MICROBIALLY INDUCED SELF-HEALING CEMENT MORTAR

Submitted in partial fulfilment of the requirements

for the degree of

Bachelor of Engineering

by

Hussain Yusuf (14CE16)

Khan Inamulhaq (14CE25)

Khan Ateef (14CE28)

Khan Shehbaz (14CE34)

Under the guidance of

Prof. Junaid Maste

Department of Civil Engineering

School of Engineering and Technology

Anjuman-I-Islam's Kalsekar Technical Campus

New Panvel, Navi Mumbai-410206

2017-18

MICROBIALLY INDUCED SELF-HEALING CEMENT MORTAR

Submitted in partial fulfilment of the requirements

for the degree of

Bachelor of Engineering

by

Hussain Yusuf (14CE16)
Khan Inamulhaq (14CE25)
Khan Ateef (14CE28)
Khan Shehbaz (14CE34)

Under the guidance of

Prof. Junaid Maste

Department of Civil Engineering
School of Engineering and Technology
Anjuman-I-Islam's Kalsekar Technical Campus
New Panvel, Navi Mumbai-410206

2017-18

CERTIFICATE

This is to certify that the project entitled **“Microbially Self-Healing Bacterial Cement Mortar”** is a bonafide work **Mr. Hussain Yusuf (14CE16), Mr. Khan Inamul Haq (14CE25), Mr. Ateef Khan (14CE28) and Mr. Khan Shehbaz (14CE34)** submitted to the University of Mumbai in partial fulfillment of the requirement for the award of the degree of **Bachelor of Engineering** in **Civil Engineering** Course conducted in **Anjuman-I-Islam`s Kalsekar Technical Campus.**

Prof. Junaid Maste

(Project Guide)

Dr. R. B. Magar

(Head of Department)

Dr. Abdul Razak Honnutagi

(Director, AIKTC)

Project Report Approval for B.E.

This project report entitled **"Microbially Self-Healing Bacterial Cement Mortar"** by "**Mr. Hussain Yusuf (14CE16), Mr. Khan Inamul Haq (14CE25), Mr. Ateef Khan (14CE28) and Mr. Khan Shehbaz (14CE34)**" is approved for the degree of "***Bachelor of Engineering"*** in ***"Department Of Civil Engineering***".

Examiners

1 ________________

2 ________________

Supervisors

1 ________________

2 ________________

Chairman (Director)

____

Date

Acknowledgment

Before we indulge into the things we would like to add a few unfeigned words for the people who are a part of our team as they have given everlasting contribution & support right from the genesis of the report till the end.

Apart from our team we are indebted to a number of people who have assisted & furnished us with constructive guidance.

We acknowledge with deep sense of gratitude towards the encouragement in the form of substantial assistance provided by each member of our team.

We would like to express our sincere gratitude towards our **Dr. Rajendra Magar** H.O.D (Civil department) for providing us project lab & the needed assistance.

We would sincerely like to thank our project mentor **Prof. Junaid Maste** for providing us the required technical guidance, stimulating suggestions in the process of preparing this report.

We also express our gratitude Mr. Sulemaan (HAAS) and Mr. Wasif (School of Pharmacy) for giving us their expertise & valuable time during the progress of this project.

Declaration

We declare that this written submission represents our ideas in our own words and where others ideas or words have been included, we have adequately cited and referenced the original sources. We also declare that We have adhered to all principles of academic honesty and integrity and have not misrepresented or fabricated or falsified any idea/data/fact/source in our submission. We understand that any violation of the above will be cause for disciplinary action by the Institute and can also evoke penal action from the sources which have thus not been properly cited or from whom proper permission has not been taken when needed.

Hussain Yusuf ______________________

(14CE16)

Khan Inamulhaq ______________________

(14CE25)

Ateef Khan ______________________

(14CE28)

Khan Shehbaz ______________________

(14CE34)

Date:

Abstract

Carbonate-producing bacteria have attracted lots of interest as a promising, natural, environmental friendly novel technique to improvement of concrete characteristics. Considerable research has been conducted on utilizing microbial-induced carbonate precipitation to mitigate several concrete problems such as crack repair, reduction and modification of porosity and permeability. Furthermore, bacterial carbonate precipitation has shown positive influences on compressive strength improvement of concrete.

Bacterial concrete is found to be superior in all aspects of durability. In previous studies it is found that bacterial concrete containing 22.5 ml of bacteria is optimum dosage.

In the meanwhile, it seems the study related to the exposure in severe environment of bacterial concrete has not been comprehensively investigated. Therefore its decided to carry out an investigation of behavior of bacterial concrete in different severe environment of Chloride and sulphate by forming mortar cubes of bacterial solution 22.5ml/cube and placed in different solution of sulphate and chloride (viz. 5g/lit, 7.5g/lit, 10g/lit, 12.5g/lit, 15g/lit) further these various samples are tested under various laboratory methods (viz. compressive strength, ultra sonic pulse velocity, SEM and mass variation). By doing so, it is found that samples under the different environmental exposures of sulphate and chloride gave the maximum compressive strength of 49.4 MPa in 5g/lit of sulphate and 53.3 MPa in 12.5g/lit of Chloride but these compressive strengths are lesser as compared to conventional cement mortar cubes under same environmental conditions. In this study growth of bacteria is also observed my using mass variation method by observing the weight of samples it is found that the bacteria is growing well in the sample. By performing Ultrasonic Pulse Velocity Test it is found that the quality of cubes are also good.

Table of Contents

Certificate **i**

Project report approval for B.E. **ii**

Acknowledgement **iii**

Declaration **iv**

Abstract **v**

Chapter 1 Introduction **1**

Chapter 2 Literature Review **5**

2.1 Literatures 5

2.2 Summary of literature review **18**

Chapter 3 Bacterial condition and physical material **19**

3.1 Selection of bacteria 19

3.2 Cultivation of bacteria 20

3.3 Safety measure for bacterial solution 21

3.4 Material 22

3.4.1 Cement 22

3.4.2 Sand 22

3.4.3 Cube mould **23**

Chapter 4 Experimental method and tests **24**

4.1 Preparation of mortar mix cube and sample labeling 24

4.2 Methods of mixing bacterial solution in to cement mortar 24

4.3 Casting of cubes 26

4.4 Curing **27**

Chapter 5 Experimental tests on bacterial mortar **28**

5.1 Mass variation test 28

5.2 scanning electron microscope (SEM) 29

5.3 ULTRASONIC pulse velocity test (UPVT) 30

5.4 Compressive strength test **30**

Chapter 6 Experimental result and discussion **33**

6.1 compressive strength 33

6.2 Ultrasonic pulse velocity 37

6.3 Mass volume percentage 38

Chapter 7 Conclusion **40**

References **42**

List of illustrations

Fig 3.1 Microphotograph of stains of Bacilius Subtilis **20**

Fig 3.2 Preparation of broth **20**

Fig 3.3 Heating the solution with help of burner **21**

Fig 3.4 Placing the solution in incubator atv 370 C **21**

Fig 3.5 Cement **22**

Fig 3.6 Sand **22**

Fig 3.7 Moulds **23**

Fig 4.1 Direct mixing **25**

Fig 4.2 Direct mixing of bacterial solution in cement mix **25**

Fig 4.3 Cement mortar cubes **26**

Fig 4.4 Vibratory machine **26**

Fig 4.5 Curing of cubes in normal water **27**

Fig 4.6 Curing of cubes in MgSO4 **27**

Fig 4.7 Curing of cubes in MgCl2 **27**

Fig 5.1 SEM **29**

Fig 5.2 Compressive strength testing **31**

Fig 5.3 Reading on compressive testing machine **31**

Fig 5.4 Sample after testing in compressive testing machine **32**

List of tables

Table 6.1 Bacterial mortar in sulphate environment **34**

Table 6.2 Bacterial mortar in chloride environment **34**

Table 6.3 Convectional mortar in sulphate environment **35**

Table 6.4 Convectional mortar in chloride environment **36**

Table 6.5: Convectional and Bacterial mortar in normal water **36**

Table 6.6 Ultrasonic Pulse Velocity **37**

Table 6.7 Mass Variation **38**

Chapter 1

Introduction

1.1 Background

Carbonate-producing bacteria have attracted lots of interest as a promising, natural, environmental friendly novel technique to improvement of concrete characteristics. Considerable research has been conducted on utilizing microbial-induced carbonate precipitation to mitigate several concrete problems such as crack repair (Van Tittelboom et al. 2010; Wiktor and Jonkers 2011), reduction and modification of porosity (Ghosh et al. 2005, 2009), and permeability (De Muynck et al. 2007a; Jonkers and Schlangen 2008; Nemati and Voordouw 2003). Furthermore, bacterial carbonate precipitation (bio deposition) has shown positive influences on compressive strength improvement of concrete (Bang et al. 2001; Ghosh et al. 2005, 2009; Jonkers and Schlangen 2008; Jonkers et al.2010; Reddy et al. 2010) and also, it also reduces water absorption and carbonation of concrete as an alternative surface treatment (De Muynck et al. 2007a, b, 2008a, b). As part of metabolism, some bacteria produces enzyme urease which catalyzes the hydrolysis of urea to generate carbonate ions without an associated production of protons which leads to $CaCO_3$ precipitation in presence of calcium ions (Chahal et al. 2012; Okwadha and Li 2011; Siddique and Chahal 2011). Therefore, bacteria cells not only provide a nucleation site for $CaCO_3$ precipitation due to their negatively charged cell walls, but also create an alkaline environment inducing further growth of $CaCO_3$ crystals (Ferris et al. 1987; Stocks-Fischer et al. 1999). One ml of urea is hydrolyzed intracellularly to 1 ml of ammonia and 1 ml of carbonate, which is presented in Eq. (1). According to Eq. (2), carbonate hydrolyses to ammonia and carbonic acid. Eqs. (3) and (4) demonstrate former products subsequently equilibrate in water to form bicarbonate, ammonium, and hydroxide ions. The latter causes pH increase resulting in the formation of carbonate ions [Eq. (5)], which in the presence of soluble calcium ions precipitate as $CaCO_3$ [Eq. (6)]. Eq. (7) is the overall reaction, which demonstrates that ammonium and calcium carbonate are the products of added urea and calcium to the system (Siddique and Chahal 2011; Van Tittelboom et al. 2010).

$$CO(NH_2)_2 + H_2O \rightarrow NH_2COOH + NH_3 \qquad \ldots\ldots (1)$$

$$NH_2COOH + H_2O \rightarrow NH_3 + H_2CO_3 \qquad \ldots\ldots (2)$$

$$H_2CO_3 \rightarrow HCO_3 + H^+. \qquad ..(3)$$

$$2NH_3 + 2H_2O \rightarrow 2NH_{4+} + 2OH^- \qquad \ldots\ldots (4)$$

$$HCO_{3-} + H^+ + 2OH^- \rightarrow CO_{3}{}^{2-} + 2H_2O \qquad \ldots\ldots (5)$$

$$CO_3{}^{2-} + Ca^{2+} \rightarrow CaCO_3 \qquad \text{……. (6)}$$

$$CO(NH_2)_2 + 2H_2O + Ca^{2+} \rightarrow 2NH_{4+} + CaCO_3 \qquad \text{…….(7)}$$

