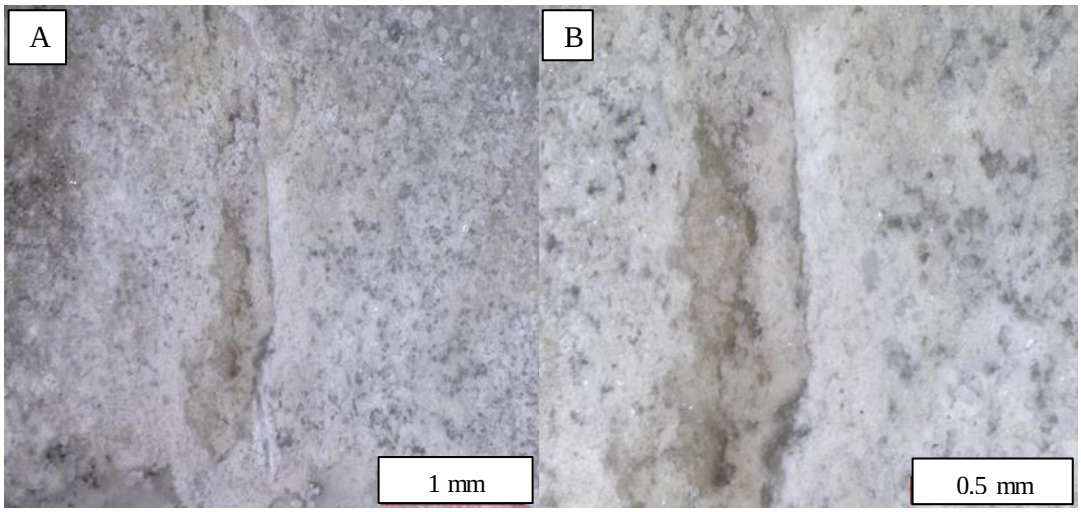


Figure 7.52: Crack after healing by injecting *B.pseudofirmus* in SHA-3 for set 5-4 at tension zone.

7.3.5.2 Initial surface absorption test (ISAT)

The results showed that rate absorbed in the surface of set 5-1 had high (ISAT) values. Figure 7.53 indicated that the average of set 5-4 during the test was close to the set 5-0 (without a crack). The graph also shows that the ISAT rate for all sets decreased with time. The rate of ISAT gradually decreased with time as the prism became saturated with water. In addition, Figure 7.53 confirmed that the efficiency of the sealing material that was produced by bacteria is of a high quality and these materials have the lowest ISAT rate compared to the set 5-1 and the sets (5-2 and 5-3).

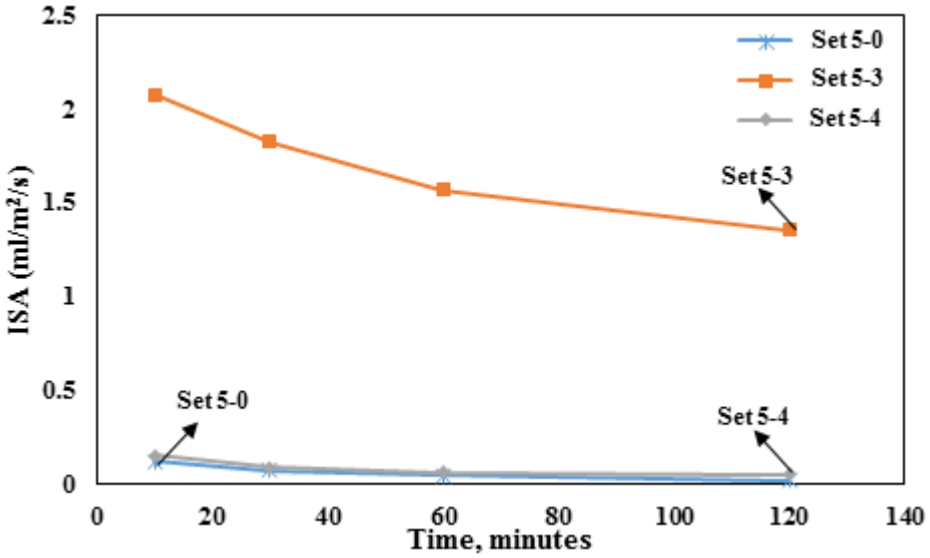


Figure 7.53: Initial Surface Water Absorption Rate for set 5-3, set 5-4 and set 5-0.

Figures 7.54 indicated that the mean ISAT average of the set 5-4 at 10 minutes was 0.152 ml/m^2/s and at two hours was 0.051 ml/m^2/s. The main ISAT average of the set 5-0 at 10 minutes was 0.122 ml/m^2/s and was 0.020 ml/m^2/s at two hours. Typically concrete/mortar is considered to have excellent resistance to ingress water according to BS 1881-5:1970[401] if it has values less than 0.24 ml/m^2/s at 10 minutes and 0.07 ml/m^2/s at two hours.

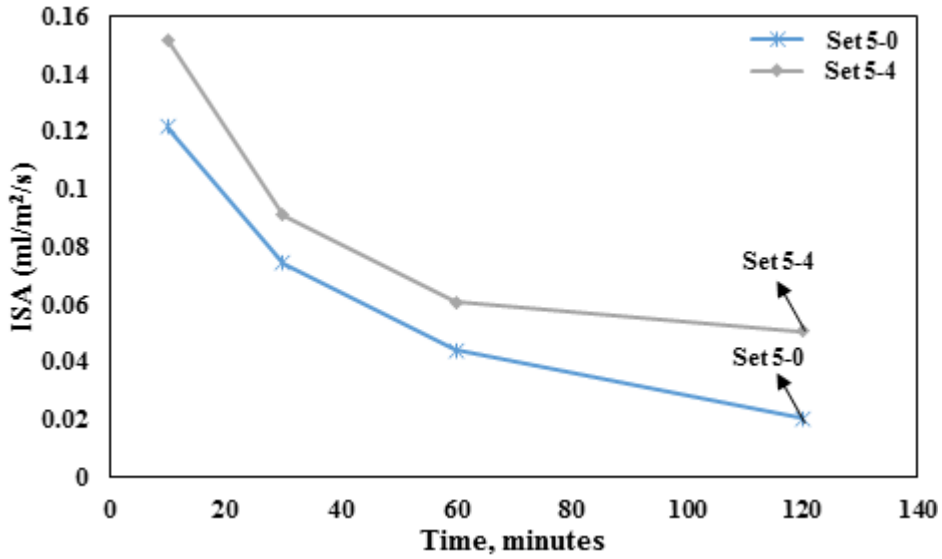


Figure 7.54: Initial Surface Water Absorption Rate for set 5-4 and set 5-0.

7.3.5.3 Permeability via water absorption

Figure 7.55 shows the average sorption coefficient (SC) of each set calculated - each set has three samples. The results revealed that the average of set 5-1 SC were the most absorbent, while the average of set 5-0 SC were the least absorbent. The *Pseudofirms* with SHA-3 sets had the ability to increase the durability of mortar by reducing the pore size in samples, whereas the average SC of its set was close to the average SC of the set 5-0. Further, the Sorptivity of set 5-3 and set 5-4 were almost similarly to that of the set 5-0, which are 2.29 $g/t^{0.5}$, 2.14 $g/t^{0.5}$, and 2.09 $g/t^{0.5}$ respectively. However, with the increase in duration of treatment crack by injecting bacteria with self-healing agent solution, set 5-3 and set 5-4 showed higher average sorption coefficient compared with that of the set 5-2 and set 5-1.

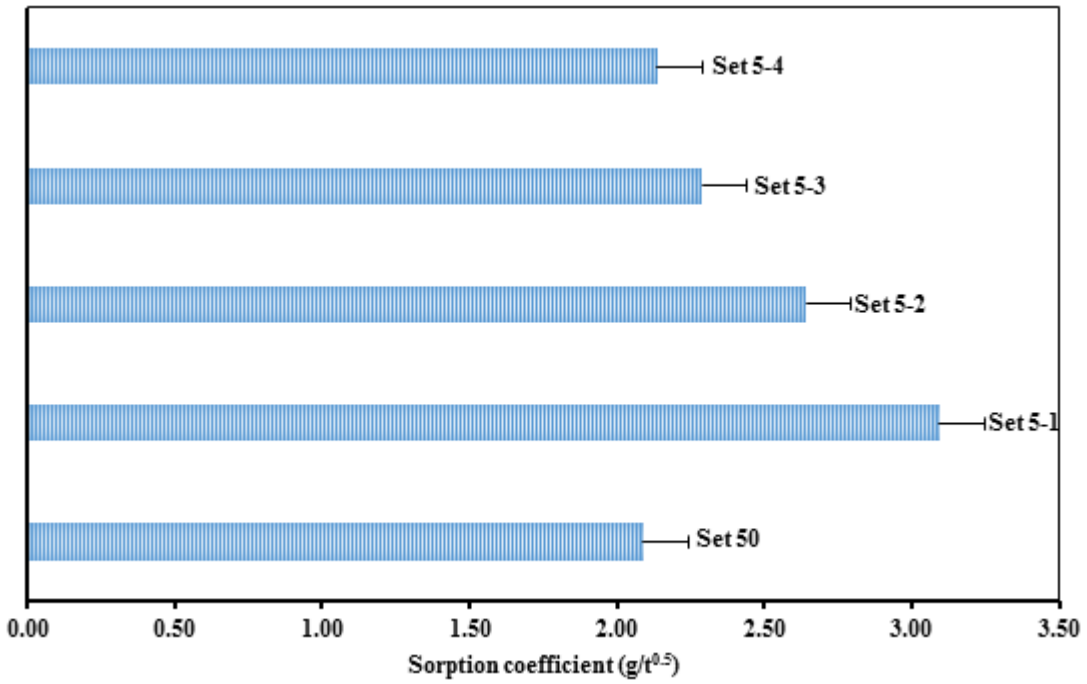


Figure 7.55: Average sorption coefficient calculated from permeability test via water absorption.

7.3.5.4 Summary

The relation between the number of feeding by *Bacillus pseudofirmus* with SHA-3 and time of healing was investigated in this section. Based on the results one can conclude that, the feeding by bacteria with SHA-3 for seven days presented a high efficiency material sealed crack completely, while the samples feeding for odd days also were sealed but the efficiency of material less quality compared to set fed for seven days continuously. The IAST analysis

presented the efficiency of materials produced by *B.pseudofirmus* with SHA-3 for seven days feeding close to the reference set. Moreover, capillary water absorption test demonstrated linear relation between feeding amount of bacteria with SHA-3 and the efficiency of sealing material produced by them into crack.

7.3.6 The effect of temperature on the ability of the three bacillus species to precipitate calcite (stage six)

As previously described, samples were prepared and divided into two groups, one of them kept at 20℃ and the other group kept at 40℃ during injection process. Those groups were treated for 28 days by directly injecting constant amounts, around 1ml bacteria+SHA-2, SHA-2, or water twice a day. The crack's treatment progress was observed by using a crack detection microscope. The observations about the progress of treatment in both categories showed no sealing inside all samples except the set 6-5 which, injected with *B.halodurans*+SHA-2. In these prisms cracks were sealed by only biofilm at the edges of the prism. The biofilm produced by set 6-5 could be due to growth of bacteria but it is not perfect to produce calcite, while it did not happen in the set 6-3 and set 6-4. These temperatures are suitable for growth the bacteria as presented in section 5.3.7 but according to the observation here all types of bacteria did not produce calcite in the cracks.

7.3.6.1 Summary

The effect of different temperatures on the ability of the three *bacillus* species to precipitate calcite was investigated in this section. Based on the results one can conclude that:

1- The observation shows that both *B.cohnii* and *B.pseudofirmus* had a stationary pattern between 25℃ and 30℃, in which they greatly grew. *B.halodurans* had a stationary pattern at 30℃ and 40℃ and the lowest growth was recorded at 25℃.

2- The ability of all types of bacteria with SHA-2 were investigated. The minimum crack width was 0.4 mm at the edges for all samples and they were treated for 28 days at 20℃ and 40℃. As a result of observation, we can say there was no healing for all types of bacteria and the most suitable temperature for bacteria to precipitate calcite was 30℃.

7.4 Conclusion

This section gathers the conclusions drawn from the experimental work reported in this chapter. The specific objectives of this work were described in the introduction of this chapter, and the experimental stages were designed to satisfy these objectives, which has led to the specific conclusion. These conclusions are drawn from the experimental results presented in the previous sections, and also from the tabulated results given in Table 7.1 and Table 7.3.

a- It was found that the three studied bacteria, *B.pseudofirmus*, *B.halodurans*, and *B.cohnii*, have the ability to generate calcium carbonate as a sealing material in the healing process.

b- According to ISAT results, the calcium carbonate by *B.pseudofirmus* is higher in quality than that produced by both *B.halodurans*, and *B.cohnii*, and *B.halodurans* produced the lowest quality of calcium carbonate than the other two bacteria.

c- *B.pseudofirmus* needs less time to start sealing as well as less time to finish sealing, which has an advantage over the other two bacteria (*B.cohnii*, and *B.halodurans*) and also has a lower precipitation time ratio (defined as the ratio between the amount of calcium carbonate precipitation to sealing time). *B.cohnii* had the ability to precipitate $CaCO_3$ with a precipitation time ratio higher than *B.pseudofirmus* and lower than *B.halodurans*.

d- Three types of bacillus had proven their ability to re-healing / re-sealing cracks over once.

e- Both *B.pseudofirmus* and *B.cohnii* can grow in temperatures that range between 25℃ and 30℃. *B.halodurans* can grow in temperatures up to 40℃, which can be used to remediate concrete cracks built in hot climate regions.

f- All the three bacteria are suitable for remediation of concrete/mortar cracks and the *B.pseudofirmus* is the most suitable bacteria for remediating concrete/mortar cracks than the other two (*B.cohnii*, and *B.halodurans*) because of its efficiency and that it is more economically viable (less time to start sealing/healing and less time to finish sealing/healing) in producing $CaCO_3$.

g- Qualitative observation specified that the ability of the three types of bacteria to seal cracks at different temperatures out of their growth range resulted in no progress for both *B.pseudofirmus* and *B.cohnii* with SHA-2 to seal cracks, while *B.halodurans* with SHA-2 produced only biofilm in the edges of the prism.

Finally, the readers are referred to the individual conclusion at the end of each section for more specific conclusions.

Chapter 8

Direct delivery systems of bacteria-based self-healing concrete

8.1 Introduction

Experimental studies have supported the application of mineral-producing bacteria in the remediation of concrete cracks, as presented in Chapter 7. The effect of SHA on physical properties such as setting time, hydration kinetic and microstructure, and mechanical properties such as developing and hardened compressive strength are presented in Chapter 6. The suitable bacteria, their ability to growth, survive on harsh environmental and their ability to produce calcite, as presented in Chapter 5.

The delivery of bacteria spores inside concrete environment has always been the most challenging task, so the main objective of this chapter to study the possibility of using successful previous delivery system used to remediate concrete cracks by using mineral agent such as epoxy[42], $Ca(OH)_2$[42], Na_2SIO_3[49], and retarder agent[66] to be used as delivery systems of bacteria spores inside concrete matrix such as vascular healing system, perlite as delivery healing system and microencapsulation delivery systems.

This chapter describes work to investigate the most efficient delivery systems of self-healing technique. This chapter covers three main stages of investigation:

- Stage one; the ability of calcium alginate beads to protect bacterial and bacterial self-healing agents during mixing and concrete hardening by using two different densities 3% and 5% was investigated.
- Stage two; divided into four parts, first part demonstrated capillary tubes as delivery system of bacteria and SHA, and parts (two, three, four) investigate the effect of viscosity on the efficiency of the vascular delivery using various materials.
- Stage three; evaluated the perlite properties, study the effect of perlite on the mortar properties such as flexural, compressive strength and porosity. Finally test the efficiency of perlite to encapsulate bacteria and bacterial self-healing agents.

8.2 Back ground

8.2.1 Calcium Alginate beads

Calcium alginate beads was produced by forming aqueous sodium alginate to aqueous calcium chloride using suitable equipment's such as a syringe to drop sodium alginate solution into calcium chloride solution. The reaction between two liquids are possible to form spherical bead particles and the chemical structures of calcium alginate are shown in Figure 8.1.

