Synthesis & Characterization of Bio-Influenced Nano/Micro-Particles for the Feasibility Investigation of Self-Healing Concrete

Submitted By

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BACHELORS IN CIVIL ENGINEERING

Year 2013-2017

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This is to certify that the

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has been accepted towards the requirements

for the undergraduate degree

in

CIVIL ENGINEERING

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ACKNOWLEDGEMENTS

In the name of Allah, the most merciful, the most compassionate all praises be to Allah, the lord of the worlds and prayers and peace be upon Muhammad (S.A.W) his servant and messenger.

The completion of this project was only possible due to unlimited blessings of almighty Allah and collaboration of many people, to whom we wish to express our gratitude.

First & foremost, we would like to thank our beloved parents and teachers for their unconditional love and support throughout our life and for strengthening us to chase our dreams.

We would like to express our profound gratitude to our supervisor Dr. Rao Arsalan, Assistant Professor, NUST Institute of Civil Engineering, for his guidance and support throughout this project and especially for his guidance and support throughout this project and especially for his confidence in us. We are grateful to him for motivating us along this arduous course. He inspired and courage us to be the best version of us. A special thanks to his countless hours of reflecting, reading, encouraging and most of all patience throughout the project.

There are number of people without whom this thesis might not have been written and to whom We are grateful indebted. We were fortunate to have an outstanding faculty in our department. We owe our sincere gratitude to Prof. Dr. Syed Ali Rizwan (former HOD Structure Dept. NICE), Dr. Imran Hashmi (AD IESE), Engr. Muhammad Fawad (Lab Engr. Structural Lab, NICE) & Ms. Sahaab Farooq (Lab Incharge, microbiology lab, IESE).

We are grateful to the PG students of IESE especially Ms. Amrah Qureshi (IESE, NUST00000118895) for preparing bacterial culture, performing various identification tests and providing technical support throughout the project execution. The authors also express their gratitude to Ms. Romana Khan (IESE, NUST), Shabila Perveen (IESE, NUST) & Rabia Akmal (IESE, NUST) for their valuable support in Microbiology lab of IESE, NUST.

Abstract

Bacteria-based self-healing concrete can be made more efficient by using immobilization techniques; hence shielding the bacteria from crushing during mixing to remain dormant till the development of concrete cracks for uniform healing of developed cracks. In this study, two intrusion approaches, direct induction and immobilization, are deliberated by using '*Bacillus Subtilis'* bacteria for self-healing concrete. Furthermore, for immobilization evaluation, two media iron oxide Nano/micro (INMP) and bentonite Nano/Micro particles (BNMP) are selected. Compressive and tensile strengths were computed at 3, 7 and 28 days. Moreover, Scanning Electron microscopy, X-ray Diffraction, X-Ray Fluorescence and Thermogravimetric Analysis are employed for monitoring self-healing efficacy. Results revealed that Immobilization through INMP media is best among all intrusions , as it can heal cracks up to 1.2mm and 85% recovery in compressive strength was observed after crack formation followed by direct intrusion with 65% strength recovery and 0.4mm crack healing. Rather, BNMP immobilization exhibited crack healing up to only 0.15mm width with 45% strength recovery. Thus, it can be concluded that media selection for immobilization is important as BMNP was least effective as compared to other methods.

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INMP	Iron oxide Nano/Micro
BNMP	Bentonite Nano/Micro particles
LWA	Light Weight Aggregate
GNP	Graphite Nano Platelets
ACI	American Concrete Institute
ASTM	American Society for Testing and Materials
CH	Calcium Hydroxide
CSH	Calcium Silicate Hydrate
SP	Superplasticizer
$Ca(OH)_2$	Calcium Hydroxide
OPC	Ordinary Portland cement

CHAPTER 1

INTRODUCTION

1.1 General

Concrete is a composite material and widely used in construction. The evidence of using concrete in construction exists dated back as 1700 BC(1). Currently, it is being obtained by mixing cementitious materials with fine aggregate and coarse aggregate in presence of water often complimented by admixtures for certain incentives (2).

Deteriorations resulting from the corrosion of steel reinforcement shortens the service life of reinforced concrete structures. Concrete has relatively low tensile strength and ductility. The reliability of any structure is primarily associated with its durability, serviceability and strength Higher compressive strength is attributing of concrete matrix, but lower efficacy in tensile strength. Concrete matrix exhibits relatively high value of compressive strength; however, its tensile strength value is low and concrete members are prone to crack on the tension side (3, 4). Concrete endures serious wear and tear throughout its predictable service life and cannot sustain under significant amounts of strain. Standard concrete bears up to approximately 0.3% strain before failure (5). Different repairing techniques are employed to heal the cracks in concrete and make it less prone to failure. Efforts are underway to form a concrete that resists deterioration and is thus durable requiring minimum or almost no maintenance (1, 3-6).

One of these efforts is self-healing concrete using microorganism. The idea of developing self-healing concrete originates from microbiology. In 2008 a microbiologist Hendrik Jonkers whilst studying how bones heal themselves using mineralization, decides to experiment a similar process in concrete. He made a two component self-healing concrete. He infused concrete with Bacillus pseudofirmus and Sporosarcina pasteurii bacteria to catalyze the conversion of organic compound into calcite(7, 8) .These bacteria are found naturally in highly alkaline lakes near volcanos and can survive up to 200 years without air and food. They form a layer of calcium carbonate over the cracks that were formed in the concrete(8)

1.2 Problem Statement

Different mechanisms of self-healing are present in literature and can be broadly categorized as autogenous or autonomous. Autogenous healing includes formation of hydration products to fill the cracks while autonomous healing is addition of bacteria, admixtures, polymers and Nano-particles (9). Most of these methods are not effective, so, self-healing through micro-organisms is used widely, because it's cost effective and gives promising results (10). Healing of concrete depends upon the production of calcium carbonate which is directly related to many factors such as pH of concrete, dissolved inorganic compounds, nucleation sites and presence of calcium ions throughout the mixture.

Moreover, it also depends upon the type of bacteria, bacteria concentration, curing procedure and material used for incorporation of bacteria (11). Problems associated with bacteria influenced self-healing concrete are; reduction in compressive strength and slower rate of self-healing with time. These may be due to lack of bonding and crushing of bacteria during mixing of concrete (12). To overcome these issues, researcher's uses different approaches for mixing Bacteria in self-healing concrete are under study. These include encapsulation of bacteria through aggregates, minerals admixtures, polymer compounds and immobilization through nano/macro particles(13,14).

1.3 Objectives:

The main objective of this research project was to find a suitable conducting media for the intrusion of bacteria so that it can sustain in the alkaline concrete environment. Water, iron oxide Nano/micro particles & bentonite Nano/Micro particles were used as a conducting media. Efficiency and survival of bacteria was observed after the cracking and healing of concrete samples.

To study the impacts of bio-influenced materials, mechanical properties and microscopic structure was observed. Effect of bacteria and conducting media was measured after the experimental program.

Further objectives of the research project include the healing efficiency in the form of crack width healed, regaining of mechanical properties and the percentage of strength gained or lost after the cracks. Activation process of bacteria was also to be observed in the project.

1.4 Organization of Report

Chapter 1 of the thesis is an introductory chapter about bio influenced self-healing concrete, objective of the study and thesis overview. In chapter two, a brief literature review is stated. Chapter three of the thesis represents the Experimental Procedure which includes materials, casting scheme and testing procedures. Chapter 4 discusses the tests carried out, the observations, test results and evaluation of test results. The conclusions based on findings of this research and recommendations for further studies are presented in Chapter 5.

CHAPTER 2

LITERATURE REVIEW

2.1 Previous studies on bio influenced self-healing practice

Numerous efforts have been taken place by researchers, for improving the efficiency of bio-influence bacterial self-healing concrete by recovering unwanted loss in concrete properties. Initially, it was suggested, use of bacteria spores embedding on silica sol–gels or inorganic oxides before mixing in concrete(10); it is a viable solution for longer bacterial life without compromising compressive strength. For survival of bacteria air entrainment agents can be used because their microspores provide cushion for survival of bacteria spores at later stages(15). Two component healing agent, calcium lactate and bacterial spores were embedded on porous expanded clay particles (EC) which were used as a replacement of conventional concrete aggregates, showed double crack healing capacity with enhancement in durability of concrete in wet conditions (9).

Bacterial spores encapsulated on hydrogel are intruded in self-healing mortar and repaired cracks up to 0.5mm width and water permeability decreases 68% (16). The potential of using polyurethane sheet and silica gel for immobilization of bacteria in self-healing concrete was investigated. Samples containing Polyurethane immobilized bacteria depicted higher strength regain (60%) and lower permeability compared to silica gel healed samples whose strength regain was only 5% (17) . The immobilization of bacteria spores was also done using microencapsulation technique melamine capsule sized 5 micron. 40-80% higher crack healing rate was achieved as compared to non-bacteria micro capsulation mixes (18). The use of Bacillus subtilis HU58 and nutrients immobilized on natural diatomite Lam Dang as control releasing agent , increases compressive and flexural strength of concrete by lowering permeability of self-healed concrete samples. They concluded that use of immobilized diatomite Bacillus Subtilis HU58 pellets (0.5% replacement) as coarse aggregates as effective as compared the solution(0.5% replacement of tap water) containing bacteria , nutrient broth and urea for modified mortar (19).