Effect of the application of ureolytic bacteria such as Bacillus Pasteurii , now reclassified as Sporosarcina Pasteurii (Siddique and Chahal 2011), B. Subtilis, and B. Sphaericus on concrete characteristics has been extensively studied. Stocks-Fischer et al. observed $CaCO_3$ crystals in sand specimens containing B. Pasteurii cells accompanied with urea and $CaCl_2$. Possible biochemical reactions in urea-$CaCl_2$ medium to precipitate $CaCO_3$ at the cell surface can be summarized as follows (Stocks-Fischer et al. 1999):

$$Ca^{2+} + Cell \rightarrow Cell - Ca^2 \qquad \text{…..(1)}$$

$$Cl^- + HNO_{3-} + NH_3 \rightarrow NH_4Cl + CO_3{}^{2-} \qquad \text{…… (2)}$$

$$Cell - Ca^2 + CO_3{}^{2-} \rightarrow Cell - CaCO_3 \downarrow \qquad \text{…… (3)}$$

Reddy et al. have reported the addition of B. Subtilis bacteria increases the compressive strength of standard grade concrete up to about 15% at 28 days, and also shows a significant improvement in split tensile strength compared to conventional concrete (Reddy et al. 2010). Chahal et al. have prepared fly ash concrete specimens employing S. Pasteurii, and attributed significant 28-day compressive strength increment of fly ash concrete to consolidation of the pores inside the concrete cubes with bacterial induced calcium carbonate precipitation (Chahal et al. 2012).

1.2 Aim of the Project

- Develop a bacterial cement mortar by introducing the bacteria of Bacillus family (Bacillus Subtilis).

- To observe the change in the properties of cement mortar such as compressive strength and mass variation by introducing the Bacteria.

- To know the quality of mortar by Ultrasonic pulse velocity test.

- To perform microstructure analysis using SEM.

- To observe the behaviour of bacteria (Bacillus Subtilis) in different environmental exposures i.e. (sulphate and chloride environment)

Chapter 2

Literature Review

2.1 Literatures

In order to carry out the project work various literatures were studied and findings obtained by the researchers were used to identify the research area, summarizations of literatures are as follows:-

A method of strength improvement of cement–sand mortar by the microbiologically induced mineral precipitation was described by **P. Ghosh et al. (2005).** A thermophilic anaerobic microorganism is incorporated at different cell concentrations with the mixing water. The study showed that a 25% increase in 28-day compressive strength of cement mortar was achieved with the addition of about 10^5 cell/ml of mixing water. The strength improvement is due to growth of filler material within the pores of the cement–sand matrix as shown by the scanning electron microscopy. The modification in pore size distribution and total pore volume of cement–sand mortar due to such growth is also noted. *E. coli* microorganisms were also used in the cement mortar for comparison, but no improvement in strength was observed.-**"Use of microorganism to improve the strength of cement mortar".**

As synthetic polymers, used for concrete repair, may be harmful to the environment, the use of a biological repair technique was investigated by K. Van Tittelboom et al. (2010). Ureolytic bacteria such as *Bacillus Sphaericus* were able to precipitate $CaCO_3$ in their micro-environment by conversion of urea into ammonium and carbonate. The bacterial degradation of urea locally increases the pH and promotes the microbial deposition of carbonate as calcium carbonate in a calcium rich environment. These precipitated crystals can thus fill the cracks. The crack healing potential of bacteria and traditional repair techniques were compared by means of water permeability tests, ultrasound transmission measurements and visual examination. Thermo gravimetric analysis showed that bacteria were able to precipitate $CaCO_3$ crystals inside the cracks. It was seen that pure bacteria cultures were not able to bridge the cracks. However, when bacteria were protected in silica gel, cracks were filled completely.-"**Use of bacteria to repair cracks in concrete.**"

Microbially enhanced calcite precipitation on concrete or mortar had become an important area of research regarding construction materials. Study examined by V. Achal et al. (2011) stated the effect of calcite precipitation induced by *Sporosarcina Pasteurii* (Bp M-3) on parameters affecting the durability of concrete or mortar. An inexpensive industrial waste, corn steep liquor (CSL), from starch industry was used as nutrient source for the growth of bacteria and calcite production, and the results obtained with CSL were compared with those of the standard commercial medium. Bacterial deposition of a layer of calcite on the surface of the specimens resulted in substantial decrease of water uptake, permeability, and chloride penetration compared with control specimens without bacteria. The results obtained with CSL medium were comparable to those obtained with standard medium, indicating the economization of the bio-calcification process. The results suggest that calcifying bacteria play an important role in enhancing the durability of concrete structures. - **"Effect of calcifying bacteria on permeation properties of concrete structures,"**

Fly ash acts as a partial replacement material for both Portland cement and fine aggregate. An innovative approach of microbial calcite precipitation in fly ash-amended concrete had been investigated. The first report by V. Achal et al. (2011) to discuss the role of microbial calcite precipitation in enhancing the durability of fly ash-amended concrete. The study investigated the effects of *Bacillus Megaterium* ATCC 14581 on compressive strength, water absorption and water impermeability of fly ash-amended mortar and concrete. Mortar specimens were used for compressive strength and water absorption tests, while concrete specimens were used for water impermeability tests. At the fly ash concentrations of 10%, 20% and 40% in mortars, bacterial cell enhanced mortar compressive strength by 19%, 14% and 10%, respectively, compared to control specimens. Treated mortar cubes absorbed more than three times less water than control cubes as a result of microbial calcite deposition. Microbial deposition of a layer of calcite on the surface of the concrete specimens resulted in substantial decrease of water uptake and permeability compared to control specimens without bacteria. Microbial cells also prevented ingress of water effectively in different concentrations of fly ash-amended concrete. Scanning Electron Micrography (SEM) analyses evidenced the direct involvement of bacteria in calcite precipitation. The approach of the present study gives us dual environment friendly advantages. First, use of fly ash-a recovered resource reduces depletion of natural resources and also reduces the energy- intensive manufacturing of other concrete ingredients, leading to savings in both energy usage and emissions of greenhouse gases. And second, use of bacterial cells to improve strength and durability of fly ash-amended concrete further provides greener and economic options. - **"Improved strength and durability of fly ash amended concrete by microbial calcite precipitation**."

Microorganism was a unique living element and had the ability to precipitate minerals through the process of bio-mineralization. The precipitation process occurred naturally and most of the precipitated products are very important compound composed of such as carbon, nitrogen, oxygen, sulphur, phosphorus and silica. So far, concrete incorporated with microorganism that able to precipitate calcium carbonate (calcite) was reported. However, little information on silica precipitation and its effect on concrete properties had been revealed. The concrete specimens were incorporated with Bacillus Subtilis silica adsorbed in their cell wall by H Afifudin et al. (2011) -. Concrete specimens with five different concentrations of Bacillus Subtilis cell with 104, 105, 106 and 107 cell/ml and control (without Bacillus Subtilis) were cast. The experimental investigation made to prove that the silica precipitated by this microorganism can enhance the concrete properties namely its compressive strength and resistance to carbonation. The microstructure of the concrete contained Bacillus Subtilis was also examined. It was found that the inclusion of Bacillus Subtilis into the concrete enhanced the compressive strength. The concentration of 106 cell/ml was found to be the optimum concentration to give most enhanced effect to the compressive strength. However the effect of including Bacillus Subtilis to the resistance to carbonation of the concrete specimen is found to be insignificant. - **"Microorganism precipitation in enhancing concrete properties**"

Cracks in concrete were the main reason for a decreased service life of concrete structures. It was therefore more advisable and economical to restrict the development of early age small cracks the moment they appear, than to repair them after they have developed to large cracks. A promising way is to pre-add healing agents to the concrete to heal early age cracks when they appear, i.e. the so-called self-healing approach was described by J. wang et al. (2012). In addition to the more commonly studied polymeric healing materials, bacterial $CaCO_3$ precipitation also has the potential to be used for self-healing. It is more compatible with the concrete matrix and it is environment friendly. However, bacterial activity decreases a lot in the high pH (>12) environment inside concrete. In this research, the possibility to use silica gel or polyurethane as the carrier for protecting the bacteria was investigated. Experimental results show that silica gel immobilized bacteria exhibited a higher activity than polyurethane immobilized bacteria, and hence, more $CaCO_3$ precipitated in silica gel (25% by mass) than in polyurethane (11% by mass) based on thermo gravimetric analysis. However, cracked mortar specimens healed by polyurethane immobilized bacteria had a higher strength regain (60%) and lower water permeability coefficient (10^{-10}–10^{-11} m/s), compared with specimens healed by silica gel immobilized bacteria which showed a strength regain of only 5% and a water permeability coefficient of 10^{-7}–10^{-9} m/s. The results indicated that polyurethane has more potential to be used as a bacterial carrier for self-healing of concrete cracks. -**"Use of silica gel or polyurethane immobilized bacteria for self-healing concrete."**

The role of bacterial cell walls of *Bacillus Subtilis* as a concrete admixture to improve the mechanical performance of concrete. The bacterial cell walls are known to mediate microbial induced carbonate precipitation, a process in which $CaCO_3$ is formed from Ca^{2+} ions and dissolved CO_2. Consistent with such knowledge, incorporation of bacterial cell walls increased carbonation of $Ca(OH)_2$ and formation of $CaCO_3$ in concrete. Furthermore, the bacterial cell walls significantly increased compressive strengths of concrete by 15% while also decreased porosity at 28 days of curing as described by R. Pei et al. (2013). As in $CaCO_3$ precipitation *in vitro* indicated that bacterial cell walls, but not dead cells, accelerated carbonation of Ca^{2+} ions in $Ca(OH)_2$ solution. Since $CaCO_3$ formed can fill up the void, decrease the porosity and increase the compressive strength in concrete, bacterial cell walls could act as a promising concrete admixture with benefits in enhancing mechanical performance and improving other carbonation-related properties.-**"Use of bacterial cell walls to improve the mechanical performance of concrete".**

