# "IMPROVEMENT IN PROPERTIES OF BULK AND SURFACE CONCRETE BY USING BACTERIA"

A

Thesis

Submitted in fulfilment of the requirement

For the award of the degree of

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**IN**

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**STRUCTURE**

By

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# CERTIFICATE

Certified that the thesis **"improvement in properties of bulk and surface concrete by using bacteria"** which is submitted by **Ms. Ekta Tripathi**, in fulfilment of the requirement for the award of the degree of Master of Technology in the **Department of Civil Engineering (CED),** Thapar University, Patiala, is a record of the candidate's own independent and original research work carried out by her under our supervision and guidance. The matter embodied in this thesis has not been submitted in part or full to any other University or Institute for the award of any degree.

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# ABSTRACT

There are number of techniques that can be used to increase strength and durability of a concrete structure. Microbial treatment is latest and found out to be effective among all the techniques.Mcirobial concrete is a revolution metabolic by product of microbially induced calcite precipitation with the help of urease, which is a hydrolyzing enzyme, gives a hopeful research in biotechnology and civil engineering for the enrichment of strength and durability of a building material and reinforced concrete structures. The main focus of this present research work is to set up an efficient, cost-effective and ecological process, which could improve strength and durability of concrete structures. At the same time the bacteria should not have any detrimental effect on the basic structure of concrete.

Bacteria used for the biological process was extracted from fully alkaline atmosphere that is by using calcium hydroxide which is the main source of cement so that the grown bacteria can easily survive in alkaline environment. In this research Bacillus bacteria which are a strain of CT-5 were inoculated from cement source. The inoculated bacterium was used with nutrient broth, urea and calcium source to make bacterial culture. The culture so obtained was mixed with basic ingredients of concrete to obtain microbial concrete. Bacteria is added to concrete at different stages of its formation, in order to check its efficacy when used in making of fresh concrete or as repair strategy of old concrete. The addition of bacteria is found out to very effective in increasing the strength and durability of concrete.

The compressive strength of the microbial concrete was monitor at 3, 7 and 28 days. The powdered sample was further obtained from the crushed specimen that was used to perform pH, EDTA, SEM-XRD for each case. About 60% of increase in compressive strength was observed by microbial treatment. Different tests have also confirms presence of calcium carbonate precipitation. Further the following approach has also found out to be efficient in reducing water permeability in concrete structure.

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| SEM | Scanning Electrom Microsope |
| XRD | x-ray diffraction |
| MICP | Microbially induced calcite precipitation |
| MIP | Mercury intrusion porosimeter |

# CHAPTER 1

# INTRODUCTION

## 1.1 OVERVIEW-

Portland cement concrete has clearly emerged as the material of choice for the material of choice for the construction in the world today. This is mainly due to low cost of materials and construction for concrete structure as well as low cost of maintenance. Therefore, much advancement of concrete technology have occurred depending on the speed of construction, the strength of concrete, the durability of concrete and the environmental friendliness of industrial material like, fly ash, blast furnace slag, silica fume, metakolin etc.

Earlier strength was the main criteria considered during construction. In recent years, it has been focussed out that not only structural safety but also durability is significant when designing building or concrete structures. However, in traditional structural design the deprivation of structures over long periods of time is not regarded as serious problem; the demolition of structures has a much greater impact on people or society. Taking these social factors in to consideration, it is obvious that we should not consider the problem of durability of building materials as a problem of the past. It is essential to develop some new eco-friendly self remediating techniques to support already designed and future constructing buildings to meet various demands to enhance their durability. Many techniques have been adopted for the improvement of strength and durability of a concrete. Microbial treatment on concrete is one of them. Microbial mineral precipitation resulting from metabolic activities of some specific microorganism in concrete to improve the overall performance of concrete has become a significant area of research.

## 1.2 WHAT IS MICROBIAL CONCRETE

The microbial concrete can be prepared by adding, curing and spraying spore forming bacteria in the concrete that are able to continuously precipitate calcite, this process of formation of calcite precipitation is called microbially induced calcite precipitation.

Carbonate precipitation by bacteria has been performed using ureolytic bacteria is considered. These bacteria are able to influence the precipitation of calcium carbonate by production of urease enzyme. This enzyme catalyzes the hydrolysis of urea to $CO_2$ and ammonia, resulting in an increase of pH and carbonate concentration in the bacterial environment. Precipitation of calcium carbonate crystals occurs by heterogeneous nucleation on the bacterial cell wall once super saturation is achieved. Microbially induced carbonate precipitation has been investigated by several researchers for the deposition of a protective surface layer, with consolidating and waterproofing properties, on the surface of ornamental stone,i.e.biodeposition.In the patented 'Calcite Bio concept' process, a layer of selected bacteria is sprayed on the surface together with specific nutrients and calcium ions (Adolphe et al.,1990).

### 1.3 MAIN INGREDIENT OF MICROBIAL CONCRETE-

The main components of microbial concrete are cement, sand, aggregate and addition of bacterial culture in any form. Addition of bacteria in any form in concrete gives microbial concrete.

### 1.4 APPLICATION IN CIVIL ENGINEERING-

Undesirable effects of bio film formation resulting in bio deterioration in civil works have gained attention and have been extensively studied, whereas this thesis reports the useful role of urease-producing microorganism to induce bio calcification in concrete cubes.

The main research on bio calcification is related to restoration of limestone on historic buildings. However, its potential application in building and construction is much wider, including soil stabilization, mortar and concrete surface protection and concrete repair. Various microorganisms have been tested, but as different research groups use different conditions and culture media it is difficult to compare the results. In this thesis an endospore-forming soil microorganism, *Bacillus pasteurii*, has been used as urease producer.

MCP has been used for crack repair in concrete (Band et al.,2001;Ramachandran et al.,2001;Bachmeier et al.,2002;Dejong et al.,2006),sand consolidation(Ferris and \Stehmeier,1992;Gollapudi et al.,1995;Stocks-Fischer et al.,1999;Nemati and Voordouw,2003),repair of calcareous monuments (Le Metayer-Levrel et

al.,1999;Tiano et al.,1999,2006;Rodriguez-Navarro et al.,2003;De Belie et al.,2006;Dick et al.,2006;Jimenez-Lopez et al.,2008),concrete compressive strength improvement(Band et al.,2001;Ramachandran et al.,2001;Ghosh et al.,2005;Jonkers et al.,2010),concrete durability improvement (De Muynck et al.,2007),selective plugging for enhanced oil recovery (Gollapudi et al.,1995),wastewater treatment(Hammes et al.,2003),and soil improvement (Whiffin et al.,2007;Ivanov and chu,2008;Dejong et al.,2010).

## 1.5 OBJECTIVE OF THESIS-

Objective of the thesis is to study the effect of addition of bacteria in different form in concrete. Concrete can be mixed and cured in bacteria. Five different mixes were used, Water mixed and water treated, Bacterial culture mixed and water treated or cured, Water mixed and bacterial culture treated or cured, Bacterial mixed and bacterial treated or cured and Bacterial mixed and bacterial sprayed respectively. The effect was studied in terms of determining compressive strength, pH, and calcium carbonate precipitation etc.The bacterial culture used was *Bacillus* which was inoculated from $CT_5$ strain. `

## 1.6 FLOW OF THESIS-

**Chapter 1** gives an introduction of the thesis, in which microbial treatment and its application in civil engineering is explained. **Chapter 2** gives the details of bio calcification, in which process of bio calcification, process of formation of calcium carbonate, nature of bacteria and improvement in durability of concrete through MCIP is explained. **Chapter 3** gives relevant literature review, in which for all parameters results obtained by different researchers are explained. **Chapter 4** explains materials and methods used during entire work. **Chapter 5** gives all the calculated result and discussion.