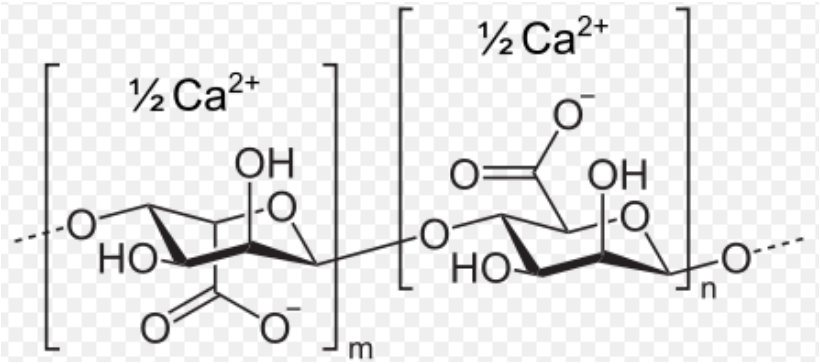


Figure 8.1: Chemical structure of calcium alginate beads[417].

The calcium alginate beads have been used in many applications such as a capsule. Many researchers used calcium alginate to encapsulate both macromolecular agents[418,419] and low molecular weight therapeutic agents[420–422] to control release both of them as an oral system of agents, also the calcium alginate beads used to capsulate *Bacillus licheniformis* KBR6 cells by immobilizing them in calcium alginate to protect bacteria during the manufacturing of tannin-acyl-hydrolase (tannase)[423].

8.2.2 Glass capillaries tubes delivery system

Various delivery systems were investigated. In the 1990's, Dry started investigating self-healing concrete[85] and polymers[41]. while, Kadam et al developed practical technique of self-repairing durable concrete[424]. Since 1950s microencapsulation has been used and has been employed in numerous construction materials[40]. White and co-workers[42,425] studied the application of chemical encapsulation for the production of self-healing polymeric materials. They conducted a number of experimental methods to release encapsulated chemical agents such as actuation, sensing and encapsulation into concrete cracks[426–429]. Many researchers

[44,54,78,80–82] have investigated the influence of capsule content, capsule size, and optimal capsule size on mechanical properties including the deflection capacity, flexural strength, and stiffness. Pelletier et al[49] investigated the self-healing properties of concrete material and corrosion inhibition such as the compressive strength recovery, ductility, the toughness and the attenuation.

The brittle hollow glass/fibres have been also used by many researchers for the last decade[47,148,149,188,262]. Whereas, Sangadji and Schlangen[2] used different techniques which tried to imitate the process of bone healing. Their experimental procedure was creating a porous network to simulate the "spongy bone" by putting porous concrete in concrete structure. They used cylindrical and beam samples for their experiment and injected healing agent manually after creating cracks on the specimens. The results showed that a macro-crack sealed and also strength of concrete was regained. While Nishiwaki et al[262] developed self-healing system for concrete by using self-diagnosis composite as a healing device. This heating device and pipe containing a repair agent made of heat-plasticity organic film was embedded in the concrete, then heating around crack by the heating device and pipes and they found that the repair agent fills up the crack and the repair agent in the crack to be hardened. Also the confirmed their experiment results by comparison with three-dimensional analysis. Sun et al[46] studied the efficiency of hollow glass fibre on the self-healing performance of micro cracks in concrete bridge by using repair agent. Moreover, Joseph et al[47] have been studied the effect of diameter and longitude of glass reservoirs on self-healing and the level of reinforcement and loading rate on the amount of self-healing. The conclusion of their experiments shows that during the first and second loading cycles healing occurs. This system of capillary glass tubes can be one channel vascular system which can carry one-component of healing agent which has been studied by Joseph et al[17],[47],[83],[84], or multi-channels system which used a combination of multi-component healing agents has been studied by Mihashi et al[76] and Dry and McMillan[12]. Therefore, when cracking occurs, this healing material is released from inside the tube or fibres and enters into the structural matrix.

One of the main mechanisms of self-healing agent's embedment in concrete was hollow fibre/glass systems. These systems present self-healing agents to the concrete through pipettes or/and tubes or/and pipes which is depending on the diameter. The philosophy of hollow fibre/glass systems is, when cracks generate due to one of structural cracks causes or due to one of non-structural cracks' cause, the tubes is broken due to tension stresses produced by

cracked mortar then, the self-healing agents release to flow into these cracks under gravity effect and capillary action as shown in Figure 8.2.

The hollow fibre/glass systems provide two types of modes, namely: active mode and passive mode as shown in Figures (8.3A and 8.3B). Both modes have embedded single and multi-tubes/fibre to heal cracks, and first application of this type was established between 1990 to 1994 by Dry[8,9,56,85,200] to investigate the ability of this system on healing cracks and reduce the permeability of beams. Different diameters of fibre/tubes have been studied by many researchers such as Mihashi's[76] used diameter of glass pipes in his research was 2mm for the outer diameter and 0.8mm for the inner diameter. While Joseph et al[190] used curved plastic tubes with 4mm for the outer diameter and 3mm for inner diameter.

8.2.2.1 Philosophy and Mechanism of the system

Due to a number of cracks randomly distributed in the structural elements, and insufficient amount of healing agent carried by microencapsulation in cementitious matrix to heal all cracks encourage a number of researchers to come up with novel idea to overcome this problem, by developing vascular networks within the concrete matrix. These tubes of voids are located in the tension side of the structural element Figures 8.2A and B. Thus, when the cracks generate due to the effect of the external loads or other the environmental effect, the tubes break and the containing sealing agents flow into cracks under the effect of gravity and capillary forces (Figure 8.2C). The healing procedure is completed when the healing agents inside the cracks is completed hardening, and sufficient strength recovery is reached as well as suitable reduction of permeability is achieved.

There were two modes passive and active studied in literature review chapter 2 associated with vascular delivery system Figures 8.3A and B. These two healing modes are differentiated by how much human intervention in their requirements to complete healing processes. A passive mode does not require any external intervention, whereas the active mode is completely depending on the human intervention to achieve a large degree of control. This mode is not the scope of this research, but this type of mode is not practical to be used in the field and will be more expensive than the passive mode.

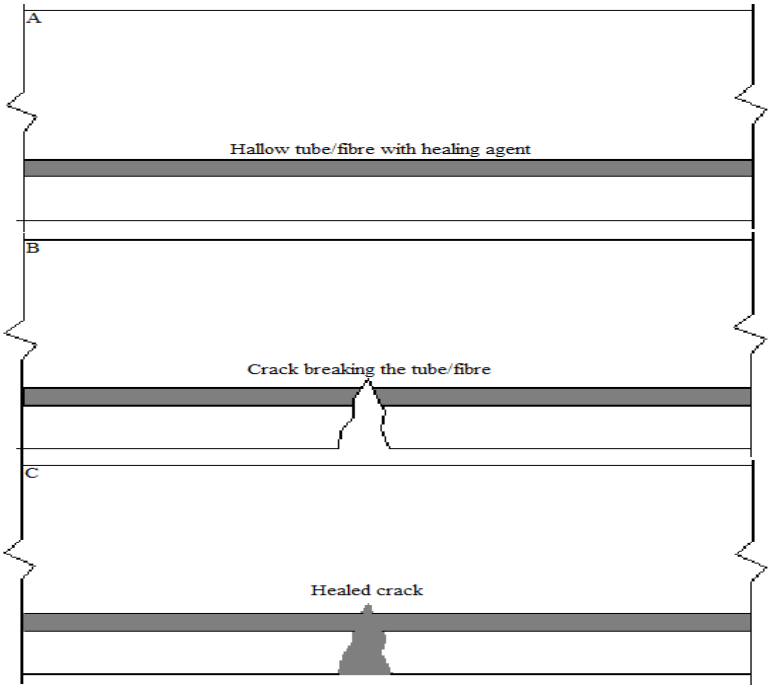


Figure 8.2: Shows the mechanisms of hollow tube/fibre system (adapted from Thao et al[37]).

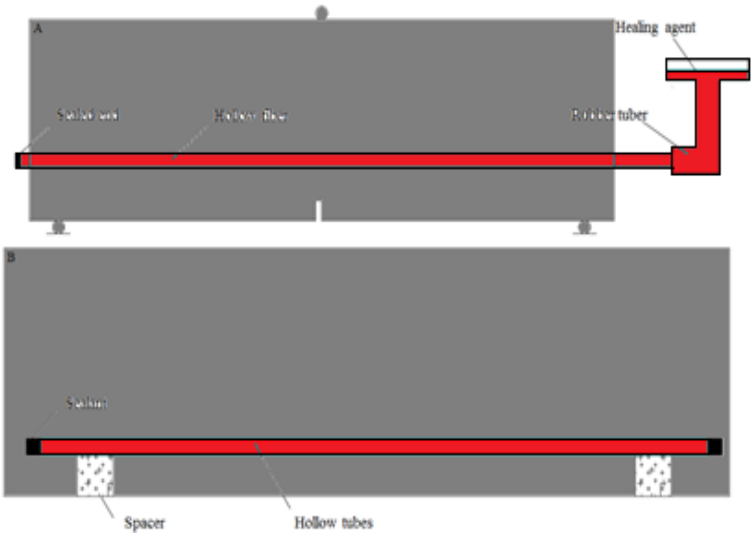


Figure 8.3: (A) Shows active mode and (B) shows passive mode (adapted from Thao et al[37]).

8.2.2.2 Vascular, healing delivery systems

Two types of the vascular healing delivery system were studied as given in chapter 2 such as vascular networks formed by some tabular material distributed uniformly within the concrete matrix and carrying the mineral healing agent. The other type is formed by tubes or voids inside the concrete matrix and these grove or channels were coated with suitable material to

insure smooth inflow and sufficiently low permeability (this type is not the scope of this research).

8.2.2.3 The factors effecting the efficiency of vascular delivery system

In order, this delivery system to work efficiently the following factors should be considered.

1- The inside and outside diameter of the using tubes.
2- The materials, which these tubes manufactured.
3- The viscosity of the healing agents.
4- The type of mode used in the testing (active or passive).
5- The pressure inside the used tubes.

8.2.3 Perlite

Perlite is a siliceous volcanic rock containing naturally occurring combination of two to six percent of water. When raw stone was in the ground and heated above 871°C, the water evaporates and the combination of perlite expands from four to twenty times of its original volume. This expansion process creates a number of cells in the vitreous particles, which have an excellent thermal conductivity of expanded perlite[430].

Perlite have been used in many aspects e.g. construction industry, air filtration, oil well treatments and other commercial and industrial aspects. The perlite used in all previous aspects due to competitive price (about $40.57 per ton of sold perlite), light weight, expands and thermal conductivity[431].

To significantly increase the functionality of bacteria and self-healing agents over time, in 2010 Wiktor and Jonkers applied two-component healing agent was protected by expanded clay particles [22]. The aim of this part of this research was to evaluate perlite as an alternative to expanded clay. The work investigated perlite properties, and the efficiency of perlite to encapsulate bacteria and bacterial self-healing agents. Finally study the effect of perlite on the mortar properties such as flexural, compressive strength and porosity.

8.3 Stage one: Calcium Alginate Beads

8.3.1 Use of Calcium Alginate Beads (CAB)

The ability of calcium alginate beads (CAB) to protect bacterial and bacterial self-healing agents during mixing and concrete hardening to prevent the premature production of calcite was investigated. We explored the effect of calcium alginate beads on fresh and hardened concrete properties as well as the ability of CAB to be used as a delivery system in mortar have been considered.

8.3.2 Materials and methods

Sodium alginate was obtained from the Biology Laboratory at Bath University. Part one; the amount of 3g of sodium alginate was weighed and dissolved in 100 mL of buffer. The buffer was made from 3mL of 1M of (30mM Tris–HCl, pH 9.5) put in 97 mL of distilled water. Then, the solution was mixed to homogeneity using a magnetic stirrer to form a 3% sodium alginate solution. The solution was stored overnight in cooling room before using it to produce calcium alginate beads. Calcium chloride ($CaCl_2$) solution with concentration of 2% was prepared. From 2g of $CaCl_2$ and 100mL of a buffer using a magnetic stirrer.

Part two; the amount of 5g of sodium alginate was weighed and dissolved in 100 mL of buffer. The buffer was made same as part one. Then, the solution was mixed to homogeneity using a magnetic stirrer to form a 5% sodium alginate solution. The solution was stored by following the same procedure in part one.

8.3.3 Preparation of calcium alginate beads

The production of calcium alginate beads was made manually using a large syringe diameter. The processing procedure involved dropping sodium alginate solution into the calcium chloride solution, in a large recipient. The gel of calcium alginate beads was formatted as a result of chemical reaction between sodium alginate solution and calcium chloride solution, as shown in Figure 8.4. Once the process of producing calcium alginate beads was completed, the beads were kept in a refrigerated room to avoid contamination.

Figure 8.4: Calcium alginate beads production.

8.3.4 Tests

In order to reach the target of this study the preliminary study was divided into two main parts as shown in Table 8.1 to investigate the behaviour of the beads with concrete (alkaline environments) and also to find their effect on the mechanical properties of concrete such as flexural and compressive strength.

Table 8.1: Design mortar with calcium alginate beads

3% & 5% calcium alginate beads concentration						
Batch	% of beads by mass (g)	W/C	Cement (g)	Standard sand (g)	Water (mL)	Beads (g)
1	0%	0.5	450	1350	225	0
2	1%(20.25)	0.5	450	1350	225	20
3	3%(60.75)	0.5	450	1350	225	61
4	4%(81.00)	0.5	450	1350	225	81

The mortar was mixed according to EN 196-1:2005[395]; sample size was 40x40x160 mm. Then, the beads added afterwards to the mix and mixed all proportions manually with a spatula until the mix became homogenous as shown in Figure 8.5. Then, both mortar batches for control and mortar with calcium alginate beads were cast into moulds and a vibrating table was used to reduce the air bubbles (Table 8.1). In this stage, reinforcement or fibre reinforcement was not used.

Figure 8.5: Shows calcium alginate beads during mix with mortar paste.

8.3.5 Results and discussion

The samples cured in a curing room at 20±2℃ with 80% relative humidity for 24 hours; then the moulds were removed. After 7 days of curing, the calcium alginate beads decreased in size in Batches 2–4 as a result of losing water during the curing process (Figure 8.6). The decrease of beads size caused holes on all sample's surface due to detachment of the beads from the mortar surface could be due to less contact surface angles between beads and mortar.

Figure 8.6: Shows calcium alginate beads mortar samples after 7 days.

On the other hand, 3% calcium alginate bead concentration (CAB) has a low density, and located on the surface of the samples as illustrated in Figure 8.7a,b. However, the minimal shrinkage of beads due to water loss as well as the lack of a uniform distribution among mortar samples meant that these could not be used to deliver bacteria and self-healing agents in mortar (part one).

The compressive strength and flexural for part one were affected by the presence of CAB in the mortar mix. However, the floating and shrinkage of calcium alginate beads in mortar samples caused a major implementation issues.

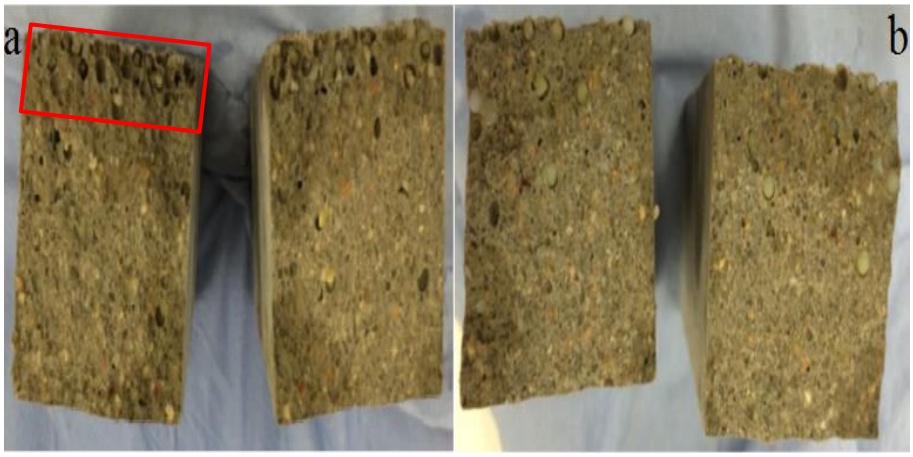


Figure 8.7: Cross-sections of calcium alginate beads in mortar samples after 7 days.

Part two; the density of the calcium alginate beads as increased from 3% to 5% to avoid the floating of beads during mixing by increasing the morality of beads Table 8.1[432]. Moreover, to avoid the shrinkage of beads size, the beads were air dried over a Teflon surface before supplying to the concrete mix for 24 hours. According to Lyn and Ying[433] to generate contact angles of the surface of the beads a Teflon surface was used to reduce the sphere deformation caused by the contact between mortar paste and beads.