The well-known fact that concrete structures are very susceptible to cracking which allows chemicals and water to enter and degrade the concrete, reducing the performance of the structure and also requires expensive maintenance in the form of repairs. Cracking in the surface layer of concrete mainly reduces its durability, since cracks were responsible for the transport of liquids and gasses that could potentially contain deleterious substances. When micro cracks growth reaches the reinforcement, not only the concrete itself may be damaged, but also corrosion occurred in the reinforcement due to exposure to water and oxygen, and possibly CO_2 and chlorides too. Micro-cracks are therefore the main cause to structural failure. One way to circumvent costly manual maintenance and repair is to incorporate an autonomous self -healing mechanism in concrete. One such an alternative repair mechanism is currently being studied by M. V. S. Rao et al. (2013) i.e. a novel technique based on the application of bio mineralization of bacteria in concrete. The applicability of specifically calcite mineral precipitating bacteria for concrete repair and plugging of pores and cracks in concrete had been recently investigated and studies on the possibility of using specific bacteria as a sustainable and concrete -embedded self-healing agent was studied and results from on-going studies were discussed. Synthetic polymers such as epoxy treatment etc. were currently being used for repair of concrete are harmful to the environment, hence the use of a biological repair technique in concrete is focused. In the present paper, an attempt is made to incorporate dormant but viable bacteria in the concrete matrix which will contribute to the strength and durability of the concrete. Water which enters the concrete will activate the dormant bacteria which in turn will give strength to the concrete through the process of metabolically mediated calcium carbonate precipitation Concrete, due to its high internal pH, relative dryness and lack of nutrients needed for growth, is a rather hostile environment for common bacteria, but there are some extremophiles spore forming bacteria may be able to survive in this environment and increase the strength and durability of cement concrete. Overview of development of bioengineered concrete using bacterial strain Bacillus Subtilis JC3 and its enhanced mechanical and durability characteristics was briefly described in this paper**.-"A sustainable self-healing construction material."**

World widely, concrete is one of the most popular construction materials because of its strong, durable and inexpensive material. It has specialty of being cast in any desirable shape but plain concrete however is porous, possesses very low tensile strength, limited ductility and little resistance to cracking. These problems become more complicated in various environmental condition to which concrete is exposed. Conventionally, a variety of sealing agent namely, latex emulsions suffer from serious limitations of incompatible interfaces, susceptible to ultraviolet radiations, unstable molecular structure and high cost. Therefore, a novel and more environmental friendly technique was proposed for treating concrete material in structure by employing bacteria induced calcium carbonate precipitation in the form of calcite by J. M. Irwan et al. (2013) - **"Concrete Repair, Rehabilitation & Retrofitting II".**

Study was carried out by A. Talaiekhozani et al. (2013) to investigate two indigenous micro-organisms that can be isolated from soil. The isolated micro-organisms could precipitate calcium carbonate. These micro-organisms were applied to design self-healing concretes. Concrete is one of the most important materials which are used to build structures. Strength and durability of concrete is very important. Hence, a lot of research in this field is being conducted. Although a few reports can be found on the use of different micro-organism to design self-healing concretes, no research had been carried out to isolate suitable indigenous micro-organisms in Malaysia. In this study two strains of microorganisms were isolated from soil. Broken concrete was treated by a medium culture (MC) containing micro-organisms. Results of this study showed that, cracked concrete could be filled by calcium carbonate after treating by a MC containing micro-organisms. However, this treatment is not very effective on the strength of concrete. Results of this study can be used to have a better grasp of biological self-healing concrete, it is extremely important to have cheap and durable materials to build concrete structures in future.-**"Application of Proteus mirabilis and Proteus vulgaris mixture to design self-healing concrete."**

The applications of concrete are rapidly increasing worldwide and therefore the development of sustainable concrete was urgently needed for environmental reasons. As presently about 7% of the total anthropogenic atmospheric CO_2 emission was due to cement production, mechanisms that would contribute to a longer service life of concrete structures would make the material not only more durable but also more sustainable. One such mechanism that receives increasing attention in recent years is the ability for self-repair, i.e. the autonomous healing of cracks in concrete. In this study we investigated the potential of bacteria to act as self-healing agent in concrete, i.e. their ability to repair occurring cracks. A specific group of alkali-resistant spore-forming bacteria related to the genus *Bacillus* was selected by H. M. Jonkers et al. (2013) for this purpose. Bacterial spores directly added to the cement paste mixture remained viable for a period up to 4 months. A continuous decrease in pore size diameter during cement stone setting probably limited life span of spores as pore widths decreased below 1 µm, the typical size of *Bacillus* spores. However, as bacterial cement stone specimens appeared to produce substantially more crack-plugging minerals than control specimens, the potential application of bacterial spores as self-healing agent appeared promising.-**"A two component bacteria-based self-healing concrete."**

Microbial induced calcium carbonate precipitation (MICCP) was a novel method for the protection of cement-based materials. This paper produced by Senthilkumar et al. (2014) deals the comparative studies on strength characteristics in microbial cement mortars which were treated by Enterobacter sp. M2 microorganism in different calcium source (calcium hydroxide, calcium acetate, calcium chloride and calcium oxide) with various curing process. The crystalline phases of calcium carbonate (*$CaCO_3$*) crystals formation and the surface morphology of cement mortar were investigated by X-ray diffraction (XRD) and scanning electron microscope (SEM). Cement mortar specimens with and without addition of bacterial species were casted and ~ 44% increase in compressive strength, ~56% in tensile strength was noticed while compared to control specimen (without bacteria). Surface treatment of specimen with bacteria resulted around ~40% decrease of water absorption and increases the resistance to water and hazard material penetration, mainly attributed to its pore blocking effects. This biological surface treatment shows promising prospect for increasing durability aspects of concrete/cement mortar.-**"Comparative studies on strength characteristics of microbial cement mortars".**

Shortcomings of conventional treatments have drawn the attention to alternative techniques for the improvement of the compressive strength. This paper reported by V. Senthilkumar et al. (2014) reports the effects of bacterial carbonate precipitation on the compressive strength of cement mortar specimens. The method of microbial mineral plugging in porous media was common in nature. Physical and biochemical properties of *$CaCO_3$* precipitation induced by Enterococcus sp. microorganism into cement mortar specimen was studied and analyzed. X-ray diffraction is used to identify the calcium carbonate (*$CaCO_3$*) crystal as calcite, vaterite, aragonite and scanning electron microscope (SEM) was used to verify these formations as white precipitation (*$CaCO_3$*) in the microbial cement mortars. In the present study a noteworthy enhancement of compressive strength 45% is observed in the Enterococcus sp. treated bio curing specimen while compared to control. - **"Fortification of compressive strength in enterococcus microorganism incorporated microbial cement mortar."**

During cement manufacturing, cement kiln dust (CKD) is generated which represents significant environment concern related to its emission, disposal and reuse due to high alkalinity. R. Siddique et al. (2014) studied the effect of bacterial (*Bacillus Halodurans* strain KG1) treated cement kiln dust on the compressive strength, water absorption and porosity (at 7, 28 and 91 days) of concrete after reducing the alkalinity. Concrete specimens were prepared with 0%, 5%, 10% and 15% untreated and treated CKD replacing cement. Test results indicated that 7.15% and 26.6% increase in strength of concrete was achieved at 28 and 91 days, respectively, with the addition of bacterial treated 10% CKD whereas reduction in water absorption (20%) and porosity (12.35%) was observed at 91 days. X-ray diffraction (XRD) and scanning electron microscopy (SEM) results suggested that in bacterial treated 10% CKD concrete increased calcium silicate hydrate and formation of non-expansive ettringite in pores dense the concrete structure resulted in increased compressive strength. - **" Influence of bacterial treated cement kiln dust on the properties of concrete."**

A paper by R. Andalib et al. (2014) provided an in-sight into a new bio-technological method based on calcite precipitation for achieving high strength bio-concrete durability. It was very clear that mineral precipitation has the potential to enhance construction material resistance towards degradation procedures. The appropriate microbial cell concentration ($30*10^5$cells/ml) was introduced on to different structural concrete grades (40 , 45 and 50 MPa) by mixing water. In order to study the durability of structural concrete against aggressive agents, specimens were immersed in different types of acids solution (5% H_2SO_4 and *HC*) to compare their effects on 60th, 90th and 120th day. In general, sulphuric acid and hydrochloric acid are known to be the most aggressive natural threats from industrial water which can penetrate concrete to transfer the soluble calcium salts away from the cement matrix. The experimental results demonstrate that bio-concrete has less weight and strength losses when compared to the ordinary Portland cement concrete without microorganism. It was also found that maximum compressive strength and weight loss occurred during H_2SO_4 acid immersion as compared to *HCl* immersion. The density and uniformity of bio-concrete were examined using ultrasonic pulse velocity (UPV) test. Microstructure chemical analysis was also quantified by energy dispersive spectrometer (EDS) to justify the durability improvement in bacterial concrete. It was observed that less sulphur and chloride were noticed in bacterial concrete against H_2SO_4 and *HCl*, respectively in comparison to the ordinary Portland cement concrete due to calcite deposition.-**"Durability improvement assessment in different high strength bacterial structural concrete grades against different types of acids."**

Cracks increase permeability affecting the durability of concrete. As they develop gradually, it is difficult to determine when to repair them. Self-healing materials can repair themselves gradually as cracks form. A study carried out by **C. Stuckrath et al. (2014)** shows, the isolated and combined effect of two self-healing agents for concrete, both based on calcium carbonate precipitation, was studied. Lightweight aggregates were impregnated with chemical and biological solution to be added as healing agents in concrete mixtures. The influence of two common chemical admixtures on the performance of the self-healing agents was also studied. All self-healing agents were able to seal cracks between 0.08 and 0.22 mm in width. The estimated effect of chemical agents on the mean healing was higher than that of biological agents. In addition, thermo gravimetric analysis suggests the precipitates were different. Admixtures had no significant influence on the performance of self-healing agents**.-"Performance of self-healing in reinforced mortar containing chemical admixture."**

Microcapsules were applied to encapsulate bacterial spores for self-healing concrete. The viability of encapsulated spores and the influence of microcapsules on mortar specimens were investigated by J. Y. Wang et al. (2014) firstly. Breakage of the microcapsules upon cracking was verified by Scanning Electron Microscopy. Self-healing capacity was evaluated by crack healing ratio and the water permeability. The results indicated that the healing ratio in the specimens with bio-microcapsules was higher (48%–80%) than in those without bacteria (18%–50%). The maximum crack width healed in the specimens of the bacteria series was 970 μm, about 4 times that of the non-bacteria series (max 250 μm). The overall water permeability in the bacteria series was about 10 times lower than that in non-bacteria series. Wet–dry cycles were found to stimulate self-healing in mortar specimens with encapsulated bacteria. No self-healing was observed in all specimens stored at 95%RH, indicating that the presence of liquid water is an essential component for self-healing.-"**Self-healing concrete by use of microencapsulated bacterial spores."**