Self-Healing Concrete approach in which they are utilizing micro-organisms such as Bacillus Pasteurii, Shewanella, E.Coli etc. to make a concrete healable. Bio-influenced self-healing concrete or Bio concrete has initially intruded micro-organism which results in mineral compounds formation which basically fills the cracks. But there is survival problem of these micro-organisms they do not survive in the alkaline cementitious environment of the concrete till the development of cracks.

Ceramsite carrier was used in a study for immobilization of bacteria and nutrients separately for successful enhancement of area repair rate upto 87.5% complimented by increase in mechanical properties (20) . The effect of intrusion method on efficiency of Bacillus subtilis in self-healing concrete was studied using three different mechanisms. These are direct intrusion, intrusion through carrier medium light weight aggregate (LWA) and Graphite nano platelets (GNP). Intrusion through GNP immobilization was found effective at early age while LWA immobilization exhibited greater crack healing at later ages. Direct intrusion of bacteria was less effective as compared to all and showed less compressive strength with lower crack healing rate(21) . Immobilized bacteria using Zeolite carrier was used in RCC and fiber reinforced mortar to compare micro structural and crack healing properties. Compressive strength was increased and chlorides ion penetration was reduced (22). In another study perlite is used for immobilization. Effect of direct induction and immobilization through expanded perlite (EP) were compared. Experimentation results proved that EP immobilized bacteria exhibit efficient crack healing (up to 0.79mm) and strength which is better than expanded clay immobilized self-healing concrete (EC) who have 0.45mm (23).

This study mainly focuses on the effect of the various immobilization media for the intrusion of bacteria in concrete and determines the effectiveness of encapsulation material for survival of bio agents and effect of addition of encapsulation on concrete properties and recovery of mechanical and durability properties. Two types of innovative addition have been synthesized for making immobilized self-healing bacteria using INMP and BNMP and their feasibility investigation was studied and characterization was done. Bentonite is cost effective, locally available and environmental friendly material. Due to its pozzolanic nature, bentonite has a lot of potential to be used in concrete. With the introduction of bentonite as a cement replacement, the workability, density and water absorption decreased (24). An effort has been made to use it as immobilization media for concrete. Other material is nano/micro Iron oxides particles which has been previously used for improving pull out strength of concrete (25), self-healing of bituminous materials (26) and for improving corrosion resistance (27).So, It was decided to study their feasibility for immobilization for bacterial self-healing concrete.

To increase the survival rate of bacteria in concrete, the bacterial spore formation, the mechanical properties of concrete and simultaneously needed organic bio-mineral precursor compounds (calcium lactate), iron oxide and bentonite was tested as the conducting media's in this study.

The vast research being carried on the self-healing depicts that there is a lot of variation in the type of bacteria, its carrier compound and concentration. All these factors have an enormous impact on the self-healing efficiency and the environmental factors as well. The objective of our study is to determine the suitable, cost effective and environmental friendly materials as a carrier compound for bacterial survivability in concrete. The effectiveness of different approaches was determined by the width and length of healed cracks. Furthermore, impact of novel compounds on the microstructural and mechanical properties of concrete were determined by Scanning Electron Microscopy (SEM) and compressive strength analysis.

2.2 Approaches to self-healing concrete

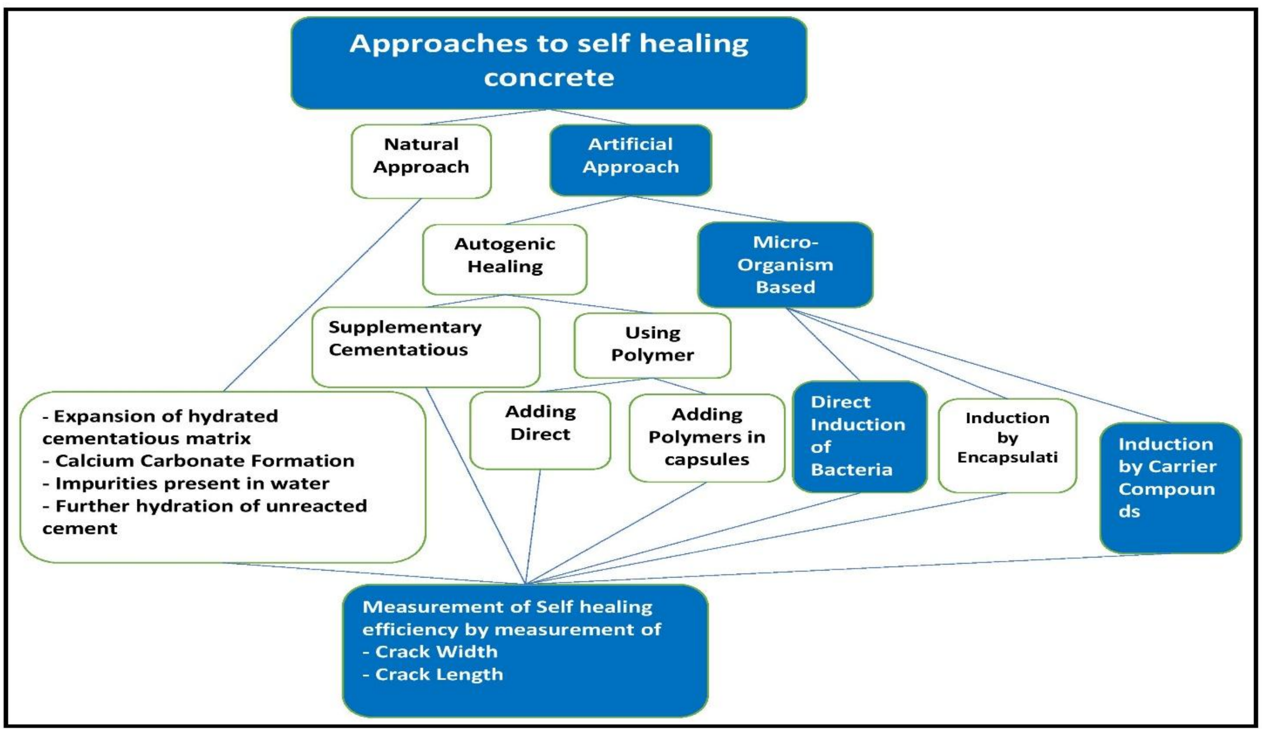
Various approaches have been utilized in the past by researchers for achieving self-healing concrete also popular as bio concrete. Basically, there are two main approaches known as natural approach and artificial one are common. In natural approach, concrete heals itself by the expansion of hydrated cementitious matrix. Some calcium carbonate is also formed after cracking due to hydration of un-hydrated matrix. Some impurities are also present in water and they fill the cracks during curing time. Further hydration of unreacted cement particles also produce some healing effect in concrete.

Second approach is artificial one and it includes autogenic and micro-organisms based self-healing. In autogenic self-healing; supplementary cementitious materials or polymers are added in cement or concrete to produce self-healing effect. In this approach polymers are added either directly or in the form of capsules.

In micro-organism based self-healing, bacteria is added in concrete either directly or by some carrier compounds. Also, capsules based approach can be used but it costs more and is not environmental friendly in some cases. Bacteria is added directly in the liquid form with its food, when concrete gets hardened, it goes into dormant phase and remains in this stage for more than 200 years. Whenever cracks appear in concrete due to any causes, bacteria get activated by water and air, starts producing calcium carbonate and ultimately tries to fill the crack with deposited

calcium carbonate.

Figure 2.1: Approaches to self-healing concrete



2.3 Bacteria based self-healing concrete:

In bacteria based self-healing concrete, bacteria is added along with its food in concrete. Bacteria need some protective layer to survive in concrete. Bacteria cannot live longer in alkaline environment of concrete and usually dies. Some alkali resistant spore forming bacteria is needed in concrete. After testing various species of bacteria, it was found that class bacillus is such type of bacteria which can survive efficiently in concrete. Bacillus subtillus was used in this research project as it can survive efficiently in concrete and it can produce spores.

2.4 Healing Mechanism:

A brief healing mechanism is explained below as expalaned by Hammes et al.

The first picture shows that the bacteria is incorporated in concrete mix. It is in dormant phase and it can survive up to two hundred years in this stage.

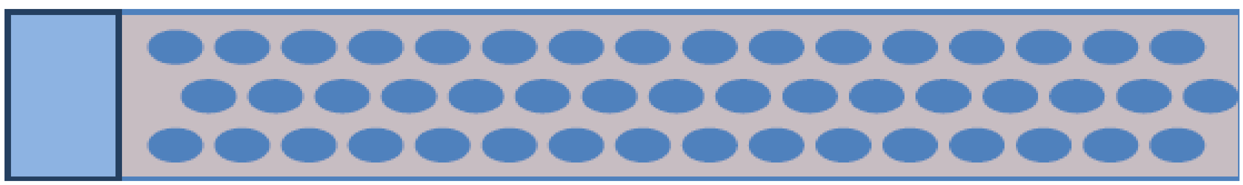


Figure 2.2: Bacteria Incorporated in concrete mix.

Second picture shows that due to some minor tensile stresses or other causes like shrinkage or temperature effects, a crack appears. As the crack appears, bacteria gets vulnerable to carbon dioxide and water from air. It starts consuming its food and starts producing calcium carbonate which fills the cracks as shown in figures.