After 24 hours of air-drying, small amount of beads had a significantly reduced in their size as shown in Figure 8.8. However, the reduction was minor for majority of beads.

Three batches of 5% CAB were casted to verify the behaviour of the beads during mixing, casting and curing of the mortar samples. It is clear from Figure 8.9 that CAB are not effective for encapsulating bacteria in concrete. This problem was not overcome by increasing calcium alginate bead concentration up to 5% the morality of beads, but they still have the same problem, which they have it with 3% calcium alginate bead concentration.

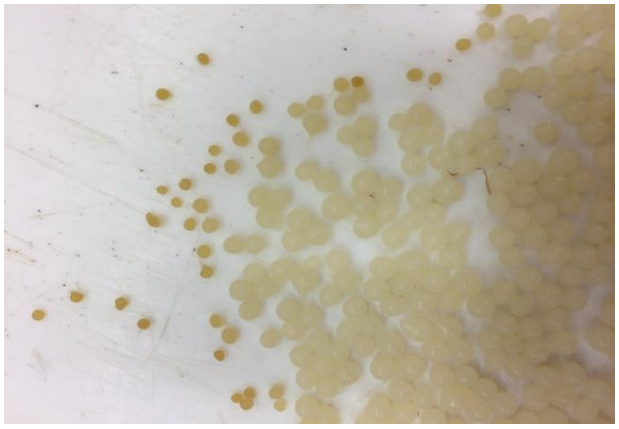


Figure 8.8: Calcium alginate beads after 24h of air-drying.

Figure 8.9: Cross-sections of calcium alginate bead mortar samples after 24 hours in the conditioning room.

8.3.6 Summary as use Calcium Alginate Beads (CAB)

Here we explored the concept of using CAB as direct delivery system for self-healing cement mortar, which was based on two main issues related to the CAB shell stiffness and its durability. The results indicated that CAB shells is very weak cannot stand shrinkage forces and are very light due to its low density which causing floating and poor distribution in mortar matrix. These two factors weaken the mortar strength and decreasing in its durability, so CAB cannot be used as delivery system in self-healing materials without further investigation, which is not scope of this research.

8.4 Stage two: Vascular tubes

8.4.1 Test preparation and methodology

The experimental set up as a passive mode. All three types of bacterial spore suspension, bacterial self-healing agents (SHA-1) were used as mentioned in chapter 5. The delivery system of capillary tubes was studied using small scale of mortar samples were designed according to EN 196-1:2005[24] and cement used was CEM II/B-V 32.5R with 0.5 W/C and EN a standard sand was used in all mortars.

8.4.1.1 Part one: Study the ability of using capillary tubes as delivery system

The capillary glass tubes were filled with water, spore suspension and SHA-1 using sterile 3ml syringes tipped with non-pyrogenic needles and almost positioning them horizontally, to avoid the adhesive and air bubbles that become trapped into the tube as a result of the capillary attractive force, then both ends were sealed by heating after packaging, and placed in mortar samples as illustrated in Figure 8.10. Water, spore suspension and bacterial self-healing agents were separately packed in capillaries glass tubes with specified dimensions as given in table 8.2. The specimens were divided into four experimental sets (three samples of each 40x40x160mm) as follow:

- Set 1-1 three control specimens were tubes filled with water only.
- Set 1-2 three specimens were tubes filled with *pseudofirmus* and SHA-1.
- In addition, three specimens were tubes filled with *cohnii* and SHA-1 is presented as set 1-3.
- Three specimens were tubes filled with *halodurans* and SHA-1 is known as set 1-4.

Then, cured in curing room at 20°C with 80% relative humidity for 24 hours followed by 9 days immersion in water at 20°C. All tubes were laid horizontally in tension zone close to the surface. All samples were subjected to an initial load (three-point load test) to generate cracks, then the samples were incubated in humid conditions for two weeks to allow healing process taking place and then, the load was applied again up to failure as a second cycle of loading.

This part considered several aspects, which effect the glass capillaries tube system such as viscosity of SHA-1 and bacteria solution (bacteria cells with buffer), distribution of tubes into mortar samples, diameter, length of tubes and encapsulation method.

Table 8.2: Properties of capillary tubes used in preliminary study

Tube outer diameter (mm)	2.0
Tube inner diameter (mm)	1.8
Wall thickness (mm)	0.2
Tube length (mm)	75
Internal volume of tube without end plugs (µl)	178

Figure 8.10: Shows capillary glass tubes preparation during casting of samples.

8.4.1.2 Part two: Investigate the effect of viscosity on the efficiency of the vascular delivery

The procedure of the experimental used in this section was established according to the part one study described in section (8.4.1.1). Sodium alginate have been used in many aspects in last century especially in the food industry to increase viscosity [434]. In this study sodium alginate was use to increase viscosity of both bacteria solution and SHA-1 solution, to facilitate their flow through the cracks. Water and sodium alginate with safranin dye were separately packed in glass tubes with measured dimensions as given in Table 8.3.

The glass tubes were sealed by heating from one end, and filled water, 1%, 3% and 5% sodium alginate with safranin dye by using sterile 3ml syringes tipped with non-pyrogenic needles and then, positioning them horizontally in the model, to avoid the adhesive and air Bubbles that might trapped into the tube as a result of the capillary attractive force. Then the

other end was sealed with a wax compound. The specimens were divided into four experimental sets as follow:

- Set 2-1 three control specimens were tubes filled with water only.
- Set 2-2 three specimens were tubes filled with 1% sodium alginate with safranin dye.
- In addition, three specimens were tubes filled with 3% sodium alginate with safranin dye is presented as set 2-3.
- Three specimens were tubes filled with 5% sodium alginate with safranin dye is known as set 2-4.

Table 8.3: Properties of glass tubes used in investigate the viscosity of delivery system

Tube outer diameter (mm)	6.2
Tube inner diameter (mm)	6.0
Wall thickness (mm)	0.2
Tube length (mm)	125
Internal volume of tube without end plugs (µl)	2826

The number of sets as well as number of samples in each set, method of curing was the same as those used in previous section (8.4.1.1). The cracks were generated by subjecting the samples to three-point flexural test.

8.4.1.3 Part three: Study different types of polysaccharides for packing SHA

The experimental was established according to the part one study described in the previous section (8.4.1.1). Polysaccharides have been used in enormous industrial applications specially in the food industry[435]. The main polysaccharide features are its ability to change the properties of aqueous environments, and also its ability to thicken, chelate, emulsion, stabilized, encapsulate, flocculation, swell and suspend, or as a gel, film and membranes. The polysaccharide properties of swell and suspend to release of healing agents out of tubes, are investigated in this section. The polysaccharide is known as absorption of water, which might push and/or increase the viscosity of bacterial and SHA-1 out of capillary into micro-crack and their properties for all experiments are described in Table 8.4. Spore suspension and SHA-1 with for autoclave and un-autoclave polysaccharides were separately packed in glass tubes with measured dimensions as given in Table 8.3. The specimens were divided into nine

experimental sets for autoclave and other nine sets for un-autoclave polysaccharides with SHA agent as follow:

- Set 3-1 three specimens were tubes filled with *pseudofirmus* and 1% Alginate with SHA-1.
- Set 3-2 three specimens were tubes filled with *pseudofirmus* and 2% Alginate with SHA-1.
- In addition, three specimens were tubes filled with *pseudofirmus* and 3% Alginate with SHA-1 is presented as set 3-3.
- Set 3-4 three control specimens were tubes filled with *pseudofirmus* and 1% Gelrite with SHA-1.
- Set 3-5 three specimens were tubes filled with *pseudofirmus* and 2% Gelrite with SHA-1.
- In addition, three specimens were tubes filled with *pseudofirmus* and 3% Gelrite with SHA-1 is presented as set 3-6.
- Set 3-7 three control specimens were tubes filled with *pseudofirmus* and 1% Xanthan gum with SHA-1.
- Set 3-8 three specimens were tubes filled with *pseudofirmus* and 2% Xanthan gum with SHA-1.
- In addition, three specimens were tubes filled with *pseudofirmus* and 3% Xanthan gum with SHA-1 is presented as set 3-9.

All samples were prepared, casted, and cured as previous two parts as shown in Figure 8.11. Then, the samples were subjected to 3-point flexural test to generate controlled crack width (0.4mm). Then all samples put in an incubator in which the SHA-1 inoculated with microorganisms (bacterial) are cultured at a constant temperature 30°C as shown in Figure 8.12.

Table 8.4: different percentage of three types of polysaccharide used to investigate the ability of them as delivery system.

Types of polysaccharides	Percentage of types of polysaccharides	Dimension of tubes	No. of autoclaved polysaccharides with bacterial self-healing agents samples	No. of unautoclaved polysaccharides with bacterial self-healing agents samples
Alginate	1% 2% 3%	As shown in Table 8.3.	Each type has 27 samples	Each type has 27 samples
Gelrite	1% 2% 3%			
Xanthan gum	1% 2% 3%			

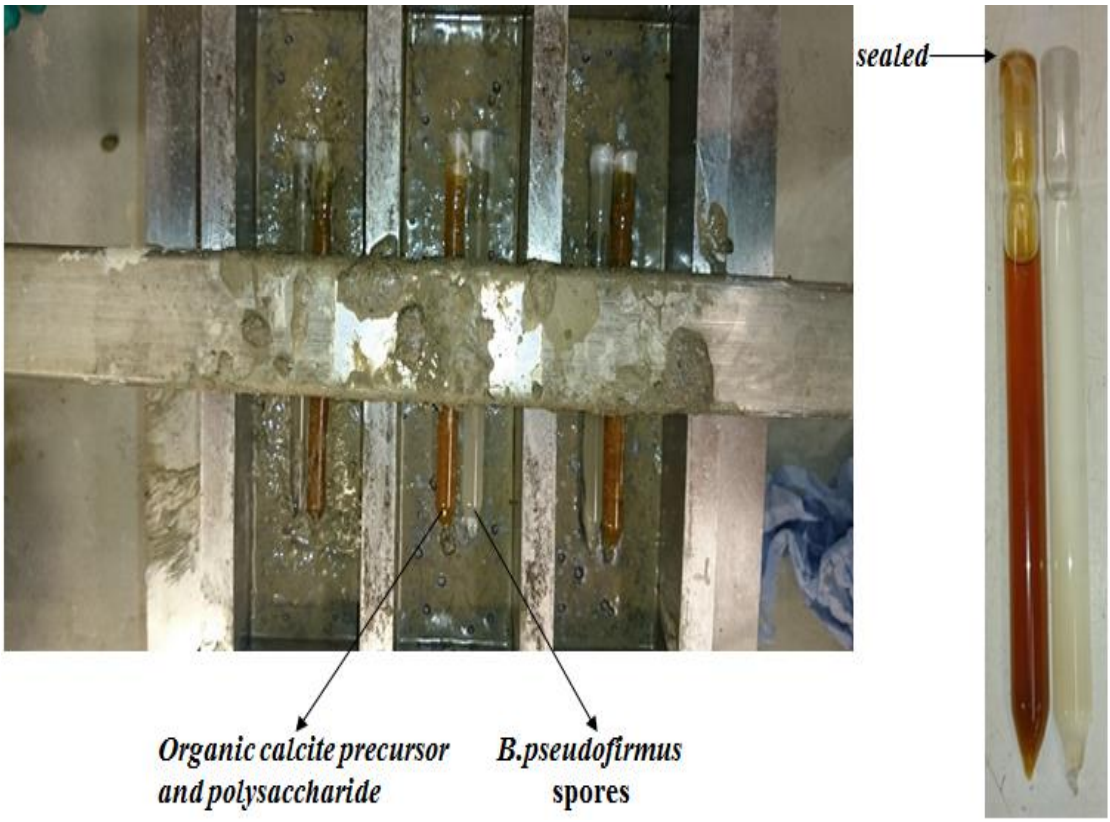


Figure 8.11: Preparation of glass tubes with bacterial and bacterial self-healing agents during sample casting.

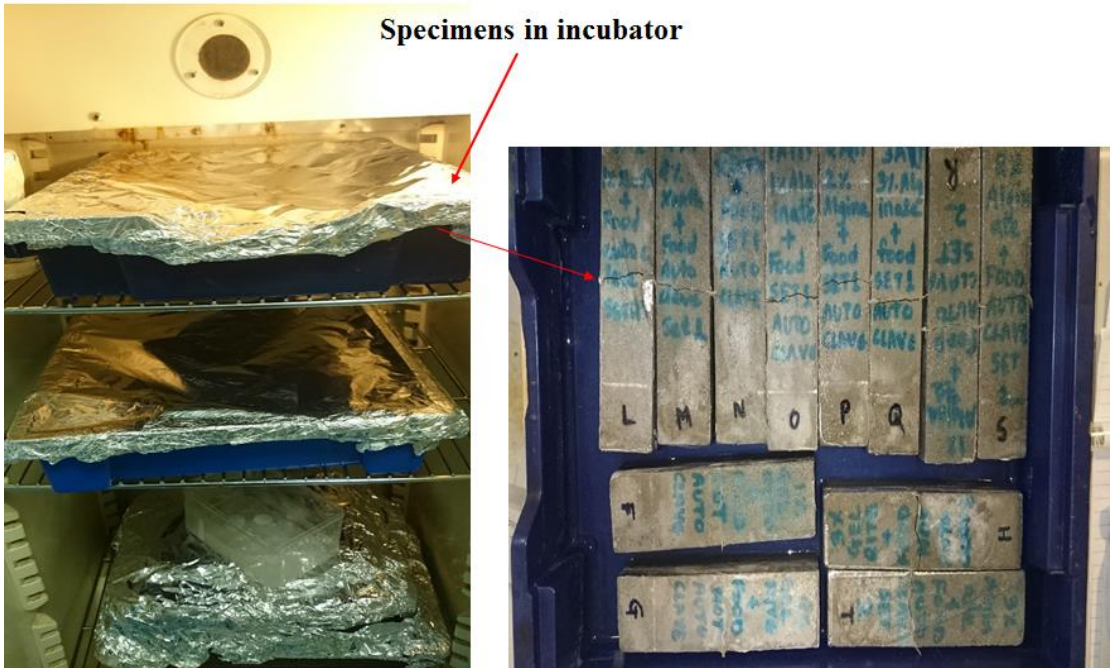


Figure 8.12: Samples with bacterial and bacterial self-healing agents incubated at 30°C.

8.4.1.4 Part four: Superabsorbent polymer used as delivery system for packed SHA

The experimental set up, number of samples, method of curing, and method of testing were the same as those presented as previous study described in section **(8.4.1.1)**, the only differences are materials used as delivery system of healing agents into crack. Superabsorbent polymer (SAP) have been used in enormous industrial applications it. The main SAP features is its ability to absorb water several thousand times of its weight and its ability of keeping water under pressure, and releasing it slowly when it became dry. The ability using swell and suspend of SAP properties to release of healing agents out of tubes is investigated as delivery system in this section.

Bacterial spore suspension, SHA-1 mixed with SAP as powder by ratio 3:1, and SHA-1 with SAP positioning at the edges of tube only as shown in Figure 8.13. were separately packed in these glass tubes and the measured dimensions of the used tubes are given in Table 8.3. The specimens were divided into two experimental sets as follow:

- Set 4-1 three specimens were tubes filled with *pseudofirmus* and SAP mixed with powder of SHA-1.
- Set 4-2 three specimens were tubes filled with *pseudofirmus* and SHA-1 with SAP positioning at the edges.