Bacterial-based self-healing is a promising solution for sustainable concrete maintenance. A study carried out by J. Y. Wang et al. (2014) shows, bacterial spores were first encapsulated into hydrogels and then were incorporated into specimens to investigate their healing efficiency. The precipitation of $CaCO_3$ by hydrogel-encapsulated spores was demonstrated by Thermo gravimetric analysis (TGA). The mortar specimens with hydrogel-encapsulated spores, showed a distinct self-healing superiority: the maximum healed crack width was about 0.5 mm and the water permeability was decreased by 68% in average. Other specimens in non-bacterial series had maximum healed crack width of 0–0.3 mm and the average water permeability was decreased by 15–55% only. - **"Application of hydro gel encapsulated carbonate precipitating bacteria for approaching a realistic self-healing in concrete."**

In this paper published by C. X. Qian et al. (2014), three bio-mineralization mechanisms were proposed to repair cement-based materials cracks. The common feature was that the three are all induced by bacterial. A type of bacterial which can decompose urea and release carbonate ions could be applied to repair micro cracks on concrete surface when combining calcium ions. But what need to be noted is that the way of repairing cracks is passive. Some Alkaliphilic bacterial spores could be added to concrete when casted and two different types of bacterial were used to realize the function of self-healing. The sources of carbonate ions made them different; the one release carbonate dioxide through its own cellular respiration, the other could transfer carbon dioxide in air to bicarbonate. Coefficient of capillary suction, apparent water permeation coefficient and area repairing rate were applied to characterize the repairing effectiveness. The tests results were that all three bio- mineralization mechanisms showed excellent repair effect to small cracks formed at early ages. When the bacteria were immobilized by Ceramsite, the self-healing effect could be improved for the cracks formed at late ages. - " **Self-healing and repairing concrete cracks based on bio-mineralization."**

Using Carbonate producing bacteria was a promising novel technique by F. Nosouhian, et al. (2015) for the improvement of concrete characteristics. Durability of concrete in harsh environment such as sulphate exposure has been constantly an important issue. The intention of the current study was evaluation h mixing water, the effect of sulphate solution exposure on durability properties of tested specimens including mass variation, volume variation, and water absorption of durability improvement of concrete containing bacteria exposed to sulphate environment. To do so, seven groups of 70-mm concrete prisms were made using two different bacterial strains accompanied with mixing water, the effect of sulphate solution exposure on durability properties of tested specimens - **"Concrete Durability Improvement in Sulphate Environment Using Bacteria".**

Concrete structures are often reinforced with steel. In order for the reinforcement to take over tensile forces, concrete has to crack. Through such cracks, water and compounds that are harmful to concrete can enter. This can cause durability issues like leakage, concrete degradation and reinforcement corrosion. In situ repair of cracks was often labor-intensive and inefficient. Preferentially, cracks were autonomously healed from the inside out in an early stage, preventing the ingress of water. This can be achieved by incorporating healing agent particles composed of nutrients and bacterial spores into the concrete matrix. The bacteria will cracks with calcium carbonate. However, a coating is needed to protect the water-soluble healing agent from water during mixing. In order to allow the bacteria access to water for activation after the concrete has hardened, such a coating should break whenever a crack occurs in the concrete. Therefore, it should adhere well to the concrete matrix. It is possible to achieve this by protecting the particles with a brittle geo-polymer coating. For this study, healing agent particles were coated with geo-polymers following different mixture recipes. Metakaolin is used as an Aluminosilicate source and sodium silicate as well as sodium aluminate is used as activator liquids. The particles are coated by granulation in a low-shear granulator. In order to improve the coating process, the operating window and the granulation mechanism were determined for all activator liquids used. Leaching and strength tests were performed by S. A. L. De Koster et al. (2015) and coated particles are incorporated in cement paste in order to determine the feasibility of application of the particles in concrete. Results show that the prepared particles are better protected from leaching than untreated particles. Using a high pressure single-fluid nozzle to improve nebulisation when coating produces more particles of the desired size than coating with a low pressure single-fluid nozzle with poor nebulisation. Furthermore, particles prepared with a high pressure nozzle sprayer perform better when incorporated into cement paste than particles prepared with a low pressure nozzle sprayer. - **"Geo-polymer coating of bacteria-containing granules for use in self-healing concrete."**

The objective of this study by E. Mostavi et al. (2015) was to evaluate a new generation of self-healing materials that hold promise for better durability and performance. The in situ polymerization method was used to develop double-walled microcapsules. The microcapsules were prepared in a single batch process containing sodium silicate as the healing agent encapsulated in double-walled polyurethane/urea-formaldehyde (PU/UF) microcapsules. Double-walled microcapsules provide enhanced durability at high temperatures compared with single-walled microcapsules while preserving adequate interfacial bonding of microcapsules. A parametric study was carried out to investigate the effect of different parameters such as agitation rate, pH, and temperature on the performance of the microcapsules and to determine the optimum microencapsulation procedure. The prepared microcapsules were then incorporated into self-healing concrete beams. To monitor the healing process of the cracks, micro cracks were created by imposing a certain magnitude of displacement in the middle of the beams. The healing process of concrete specimens was monitored and quantified using portable ultrasonic non-destructive digital indicating tester (PUNDIT). Results showed that lower pH and higher agitation rate and curing temperature improve the formation of microcapsule shells. Measurements of ultrasonic wave transmission time through the concrete specimens containing different contents of microcapsules were analyzed to quantify the healing rate. It was found that the healing rate in concrete beams with 5% microcapsules was higher in the first week in comparison with specimen containing 2.5% of microcapsules. **- "Evaluation of self-healing mechanisms in concrete with double-walled sodium silicate microcapsules."**

Using carbonate-producing bacteria was a promising novel technique by F. Nosouhian et al. (2016) for the improvement of concrete characteristics. Durability of concrete in harsh environments such as sulfate exposure has been constantly an important issue. The intention of the current study was evaluation of durability improvement of concrete containing bacteria exposed to sulfate environment. To do so, seven groups of 70-mm concrete prisms were made using two different bacterial strains accompanied with mixing water; the effects of sulfate solution exposure on durability properties of tested specimens including mass variation, volume variation, water absorption, and compressive strength were then determined. Furthermore, seven groups of concrete discs with 100 mm diameter and thickness of 50 mm were prepared from the aforementioned batches to investigate the chloride permeability of bacterial concrete by rapid chloride permeability test (RCPT). The results indicated that bacteria incorporation in concrete reduces mass variation, volume variation (in higher ages), and water absorption; it also increases the compressive strength of the specimens. The results also showed that the 28-day compressive strength of the bacteria- containing concretes is about 20% more than that of the control specimens. Moreover, bacterial concrete have lower chloride penetration in comparison with the control specimens. **- "Concrete durability improvement in a sulfate environment using bacteria."**

Crack formation and progression under tensile stress was a major weakness of concrete. These cracks also make concrete vulnerable to deleterious environment due to ingress of harmful compounds. Crack healing in concrete can be helpful in mitigation of Bacteria *Bacillus subtilis*. Bacteria were introduced in concrete by direct incorporation, and thorough various carrier compounds namely light weight aggregate and graphite Nano platelets. In all the techniques, calcium lactate was used as an organic precursor. Development and propagation of cracks in concrete. This paper published by W. Khaliq et al. (2016), presents the process of crack healing phenomenon in concrete by microbial activity of Specimens were made for each mix to quantify crack healing and to compare changes in compressive strength of concrete. Results showed that bacteria immobilized in graphite nano-platelets gave better results in specimens pre-cracked at 3 and 7 days while bacteria immobilized in light weight aggregates were more effective in samples pre-cracked at 14 and 28 days. In addition, concrete incorporated with bacteria immobilized in light weight aggregate, also exhibited significant enhancement in compressive strength of concrete. - “**Crack healing in concrete using various bio-influenced self-healing techniques.”**

One of the most common problems developed in concrete is about cracks. Cracks are unavoidable in concrete and make the concrete elements weak. Cracks allow water and other salts to seep through them and make the concrete weak and reduce the life of concrete. Corrosion of steel due to salts weakens the reinforced concrete in tension as well. So there is need to develop the methods for curing the cracks and regaining the strength of concrete structures. Currently synthetic polymers can be used to repair the cracks which are harmful to environment which lead to develop biological treatment techniques. In this study, a biological repair technique was used in which bacteria of 10$_5$cells/ml were mixed with concrete to heal the cracks. The experiments were carried out to evaluate the effect of Bacillus Subtilis on the compressive strength, Tensile strength and Flexural test for 3, 7 and28 days. In addition to above technique fly ash was partially added in the place of cement. The fly ash (0, 10 and 30 %) was added by weight of cement in concrete mix and experiments were carried out. The experimental results show that 10 % fly ash replaced concrete with and without bacteria has more strength when compared to conventional concrete and was studied by R. Sri Bhavana, P. Polu Raju and S S Asadi in year 2017

Influence of bacteria on strength and permeation characteristics concrete incorporating silica fume (SF) as a substitution of cement had been investigated. The cement was partially substituted with 5, 10 and 15% SF and with constant concentration of bacterial culture, 105 cfu/mL of water. Cement was substituted with silica fume in concrete by weight. At 28 d, nearly 10–12% increase in compressive strength was observed on incorporation of bacteria in SF concrete. At 28 d, the compressive strength of concrete increased from 32.9 to 36.5 MPa for SF, 34.8 to 38.4 MPa for SF5, 38.7 to 43.0 MPa for SF10 and 36.6 to 40.2 MPa for SF15 on addition of bacteria. Water absorption, porosity and capillary water rise reduced in the range of 42–48%, 52–56% and 54–78%, respectively, in bacterial concrete compared to corresponding non-bacterial samples at 28 days. Reduction in chloride permeability of bacterial concrete was observed and the total charge passed through bacterial concrete samples reduced by nearly 10% compared to nonbacterial concrete samples at 56 d of age. At 28 d, total charge passed through concrete reduced from 2525 to 1993 C for SF, 1537 to 1338 C for SF5, 961 to 912 C for SF10 and 1186 to 1174 C for SF15 on addition of bacteria. Calcite precipitation on addition bacteria and confirmed by SEM and XRD analysis was considered as the reason for improvement in properties of concrete. Economic study of bacterial SF concrete had also been carried out in the present work. The Benefit/Cost Ratio of bacterial SF concrete got reduced with the increase in SF quantity. Compared to control concrete, bacterial SF concrete containing 10% silica fume demonstrated highest benefit in improvement in its properties and corresponding highest Benefit/Cost Ratio and was studied by Rafat Siddique, Anita Rajor and colleagues in year 2017 as **"Effect of bacteria on strength, permeation characteristics and micro-structure of silica fume concrete"**

2.2 Summary of literature review

Based on the above literature study it was found that various work has been conducted in regards with comparison between conventional and bacterial concrete. Also some literature express the maximum width of cracks healed by the bacterial concrete, some of them highlighted the effect on bacterial concrete in various environmental conditions. But no literature so far indicate the various dosage of environment exposure in which bacteria can functions more efficiently, therefore it needs to be addressed.