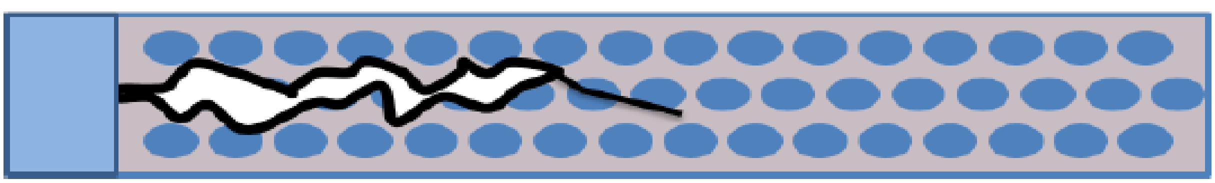


Figure 2.3: Crack produced in concrete due to tensile stresses

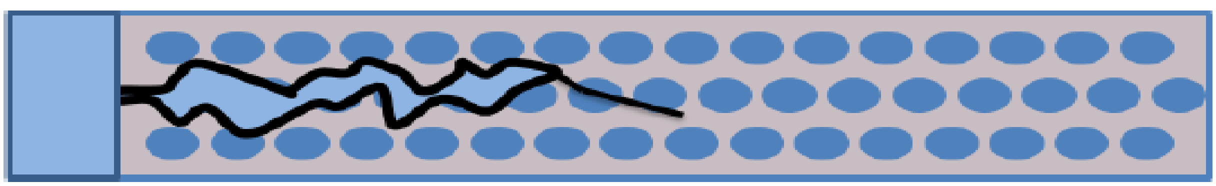


Figure 2.4: Water is deliberately entered in the cracks.

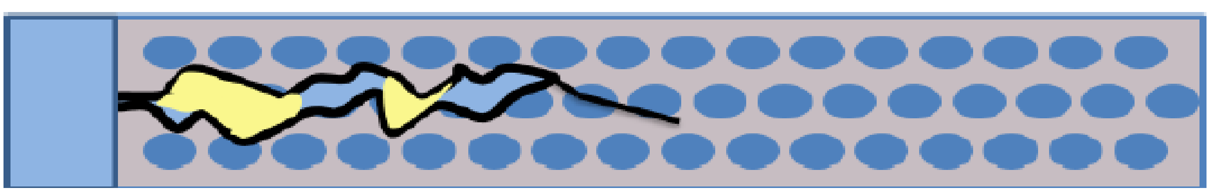


Figure 2.5: CaCO3 is produced which heals the cracks.

CHAPTER 3

Experimental Program

The following materials were used for this experimental program.

3.1 Materials

3.1.1 Micro-organism and their cultivation

Bacillus Subtilis is gram positive (having peptidoglycan layer) bacteria commonly found in soil (28). It has rod-shaped structure, can form protective endospores in the alkaline environment and endure extreme pH, temperature and high salinity (29).

The bacterial culture was incubated in the controlled microbiology laboratory. For growing of bacteria medium was selected as blank. The blank solution was used as a reference, based on which absorbance of bacterial solution was measured in the spectrophotometer standard. The 0.5 ml quantity of blank solution was placed in spectrophotometer with a selected wavelength of 600nm. After reading of the blank solution by the machine, 0.5 ml of bacterial solution was used as replacement with the same wavelength of 600 nm was used. Based on which the concentration of bacteria in the solution measured using the expression $Y = 8.59 \times 10^7 X^{1.3627}$ (30). Where Y is the bacterial concentration per mL and X is the reading at OD_{600}.

With spectrophotometer, the bacterial concentration was found to be 2.3×10^8 cells/ ml. From these results, spore concentration in samples was kept equal to 2.3×10^8 cells/cm3 of concrete mixture.

Figure 3.1: Prepared Bacterial Culture

Before preparing and making it fresh, the culture was sent to South Korea for the identification and verification. ATCC number of the bacillus subtillus used in this project is 11774. ISO 9001:2008 certification and ISO/IEC 17025:2005 accreditation were used for the manufacturing of Each ATCC genuine culture and represents a direct, minimal passage descendent of the original material deposited with ATCC, handled only by ATCC. These products are backed by guarantee and covered by the technical support.

3.1.2 Conducting media

a) Iron oxide

Iron oxide was used as a protective material for bacteria in concrete. Availability of hematite α-$F_{e_2}O_3$ is more common in Pakistan. Iron-oxide in millimeter size was obtained from Heavy Mechanical Complex Taxila.

To obtain the Nano/Micro particles of iron oxide (INMP), planetary ball mill, Standard was used. It has very low efficiency and approximately 25 grams powder was yield after 4-5 hours of running machine. Then Soil compactor standard was used to grinding purpose. Material was placed under the hammer of soil compactor. Every process, iron oxide was passed through set of sieves i.e. Seive#50, Seive#100 and Sieve#200. Materials passing through sieve#200 having average particle size of 6.69 µm micron was used for the protective coating of bacteria. Particle analysis in shown in figure 2.

Iron oxide in mm size Planetary ball mill Soil Compactor Iron oxide mean size 6.63µm

The bentonite clay was also used as a carrier for bacterial. The particle size ranges in between 20-70 micro meter having average particle size 6.129 µm. The bentonite of Jahangerah shown on figure (KPK, Pakistan) was utilized in our study.

Figure 3.2 Bentonite Powder

Particle size analysis of powdered bentonite and iron-oxide were performed.