## 1.7 CONCLUDING REMARK-

Microbial treatment of concrete is a revolutionary research in the field of biotechnology connecting civil engineering. It gives positive aspect in terms of

Strength and durability to a concrete structure.MCIP is the result metabolic activities of various micro-organisms which improve overall behaviour of concrete by forming a calcite layer on the concrete surface.

# CHAPTER 2

# BIO CALCIFICATION

## 2.1 INTRODUCTION-

In nature, bio mineralization is the process by which living organism precipitate inorganic minerals in the form of skeletons, teeth etc. Two different process of mineral formation can be distinguished .The first occur in numerous animals as an "organic matrix mediated process". The second, exemplified by some bacterial species and algae, is characterizes by bulk extracellular and intracellular formation.

## 2.2 CALCIUM CARBONATE-

Calcium carbonate is one of the most common and widespread mineral on Earth, constituting 4% by weight of Earth's crust. It is naturally found extensive sedimentary rock masses, as limestone, marble and calcareous sandstones in marine ,freshwater and terrestrial environments (Klein and Hurlbut,1999;Hammes and Verstraete,2002).Bacterial contribution to these extensive formations had been suspected for some times but remained controversial until recent investigations involving the microbial pathways and the required precipitation conditions, indicated that bacteria have the potential to far exceed the abiotic contribution to calcium carbonate deposition in most environments on Earth (Castanier et al., 2000). In addition to this, the huge limestone formations on the sea bottom resulted from great thicknesses of calcareous material from pelargic skeletal organisms (such as cocco lithophores and foraminifera) (Klein and Hurlbut, 1999; Morse, 2003). It has been shown that the cellular organelles that are largely responsible for the production of carbonate shells in eukaryotic organisms are the mitochondria (or chloroplasts). These organelles are widely considered as primitive endosymbiotic bacteria, which further supports the bacterial contribution to carbonate precipitation (Castanier et al., 2000).

These facultative bacteria are able to precipitate calcite through the enzymatic hydrolysis of urea. The microbial urease enzyme hydrolyzes urea to produce dissolved ammonia, dissolved inorganic carbon and $CO_2$, and the ammonia released in the surroundings subsequently increases pH, leading to accumulation of insoluble

$CaCO_3$ in a calcium rich environment .Quantitatively, 1 mol of urea is hydrolyzed intracellular to 2 mol of ammonia *(Esq. (2.1) and (2.2)).*

$$CO(NH_2)_2 + 2H_2O \text{ — } 2NH4^{+} + CO_3^{2-} \quad (2.1)$$

$$CO(NH_2)_2 + 2H_2O + Ca^{2+} \text{ — } 2NH_4^{+} + CaCO_3 \quad (2.2)$$

This reaction occurs under the influence of natural environmental factors that control the activity of the urease enzyme. Factors such as the type of bacteria, bacteria cell concentration, ionic strength and pH of the media may have a significant impact on MCP.

## 2.3 UREASE AND MICROBIOLOGICALLY INDUCED CALCITE PRECIPITATION-

Microbiologically induced calcite precipitation generally results from a series of complex biochemical reactions involving urease. The commercial demand for urease is not high and currently, urease is only available in industrial quantities from Roche for use in the diagnostic and high technology specialist ceramics fields (Gauckler and Baader, 1999; Roche, 2001). It is thus expensive and is of a higher purity than is required for bio cementation. In general, four modes of regulation exist for the synthesis of urease in microbial systems (Mobley and Hausinger, 1989; Mobley et al., 1995).

- Constitutive, where a constant enzyme activity is expressed per cell, independent of external conditions.
- Inducible, where a background level of enzyme activity is expressed per cell which can be induced by the presence of an inducer molecule (e.g. urea) or other environmental condition.
- Repressible, by the presence of ammonia or ammonia precursors including urea. This synthesis is de-repressed (i.e. enzyme activity increases) under nitrogen limiting conditions.
- Developmental, where an organism in different developmental stages has variable expression of urease (Falkinham III and Hoffman, 1984).

Urea is the chief nitrogenous waste produced by vertebrates and is a major nitrogen resource in aquatic and soil ecosystems. In response to the widespread availability of urea in the environment and the universal requirement for nitrogen, a diverse section of the biota has evolved with the ability to hydrolyse urea, through the action of urease. Urease occurs in many bacteria, several species of yeast and a number of higher plants including jack beans (Canvalia ensiformis) (Dixon et al., 1980), soybean leaf and seed (Glycine max) (Kerr et al., 1983), pigweed (Chenopodium album) (El-Shora, 2001) and mulberry leaf (Morus Alba) (Hirayama et al., 2000).

An ideal microbial source of urease for bio cementation must be tolerant to high concentrations of urea and calcium. The organism should also have a high level of urease activity that is either constitutively produced (i.e. a constant amount of enzyme is expressed per cell) or can be reliably induced.

## 2.4 NATURE OF BACTERIA-

The bacteria should posses high ureolytic efficiency, alkalophilic (optimum growth rate occurs at pH around 9, and no growth at all around pH 6.5), non-pathogenic, and posses the ability to deposit calcite homogeneously on the substratum. The bacteria should have high negative zeta-potential (Dick et al.,2006;De Muynck et al.,2007) to promote adhesion and surface colonization, and produce enormous amount of urease enzyme in the presence of high concentrations of ammonium (Kaltwasser et al.,1972;Friedrich and Magasanik,1977) to enhance both the rate of ureolysis and MCP (Nemati and Voordouw,2003).

Urease–catalyzed ureolysis like any other enzymatic reaction is temperature dependent. However, the optimum temperature ranges from $20^0$C to $37^0$C depending on environmental conditions and concentrations of other reactants in the system. Ferris et al.(2003), Memati and Voordouw (2003) ,and Mitchell and Ferris(2005) reported that increasing the temperature from $15^0$C to $20^0$C increased rate of ureolysis,$k_{urea}$ 5 times and 10 times greater than $k_{urea}$ at $10^0$C.It can therefore be emphasized that increasing temperature within the optimum range enhances rate of ureolysis.

## 2.5- PROCESS OF FORMATION OF CALCIUM CARBONATE-

The precipitation of calcium carbonate relates to four key factors:

- The concentration of dissolved inorganic carbon
- The pH
- The concentration of calcium ions
- The presence of nucleation sites and crystal nucleation development.

One of the most acceptable hypotheses for calcium carbonate precipitation is that calcium ions are not used by microbial metabolism, and hence accumulate in the extracellular medium. Calcium carbonate can be produced by two different pathways: passive or active.

**2.5.1 The nitrogen cycle-** Involves ammonification of amino-acids, degradation of urea and uric and dissimilatory reduction of nitrates, contributes to passive calcium carbonate formation.Ureolytic bacteria can hydrolyze urea producing ammonia and $CO_2$.The high pH around the cells in the presence of available $CO_2$ and calcium ions allows calcium carbonate precipitation in the presence of ammonia *(Eqs 2.3).*

$$CO(NH_2)_2 + 2H_2O \quad — \quad CO_3^{2-} + 2NH_4^{+} \qquad (2.3)$$

**2.5.2 The sulphur cycle-** Also contributes to passive calcium carbonate precipitation by dissimilatory reduction of sulphates.

**2.5.3 In active-** Calcium carbonate production the mechanism is not clear but is probably initiated by ion exchange through the cell membrane, by activation of calcium and magnesium ion pumps or channels, probably couple.

