STUDY ON MECHANICAL PROPERTIES OF CEMENT MORTAR BY THE ADDITION OF UREOLYTIC BACTERIA

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MAY 2015

STUDY ON MECHANICAL PROPERTIES OF CEMENT MORTAR BY THE ADDITION OF UREOLYTIC BACTERIA

A thesis

Submitted by

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(213CE2075)

In partial fulfilment of the requirements for

the award of the degree

of

MASTER OF TECHNOLOGY

In

STRUCTURAL ENGINEERING

Under the Guidance of

Prof. PRADIP SARKAR

DEPARTMENT OF CIVIL ENGINEERING
NATIONAL INSTITUTE OF TECHNOLOGY, ROURKELA 769008
May 2015

NATIONAL INSTITUTE OF TECHNOLOGY

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CERTIFICATE

This is to certify that the thesis entitled "STUDY ON MECHANICAL PROPERTIES OF CEMENT MORTAR BY THE ADDITION OF UREOLYTIC BACTERIA**"** submitted by **Aparna K Sathyan** in partial fulfilment of the requirement for the award of **Master of Technology** degree in **Civil Engineering** with specialization in **Structural Engineering** to the National Institute of Technology, Rourkela is an authentic record of research work carried out by her under my supervision. The contents of this thesis, in full or in parts, have not been submitted to any other Institute or University for the award of any degree or diploma.

Project Guide

Prof. PRADIP SARKAR

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ACKNOWLEDGEMENTS

First and foremost, praises and thanks to the God, the Almighty, for his showers of blessings throughout my work to complete the research successfully.

I would like to express my sincere gratitude to my guide **Dr. Pradip.Sarkar** for enlightening me with the first glance of research, and for his patience, motivation, enthusiasm, and immense knowledge. His guidance helped me in all the time of research and writing of this thesis. I could not have imagined having a better advisor and mentor for my project work. It was a great privilege and honour to work and study under his guidance. I am extremely grateful for what he has offered me. I am extending my heartfelt thanks to his wife, family for their acceptance and patience during the discussion I had with him on research work and thesis preparation.

Besides my advisor I extend my sincere thanks to **Dr. Robin Davis P,** and all faculties in **Structural Engineering** Department, NIT Rourkela for their timely co-operations during the project work. I extend my sincere thanks to **Dr. Surajit Das ,** and students in **Life science and Biotechnical Engineering** Department for the supports throughout my research work ,

It gives me great pleasure to acknowledge the support and help of **Shemin T John**, and **Rohini B** for their help throughout my research work.

Last but not the least; I would like to thank my **family,** for supporting me spiritually throughout my life and for their unconditional love, moral support and encouragement.

So many people have contributed to my research work, and it is with great pleasure to take the opportunity to thank them. I apologize, if I have forgotten anyone.

Aparna K Sathyan

ABSTRACT

Keywords: *bacterial concrete, compressive strength, calcite precipitation, cement mortar, microstructure*

In the present scenario where the constructions are increasing, the need to find a supplementary cementing material for the improvement of strength and which has less environmental effects is of great significance. Ureolytic bacteria are the ones which can improve the strength of cement mortar by the precipitation of calcium carbonate in the presence of urea and a calcium source. In the present study *Bacillus sphaericus* is used to check its applicability in this regard. Various tests like consistency and initial setting time are done to find out the effect of bacterial solution on cement. Tests such as compression strength and sorptivity test are used in the present study to identify the variation in the mechanical properties of cement mortar. To know the mineralogy and morphology of the calcium carbonate precipitated by the bacteria XRD and FESEM analysis are carried out.

Compressive strength (at 7-day and at 28-day) of mortar cube found to be increasing with the increase of bacteria concentration up to 10^7 cells/ml The optimum doses of bacteria found to increase the average compressive strength by 58% (at 7-day) and 23% (at 28-day) over the control specimen. The more increase in strength after 7 day curing may be due to the presence of nutrient medium and it getting depleted as it reaches 28 days and causing death of bacteria The minimum cumulative water absorption is obtained for a cell concentration of 10^9 cells/ml The mineralogy and morphology of the calcium carbonate precipitated by the bacteria test was able to confirm that the bacterially precipitated calcium carbonate is calcite and is having lamellar rhombohedra or hexagon shape

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CHAPTER 1

INTRODUCTION

1.1 BACKGROUND AND MOTIVATION

Concrete is considered as one of the most important building materials in the construction sector in the world. Improvement in concrete technology can be achieved through its strength improvement and its enhancement in durability using pollution-free and natural methods. As the construction industry is progressing, the usage of cement is also increased exponentially as we are in search of stronger and durable structures. This increases the cement productivity globally and in turn increase the carbon dioxide emission to the atmosphere. We need to find a technique which can increase the strength and durability of structures without increasing the use of cement for a better future.

Supplementary cementing materials (SCMs) are often used in concrete mixes to reduce cement contents, improve workability, increase strength and enhance durability through hydraulic or pozzolanic activity. Silica fume and fly ash are commonly incorporated in concrete as partial cement replacement. All building materials are porous. This porosity of the building material along with penetration of moisture and other harmful chemicals such as acids, chlorides and sulphates adversely affect the concrete and reduce the structures strength and life. An additive that seals the pores and cracks and thus reduces the permeability of the structure, would immensely improve its life. Conventionally, a variety of sealing agents such as latex emulsions suffer from serious limitations of incompatible interfaces, susceptibility to ultraviolet radiations, unstable molecular structure and high cost.

One of the encouraging biomimetic processes in nature is done by soil-thriving bacteria [15]. It converts sand to sandstone. Later, it was found out that, a calcite precipitating bacteria, Bacillus pasteurii, was responsible for the binding agent production for this conversion. This

mineral deposition technique can answer for the natural method for the sealing of pores and cracks of concrete and mortar. Biomineralization is defined as a biologically induced precipitation in which an organism creates a local micro-environment with conditions that allow optimal extracellular chemical precipitation of mineral phases. This can be observed in many biological species living in various natural environments such as soil, geological formations, fresh water biofilms, hot springs, saline lakes and oceans. The exact mechanism behind the microbial calcium carbonate precipitation is not found till date.

The motivation of the present work is to study the effect due to addition of Ureolytic bacteria in the microstructure, compressive strength and capillary water absorption of cement mortar.

1.2 OBJECTIVES AND SCOPES

Based on the conclusions of literature review presented in Chapter 2 the main objective of the present study is identified as to improve the engineering properties of normal strength cement mortar using a single bacterial species. This main objective is divided in to following sub-objectives:

a) To study the variation of compressive strength of cement mortar with bacteria
b) To study the setting time of cement in the presence of bacteria
c) To study the capillary water absorption (sorptivity) of cement mortar using bacteria
d) To study the effect of bacterial culture medium on the setting time of cement.

1.3 METHODOLOGY

Following step by step methodology is adopted to achieve the above mentioned objectives:

i) Literature Review (on properties of fresh and hardened concrete, Bacterial Concrete)
ii) Select an Ureolytic bacteria by trial
iii) Culture the selected bacteria

iv) Estimation of setting time of cement with bacteria

v) Prepare mortar cubes with by varying the dose of bacteria

vi) Find the compressive strength, capillary water absorption of hardened mortar

vii) Study the variation of compressive strength and arrive at an optimum dosage of bacteria

viii) Study the morphology using the images obtained from Field emission scanning electron microscope (FESEM) for the samples used for compressive strength

ix) Study the X-ray diffraction of the samples used for compressive strength to obtain the compounds

1.4 ORGANISATION OF THE THESIS

This introductory chapter presents the background, objectives, scopes and the methodology of the present study.

Chapter 2 presents the mechanism and literature review of the present study

Chapter 3 deals with the experimental works and the respective results obtained from the same

Chapter 4 gives the results and discussions of the study

Chapter 5 presents the conclusion of the present study

CHAPTER 2

LITERATURE REVIEW

2.1 GENERAL

Researches on the application of bacteria on concrete has started nearly two decades back. This Chapter briefly discuss about various types of bacteria, mechanism of calcite precipitation, previous studies on the application of bacteria.

2.2 INTRODUCTION TO BACTERIA

Bacteria are prokaryotic microorganisms. They are a few micrometres in length and have a number of shapes, ranging from spheres to rods and spirals. Bacteria are present in most of its habitats in universe. They inhabit soil, water, acidic hot springs, radioactive waste, and the deep portions of Earth's crust. We can see them living in symbiotic and parasitic relationships with plants and animals.

There are approximately 5×10^{30} bacteria on Earth, forming a biomass which exceeds that of all plants and animals. Bacteria plays vital role in recycling nutrients such as in nutrient cycles fixation of nitrogen from the atmosphere and putrefaction. In the biological communities surrounding hydrothermal vents and cold seeps, bacteria it provide the nutrients needed to sustain life by converting dissolved compounds such as hydrogen sulphide and methane to energy for biological communities surrounding hydrothermal vents and cold seeps. All the bacteria living in the earth has not been characterised. The bacteria that can be grown in the laboratories will be only half of the phyla of bacterial of bacterial species. The branch of microbiology that studies bacteria is called bacteriology.