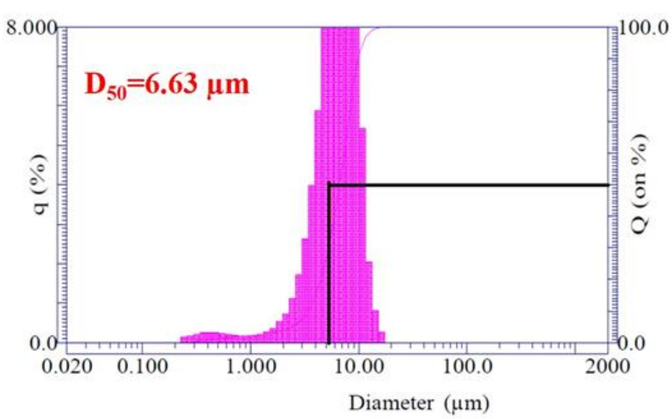


Figure 3.3: PSA of Bentonite

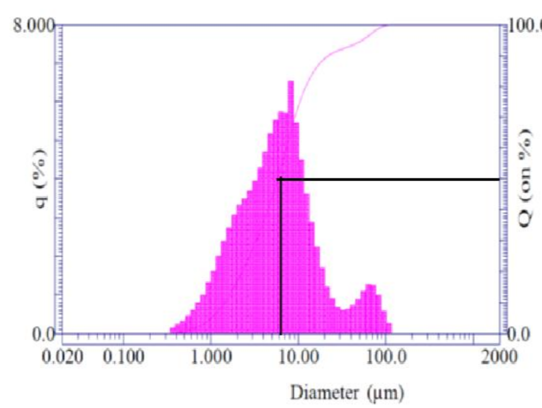


Figure 3.4: PSA of Iron-Oxide

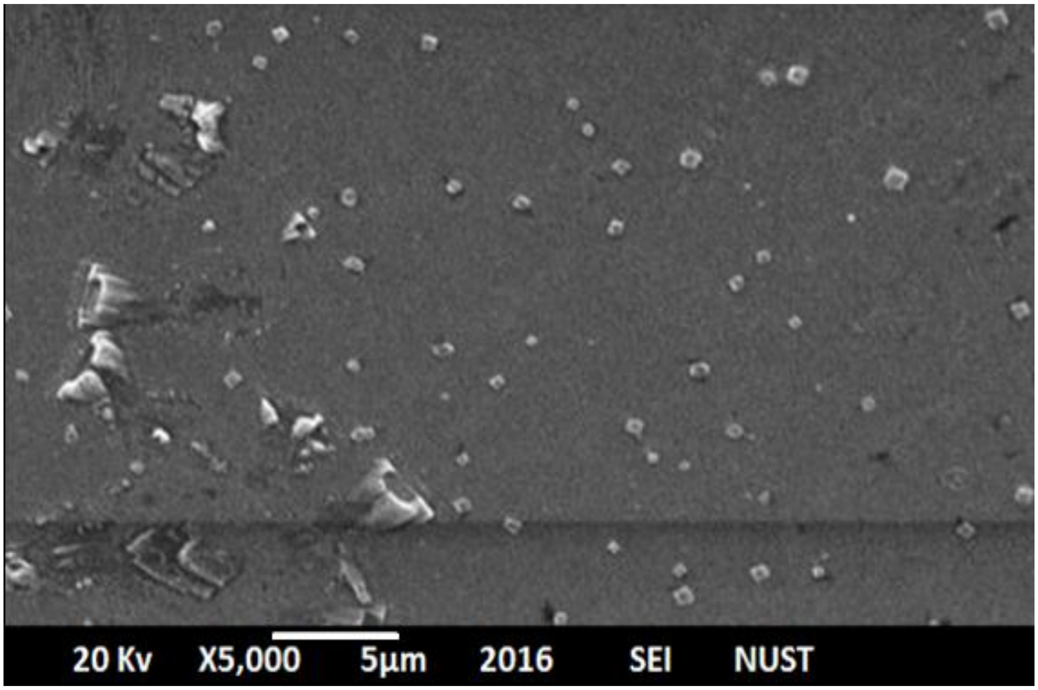


Figure 3.5: SEM image of bacterial spores in Nutrient Broth

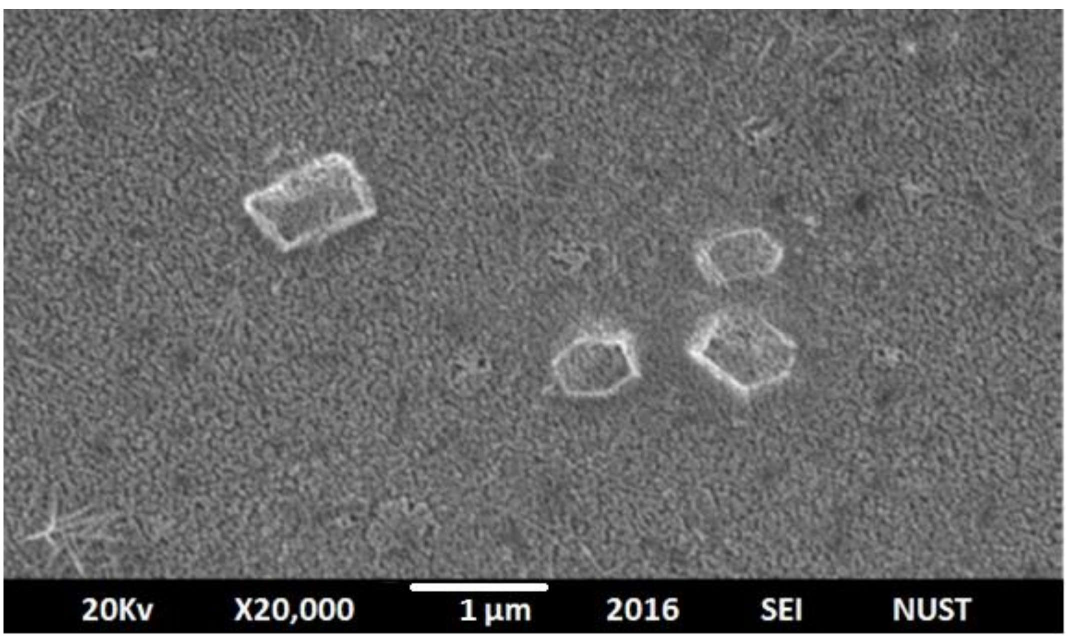


Figure 3.6: SEM image of bacterial spores with Iron-Oxdie

The above SEM image is of bacterial culture with iron oxide as a protective layer. Bacterial culture forms a protective colony with iron oxide and can survive the harsh alkaline environment of concrete with this protective layer.

Chemical compositions of CEMI, iron oxide and bentonite are listed in Table 1.

Table 3.1: Chemical and physical properties of materials used

		Chemical composition (%)	
		(Cement I)	(Bentonite)
Silicon dioxide (SiO_2)	19	54.55	
Aluminum oxide (Al_2O_3)	9.87	20.19	
Ferric oxide (Fe_2O_3)	3.46	8.60	
Calcium oxide (CaO)	60.0	7.28	
Sodium oxide (Na_2O)	0.84	1.27	
Magnesium oxide (MgO)	1.63	4.20	
Phosphorus pentoxide (P_2O_5)	.068	1.107	
Potassium oxide (K_2O)	1.19	3.92	
Sulfur trioxide (SO_3)	2.63	--	
Titanium oxide (TiO_2)	--	0.91	
Zinc oxide (ZnO)	--	0.17	
Physical Properties			
Specific gravity	3.05	2.82	4.8-5.26
Blain fineness (cm^2 /gm)	µm	4800	--
Initial setting time (mins)	1720	--	--
Final setting time (mins)	95	--	--
Average particle size	20	4.75 µm	6.63 µm
Loss on Ignition	360	5.428	0.1-0.3
	1.03		

3.2 Bacterial Growth Curve:

Bacterial growth curve is to study the distinct stages of bacterial culture growth. This growth curve was prepared in controlled microbiology lab (IESE, NUST).

The bacterial culture growth has four distinct phases.

i. **Lag Phase**

When a microorganism is intruded into the fresh medium, they require some time to adapt with the pristine environment. This phase is known as Lag phase; in which bacteria are maturing them but do not have ability to divide and reproduced. During this bacteria prepare themselves for synthesis of RNA, enzymes and other molecules formation. The length of log phase was almost 2 hours for Bacillus Subtilis used in this study as shown in figure.

ii. **Exponential phase**

This phase is characterized by cell doubling. The organisms begin the DNA replication by binary fission at a constant rate also the metabolic activity increases. The developing media is exploited at the maximal rate. The number of bacteria increases logarithmically and exponentially and finally the single cell splits into two, which rectify into four, eight, sixteen, thirty-two and so on (That is 2^0, 2^1, 2^2, 2^3.........2^n, n is the number of generations). This is longest phase consisted of 48 hours. The time taken by the bacteria for doubling during a definite time is termed as the generation phase.

iii. **Stationary phase**

This phase is due to growth limitation of bacteria causes by depletion of necessary nutrients, accumulation of waste materials, toxic metabolisms and formation of an inhibitory product which changes the pH and temperature of solution. When growth rate and death rate become equal, it is termed as stationary phase. The result is a “plane,” horizontal linear part of the curve. Its duration was 16 hours in prepared sample.

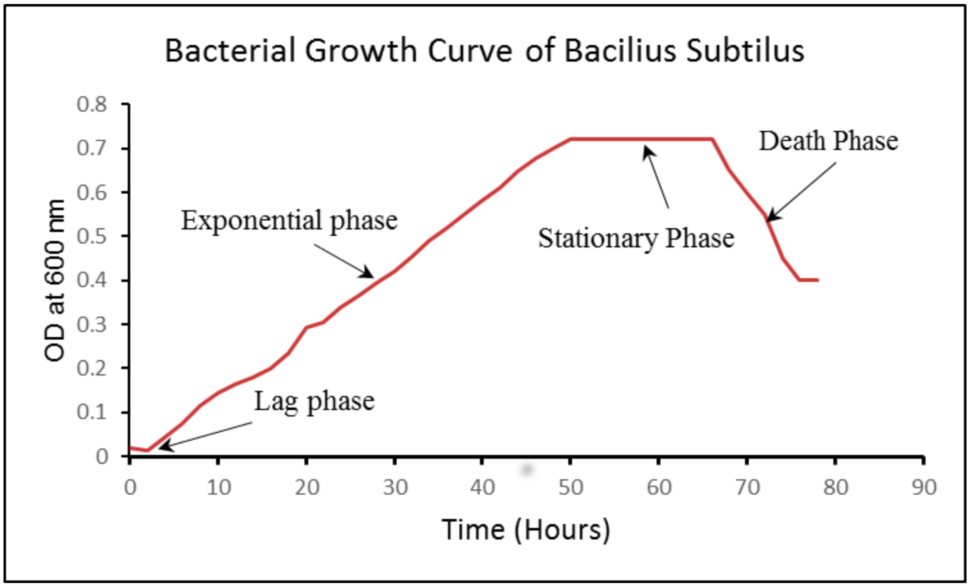


Figure 3.7: Different phases of growth of a bacteria

iv. **Death Phase**

During this, the bacterial culture entirely loses its ability to reproduce. Single bacteria begin to decease due to the unfavorable situations and the death is fast and at uniform rate. Death phase is caused by unviability of nutrients, unfavorable environmental conditions and the subsequent accumulation of metabolic waste products. Its duration was 10 hours as you can see in figure. Organisms can survive in the environment by producing endospores which can resist this condition.

3.3 Mix proportions

Four diverse types of mix are used for the study. Ordinary Portland cement (CEM-I) in accordance to ASTM C 150-07 was used for subject investigation. The mix was designed for a compressive strength of 4000 psi. A constant water cement ratio (W/C) of 0.4 was used for all the formulations using 1% super plasticizer. Calcium lactate added as 1% by cement weight into the solution (bacterial spore + nutrient broth + carrier medium). How much grams sample using mix 1.1.9.2.2:

The control samples in which there is no bacterial spore specimen incorporation are termed as "CM". In ''Mix A" samples, bacteria were intruded directly by adding the bacterial solution in water during mixing of concrete, without using any protective immobilized media. The samples in which the bacterial incorporation is done with the iron oxide as protective carrier are designated as "Mix B". The samples in which the bacterial incorporation is done with the bentonite as protective carrier material are termed as "Mix C".

Before mixing in concrete the (iron oxide + Nutrient Broth) were autoclaved for 2 hours and then placed in the incubator for 48 hours. Similarly,

For Mix B and Mix C Iron oxide and bentonite mixed with nutrient broth in the separate bottles was placed in autoclave and exposed to elevated temperature and pressure for 15-20 mints. Then a single colony of B. Subtilis was inoculated into the solution under the sterile condition. After the inoculation processes, the solution was placed in incubator at 37◦C for 48 hrs. Absorbance of incubated bacterial culture was measured using the spectrophotometer at wavelength 600 nm. The expression Y = 8.59 x 107X1.3627 was used for the further calculation to determine the concentration of bacterial solution which was $2.3x10^8$ (unit)

Table 3.2: Mix proportions

Notation	Medium	Proportioning	Super Plasticizer	W/C Ratio	Bacterial Concentration
Mix A	Control	1:1.9:2.2	1%	0.4	2.3 x 10*8
Mix B	Water				
Mix C	Iron Oxide				
Mix D	Bentonite				

3.4 Casting of specimens and testing regimes

Cylindrical specimen of diameter 4 in and height of 8 in were prepared as shown in figure and removed from molds after 48 h of casting and then placed for curing under normal controlled conditions. For all mix types, similar samples were casted. For the assessment of compression, flexure and healing analysis, 3 samples for each mix were casted and total of 108 samples were prepared in total.