## 2.6 IMPROVEMENT IN DURABILITY OF CONCRETE THROUGH MCIP-

It's safe to say that without microbes, biotechnology would be an extremely limited science. They not only provide the foundation for much of the basic research involved in biotechnology, they help to create many of the processes which are integral to this science. Ubiquitous in nature, the world of microorganisms fascinates everyone. Man

has made many inventions in every century to harvest the potential of these tiny inhabitants of nature to his best advantage. Starting from medical science to agricultural science, man has come a long way in developing protective strategies to enhance the durability of building materials in every way possible. Microbiologically Induced Calcite Precipitation (MICP) has gained interest in the last 20 years, particularly with regard to the potential role marine systems may play as 'carbon sinks' for the increasing global production of $CO_2$. Three main groups of organisms exist that can induce MICP through their metabolic processes:

- Photosynthetic organisms such as cyanobacteria and algae that remove $CO_2$,
- Sulphate reducing bacteria that are responsible for the dissimilatory reduction sulphate and
- Several organisms that are involved in the nitrogen cycle (Castanier et al., 1999; Hammes and Verstraete, 2002).

The most common form of MICP in aquatic environments is caused by photosynthetic organisms (McConnaughey and Whelan, 1997). The metabolic processes of algae and cyanobacteria utilize dissolve $CO_2$ *(Eq2.4),* which is in equilibrium with $HCO_3^-$ and $CO_3^{2-}$ *(Eq2.5).* The removal of $CO_2$ induces a shift in this equilibrium, and results in an increase in pH *(Eq2.6)* (Ehrlich, 1998). When this reaction occurs in the presence of calcium ions, calcium carbonate is produced *(Eq 2.7)* (Hammes and Verstraete, 2002).

$$CO_2 + H_2O \quad — \quad (CH_2O) + O_2 \tag{2.4}$$

$$2\,HCO_3^- \quad — \quad CO_2 + CO3^{2-} + H_2O \tag{2.5}$$

$$CO_3^{2-} + H_2O \quad — \quad HCO_3^- + OH^- \tag{2.6}$$

$$Ca_2^+ + HCO_3^- + OH^- — \quad CaCO_3 + 2H_2O \tag{2.7}$$

Calcite can also be precipitated by heterotrophic organisms, by the production of carbonate or bicarbonate and modification of the environment to favour precipitation (Castanier et al., 1999).The abiotic dissolution of gypsum ($CaSO_4.2H_2O$)*(Eq2.8)* provides an environment that is rich in both sulphate and calcium ions. In the

presence of organic matter and absence of oxygen, sulphate reducing bacteria can reduce sulphate to $H_2S$ and release $HCO_3^-$ *(Eq 2.9)* (Ehrlich, 1998; Castanier et al., 1999; Wright, 1999). If $H_2S$ then degasses from the environment, this results in an increase in pH and favours the precipitation of calcium carbonate (Castanier et al., 1999).

$$CaSO_4.2H_2O \quad — \quad Ca_2^{+} + SO_4^{2-} + 2H_2O \qquad (2.8)$$

$$2(CH_2O) + SO_4^{2-} \quad — \quad HS^- + HCO_3^- + CO_2 + H_2O \qquad (2.9)$$

## 2.7 COULD BACTERIA BREATHE NEW LIFE INTO AN ANCIENT CONSTRUCTION MATERIAL?

The idea to use bacteria for repairing concrete is not entirely new. Even though concrete is highly alkaline and a hostile environment for most microorganisms, some alkalophilic *Bacillus* species have been shown to fill cracks in concrete by producing calcite (CaCO3). These bacteria, however, have to be applied manually to the crack, which means that regular inspection of the structure is still needed. In a recent explained: "When incorporated in the concrete, the bacteria are in a dormant (spore) state. This only changes when a crack develops and water starts to seep in. The water awakens the bacteria, which get to work and start oxidizing calcium lactate, forming calcite in the process." The insoluble CaCO3 precipitates on the crack surface, thus plugging and repairing the crack. As a bonus, the combination of high pH and bacterially produced CO2 causes the formation of even more carbonate.

## 2.8 CONCLUDING REMARK-

Urea added in the bacterial culture disintegrates in to ammonia and $CO_2$. $CO_2$ when reacted with water present in the solution forms bi carbonates ($HCO_3$).Bi carbonates when reacted with calcium source present in culture it forms calcium carbonate ($CaCO_3$).This is the chemical process involved in the formation of calcite layer on the concrete surface. The bacteria inoculated from the cement source must have pH lie in between 9-12, non-pathogenic and capable of producing calcite layer.

# CHAPER 3

# LITERATURE REVIEW

Microbiologically induced calcite precipitation (MCIP) is a technique that comes under a broader category of science called bio mineralization. It is a process by which living organism form inorganic solids. MCIP is highly desirable because the calcite precipitation induced as a result of microbial activities, is pollution natural. The promising results of this process encouraged different research groups from the world to promote MCIP. The goal of this review is to provide an in-depth knowledge about the different key factors to understand overall process. In this review parameter to study durability of concrete structure is presented. Finally MCIP has been explored for the enhancement of durability of concrete structure.

## 3.1 GENERAL-

This chapter represents through review of literature on use of microorganism to improve durability and strength of concrete. Microbial treatment improves both strength and durability of concrete.

## 3.2 COMPRESSIVE STRENGTH IMPROVEMENT BY MICROBIAL TREATMENT-

**Ghos et al. (2003)** describes a method of strength improvement of cement-sand mortar by the microbiologically induced mineral precipitation. A thermophilic anaerobic microorganism is incorporated at different cell concentrations with the mixing water. Microorganism of different concentration was added to mortar via the mixing water for his study. The cement sand ratio used was fixed at 1:3(by weight), and water to cement ratio was fixed at 0.4.A cube mould of 70.6 mm was used. Seven concentrations ranging from 10 to $10^7$ per ml of mixing water were incorporated both for aerobic and *E-coli* microorganism. 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 *(Table 3.1).*The strength improvement is due to growth of filler material within the pores of the cement –sand matrix. 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 comparisons, but no improvement in strength was observed as shown in *Table3. 2.*

***Table 3.1- Effect of anaerobic microorganism addition on mortar strength, (Ghos et al. (2003)).***

| **Cell Conc/ml of water** | **Average mortar compressive strength MPa** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **3 days** | | **7days** | | **14 days** | | **28 days** | |
| | **Strength** | **% Relative Increase With control** | **Strength** | **% Relative Increase With control** | **Strength** | **% Relative Increase With control** | **Strength** | **% Relative Increase With control** |
| Nil | 8.67 | - | 12.60 | - | 16.00 | - | 23.13 | - |
| 10 | 8.68 | 0 | 12.74 | 1.11 | 16.21 | 1.31 | 24.21 | 4.66 |
| $10^2$ | 8.76 | 1.04 | 12.87 | 2.14 | 16.44 | 2.7 | 25 | 8.08 |
| $10^3$ | 8.80 | 1.49 | 12.98 | 3.01 | 16.87 | 5.43 | 25.40 | 9.81 |
| $10^4$ | 8.89 | 2.53 | 13.4 | 6.34 | 17.10 | 6.87 | 25.44 | 9.98 |
| $10^5$ | 9.34 | 7.73 | 14.70 | 16.67 | 19.50 | 21.87 | 28.98 | 25.29 |
| $10^6$ | 9.20 | 6.11 | 13.80 | 9.52 | 17.50 | 9.38 | 26.52 | 14.65 |