Bacteria display a wide diversity of shapes and sizes, called *morphologies*. Typical size of Bacterial cells are 0.5–5.0 micrometres in length. Most bacterial species are either spherical, called *cocci* (*sing*. coccus, from Greek *kókkos*, grain, seed), or rod-shaped, called *bacilli* (*sing*. bacillus, from Latin *baculus*, stick). Some bacteria, called *vibrio*, are shaped like slightly

curved rods or comma-shaped; others can be spiral-shaped, called *spirilla*, or tightly coiled, called *spirochaetes*. There are some species even have tetrahedral or cuboidal shapes. More recently, bacteria were discovered deep under Earth's crust that grow as branching filamentous types with a star-shaped cross-section. The large surface area to volume ratio of this morphology may give these bacteria an advantage in nutrient-poor environments in the crust of earth

2.3 MECHANISM OF CALCIUM CARBONATE PRODUCTION

Biomineralization is defined as a biologically induced precipitation in which an organism creates a local micro-environment with conditions that allow optimal extracellular chemical precipitation of mineral phases. The precise role of the microbes in the carbonate precipitation process is still not clear. Almost all bacteria are capable of calcium carbonate precipitation [1]. Bacteria are known to hydrolyze urea by urease for the purposes of: (1) increasing the ambient pH, (2) utilizing it as a nitrogen source, and (3) using it as a source of energy [5]. Recently, in concrete the microbial precipitation of calcium carbonate for the improvement of properties has become a new area of research.

The microbial precipitation of $CaCO_3$ is determined by several factors including: the concentration of dissolved inorganic carbon, the pH, and the concentration of calcium ions and the presence of nucleation sites. The first three factors are provided by the metabolism of the bacteria while the cell wall of the bacteria will act as a nucleation site. The bacteria used in this research produce urease which catalyses the hydrolysis of urea ($CO(NH_2)^2$) into ammonium (NH_4^+) and carbonate (CO_3^{2-}). First, 1 mol of urea is hydrolysed intracellular to 1 mol of carbamate and 1mol of ammonia (Eq. 1). Carbamate spontaneously hydrolyses to form additionally 1mol of ammonia and carbonic acid (Eq. 2). These products subsequently form 1mol of bicarbonate and 2mol of ammonium and hydroxide ions (Eqs. 3 and 4). The last 2

reactions give rise to a pH increase, which in turn shifts the bicarbonate equilibrium, resulting in the formation of carbonate ions (Eq. 5)

$$CO(NH_2)_{2+}\ H_2O \rightarrow NH_2COOH + NH_3 \quad (1)$$

$$NH_2COOH + H_2O \rightarrow NH_3 + H_2CO_3 \quad (2)$$

$$H_2CO_3 \leftrightarrow HCO_3^- + H^+ \quad (3)$$

$$2\ NH_3 + 2\ H_2O \rightarrow 2NH_4^+ + 2OH^- \quad (4)$$

$$HCO_3^- + H^+ + 2NH_4^+ + 2OH^- \rightarrow CO_3^{2-} + 2NH_4^+ + 2\ H_2O \quad (5)$$

Since the cell wall of the bacteria is negatively charged, the bacteria draw cations from the environment, including Ca^{2+}, to deposit on their cell surface. The Ca^{2+}-ions subsequently react with the CO_3^{2-}-ions, leading to the precipitation of $CaCO_3$ at the cell surface that serves as a nucleation site (Eqs. 6 and 7).

$$Ca^{2+} + Cell \rightarrow Cell\text{-}Ca^{2+} \quad (6)$$

$$Cell\text{-}Ca^{2+} + CO_3^{2-} \rightarrow Cell\text{-}CaCO_3\downarrow \quad (7)$$

The schematic diagram showing mechanism of calcite precipitation is given in Fig 1.

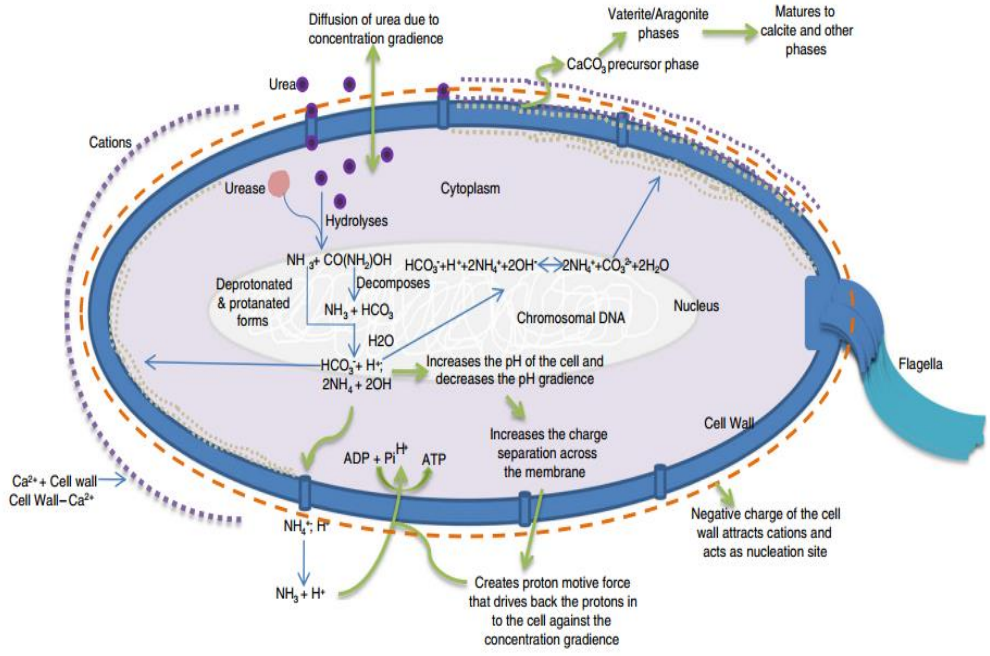

Fig 1 Mechanism of calcite precipitation by bacteria

2.4 PREVIOUS STUDIES ON APPLICATION OF BACTERIA

The application of ureolytic bacteria for calcite precipitation and related improvement in strength can be used in limestones restoration works [15] and sand and soil column consolidation. The restoration works of decayed limestones (Euville stone which has large water absorption ability) are studies by Dick et *al.* (2006) [15] by using five different strains of Bacillus sphaericus group. His studies show that even after putting the limestone cubes for curing in bacterial solution no significant growth of calcium carbonate was observed on the cube surface. Only after adding calcium source (calcium chloride) layer of calcite was observed over the surface.

The improvement of soil bearing capacity by microbial calcite precipitation and without effecting the permeability of fluids is shown by Whiffin et *al.* (2007) [16] .The property of not effecting the permeability of soil makes the microbial soil treatment unique.

Bang et *al.* (2001) [12] *Bacillus pasteurii* immobilised in polyurethane is used in the study. The polyurethane provide protection to the bacterial cells from the extremely alkaline environment of concrete, while serving as nucleation sites for calcite crystals The variation in the amount of precipitation of calcite by free cells and immobilised cells is little. The compressive strength of the mortar cubes are increased considerably after 7 days of curing in urea calcium chloride medium. Calcite in polyurethane showed little effect on the improvement of elastic modulus and tensile strength of the polymer. This can be accounted for the deterioration of chemical interaction between the calcite and the form after immersion on the medium

Bachmeier et *al*. (2002) [19] uses urease enzyme produced by *Bacillus pasteurii* immobilised in polyurethane (PU) for calcium carbonate production. Here also recombinant *E.coli* are also used for calcite production. It is suggested that use of immobilised enzyme is more

recommended than using bacterial cells. The viability of enzyme immobilised in the PU is to be studied further. SEM images show well organised calcite crystals

De Muynck et *al.* (2007) [7] the improvement of resistance of mortar specimens to degradation processes is studied here. *Bacillus sphaericus* is used here. The durability of the treated substrate has been studied by measuring the resistance to carbonation and chloride ingress. Precipitation of calcite was quantified by X-ray diffraction (XRD) analysis and morphology observed by SEM. The presence of a newly formed layer of calcite on the surface of the mortar specimens is observed. Mortar cubes were used for the accelerated carbonation tests and capillary water suction experiments. Cylindrical specimens were drilled out of mortar slabs for chloride migration and for water vapour diffusion tests..Bacterial calcite on the surface of the specimens resulted in a decrease of capillary water uptake and permeability towards gas. This bacterial treatment resulted in a limited change of the chromatic aspect of mortar and concrete surfaces.

De Muynck et *al.* (2008) [9] the use of pure cultures of *Bacillus sphaericus* and mixed cultures of ureolytic bacteria is studied here. The effect of using different kinds of nutrients on the treatment is also studied here. The capillary rise and gas permeability is decreased by the treatment. Pure culture of bacteria shows more decrease. The morphological variation is seen in the SEM images for calcite is accounted for usage of various cultures and hence the variation in the levels of urease enzyme. The capillary rise and gas permeability results for pure Bacillus sphaericus are comparable with conventional water repellents

Achal et *al*. (2009) [5] enhanced urease activity and calcite production of phenotypic mutants of *Sporosarcina pasteurii* (MTCC 1761) developed by UV irradiation is studied here. Improved calcite precipitation is studied by mixing bacterial medium with sand columns. Precipitated calcite is measured by the EDTA titration method. The morphology and chemical

constituents of the bacteria and sand consolidated column is analysed with SEM-EDX and XRD.

Achal et *al*. (a) (2011) [6] the durability of concrete or mortar is examined in the study. The effect of calcite precipitation induced by *Sporosarcina pasteurii* is analysed. An inexpensive industrial waste, corn steep liquor (CSL), from starch industry is used as nutrient source for the growth of bacteria and calcite production, and the results obtained compared with those of the standard commercial medium. Cement mortar cubes of 70.6 mm is used, as per IS 4031-1988. Water uptake, permeability, and chloride permeability tests are done with bacteria cultured in CSL medium and it is found out that the results are compactable with those bacterial cultured using commercial medium and thus indicating the economization of the bio calcification process.