Chapter 3

Bacterial Conditions & Physical Materials

3.1 Selection of Bacteria:-

There are various types of Bacterias that can be used in the concrete such as B. Subtilis, B. Pasteurii, B. Cohnii, B. Licheniformis etc. We have selected ***Bacillus Subtilis*** since this bacteria produces Calcium Carbonate and due to ease of availability from pharmacy department of AIKTC, we have used it for our furture investigation. It is also formally known as **Hay bacillus** or **grass bacillus,** is a Gram-positive, catalane-positive bacterium, found in soil and the gastrointestinal tract of ruminants and humans. A member of the genus *Bacillus*, *B. subtilis* is rod-shaped, and can form a tough, protective endo-spores, allowing it to tolerate extreme environmental conditions. *B. subtilis* has historically been classified as an obligate aerobe, though evidence exists that it is a facultative aerobe. *B. subtilis* is considered the best studied Gram-positive bacterium and a model organism to study bacterial chromosome replication and cell differentiation. It is one of the bacterial champions in secreted enzyme production and used on an industrial scale by biotechnology companies.

The microphotograph of strains of Bacillus Subtilis is shown in fig. 1 below

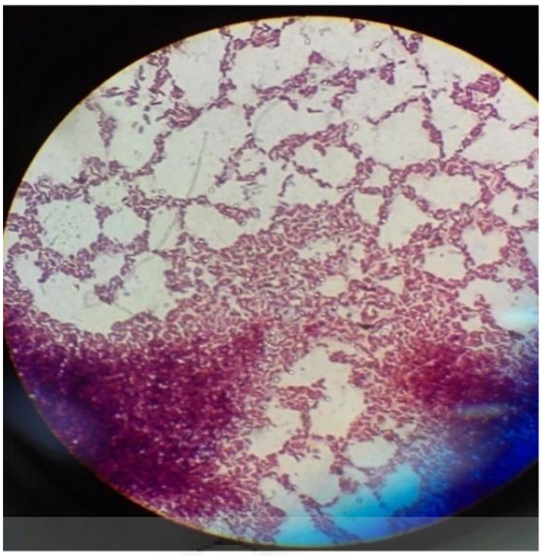


Fig 3.1: Microphotograph of strains of Bacillus Subtilis

3.2 Cultivation of Bacteria

Preparation of bacterial solution

We prepared Nutrient broth solution by addition of Nutrient broth and water. After heating the solution it was placed in Autoclave machine for sterilization. Two colonies of the bacteria were inoculated into nutrient both of 350 ml in 500ml We added bacteria subtilis in the solution with the help of micron loop. Then we placed the solution in Incubator at 37^0 for 3 days to achieve significant growth

Fig 3.2: Preparation of broth

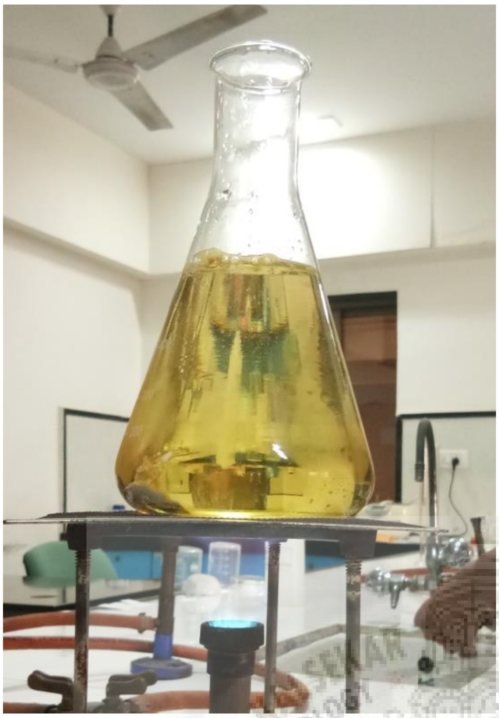


Fig 3.3: Heating the solution with help of burner

Fig 3.4: Placing the solutions in Incubator at 37^0C

3.3 Safety Measures for Bacterial Solution:-

Bacteria are harmful for the health and it may lead to diseases, therefore precautions must be taken. It is compulsory to use gloves while dealing with the bacterial solution. The flask must be heated before pouring the bacterial solution. The whole procedure must be done between the two candles so that the bacterium doesn't get contaminated by the interference of the other bacteria's present in the environment.

3.4 Material

3.4.1. Cement - Pozzolana Portland cement which is available in local market is used in the investigation. The cement used has been tested for various properties as per IS: 1489 (part 1) 1991 having specific gravity 2.90.

Fig 3.5: Cement

3.4.2. Sand - In our investigation we had used the Gujarat sand confirming the zone III according to IS- 383.Specific gravity of sand was found out to be 2.60.

Fig 3.6: Sand

3.4.3 Cube Moulds - The cube Moulds (*70.6* **x** *70.6* **x** *70.6 mm*) was placed in position on an even surface. All the interior faces and sides were coated with mud oil to prevent the sticking of concrete to the Moulds.

Fig 3.7: Moulds

Chapter 4

Experimental Methods & Test

4.1 Preparation of mortar mix cubes and sample labeling

Cement mortar cubes were prepared by taking Cement-Sand ratio as **1:3**. The Water-Cement ratio was taken as 0.45 with the help of *IS Code 2250*. Totally 66 cube samples were prepared, viz. 33 convectional cement mortar cubes and 33 Bacterial cement mortar cubes.

4.2 Methods of mixing bacterial solution into cement mortar:

There are different methods of mixing the bacterial solution in the concrete which are viz.

(a) Direct Mixing

(b) Indirect Mixing

(c) Injection methods

We have used **Direct Method** of mixing bacteria into the cement mortar. In our investigation we have adopted the direct method in which, firstly the measuring jars were sterilized in oven for a temperature of about $100^0C$for 5 min. After 5 min once it gets slightly cooled, the bacterial solution is poured form the flask in the measuring jar. The flask is firstly heated under the candle before pouring it into the jar, so that the bacterium doesn't get contaminated by the other bacteria's present in the environment.

Fig 4.1: Direct mixing

Fig 4.2: Direct mixing of bacterial solution in cement mix

4.3 Casting of cubes

Once the mortar cubes are completely mixed, they are poured in moulds. Compaction is done by the mortar cube vibrator machine. Mortar cubes were removed from the Moulds after 24 hours and they were put into the curing tank in different environments.

Fig 4.3: Cement mortar cubes

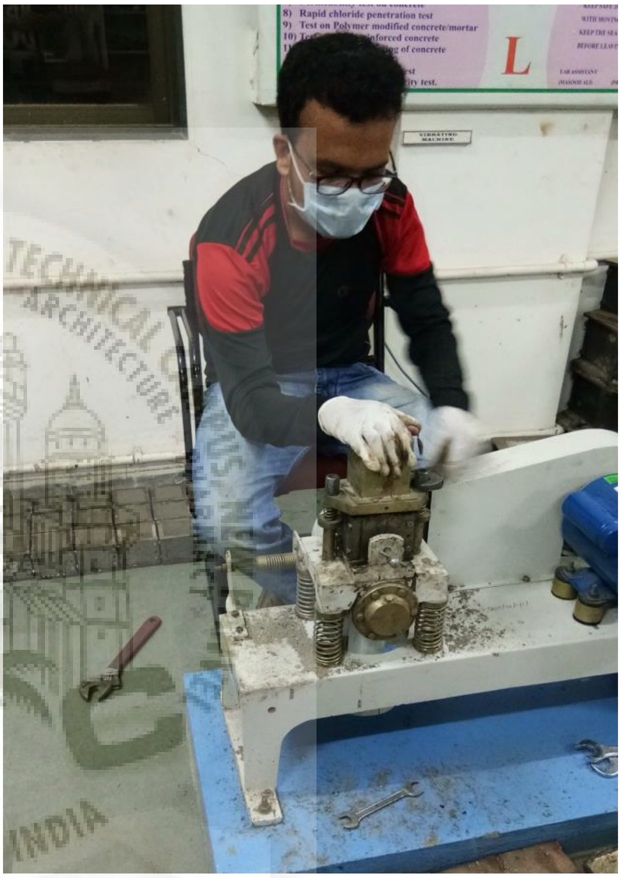


Fig 4.4: Vibratory machine

4.4 Curing

Curing has been done in three different environments for 14 days, viz.
Normal water, Sulphate environment (Magnesium Sulphate $MgSO_4$) and Chloride environment (Magnesium Chloride $MgCl_2$) with different dosages

$MgSO_4$	5.0 g/L	7.5 g/L	10.0 g/L	12.5 g/L	15.0 g/L
$MgCl_2$	5.0 g/L	7.5 g/L	10.0 g/L	12.5 g/L	15.0 g/L

Fig 4.5: Curing of cubes in normal

Fig 4.6: Curing of cubes in $MgSO_4$

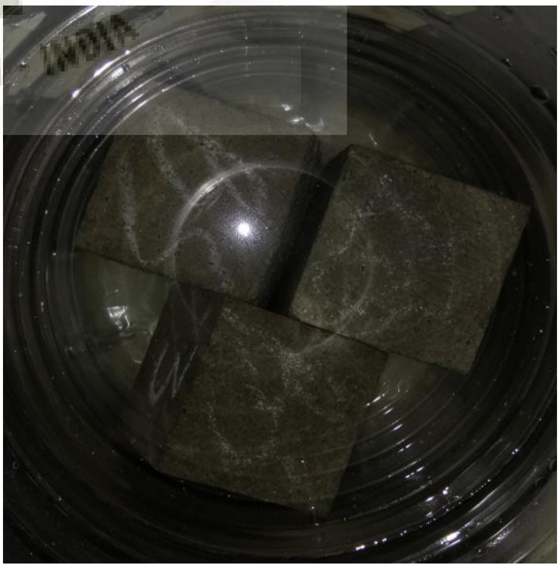


Fig 4.7: Curing of cubes in $MgCl_2$

Chapter 5

Experimental test on bacterial mortar

Various tests are performed on bacterial cement mortar in order to get the results in various forms. These experimental methods are summarized below.

5.1 Mass Variation Test

Mass changes could be an indicator determining the durability of hardened concrete in harsh environments such as Sulphate and Chloride exposure. Leaching and Sulphate penetrations are among reasons that cause mass changes of concrete in sulphate environment. Leaching actually generates loss of mass while the sulphate penetration causes mass increase.

After determination of dry weigh of the specimens at the ages 14 days mass variations of immersed specimens were calculate using the following formula.

$$\text{Mass variation (\%)} = \frac{m_2 - m_1}{m_1} \times 100$$

5.2 Scanning Electron Microscope (SEM)

The Morphology and mineralogical composition of the deposited calcium carbonate crystals were investigated using scanning electron microscope (SEM). SEM micrographs were obtained using a jell JSM 5600 LV model Philips XL 30 attached with EDX unit, with accelerating voltage 30K.V., magnification 10x up to 400000x and resolution for W.(3.5 nm). Samples surface were first coated with carbon then with gold.