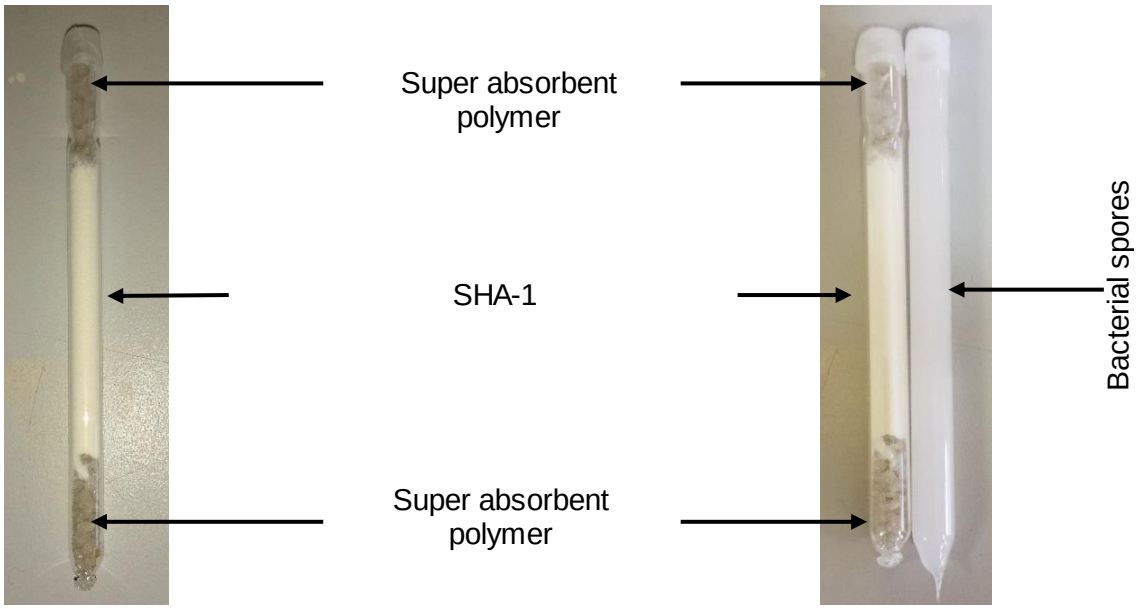


Figure 8.13: Packed bacterial self-healing agents with SAP at edges.

All samples have dimension 40x40x160mm were prepared according to the BS EN 196-1[395], the mortar mixture is poured into moulds in 3 layers and subjected to vibration each layer to expel air more in the mixture and then tubes were inserted after first layer as shown in Figure 8.14.

Figure 8.14: Preparation of glass tubes with bacterial and bacterial self-healing agents during casting of samples.

All samples were cured in curing room at 20°C and relative humidity was 80% in a curing room for 24 hours followed by 9 days' immersion in water at 20°C. All samples of crack were controlled at 0.4 mm by using small piece of fibre (spacers) when they were subjected to three-point flexural test. Then all samples put in an incubator after inject them once by Distilled water, in which SHA-1 inoculated with microorganisms (bacterial) are cultured at a constant temperature 30°C for more than one month.

8.4.2 Results and discussion

8.4.2.1 Observations about the delivery system mechanism using capillary tubes (part one)

The tested samples were inspected visually, and they found all the tested tubes were broken due to the tension strength generated at lower face of surface caused by flexural strength and also it was noticed no evidence of healing on all samples, whilst it was observed also during and after the test that the amount of spores suspension and SHA-1 release from the broken tubes were not sufficient to produce an enough amount of self-healing materials. Moreover, it was found that a considerable amount from both bacteria spores and SHA-1 were remain inside capillary glass tubes even after they were broken, and some of these tubes' cross-sections were blocked by inorganic materials preventing the healing materials to come out. These organic materials could be due to precipitate of bacteria with SHA-1.

It was concluded from the results of this part that the small diameter of tubes, atmosphere pressure on the cross section of tubes, capillary action between glass tube and liquid and tension surface of liquid into cross section of breaking tube were greater than both gravitational force and attractive force of capillary due to the opening crack as illustrated in Figure 8.15. Which allowed, only a limited amount of bacteria spores and SHA-1 were released into the crack zone.

In order to avoid all these issue, glass tubes were used in mortar samples for the further experimental setup (section 8.4.1.2) had larger diameter and longer than the previous experiment. This meant that amount of bacteria spores and SHA-1 are increased by fifteen times due to find out the volume of tube according to its cross section and its length and the atmosphere pressure, capillary action and tension surface of liquid across the cross section of breaking glass tube are neglected.

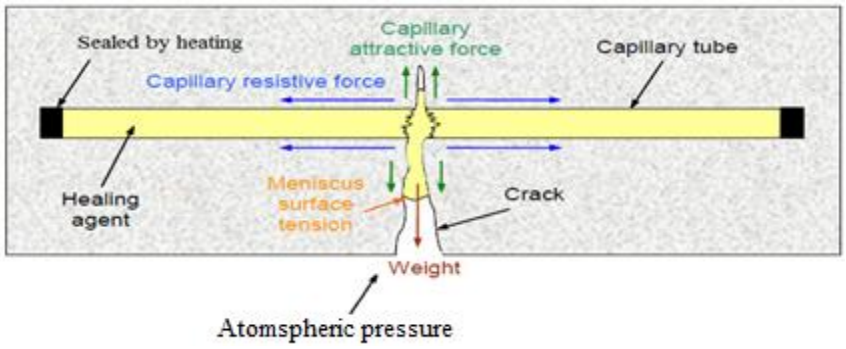


Figure 8.15: Effect of forces on capillary glass tubes (adapted from Joseph et al[47]).

8.4.2.2 Observations about the work efficiency of the delivery system using sodium alginate with safranin dye (part two)

Figure 8.16A shows the crack-taking place and water leakage due to broken of glass tubes. While, Figure 8.16B shows all water released from tube, meant that a crack width was enough to allow solution inside tube come out, thickness of tube is suitable to resist internal stresses of mortar and the bond between glass tube and mortar is sufficient.

Figure 8.16C shows small amount of 3% sodium alginate gel with safranin dye egress from tube cross section. Which means that viscosity of sodium alginate was very high, even other two percentage has the same results. Therefore, sodium alginate is not suitable to be used as a delivery system of bacterial in concrete.

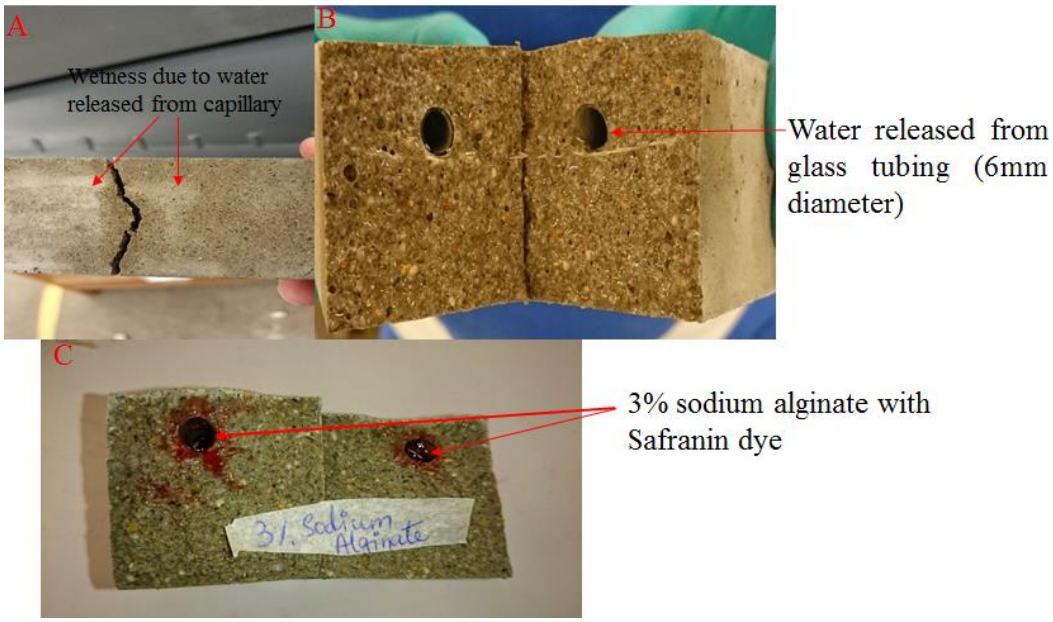


Figure 8.16: Shows mechanism of delivery system using water and 3% sodium alginate.

8.4.2.3 Some observation about the work efficiency of the delivery system using different types of polysaccharides (part three)

Three sets of polysaccharides with bacterial and SHA-1 sets were incubated at a constant temperature 30°C as shown in Figure 8.12. these sets were monitored once a week using a crack detection microscope with magnification x40 which supplied from C&D (Microservies) Ltd for three continuous months.

From inspection, it was clear that glass tubes were broken due to the applied flexural strength, which caused tensile failure at lower alkali resistant fibreglass mesh. There was no evidence of healing on all samples during incubation period. Moreover, it was found that both bacteria spores tube and SHA-1 tube with one type of polysaccharides had a considerable amount remain from bacterial self-healing agent and bacterial spores inside glass tubes even after they were broken, and one of these tubes cross section was blocked by gel materials due to viscosity of polysaccharides materials or the width of crack was too narrow to allow polysaccharides materials with SHA-1 come out. The other glass tube cross section was blocked due to inorganic materials precipitate from react bacteria spore suspension with bacterial self-healing agents.

8.4.2.4 Observation about delivery system mechanism using Superabsorbent polymer (part four)

The images by using CT scan show a crack pass through tubes, and both tubes were broken and bacterial spore suspension, bacterial self-healing agents came out from tube as shown in Figure 8.17.

From the visualization and CT scan, images it was concluded that the breaking occurred due to the flexural strength caused tensile failure, and there was no evidence of healing on all samples, whilst it was also observed during and after the test that the amount of spore suspension released were not sufficient to produce an enough amount of self-healing materials.

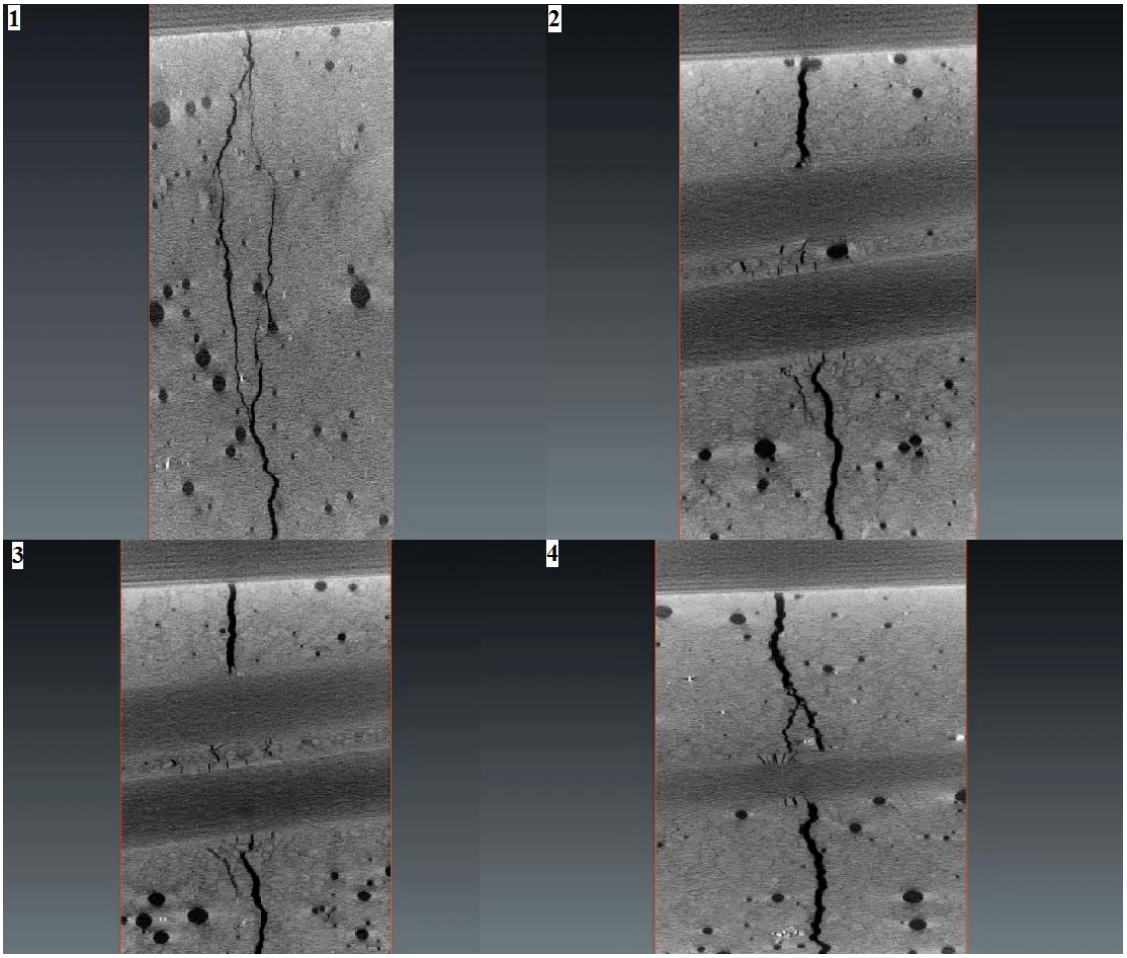


Figure 8.17: CT scan for glass tubes in different positions with bacterial and bacterial self-healing agents.

8.4.3 Summary of use vascular tubes

The results obtained in the four delivery self-healing for remediation of concrete cracks tests are insufficient to prove or disprove the hypothesis that the three modes of delivery system for bacteria and their agent to be delivered directly to remediate concrete cracks. In fact, the experimental results from these four delivery system indicated that the self-healing mechanism was not activated in four model of the delivery systems, however, the observation to the experimental output indicated that a considerable amount remain from bacterial self-healing agents and bacteria spores inside glass tubes even after they were broken and some of these tubes their cross section were blocked by these materials.

In spite some effecting variable on the delivery system model were studied like tube length and diameter, viscosity of the bacteria agent, and the ability of glass tubes to resist internal

stress due to mortar hardened were also studied, but no positive results were reached. However, using a passive mode as delivery system for hidden pipes in concrete gave only low self-healing efficiency in all four tested delivery system due to the negative pressure forces generated by the capillary tube action due to the effect of its ends. Under these conditions, the driving forces to the bacteria and their agent namely capillary attractive forces of the cracks and gravitational force applied to the out coming bacteria agent from the sealed pipe were not sufficient to overcome the resisting forces produced by the sealed tube boundary and its negative pressure force came from the sealed ends. Thus, in order to increase and facilitate the flow of bacteria agent from inside the pipe into cracks an active mode is necessary to overcome this issues.

The results obtained indicate that additional experiments and analysis of the effecting variables were needed to gain additional insight into the studied delivery systems, and that a new research plan based in these experimental observations and results was needed to cover all effecting variable and to find the most influential one in order to come up with new workable direct delivery system to the bacteria self-healing agent.

To this end, the research presented in this section was to explore whether the used delivery system using chemical agents can be used as delivery system to the bacteria self-healing agent, and the found results gave negative answer, so as mentioned above more intensive research is needed in this area which was not the task of this thesis

8.5 Stage three: Perlite

This stage was divided into four main parts; First part was evaluated the physical and mechanical properties of uncoated perlite and the distribution of perlite inside the mortar.

Second part was studied the physical and mechanical properties of coated perlite.

Third part was investigated the efficiency of perlite to encapsulate bacteria and bacterial self-healing agents and their ability to produce calcite outside perlite.

Fourth part was demonstrated the ability of bacteria and SHA inoculate into perlite to heal cracks in cement mortar.

8.5.1 Investigate physical and mechanical properties of pure perlite (uncoated) and studying the effect of these properties on mortar properties (part one)

8.5.1.1 Materials and methods

The delivery system of perlite was studied by using small scale of mortar samples, which were designed according to EN 196-1:2005[24] and the used cement was CEM II/B-V 32.5R with 0.5 W/C, which was used a tap water for all the experiments. EN standard sand was used in all mortars, and five sets of three samples each were prepared and studied, one set was taken as control (0.0% of perlite) and the other sets were prepared with different percentage of perlite as given in Table 8.5. The aim of this experiment is to study the effect of % of perlite as supplement of sand on concrete mortar such as its workability, compressive strength, and physical properties.

Table 8.5: Mix proportions of test specimens using uncoated perlite as supplement of sand.

	Mix design				
	Perlite to sand ratio (vol.)	Cement (g)	Perlite (g)	Sand (g)	W/C ratio
Control	0.00%	450	0.0	1350	0.5
Batch 1	0.50%	450	27	1266	0.49
Batch 2	1.5%	450	56	1000	0.46
Batch 3	2.5%	450	62	650	0.43

8.5.1.2 Physical and mechanical properties of uncoated perlite

The physical and mechanical properties of uncoated perlite, such as bulk density, apparent density and absorption capacity are given in Table 8.6. All the physical and mechanical properties were tested according to BS EN 1097[398].

Table 8.6: Physical properties of uncoated perlite.