Achal et *al*. (b) (2011) [11] *Bacillus sphaerius*, isolated from commercially available cement is used in the study. Compressive strength test and water absorption test are done here and improvement of compressive strength by 36% and water absorption is reduced six times by the application of the bacteria in the mortar cubes. Nutrient broth-urea medium is used as curing solution in the study

Wiktor et *al*. (2011) [3] uses the bio-chemical two-component self-healing agent consisted of a mixture of calcium lactate and bacterial spores both embedded in expanded clay particles. Spores of a bacterial isolate obtained from alkaline lake soil (Wadi Natrun, Egypt) were used which have an resemblance od 98.7% homology to *Bacillus alkalinitrilicus*. The crack healing capacity of aged concrete specimen is studied here. The variation in the oxygen consumption rates of bacterial specimen and control specimen is observed and shows that active bacteria remain viable and functional several months after concrete casting. According to EDAX and FT-IR analysis gives a mixture of calcite and aragonite, displayed primarily deformed rhombohedra and needle-like morphologies.

Chahal et *al.* (2012) [4] studies the effect of the *Sporosarcina pasteurii* on the compressive strength, water absorption and rapid chloride permeability of concrete made with silica fume (cement was replaced with 5% and 10% of silica fume by weight).the bacterial concentrations used are 10^3, 10^5, 10^7 cells/ml. Compressive strength (BIS: 516-1959), Water absorption and porosity (ASTM 642), Rapid chloride permeability test (ASTM C 1202) are the tests done in the study and the results shows that there is significant increase in the compressive strength with 10% silica and the optimum dosage of 10^5 cell/ml of bacteria. It is said that with the increase of bacterial concentration the matrix integrity is disrupted and hence the strength is decreased. XRD and EDAX data are given to support the observations.

Majumdar et *al.* (2012) [8] bacterial protein produce by bacteria closely related with the *Thermoanaerobactor fermicutes* is used in the study. This is directly mixed with the mortar and cubes of size 70.6x70.6x70.6 mm are casted .crack repair test, sulphate resistance test, and water absorption test, flexural (The dimension of the standard beam was 200 mm × 50 mm × 50 mm. The beams were cured for 28 days under water and their flexural strength were determined in 4-point condition.) tests are also conducted in addition to the compression tests. Maximum increase in compressive strength was observed in bacterial concentration of 10^5 cells/ml and increment was 42.4% after 120 days of water curing. The maximum flexural strength increment was 33% with 3 μg/g bioremediase protein incorporated samples.

Annamalai et *al.* (2012) [18] introduces the use of Bio Caulk (Bio + Caulk = biological material + paste used as a sealant). Bacterial calcite produced by *Bacillus sphaericus* was separated and was used as sealing agent by itself and also by mixing with carbon nanotubes. This calcite produced was mixed with flyash and was used as sealing agent. The variation in compressive strength with the use of conventionally used sealing agents, Bio Caulk, and Bio

Caulk with carbon nanotubes are studied and the mortar cubes with carbon nanotubes with calcite showed maximum increase in the compressive strength of 45%

Achal et *al.* (2013) [10] the bio cementation ability of a *Bacillus sphaericus* to seal cracks is demonstrated. The reduction in porosity and chloride permeability was studied with bacterial strain. The crack healing capability is also checked and visualisation of amount of calcite deposits is done by using scanning electron microscope. Cubes of size 70.6x70.6x70.6mm are used to evaluate the improvement in compressive strength by the application of bacterial strain. Cubes are cured in nutrient broth-urea medium. 40% increase in compressive strength is observed in microbial remediated cubes and significant crack healing of 13.4mm wide ones are also seen. It results in the decrease in water and chloride ion.

Sujatha et *al.* (2014) [13] ureolytic bacteria was isolated from ant hill and was cultured for the study. Mortar cubes of 70.6x70.6x70.6mm was casted and bacteria was administered into the mortar through curing water. Here control specimen and one batch of bacterial where cured in tap water and another batch of bacterial cubes where cured in 1g urea/L. Compressive strength after 28 days of the bacterial cement mortar is also found to increase up to 18% when cured with water containing urea and up to 12% when cure with water.

Maheswaran et *al.* (2014) [20] comparison of ureolytic activity of *Bacillus cereus and Bacillus pasteurii* is done here. The bacterial mortar cubes are casted by replacing entire volume of water was replaced with phosphate buffered saline (PBS) suspended bacteria. Curing of bacterial cubes where carried out in nutrient solution. The test results shows an increase of 38% compressive strength using *Bacillus cereus* at bacterial cell concentration of 10^6 cells/ml and 29% increase in the case of *Bacillus pasteurii* over the control cement mortar specimen at bacterial concentration of 10^5 cells/ml. *Bacillus cereus* incorporated mortar cubes show significant decrease in chloride permeability. X-ray diffraction, scanning electron microscope,

thermogravimetric analysis and Fourier transform-infrared spectroscopy are used to confirm the bacterial calcite precipitation

2.5 SUMMARY

A brief introduction about various types of bacteria, the mechanism of the calcite production is presented in this Chapter. Many previous research efforts are discussed in this Chapter to apply the bacteria in the cement mortar and concrete. Many of the past studies focussed on the compressive strength of concrete. There are only few studies conducted on the application of bacteria in setting time of cement, and also in the capillary water absorption.

CHAPTER 3

EXPERIMENTAL WORK AND RESULTS

3.1 INTRODUCTION

An experimental program was planned to study the effect of bacteria on the different engineering properties of cement mortar. This experimental program has four major parts: (i) selecting the appropriate bacterial species suitable for cement mortar and culture of bacteria, (ii) casting and curing of specimens (cement mortar), (iii) evaluation of mechanical properties of hardened cement mortar and (iv) characterisation studies on hardened cement mortar. This chapter presents the details of these four phases of experimental program carried out as part of this project and the results obtained from the same.

3.2 SELECTION OF BACTERIAL SPECIES

A number of bacterial species were reported in literature to improve different properties of concrete and cement mortar. However, the present study requires a bacteria which is non-contagious, that survive in the alkaline concrete like environment and that must be capable of producing calcium carbonate through the metabolism. The single celled eukaryotes like bacteria and other microbes can live and reproduce only if they have certain range of environmental conditions. These are temperature, pH, osmotic pressure, dissolved gases and water availability. The pH of the fresh concrete is in the range of 11.5 to 13 and there will be rise in temperature because of heat of hydration. The ureolytic bacteria used in this study should be alive in these alkaline environment and also have temperature tolerance. Two different non-contagious ureolytic bacteria (*bacillus*), namely, *Bacillus cereus* and *Bacillus sphaericus* are tested in this study to check its survival in a concrete-like environment.

3.2.1 Testing of survival of *Bacillus cereus* in concrete-like environment

The following steps are involved in testing the tolerance of this bacteria in concrete-like environment.

i. 200 ml of nutrient medium (Luria Bertani broth) was prepared for the culture of bacteria.

ii. It was then transferred to 12 fresh clean test tubes and its volume was made to 10ml by adding NaOH to increase the pH in the test tubes. (pH of the medium was then found to be 8, 9, 10, 11, 12, and 12.5 in two sets of test tubes respectively).

iii. After the preparation of media, the test tubes were sealed with cotton plug and then sterilized using autoclave.

iv. After autoclaving, *Bacillus cereus* bacteria from the mother culture was scraped and added to the test tubes and mixed well.

v. It was then incubated for 24 hours at 37ºC and 50ºC.

After incubation of 24 hours at different temperature and different pH level the growth of the bacteria was tested for each of 12 test tubes through turbidity of the solution. The results of this test are listed as follows:

i) Growth of bacteria was not observed in the any of the six cultures incubated at 50ºC

ii) At 37ºC, growth was observed only in cultures with pH 8 and 9

Table 3.1 presents the result of the growth of *Bacillus cereus* in different cultures. It can be seen from the table that the bacteria could survived only in the cultures with pH 8 and 9. However, the pH of fresh concrete (or cement mortar) is in the range 11.5 to 13, which means that *Bacillus cereus* may not survive in concrete-like environment. Therefore some other species of Bacillus which have a pH tolerance of 11.5 to 13 should be used.

Table 3.1: Temperature and pH tolerance of *Bacillus Cereus*

pH	Presence of Bacteria	
	cultures incubated at 37ºC	cultures incubated at 50ºC
8	+	-
9	+	-
10	-	-
11	-	-
12	-	-
12.5	-	-
+ presence of bacteria; - Absence of bacteria		

Table 3.2: Temperature and pH tolerance of *Bacillus sphaericus*

pH	Presence of Bacteria	
	cultures incubated at 37ºC	cultures incubated at 50ºC
8	+	+
9	+	+
10	+	+
11	+	+
12	+	+
12.5	+	+
+ presence of bacteria; - Absence of bacteria		

3.2.2 Testing of survival of *Bacillus sphaericus* at in concrete-like environment

As the trial of *Bacillus cereus* failed a different species of the Bacillus group *Bacillus sphaericus* was considered next. The same procedure described in the previous section was followed to test *Bacillus sphaericus*. Growth of bacteria was observed in the cultures incubated at both 37ºC and 50 ºC for all the pH value from 8 to 12.5. Table 3.2 presents the temperature and pH tolerance of *Bacillus sphaericus.* It can be seen from the table that the bacteria could survive the pH range of 8 to 12.5 at both 37^0C and 50^0C. Therefore it can be concluded that

this Bacillus species can be suitable for fresh concrete (or cement mortar) which has pH about 11.5 to 13. The above result shows that, this species can survive the temperature in concrete (or cement mortar) arising out of the heat of hydration.

