BIO-INSPIRED SELF-HEALING CEMENTITIOUS MORTAR USING BACILLUS SUBTILIS IMMOBILIZED ON NANO/MICRO ADDITIVES

By

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This is to certify

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Has been accepted towards the partial fulfillment

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Thesis Supervisor

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THESIS ACCEPTANCE CERTIFICATE

Certified that final copy of MS thesis written by **Mr. Siraj ud din**, Registration No. **NUST201463407MSCEE15214F**, of MS Structural Engineering 2014 Batch (NICE) has been vetted by undersigned, found completed in all respects as per NUST Statutes/Regulations, is free of plagiarism, errors, and mistakes and is accepted as partial fulfilment for award of MS/MPhil degree. It is further certified that necessary amendments as pointed out by GEC members of the scholar have been incorporated in the said thesis.

Signature_____

Name of Supervisor Dr. Rao Arsalan Khushnood

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Date: _____

Signature (Dean/Principal) _____

Date:_____

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ABSTRACT

High strength, durability and low cost make concrete one of the most used construction material in the world. In the service life span of cementitious composites, the generation of cracks is inevitable. These micro level cracks hamper the durability while their transformation into macro level causes problems of structural integrity and capacity reduction. For maintaining serviceability of the structures repair/maintenance has to be undertaken which is usually costly, difficult and environmentally unfriendly. Therefore, the inclusion of nano/micro sized self-healing materials may be beneficial not only in terms of automatic cracks healing but improved durability, cost effectiveness and eco-friendly.

Self-healing concrete repairs/mends the crack by utilizing compounds, resins and microorganisms added to concrete in the mixing stage. Bio-inspired self-healing cementitious composites has the capability of repairing cracks by producing chemical products from the microorganisms and precursor compound incorporated during mixing phase. The incorporated bacteria remain dormant in the matrix until cracking occurs and water seeps in the cracks. The ingress water activates the bacteria, which utilize the precursor compound to fill cracks hence inhibiting strength loss and increasing durability of concrete structures.

In the present research, Bacillus subtilis bacteria were grown and incorporated in mortar specimens using various nano and micro sized carrier particles of iron oxide, limestone and siliceous sand. The resulting cement composites were analyzed for their crack healing capabilities and also for mechanical characteristics, microstructure and phase configuration. The results indicated that the iron oxide particles as carrier material are more efficient in crack healing throughout the life span of cement mortar composites whereas limestone and siliceous sand particles produced promising results at initial age (3 and 7 days) and later age (28 days),

respectively. All the incorporation techniques exhibited an enhanced compressive strength as compared to control.

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1. INTRODUCTION

1.1. General

Self-healing concrete is a type of concrete that has the capability of repairing its own cracks. The cracks are self-healed by production of mineral from intentionally added bacteria, chemical compounds or resins. This study investigated the self-healing of cracks by the addition of mineral producing bacteria in concrete known as bio-influenced self-healing concrete or in short bio concrete. In earlier studies, bacterial solution was applied externally by spraying on the cracks which was less effective and in some cases, impractical. Also, this method cannot be categorized as truly self-healing. Therefore, in recent years bacteria and the organic precursor compound are added to concrete during mixing stage.

Concrete is one of the most commonly consumed building material in the world. The consumption of concrete is about 2.5 metric tonnes (more than one cubic meter) per person alive every year (Van Oss, 2005). Strength, durability and low cost are some of the reasons for this extensive use of concrete. Although concrete has many advantages but it also has some drawbacks as well. One of the main drawback of concrete is low tensile strength which renders it vulnerable to formation and propagation of cracks. The tensile stresses can be induced by tensile forces, plastic shrinkage and/or expansive reactions (Mehta and Monteiro 2006). This vulnerability of concrete to crack under tension not only causes reduced strength but also renders concrete exposed to the adverse effects of environment. Cracking in concrete is a major concern as it can never be fully avoided. Although the micro cracks do not affect the strength of concrete greatly but they make concrete permeable to harmful chemicals. This ingression of harmful chemicals may cause long term concrete matrix deterioration and corrosion of steel reinforcement (Reinhardt and Jooss 2003). This corrosion of steel reinforcement results in increased crack width and length thus causing strength and stiffness loss in concrete structures (Lea and Hewlett 2001). This deterioration of concrete and steel reinforcement also causes high repair and maintenance cost. (Federal Highway Administration 2001) of United States of America reported in 2001 that the maintenance of concrete highway bridges cost an amount of 4 billion dollars annually. United Kingdom spends around 45% of the annual construction budget on repair and maintenance of already constructed concrete structures (De Rooij and Schlangen, 2001).

These problems made scientists and researchers to find a method for countering the crack propagation in concrete, thus improving the durability of concrete structures. Different strategies were investigated to cater for the drawback of cracking in concrete. These strategies included use of epoxy systems, acrylic resins and silicone based polymers. Although these methods helped in mediating the cracking problem but most of the materials were not compatible with concrete, unfriendly to environment and costly (Vekariya and Pitroda 2013).

Recent studies reveal that bio-inspired self-healing concrete or bio concrete is proving to be a more feasible solution to the problem of cracking in concrete structures. The healing mechanism of bio concrete comprises of two components, a suitable bacteria and calcium based nutrient compound. This process of self-healing increases the structure durability and minimizes the manual maintenance and repair required for structures. The method of bio concrete for self-healing also decreases the use materials dangerous to the environment and enhances the compressive strength of concrete (Vekariya and Pitroda 2013).

In bio concrete the bacteria are added to concrete in dormant condition along with the precursor organic compound as nutrient. The bacteria remain inactive in concrete matrix until the formation of cracks which permits the ingression of water in concrete. The bacteria are activated when come in contact with water and start to feed on the organic compound. The organic compound is converted to calcium carbonate via the metabolic activity of bacteria. The calcium carbonate produced fills the cracks which results in decreased permeability in concrete hence increasing the durability of concrete.

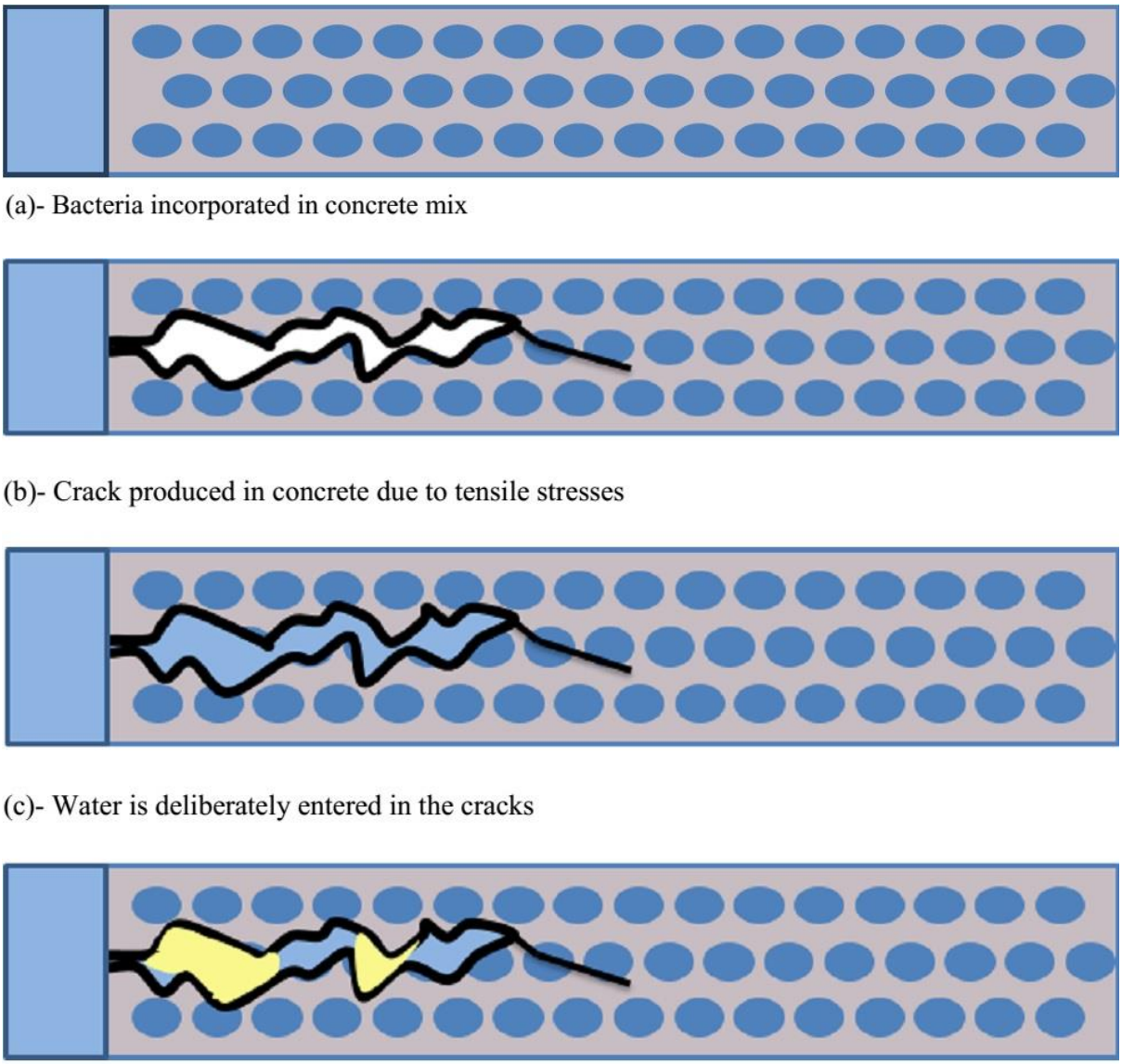

Figure 1.1 Process of self-healing of cracks through bacteria

The process of self-healing of concrete depends on different factors such as pH value of concrete, availability of calcium ions, presence of nucleation sites and the amount of dissolved inorganic carbon (Hammes et al. 2003). Furthermore, other parameters such as bacteria type, bacterial concentration in the matrix, different materials used for immobilization of bacteria and different curing procedures also affect the self-healing process in concrete.

The bacteria utilized as self-healing agent should have the ability to remain active and perform healing of cracks for a long period, preferably total life of the structure. It should also resist the high alkalinity of concrete matrix. Additionally, the incorporation of bacteria should not negatively affect other mechanical properties of concrete.

There are many species of gene bacillus that meet these requirements. These bacteria have the ability of forming spores in unfavorable conditions and become dormant. They become active when come in contact with water ingress through cracks. After activation, they start feeding on the nutrient compound and produce calcium carbonate.

In addition to the selection of bacteria, selection of the organic compound as a nutrient is also of significant importance. Most often two types of nutrient compounds are used i.e. calcium lactate ($CaC_6H_{10}O_6$) and urea ($CO(NH_2)_2$). However, use of urea requires rich calcium environment to produce calcium carbonate.

1.2. Objectives

The major aim of this investigation is to study the self-healing response of mortar when bacteria are immobilized using various techniques. The objectives are defined as follow:

- Study the viability of bacteria in mortar and its capability of healing cracks.
- Compare the performance of different carrier materials used for the immobilization of bacteria.
- Studying effects of bacteria and nutrient compound on the strength of concrete.

In this study three different methods were used for the immobilization of bacteria in mortar. By immobilizing bacteria in siliceous sand particles, incorporation of lime stone particles containing bacteria and iron oxide nanoparticles coated with bacteria. The cement composites were tested in compression machine for compressive strength

evaluation. The resulting cement composites were also analyzed for their crack healing capabilities and mechanical characteristics and microstructure.

To achieve the above mentioned objectives many different tasks were performed. Firstly, literature review was done. Samples formulations were determined and specimens were prepared using those formulations. Specimen testing and acquisition of data setup were decided to get the results. The data acquired was used to compare the performance of different immobilization techniques and their impact on the compressive resistance of mortar specimens.

1.3. Organization of Report

Chapter 1 of the report is introduction of the self-healing concrete, objectives and thesis review. Chapter 2 includes literature review on bio concrete. Materials, preparation of the specimens and testing procedures are discussed in chapter 3 of the report. Chapter 4 comprises of different tests performed, observations, test results and evaluation of test results. Conclusion and findings of this research are presented in chapter 5. This chapter also includes recommendations for further studies.

2. LITERATURE REVIEW

2.1. General

Bio-inspired self-healing concrete is becoming a viable solution to the problem of durability in concrete structures. Bio concrete also helps in the reduction of carbon dioxide production by cement industries. All these advantages have made bio inspired self-healing concrete a main attention of the research studies in current years. The focus of these studies was to evaluate the impact of different aspects on the performance of self-healing concrete. Some of these factors include type and concentration of bacteria, precursor organic compound and different techniques used for the immobilization of bacteria in the concrete mix.