***Table 3.2-Effect of E.Coli microorganism addition on mortar strength, (Ghos et al. (2003)).***

| **Cell conc/ml Of water** | **Average mortar compressive strength MPa** | | | |
|---|---|---|---|---|
| | **3 days** | **7days** | **14 days** | **28 days** |
| Nil | 8.72 | 12.58 | 16.32 | 23.13 |
| 10 | 8.68 | 12.60 | 16.33 | 23.00 |
| $10^2$ | 8.74 | 12.44 | 16.28 | 23.11 |
| $10^3$ | 8.44 | 12.53 | 16.23 | 23.14 |
| $10^4$ | 8.58 | 12.61 | 16.00 | 23.10 |
| $10^5$ | 8.71 | 12.56 | 16.34 | 23.13 |
| $10^6$ | 8.18 | 12.49 | 16.29 | 23.60 |
| $10^7$ | 8.73 | 12.66 | 16.41 | 22.50 |

**Jagadeesha et al. (2013)** reported on an experimental investigation carried out on experimental investigation carried out on mortar cubes which were subjected to bacterial precipitation by different bacterial strains and influence of bacterial calcite precipitation on the compressive strength of mortar cube on 7, 14 and 28days of bacterial treatment .Three bacterial strains Bacillus Flexus, isolated from concrete environment, Bacillus pasturii and Bacillus sphaericus were used. The cubes were immersed in bacterial culture medium for above mentioned days with controlled cubes immersed in water and was tested for compressive strength. The compressive strength of mortar cubes tested for compressive strength at 7,14 and 28 days for different bacteria in different calcium source and for control mortar cubes are tabulated in *table 3.3 and 3.4*.It was observed that the compressive strength of cement mortar cubes showed significant increase up to 18% *(Table 3.5)* compared to control specimens. Compared to other bacterial species *Isolate -1* has shown maximum increase in compressive strength, followed by Bacillus *pasteurii* and *Bacillus sphaericus*.The specimen which showed increase in the compressive strength has exhibited the precipitation even inside fractured surface *(Figure 3.1)*, which clearly indicates that the calcite deposition has not only occurred on the surface but also on the interior f the specimen through the microscope pores. However, the increase of compressive as compared to control specimen was reduced on $14^{th}$ day and there was a further reduction in the percentage increase in $28^{th}$ day.

***Table 3.3- compressive strength of mortar specimens, (Jagadeesha et al. (2013)).***

| **Species** | **Compressive strength MPa** | | | | | |
|---|---|---|---|---|---|---|
| | **Day3** | | **Day7** | | **Day28** | |
| | **$CaCl_2$** | **$CaNO_3$** | **$CaCl_2$** | **$CaNO_3$** | **$CaCl_2$** | **$CaNO_3$** |
| **Isolate-1** | 38.60 | 35.70 | 46.50 | 45.20 | 59.8 | 57.2 |
| **B.pasteurii** | 37.40 | 34.60 | 45.70 | 42.80 | 58.0 | 55.4 |
| **B.sphaericus** | 36.20 | 34.00 | 45.00 | 42.70 | 58.0 | 55.8 |

***Table3. 4 – Compressive strength of control specimens, (Jagadeesha et al., (2013)).***

| Control Specimen | Compressive strength , MPa | | |
|---|---|---|---|
| | Day 7 | Day 14 | Day 28 |
| | 32.7 | 41.5 | 54.5 |

***Table 3.5 -Percentage increase in compressive strength, (Jagadeesha et al. (2013)).***

| Bacteria | Percentage Increase In Compressive Strength | | | | | |
|---|---|---|---|---|---|---|
| | 7 Day | | 14 Day | | 28 Day | |
| | $CaCl_2$ | $CaNO_3$ | $CaCl_2$ | $CaNO_3$ | $CaCl_2$ | $CaNO_3$ |
| **Isolate** | 18.00 | 9.20 | 12.00 | 8.90 | 9.72 | 4.95 |
| **B.pasteurii** | 14.40 | 6.00 | 10.10 | 3.14 | 6.42 | 1.65 |
| **B.sphaericus** | 10.70 | 4.00 | 8.50 | 2.90 | 6.42 | 2.36 |

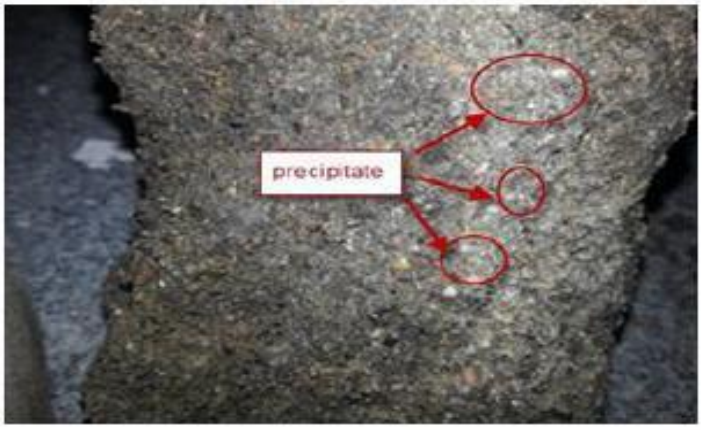



***Fig3. 1-Precipitate on fractured surface treated with Isolate-1(Jagadeesha et al. (2013).***

**Maheswaran et al. (2007)** presents detail of experimental studies carried out on cement mortar using Bacillus cereus and Bacillus pasteurii in different cell concentrations. The cement used for mortar/concrete was 53 grade ordinary Portland cement (OPC) .The grade -2 sand and the locally available coarse aggregate with equal proportion of 12.5 and 20mm size. Ordinary potable water was used for control mortar and concrete, while the entire water was replaced with phosphate buffered saline (PBS) bacteria for the test specimens, with bacteria incorporation as described in the subsequent section. The starin B-pasteurii, Microbial type culture collection,

MTCC 1761 and new strain B.cereus (CS-I) grown in the NB-Urea medium were used. Cell concentration of $10^5$ to $10^7$ cells/ml was prepared along with its culture medium and then the prepared micro organisms were incorporated in cement mortar and concrete to study the strength characteristics. The compressive strength of cement mortar cube specimens of 70.6mm size with water binder ratio appropriate to standard consistency measurement was determined after 5,7,14,21 and 28 days of curing. Test result showed *(Table3.6,3.7)* that the addition of bacterial cultures of both species enhanced the compressive strength of cement mortar due to the bio-mineralization of calcium carbonate in the cement mortar matrix. The test results revealed 38% increase in compressive strength using B.cereus and 29% increase in the case of B.pasteurii over the control cement mortar.