Loose bulk density	121.6 kg/m^3
Apparent density	291.8 kg/m^3
Absorption Capacity	146.32%
Surface Moisture	1.00%
Moisture Content	0.4%

While the distribution of particle size of perlite was tested by sieve analysis test according to BS EN933[399]. Amount of 100g of perlite was poured into the top of a group of a nested sieve, which has 4-mm screen openings on the top and a pan at the base. Then a group of nested sieves shaken for period of time by mechanical means, the perlite retained on each sieve was weighed and is given in Table 8.7.

Table 8.7: Sieve analysis results of perlite according to the limits of BS EN 933[399].

Sieve size (mm)	Cumulative mass retained (g)	Percentage retained	Cumulative percent retained	Percentage of passing
4.00	6.77	6.78	6.78	93.22
2.00	43.59	43.69	50.47	49.53
1.00	32.91	32.98	83.45	16.55
0.50	10.96	10.98	94.43	5.56
0.25	1.84	1.84	96.27	3.72
0.20	3.74	3.75	100.00	0.00
Pan	0.22			
Total	100			

8.5.1.3 Observation of uncoated perlite distribution inside mortar samples

Four groups of mortar samples were studied as given in Table 8.5. Perlite expands from four to twenty times of its original volume when it is immersed in water; therefore, expanded perlite could be used to deliver healing agents. All samples were prepared as mentioned in section 8.5.1.1. The mortar mixture was poured into moulds in three layers and subjected to vibration to expel more air into mixture. Compressive strength tests were carried out on cured samples at 7, 14, 21 and 28 days.

8.5.1.4 Effect of uncoated perlite percentage on strength of mortar

Four groups of mortar prisms prepared in accordance with EN 196-1 with addition of 0, 0.5, 1.5 and 2.5% of perlite by volume of standard sand and cured in curing room at 20°C and relative humidity was 80% for 24 hours followed by immersion in water at 20°C for the rest of curing period as shown in Table 8.5. The prisms were tested at 7, 14, 21 and 28days.

8.5.2 Investigate physical and mechanical properties of coated perlite and effect of these properties on mortar properties (Part two)

To verify the suitability of perlite in terms of its ability to prevent the nutrients from being released into the concrete, initial tests were carried out in which safranin as presented in section 8.5.2.3, a dye commonly used to stain microbial cells, was added to the perlite. These stained perlite were then added to mortar. On inspection of cut faces from the hardened faces, it was clear that there was substantial leakage of the dye from the perlite. Further trials considered a number of coatings that could be used to prevent dye leakage. It was found that a double layer of protection: consisting of a layer of sodium silicate and a layer of Portland fly ash cement prevented leakage of the dye. The sodium silicate coating was applied by soaking the impregnated perlite in sodium silicate solution until the perlite was completely wet. The perlite was then dried at 20°C for 24 hours. A second layer of sodium silicate was then applied to the perlite, as above, followed by the application of dry cement to the wet sodium silicate surface. The perlite was then cured in water for 48 hours.

8.5.2.1 Materials and methods

The samples of mortar were designed according to EN 196-1:2005[24] and the procedures of preparing all samples were followed the same steps in section 8.5.1. Six sets of three samples each were prepared and studied, one set was taken as control (0.0% of coated perlite) and the other sets were prepared with different percentage of coated perlite as given in Table 8.8. The aim of this experiment is studying the effect of coated perlite percentage on concrete mortar such as its workability, compressive strength, and physical properties.

Table 8.8: Mix proportions of test specimens using coated perlite as supplement of sand.

Mix design					
	Perlite to sand ratio (vol.)	Cement (g)	Coated perlite (g)	Sand (g)	W/C ratio
Control	0.00%	450	0.0	1350	0.5
Batch 1	5.0%	450	121	1282	0.5
Batch 2	10.0%	450	175	1217	0.5
Batch 3	15.0%	450	242	1150	0.5
Batch 4	20.0%	450	303	1082	0.5
Batch 5	25.0%	450	364	1015	0.5

8.5.2.2 Physical and mechanical properties of perlite

The properties of the coated perlite (in the absence of bacteria and nutrients) are given in Table 8.9. Based on comparison of the density of the coated and uncoated perlite it was estimated that the mass of the coating was approximately 80% of the overall mass of the coated perlite. Further, this technique was used to coat encapsulated of nutrients (calcium acetate and yeast extract) and bacteria spores (*B. pseudofirmus*) encapsulated was also coated.

Table 8.9: Physical properties of coated perlite.

Loose bulk density	476 kg/m^3
Apparent density	1050 kg/m^3
Absorption Capacity	15.7%

The coated perlite particle size distribution was tested according to BS EN933[399] 'Tests for geometrical properties of aggregates. Determination of particle size distribution. Sieving method'. Amount of 100g of coated perlite was poured into the top of a group of nested sieve, which has 4-mm screen openings in the sieve on the top and a pan at the base. Then a group of nested sieves shaken for ten minutes by mechanical means, the coated perlite retained on each sieve was weighed and is given in Table 8.10.

Table 8.10: Sieve analysis results of perlite according to the limits of BS EN 933.

Sieve size (mm)	**Cumulative mass retained (g)**	**Percentage retained**	**Cumulative percent retained**	**Percentage of passing**
4.00	17.00	17.08	17.08	82.92
2.00	81.56	81.93	99.01	0.99
1.00	0.66	0.66	99.67	0.33
0.50	0.05	0.05	99.72	0.28
0.25	0.08	0.08	99.80	0.20
0.20	0.20	0.20	100.00	0.00
Pan	0.45			
Total	100			

8.5.2.3 Effect of coated perlite percentage on strength of mortar

Six groups of mortar prisms prepared with addition of 0, 5.0, 10.0, 15.0, 20.0 and 25.0 % of perlite by volume of standard sand as presented in Table 8.8 to assess the effect of the coated

perlite (containing nutrients) on the mechanical properties of the concrete. After mix was made followed by cured samples in curing room at 20°C and relative humidity was 80% for 24 hours followed by immersion in water at 20°C for the rest of curing period. The prisms were tested at 3, 7 and 28 days to ensure that the nutrients were well encapsulated and that the process had not affected the early-age mechanical properties of the concrete mortar.

8.5.3 Investigate the efficiency of perlite to encapsulate bacteria and bacterial self-healing agents and their ability to produce calcite outside perlite (Part three)

The nutrients (calcium acetate and yeast extract) was encapsulated separately from the bacteria spores (*B. pseudofirmus*) to minimize the potential for germination before a crack is formed. The perlite was impregnated with the nutrients and bacteria by soaking the perlite in the appropriate volume of solution until all solution was absorbed. The composition of the perlite is shown in Table 8.11.

Table 8.11: Composition of uncoated perlite, per g of perlite, after "impregnation" with bacterial agents.

	Calcium acetate, g	Yeast extract, g	Spores (B. *pseudofirmus*)
Perlite with nutrients	0.3	0.03	-
Perlite with spores	-	-	approx. 4.1×10^9

8.5.3.1 Survivability of bacteria spores inside perlite

Tests were also carried out to ensure that the viability of spores (ability to germinate) was retained after impregnation in the perlite. Experiments were conducted under sterile/aseptic conditions by crushing perlite impregnated with *B. pseudofirmus* spores at 0, 3, 15, 30 and 90 days. 1g of each sample was obtained at each time period and serially diluted (10^{-1}-10^{-9}) in test tubes. These were then vortexed for two minutes to provide homogeneity. The viability of spores in terms of colony forming units (CFU) was then determined.

8.5.3.2 Investigate the ability of bacteria and bacterial agent ingredient inside perlite to produce calcite

Two types of experimental sets were prepared in the laboratory to assess the ability of both (*B. pseudofirmus*, bacterial agent ingredient) to produce calcite outside perlite. It was important to ensure that both *B. pseudofirmus* and bacterial agent ingredient were enough to produce calcite when the crack cross a lightweight aggregate (perlite) due to adverse

influence of environment on concrete. The number of bacteria and amount of bacterial agent ingredient are provided in Table 8.11.

Perlite was impregnated by soaking it into *B. pseudofirmus* with buffer for the first set and bacterial agent solution for the second set until the perlite was completely wet. The perlite was then dried at 20°C for 24 hours. First set of experiment was taken 2.5 g of grinding impregnated perlite by *B. pseudofirmus* mixed well with 25 mL of LB acetate growth medium and was incubated at 30°C on an orbitary shaker for 15 days. Second set of experiment was prepared by taking 2.5 g of grinding impregnated perlite by *B. pseudofirmus* mixed well with three times of its weigh of grinding impregnated perlite by bacterial agents and keep them in 75 mL buffer solution, as above.

After 7 days, 15 days, 10 mL first set, and 30 mL second set were centrifuged at 10,000 rpm for 15 minutes. The precipitation was washed two time with distilled water. The precipitation was then oven dried at 50°C for three days to obtain a dry powder.

8.5.4 Prepare mortar samples with inoculation perlite (part four)

The mixes proportion were designed as previous parts. The standard sand (without self-healing agents) was reduced to permit the use of perlite as a replacement, despite the fact that the perlite was coated with some unhydrated cement. The mortar mix used was given in Table 8.12. All samples edges were coated by fibre resistance fire to resist the stresses due to tensile load and to keep the sample as one unit. A tensile splitting strength test was applied to each prism after a 28-day curing period in order to create a single crack at the centre of the sample. For all samples, the crack width was measured using a linear variable differential transformer (LVDT) sensor as shown in Figure 8.18 and the crack width was increased by a speed of 0.025 mm/s until a crack width of 0.35 mm was reached. This can be seen in Table 8.13.

Additionally, the mortar prisms were placed on plastic support (spacers 10-15 mm) above water by approximately 2.0-5.0 mm from the bottom of the bucket. The specimens were fed once by water in first 24 hours, directly into the cracks, by sterile 3ml syringes that had non-pyrogenic needles. The mortar blocks were then stored in an incubator at 30°C for 165 days. Then after healing, the cracks were measured again by microscope. Then, the initial surface absorption test described in BS 1881-208[436] was used as measure of the efficiency of the healing material(s) to exclude water.

Table 8.12: Mix designs for mortar samples

Mix Set	Perlite content (% by volume of sand)	CONSTITUENTS, g				
		Water	Cement (CEM II/B-V 32.5N)	Standard sand	Coated perlite (with nutrients)	Coated perlite (for spores)
Set 4-0	0	225	450	1350	0	0
Set 4-1	0	225	450	1350	0	0
Set 4-2	20	225	450	1080	306	0
Set 4-3	20	225	450	1080	275	31
Set 4-4	20	225	450	1080	245	61
Set 4-5	20	225	450	1080	214	92
Set 4-6	20	225	450	1080	184	122
Set 4-7	20	225	450	1080	153	153

Table 8.13: LVDT measurement of crack width

Damage introduction	(t= 28 days)	temperature =19.4°C		humidity=40%
Specimen	W1 (μm)	W2 (μm)	% coated perlite with nutrients	% coated perlite with bacteria spore
Set 4-1	357	187	0	0
Set 4-2	350	68	100%	0%
Set 4-3	356	110	90%	10%
Set 4-4	353	72	80%	20%
Set 4-5	354	96	70%	30%
Set 4-6	355	108	60%	40%
Set 4-7	351	102	50%	50%

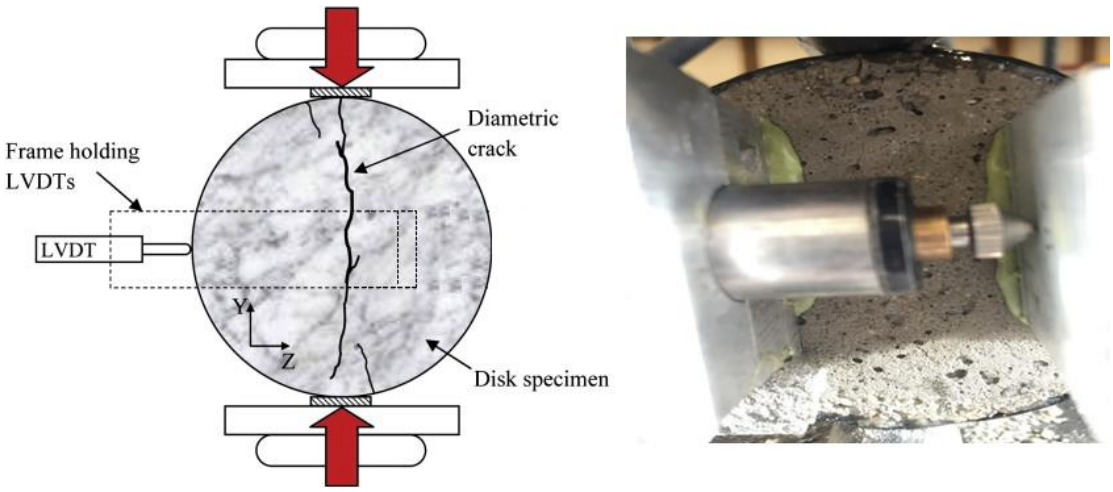


Figure 8.18: 3-point-bending test with LVDT sensor.

8.5.5 Results and discussion

8.5.5.1 Observation of uncoated perlite distribution inside mortar samples (part one)

From inspection of all perlite batch samples, it was clear that the perlite particles were not well distributing among the sample section as shown in Figure 8.19A and these perlite were mainly concentrated at the top of the sample cross section as shown in Figure 8.19B. This was probably due to the high amount of perlite or/and high W/C ratio and lightweight of perlite which prone them to floating as result of vibration.

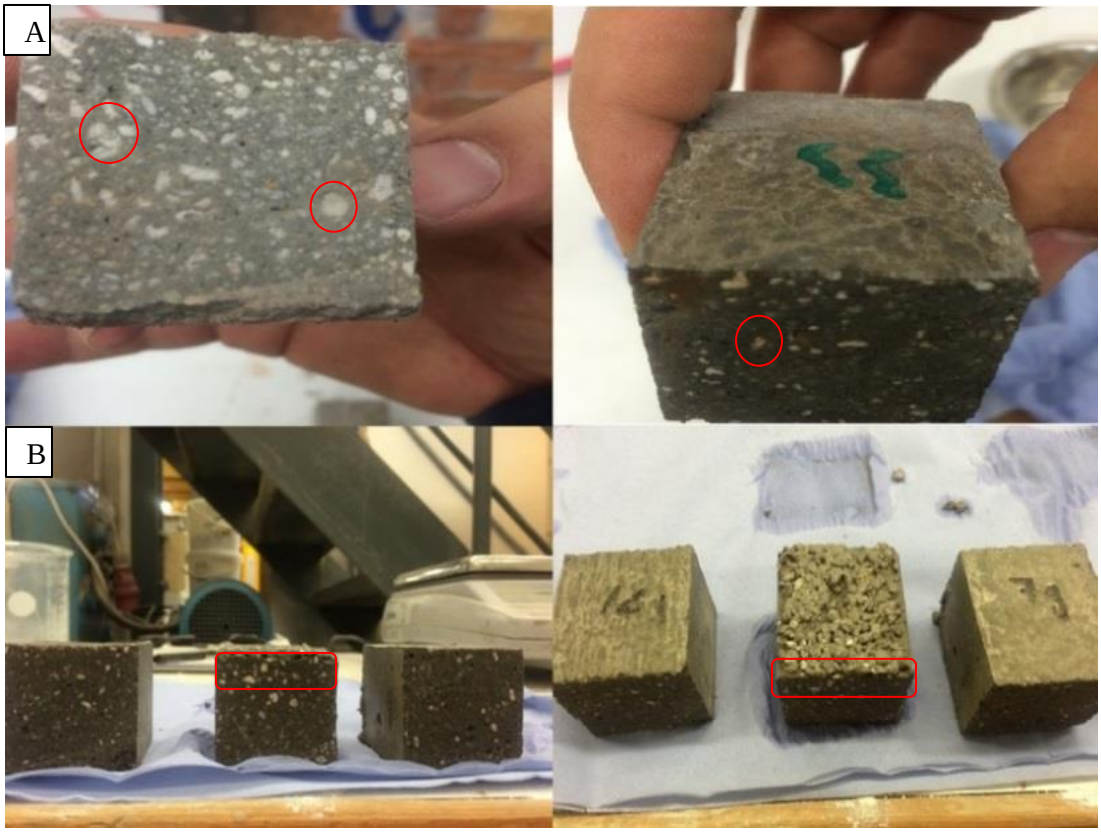


Figure 8.19: Perlite samples cross section-red circles indicate perlite particles. In addition, the red square shows floating perlite.