2.2. Previous Studies on Bio Influenced Self-Healing Concrete

There are several studies that have been conducted on bio-inspired self-healing process of concrete as well as mortar which is the focus of this study. Some of the important studies are summarized below:

(Ramachandran, Ramakrishnan, and Bang 2001) have studied the impact of type and concentration of bacteria on the compressive strength of cement mortar. Also, a comparison was made between stiffness and compressive strength of mortar beams and cubes. The variation in their values with different crack depths and bacterial concentrations is used to determine the effectiveness of bacteria in repairing concrete cracks. For this purpose, two types of Portland cement mortar cubes and beams of dimensions 50 mm and 25x25x150 mm respectively were casted. One set contained different concentration of *Bacillus pasteurii* while the other set included 3.175 mm wide cracks with varying depths. The cracks in the specimen were filled with sand mixed with *Bacillus pasteurii* having a concentration of $3.8x10^9$ cells/cm^3. Curing of samples was

carried out in urea and $CaCl_2$ solution for 28 days. The cracks in control specimen were unfilled and left open to air.

To determine the compressive strength samples were tested in the compression machine. SEM and XRD were also performed to quantify the crack healing ability of bacteria. Test results showed that the lower concentrations of *Bacillus pasteurii* increased the compressive strength of mortar. Cracks with shallow depth showed more healing as compared to deep cracks. Figure 2.1 shows the result of compressive tests at varying concentrations of bacteria. Figure 2.2 depicts the increase in the compressive strength of mortar with varying crack depths.

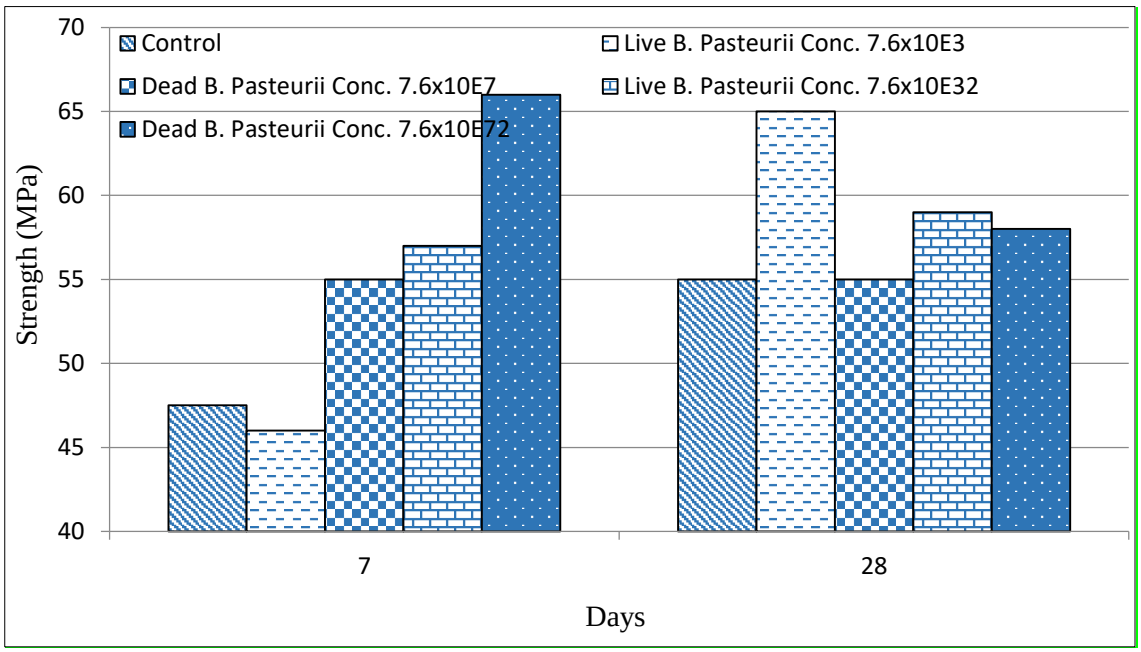

Figure 2.1 Compressive strength of cement based mortar under various self-healing conditions

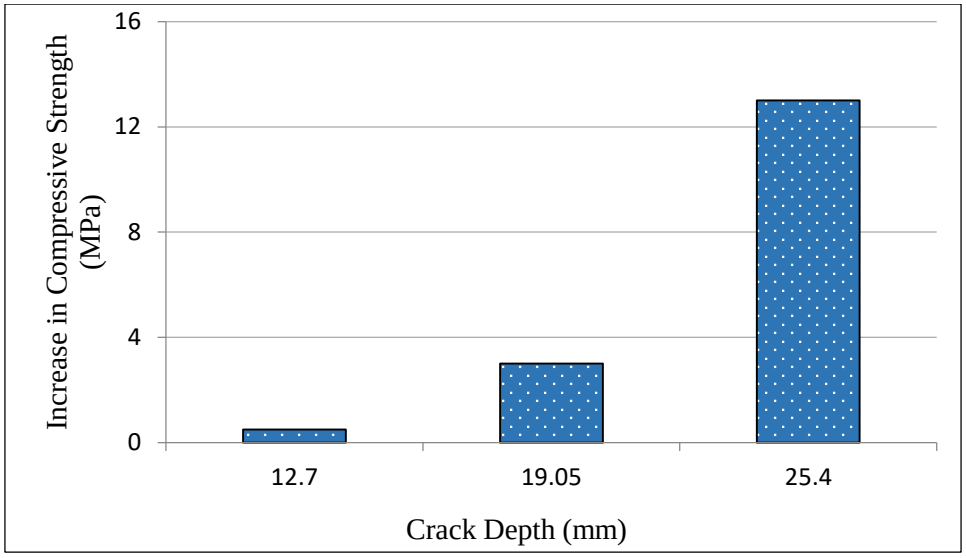

Figure 2.2 Increase in strength of cement mortar with of cracks of different depths

(Ghosh et al. 2005) conducted research to determine the impact of varying concentration of anaerobic bacteria on the compressive strength of cement mortar. The bacteria used in the study were *Shewanella* species and *Escherichia coli*. To accomplish this task, mortar cubes of dimension 70.6 mm were made containing different concentrations of both bacteria ranging from zero to 10^7 cells per ml of mixing water. For all bacterial concentrations, increase in compressive strength was observed. For *Shewanella* the maximum compressive strength was reported at 10^5 cells per ml. The increase was observed to be 25% at 28 days. This enhanced compressive strength was attributed to improved pore size distribution confirmed by SEM and MIP tests performed on the samples. Further increase in the concentration of bacterial solution decreased the compressive strength increment. Although *E. coli* showed increased compressive strength of mortar but the increment was insignificant. Figure 2.3 shows the compressive strength at various concentrations of bacteria.

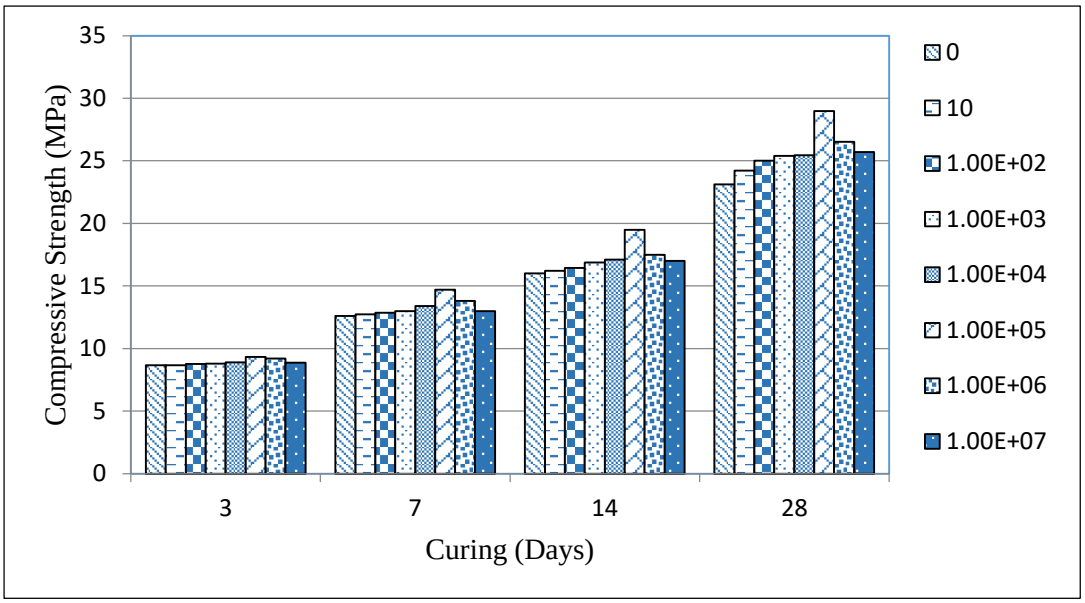

Figure 2.3 Effect of bacteria addition on the strength of mortar

(Schlangen and Jonkers 2008) studied the healing capacity of two component healing system comprising of bacteria and organic precursor compound. The bacteria used in the study were *Bacillus pseudofirmus*. The precursor organic compounds used were peptone, calcium lactate and calcium glutamate. Cement paste cubes of 4 cm were prepared using OPC. The water cement ratio was kept at 0.4. Specimen without any addition acted as control whereas for other specimen organic compounds was incorporated in a quantity of 1% by weight of cement. In addition, bacterial cement specimens were also prepared by incorporating bacterial suspension having cell concentration of 5.8 x 10^8 cells/cm^3. For crack healing potential two types of specimens, one with single healing agent (Bacteria only) and other with two healing agents (Bacteria and Calcium lactate) were casted. Compression test results reported that the addition of healing agents may affect the compressive strength negatively. Compressive strength of peptone specimen was already lower than 50% of the control after 7 days of curing which continued to drop with further curing. Calcium lactate was the only healing agent which did not affect the compressive strength significantly. Figure 2.4 shows the strength comparison of different specimen.

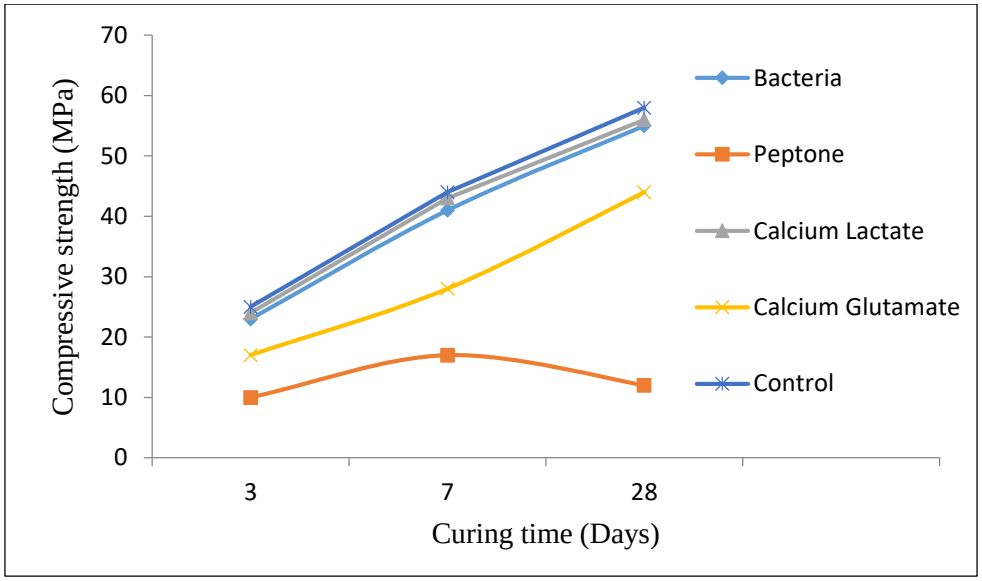

Figure 2.4 Compressive strength development of cement past containing bacteria

(Van Tittelboom et al. 2010) studied the efficiency of bacteria to heal cracks and compared the results with conventional crack repairing techniques. For this purpose, different tests such as water permeability tests, ultrasound transmission and visual observations were performed. Cracks were induced using split tensile technique and by forming grooves using copper wires. Crack healing by *Bacillus sphaericus* incorporated through silica gel showed an increase in the ultrasound pulse velocity.

The results of water permeability are presented in Figure 2.5. Crack healing by bacteria immobilized in silica gel showed the least value of water permeability coefficient (K) as compared to other samples. The maximum decrease in transmission time, determined by ultrasonic pulse velocity test is observed for samples treated with bacterial solution immobilized in silica gel.