***Table 3.6- Compressive strength for wild strain CS-1 incorporated mortar, (Maheswaran et al. (2007)).***

| **Compressive strength (MPa)** | | | | |
|---|---|---|---|---|
| **Days** | **Control** | **$10^5$ cells/ml** | **$10^6$ cells/ml** | **$10^7$ cells/ml** |
| 5th | 15.34.65 | 24.9 | 25.54 | 30.59 |
| 7th | 17.93 | 25.65 | 31.41 | 34.9 |
| 14th | 22.77 | 32.8 | 38.8 | 38.32 |
| 21th | 25.67 | 40.4 | 47.8 | 42.3 |
| 28th | 36.64 | 43.47 | 50.52 | 44 |

***Table 3.7- Compressive strength for MTCC 1761 incorporated cements mortar, (Maheswaran et al. (2007)).***

| **Compressive strength (MPa)** | | | |
|---|---|---|---|
| **Days** | **Control** | **$10^5$ cells/ml** | **$10^7$ cells/ml** |
| 5th | 15.34 | 18.65 | 31.3 |
| 7th | 17.97 | 28.65 | 33 |
| 14th | 22.77 | 36.43 | 40.58 |
| 28th | 36.34 | 46.88 | 41.19 |

**Gavimath et al. (2012)** investigated the potential application of bacterial species i.e. B.sphaericus to improve the strength of cement concrete. Natural river sand well graded passing through 4.75mm sieve was used to find the compressive strength of mortar cubes. Portland cement of 43 grades available in local market is used in the investigation having specific gravity of 3.15.They have made an attempt to incorporate dormant but viable bacteria *(B.sphaericus)* in the concrete matrix which will contribute to the strength 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, however, is due to high internal pH, relative dryness and lack of nutrients needed for growth rather hostile environment for environment and increase the strength and durability of cement concrete. The cubes were prepared for concrete mix with and without addition of microorganism. The size of the cubes were taken as 100mm×100mm×100mm.From the tests, it was observed that the concrete specimen prepared by incorporating the microorganism yielding higher strength as compared to conventional concrete. The result of the compressive test on conventional and bacteria concrete is indicated in *Table 3.8.*The result of compressive test with and without addition of B.sphaericus is shown in *Table3. 9.* From the observation it is revealed that there is an increase in compressive strength of 30.76%, 46.15% and 32.21% at $3^{rd}$, 7th and $28^{th}$ day respectively while using B.sphaericus bacteria compared to conventional concrete. They found that incorporation of spore forming bacteria of the species Bacillus will not negatively affect the compressive and split tensile strength of the cement concrete.

***Table 3.8- Result of the compressive test with and without addition of microorganism, (Gavimath et al. (2012)).***

| Sr. No | Types of Bacteria | Compressive strength | % increase |
|---|---|---|---|
| 1 | Without bacteria | 3.94 | - |
| 2 | B.sphaericus | 36.28 | +51.54 |

***Table 3.9- Result of the compressive tests with and without addition of B.sphaericus, (Gavimath et al. (2012)).***

| No. of Days | Compressive strength of conventional concrete cube, N/mm2 | Compressive strength of B.sphaericus concrete cube, N/mm2 | % increase in strength |
|---|---|---|---|
| 3 | 19.24 | 25.16 | 30.76 |
| 7 | 23.66 | 34.58 | 46.15 |
| 28 | 34.52 | 45.72 | 32.21 |

**Ramachandran et al. (2001)** describes an innovative biotechnology utilizing micro-biologically-induced mineral precipitation induced by Bacillus pasteurii was studied in two types of Portland cement mortar specimens: one prepared from mixing with micro-organism, and the other with simulated cracks filled with microbial mixture. Portland cement mortar beams of dimensions 25.4×25.4×125mm containing no cells were cast in which the same amount of water used instead of phosphate buffer. The specimen were cured in water for 28days and then left exposed to air. The width of the cut was maintained constant at 3.175mm, while the depths of cut were 3.175 and 9.525mm .For each crack depth, a total of 10 specimens was prepared. The first five specimens were used as control without filling in the crack and left exposed to air. The cracks in the remaining five specimens were filled with mixture of sand and B.pasteurii.The sand mixed with bacteria suspension to a final concentration of $3.8\times10^{9}$ cells/$m^{3}$ was forced in to the crack with in a thin knife edge. Then the specimen beams with bacteria were placed in a tray containing Urea-$CaCl_2$ medium and cured for 28 days separately. The medium was replaced after 14 days.

Compressive strength values of cubes prepared in phosphate buffer and saline solution are compared in *Fig3.2.* Portland cement mortar cubes prepared in saline have shown an overall decrease in compressive strength in the presence of cells. The decrease in compressive strength of the cubes containing saline may be due to presence of chloride ions in the solution. The compressive strength of mortar cubes prepared in the phosphate buffer was consistently higher than the strength of saline – prepared cubes at various concentrations of cells laden in the cubes. Thus; phosphate buffer solution was used in all the other tests. The cubes prepared with phosphate

buffer, however showed a tendency to decrease slightly in straight at higher concentrations of cells.

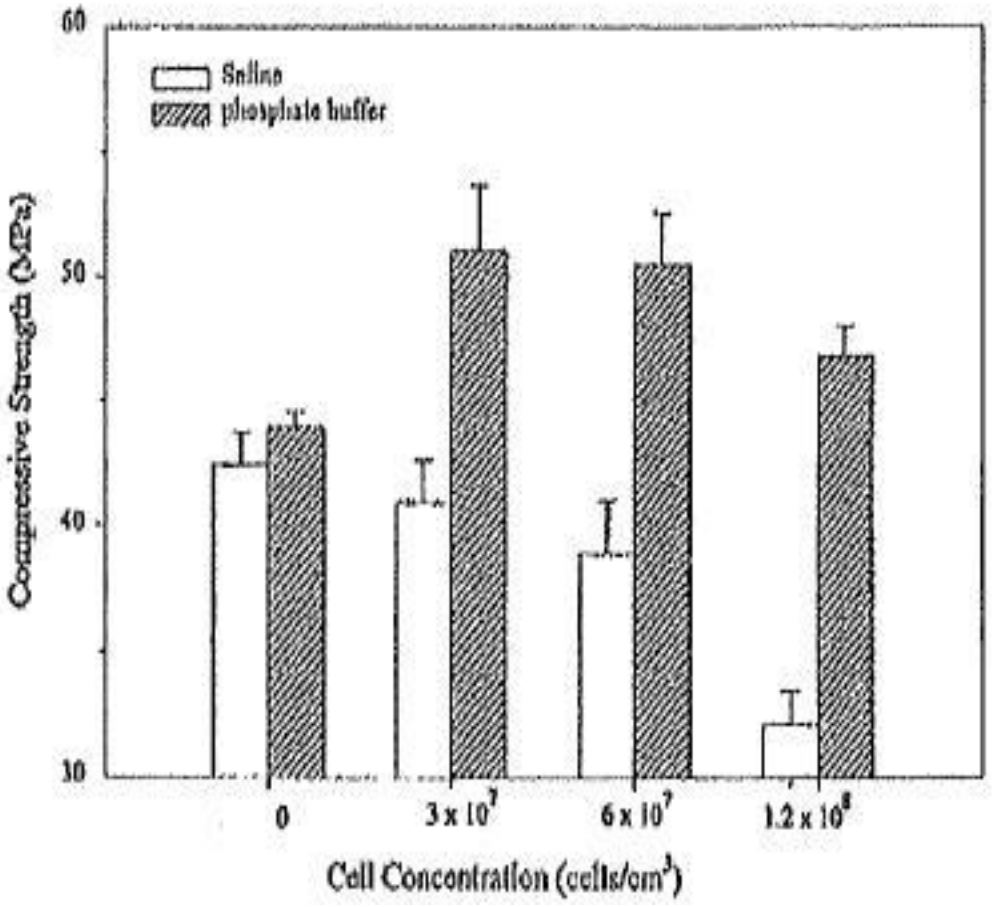



***Fig3. 2- Effects of saline and phosphate buffer on compressive strength of Portland cement mortar cubes containing various concentration of B.pasteurii,* (Ramachandran et al. (2001))**

***Ghos et al. (2009)*** studied on Microbial activity on the microstructure of bacteria modified mortar.OPC 43 and standard sand were used for the study. Standard mortar cubes (70.6mm×70.6mm×70.6mm) by mixing with bacteria were cast. Bacteria cell concentrations were used as $0\text{-}10^7$ cells/ml water. For mortar preparation, the cement to sand ratio was taken as 1:3 and water to cement ratio was fixed 0.4.All the specimens were cured under water after 24h of casting. The compressive strengths of the mortar cubes were determined at 3,7,14 and 28 days of curing. *Fig3. 3* shows a bar diagram of the compressive strength of the mortar cubes having different bacterial cell concentrations at different days (3,7,14 and 28days) of water curing. This increment is

maximized at the bacterial cell concentration of $10^5$cells/ml, which is in agreement with the earlier results.