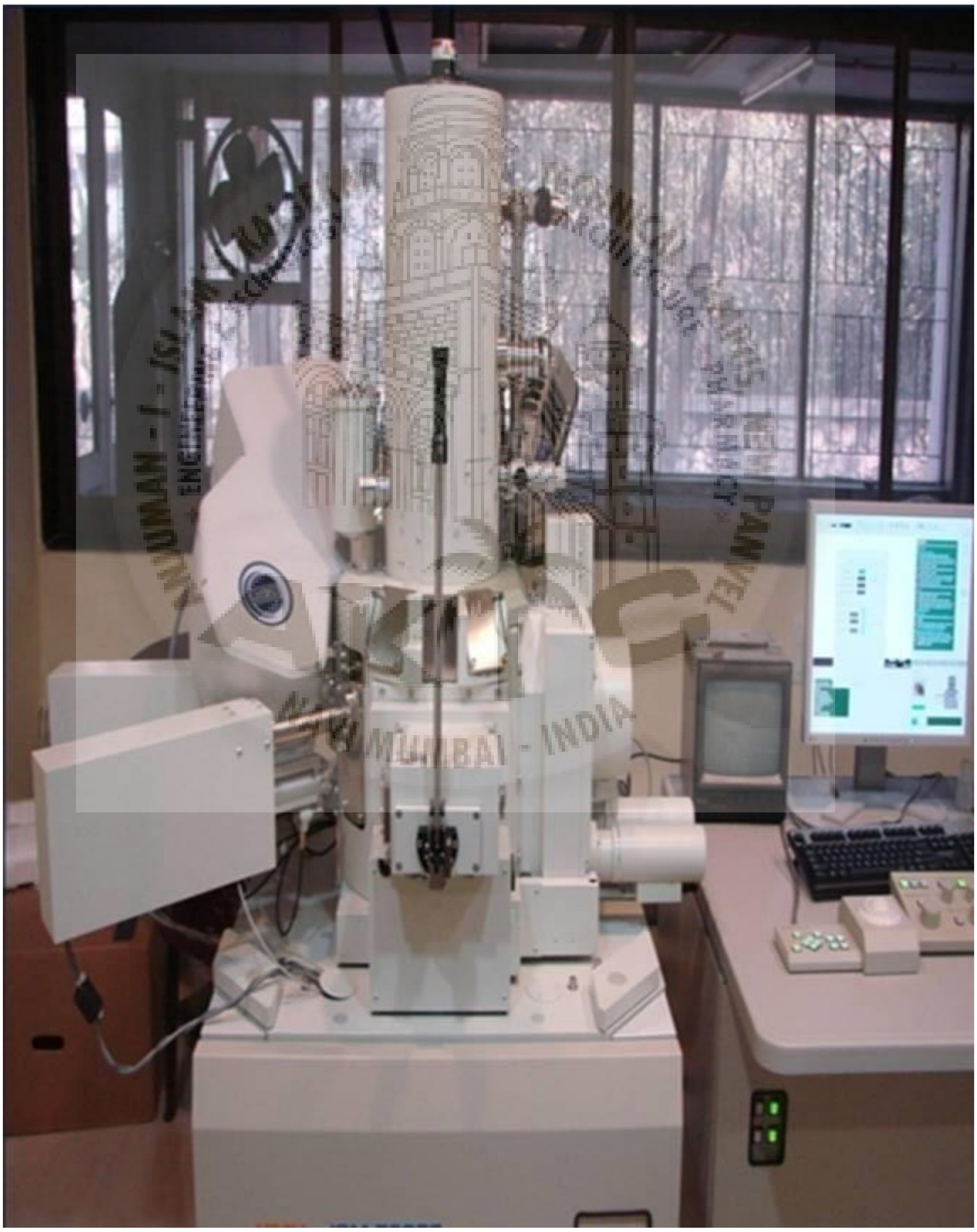


Fig 5.1 : SEM

5.3 Ultrasonic Pulse Velocity Test (UPVT)

This method consists of producing an ultrasonic longitudinal pulse by an electro acoustical transducer which is held in contact with one surface of the freshly placed concrete member under test. After traversing a known distance in the concrete, the pulse to be measured from which the pulse velocity timing circuit enables the transit time of the pulse to be measured from which the pulse velocity is calculated. This procedure is called the "**Ultrasonic method**." Ultrasonic pulse velocity test is generally carried out to determine the presence of voids in the internal structure of concrete by means of passing the ultrasonic rays through the body on mortar and also to know the denseness of the concrete structure All the respective bacterial mortar and Conventional mortar samples with the environmental dosage (sulphate and chloride) viz. 5 g/l, 7.5 g/l, 10 g/l, 12.5 g/l and 15 g/l were tested at IIT Bombay.

5.4 Compressive strength test

The mortar samples were removed from the tank after their respective days of curing. The cubes were allowed to dry under the Laboratory condition. Once the cube were completely dried, placed under the compressive testing machine with an intention to get the compressive strength of mortar samples. The entire sample specimen tested under compressive testing machine.

After removing the specimen from water over specified curing time and wiped out excess water from the surface. Clean out the bearing surface of the testing machine. The various sample specimens were placed one after another in the machine in such a manner that the load shall be applied to the opposite sides of the cube cast. The specimen centrally aligned on the base plate of the machine. The load gradually applied without shock and continuously at the rate of 5.2 KN/sec till the specimen fails. The maximum load recorded and any unusual features in the type of failure noted down. Mortar cubes placed in the CTM machine before crushing and after crushing shown in fig below. Readings of each bacterial mortar sample placed in different curing environments viz. Conventional, Sulphate and Chloride were taken after 14 days of curing.

Fig 5.2: Compressive Strength Testing

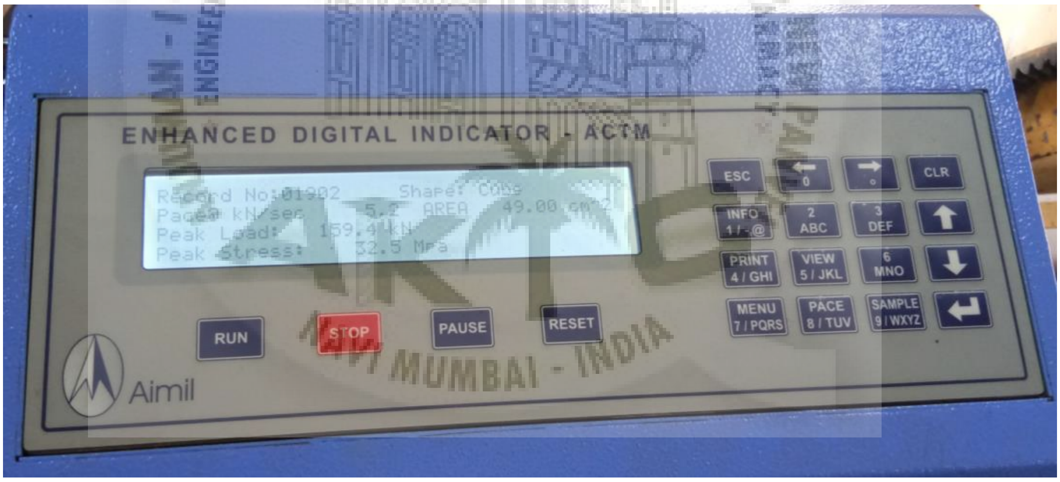


Fig 5.3: Reading on compressive testing machine

Fig 5.4: Sample after testing in compressive testing machine

Chapter 6

Experimental Results and Discussion

Various tests were conducted to know the characteristics of the concrete cube. The test was conducted to investigate the optimum dosage of the sulphate and chloride solution under which the cube attains its maximum strength.

6.1 Compressive Strength:-

Compressive strength of concrete cube was carried out after curing period of 14 days. The results so obtained are tabulated below with their respective graph.

Table 6.1: Bacterial mortar (Sulphate environment)

Dosage of $MgSO_4$	Compressive strength of mortar after 14 days (MPa)				
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
5 g/l	40.0	34.6	34.3	37.5	38.5
7.5 g/l	31.7	34.6	39.3	34.3	34.9
10 g/l	34.5	38.2	32.5	34.6	35.1
12.5 g/l	33.8	35.1	31.9	32.8	33.4
15 g/l	29.5	31.8	31.9	30.9	31.2

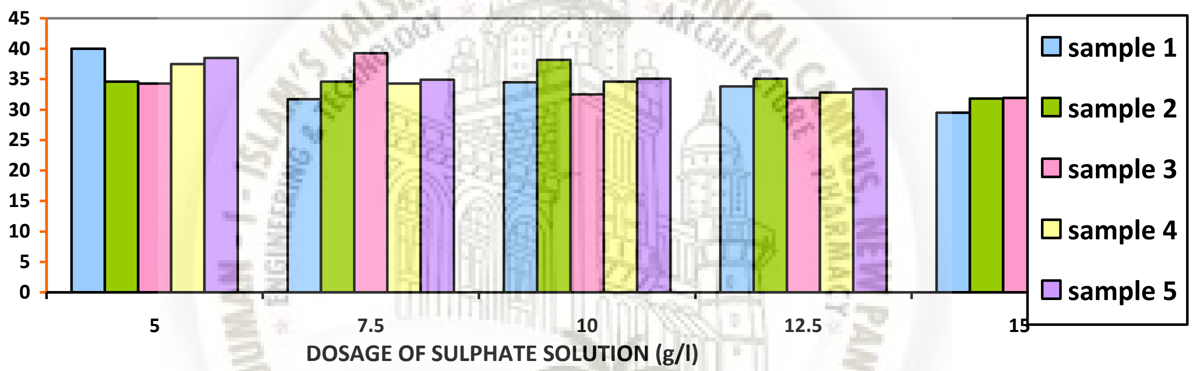


Table 6.2: Bacterial mortar (Chloride environment)

Dosage of $MgCl_2$	Compressive strength of mortar after 14 days (MPa)				
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
5 g/l	45.7	45.9	44.8	44.1	45.7
7.5 g/l	45.9	47.9	46.1	43.5	44.9
10 g/l	44.1	39.8	40.1	35.4	39.5
12.5 g/l	47.4	43.7	49.4	47.9	46.9
15 g/l	45.9	39.6	44.3	43.2	44.5