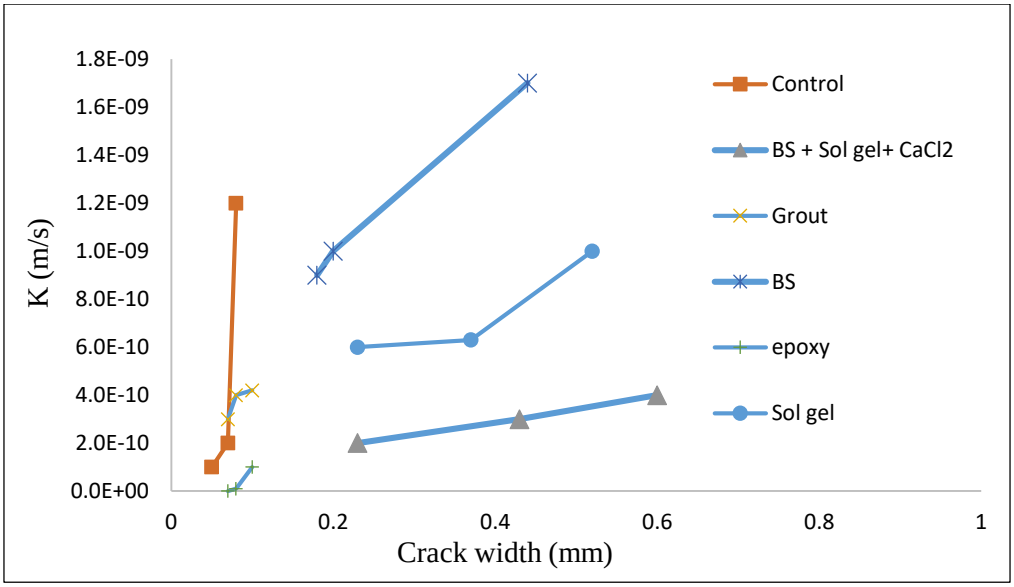

Figure 2.5 Water permeability of concrete samples against various crack widths

An experimental program was devised (Khaliq and Ehsan 2016) to check the self-healing in concrete. The program included incorporation of *Bacillus subtilis* using different carrier compounds. Four different formulations were casted for the above objective. Bacteria were incorporated in concrete directly, through light weight aggregate and Graphite Nano platelet. No bacterial solution was added to the control specimen. Mix proportions for all four formulations are shown in the Table 2.1.

Specimens	Mix 1	Mix 2	Mix 3	Mix 4
Cement (kg/m^3)	370	370	370	370
Fine aggregate (kg/m^3)	840	840	840	840
Coarse aggregate (kg/m^3)	990	990	990	990
Water cement ratio	0.40	0.40	0.40	0.40
Super plasticizer (%)	1	1	1	1
Calcium lactate (kg/m^3)	18	18	18	18
Bacteria (lit/m^3)	0	6.33	6.33	6.33
Incorporation technique	None	Direct	By LWA	By GNP

Table 2.1 Mix design of all specimens

Specimen of 150 mm dia and 300 mm height were analyzed for strength while for self-healing efficiency specimen of 150 mm dia and 100 mm height were used. SEM and XRD analysis were also performed to study the microstructural changes. Results showed that samples with GNP incorporation showed better performance for the early age pre-cracking while for samples with pre-cracking at 14 and 28 days LWA incorporation showed enhanced healing. These results are shown Figures 2.6 and 2.7.

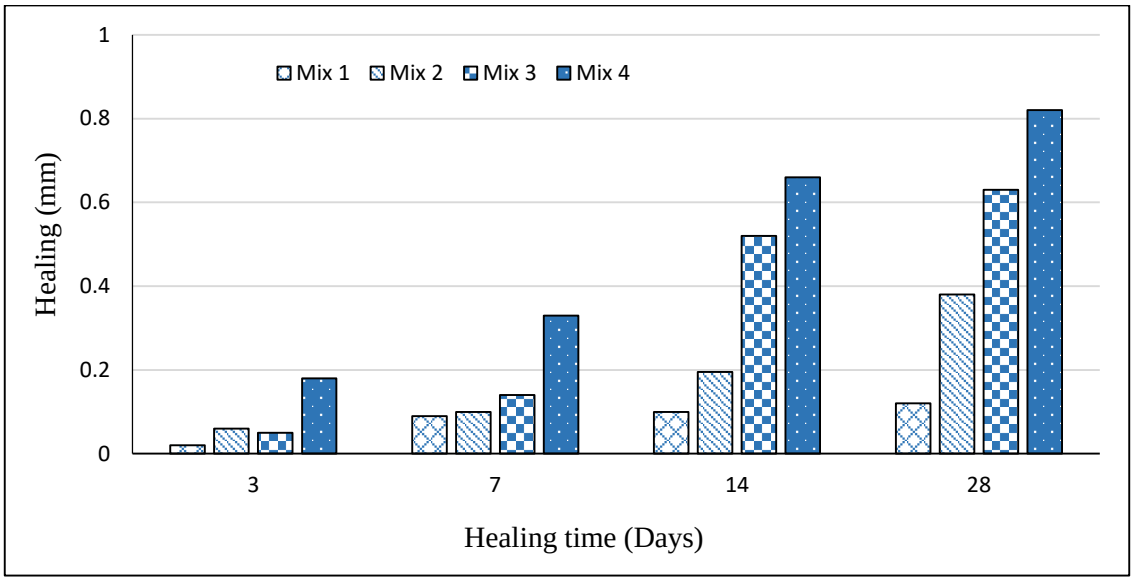

Figure 2.6 Crack healing in specimens pre-cracked at 7 days

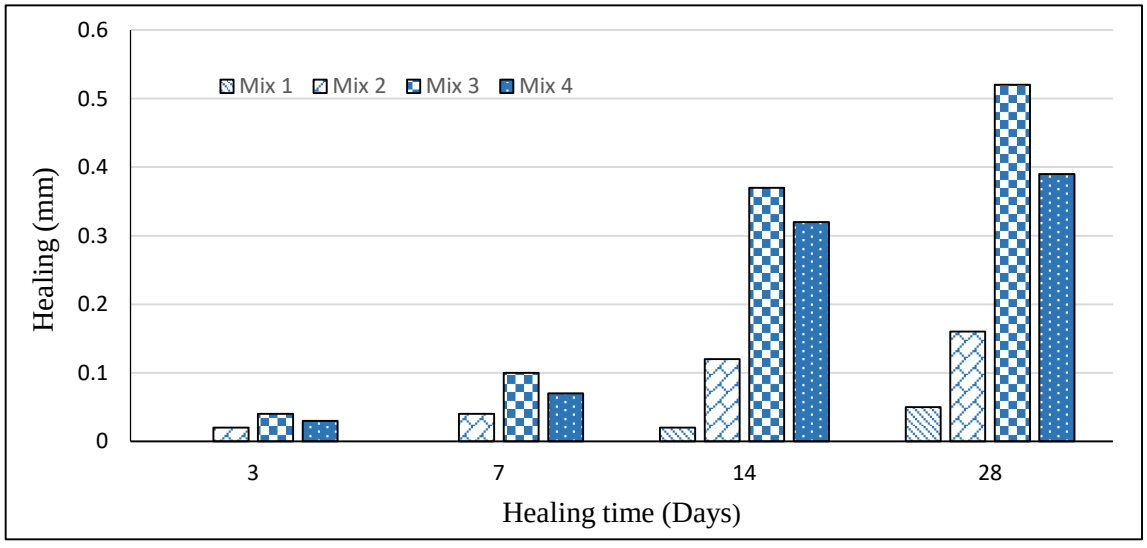

Figure 2.7 Crack healing in specimens pre-cracked at 28 days

(Wang et al. 2012) evaluated the self-healing efficiency of *Bacillus sphaericus* bacteria by immobilization in silica gel and polyurethane foam. The efficiency of bacteria in self-healing of cracks was determined by evaluating regain in the strength of the pre-cracked mortar specimens and the decrease in the water permeability. For this purpose, two types of mortar specimens were made, prisms (40 mm x 40 mm x 160 mm) to study the regain in compressive strength and cylinders (ϕ = 80 mm, H = 22 mm) for investigating the water permeability. The water cement and sand to cement ratio were kept at 0.5 and 3 respectively. Immobilized bacteria were incorporated into mortar specimens using glass tubes (length = 40 mm, dia = 3 mm) carrying the healing agents. Pre-cracking of specimens was carried out by subjecting to controlled loading and a crack width of 0.5 mm was induced. Prisms were reloaded after one week of curing to determine the regain in compressive strength. The efficiency of bacteria in self-healing of cracks was determined from the comparison of peak loads during first and second loading cycles. The cylinders were tested for water permeability and values were recorded at regular time intervals for 30 days of test.

The strength regain of specimens with PU immobilized bacteria was higher about 50% to 80% as compared to specimens with silica gel incorporated bacteria with strength regain of less than 5%. The strength regain in the control specimen was zero. Results of the strength regain of different specimens are shown in the Figure 2.8.

Test results showed that the coefficient of water permeability k slowly achieved a steady value over the 30 days of testing. For control samples the values of k ranged from 4×10^{-6} to 7×10^{-7} m/s. For specimens with silica gel incorporated bacteria the situation was same as the control specimens and the final value of k was in the range of 10^{-6} m/s. This result indicates that the silica gel has a very limited role in decreasing the water permeability of the cracked specimen. The value of k for specimens with PU immobilized

bacteria was about 6 x 10^{-11} to 7 x 10^{-11} m/s. The comparison of different specimens is shown in the Figure 2.9.

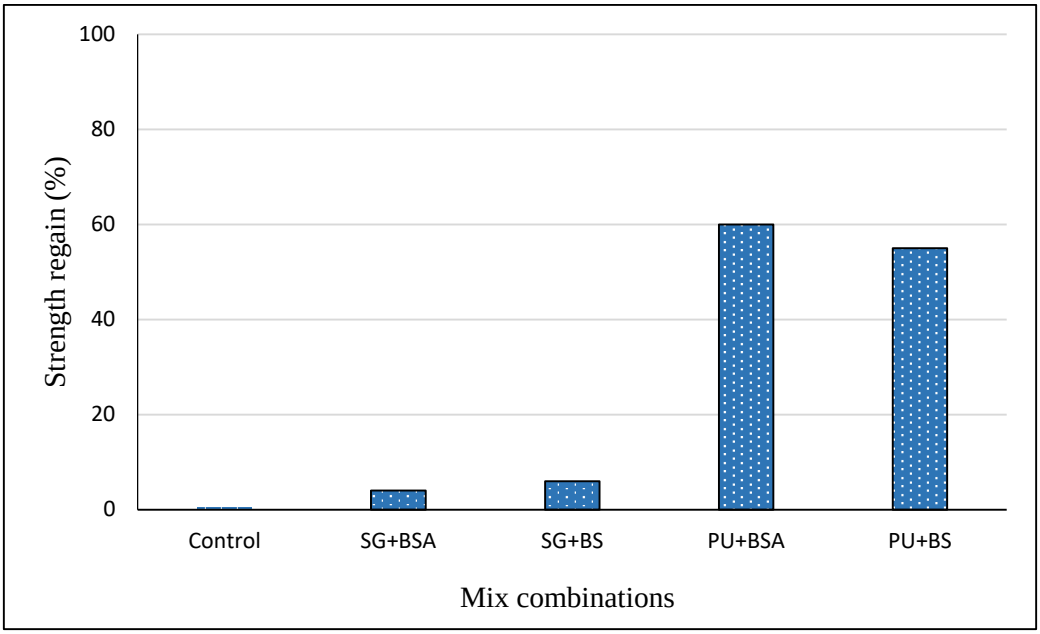

Figure 2.8 Strength regain percentage for different mix combinations

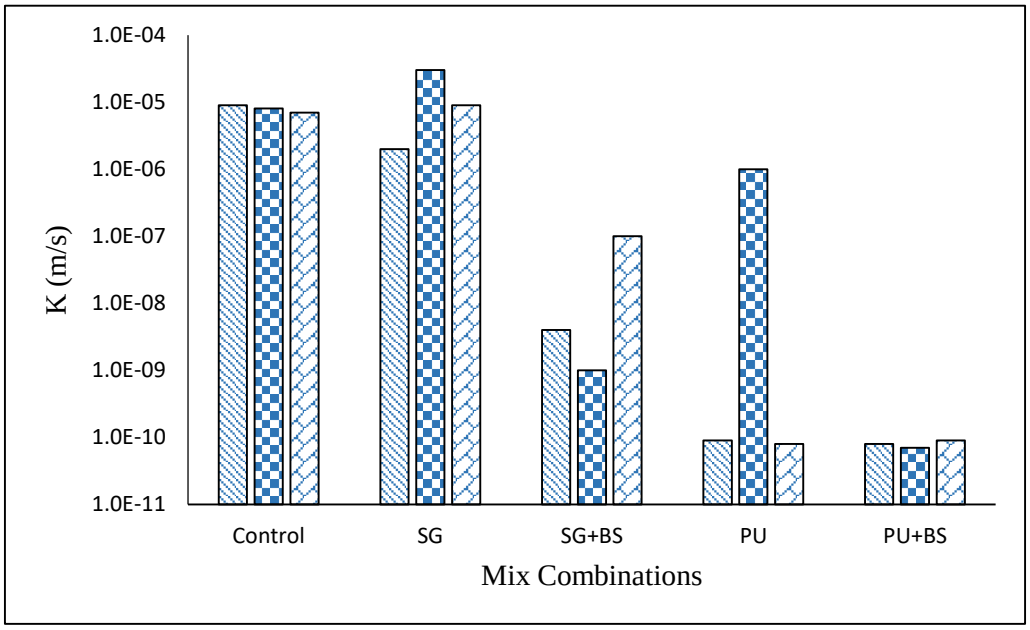

Figure 2.9 Water permeability of different cylinders after being repaired by different techniques