Samples were pre-cracked at 3, 7 and 28 days for the observation of structural as well as non-structural cracks for healing measurements. For the development of structural cracks, the samples were subjected to compressive test machine under precise and cautious compressive loading conditions till visible cracks appeared on the exterior. Cracks of different aperture were measured on the specimens by using Crack Width Measuring Microscope (CWM) standard and structural cracks were marked for further investigation of self-healing.
The pre-cracked samples were again placed for curing under controlled conditions and crack width was measured on fixed breaks of 3, 7 and 28 days and comparison was made with the original crack width as a measure of self-healing efficiency.
Total of 108 cylinders were casted as shown.

Table 3.3: Casting scheme of cylinders

TESTS	**Mix: 1**	**Mix: 2**	**Mix: 3**	**Mix: 4**
Samples for compression analysis				
3 Days	3	3	3	3
7 Days	3	3	3	3
28 Das	3	3	3	3
Samples for flexural analysis				

3 Days	3	3	3	3
7 Days	3	3	3	3
28 Days	3	3	3	3
Samples for Healing Analysis (Pre-Cracking)				
3 Days	3	3	3	3
7 Days	3	3	3	3
28 Days	3	3	3	3

The effect of self-healing efficacy can be achieved through different methods and also involve measuring compressive strength as per ASTM C 39. Compressive strength tests were performed on specimens at the age of 3,7and 28 days of curing and after the 28 days of healing of pre-cracked samples. Fig.7 shows prepared specimens.

Proper tamping is carried out to ensure the proper compaction of specimens

Figure 3.8: Casting of 4x8"samples

Figure 3.9: Samples casted for the compression and healing analysis

CHAPTER 4

RESULTS AND DISCUSSION

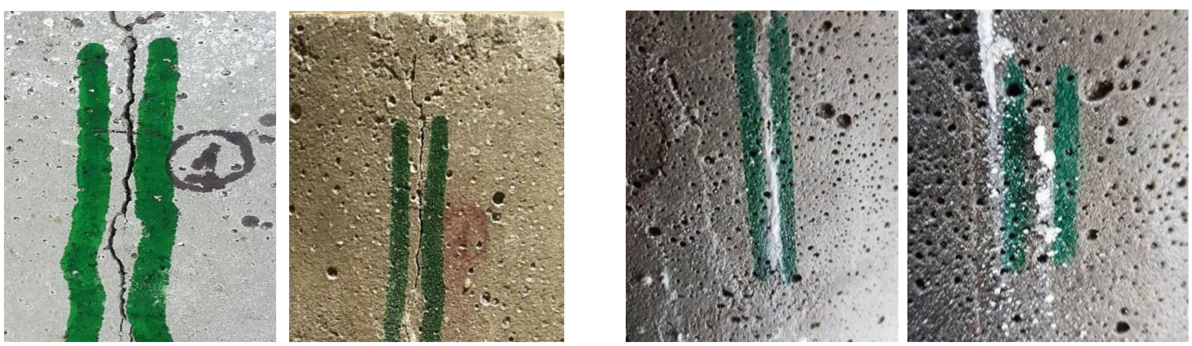
For the assessment of Self-Healing efficiency, many tests were conducted and results are presented and discussed here. These results include Visual measurement of Cracks, Micro-structural Study through SEM and EDX, Micro-Analytical Study through XRD, Thermal Evidence by TGA, Chemical Study by XRF and Mechanical Evidences by compressive strength tests.

4.1 Visual Evidence:

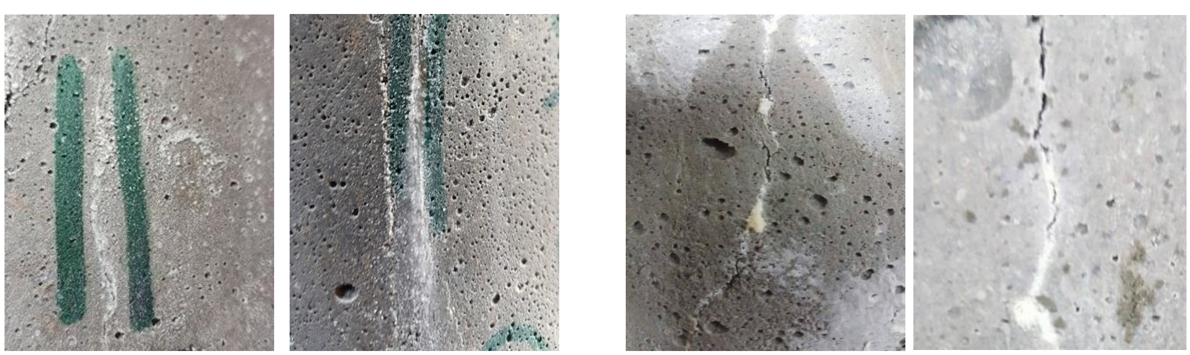
Our first assessment criteria were visual measurement of cracks that is there any visible Calcium Carbonate produced and measurement of crack width by using Crack width measuring Microscope.

Two out of three samples were Pre-Cracked at 80% of their compressive strength for the measurement of non-structural cracks and one sample was ultimately loaded for the structural cracks.

Pre-cracked specimens were examine at the specified time of 3,7 and 28 days to measure the efficiency of self-healing process and the width of healed cracks by use of crack width measuring micro-scope. The healing was measured in millimeter as the subtraction of initial crack and healed crack width at specified time.



Negligible Healing in control sample (CM)   Partial healing in water inducted samples (Mix A)



Uniform Healing in samples with iron oxide (Mix B)   Partial healing in samples with bentonite (Mix C)

Figure 4.1: Shows the healing efficiency of all four Mixes

In control Sample, there isn't any sign of Calcium carbonate as it does not contain any intruding media *'Bacillus Subtilis'* and it showed minimum crack healing. This is due to the hydration process of cement particles and carbonation of calcium hydroxide is responsible for the precipitation of calcium carbonate crystals, so it can heal cracks of smaller width.

INMP served as the best conducting media and best protective for micro-organism *'Bacillus Subtilis'* and showed uniform and maximum crack healing efficiency. INMP in Mix B was completely distributed in the cementitious matrix and was responsible for efficient and uniformly healing of cracks.

Now consider other two formulations Mix A and Mix C, partial and non-uniform healing was observed. Cracks up to 0.44mm was healed using water as the conducting media in Mix A. Water present in the micro and macro pores of the concrete has evaporated and bacteria in these pores couldn't able to survive for the healing of cracks. Only water present in the gel pores of concrete has protected the bacteria and was responsible for the partial and non-uniform healing of cracks.

In Mix C, using BNMP particles as the conducting media, non-from and partial healing was observed. At early age, healing efficiency was present but with the passage of time it continues to reduce due to the reactivity inside the cementitious material because of the pozzolanic nature of bentonite.

Results of crack healing efficiency are presented in the figure 6.