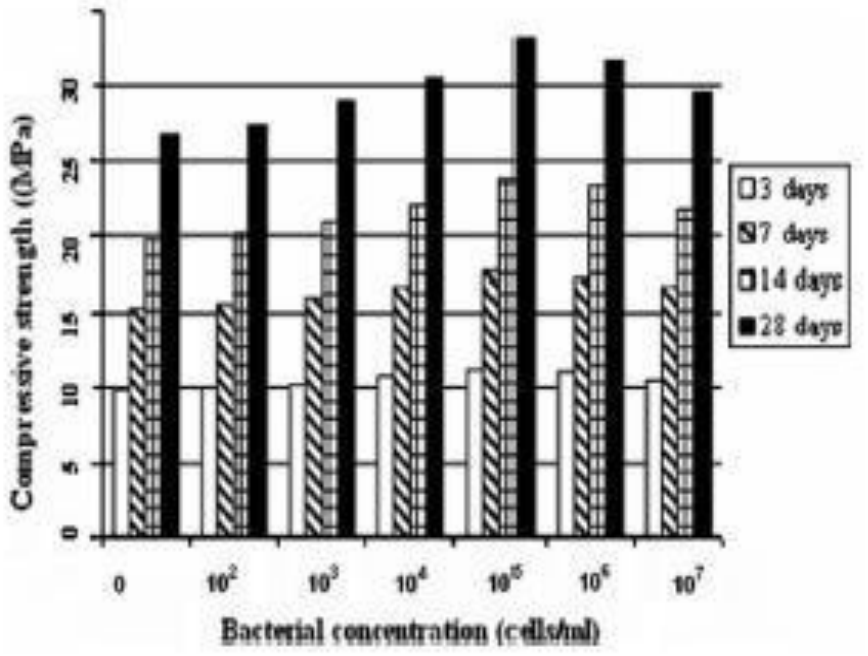



***Fig 3.3 -Compressive strength of mortar vs. concentration at different ages, (Ghos et al. (2009 ).***

## 3.3 CHARACTERIZATION STUDIES TO CONFIRM CALCITE PRECIPITATION

### 3.3.1 XRD/SEM

**Ramchandran et al. (2001)** depicts the profiles of XRD patterns of three Portland cement mortar samples 1) mixed and cured in water; 2) mixed in phosphate buffer and cured in Urea-$CaCl_2$ medium; 3) mixed with B-pasteurii suspended in phospahte buffer and cured in Urea-$CaCl_2$ medium. The XRD pattern was determined by scanning from 5 to 60 degrees,2 theta using a vertical x-ray diffractometer.All cement mortar cubes tested contained similar proportions of the major cement material, portlandite, qurtz, oligoclase and calcite. Crystal fractions in cement mortar samples and the ratio of calcite to a total of quartz and oligoclase are included in *table 3.10*. Ratios of calcite to quartz and oligoclase, both of which originate from sand, not from Portland cement were considered to be meaningful. In absence of bacteria, the average ratio value of calcite to these sand components was 0.20 and 0.29 for the sample cured in water and in Urea-$CaCl_2$ medium, respectively.In the presence of

bacteria, the ratio from the sample containing *B-pasteuri*, which was cured in Urea-$CaCl_2$ medium, was 0.30.The result indicate that XRD analysis detect an insignificant increase of calcite precipitation in cell-laden Portland cement mortar as shown in *Fig 3.4*.

***Table3. 10-XRD quantitative analysis of Portland cement mortar samples, (*Ramchandran et al. (2001))**

| | **Portlendite $Ca(OH)_2$** | **Quartz $SiO_2$** | **Oligoclase (Na, Ca) $Al(Al,Si)Sl_2O_8$** | **Calcite $CaCO_3$** | **Calcite/ Quartz+Oligoclase** |
|---|---|---|---|---|---|
| Sample 1* | 0.22 | 0.62 | 0.03 | 0.13 | 0.20 |
| Sample $2^t$ | 0.19 | 0.61 | 0.02 | 0.18 | 0.29 |
| Sample $3^t$ | 0.26 | 0.54 | 0.03 | 0.17 | 0.03 |

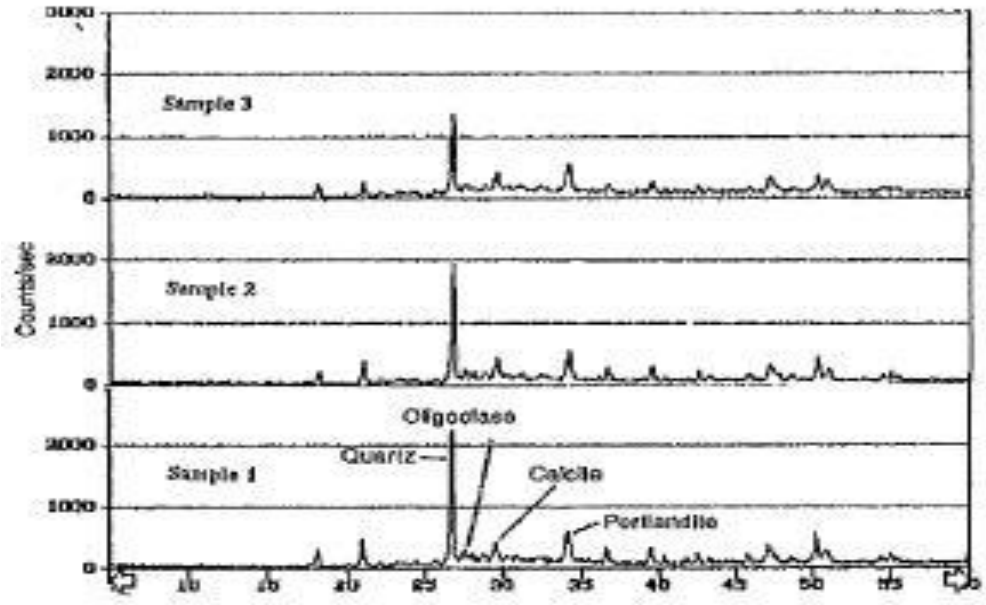



***Fig3. 4-X-ray diffraction patterns for cement mortar samples, (*Ramchandran et al. (2001)).**

**Maheswaram et al. (2014)** Depicts the XRD shown in *Fig 3.5* for the bacterial incorporated mortar samples CS-I and MTCC 1761 and control mortar, explained that the principal calcite peaks can be observed at $29.41^0$, $35.97^0$ and $57.4^0$ The starin B-pasteurii, Microbial type culture collection.MTCC 1761 and new strain B.cereus (CS-I) grown in the NB-Urea medium were used. Continuous scan from $10^0$ to $70^0$ 2 theta

in step width of $0.02^0$ time of 0.5 s/step was performed on less than 25 µm size powder sample were used. In the intensity mapping of the characteristic peaks of calcite; it is found that higher calcite intensity peaks with reference to International Crystal Diffraction Database (ICDD) were formed for the both bacterial incorporated specimens, especially more in the CS-I incorporated specimens *(Fig 5)* This is an indication of high percentage of calcite in the bacteria incorporated specimens, which could be attributed to an active transformation of the unstable calcium ions in to stale $CaCO_3$.This wild strain is able to produce higher amount of calcite, thus resulting in insignificant higher compressive strength percentage (38%) higher than the control.