(Wiktor and Jonkers 2011) performed tests to study the crack healing capacity of bacteria based self-healing concrete. For this study bacterial spores were used as self-healing agent with a precursor organic compound calcium lactate immobilized in expanded clay

particles. The bacterial spores used in the study were alkali resistant soil bacteria named *Bacillus alkalinitrilicus*. Reinforced mortar prisms with dimension of 4 x 4 x 16 cm were prepared. Each specimen contained a Zinc coated steel bar placed horizontally in the mold and extending 5 cm from either side of the mold. Table 2.2 shows mixing proportions.

Ingredients	Weight (g)
Cement	384
Water	192
Fine aggregate (0.125-1 mm)	929
LWA (1-4 mm)	292
W/C ratio	0.5

Table 2.2 Mix proportions of specimens

After 56 days of curing, cracks were induced in reinforced mortar prisms by controlled tensile force. The induced cracks varied from 0.05 to 1 mm in width. Optical observation, SEM and oxygen consumption tests were conducted to evaluate the self-healing performance. Optical observation was conducted by stereomicroscope and the results for control and bacteria based specimens before and after 100 days of immersion in tap water are shown in the Figure 2.10. Despite a larger crack width, cracks in bacteria based specimens were completely healed as compared to control specimens.

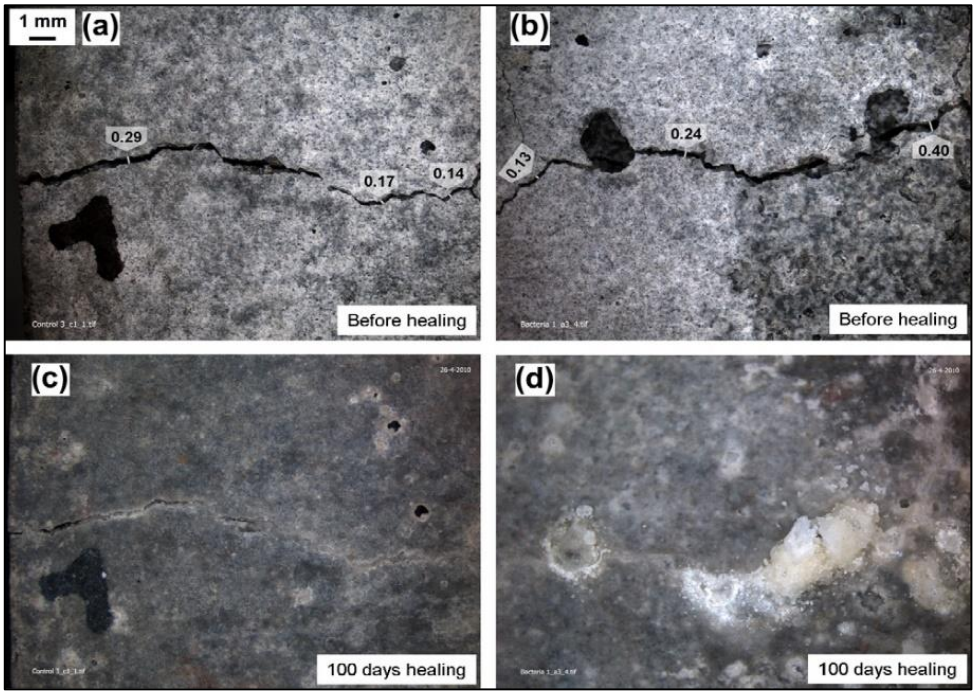

Figure 2.10 SEM images of crack healing in control and Bio chemical agent based specimens.

As the crack width varied along the length of each crack. Therefore, to quantify the crack healing efficiency, crack width was measured each week at regular intervals along the length of cracks. Healing efficiency for each location was calculated using

Healing % = (Cwi – Cwt / Cwi) x 100

Where Cwi is the initial crack width and Cwt is width at time t. A total of 150 measurements were made in both control and bacteria based mortar specimens. Results are presented in the Figure 2.11.

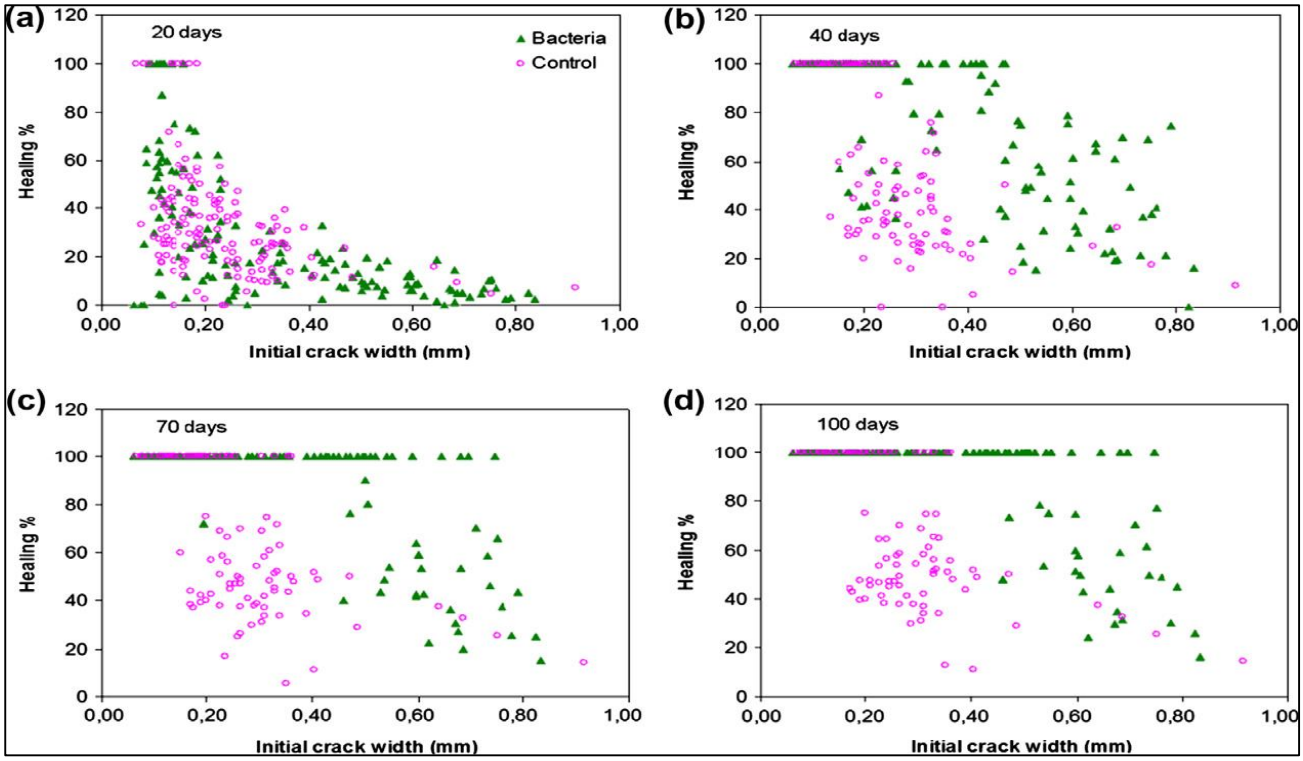

Figure 2.11 Crack healing as a percentage of initial crack width for control and bio chemical agent based specimens

(De Belie and De Muynck 2008) investigated the crack healing potential of bio deposition treatment of concrete. The bacterial culture used for the study was *Bacillus sphaericus.* The study was carried out by creating standardized cracks of 0.3 mm using thin copper plates in concrete specimens of 160 x 160 x 70 mm. Realistic cracks were induced by performing split tensile test on specimens wrapped in fiber reinforced polymer. The curing of cracked samples was done in a nutrient solution containing $CaCl_2$ or $Ca(NO_3)_2$. Bacterial strains were incorporated in concrete by immobilization in silica gel. The crack healing efficiency was checked by conducting visual inspection, ultrasound and water permeability tests.

Results of visual inspection and ultrasound tests showed complete healing of cracks up to 0.3 mm wide and 10 mm deep. Water permeability test confirmed that 0.6 mm wide cracks were completely healed by the process of bio deposition. Furthermore, water permeability tests also depicted that epoxy, BS+sol-gel+$CaCl_2$ and BS+sol-

gel+Ca(NO_3)$_2$ were most effective in the reduction of water permeability. Results are shown in the Figure 2.12.

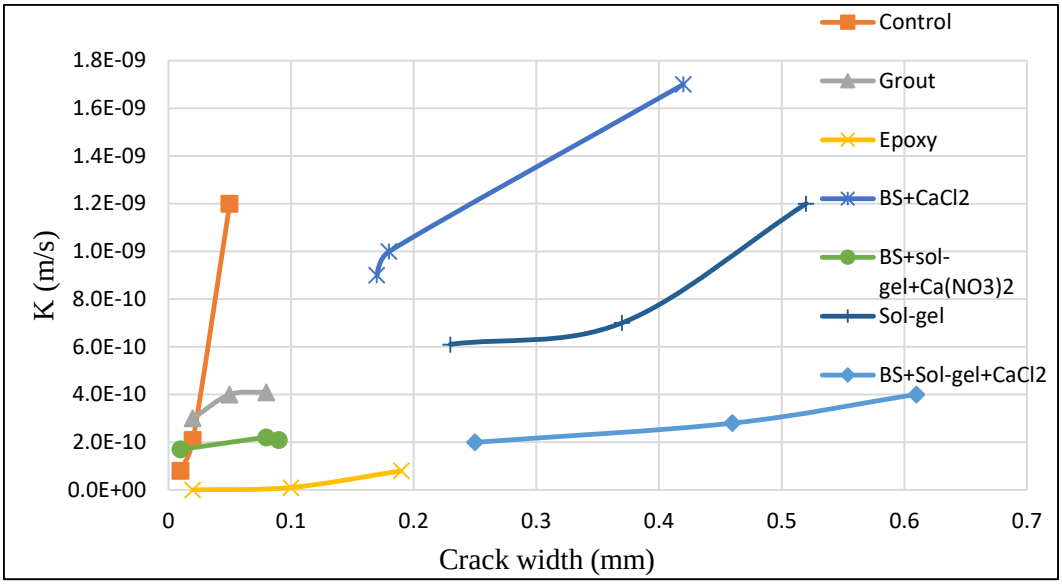

Figure 2.12 Water permeability of concrete samples against various crack widths

In addition to the type and concentration of bacteria, selection of carrier material also plays a significant role. In the production of self-healing concrete, strength of concrete and viability of bacteria are mainly dependent on the carrier material. Following are some studies reporting research related to carrier materials.

(Gadea et al. 2010) conducted a study on making of lightweight cement based mortar using polyurethane foam waste (PFW). PFW was grounded to the size less than 4 mm and was used as replacement of fine aggregate. CEM-I 42.5 R and CEM-IV 42.5 N were used in the making of lightweight mortar. Gadea checked different mechanical properties such as workability, permeability and strength for each replacement level. Results shown in Figure 2.13 depicted that inclusion of PFW caused an increase in the workability of mix. At 100% replacement level the increase in workability was found to be 120 min. While PFW played a positive role in increasing workability of the mix, its effects on flexural and compressive strength were devastating.