3.2.3 Test for $CaCO_3$ precipitation in agar plate state

Section 2.2 presents the mechanism of microbial precipitation of calcium carbonate ($CaCO_3$) which is responsible for strength in concrete (or cement mortar). In order to confirm that selected bacterial species is capable of producing calcium carbonate, following standard test [23] has been undertaken using Calcite Precipitation Agar (CPA). CPA is a solid medium for screening of bacterial precipitation of calcium carbonate. Steps involved to carry out this test are as follows:

i. 0.6g of Nutrient broth, 5.7g of $CaCl_2$; 0.424g of $NaHCO_3$; 2.0g of NH_4Cl; 3.0g of Agar, 190ml of distilled water was weighted and taken in a 200ml conical flask
ii. All media components were autoclaved
iii. After autoclaving urea is added to the medium.
iv. 20µl of broth culture was inoculated in the center of a plate, and then incubated at 30°C for 6 days.

Fig. 3.1 presents the precipitation of some material on the plate at points A, B, C and D. To characterise this material, the precipitation is observed in a field emission scanning electron microscope (FESEM). Fig. 3.2 presents the FESEM images of the precipitation. The rod shaped bacteria and the crystalline calcite produced by the bacteria are marked in Fig. 3.2. This proves the evidence of the formation of calcite precipitated by bacteria.

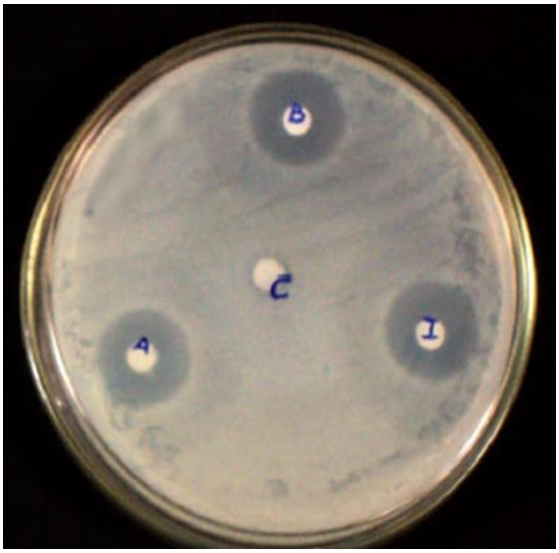

Fig. 3.1: Calcite precipitation on plate

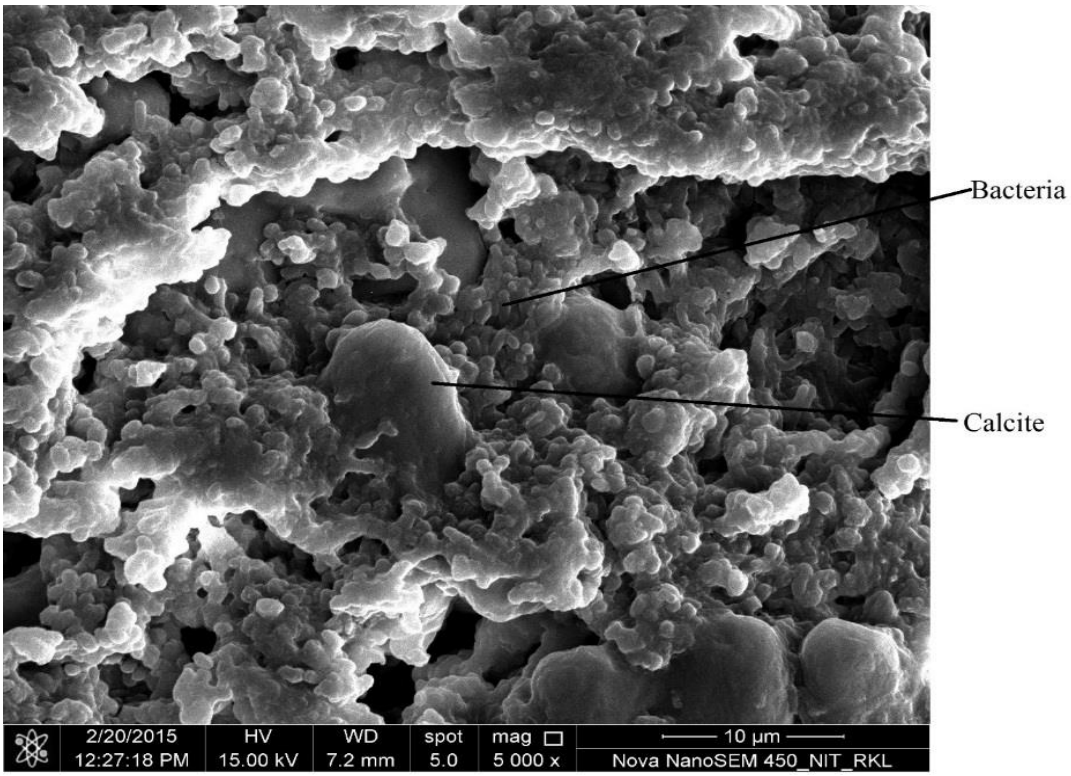

Fig 3.2 FESEM images of the calcite precipitated

3.3 CASTING AND CURING OF SPECIMENS

Basic tests of cement (such as normal consistency and setting time) were carried out before casting of the test specimens. This is done to check any possible change in the characteristics of cement paste due to addition of bacterial solution. Specimens of mortar cube of 70.6mm size were casted as per Indian standard IS 4031-1988 for compressive strength and sorptivity test.

3.3.1 Tests on Cement

The standard tests conducted on cement are given below:

- Standard tests on cement is conducted for the consistency of cement as per Indian standard IS 4031 Part 4 (1988). The consistency of the cement paste was found to be 32%. The details of the consistency test on cement performed is explained in the APPENDIX A.
- Initial and final setting time of cement paste with and without bacteria was found out as per Indian standard IS 455 (1989). The method of testing and other details of the test are summarised in APPENDIX A. The values of setting time obtained are presented in the Table 3.3. It can be seen from the table that the presence of bacteria does not have considerable effect on the setting time.

Table 3.3: Setting time of the cement paste

Specimen	Initial Setting time (minutes)	Final Setting time (hrs)
Cement paste with tap water	52	6:00
Cement paste with tap water and bacteria solution	50	6:07

3.3.2 Details of Mortar Cube Test Specimens

As one of the objective is to study the variation of compressive strength of mortar cubes with various concentration of bacteria, a cement to sand ratio of 1:6 and water cement ratio of 0.55 are considered to prepare the mortar cubes. Accordingly, the amount of cement, sand and water are calculated as shown in the Table 3.4. The table presents identifications for different mortar cubes such as control, B1, B2, B3, B4 and B5 with number specimens casted and its constituents. The specimens B1-B5 is prepared by replacing appropriate amount of water with bacterial solution to get the desired level of concentration of bacteria in water. Section 3.3.2.1 presents the procedure to prepare the cell culture whereas Section 3.3.2.2 presents the detailed procedure to prepare the mortar cubes.

Table 3.4: Details of test specimen for mechanical properties of mortar

Mortar cube ID	Bacteria concentration (cells per *ml*)	Number of specimen for			Mix proportion			Curing Soln.
		7 day comp. strength	28 day comp. strength	Capillary water absorption	Cement (kg)	Sand (kg)	Water (ml)	
Control	0	3	3	3	0.13	0.77	72ml	†
Control	0	0	3	0	0.13	0.77	72ml	Ø
B1	10^5	3	3	3	0.13	0.77	72ml*	Ø
B2	10^6	3	3	3	0.13	0.77	72ml*	Ø
B3	10^7	3	3	3	0.13	0.77	72ml*	Ø
B4	10^8	3	3	3	0.13	0.77	72ml*	Ø
B5	10^9	3	3	3	0.13	0.77	72ml*	Ø

* indicates that the volume of water includes bacteria and culture medium
† indicates tap water as curing solution
Ø indicates a mix of tap water, urea and calcium chloride as curing solution

The mortar cubes are de-moulded after 48 hours as indicated in Section 3.3.2.2 and then placed in curing solution of tap water, urea and calcium chloride. 2% of urea (in terms of volume of total water) was added in to solution to activate the urease enzyme used for the metabolism of

bacteria. Calcium chloride of 25 mM/lit was added to supply a source of calcium to the system in order bacteria can produce the desired $CaCO_3$. One set of control specimen were also cured in tap water without any admixture (urea and calcium chloride) for reference.

3.3.2.1 Preparation of cell culture for bacterial mortar cubes

i. 500ml of Luria Bertani broth was prepared in two fresh clean conical flask of 1l
ii. After autoclaving the nutrient medium bacteria was inoculated into it and incubated for 24hours

This bacteria culture replaces the water in bacterial mortar cubes

3.3.2.2 Preparation of mortar cubes of 1:6 cement to sand ratio

i. Take required amount of cement and sand and mix them dry thoroughly.
ii. Add the calculated volume of water to the dry mix of cement and sand and mix thoroughly fro not more than 4 minutes.(potable water was used for the preparation of control specimen and bacterial medium was used for the preparation of bacterial concrete cubes)
iii. Place the mortar in the mould and mount it in the holder of the vibrating machine and clamp it in position
iv. Fill the mould with required amount of mortar during vibration and the vibration should be done as per specified speed to attain the required compaction.
v. After attaining required compaction, remove the mould from the holder and keep it in a place 48 hours for setting .

At the end of 48 hrs remove the cube from the mould and immediately submerge in water for attaining the required curing

3.4 TESTING OF SPECIMEN FOR MECHANICAL PROPERTIES

Specimens were tested after 7-days and 28-days of curing for Compressive Strength and capillary water absorption and the effect of bacteria (*Bacillus sphaericus*) on these two mechanical properties are studied. The following section presents the results of these two tests.

3.4.1 Compressive Strength Test on Cement Mortar

All the mortar cubes are tested in a load controlled universal testing machine to obtain the unidirectional compressive strength obtained at 7 days and 28 days as shown in Table 3.5. The same results are also plotted in Figs. 3.3 and 3.4 for 7-day and 28-day compressive strength respectively. It can be observed from the table and the figures that as the cell concentration increases the compressive strengths at both 7 days and 28 days increases initially and then decreases. The maximum strength occurs at a cell concentration of about 10^7 cells/ml and hence this cell concentration can be treated as Optimum dosage.