8.5.5.2 Effect of uncoated perlite percentage on strength of mortar (part one)

The results were indicated that the strength development decreased with the increasing of perlite percentage in the mortar as shown in Figure 8.20. Overall, the 0.5% and 1.5% results closed to control results, while for 2.5%, it incredibly decreased. This graph, which presented the amount of perlite that effect on compressive strength when it exceeded 1.5%.

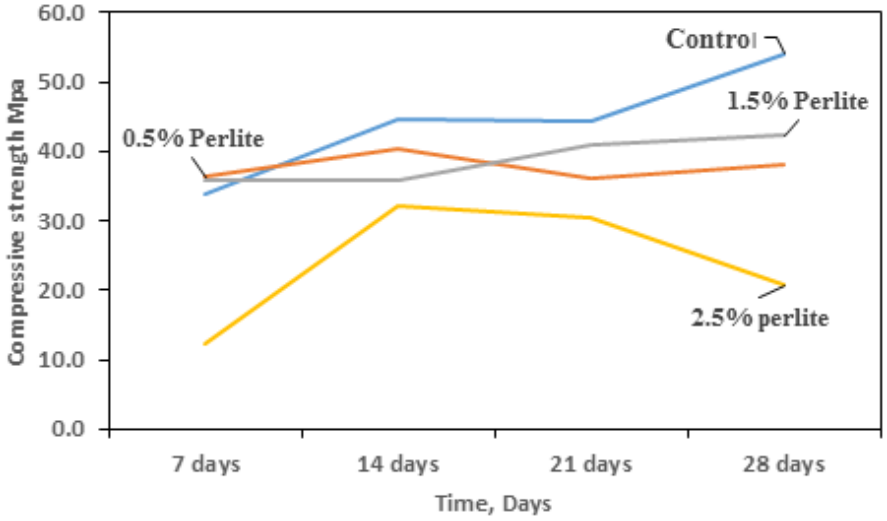


Figure 8.20: Variation of compressive strength of perlite samples with time (% by volume).

8.5.5.3 Effect of coated perlite percentage on strength of mortar (part two)

The results indicated that the strength development decreased with the increasing of coated perlite percentage in the mortar as shown in Figure 8.21. Overall, up to 10% of coated perlite was slightly effect on compressive strength, whereas compressive strength of 10% samples less than control samples by around 25%.

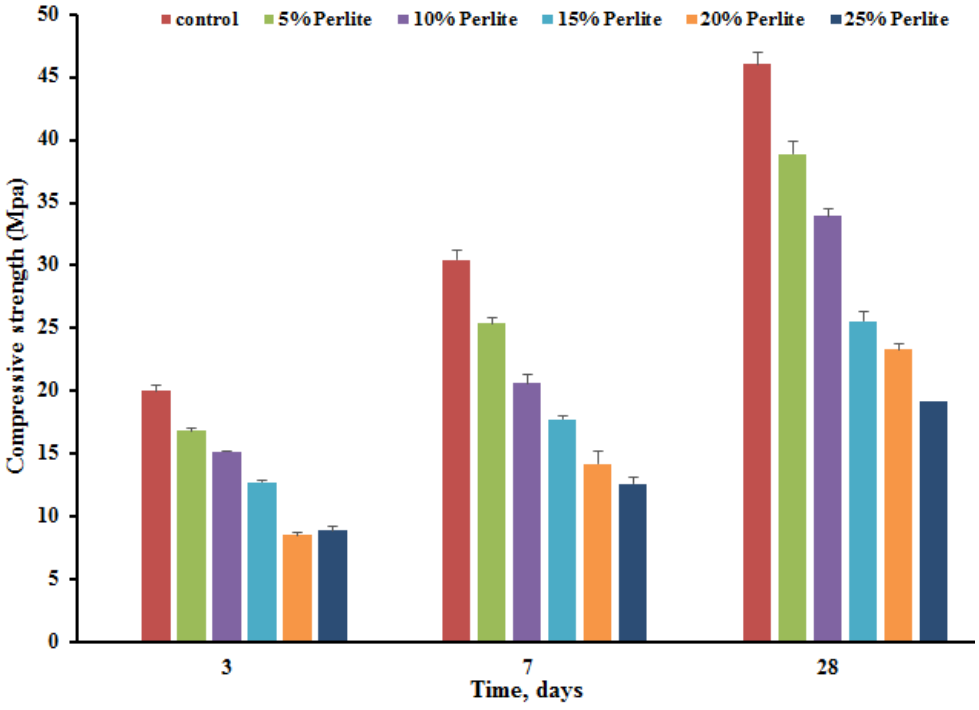


Figure 8.21: Effect of coated perlite on compressive strength development (% by volume).

8.5.5.4 Survivability of bacteria spores inside perlite (part three)

The results indicated that whilst the number of viable spores may have decreased steadily over a period of 90 days, from approximately 10 x 10^9 to 2 x 10^9 (Figure 8.22), this reduction in viable spores was only around 0.8% of the initial number.

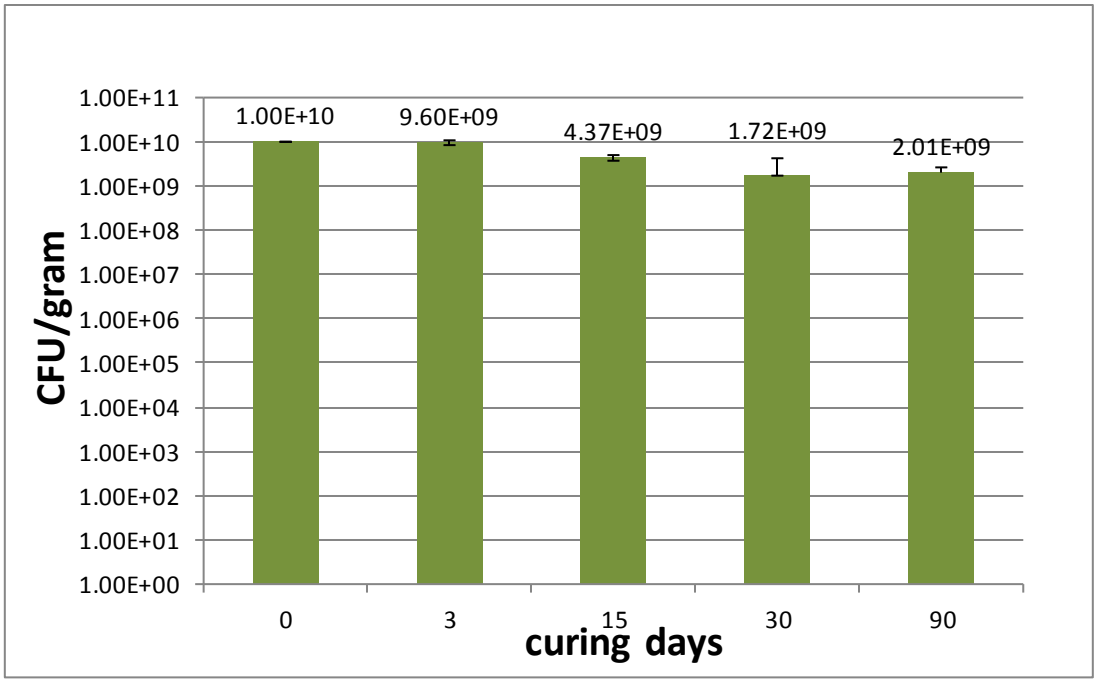


Figure 8.22: Viability of spores after impregnation in perlite.

8.5.5.5 Investigate the ability of bacteria and bacterial agent ingredient inside perlite to produce calcite (part three)

The amount of calcium carbonate was investigated by Fourier transform infrared spectroscopy (FTIR) analysis as shown in Figures (8.23&8.24).

In order to understand the ability of bacteria and bacterial agents come out from perlite and have ability to produce calcite, FTIR analysis was carried out. Figure 8.23 shows that pure perlite (reference), perlite impregnation with *B. pseudofirmus* mixed with LB acetate growth medium, perlite impregnation with bacterial agents mixed with perlite impregnation with *B. pseudofirmus* and diluted in buffer and calcite as a reference curve. The spectrum reveals the main peak that evidence for presence of calcite at 1450 cm^{-1}. Both samples of first set and second set shows that there was small amount of calcite have produced in both sets. However, this amount of calcite was increased in both sets with time as shown in Figures 8.23 and 8.24

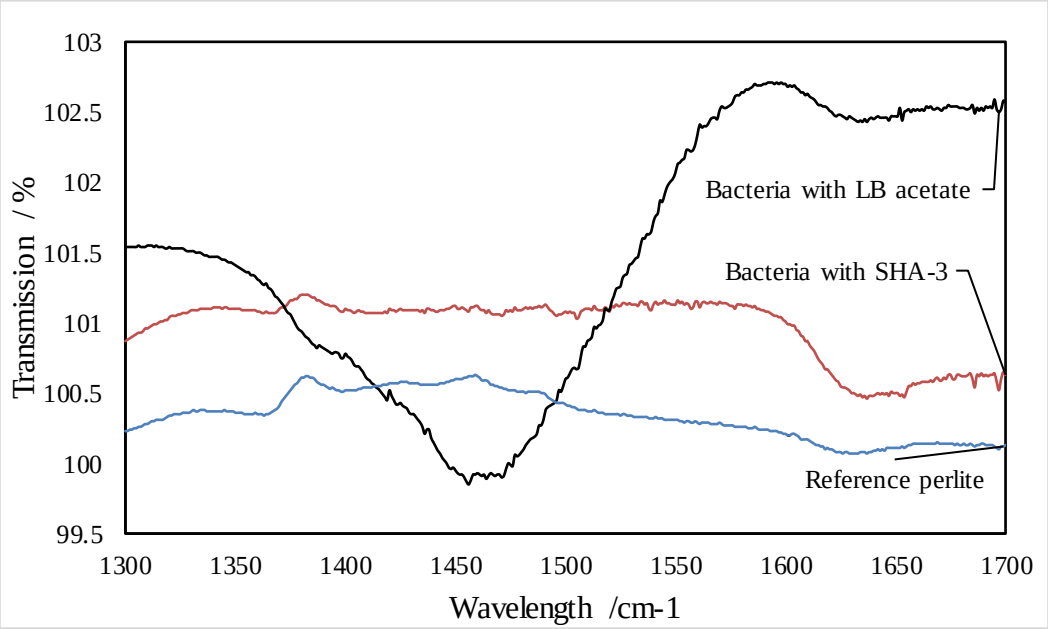


Figure 8.23: Infrared Spectrum of impregnation perlite and calcite reference after 7 days.

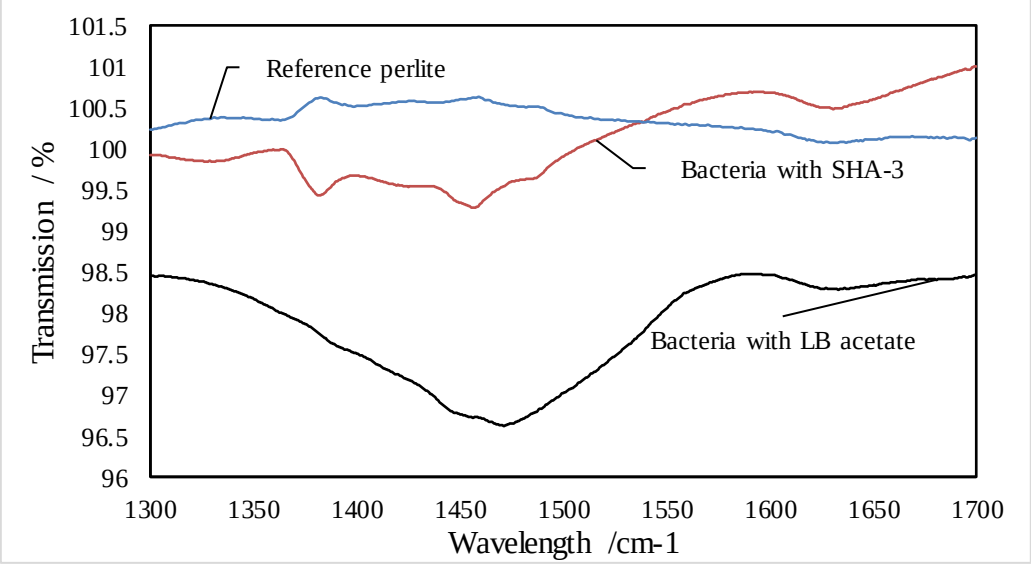


Figure 8.24: Infrared Spectrum of impregnation perlite and calcite reference after 14 days.

8.5.5.6 Observation of the healing in cracks of disc samples (part four)

The efficiency of the materials produced by *B. pseudofirmus* with bacterial agent in perlite was investigated. Figure 8.25 showed healing treatment after certain healing time. Microscope images revealed that set 4-1, set 4-2, set 4-3, set 4-4, and set 4-7 after duration of healing showed non or partial healing. However, microscope images of set 4-5, and set 4-6 showed improved healing compared to the rest samples.

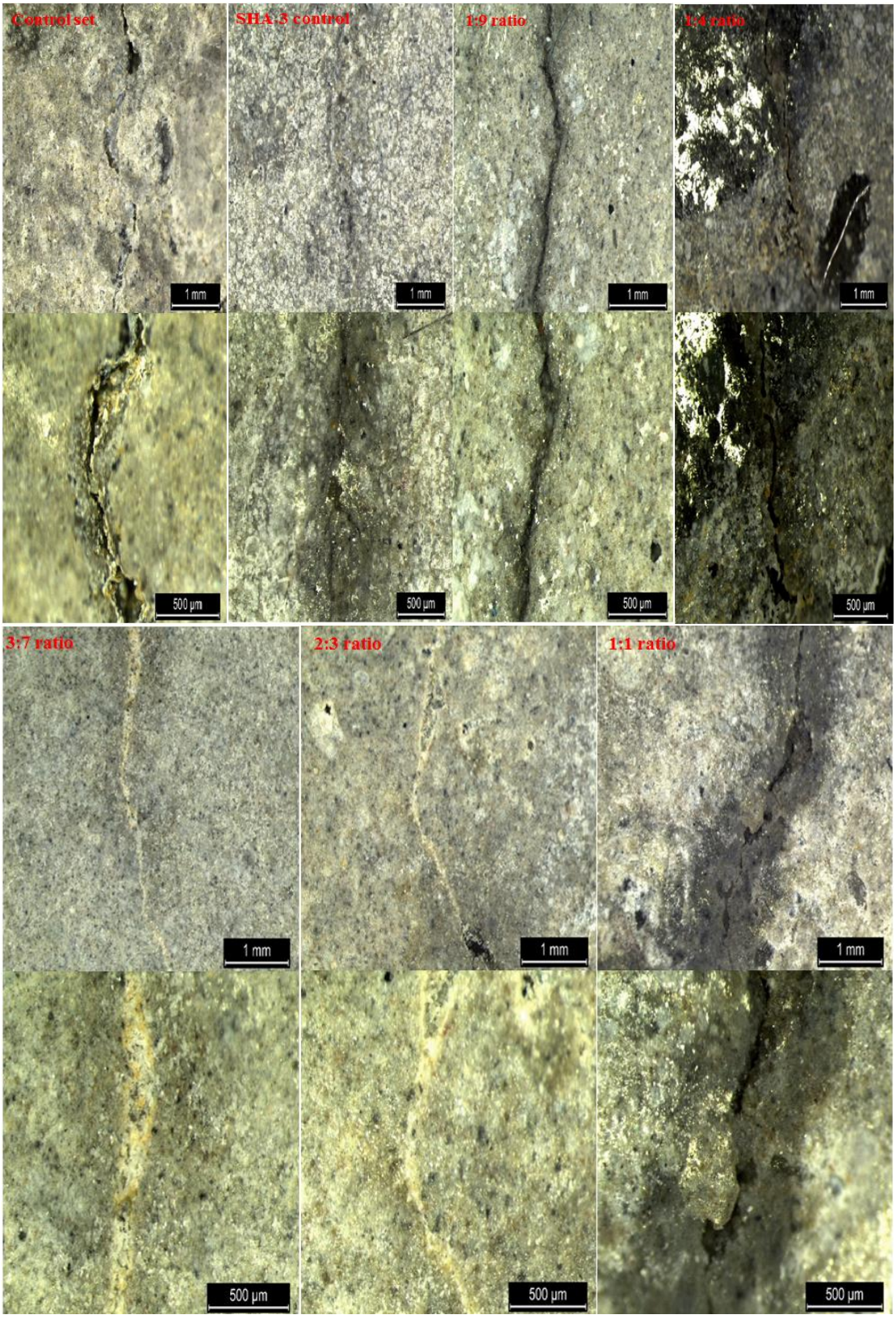


Figure 8.25: Typical microscope observations of cracks healing process after 165 days.