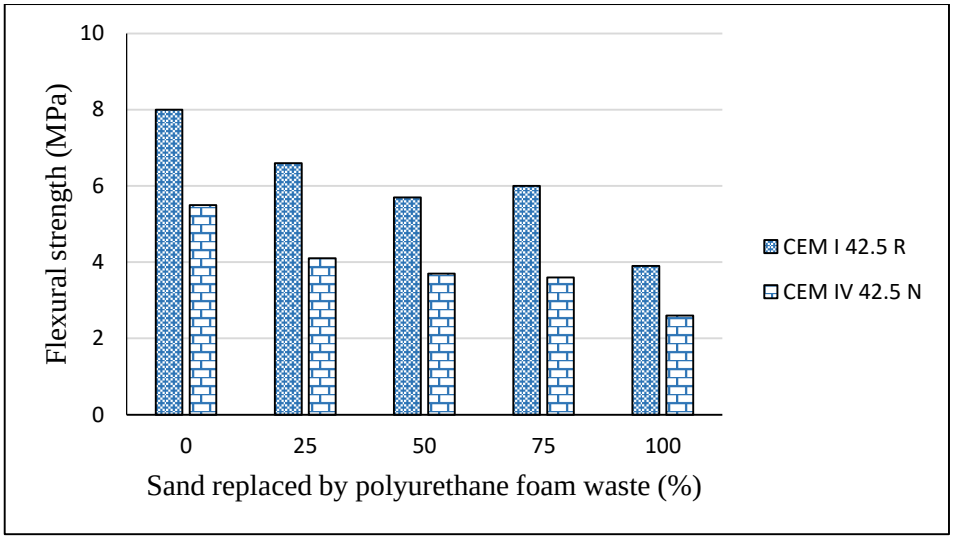

Figure 2.13 Flexural strength at various level of replacement of polyurethane foam

(Sikora et al. 2016) studied nano-Fe_3O_4 as an admixture and its effect on the mechanical properties and microstructure of cementitious composites. Cement was replaced by Nano-Fe_3O_4 at replacement level of 1 to 5%. During this study mortar specimen (40 x 40 x 160 mm) were casted using rapid hardening Portland cement type I 42.5 R. After 28 days of curing, flexural and compressive strength tests were performed on the mortar specimen. Mercury intrusion porosimetry was used for the pore structure characterization. SEM and EDX spectroscopy were also performed.

Results showed that Fe_3O_4 nanoparticles acted as a filler material, thus improving the microstructure and decreasing porosity. The addition of Fe_3O_4 nanoparticles had no effect on the rate of hydration and main hydration products. The reduced porosity attributed to increased compressive strength (up to 20%). This study also showed that 3% was the optimum level of replacement for both mechanical and microstructural properties. Results are shown in Figure 2.14.

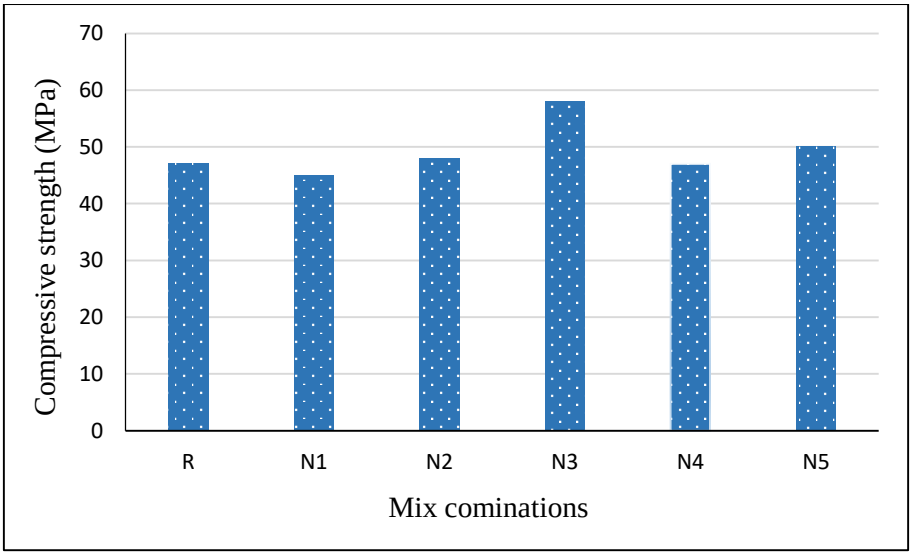

Figure 2.14 Compressive strength of mortar after 28 days of curing

The studies presented shows that there is variation in percent healing, threshold crack healing and compressive strength. These variations can be attributed to different factors such as type of bacteria used, their concentration, type and amount of nutrient compounds, material used for the immobilization of bacteria and various curing techniques used. For self-healing concrete to be practical, it is required to find the most effective bacteria along with its optimum concentration, a suitable carrier material and a host precursor compound.

2.3. Summary

This chapter of the thesis report included a review of the past studies on self-healing techniques. It was observed from the studies that a limited number of bacteria types and carrier materials have been investigated. The objective of this investigation is to evaluate the efficiency of *Bacillus subtilis* as a self-healing agent. This study will also focus on the performance of iron oxide particles, limestone powder and siliceous sand as carrier compounds in self-healing concrete.

3. MATERIALS AND EXPERIMENTAL PROGRAM

3.1. General

In recent years, many studies have been conducted on the emerging and promising technique of bio-inspired self-healing concrete. These studies used different groups of bacteria and protective carrier compounds, however the variation in amount of self-healing and compressive strength showed that there is still a need of further investigation on new alternatives in the form of both bacteria and protective carrier materials. Evaluation of the ideal healing bacteria and protective material can be helpful in the optimization of the self-healing process in bio-inspired concrete. In this study mortar specimens, incorporated with precursor organic compound and carrier materials impregnated with bacteria, were casted. The specimens were checked for compressive strength and pre-cracking technique were applied for healing studies. Details of the different formulations used in the study are provided in the Table 3.1.

Formulation	Dimension (in)
F-1	2 x 2 x 2
F-2	2 x 2 x 2
F-3	2 x 2 x 2
F-4	2 x 2 x 2

Table 3.1 Description of test specimens

Formulations names are used to differentiate the incorporation techniques used for the addition of bacteria in the mortar specimens. F-1 represents control specimens which contain no bacteria and precursor organic compound. F-2 is used to represent the mortar specimens containing bacteria incorporated through Iron oxide particles. Bacterial

incorporation through limestone particles is represented by F-3, whereas, F-4 is used to denote bacterial inclusion by siliceous sand particles.

For compressive strength of mortar, specimens were tested in accordance to ASTM C-109 standards and ASTM 2809 standard was used for the scanning electron microscopy of the specimens. This chapter includes the description of the materials and sample preparation methods used in the study. Experimental setup including testing procedures is also discussed in this chapter.

3.2. Materials

ASTM C150 Type-I ordinary Portland cement (grade 53) was used along with normalized siliceous sand having fineness modulus of 2.02 and specific gravity of 2.65 in the preparation of cement mortar composite samples. The average particle size 'D_{50}' of cement was 16.40 μm with a density of 3.17 g/cm^3. The chemical composition of cement was determined by X-Ray Fluorescence (XRF) and is shown in Table 3.2. The self-healing microorganisms *Bacillus subtilis* were grown locally. In total, three carrier materials were investigated namely iron oxide, limestone and siliceous sand particles. The detailed procedure of microorganism production and their utilization is discussed in the following paragraphs.

3.2.1. Production of Microorganisms

Freshly prepared cementitious composites exhibit high alkalinity. It may be attributed to calcium hydroxide content which is the second most produced hydration product after calcium silicate hydrate (CSH) gel. Capillary water in fresh concrete has a pH value in the range of 11 to 13. Therefore, it is immensely important that self-healing microorganisms must have the ability to adjust within the alkaline environment while producing large amounts of calcium carbonate independent of the calcium ion concentrations in the composite matrix (Rao et al., 2013; Van Tittelboom et al., 2010;

Schlegel and Zaborosch, 1993). In the present study, *Bacillus subtilis* were chosen as self-healing agent as they satisfy the required conditions to survive in the unfavorable conditions of mortar matrix. *Bacillus subtilis* is gram-positive bacteria, which have the capability of forming spores when the environment is harsh and unfriendly. This ability of bacteria to form spores, protects the bacteria from mechanical stresses and alkalinity of the mortar matrix (Schlegel and Zaborosch, 1993).

Bacillus subtilis was regenerated by adding 1 ml of frozen glycerol to 5 ml of 0.9% autoclaved saline solution. The sporulation of bacteria was performed using standard protocols. Fresh *Bacillus subtilis* colony was inoculated in 15 ml of LB media (5 g of NaCl, 5 g of trypton and 2 g of LG broth in 1000 ml of water) and the mixture was kept for incubation for 6 to 8 hours at 37 ºC shaking at a rate of 200 rpm. Difco sporulation medium (DSM) was prepared by mixing 1.5 g of meat extract, 2.5 g of peptone, 0.25 g $MnSO_4$, 0.1 g of KCl and 0.5 ml of $MgSO_4$(1M) in 500 ml of water and autoclaved for sterilization. After sterilizing the media 0.25 ml of 1 molar $CaCl_2$ solution and 0.5 ml of 1 mM $FeSO_4$ was added to the media. A mixture of 2.5 ml of inoculated LB media and 500 ml of DSM was incubated for 4 days at 37 ºC while shaking at 200 rpm. Cells were then washed 8 to 10 times to remove any remaining nutrients and lyse vegetative cells.

A bacterial growth curve showing the rise and fall in bacterial population with time was plotted as shown in Figure 3.1 in which different phases of bacterial growth may be distinguished clearly. In lag phase of the curve, bacteria adapt to the growth conditions. Exponential phase also known as log phase is characterized by the doubling of cells. The growth is proportional to the number of bacteria present. After the depletion of nutrients in the medium, bacterial growth enters the stationary phase in which growth and death rates are equal and finally in the death phase, decline of bacterial population can be observed.

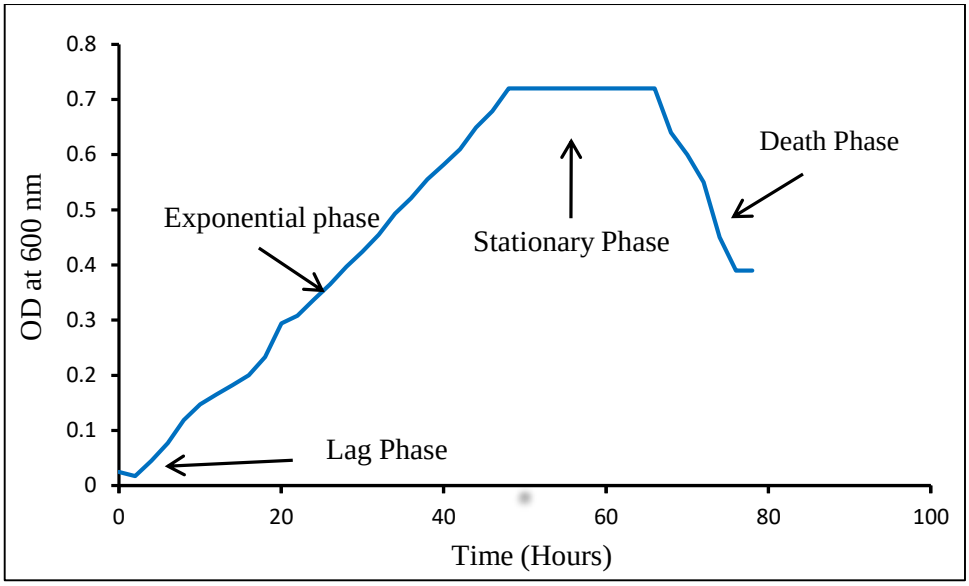

Figure 3.1 Bacterial growth curve of Bacillus subtilis

Concentration of bacterial solution was determined using a spectrophotometer. To perform the test, medium used for the bacterial growth was selected as blank and used as standard for the measurement of optical density of bacterial solution. At first 0.5 ml of standard blank solution was placed in spectrophotometer and 600 nm wavelength was selected. After the machine was calibrated using blank solution, 0.5 ml of bacterial solution was placed in spectrophotometer and again 600 nm wavelength was used to conduct the optical density test. The bacterial concentration was determined using the expression $Y = 8.59 \times 10^7 X^{1.3627}$ where Y is the concentration of bacterial cells per ml and X is the reading from spectrophotometer at OD_{600}. Using the reading from spectrophotometer, bacterial concentration was calculated to be 2.8×10^8 cells/ml of the solution. The spore concentration of bacteria in concrete was maintained at 6×10^6 cells/cm^3 of concrete mixture.