Table 3.5 Effect of bacteria on compressive strength

Cell concentration (cells/ml)	Mean compressive strength at 7 days (MPa)	Percentage increase (%)	Mean compressive strength at 28 days (MPa)	Percentage increase (%)
0 (Control)	3.44	-	5.90	-
10^5	4.46	29.65	6.98	18.30
10^6	5.34	55.23	7.02	18.98
10^7	5.44	58.23	7.28	23.38
10^8	4.91	42.73	6.19	4.90
10^9	4.71	36.90	6.10	3.38

Two control specimen of same mix proportion ware cured in two different curing solution: (i) normal tap water and (ii) mix of tap water, urea and calcium chloride. The purpose of this test

was to rule out any possible effect of curing solution on the properties of specimen without bacteria. The test results indicate no significant differences in compressive strength and capillary water absorption among the two control specimens.

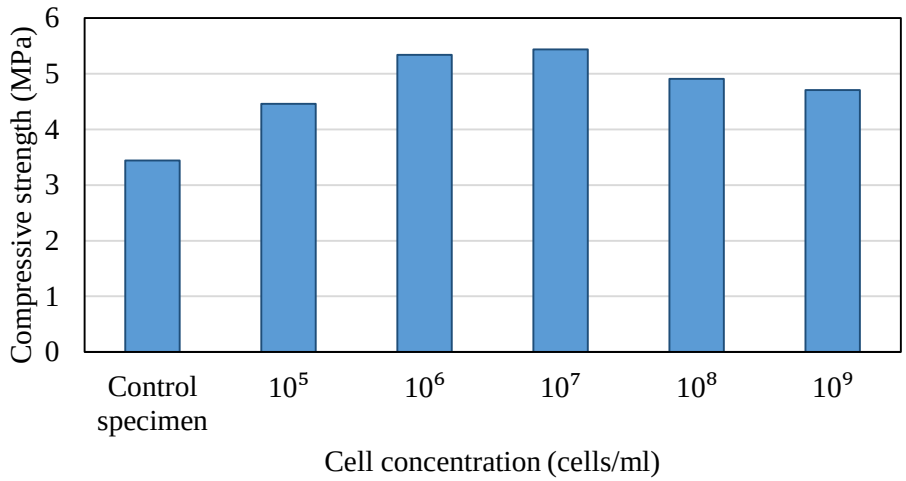

Fig 3.3 Variation of compressive strength with variation in cell concentration – at 7 day

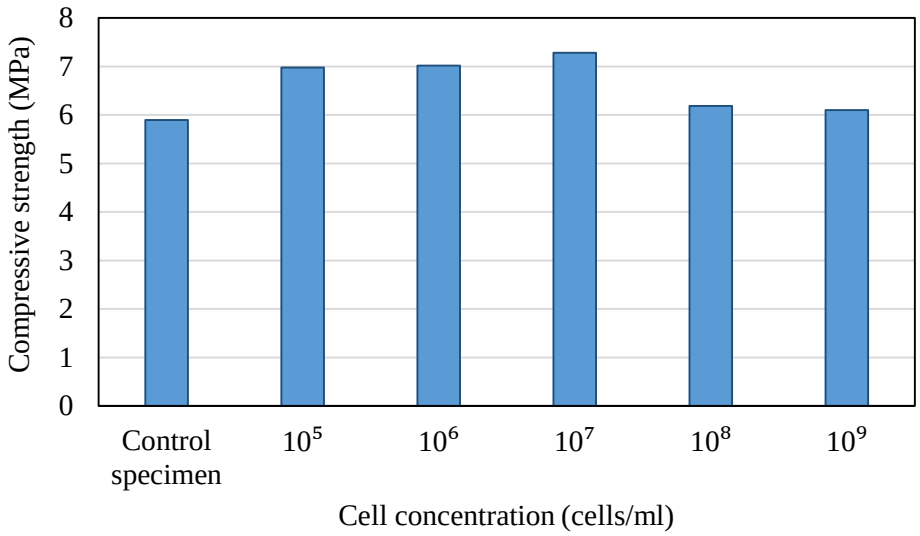

Fig 3.4 Compressive strength – cell/ml graph for 28 day curing

3.4.2 Sorptivity Test on Cement Mortar

Sorptivity (S) is a material property which characterizes the tendency of a porous material to absorb and transmit water by capillarity. The capillary water absorption is called the sorptivity, which can be considered as a measure of the durability of concrete. This of the can be determined by the measurement of the capillary rise absorption rate on reasonably homogeneous material. The cumulative water absorption (per unit area of the inflow surface) increases proportionally with the square root of elapsed time (t) as follows [22]:

$$I = S\sqrt{t} \tag{1a}$$

$$S = \frac{I}{\sqrt{t}} \tag{1a}$$

Where; S = sorptivity in mm, t = elapsed time in min.

Here the cumulative water absorption per unit area of the inflow surface can be calculated as follows:

$$I = \frac{\Delta w}{A \times d} \tag{2}$$

Where, Δw = change in weight of cube after the elapse time = $w_2 - w_1$; w_1 = oven dry weight of cylinder in grams; w_2 = weight of cubes after t time capillary suction of water in grams, A = surface area of the specimen through which water penetrated; and d = density of water

The steps involved in the test are as follows [22]

i) The specimen was cured for 7 days and then dried in oven at a temperature of 100°C for a period of 24 hours.

ii) After drying in oven the flow from the peripheral surface of the cubes is prevented by sealing it properly with non-absorbent coating (knife putty filler)

iii) Cubes are immersed in the water with water level not more than 5 mm from the bottom of the cube after the filler dries out

iv) The quantity of water absorbed in time period of 30 minutes, 1, 2, 4, 6, 12, 24, 36, and 48 hours was measured by weighting the specimen using a weighting balance with a precision of 0.1 g. Surface water on the specimen was wiped off with a dampened tissue and each weighting operation was completed within 30 seconds

Fig 3.5 cubes arranged for sorptivity test

Table 3.6 Cumulative water absorption for various concentrations of bacteria

Time of Soaking, t (hours)	Cumulative water absorption (mm)					
	Control specimen	10^5	10^6	10^7	10^8	10^9
0.5	9.56	8.22	8.89	10.96	8.22	6.82
1	9.69	8.62	9.09	11.16	8.42	7.15
2	10.03	9.09	9.56	11.43	8.82	7.55
4	10.49	9.63	9.96	11.83	9.29	7.95
6	10.7	9.96	10.06	12.23	9.76	8.29
12	10.9	10.16	10.46	12.43	10.03	8.62
24	11.43	10.56	11.1	13.04	10.56	9.22
36	11.56	10.76	11.16	13.1	10.49	9.29
48	11.77	10.96	11.3	13.24	10.63	9.29

Fig. 3.5 presents the cubes arranged for sorptivity test. The data analysed from the above test is given in the Table 3.6. The graphical representation of the same is given in Fig. 3.6. It can be observed that as the bacterial cell concentration increases the cumulative water absorption is also increasing and reaching a maximum value at 10^7 cells/ml and then goes on decreasing. The minimum cumulative water absorption is obtained for a cell concentration of 10^9 cells/ml

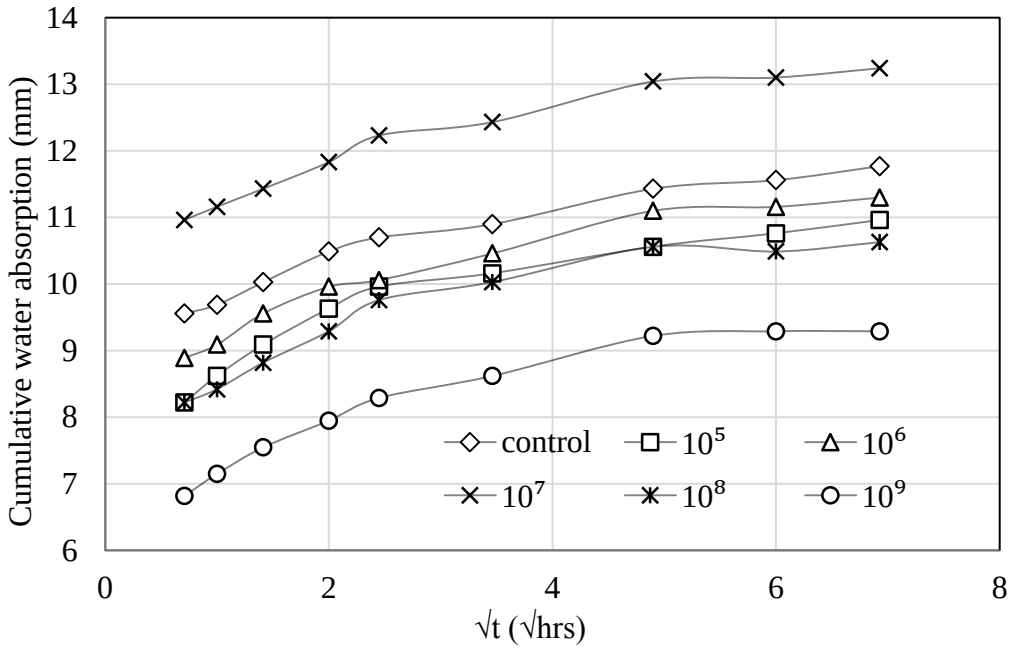

Fig 3.6: Cumulative water absorption for various cell concentrations

3.5 CHARACTERIZATION STUDIES

Various characterization techniques/ methods are used to analyse the formation of calcite by means of bio-mineralization. These techniques are specialized or involve all modes of microbial analysis like imaging, diffraction and spectroscopy, including X-rays, neutron or electron as primary radiation. Samples were collected from the tested mortar in the form of powders and/or broken pieces to conduct the above studies

3.5.1 X-Ray Diffraction Spectrometry

The crystallinity of the calcium carbonate can be found out using X-Ray diffraction spectrometry. Calcium carbonate can exist in three polymorphic forms. They are calcite, aragonite and vaterite. Calcite is the most stable and the least soluble one among the three [14] Mineralogical composition of the deposited $CaCO_3$ crystals were analysed by X-ray diffraction

3.5.1.1 X-Ray diffraction spectrometry on the layer formed over the curing water

Fig. 3.7 shows the bacterial cube specimens in curing solution. It can be observed in this figure that a layer is formed over the surface of water. When this layer was dried it gave a white colour powder. The XRD analysis of this layer was carried out and results are presented in Fig. 3.8. It can be observed that all the peaks obtained from the graph was of calcite. Thus we can conclude that the layer was calcite which was produced by bacteria. This layer was not observed on the curing solution with control specimen in absence of bacteria. This results was helpful to gain confidence on the precipitation of calcite by the metabolism of bacteria.