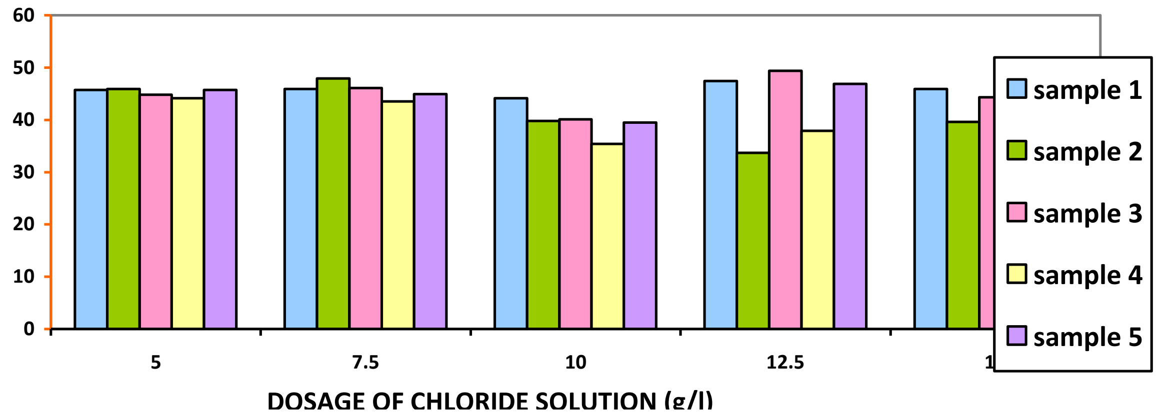


Table 6.3: Convectional mortar (Sulphate environment)

Dosage of $MgCl_2$	Compressive strength of mortar after 14 days (MPa)				
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
5 g/l	39.8	46.0	46.5	44.2	45.8
7.5 g/l	42.7	39.1	39.4	40.5	41.2
10 g/l	46.4	46.7	39.4	40.1	45.2
12.5 g/l	40.7	37.9	53.3	47.1	45.6
15 g/l	50.4	51.2	43.2	40.2	44.9

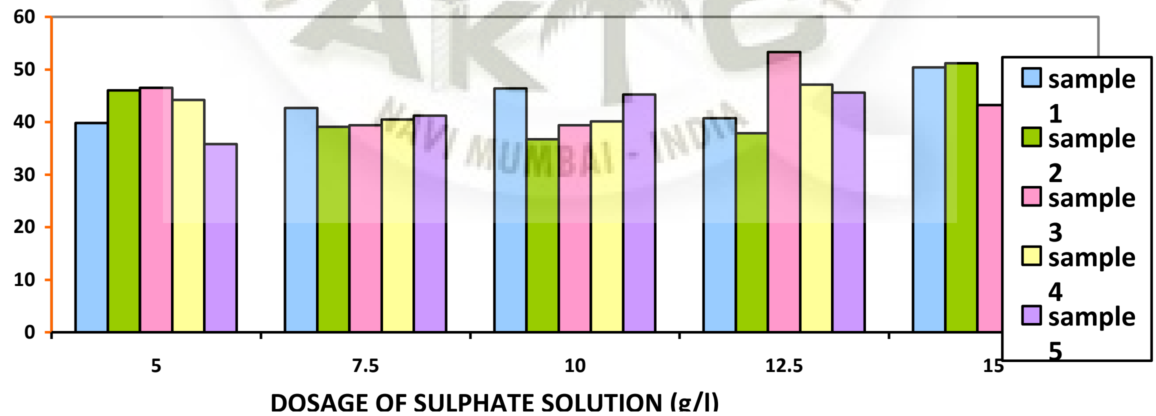


Table 6.4: Convectional mortar (Chloride environment)

Dosage of $MgCl_2$	Compressive strength of mortar after 14 days (MPa)				
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
5 g/l	41.9	49.3	47.5	48.6	48.9
7.5 g/l	39.2	46.1	44.3	43.9	40.5
10 g/l	40.9	42.3	41.9	38.7	45.2
12.5 g/l	37.3	39.7	42.1	39.2	38.6
15 g/l	39.6	43.5	36.9	37.7	40.6

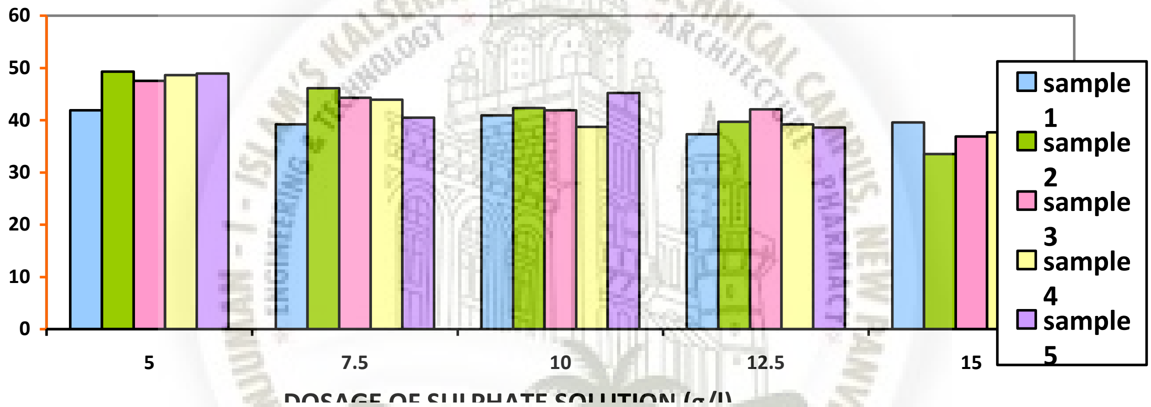
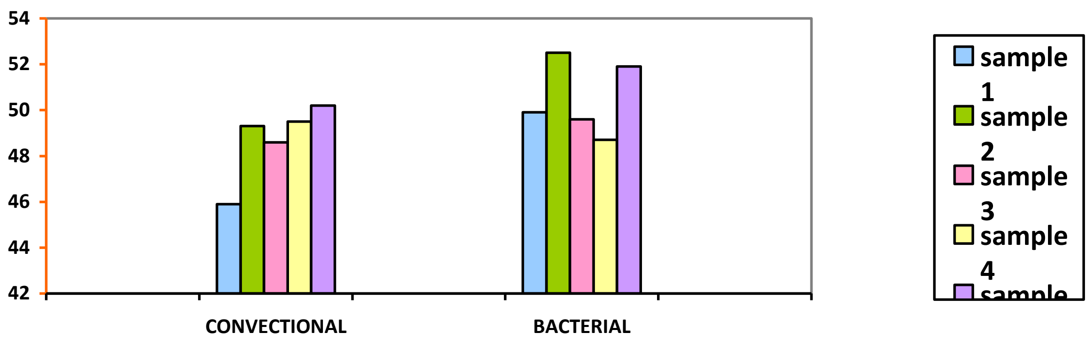


Table 6.5: Convectional and Bacterial mortar in normal water

Curing Envn.	Compressive strength of mortar after 14 days (MPa)					
	Type of mortar	1	2	3	4	5
Normal water	Convectional	45.9	49.3	48.6	49.5	50.2
	Bacterial	49.9	52.5	49.6	48.7	51.9



6.2 Ultrasonic Pulse Velocity

Ultra sonic pulse velocity test was carried out to know the presence of voids in the internal structure of the concrete cubes. The results so obtained after conducting the test are tabulated below.

TABLE 6.6 **Ultrasonic Pulse Velocity**

No.	Property of mortar	RCC Member	Prob. Distance (mm)	Time Micro (sec)	Velocity (Km/sec)	Probing Method
1	**CONVECTIONAL MORTAR**					
a)	**Sulphate solution**					
	5 g/l	Cube	70	17.99	3.90	Direct
	7.5 g/l	Cube	70	18.8	3.72	Direct
	10 g/l	Cube	70	19.9	3.51	Direct
	12.5 g/l	Cube	70	18.8	3.72	Direct
	15 g/l	Cube	70	19	3.69	Direct
b)	**Chloride solution**					
	5 g/l	Cube	70	18.1	3.87	Direct
	7.5 g/l	Cube	70	19.2	3.65	Direct
	10 g/l	Cube	70	19.1	3.67	Direct
	12.5 g/l	Cube	70	19.9	3.52	Direct
	15 g/l	Cube	70	21.1	3.31	Direct
2	**BACTERIAL MORTAR**					
a)	**Sulphate solution**					
	5 g/l	Cube	70	18.7	3.75	Direct
	7.5 g/l	Cube	70	20.3	3.45	Direct
	10 g/l	Cube	70	19.1	3.66	Direct
	12.5 g/l	Cube	70	18.0	3.91	Direct
	15 g/l	Cube	70	18.1	3.87	Direct
b)	**Chloride solution**					
	5 g/l	Cube	70	18.5	3.79	Direct
	7.5 g/l	Cube	70	19.1	3.67	Direct
	10 g/l	Cube	70	17.9	3.90	Direct
	12.5 g/l	Cube	70	18.6	3.77	Direct
	15 g/l	Cube	70	19.5	3.59	Direct

3	Normal Water					
a)	Convectional mortar	Cube	70	17.7	3.95	Direct
b)	Bacterial mortar	Cube	70	17.6	3.97	Direct

6.3 Mass Volume Percentage

The mortar samples were weighed before and after curing. The readings in the variations in their mass are summarized in the following table.

Table 6.7: Mass Variation

Sr. No	CONVECTIONAL MORTAR			BACTERIAL MORTAR		
	m_2 (g)	m_1 (g)	Mass variation %	m_2 (g)	m_1 (g)	Mass variation %
1	791	782	1.1378	805	794	1.3664
2	796	785	1.3819	814	797	2.0884
3	799	787	1.5018	809	795	1.7305
4	800	791	1.125	817	805	1.4687
5	805	794	1.3664	812	794	2.2167
6	803	788	1.8679	818	801	2.0782
7	804	792	1.4925	819	802	2.0757
8	797	781	2.0075	819	800	2.3199
9	810	795	1.8518	821	802	2.3145
10	807	794	1.6109	817	800	2.0807
11	798	779	2.3804	819	798	2.5641
12	798	782	2.0050	813	793	2.4600
13	797	784	1.6311	814	799	1.8427
14	803	788	0.6030	814	801	1.5970
15	791	792	1.3698	817	803	1.7135
16	800	780	1.3906	810	794	1.9153
17	805	794	0.7500	819	809	1.2210
18	798	798	0.8695	827	814	1.5719
19	813	786	1.5037	815	802	1.5950
20	813	794	2.3370	825	804	2.5454
21	803	791	1.4943	819	803	1.9536

22	808	799	1.1113	822	812	1.2166
23	809	794	1.8541	827	808	2.2974
24	807	795	1.6109	818	802	1.9559
25	801	790	1.3732	823	804	2.3086
26	800	784	2.0000	818	799	2.3227

Chapter 7

Conclusion

Compressive strength of bacteria –containing mortar in sulphate and chloride environment was experimentally investigated in the current study. Moreover UPVT results and SEM examination microphotographs are provided. Based on the experimental results and testing of the present study considering used dosage of suphate and chloride solution, following conclusions can be drawn:

1. Bacterial cement mortar is found to be superior to that of conventional cement mortar in aspect of compressive strength in normal environment conditions.
2. Among the different dosages of sulphate and chloride solution in which bacterial cement mortar is cured at the age of 14 days, its shows that bacterial concrete provide maximum compressive strength of 36.98 Mpa in 5g/lit of sulphate solution and 47.06 MPa in 12.5g/lit of chloride solution.
3. Overall comparison in between Bacterial cement mortar and conventional cement mortar, conventional cement mortar is dominating in these two severe exposure environment i.e Sulphate and Chloride.