Figure 3.2 Bacterial solution

3.2.2. Carrier Materials

3.2.2.1. Iron oxide Nanoparticles

Protocol from (Horák et al. 2007) was used for the synthesis of iron oxide nanoparticles. A solution of $FeCl_2$ and (0.2 mol/L) and $FeCl_3$ (0.2 mol/L) in 1:2 molar ratio was prepared. NH_4OH (0.5 mol/L) was added dropwise to the solution till the pH became 12. The resulting product was washed with distilled water to remove excess ammonium ion or until the pH has been neutralized. The colloidal mixture was then sonicated for 5 minutes. An aqueous solution of NaClO (5 wt %) was added to the mixture in the presence of sodium citrate solution (0.1 M) to oxidize the particles. After oxidation, the mixture was repeatedly washed and sonicated. Finally, aqueous D-mannose solution (20 wt %) was added to iron oxide mixture. The resulting product was centrifuged at 14000 rpm for three minutes and then washed with 10 ml of water.

Figure 3.3 Iron oxide nanoparticles colloidal solution

The size and shape of iron oxide particles were investigated using scanning electron microscopy. The SEM image presented in Figure 3.4 showed that the iron oxide particles were spherical in shape with particle size ranging from 15 to 22 nm. The average particle size 'D_{50}' was recorded to be 18.5 nm.

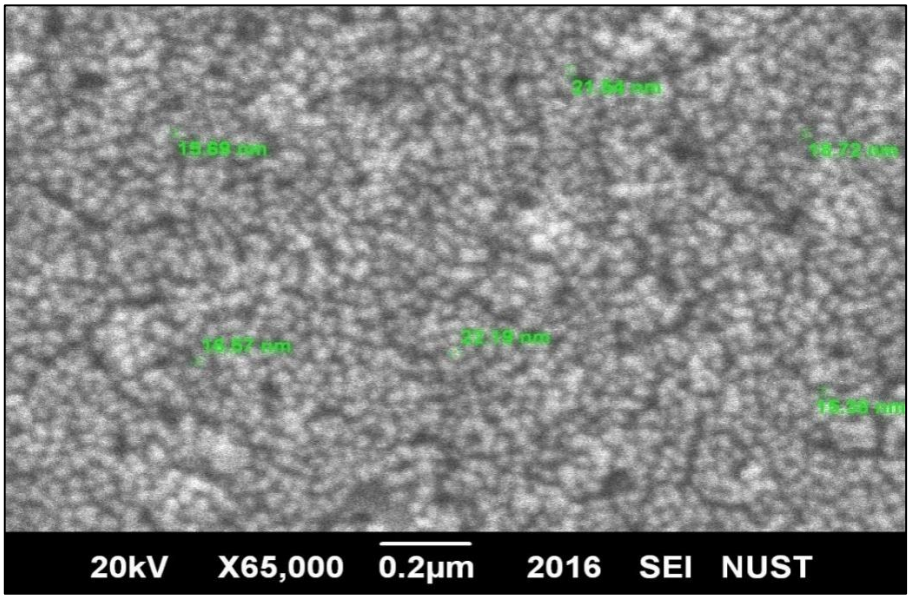

Figure 3.4 SEM image of Iron oxide nanoparticles at X 65,000 magnification showing nanoparticles.

3.2.2.2. Limestone Particles

Limestone particles (LSP) were used as carrier material for the immobilization of bacteria in mortar samples. Particle size analysis and absorption tests were performed on the LSP used in this study. Size analysis showed that the average particles size of the LSP was 22.3 µm with a surface area of 3048 m^2/kg. The absorption test was performed by soaking the 50 g of LSP in water for 24 hours. The water was then filtered off and the wet weight of the sample was noted. The sample was oven dried for 24 hours and the dry weight was measured. The absorption capacity of LSP was found to be 26 %. X-Ray Fluorescence (XRF) results summarized in Table 3.2 shows the chemical composition of limestone particles.

Table 3.2 Chemical composition of OPC and LSP (%)

Parameters	**CaO**	**SiO_2**	**MgO**	**Al_2O_3**	**Fe_2O_3**	**MgO**	**LOI**
OPC	65.00	19.19	2.23	4.97	3.27	2.23	3.84
LSP	52.67	3.00	0.67	0.69	0.27	0.67	42.24

Figure 3.5 Limestone particles

3.2.2.3. Sand

Lawrencepur sand was utilized for this study. The fineness modulus of sand was determined in accordance to ASTM C-136 and found out to be 2.02 with an average particle size (D_{50}) of 0.40 mm. The specific gravity of sand was recorded to be 2.65. Like LSP, water absorption capacity of sand was also determined. ASTM C-128 was adopted for the absorption capacity of sand. Sand was soaked in water for 24 hours and then dried to SSD conditions using a heat gun. The weight of the sample in SSD condition was measured and then placed in the oven for 24 hours and the dry weight of the sample was recorded. Test result showed the absorption capacity of water to be 2.4 %.

Figure 3.6 Siliceous sand

3.2.3. Mix Proportion

In this study four different types of formulations were used. The cement used in the study was ordinary Portland cement (OPC) type – I conforming to ASTM C 150-07. The mix contained 933 kg/m3 of OPC, 1400 kg/m3 of fine aggregate and 18.7 kg/m3 of calcium lactate. A constant water cement ratio of 0.4 was used for all mixes. Cement was checked

for normal consistency and initial and final setting time conforming to ASTM C191-11 and C187-11 respectively.

Specimens containing no bacterial spores were named as “F-1 The mix with iron oxide particles as protective carrier material was labeled as “F-2”. Iron oxide particles were attached to the bacterial spores by mixing the aqueous solution of bacterial spores with iron oxide colloidal solution. The specimens of “F-3” contained bacteria immobilized on limestone particles. Limestone particles were soaked in bacterial solution for 24 h before mixing into the mortar. Specimens containing bacterial spores incorporated by siliceous sand particles were designated as “F-4” and like limestone particles, siliceous sand was soaked for 24 h before using in the mix. The mixing regime was kept uniform for all the mixes and the details of the prepared formulations are summarized in the Table 3.3.

Table 3.3 Mix design of different formulations

Specimens		**F-1**	**F-2**	**F-3**	**F-4**
Cement	Kg/m^3	930	930	930	930
Fine Aggregate	Kg/m^3	1400	1400	1400	1400
Water Cement ratio		0.4	0.4	0.4	0.4
Calcium Lactate	Kg/m^3	18.7	18.7	18.7	18.7
Bacterial solution	$Liter/m^3$	0	7.6	7.6	7.6
Incorporation Technique		None	Iron oxide Particles	LSP	Siliceous sand

3.2.4. Test Specimens

Test specimens were prepared in Hobart mixer in accordance to ASTM C-305 and tamped to ensure proper compaction. Specimens were de-molded after 24 hours and were placed for curing. For all mix types, cubes of 50 mm were prepared. Specimens were

checked for compressive strength at 3, 7, 14 and 28 days of curing and the average value of three specimens was taken as compressive strength. For healing investigation three samples were cracked at 3, 7, 14 and 28 days of curing for each mix and were further investigated for healing measurement. The microstructural matrix of the specimens was also studied using Scanning Electron Microscope (SEM). Samples were also tested using Energy Dispersive X-ray analysis (EDX) and Thermogravimetric analysis (TGA) to investigate the mineral formation and its chemical composition.

Figure 3.7 Casting of samples

3.2.5. Test Procedure

Specimens were checked for compressive strength at 3, 7, 14 and 28 days of curing. The average value of three specimens was utilized as the compressive strength. For compressive strength test, specimens were taken out of the curing tank and surface was wiped off. Samples were tested in compression machine. The test was carried out in accordance to ASTM C-109.

For healing investigation, specimens were carefully loaded up to 80% of the compressive strength of the sample and then unloaded. This application of load caused internal cracking in the specimen. After initial pre-cracking, samples were placed in water and

healing was checked by the amount of compressive strength regained after 28 days of curing. Average value of three specimens was taken for healing measurements.

In addition to the above tests, samples were collected from the specimens pre-cracked at 7 and 28 days after healing measurement and were analyzed using scanning electron microscope (SEM). Samples were also subjected to EDX and TG analysis.

Results from all of the above tests were recorded. The results were compiled and analyzed to compare the efficiency of the different incorporation techniques. Results of the tests and their comparison along with related discussion are included in chapter 4 of this report.

4. RESULTS AND DISCUSSION

4.1. General

In this section, test results pertaining to the compressive strength, strength gain in pre-cracked samples, microstructural analysis via SEM and chemical composition via EDX & TG analysis are presented in detail.

4.2. Compressive strength analysis

Compressive strength results for mortar specimens are shown in the Figure 4.1. An increased compressive strength was noticed for all formulations.

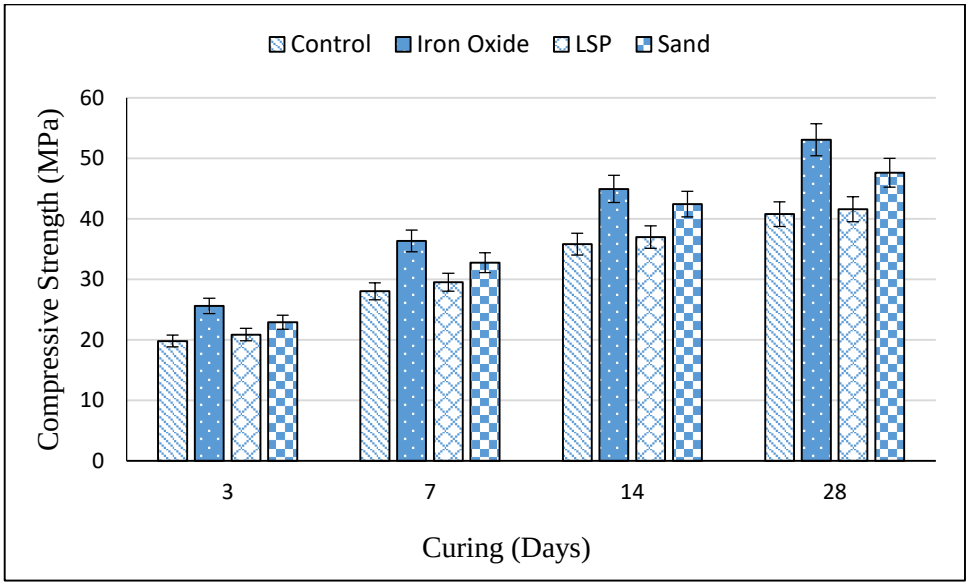

Figure 4.1 Compressive strength of mortar at different curing periods

Sample with iron oxide particles used for the incorporation of bacterial spores showed maximum compressive strength of 53.07 MPa, which is 23% increase in compressive strength over controlled specimens. This increased compressive strength is in agreement with the results of the study carried out by (Sikora et al. 2016). The increased compressive strength of the mortar specimen can be attributed to three phenomena. Firstly, due to their strong electrostatic attraction and greater surface area, a more rapid setting and hardening of the specimen is obtained. Secondly, due to the ultrafine size of the iron oxide particles,

they can also act as a filler material and therefore lead to a more compact microstructure. Thirdly, the nano-sized immobilizing media is capable to homogeneously disperse the injected microbes in the entire matrix contributing in the effective sealing of developed nano/micro cracks. These three phenomena are responsible for the decrease in the amount of pores and increase in the density of the matrix which in turn leads to increased compressive strength of the resultant mix.

Compressive strength increase in the specimens with siliceous sand as carrier material was recorded to be 14%. This strength increase of the specimen is in accordance with the previous studies and may be attributed to the incorporation of the calcite producing bacteria in the mix (Krishnapriya, Venkatesh Babu, and G. 2015; Park et al. 2010). The continual formation of calcium carbonate and the presence of calcium lactate added as a precursor organic compound made the internal microstructure of mortar more compact and therefore, resulted in the enhancement of the compressive strength. The comparison of this result with the study performed by (Ramachandran, Ramakrishnan, and Bang 2001) shows that *Bacillus subtilis* is a better choice as compared to *Bacillus pasteurii* which showed no significant strength increase.

The addition of Limestone particles as a carrier material for the immobilization of microbes showed a slight increase in compressive strength. Limestone particles does not have any pozzolanic properties and act as filler material (Bederina, Makhloufi, and Bouziani 2011). They provide nucleation sites for the growth of hydration products and improve the microstructure of the matrix. This denser microstructure resulted in increased compressive strength of the specimens (Naik, Canpolat, and Chun 2003). The microbial action seems to be little effective in conjunction with LSP.