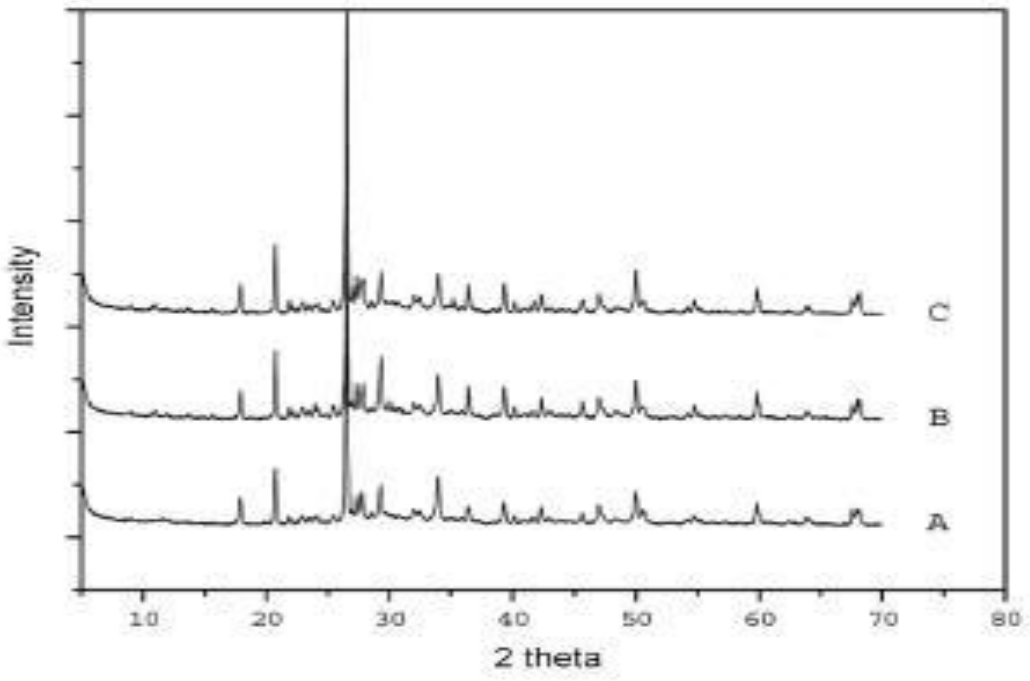



***Fig3. 5-XRD of control samples (A), CS-I with $10^6$ cells/ml (B) and MTCC 1761 with $10^5$ cells/ml (C), (Maheswaram et al. (2014)).***

**W.De Muynck et al. (2007)** studied on the treatment of the mortar cubes with bacteria and calcium source resulted in the presence of crystalline layer on the surface shown in *Fig 3.7I and 3.7II vs. Fig 3.7III* .The morphology and mineralogical composition of the deposited CaCO3 crystals were investigated with SEM and XRD.Scanning angle was 3 to $60^0$ and 2 theta. The type of bacterial culture and the nutritional composition had a profound impact on crystal size and morphology *(Fig 3.7I and 3.7II) .Fig 6* results indicated the presence of a newly formed layer on the

surface of the mortar specimens, consisting mainly of calcite. Beside calcite small quantity of vaterite were also detected.

Crystal obtained in the presence of mixed ureolytic culture (*Fig 3.7 II*) were larger in size (up to 100 µm diameter) compared to those obtained with *B-spharicus* (up to 20 µm diameter) *(Fig3.7I).*Calcite rhombohedral crystal disappeared in the presence of nutrient broth *(Fig 3.7I and3. 7II b, d)* and although they are clearly present in its absence *(Fig 3.7I and3. 7II a, c)*. Granular grains (presumably vaterite) appeared in the presence of nutrient broth. The result from XRD analysis confirmed the presence of vaterite crystal. (*Fig3.6).*The impact of type of calcium source on crystal morphology was more pronounced in the case of mixed cultures. In the absence of nutrient broth, the presence of calcium chloride resulted in rhombohedral crystals *(Fig.3.7I and 3.7II a)* while granular grains were present in the case of calcium acetate. As indicated by arrows in the inserted *figures*, several calcite crystals show rod-shaped holes, indicating possible bacterial mediation of precipitation.

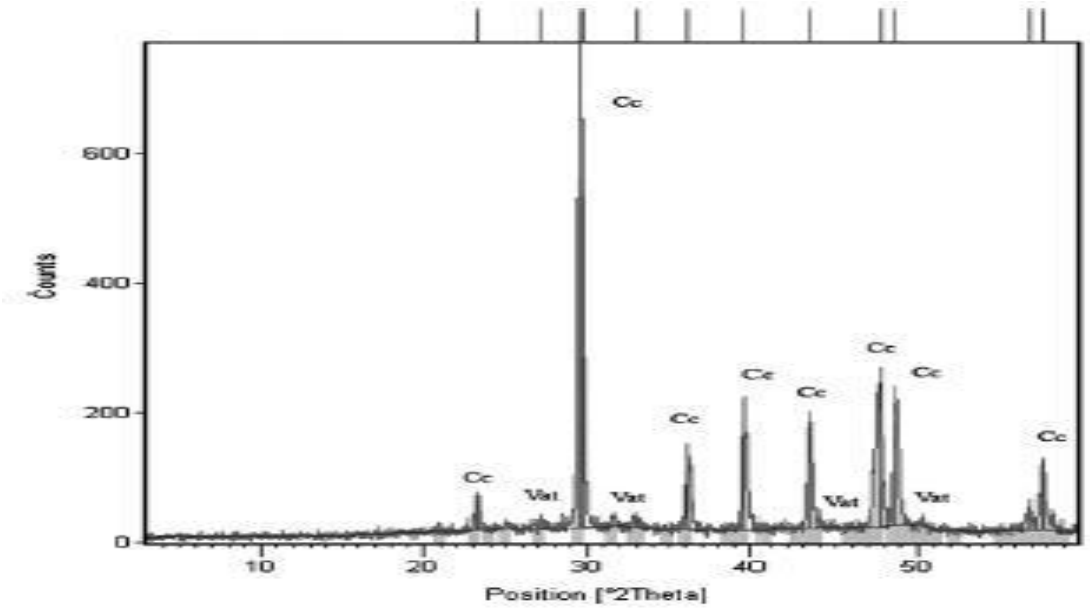



***Fig 3.6- XRD patterns of the crystalline layer present on the surface of mortar cubes treated with mixed cultures, nutrient broth and calcium chloride,* (W.De Muynck et al. (2007).**