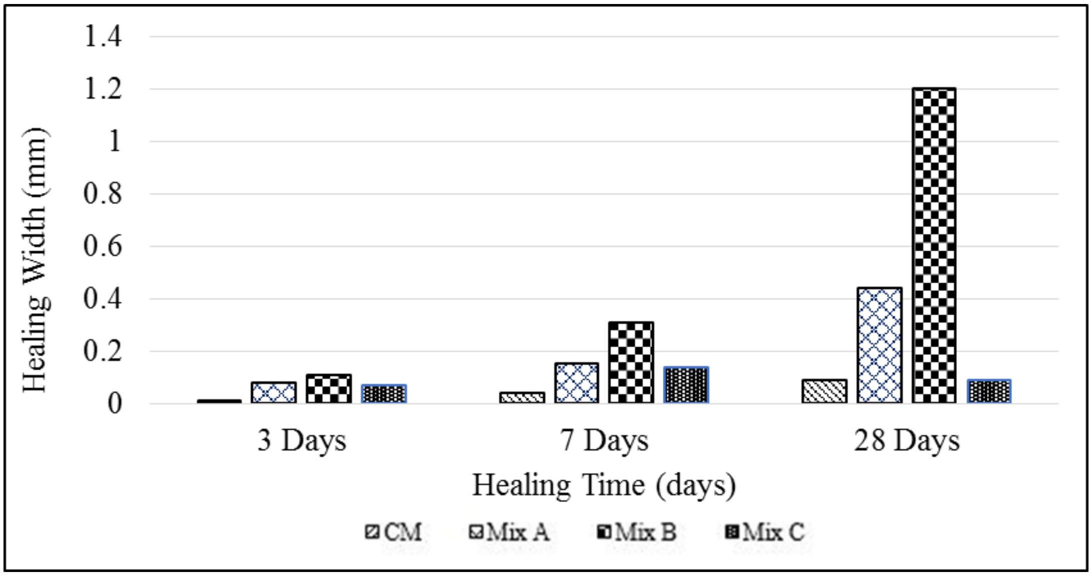


Figure *4.2*: Crack healing of specimens of all four formulations

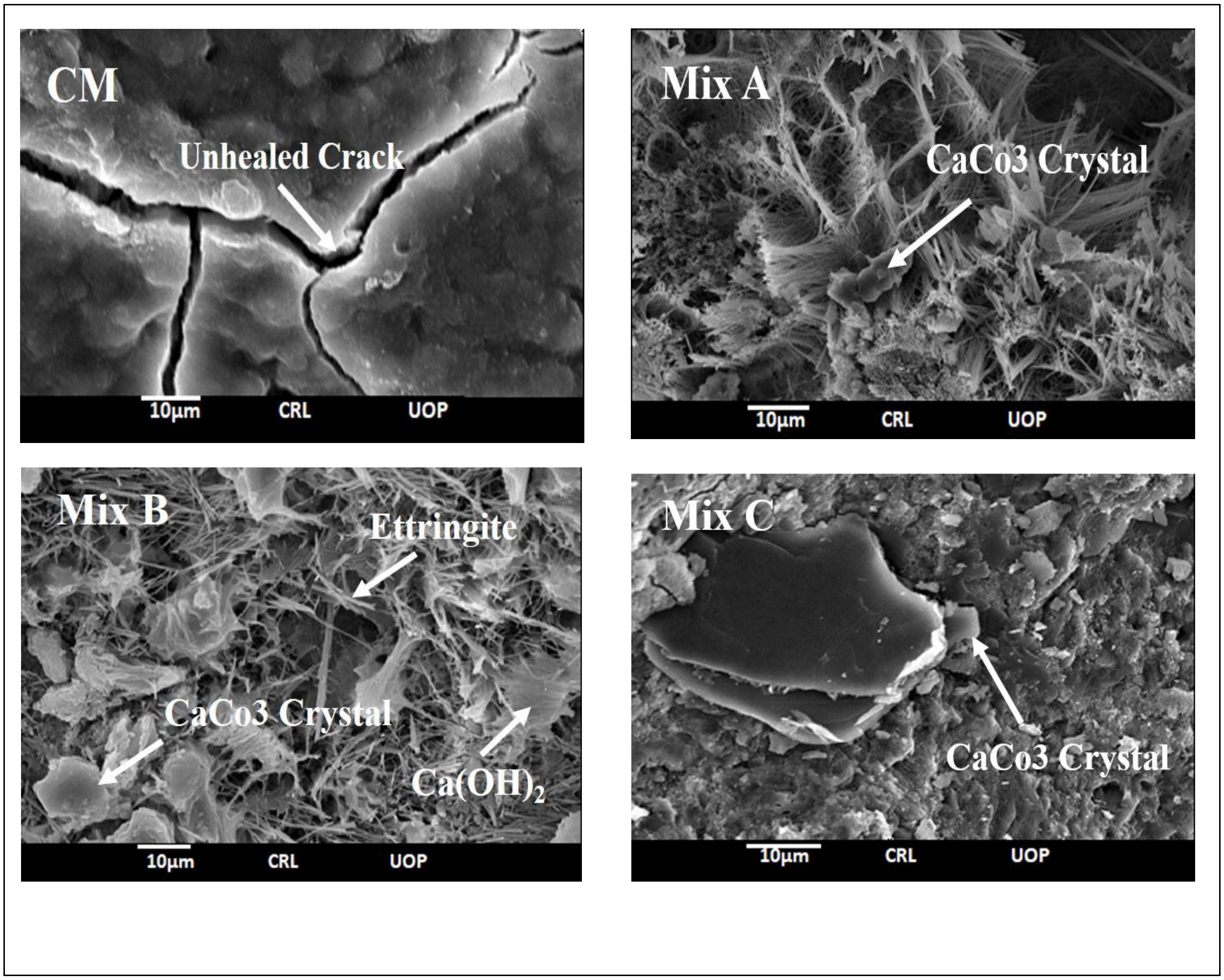
4.2 Scanning Electron Microscopy:

In addition to the results attained by detail visual examination of cracks in concrete samples of all four formulations the healing powder scratched with great care was put under examination to scanning electron microscopy analysis. The objective of SEM analysis is to study the micro structural changes in the all four different mixes as a result healing action. The SEM analysis was performed after 28 days of crack healing.

As calcium carbonate formation is a responsible factor for the crack healing therefore the formation of calcium carbonate crystals was the focus of the study. CaCo3 crystals are developed in three different formulae which are named calcite, aragonite and vaterite (31) CO^2 is available for carbonation process.

Furthermore, as portlandite $Ca(OH)^2$ is dissolved in water. Each time it encounters saturated water it gets dissolved in it, leaving less CaOH on the contact surface to convert in CaCO3. In self-healing concrete, the procedure is quite different due to the presence of calcium lactate and bacteria.

Figure 4.3: Scanning Electron microscopy (SEM) analysis of 28 days healed samples



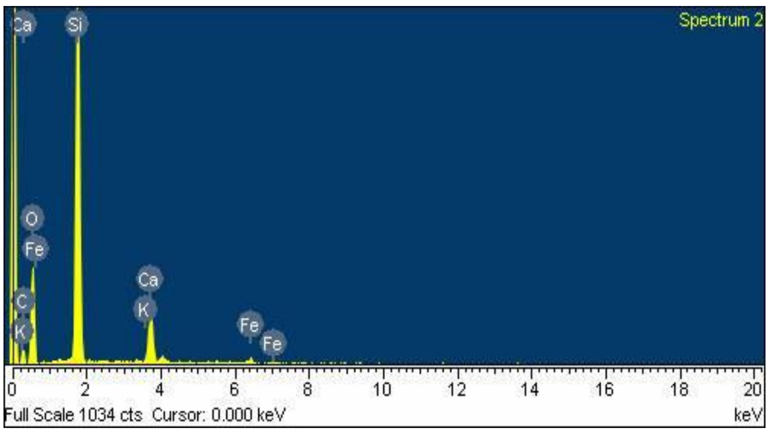
Calcium lactate is directly into CaCO3 by bacteria and as result of this metabolic reaction CO2 is produced which reacts with calcium hydroxide instantaneously, resulting in production of more CaCO3 (32).

Higher content of Calcium carbonate crystals is present in the specimens containing iron oxide as a conducting media (MIX 3). The amount of calcium carbonate produced in the Mix B and Mix C is comparatively less.

4.3 Energy Dispersive X-ray Analysis

The energy dispersive X-Ray Analysis of the powdered form of iron oxide shows its chemical properties. Percentage weight of elements depict that the hematite powder consists of 3.68% carbon. 28.35 % Oxygen, 0.42% silicon and 67.55% iron oxide.

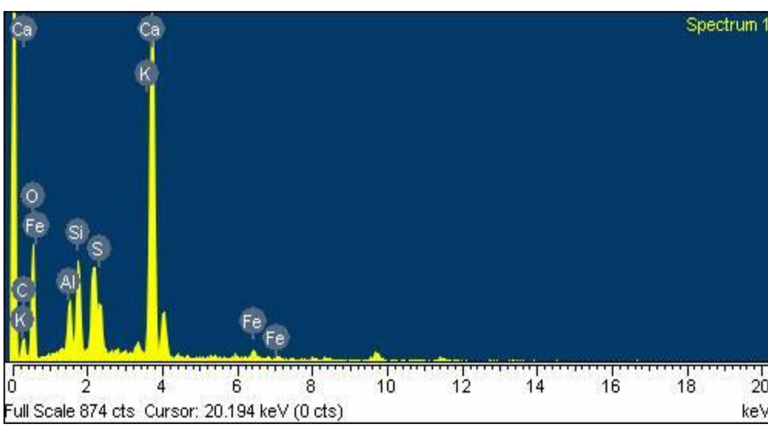
Energy dispersive X-Ray spectrum of Bentonite clay shows that the sum of percentage weights of Silicon dioxide (SiO_2), Aluminum Oxide (Al_2O_3) & Iron oxide (Fe_2O_3) is more than 75% by the total weight. Hence it proves that Bentonite clay is a pozzolana material. Shown below



Element	Weight %	Atomic %
C	19.05	28.05
O	44.38	49.84
Si	28.37	18.15
K	0.26	0.12
Ca	6.61	2.96
Fe	1.33	0.43
Total	100	

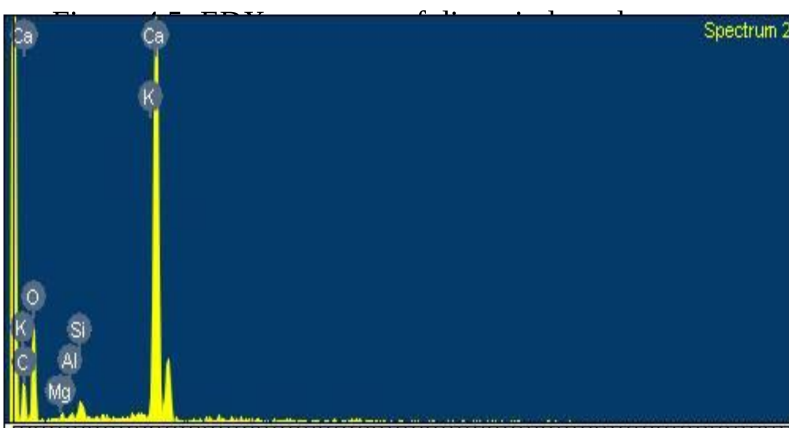
Figure 4.4: EDX spectrum of control sample