Fig 3.7 Bacterial cubes in curing solution

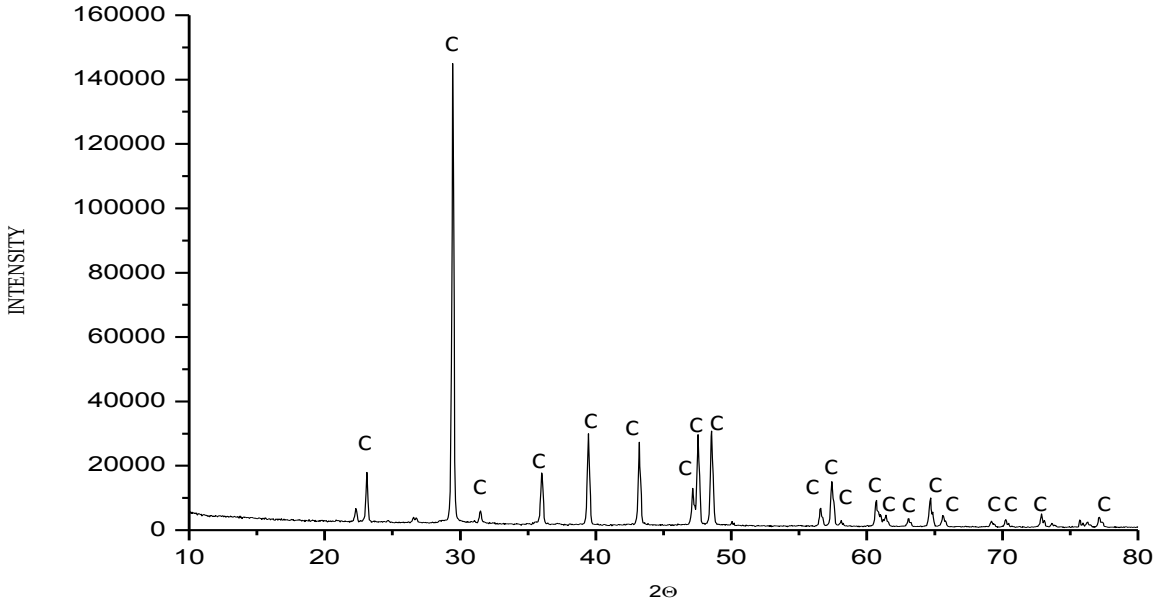

Fig 3.8: XRD for the surface layer found over water for curing (C represents calcite)

3.5.1.2 X-Ray diffraction spectrometry on mortar cubes

From the specimes used for compressive strength testing small amount of sample was collected from the core of the cube and sieved through 100µ IS sieve and was tested by XRD. The result obtained was analysed using XPert High Score software and the result was plotted using Origin Pro software. It can be observed from the Fig 3.9 that the number of calcite peaks are more in bacterial mortar cube sample and less in control sample. The increase in number of peaks signifies that the presence of calcite is more in bacterial cubes than in control cubes [4] .This responsible for the increase in compressive strength of bacterial mortar cubes

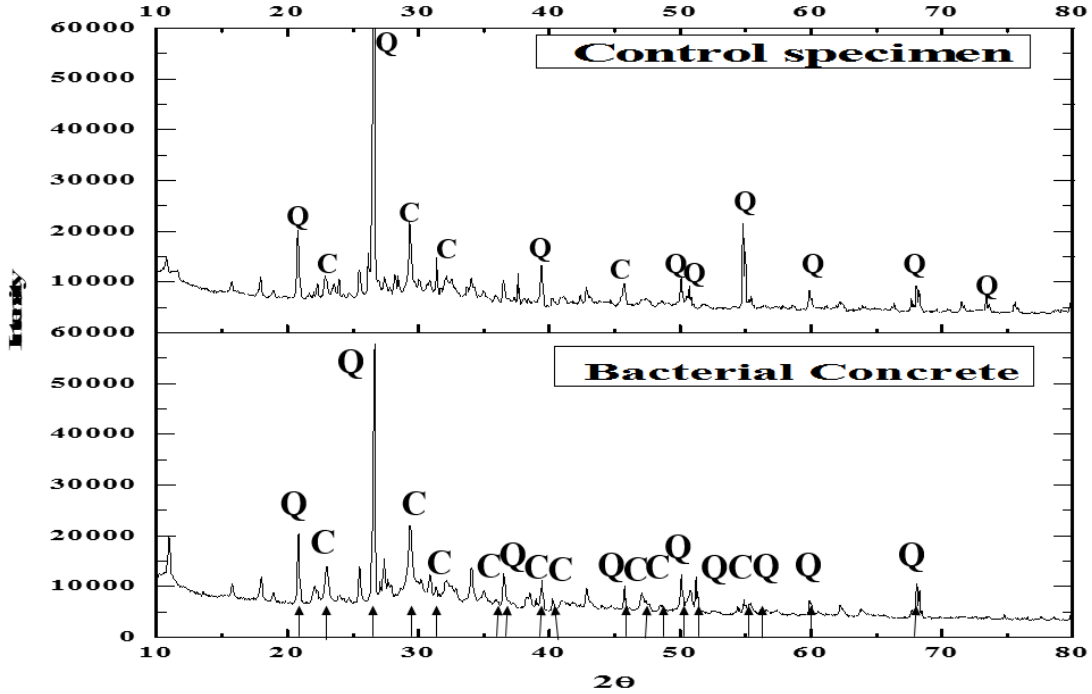

Fig 3.9 XRD for the mortar cube sample with bacteria and control cubes (‘Q’ represents quartz or silica and ‘C’ represents calcite)

3.5.2 FESEM on Mortar Cubes

The morphological, qualitative and semi-quantitative analysis of the deposited $CaCO_3$ crystals were investigated with scanning electron microscopy coupled with Energy Dispersive X-Ray. Samples were gold coated with a fine coater prior to examination

The images here are of same magnification, it can be observed from the Fig 3.10 rod shaped impression which is consistent with the shape of Bacillus sphaericus on rhombohedral hexagon crystal microstructures. Thus it can be inferred that the structure of calcite crystals precipitated by bacteria is rhombohedral hexagon. From Fig 3.11 and 3.12 which corresponds to samples collected from the core of mortar cubes used for compressive strength study after 7 day and 28 day curing, the amount of calcite crystals is more in the bacterial mortar cubes than in the control cubes. It is the presence of this crystalline calcite that lead to the improvement of compressive strength