8.5.5.7 Initial surface absorption test (ISAT)

Figure 8.26 showed the relation between initial rates of surface absorption with time. As is typical of mortars the rate of surface absorption reduces with time due to progressively increases of the moisture content of the mortar during the test. The mean initial surface absorption of the non-cracked mortar (set 4-0) at 10 min was 0.16 ml/m^2/s and at 2 h was 0.05 ml/m^2/s. Typically mortars are considered to have excellent resistance to ingress of water if they have values less than 0.25 and 0.07 ml/m^2/s at 10 min and 2 h respectively[437]. These properties were evident in the non-cracked mortars.

The four cracked sets, set 4-1, set 4-2, set 4-3, and set 4-4 had very high initial water surface absorption values. The water treated set 4-1, had initial surface absorptions of 4.42 and 2.03 ml/m^2/s at 10 min and 2 h; the set 4-3 gave values of 0.65 and 0.20 ml/m^2/s; the set 4-2 gave values of 0.53 and 0.20 ml/m^2/s; and the set 4-4 gave values of 0.57 and 0.18 ml/m^2/s, respectively. These values reflect that there was minimal resistance to water ingress. Absorption values greater than 0.5 and 0.15 ml/m^2/s are normally regarded as high. In both cases, it is likely that water was flowing through the crack without obstruction, rather than engrossing *via* absorption.

The other test sets, in which *B. pseudofirmus* with medium ratio, gave in contrast initial surface absorption values ranged from 0.36 ml/m^2/s for set 4-7 to 0.22 ml/m^2/s for set 4-6 at 10 min and ranged from 0.12 ml/m^2/s for set 4-7 to 0.06 ml/m^2/s for set 4-6 at 2 h. These results clearly show that bacterial activity within the created cracks has resulted in a compound(s) that prevents flow of water through the specimen. The conferred resistance to water absorption is similar to that of the surrounding intact mortar.

8.5.6 Concurrent study on effect of adding perlite to the based self-healing concrete at University of Bath

Concurrent research conducted at the university of Bath further explored the application of coated perlite as a delivery system the bacteria based self-healing concrete. This study explored a completely innovative and untested idea before. It represented the first attempt of testing full scale proto type concrete panels in the field using perlite as a part of coarse aggregate to enhance the performance of direct delivery system to the bacteria self-healing concrete (Figure 8.27). Subsequently the study has been reported in Paine et al[438]

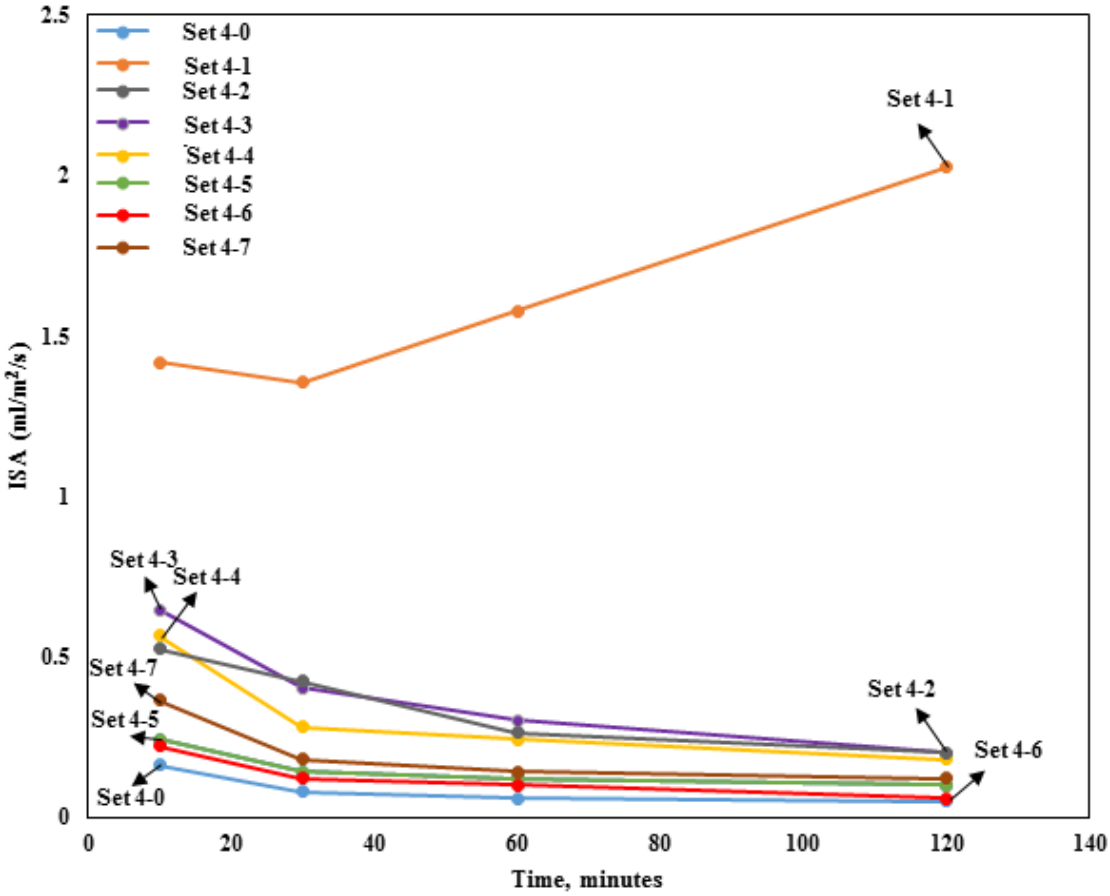


Figure 8.26: Initial Surface Water Absorption Rate for sets (4-0 to 4-7).

Figure 8.27: The five panels cast. The bacteria-based self-healing concrete panel is the third from the left.

8.5.7 Summary on use of perlite

The results indicated that the perlite aggregate was very weak in its mechanical properties than the mortar made from conventional aggregate; this gave the perlite aggregate an

advanced that the cracking passed thought it and would lead to release of the self-healing agents.

A special coating has been a developed that prevents the self-healing agents release into the mortar prior to cracking. The physical properties of perlite aggregate indicated that this type of aggregate capable to be delivery system of bacteria self-healing aggregate.

The biology investigations results demonstrated that the number of viable spores decreased steadily during the period of 90 days by about 0.8% of the initial number, which mean that the perlite is capable to carry bacteria for long time without effect of bacteria. In addition, results showed that ability of bacteria with SHA to produce enough amount of calcite outside the perlite.

8.5.8 Overall summary of encapsulate techniques

This study investigated three different encapsulate techniques within cement mortar. Results of these sections presented herein to provide a better understand of behaviour of three systems as follow:

1- Calcium Alginate Beads (CAB)

   Two main factors were investigated to demonstrate the ability of CAB used as delivery system; the proprieties of CAB shell and its durability. Results showed that CAB is very weak and very light due to low density, which cause decrease in their size, floating on the surface of mortar and poor distribution in mortar matrix.

2- Vascular tubes

   In spite some effect variables of passive mode such as tube length and diameter, viscosity of bacteria solution and their agents (SHA), and the ability of glass tube to resist internal stresses were investigated. Results showed that no sufficient data was reached. In order, to avoid all variables mentioned above an active mode is necessary to investigate.

3- Perlite

The objective of this section of this research was to investigate that physical and mechanical properties of perlite, physical and mechanical properties of coated perlite, demonstrated the ability of perlite incubator bacteria and its agents, and finally study the ability of perlite as a bacteria and its agent's incubator.

Results showed that from the mechanical properties of perlite is very weak, which gave the priority to crack to pass through it. In spite of the fact that the grains won essentially on the upper part of the cross area, the appropriation of them over the structure was practically acceptable and this issue was settled with the utilization two sodium silicate layers with dry cement in second layer of sodium silicate to increase the density of perlite and this technique gave promising results. In addition, it showed its ability to carry both bacteria and SHA for long time and produce calcite out of perlite. The results demonstrated that ability of this system to heal cracks.

It is clear from above the most suitable delivery system was perlite.

Chapter 9
SUMMARY, CONCLUSIONS AND RECOMMENDED FURTHER WORKS

9.1 Introduction

Several issues addressed and raised in this research, which includes sealing and healing of hardening cracked concrete using three different types of bacteria and provides insight towards understanding the effect of adding bacteria with or without their agent to concrete properties performance.

The summary of overall study including the results of the literature review and experimental results analysis are presented in this chapter. In addition, this chapter brings together the conclusions drawn from research work reported in this thesis and given in the previous chapters, and recommends appropriate further future work for continued development of the delivery systems in concrete using bacterial healing techniques. For more specific conclusions for each segment of this study, the reader is referred to the end of each chapter.

9.2 FINDINGS

The objective of this research is broadly divided into five phases to answer five main questions, which were raised from extensive literature review given in chapter 2 and Chapter 3.

The first phase of this study is to investigate the possibility to use two-stage bio-concrete/mortar (the first stage to make concrete/mortar components to be mixed with bacteria agent ingredient first then after the concrete/mortar hardens, the cracks are generated and the bacteria are injected in the cracks as second stage) to remediate the concrete/mortar cracks.

The second phase of this study was to evaluate the effect of bacterial self-healing agents (SHA-1) in the properties of fresh concrete. SHA-1 consists of about ten components; each component has its own effect on concrete matrix. An experimental work is intensively carried out and its results are presented in chapter 6.

The third phase of this research was to evaluate whether *B.cohnii, B.halodurans* and *B.pseudofirmus* are capable to seal the concrete cracks or not and which one of them will produce most efficient calcium carbonate to guarantee the strength and durability of the healed materials into concrete cracks. An intensive experimental work is carried in this research and the results are presented in chapter 7.

The fourth phase of this work was to develop new medium SHA-3 and to study the ability of sealing cracks after intensively investigating its properties from biological aspects.

The fifth phase of this work was to study whether the previously used delivery systems containing chemical agents can be used as delivery system containing bacteria and its agent in order to make direct remediation to concrete cracks and to provide an adequate durability to the environment effects. Several types of delivery systems were experimentally studied, some obstacles were recorded, and valuable recommendations were given in chapter 8 for future work.

9.3 SUMMARY

9.3.1 PHASE 1: Two stage bio-mortar (TSBM)

The focus of this research presented in this phase was to investigate whether concrete mortar mixtures and different bacterial agent ingredient as bio-cement mortar or two-stage cement bio-mortar can have adequate durability and strength for remediating concrete cracks. Essentially, in this phase of research, studies were conducted in two stages where each stage was divided into several tasks, each of which has been described in detail in chapter 6.

In order to come up with environmental-friendly materials to remediate concrete mortar cracks, microbiologically-induced calcite-precipitation is the most efficient material used in repairing technique. In this stage, three types of bacteria (*B.cohnii, B.halodurans* and *B.pseudofirmus*) were studied to explore their suitability in high alkaline environment to precipitate calcite. This stage is divided into two tasks:

9.3.1.1 Stage one

This stage described how chemical composition and physical characteristics of cement is used in this research as two-stage bio-cement mortar with bacteria agent ingredient. This was

analyzed in an attempt to identify parameters that could lead to reduction in the efficiency of the chosen bacteria. This stage is divided into two tasks as follows:

TASK 1

In this task, the ability of bacteria to generate crack healing in bio-cement mortar was studied. The results found indicated that the healed material by each of three bacteria were very weak and not uniformly distributed along the crack. Probably, due to poor distribution of bacterial agent ingredient in bio-cement mortar, this may lead to shortage of bacteria food in or near the crack or perhaps the provided amount of ingredient used in two-stage bio-cement mortar was not enough to ensure the amount and the quality of the healed materials. Finally, the deficiency of the healed material could be due to the chemical reaction of the bacterial agent ingredient with cement in mortar matrix. As conclusion of this stage, a more experimental study is needed in order to find the actual effect of each bacterial agent ingredient components on the rate of hydration of fresh bio-mortar.

TASK 2

As result of the task 1 suggests that the deficiency of the healed material could be due to the amount of agent provided in bio-cement mortar mix, so the aim of this task is to increase the amount of agent to bio-cement mortar. It was observed that all samples for both first and second test sets were dissolved in the water, which means the more ingredient of SHA-1 is added to bio-cement mortar the more retarded to the cement happen and less strength is obtained.

9.3.1.2 Stage two

This stage described how chemical composition of SHA-1 and physical characteristics of cement is investigated in this research. This was analysed in an attempt to identify parameters that may lead to identify which ingredient(s) effect on cement matrix. This stage is divided into three tasks as follows:

TASK 1

The effect of SHA-1 on setting times (initial and final) of cement paste were studied in this task. The results indicated that significant retardation to cement past was recorded especially

with SHA-1 and Na-citrate and the initial and final setting time were delayed by 300 minutes and 400 minutes respectively.

TASK 2

In this task, the effect of SHA-1 on compressive strength for hardened concrete was studied. It was shown that the compressive strength of mortar immersed in the medium decreased by a round 25% to 30 % of their strength as compared to the control samples strength. This decrease of compressive strength of the mortar could be due to high concentration of some component of SHA-1 such as yeast extract 12.12 %, sodium citrate 18.65 %, and calcium acetate 55.94 %.

TASK 3

The effect of medium on development strength of bio-mortar was studied. It was shown that "the more percentage of SHA-1 is added to the mortar mix the less compressive strength of the mortar is obtained."

As conclusion, the study of the effect of each component of self-healing agent on hydration kinetics of the cement is essential.

9.3.2 PHASE 2: Effect of SHA-1 ingredients on hydration kinetic of cement

The chemical reaction between the cement components and the SHA-1 is experimentally investigated in this phase in order to single out SHA-1 that affects the concrete properties. It should be known that in phase 2, the used concrete mixture design components are the same as the mixture components given in phase 1.

The effect of SHA-1 on hydration as function of time was investigated. After several trials, the optimum percent was 0.1% of SHA-1, which slightly retards in cement hydration but this percent (0.1%) of bacterial agent (SHA-1) is not suitable to provide enough food for bacteria to generate self-healing for precipitations.

Since the SHA-1 is composed from ten components, thus the grouping and regrouping of the elements and the found results from these experimental investigations indicated that the sodium citrate was the most effective item of SHA-1 on concrete matrix. The conclusion

reached after several experimental works that the optimum percent of sodium citrate that has no effect on the rate of hydration of cement was 0.05%.

This percentage was used with other SHA-1 ingredient value (0.1, 0.3, and 0.5%) known as modified SHA-1 and were tested experimentally. The results found showed no effect on concrete properties.

9.3.3 PHASE 3: Investigate the ability of all three types of bacteria to seal cracks

In this phase, all three types of bacteria were studied for remediating cracks in hardening mortar, and selecting the most suitable one. The selection of the most efficient bacteria for sealing the concrete cracks depends on many factors that can be expressed into the following expression:

$$E = f(t_1, t_2, Cp, T, QC, V)$$

Where:

E denotes the amount and the quality of produced healing/sealing materials.

t_1, t_2 denotes the start and the finish time of healing respectively.

Cp denotes the amount of calcium carbonate healed by each bacterium.

T denotes the range of temperature required for growth of each bacterium.

QC denotes the quality of the healed calcium carbonates.

V denotes the viability of each bacterium in alkaline environment (like concrete).

This phase is divided into five TASKS as follows:

TASK 1 (t)

In this task the start healing time (t_1) and the finish healing time (t_2) is monitored and recorded by preparing five sets (with 3 samples each).

The found results were monitored by microscope. The test results indicated that *B.pseudofirmus*+SHA-2 started healing (t_1) over six days and finish healing (t_2) after 12 days. The total time taken by this type of bacteria and its agent to heal the total cracks was six days, but the *B.cohnii*+SHA-2 takes 13 days to start healing(t_1) and 19 days (t_2) to finish healing with total healing time of six days. The *B.halodurans* takes 19 days (t_1) to start healing and 25 days (t_2) to finish healing with total healing time is six days. In spite of the fact that all three bacteria took the same time for healing the total cracks, but *B.pseudofirmus* has an advantage

upon the other two by its quick start and finish time. This will lead to reduce the total cost of remediation of concrete cracks.

Task 2 (Cp)

The amount of calcium precipitated (Cp) is studied in this task, by all three types of bacteria in flasks by adding different type of agents (SHA).