4.3. Compressive strength regain

Self-healing efficiency of different incorporation techniques was mechanically

investigated by determining the amount of regain in the compressive strength of specimen after being subjected to the predefined preloading i-e 80% of 'f'c'. Result of compressive strength regain is shown in Figure 4.2 while Figure 4.3 displays percentage of compressive resistance regained with reference to ultimate compressive resistance of the corresponding formulation as reported in Figure 4.1. The compressive strength regain for samples pre cracked at 3 days showed a maximum strength regain for samples carrying iron oxide particles as carrier material. This high healing efficiency of iron oxide can be attributed to its particle size. The nano particle size of iron oxide causes it to behave as filler material and ensures its even distribution throughout the mixture. Since iron oxide particles are coated with bacteria, so it also helps in the uniform and even distribution of bacterial medium throughout the mixture. Thus availability of bacteria is ensured at crack sites (bederina et al. 2011; Wiktor and Jonkers 2011). Limestone particles showed maximum regain of compressive strength after iron oxide particles. Although limestone particles also acted as filler material but its distribution was not as uniform as compared to iron oxide particles due to the relative coarser size of its particles. Similar trend was followed by samples containing siliceous sand as a carrier material and showed the least value of compressive strength regain among different incorporation techniques. All bacteria containing samples showed a greater gain of compressive strength as compared to control samples. Control samples also showed regain of compressive strength which can be attributed to the hydration of unhydrated cement grains at early age and carbonation of calcium hydroxide to produce calcium carbonate crystal which can heal cracks (Schlangen and Jonkers 2008; Reis et al. 2011).

Samples pre-cracked at 7 days of curing showed a similar trend in the compressive strength regain as seen in the specimens pre-cracked at 3 days of curing. F-2 showed the maximum regain of compressive strength as compared to other incorporation techniques.

This regain was attributed to smaller particles size of iron oxide which ensures effective filling of pores and even distribution of bacteria throughout the matrix. Also it exhibits better compatibility to retain *Bacillus subtilis* microbes in dormant stage till the development of cracks. Compressive strength regain was recorded to be 91.3%. F-3 containing limestone particles as a protective material regain of compressive strength was observed to be 77.6% while the value of compressive strength regain for F-4 was 72.9%. Again, control samples showed lower values of compressive strength regain as compared to the samples containing microbes.

Compressive strength regain for samples pre cracked at 14 days of curing was maximum for F-2 carrying iron oxide particles as immobilizer. This strength regain is due to fine particles size as discussed above and the ability of iron oxide particles to provide a better cover to bacteria by forming a protective layer around the bacterial spores as shown in Figure 4.3. The strength regain was observed to be 79.9%. F-2 was followed by F-4 containing siliceous sand as carrier material instead of LSP like before. This greater healing in siliceous sand incorporated bacteria can be attributed to the fact that siliceous sand provide a better cover to bacteria as compared to LSP due to their relative coarser particle size (Khaliq and Ehsan 2016). The viability of bacteria is reduced in samples containing LSP as a carrier material. The strength regain for siliceous sand was reported to be 69.1% while regain for LSP was 57.8%. There was a little strength regain in control samples as the hydration reaction has continued for a good period and there is less unhydrated cement available for natural healing.

Samples pre-cracked at 28 days of curing followed the same trend as of samples pre-cracked at 14 days. The more strength regain of F-2 is due to finer particle size and a better protective cover for bacteria. F-4 showed a higher regain of compressive strength as the siliceous sand particles provide better protection to bacterial spores than LSP which

was incorporated in F-3. Again, control specimen showed the least regain of compressive strength as compared to the formulations containing ‘Bacillus subtilis’ microorganisms.

It was observed that the strength regain for each incorporation technique decreases with increase in the pre-cracking age. The decrease in healing mechanism may be associated with two possible phenomena. The first one relates to the decline in natural healing at later ages beyond 7 days due to almost 100% hydration of cementitious grains. The second phenomenon links with the establishment of dense and stronger microstructure at later ages, which tends to reduce the activity of bacterial spores in the composite matrix. The decrease in compressive strength regain was minimum for F-2 carrying iron oxide particles as protective carrier material. This indicates that iron oxide particles are much compatible immobilizer of *Bacillus subtilis* microbes into cementitious environment. LSP showed relative better results for early age pre-cracking but in denser microstructure, it was not able to protect bacteria. This was due to the fact that LSP are relatively soft and not strong enough to resist the pressure resulting from the denser microstructure. Siliceous sand, being harder and coarser as compared to LSP, provides better cover in the denser microstructure (P. Thongsanitgarn,W. Wongkeo, S. Sinthupinyo 2012).

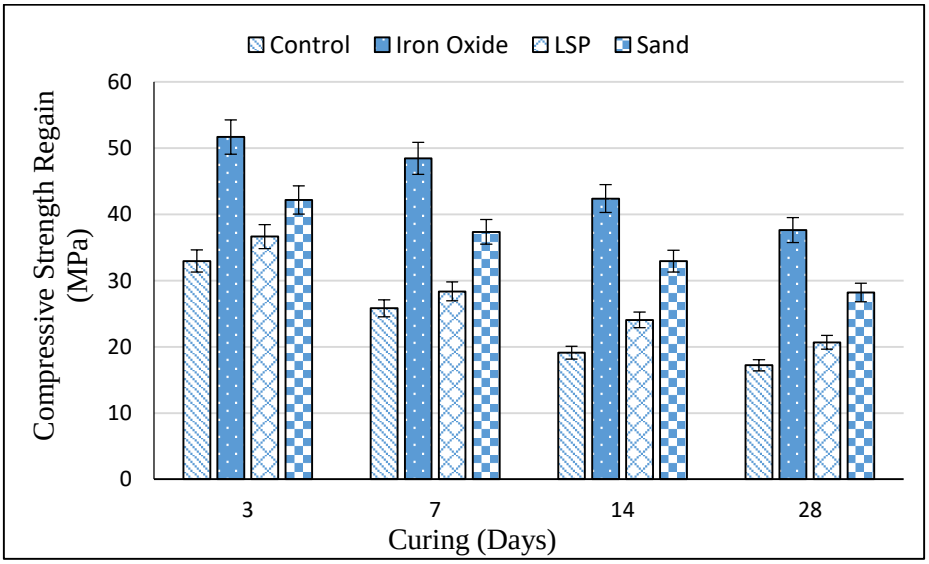

Figure 4.2 Compressive strength regain for samples pre-cracked at different ages of curing.

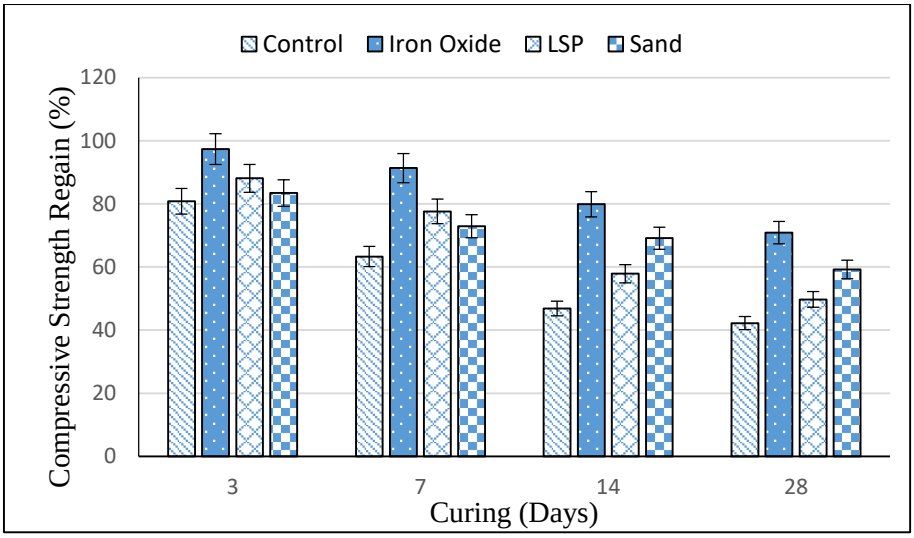

Figure 4.3 Percentage compressive Strength regained with reference to ultimate compressive Strength

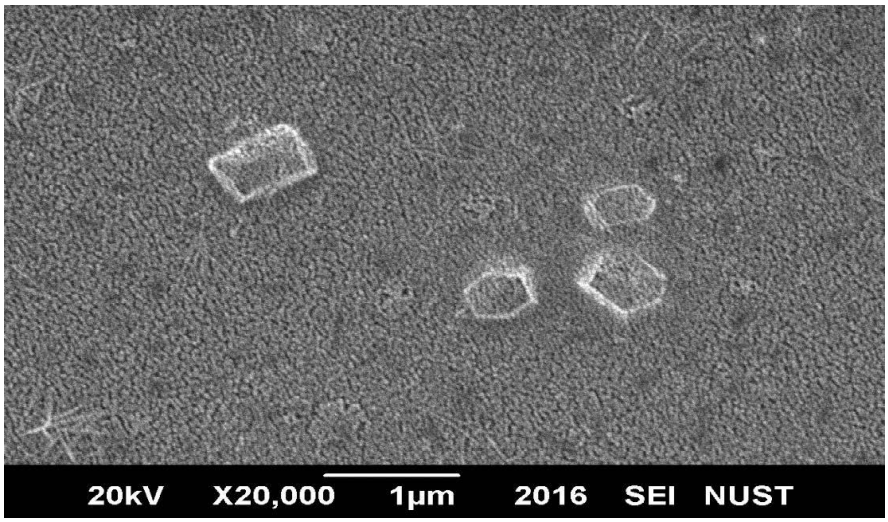

Figure 4.4 SEM image of bacterial spores covered by iron oxide particles

4.4. Microstructure analysis

Scanning electron microscopic analysis of the specimens with different incorporation technique was carried out to examine the microstructure for any possible signs of calcite precipitation. Specimens pre-cracked at 7 and 28 days of curing were selected for SEM observations after 28 days of healing process.

The main focus of micro-investigation is to observe the production of calcium carbonate crystals that can be a gauging factor of the self-healing efficiency using different immobilizers. There are three different forms of calcium carbonate crystals namely calcite, aragonite and vaterite. Among these different forms, calcite is the most stable

form of calcium carbonate (Rao et al., 2013). The chemical reaction for the formation of calcium carbonate by *Bacillus subtilis* microorganisms is shown below in equation (1), in which calcium lactate reacts with oxygen and converted into calcium carbonate , carbon dioxide gas and water (Zhang et al. 2017).

$$CaC_6H_{10}O_6 + 6O_2 \rightarrow CaCO_3 + 5CO_2 + 5H_20 \qquad (1)$$

Figures 4.4 and 4.5 show the microstructure of F-4 having siliceous sand as a carrier material. Production of the rhombohedral calcite can be clearly seen in the micrographs resulted by microbial activity (Yau and Vekilov 2001). Figure 4.4 shows the micrograph for sample pre-cracked at 7 days while Figure 4.5 is for the sample pre-cracked at 28 days of curing. The microstructure of specimens containing LSP as incorporation material is shown in Figures 4.6 and 4.7. Formulation F-2 containing siliceous sand as an immobilizer is shown in Figures 4.8 and 4.9. All the micrographs clearly reveal evident signs of calcite precipitation along the developed cracks in the fractured specimens.