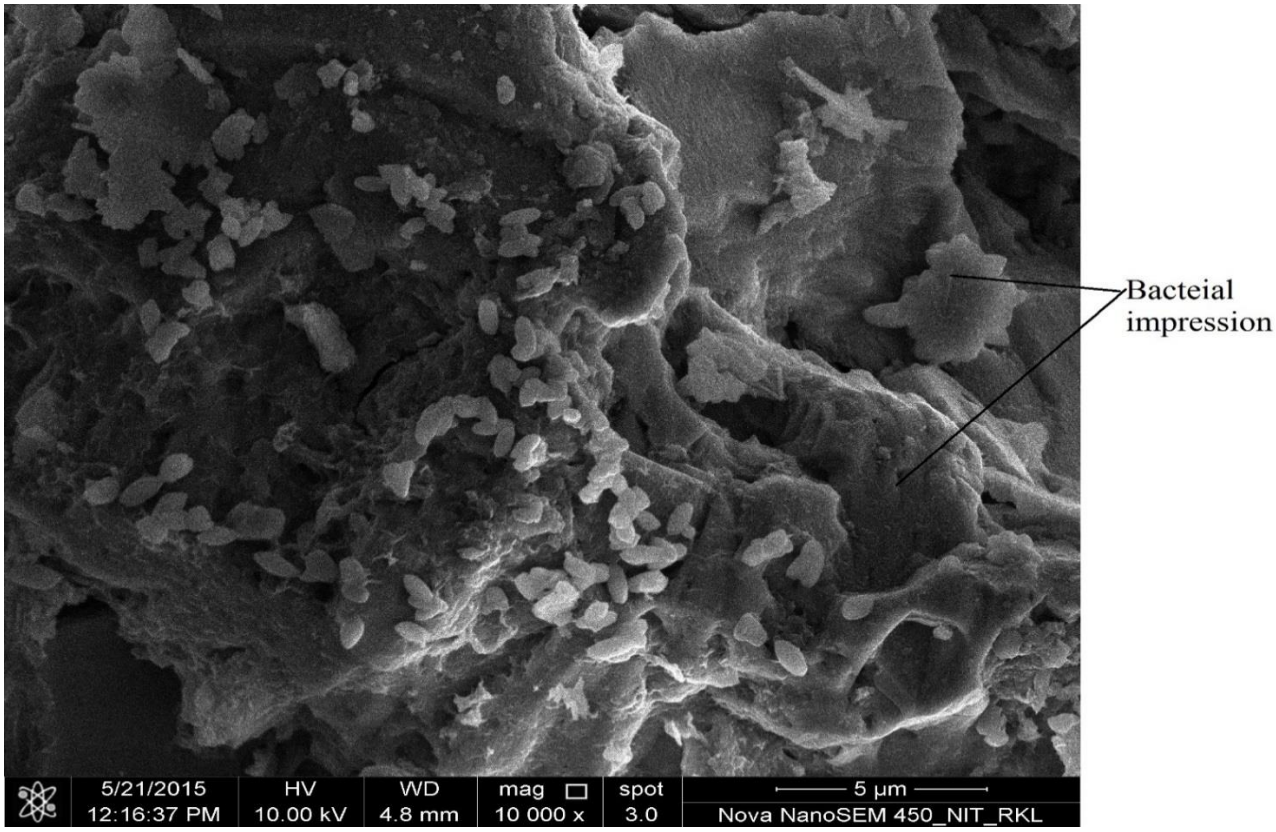

Fig 3.10 FESEM image showing bacterial impression on calcite crystals

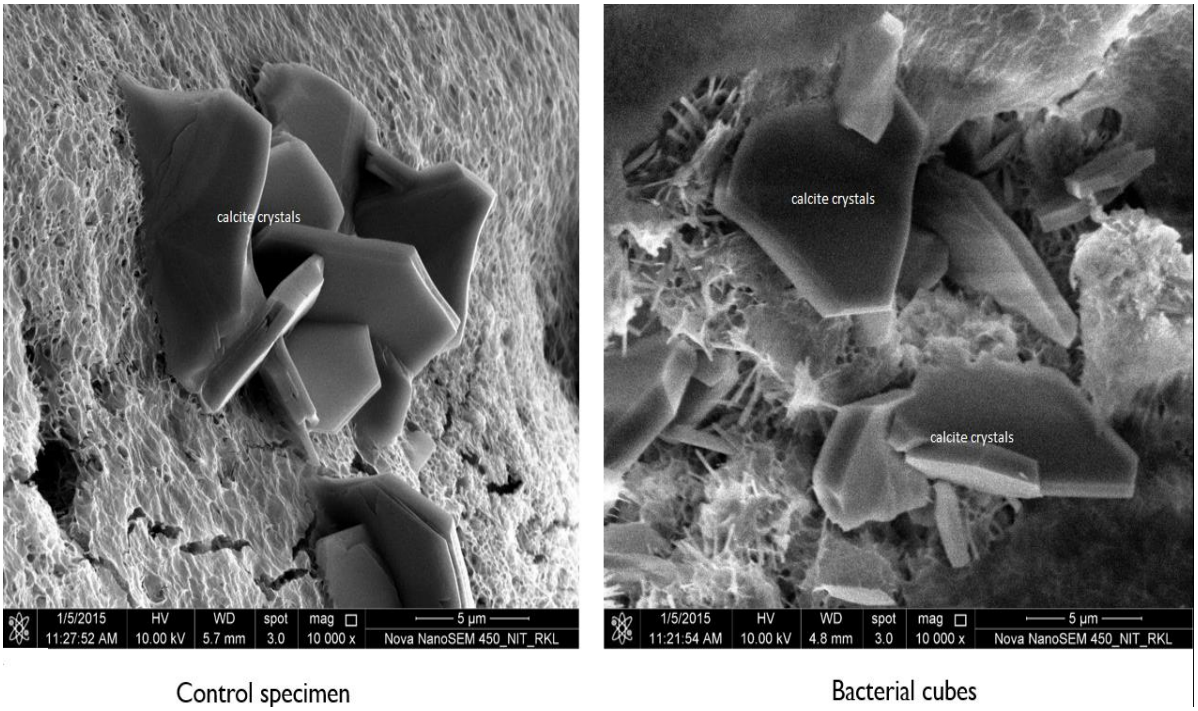

Fig 3.11: FESEM image of cubes after 7 day curing

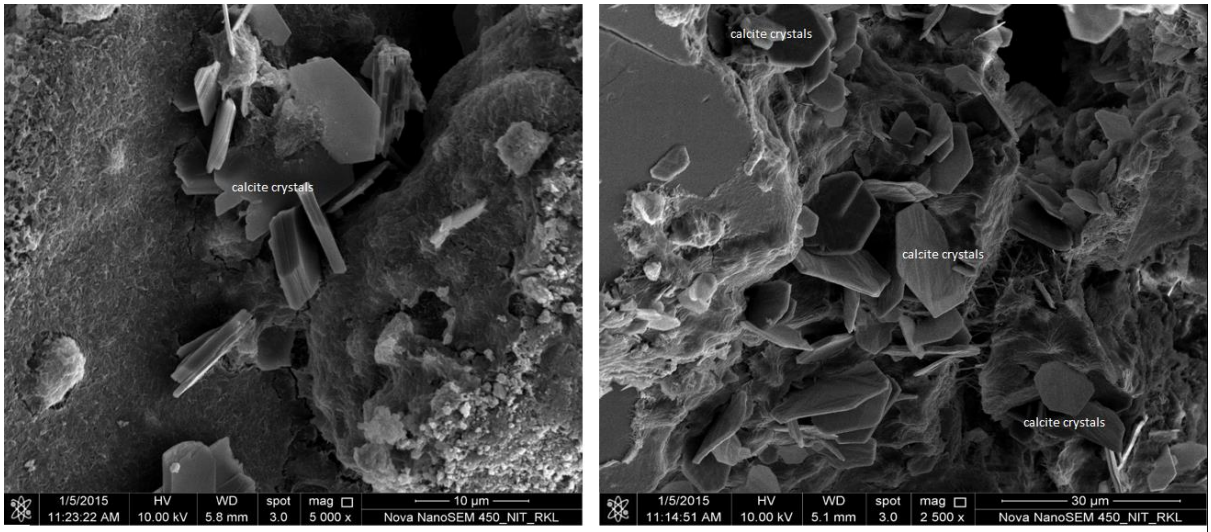

Fig 3.12: FESEM images of cubes after 28 day curing

To observe the continuous variation in the microstructure of the cement mortar due to the presence of bacterial calcite precipitation, bacterial mortar cubes where casted and was analysed by FESEM for 7, 14 and 28 days and it was observed that needle shaped structures are seen more in concentration and growing as the number of days of curing increases and are less when it reaches a curing period of 28 days. We can see that the lamellar rhombohedra structures concentration increases are as the curing period increases. Figs. 14-16 shows the FESEM image of mortar cubes after 7, 14 and 28 days curing respectively.