The found results (verified by SEM results) indicated that the p/t (amount of calcium precipitation/time of healing) for *B.pseudofirmus* was more than p/t of *B.cohnii, B.halodurans*. It was also found that the *B.halodurans* needs more time to produce enough calcium carbonate when SHA-2 or SHA-3 agent added to it. Finally, one can easily conclude from this task that the *B.pseudofirmus* has an advantage on p/t upon the other two bacteria.

Task 3 (QC)

The quality of calcium precipitated by each bacteria was tested by using initial surface absorption (ISAT) or permeability test that was done according to BS 1881-5: 1970.
The test results indicated that the samples healed by *B.pseudofirmus* reduced the permeability more than those healed by both *B.cohnii* and *B.halodurans* and the lowest amount of heal material to complete healing crack was given by *B.halodurans*. Moreover, these results were compatible to those results found by SEM and EDX tests and presented in chapter 7.

Task 4 (T)

In this task, the investigation of ability of three-*bacillus* species strain on dynamic growth with potential for incorporation into self-healing concrete is presented. The assessment of dynamic growth of *B.cohnii, B.halodurans* and *B.pseudofirmus* were measured over time at particular temperature. The range of temperature was taken from 25℃ to 45℃ for this investigation. It was clear from these results presented in chapter 5, that the temperature span for each type of bacteria was reached, which was between 25℃ to 30℃ for both *B.pseudofirmus* and *B.cohnii*. Moreover, for *B.halodurans,* its span temperature for growth was between 30℃ and 40℃. The ideal temperature for these three bacteria to grow is 30℃. However, the three *bacillus* strain were tested experimentally at these temperatures growth span and found that these three bacteria demonstrated their ability to produce calcite in self-healing concrete process.

Task 5 (v)

In this task, the survivability of bacteria spores inside the cement paste is investigated and the result is presented in section 5.3.1. The viability of spores was monitored in terms of CFU and the results indicated that the number of viable spores decreased steadily during the period of 93 days. The processes of cement hydration have minor effect on the survivability of each bacteria spores. In addition, it was observed that high alkalinity environment has no effect on survivability of the number of bacterial spores. It was noted that these spores did survived with the process of mixing, high pH, setting and hardening cement paste, for a period of more than 3-months, which gave a good indication that these spores (for the tested three types of bacteria) can be used in bio-concrete/bio-cement mortar.

Conclusion of this phase

It is clear from the results of these five tasks that all the studied three types of bacteria (*B.cohnii, B.halodurans* and *B.pseudofirmus*) are capable to produce calcium carbonate in self-healing process. This makes the three bacteria capable to remediate the concrete/mortar cracks. One can conclude from this study that the *B.pseudofirmus* is the most suitable, efficient and economical bacteria for remediation of concrete/mortar cracks.

9.3.4 PHASE 4: The most suitable SHA with *B.pseudofirmus* that would reduce the time of sealing, produce more amount of calcite

Based on the investigation from previous phases, the most suitable bacteria is *B.pseudofirmus*. Both medium SHA-1 and SHA2 have taken long time with three types of bacteria to remediate the micro cracks. Hence, further investigation considered in biology lab at University of Bath to develop suitable medium that would reduce the time of sealing, produce more amount of calcite and not be harmful on the concrete properties[100]. It was found that SHA-3 is the most suitable as presented in chapter 5. Further experimental investigation has done by using it with *B.pseudofirmus* to remediate cracks in cement mortar samples that follows the same steps of preparation of the previous samples. The results showed that the complete period of healing the crack was six days and the sealing started from second day of treatment. Results in chapter 7 showed that the ability of SHA-3 to re-healing cracks more than once. Moreover, the growth condition of SHA-3 was more favourable as compared to SHA-1 and SHA-2 as presented in chapter 5.

9.3.5 PHASE 5: Delivery system for bacteria and their SHA

In general, there are two approaches for delivering bacteria and their bacterial agent ingredients to remediate concrete cracks. The first technique is based on addition of a repaired material to concrete cracks from outside to inside of structural element cracks as experimentally studied in chapters (6&7). The second approach consists of embedding repairing material inside the concrete members. Therefore, when the structural element is subjected to the cracks due to the applied loads, then the embedded repairing material is activated. This type of system is called direct delivery system. The aim of this phase is to give a summary of the results. This summary comes from an investigation to the several delivery systems (chapter 8) used to deliver bacteria and their agent into the concrete/mortar cracks directly, which have been studied in literature for delivering the mineral agents like epoxy. This phase is divided into three tasks as follows:

TASK 1

Three groups of mortar prisms were prepared with the addition of 1%, 3%, and 4% of calcium alginate beads (CAB) (3% minorities) by mass of cement. Due to floating of CAB on the mortar surface as a result of its low density, its ability to absorb water, and its inefficiency, the morality water was increased from 3% to 5% to enhance its density. Moreover, the beads have been dried overnight to avoid the expansion with water adsorption, and some modification in the preparation methods of CAB, they still have the same problem of floating and water absorption. Therefore, the usage of CAB as a delivery system of bacteria in concrete were discarded.

TASK 2

The glass capillary tubes had been investigated as delivery system of bacteria in self-healing concrete. Here, the major obstacles were the viscosity of self-healing materials and the temperature of self-healing materials, which dependence on self-healing viscosity. The study was divided into four main categories of polysaccharides (autoclaved and un-autoclaved) packed with SHA-1 in glass capillary tubes and superabsorbent polymer.

It was found that the three types of polysaccharides were sticky on the face of capillary tube that prevented bacterial self-healing agents from coming out through broken tube for 3% while the concentration of 1% polysaccharides gave low viscosity that made the materials

came out during the cracks generation. On the other hand, superabsorbent polymer was used with bacterial self-healing agent as power and it is used on both ends and mixed with SHA-1 in whole tube. It gave promising results, but the bacterial self-healing agents were not enough to heal cracks and also the expansion of limit polymer was preventing all the agents to come out from the tube to the surface of the crack.

TASK3

The expanded perlite had been investigated to be used as delivery system of bacteria in self-healing concrete. The research was divided into four main categories. First category was dealing to investigate the effect of perlite on fresh and hardened concrete properties. Second category was dealing to investigate the effect of coated perlite on fresh and hardened concrete properties. Third category was to demonstrate the ability of perlite to impregnate and encapsulate both bacteria and bacteria agent and the fourth category was to study the ability of perlites to be used as a delivery system in concrete.

First category results show that perlite was well distributed inside cement mortar. It had slight effect on compressive strength when the percentage of perlite did not exceed 1.5% of sand volume.

Second category results presented that the ability of perlite to prevent the bacterial agent ingredient from being released into the mortar was weak. Therefore, further trials consider a double layer of coating: consisting of a layer of sodium silicate and a layer of Portland fly ash cement, which prevented leakage from perlite. Moreover, the mortar samples with 5%, 10%, 15%, 20%, and 25% of perlite by sand volume decreased the compressive strength.

Third category the viability of spores (ability to germinate) was retained after impregnation in the perlite for 90 days and the reduction in viable spores was only around 0.8% of the initial number. The ability of bacteria and bacterial agent ingredient inside perlite to produce calcite was investigated and the results by FITR indicated that there is an ability in both bacteria and their agent to produce the calcite when the crack impregnated through the perlite.

Fourth category the ability of perlite with different ratio (bacteria: SHA-3) inside mortar disks were studied. The results presented that the sufficient ratios were 2:3 and 3:7 to heal cracks. Results of visualization and ISAT showed that both sets results close to reference result, which mean the materials healed cracks, were high quality.

9.4 OVER ALL CONCLUSIONS FROM THIS RESEARCH

1- Based on the study about "the remediation of concrete cracks by using bacteria self-healing technique", it can be recommended that the use of SHA-1 for healing the concrete cracks at the current levels of its ten components is harmful to the concrete properties. The reasons for this conclusion are evident in the entire study and discussed in chapter 6.

2- Out of total often-different combinations of (SHA-1) ingredients studied in this research, only one element, i.e., sodium citrate showed its influence on concrete properties, when its contribution to the total of (SHA-1) is more than 0.05%. Moreover, when this optimum percentage of sodium citrate (0.05%) was used with all other ingredient values (0.1%, 0.3%, and 0.5%) without changing their values, the found results indicated that the used optimum percentage (0.05%) of sodium citrate had no effect on concrete properties. This indicates low probability of bio-concrete as performance worst.

3- Growth *B.pseudofirmus* in SHA-3 gives a high rate as compared to SHA-1 and SHA-2 due to the high concentration of calcium acetate. The amount of calcium carbonate from *B.pseudofirmus* with SHA-3 is higher than *B.pseudofirmus* with SHA-1 (around 23% by weigh) and is higher than *B.pseudofirmus* with SHA-2 (around 91% by weigh). The found results were monitored by microscope results that indicated that the *B.pseudofirmus* with SHA-3 healed cracks within seven days.

4- The compression study between three different bacteria for their ability of remediating the concrete cracks indicated that all three bacteria are capable of producing good quality carbonate calcium. They had efficient growth and viability in alkaline environment, but they are different in starting and finishing healing time. The optimum selection order of the studied bacteria according to their time of healing, produced amount of healed materials and other factors as presented in chapter 7 are as follows: *B.pseudofirmus*, *B.cohnii* and *B.halodurans*.

5- The proposed perlite as a lightweight aggregate to be used as delivery system of bacteria based self-healing agent in concrete mortar was studied and presented in chapter 8. The found results indicated that the developed coated perlite prevents the release of self-healing agents in concrete during mixing and handling prior to cracking and gave an indication that this type of aggregate had good potential to be suitable delivery system to the bacteria base self-healing agent.

6- The results obtained from modeling of vascular tubes to be used as direct delivery system to the bacteria based self-healing agent were inconclusively improving or disproving the

hypothesis that the joint movement of bacteria and its agent from the hidden pipes inside the concrete due to the action of crack can improve the self-healing properties of concrete. The reason of this inconclusiveness was that the self-healing mechanism was not activated during the self-healing three-point bending test to generate cracks and break the tubes due to several parameters, such as pipe length, its outside diameter, viscosity of the healing agents, type of mode used in testing (active or passive) and others, which could influence the system performance for remediating the cracks in hardened stage of concrete using bacteria self-healing technique.

9.5 RECOMMENDED FURTHER RESEARCH TOPICS

It became clear during the course of this research that there is much scope for further research, which includes not only the remediation of concrete cracks by using bacteria self-healing, but also the delivery systems of bacteria and their agent in concrete cracks in general.

In this section, the suggested further work can be divided into two groups. The areas for possible research on remediation of concrete cracks by using bacteria, and the effect of bacteria agent in cement based materials as first group. Some ideas are presented for future research which would be considered necessary for developing a delivery system for bacteria and its agent to the target concrete cracks, for remediating them as second group of research.

1- It was found in chapter 6 that the most influenced item from bacteria agent on properties of concrete mortar is the sodium citrate and its optimum percent was 0.05%. Therefore, by using this percentage the sodium citrate has no effect on cement base concrete properties. As a future research, it is recommended to develop a concrete mixture by introducing the bacteria agent as a factor as well as water cement ratio and total aggregate to cement ratio taking into consideration the optimum percentage of sodium citrate is 0.05%.
2- The remediation of concrete cracks by using bio-mortar (two-stage bio-mortar remediation) in condition that the sodium citrate in bio-concrete does not exceed its optimum percent (0.05%) could be another topic of research.
3- It was found as result of experimental investigation in chapter 5 that the growth of *B.halodurans* can be continued up to 40°C. It is recommended to carry on research using this type of bacteria for remediating cracks, and the results should be simulated in the hot countries climate.

4- The majority of researchers tested the reliability of the healed cracks for one cycle. Another possible research project would be the determination of the effect of reloading (testing) on the durability of the healed cracks to more than one cycle of healing and loading.
5- Intensive research is needed on the healed material to find its reliability in case of creep, stress, and properties of the healed sections (net area, moment of inertia, and centroid).
6- The results obtained from using vascular tubes to deliver bacteria and their agent to the generated concrete cracks were not successful due to many obstacles. One of which is an activated passive mode to be considered in delivery system. Thus, another research project could reuse this vascular tubes mode but the active mode should be used instead.
7- The calcium alginate beads (CAB) was primarily studied for any possibility to use as delivery system for bacteria and their agent to the concrete crack. Intensive a research for this material is needed especially for mechanical properties (stress, rapture) of shell, increasing the weight of the CAB to avoid floating, and its shrinkage.
8- Another promising area of research is about self-curing and self-healing concrete by using perlite lightweight aggregate as delivery system for bacteria and their agent as well as water to form complete unified self-curing and self-healing concrete.
9- One final suggestion for further research is to use a unified method of testing of the healing material and development of the unified international standard is highly recommended.

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APENDIX

1- Fig No.	Symbol	Reference number
Fig 2.7	A2.7	44,78,79,22,49,80,76,81,82,83,50,84,53
	B2.7	85,48,47,86,87,46,88,89,45,20
Fig 2.8	A2.8	44,78,79,22,49,80,76,81,82,83,50,84,53, 85,48,47,86,87,46,88,89,45,20
	B2.8	90,47,46,9,2,91
Fig 2.9	A2.9	92,93,94,95,96,97,98,99,100,20,101
	B2.9	102,103, 17,104,105, 106,18,107,108,109,105,110,100,111,112,113
Fig 2.10	A2.10	114,115,116,117,80,118,119,23,120,121,122,123,124,125
	B2.10	126,77,127,23,128,22,129,130,131
	C2.10	132,20,133
Fig 3.3	A3.3	7,35,36,63,64,65,25,134,135,136,6,137,138,26,139,140,141,68,39,142,143
	B3.3	140,48,144,145,146,36,70,147,148,84,149,90,150,151,152,153,47,3,154,155,156,157,158,159,160161,53,78,162,71,163,164,165,166 167,168,169,170,171,172,173,174,175,67,176,72,73,177,178,77,179,180,181,182,183,184,185,186,187,188,189
	C3.3	11,86,190,87,46,37,191,192,193,194,89,128,129,22,51,79,195,49,80,67,81,82,196,83,45,197,20,198,47,8,9,199,55,200,201,2,57 58,91,202,203,61,12,(204,205,206,207,208,209,210,211,212,213,214,215,216,217,218,219,220,221),(222,223,224,225,226,227,228, 229,230,231,232,233,129,80,20,77,101,100,119,234,23,120,121,122,123,124,98,235,97,236),(189,237,238,239,240,241,242,243,244,245, 246,247,248,249,250,251,252,253,254,255,256,257,258,259,260,261,262,263,264,265,266)
Fig 3.2	A3.2	7,35,36,63,64,65,25,134,135,136,6,137,138,26,139,140,141,68,39,142,143,140,48,144,145,146,36,70,147,148,84,149,90,150, 151,152,153,47,3,154,155,156,157,158,159,160161,53,78,162,71,163,164,165,166,167,168,169,170,171,172,173,174, 175,67,176,72,73,177,178,77,179,180,181,182,183,184,185,186,187,188,189,204,205,206,207,208,209,210,211,212, 213,214,215,216,217,218,219,220,221,189,237,238,239,240,241,242,243,244,245,246,247,248,249,250,251,252,253,254,

		255,256,257,258,259,260,261,262,263,264,265,266
	B3.2	222,223,224,225,226,227,228,229,230,231,232,233,129,80,20,77,101,100,119,234,23,120,121,122,123,124,98,235,97,236
Fig2.11	I	44,267,147,73,79,80,23,204,239,240,218,219,220,246,257,268,269
	II	147,148,172,73,129,22,79,80,270,189,214,219,256
	III	165,73,229,268,271,261
	IV	45,272,256,231,269
	V	69,2,50,229,231
	VI	187,22,271
	VII	3, 214
	VIII	76,238
	IX	48
	X	147
	XI	44,65,273,147,148,274,166,172,177,58,241,244,221
	XII	134,26,129,45,20, 213,275,231
	XIII	146,50,245,261
	XIV	155,71,275,251
	XV	49,80,56,243
	XVI	166,83,230
	XVII	276,277
	XVIII	165,207
	XIX	275
	XX	278

	XXI	85,139,142,150,47,50,49,81,196,9,227,213, 214,247,262,264
	XXII	36,147,148,279,216,244,246,221,261
	XXIII	46,193,45,280,281,240,208,242,260
	XXIV	83,183,206, 213,228,229,244
	XXV	64,36,134,135,182,275,206
	XXVI	64,282,283
	XXVII	193,284
	XXVIII	193,256