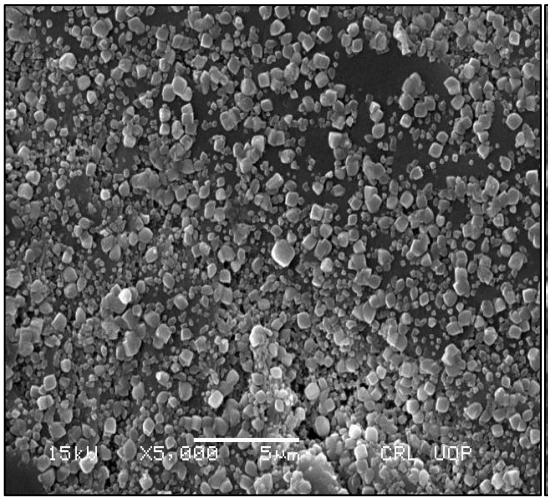

Figure 4.5 SEM image of F-4 specimen pre-cracked at 7 days

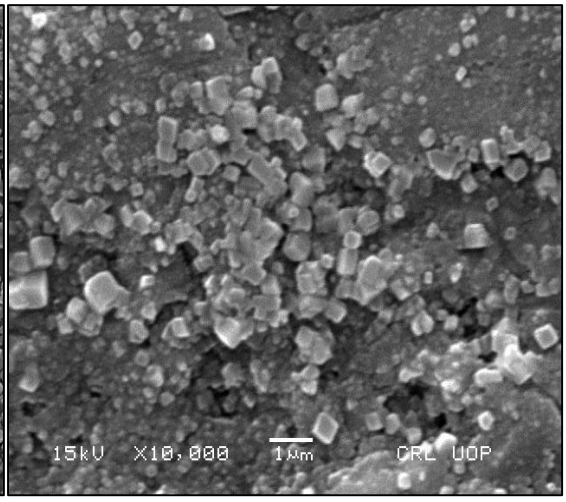

Figure 4.6 SEM image of F-4 specimen pre-cracked at 28 days

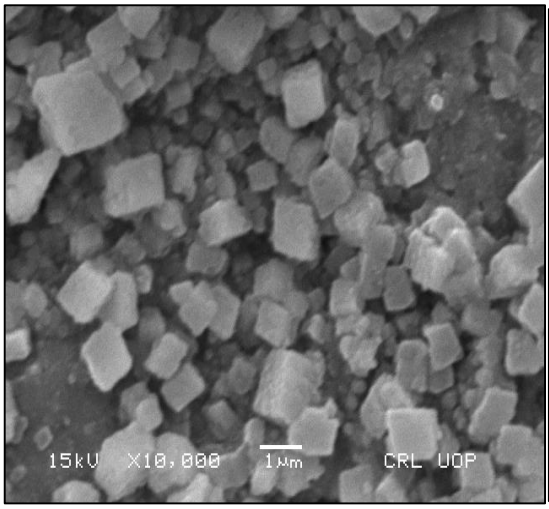

Figure 4.7 SEM image of F-3 specimen pre-cracked at 7 days

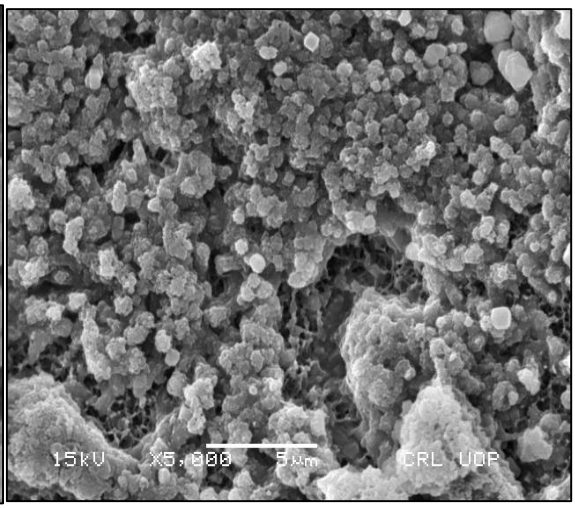

Figure 4.8 SEM image of F-3 specimen pre-cracked at 28 days

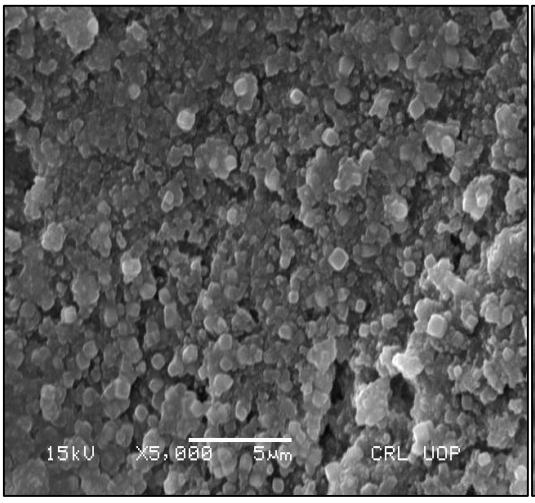

Figure 4.9 SEM image of F-2 specimen pre-cracked at 7 days

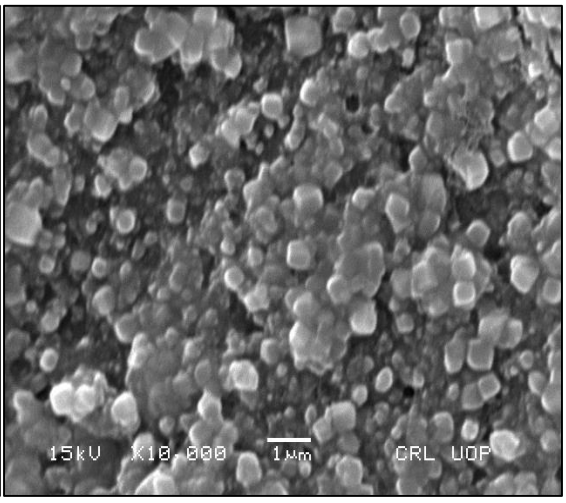

Figure 4.10 SEM image of F-2 specimen pre-cracked at 28 days

4.5. Energy dispersive x-ray analysis

In order to have a better understanding of the self-healing process with the analyzed immobilization techniques, the samples pre-cracked at 7 and 28 days of curing were characterized via EDX analysis to evidence the formation of calcium carbonate crystals by the immobilized *Bacillus subtilis* microbes. The EDX results of samples pre-cracked at 7 days are presented in Table 4.1. The analysis indicated maximum $CaCO_3$ formation for F-3 containing LSP as carrier material. F-3 was followed by F-4 containing siliceous sand as the protective immobilizing media. Least amount of $CaCO_3$ crystals were

produced in F-2 with iron oxide particles as the immobilizer. Although F-2 showed maximum strength regain, its calcite production was least of all incorporation techniques. This anomaly may be attributed to the fact that in F-2 bacterial spores were covered by iron oxide particles as shown in Figure 4.4 and had less exposure to ingress water as compared to LSP and siliceous sand which resulted in the lower production of calcite. The higher strength regain of F-2 is due to the particle size of iron oxide particles. Being small in size they act as filler and resist the cracking at nano level. Hence for same amount of loading, F-2 samples had less number of cracks as compared to other incorporation techniques (Reis et al. 2011). This fact also contributed to the less production of $CaCO_3$ crystals.

Table 4.1 EDX result of sample pre-cracked at 7 days of curing

Formulation	**Chemical composition weight %**				
	$CaCO_3$	SiO_2	$CaSiO_3$	Al_2O_3	Fe
F-2	10.73	46.22	22.51	3.15	2.29
F-3	14.51	44.87	31.41	1.03	1.61
F-4	13.64	45.64	24.54	2.18	1.81

The EDX results for samples pre-cracked at 28 days are summarized in Table 4.2. F-4 specimen having siliceous sand as bacterial carrier showed maximum production of $CaCO_3$ crystals, followed by F-3 with LSP as the immobilization media. Once again least production of calcite crystals was observed for F-2 with iron oxide particles as protective material. The higher yield of calcium carbonate crystals in F-4 can be attributed to coarse and hard nature of the siliceous sand particles as compared to LSP. As the microstructure

was developed and dense at 28 days of curing, LSP might not be able to protect the immobilized microbes leading to the lower yield of calcite crystals. It was also observed that F-2 samples almost had the same amount of calcite production for pre-cracking age of 7 and 28 days whereas F-4 and F-3 samples showed a decrease in the calcite production from 7 to 28 days of pre-cracking. This decrease was an indicator of the carrier material efficiency in providing cover to bacterial spores. The decrease was significant for F-3 samples having LSP as carrier material. F-4 showed a slight decrease in calcite production which was due to the decreased viability of bacteria. There was no decrease of calcite production in F-2 samples carrying iron oxide particles as protective material as mentioned earlier. Thus, it can be inferred that iron oxide particles were more efficient in protecting bacterial spores as compared to other immobilization techniques.

Table 4.2 EDX result of sample pre-cracked at 28 days of curing

Formulation	**Chemical composition weight %**				
	$CaCO_3$	SiO_2	$CaSiO_3$	Al_2O_3	Fe
F-2	10.49	44.06	19.80	2.08	8.22
F-3	10.99	48.72	19.87	2.34	2.06
F-4	11.68	46.51	22.97	3.24	1.35

4.6. Thermo-gravimetric Analysis

To further evidence the microbial activity by chemical means, thermo-gravimetric analysis was performed on the powdered cementitious material recovered from the healed crack. Thermo-gravimetric analysis measures the loss of mass of sample with increasing temperature. This loss of mass helps in identifying different chemical compounds based on their decomposition temperature. The decomposition temperature for $CaCO_3$ crystals

ranges from 600 to 850 ˚C as reported by (Halikia et al. 2001). The decomposition reaction of $CaCO_3$ is as follow.

$$CaCO_3\ (s) \longrightarrow CaO(s) + CO_2\ (g) \qquad (2)$$

TGA results are given in Figure 4.11 displaying significant loss in the temperature range of 600-850 ˚C which is an endorsement to calcite precipitation in developed cracks. Hence, it can be stated that the investigated immobilization media effectively preserved *Bacillus subtilis* microbes till the generation of cracks.

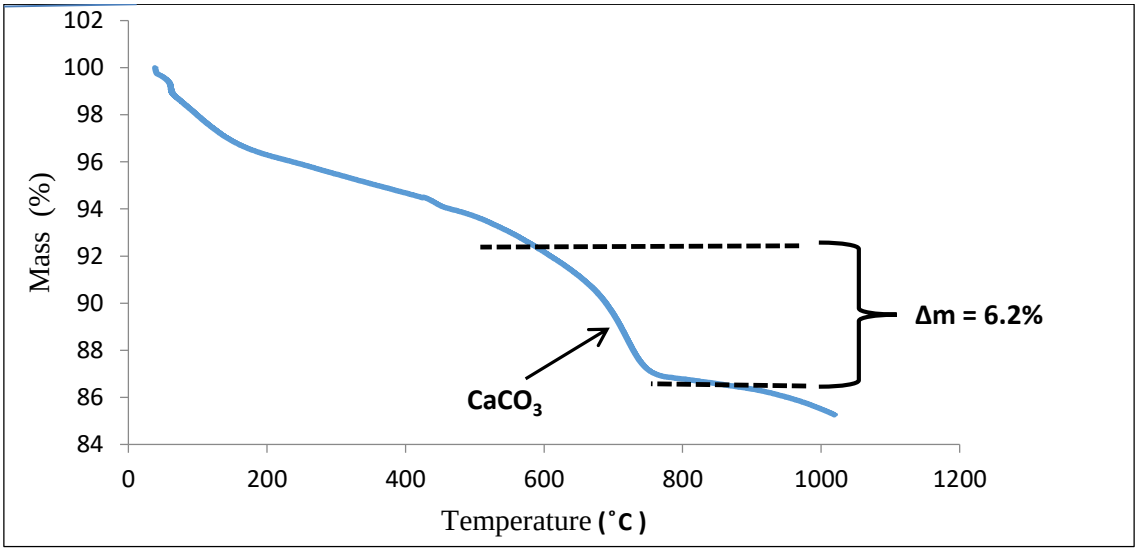

Figure 4.11 TGA curve of mortar sample

5. CONCLUSIONS AND RECOMMENDATIONS

5.1. General

The focus of this research is to evaluate and compare the self-healing efficiency of different incorporation techniques used for the addition of bacteria in mortar. Parameter used for determining the efficiency of different incorporation techniques included effects on compressive strength, regain in compressive strength after pre-cracking, micro structure development and the formation of calcium carbonate. Microstructure was studied using scanning electron microscopy while the formation of calcium carbonate was determined using EDX and TGA analysis.

5.2. Conclusions

The addition of *Bacillus subtilis* bacteria to cement mortar increased the compressive strength of the specimens that varies with the media used for immobilization. The *Bacillus subtilis* microbes possess strong potential for effective healing of the nano/micro scale structural/nonstructural cracks in cementitious composites by producing $CaCO_3$ crystals during their microbial activity as evidenced in scanning electron micrographs, energy dispersive x-ray spectrographs and thermogravimetry. The self-healing efficiency of iron oxide particles was higher as compared to limestone particles and siliceous sand grains as the immobilizer. The carrier materials having smaller particle size performed better in terms of self-healing at early ages however for later ages, carrier particles with larger size were more efficient. The contradicting behavior of nano-sized iron oxide particles contributing in effective healing at later ages is attributed to the formation of protective layer around bacterial spores.

5.3. Recommendations

Although this study has increased our understanding about self-healing process and effect of different incorporation techniques on the crack healing efficiency, there is still a need for further investigation to have better understanding of the process. Following are some of the recommendations for further investigation in the field.

- There is still a need to explore different types of bacteria and determine their optimum concentration to have maximum production of the calcium carbonate crystals.
- Further research is required for the understanding of activation mechanism of bacteria and techniques to control them.
- Durability of bacteria needs to be checked beyond 28 days.

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