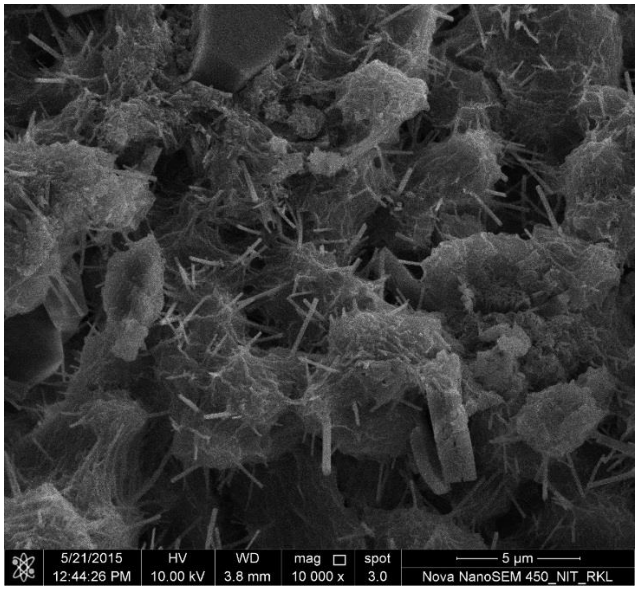

Fig 13 FESEM images of cubes after 7 day curing

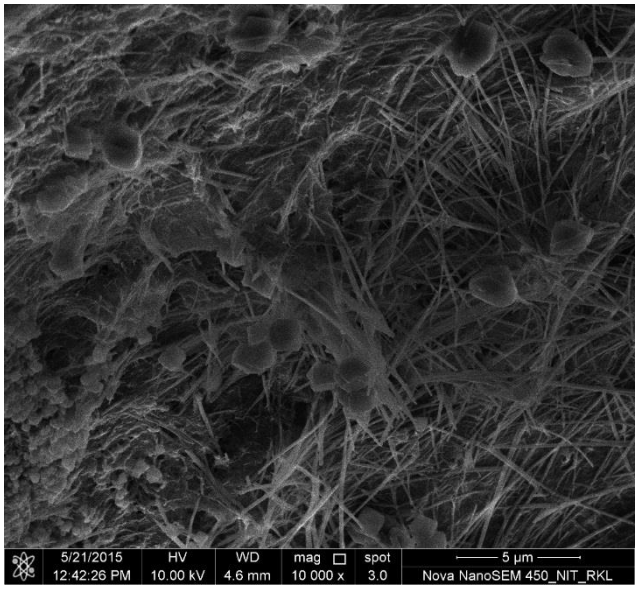

Fig 14: FESEM image of mortar cube after 14 days of curing

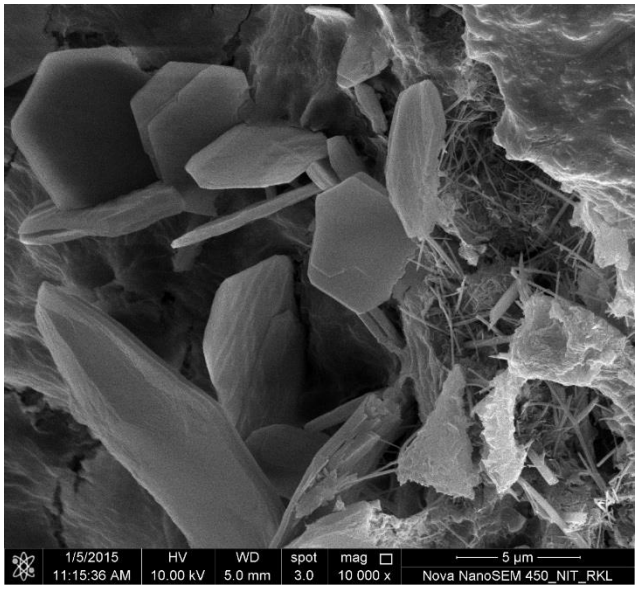

Fig 15: FESEM image of mortar cube after 28 days of curing

CHAPTER 4

SUMMARY AND CONCLUSION

4.1 SUMMARY

The objective of this study was defined to improve the engineering properties of normal strength cement mortar using a single bacterial species. There are many bacteria reported in literature which can improve the strength, durability and other mechanical properties of concrete and cement mortar. *Bacillus cereus* was first tried for this purpose. However, this species found not suitable as it has a less temperature and pH tolerance. *Bacillus sphaericus* then found to be suitable for concrete-like environment and selected for further studies. This bacterial species is also found to capable of producing calcium carbonate which is responsible for improved properties of concrete and cement mortar.

To study the variation of compressive strength and capillary water absorption of cement mortar cubes with various concentration of bacteria, a cement to sand ratio of 1:6 and water cement ratio of 0.55 are considered to prepare the mortar cubes. Accordingly, different mortar cubes with bacterial concentration of 0, 10^5, 10^6, 10^7, 10^8, and 10^9 cells/ml are prepared. The mortar cubes then cured in curing solution of tap water, urea and calcium chloride. 2% of urea (in terms of volume of total water) was added in to solution to activate the urease enzyme used for the metabolism of bacteria. Calcium chloride of 20 μg/lit was added to supply a source of calcium to the system in order bacteria can produce the desired $CaCO_3$. Hardened specimens were tested after 7-days and 28-days of curing for Compressive Strength and capillary water absorption and the effect of bacteria (*Bacillus sphaericus*) on these two mechanical properties are studied.

Various characterization techniques are used to analyse the formation of calcite due to bio-mineralization. These techniques involve all modes of microbial analysis like imaging, diffraction and spectroscopy, including X-rays. Samples were collected from the tested mortar in the form of powders and/or broken pieces to conduct the above studies.

4.2 CONCLUSIONS

The important conclusions drawn from the above study are listed as follows:

(i) To improve the properties of cement mortar or concrete the appropriate bacteria should be selected judiciously. For example, *Bacillus cereus* could not survive in the given environment whereas another Bacillus species *Bacillus sphaericus* survived.

(ii) Addition of bacteria alone cannot improve the properties of concrete/cement mortar. Ureolytic bacteria requires urea and a source of calcium to produce $CaCO_3$.

(iii) *Bacillus sphaericus* found to be not altering the normal consistency and setting time of the cement paste.

(iv) Compressive strength (at 7-day and at 28-day) of mortar cube found to be increasing with the increase of bacteria concentration up to 10^7 cells/ml. However, further increase of bacteria concentration found to reduce the compressive strength of cement mortar.

(v) The optimum doses of bacteria found to increase the average compressive strength by 58% (at 7-day) and 23% (at 28-day) over the control specimen. The more increase in strength after 7 day curing may be due to the presence of nutrient medium and it getting depleted as it reaches 28 days and causing death of bacteria [20]

(vi) In order to see if the increase in strength is due to the addition of urea and calcium chloride in curing water one set of control cubes were cured in the same solution and it was found that there was negligible (4%) variation in the strength.

(vii) The minimum cumulative water absorption is obtained for a cell concentration of 10^9 cells/ml. Optimum dose of bacterial cell concentration found to increase the cumulative water absorption over the control specimen.

(viii) The morphology of the bacterial calcite was found out by FESEM. It shows the direct involvement of bacteria in calcite production. We can see rod shaped impressions which is consistent with the dimensions of the bacteria on the calcite crystals. This is matching with the previous study [20, 12, 18].

(ix) It can be seen that the calcite crystals are lamellar rhombohedra or hexagonal in shape and needle shaped aragonite crystals of calcium carbonate which are the precursors of calcite. This shows the system allows for the continuous formation of calcite [20]. It can be clearly seen that the rod shaped ones are more in 7 day cured cubes and hexagonal ones are less and as days increases the hexagonal crystal concentration increases. From the literatures [20] it can be noted that the rod shaped structures are aragonite crystals of which are the precursors of formation of calcite crystals

(x) The XRD analysis was conducted for bacterial and control mortar cubes. The XRD result after 28 day curing shows the presence of more calcite peaks in bacterial mortar sample than the control specimen. Presence of more calcite peaks signifies the presence of more calcite in the sample [5].

(xi) A layer was observed to be formed over curing water of bacterial specimen after a few days. The XRD analysis of this layer confirmed that this layer is of calcite. This layer was not observed on the curing solution with control specimen. It can be

concluded from this information that calcite was produced by bacteria which is responsible for the improved compressive strength of mortar cube.

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APPENDIX - A

A.1 TEST FOR STANDARD CONSISTENCY OF CEMENT

Standard consistency test of the cement as per IS: 4031 Part 4 [24]

i. Take 300 g of cement and place it in the tray.

ii. Mix about 25% water by weight of dry cement thoroughly to get a cement paste. Total time taken to obtain thoroughly mixed water cement paste i.e. “Gauging time” should not be more than 3 to 5 minutes.

iii. Fill the Vicat mould, resting upon a glass plate, with this cement paste.

iv. After filling the mould completely, smoothen the surface of the paste, making it level with top of the mould.

v. Place the whole assembly (i.e. mould + cement paste + glass plate) under the rod bearing plunger.

vi. Lower the plunger gently so as to touch the surface of the test block and quickly release the plunger allowing it to sink into the paste.

vii. Measure the depth of penetration and record it.

viii. Prepare trial pastes with varying percentages of water content and follow the steps (ii to vii) as described above, until the depth of penetration is 5 to 7 mm from the bottom.

Calculate percentage of water (P) by weight of dry cement required to prepare cement paste of standard consistency by following formula, and express it to the first place of decimal.

$$P = \frac{W}{C} \times 100$$

Where,

W = Quantity of water added

C = Quantity of cement used

A.2 TEST FOR INITIAL SETTING TIME AND FINAL SETTING TIME

Test for initial setting time and final setting time as per Indian Standard IS 4031 (Part 5):1988 [25] and IS 455: 1989 [26]. Initial setting time is that time period between the time water is added to cement and time at which 1 mm square section needle fails to penetrate the cement paste, placed in the Vicat's mould 5 mm to 7 mm from the bottom of the mould.

Final setting time is that time period between the time water is added to cement and the time at which 1 mm needle makes an impression on the paste in the mould but 5 mm attachment does not make any impression.

A.2.1 Test Bock Preparation

i. Take 400 g of cement and prepare a neat cement paste with 0.85P of bacterial solution by weight of cement. (where P is the consistency)

ii. Gauge time is kept between 3 to 5 minutes. Start the stop watch at the instant when the water is added to the cement. Record this time (t_1).

iii. Fill the Vicat mould, resting on a glass plate, with the cement paste gauged as above. Fill the mould completely and smooth off the surface of the paste making it level with the top of the mould. The cement block thus prepared is called test block.

A.2.2 Initial Setting Time

i. Place the test block confined in the mould and resting on the non-porous plate, under the rod bearing the needle.

ii. Lower the needle gently until it comes in contact with the surface of test block and quick release, allowing it to penetrate into the test block.

iii. In the beginning the needle completely pierces the test block. Repeat this procedure i.e. quickly releasing the needle after every 2 minutes till the needle fails to pierce the block for about 5 mm measured from the bottom of the mould. Note this time (t_2).

A.2.3 Final Setting Time

i. For determining the final setting time, replace the needle of the Vicat's apparatus by the needle with an annular attachment.

ii. The cement is considered finally set when upon applying the final setting needle gently to the surface of the test block; the needle makes an impression thereon, while the attachment fails to do so. Record this time (t_3).

Calculation

Initial setting time = t_2-t_1

Final setting time = t_3-t_1

Where,

t_1=Time at which water is first added to cement

t_2=Time when needle fails to penetrate 5 mm to 7 mm from bottom of the mould

t_3=Time when the needle makes an impression but the attachment fails to do so.

A.3 CALCULATION FOR RAW MATERIALS MORTAR CUBES

Mortar cubes of 1:6 cement to sand ratio is prepared.

Standard consistency of cement = 32%

Density of mortar cube = 2162 kg/m^3

Water cement ratio =0.55

Quantity for cubes

Volume of 6 cubes = 2.1108 x10^{-4} m^3

Weight of 6 cubes = volume x density

$$= 2.1108 \times 10^{-4}\ m^3 \times 2162 = 4.647kg$$

Weight of sand required for 6 cubes = $\frac{6}{7}$ x 4.5635= 4.647 kg

Weight of cement required for 6 cubes = $\frac{1}{7}$ x 4.5635 = 0.7824 kg

Weight of water required for 6 cubes = 0.6x.653 = 0.430 kg