Table 4.1: EDX analysis of control sample



Element	Weight %	Atomic %
C	7.16	12.31
O	49.07	63.33
Al	2.82	2.16
Si	5.25	3.86
S	1.59	1.03
K	0.84	0.44
Ca	31.41	16.18
Fe	1.86	0.69
Total	100	

ect inducted



Element	Weight %	Atomic %
C	10.40	17.49
O	48.69	61.46
Mg	0.47	0.39
Al	0.39	0.29
Si	0.83	0.60
K	0.35	0.18
Ca	38.87	19.58

Figure 4.5: EDX spectrum of sample with

Iron oxide as carrier

Table 4.3: EDX spectrum of sample with

Iron oxide as carrier

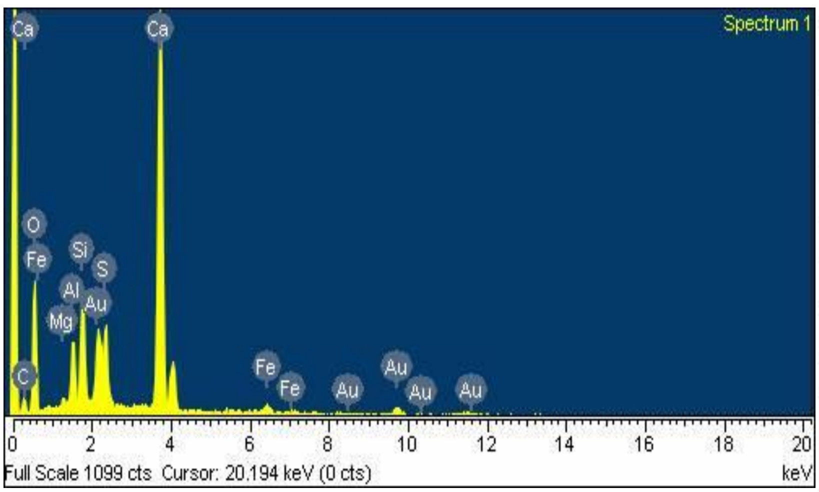


Figure 4.6: EDX spectrum of sample with

Bentonite as carrier

Element	Weight %	Atomic %
C	4.78	8.34
O	49.99	65.55
Mg	0.47	0.40
Al	3.42	2.66
Si	5.66	4.23
S	2.91	1.90
Ca	31.18	16.32
Fe	1.59	0.60
Total	100	

Table 4.4: EDX spectrum analysis of sample

With bentonite as carrier

EDX spectrum of control sample shows that Calcium is present in the mix and its amount is comparable to the amount present in the normal concrete.

EDX spectrum of direct inducted bacterial sample shows that calcium is deposited in the mix in relatively higher amount than the control samples. The enhanced deposition of calcium oxide shows that the bacteria in concrete has consumed its food calcium lactate and produced calcium carbonate.

When iron oxide was used as a carrier compound for bacteria in concrete the amount of calcium deposited was higher as compared to the control samples. Percentage weight analysis of samples with iron oxide shows that bacteria survived efficiently and produced more calcium carbonate.

Bentonite has shown comparable results as iron oxide as conducting media. It depicts that bacteria survived in larger number in these conducting media's and produced more calcium carbonate.

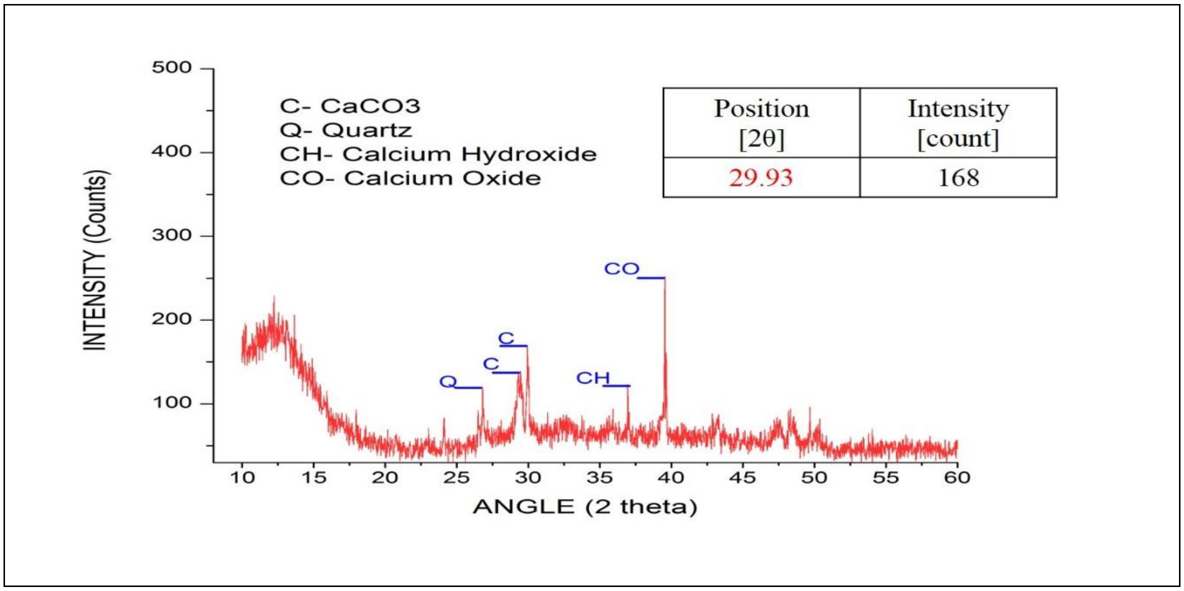
4.4 X-Ray Diffraction Analysis

For understanding of bio-concreting and to confirm the development of calcium carbonate in the specimens, the healing compounds formed in the cracks were placed under the analysis of XRD test. The calcium carbonate formed in the cracks was scratched carefully and was placed in the XRD apparatus. X-ray target was selected as Copper (Cu) due to its ability to be kept cool easily, as it has high thermal conductivity, which produces strong Kα and Kβ lines.

The readings were recorded at a wavelength of 1.54 Å and different demonstrative peaks were obtained as shown in Figure: 4.7. It can be seen from the Figure: 6 that peaks obtained at 2theta (2θ) value of 29.93^{o} which is quite close to the 2θ of 29.455^{o} of pure calcite as detected by Harrington (33).

Powder extracted by scratching off process have some impurities which results in the slight difference in 2θ value. This indicated that the material formed in the cracks is calcium carbonate in nature and is comparable with the results obtained from previous researches.

Figure 4.7: XRD analysis of healing compound produced in the cracks



4.5 X-Ray Fluorescence Analysis

X-ray fluorescence test of a scratched portion of a healed concrete sample shows the deposition of calcium oxides in the crack. The detail evidence is given below.

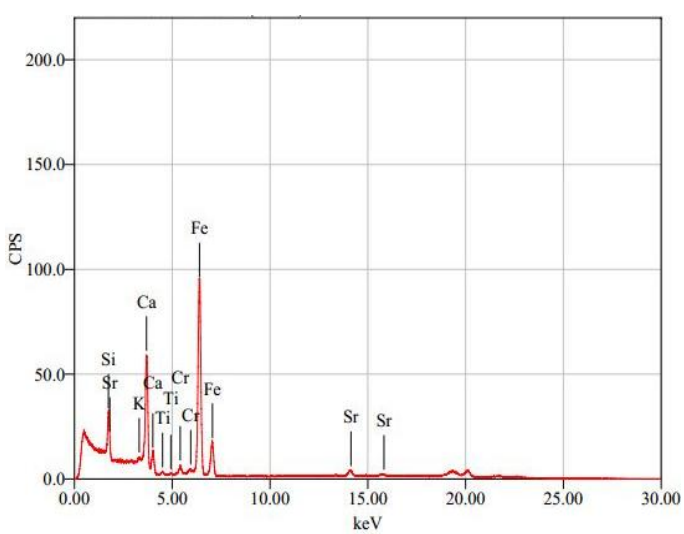


Figure 4.8: XRF analysis of scratched powder fro the healed crack

Element	Mass (in %)	Mol (in %)	Intensity (cps/mA)
SiO2	27.03	35.78	15634
K2O	1.13	0.95	1664
CaO	29.03	41.17	48171
TiO2	0.93	0.92	1283
Cr2O3	1.53	0.80	3744
Fe2O3	39.33	19.59	103658
SrO	0.99	0.76	3719

Table 4.5: XRF analysis of scratched powder from the healed crack

XRF analysis gives the chemical characterization and it is clearly evident from the results that a reasonable amount Calcium oxide 29.03% by mass is present in the sample which was scratched from healed crack.

4.5 Thermogravimetric Analysis

Thermogravimetric analysis (TGA) is one of the method of thermal analysis which is used to examine the changes in physical and chemical properties of materials. It is a function of time with constant temperature and weight loss or a function of increasing temperature (with constant heating rate). Likewise, TGA can offer information about chemical processes including decomposition chemisorptions and solid-gas reactions (21).