4. Ultrasonic pulse velocity test results also shows that the quality and uniformity of the cubes are also good.
5. The Behavior of the bacteria (B.subtilis) in normal environment is quite efficient as compared to severe exposure environment
6. It seems that incorpating calcium precipating bacteria in cement mortar with different dosage of severe environment solution i.e. (sulphate and chloride) is not capable of improving mortar durability as compared to normal conventional cement mortar as it makes the mortar denser and hence less permeable microstructure that leads to improvement of durability features of concrete

REFERENCES

- A. Rahman, M. Sam, N.F. Ariffin, M. Warid, H.S. Lee, M.A. Ismail, M. Samadi,*Performance of epoxy resin as self-healing agent*, JurnalTeknologi 16 (2015)9–13.

- A. Talaiekhozan, A. Keyvanfar, A. Shafaghat, R. Andalib, M.Z.A. Majid, M.A.Fulazzaky, *A review of self-healing concrete research development*, J. Environ.Treat. Tech. 2 (1) (2014) 1–11.

- C.X. Qian, M. Luo, L.F. Ren, R.X. Wang, R.Y. Li, Q.F. Pan, H.C. Chen, *Self-healingand repairing concrete cracks based on bio-mineralization*, Key Eng. Mater.629–630 (October) (2014) 494–503.

- Dry, C.M., *"Three designs for the internal release of sealants, adhesives, and waterproofing chemicals into concrete to reduce permeability"*, Cement and Concrete Research 30(12) (2000) pp. 1969-1977.

- E. Schlangen, S. Sangadji, *Addressing infrastructure durability andsustainability by self-healing mechanisms – recent advances in self healingconcrete and asphalt*, Procedia Eng.54 (2013) 39–57.

- Granger, S., Loukili, A., Pijaudier-Cabot, G. and Behloul, M., "Self-healing of cracks in concrete: from a model material to usual concretes", Proceedings of the 2nd international symposium on advances in concrete through science and *engineering*. Quebec city, Canada, 11-13 September 2006.

- H. Afifudin, M.S. Hamidah, H. Noor Hana, K. Kartini, *Microorganismprecipitation in enhancing concrete properties*, Appl. Mech. Mater. 99–100(2011) 1157–1165.

- H.M. Jonkers, A. Thijssen, G. Muyzer, O. Copuroglu, E. Schlangen, *Application ofbacteria as self-healing agent for the development of sustainable concrete* Ecol. Eng. 36 (2) (2010) 230–235.

- H.M. Jonkers, E. Schlangen, *A two component bacteria-based self-healingconcrete, in: Concrete Repair, Rehabilitation & Retrofitting II*, Taylor &FrancisGroup, 2009, pp. 215–220. ISBN 978-0-415-46869-3.

- J. Wang, J. Dewanckele, V. Cnudde, S. Van Vlierberghe, W. Verstraete, N. DeBelie, *X-ray computed tomography proof of bacterial-based self-healing in concrete*, Cement Concr. Compos. 53 (2014) 289–304.
- J. Wang, K.Van. Tittelboom, B.N. De, W. Verstraete, *Use of silica gel orpolyurethane immobilized bacteria for self-healing concrete*, Constr. Build.Mater. 26 (1) (2012) 532–540.
- J.M. Irwan, N. Othman, *An overview of bioconcrete for structural repair*, Appl.Mech. Mater. 389 (2013) 36–39.
- J.Y. Wang, D. Snoeck, S. Van Vlierberghe, W. Verstraete, B.N. De, *Application ofhydrogel encapsulated carbonate precipitating bacteria for approaching arealistic self-healing in concrete*, Constr. Build. Mater. 68 (2014) 110–119.
- J.Y. Wang, H. Soens, W. Verstraete, B.N. De, *Self-healing concrete by use of micro encapsulated bacterial spores*, Cem. Concr. Res. 56 (2014) 139–152.
- J.Y. Wang, W. Verstraete, B.N. De, *Enhanced self-healing capacity incementitious materials by use of encapsulated carbonate precipitatingbacteria: from proof-of-concept to reality*, in: 8th International Symposiumon Cement & Concrete Proceeding, 20–23rd Sept. 2013. Nanjing, PR China.
- Joseph, C. *"Experimental and numerical study of fracture and self-healing of cementitious materials"*, PhD thesis, Cardiff University, (2008).
- K. Van Tittelboom, N. De Belie, *Self-healing in cementitious materials—areview*, Materials 6 (2013) 2182–2217.
- K. Van Tittelboom, N. De Belie, W. De Muynck, W. Verstraete, *Use of bacteria torepair cracks in concrete*, Cem. Concr. Res. 40 (1) (2010) 157–166.
- M.V.S. Rao, V.S. Reddy, M. Hafsa, P. Veena, P. Anusha, *Bioengineered concrete –a sustainable self-healing construction material*, Res. J. Eng. Sci. 2 (6) (2013)45–51.

- N.Z. Muhammad, A. Keyvanfar, M.Z.Abd. Majid, A. Shafaghat, J. Mirza,Waterproof performance of concrete: *a critical review on implemented approaches*, Constr. Build. Mater. 101 (2015) 80–90,.
- Neville, A.M. (1996) *Properties of concrete* (4th edition). Pearson Higher Education, Prentice Hall, New Jersey.
- Neville, A.M. (2002) *Autogenous healing - A concrete miracle?Concrete Int*24(11):76-82.
- Nishiwaki, T., Mihashi, H., Jang, B-K. and Miura, K., *"Development of self-healing system for concrete with selective heating around crack"*, Journal of Advanced Concrete Technology, Vol. 4, No. 2 (2006) 267-275.
- P. Ghosh, S. Mandal, B.D. Chattopadhyay, S. Pal, *Use of microorganism to improve the strength of cement mortar*, Cem. Concr. Res. 35 (10) (2005) 1980–1983.
- R. Andalib, M.Z.A. Majid, A. Keyvanfar, A. Talaiekhozan, M.W. Hussin, A.Shafaghat, M.A.L.I. Fulazzaky, *Durability improvement assessment in differenthigh strength bacterial structural concrete grades against different types of acids*, Sadhana 39 (6) (2014) 1509–1522.
- R. Pei, J. Liu, S. Wang, M. Yang, *Use of bacterial cell walls to improve themechanical performance of concrete*, Cement Concr. Compos. 39 (2013) 122–130.
- R. Siddique, A. Rajor, *Influence of bacterial treated cement kiln dust on theproperties of concrete*, Constr. Build. Mater. 52 (2014) 42–51.
- S. Granger, A. Loukili, *Mechanical behavior of self-healed ultra- high performance concrete : from experimental evidence to modeling*, in: 3rd International Conference on Construction Materials: Performance, Innovations and Structural Implications (ConMat’05), Vancouver, Canada, 22–24th August 2005

- S.A. Abo-El-Enein, A.H. Ali, F.N. Talkhan, H.A. Abdel-Gawwad, *Application ofmicrobialbiocementation to improve the physico-mechanical properties of cement mortar*, HBRC J. 9 (1) (2013) 36–40, http://dx.doi.org/10.1016/j.hbrcj.2012.10.004.

- S.A.L. De Koster, R.M. Mors, H.W. Nugteren, H.M. Jonkers, G.M.H. Meesters, J.R.vanOmmen, *Geopolymer coating of bacteria-containing granules for use inself-healing concrete*, Procedia Eng. 102 (2015) 475–484.

- S.S. Bang, J.K. Galinat, V. Ramakrishnan, *Calcite precipitation induced bypolyurethane-immobilized Bacillus pasteurii*, Enzyme Microb. Technol. 28 (4–5) (2001) 404–409.

- Schlangen, E. and Joseph, C., *"Self-healing processes in concrete"*, In SK Ghosh (Ed.), *Self-healing materials: fundamentals, design strategies and applications*. Weinheim, Wiley-vch. (2008) pp. 141-182

- T. Hazelwood, A.D. Jefferson, R.J. Lark, D.R. Gardner, *Numerical simulation of the long-term behavior of a self-healing concrete beam vs standard reinforced concrete*, Eng. Struct. 102 (2015) 176–188.

- Talaiekhozan, M.A. Fulazzaky, A. Keyvanfar, R. Andalib, M.Z.A. Majid, M.Ponraj, R.B.M. Zin, C.T. Lee, *Identification of gaps to conduct a study onbiological self-healing concrete*, J. Environ. Treat. Tech. 1 (2) (2013) 62–68.

- Ter Heide, N., Schlangen, E. and van Breugel, *"Experimental Study of Crack Healing of Early Age Cracks"*, In Proceedings KnudHøjgaard Conference on Advanced Cement-Based Materials, Technical University of Denmark, June 2005

- V. Achal, A. Mukherjee, M. Reddy, *Microbial concrete: way to enhance the durability of building structures*, J. Mater. Civ. Eng. 23 (2010) 730–734.

- V. Achal, A. Mukherjee, M.S. Reddy, *Effect of calcifying bacteria on permeationproperties of concrete structures*, J. Ind. Microbiol. Biotechnol. 38 (9) (2011).

- V. Achal, X. Pan, N. Özyurt, *Improved strength and durability of fly ashamendedconcrete by microbial calcite precipitation*, Ecol. Eng. 37 (4) (2011)554–559.

- V. Senthilkumar, T. Palanisamy, V.N. Vijayakumar, *Comparative studies onstrength characteristics of microbial cement mortars*, Int. J. ChemTech Res. 6(2014) 578–590. ISSN: 0974-4290.

- V. Senthilkumar, T. Palanisamy, V.N. Vijayakumar, *Fortification of compressivestrength in enterococcus microorganism incorporated microbial cementmorta*, Int. J. ChemTech Res. 6 (1) (2014) 636–644. ISSN: 0974-4290.

- V. Wiktor, H.M. Jonkers, *Field performance of bacteria-based repair system:pilot study in a parking garage*, Case Stud. Constr. Mater. 2 (2015) 11–17.

- W. De Muynck, N. De Belie, W. Verstraete, *Microbial carbonate precipitation inconstruction materials: a review*, Ecol. Eng. 36 (2) (2010) 118–136.

- W. Khaliq, M.B. Ehsan, *Crack healing in concrete using various bio influencedself-healing techniques*, Constr. Build. Mater. 102 (2016) 349–357.

- W. Tang, O. Kardani, H. Cui, *Robust evaluation of self-healing efficiency incementitious materials – a review*, Constr. Build. Mater. 81 (2015) 233–247.