Powder was scratched from healed sample and thermal gravimetric analysis was conducted upon it. The Figure: 8 shows the degree of conversion. The theoretical decomposition reaction for synthetic calcite is as follows:

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

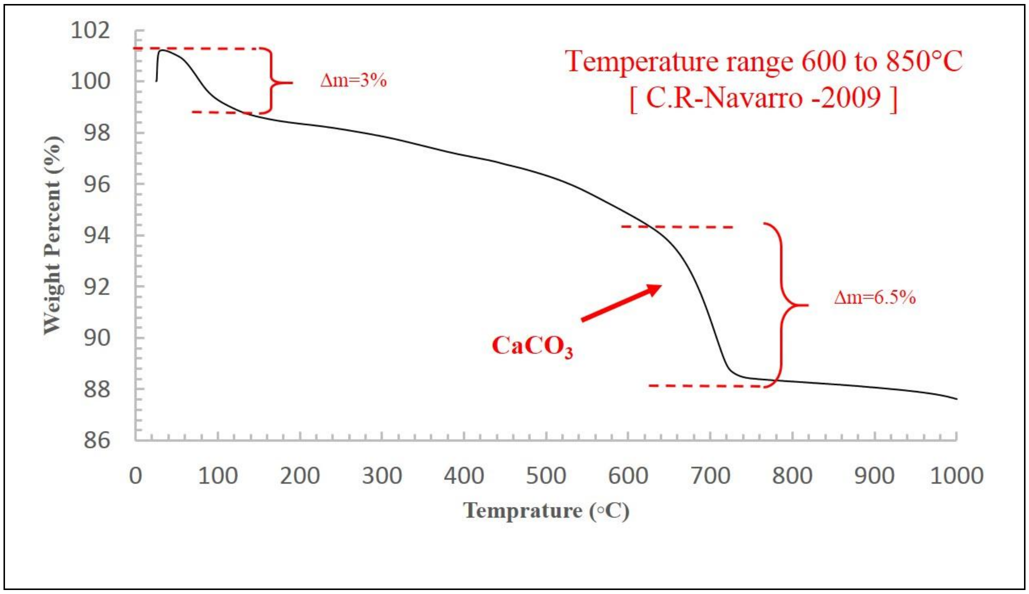


Figure 4.9: Thermo gravimetric analysis of scratched powder from healed cracks

According to Gunasekaran the thermal decomposition range for pure calcites is between 600 to 850 degrees Celsius (34). Here in Figure: 8 the major weight loss occurs in this temperature range indicates that the scratched powder is CaCO3.

4.6 Compressive Strength Analysis:

Results from Compressive Strength of concrete specimens are presented in fig. All bacterial incorporation technique irrespective of the conducting media resulted in increase in compressive strength test of the specimens.

Samples with INMP as conducting media showed the maximum compressive strength of 37MPA after 28 days of curing which is almost 21% more than the control sample. The increased compressive strength is in agreement with the results of the study carried out by P. Sikora (35).

INMP has served as the best conducting media for bacteria *Bacillus Subtilis* and micro-structure of the concrete has been improved, inherent cracks has been healed and concrete with dense micro-structure is obtained which is more durable. Also, due to the ultrafine size of INMP, they acted as a filler material and more compacted micro-structure is obtained.

Compressive Strength of the specimen using Water as carrier compound also showed slight increment in the values and increase was noted to be about 8%. The strength increase is in accordance with the previous studies and it is justified due to the formation of pure calcite due to presence of Micro-Organisms and internal micro-structure of the concrete specimen has been improved. The comparison of this result is with the study performed by the Ramachandran that Bacillus subtilis is a better choice as compared to Bacillus Pasteurii which showed no significant strength increase.(30)

Specimen incorporated with bentonite as carrier compound showed no change in compressive strength as compared to control sample. This can be justified with the pozzolanic nature of Bentonite. It is a pozzolanic material and after 28 days it starts showing reactivity inside the cementitious matrix and that reactivity is affecting its healing efficiency.

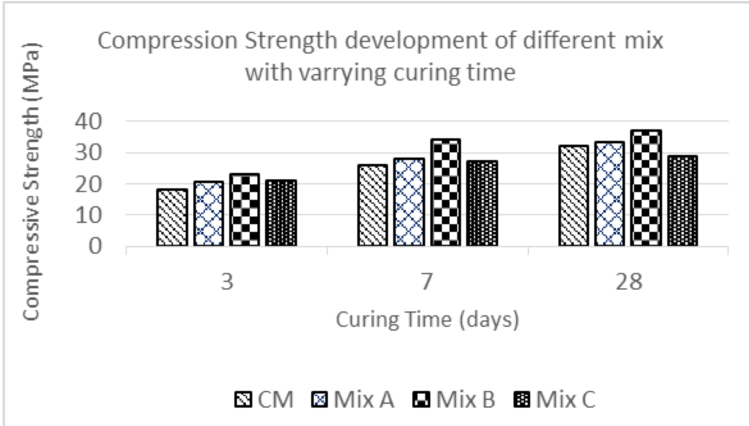


Figure 4.10: Compressive strength analysis

Figure 1: Compressive strength development with different bacteria incorporation techniques

Then the samples were pre-cracked up to 80% of their initial compressive strength and then placed in curing tank. After 3, 7 and 28 days of curing there were again examined for compressive strength test analysis and results reveled that Mix A has been able to regain up to 85% of its initial compressive strength after 28 days of curing. Mix B has been able to gain up to 65% of initial strength after 28 days of curing. Other two Mixes CM and Mix C shows approximately 45% of their initial strength after 28 days of curing. These tests conclude that Mix B is the best conducting media for micro-organism and provided the best protection to the bacteria '*Bacillus Subtilis'*.

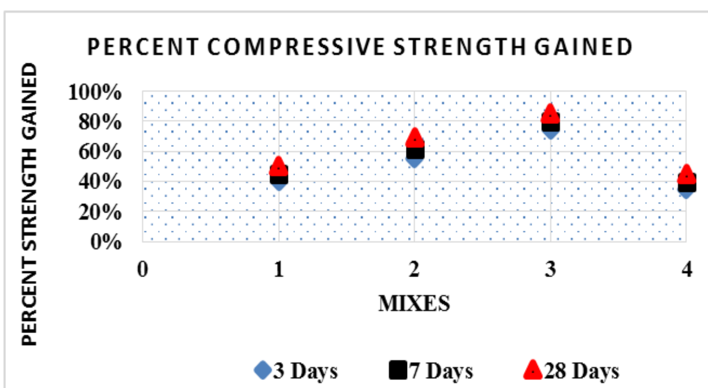


Figure 4.11: percentage regain of strength after pre-cracking and curing

4.7 Split tensile strength:

An indirect evaluation has been done to analyze the bond strength between deposited media and the adjacent matrix. Flexure strength values did not get disturb due to the intruding media but values were increased as compared to conventional concrete. An increment in flexure strength clearly indicates that there is some kind of bond present between the adjacent matrix and the deposited media that is calcium carbonate.

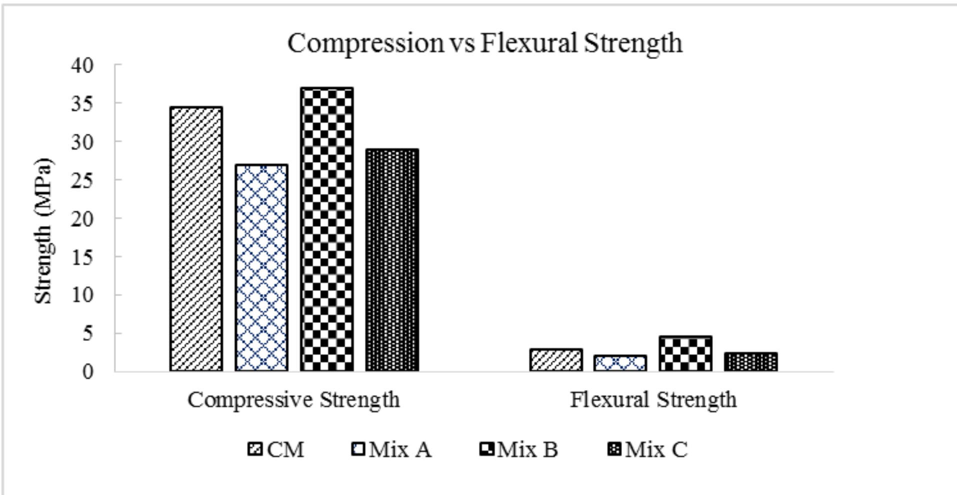


Figure 4.12: A comparison of compressive and flexural strength

CHAPTER 5

CONCLUSIONS and Recommendations

5.1 Conclusions

In this study, two types of Nano/micro sized particle as innovative carriers media have been synthesized for investigation their potential feasibility use as bacteria immobilization for self-healing cementitious materials and compared with direct induction of bacteria. Following conclusions are derived from results based upon their crack healing efficiency and microstructural properties.

1. INMP gave promising results by healing cracks up to 1.2mm and regaining compressive strength up to 85% after crack formation, because of uniform dispersion of bacteria.
2. Direct induction of in concrete healed the cracks up to .44mm with 65% strength recovery and gave better result Than BNMP immobilized self-healing Concrete.
3. BNMP showed least crack healing efficiency only up to .2mm along 45% strength regain. It may due to its pozzolonic nature and its reaction with cement hydrates.
4. Selection of immobilized material for self-healing agents is key parameter as some materials like BNMP reduces the crack healing efficiency. A further study is required to investigate the self-healing properties of concrete at later stages. Other materials can be utilized for supporting our arguments.

5.2 Recommendations

Given below are some of the recommendations for further research in this area.

- Iron-Oxide Nano/Micro Particles be used as the protective coating of micro-organisms to have maximum healing efficiency.
- Study regarding the survival of bacteria in pozzolanic & cementitious material need to be done in detail.
- Further study is required for the detailed understanding of activation process of bacteria

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