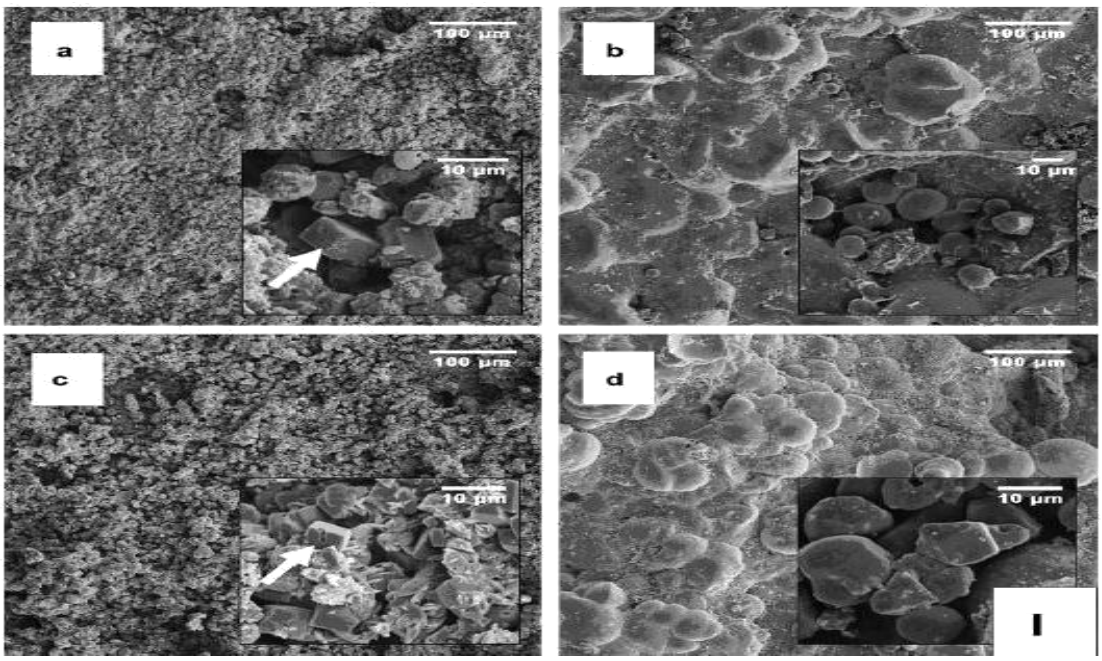
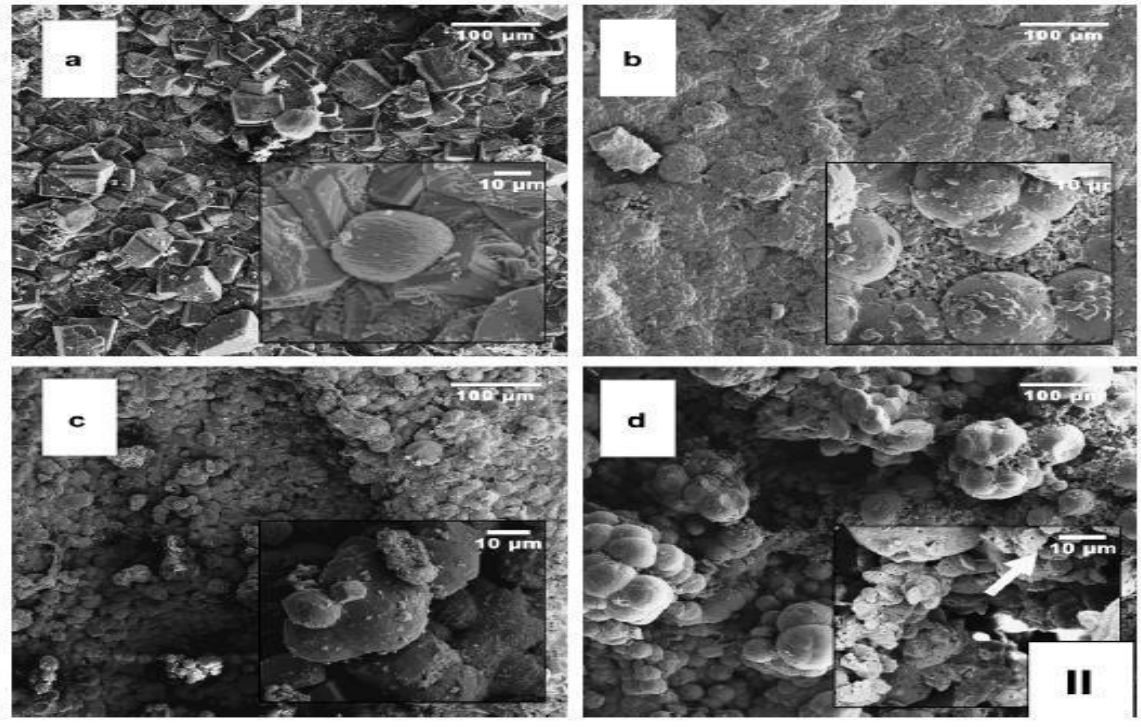
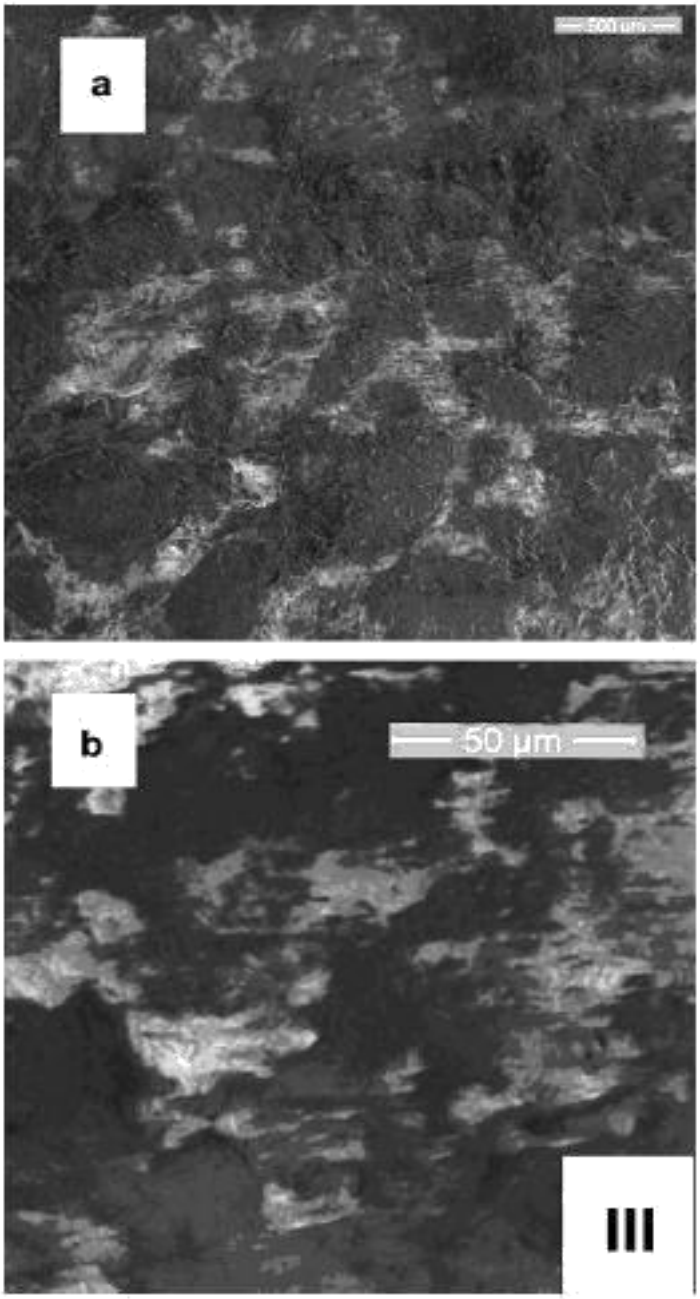



***Fig 3.7- SEM of CaCO3 crystal on the surface of mortar specimens treated with (1) Bacillus sphaericus (2) ureolytic mixed cultures, (W.De Muynck et al. (2007)).***






**Okwadha et al. (2010)** depicted that XRD and SEM analysis of the precipitated calcium carbonate powder are given in *Fig3. 8*. The $CaCO_3$ precipitate is composed of calcite and vaterite crystals *(Fig3. 8a)* but predominantly calcite with rhombohedral crystalline structure *(Fig 3.8b)*. The peaks obtained through XRD shows that the elemental composition of the precipitate is mostly calcium, carbon and oxygen .This is further evidence that the precipitate formed is calcium carbonate.

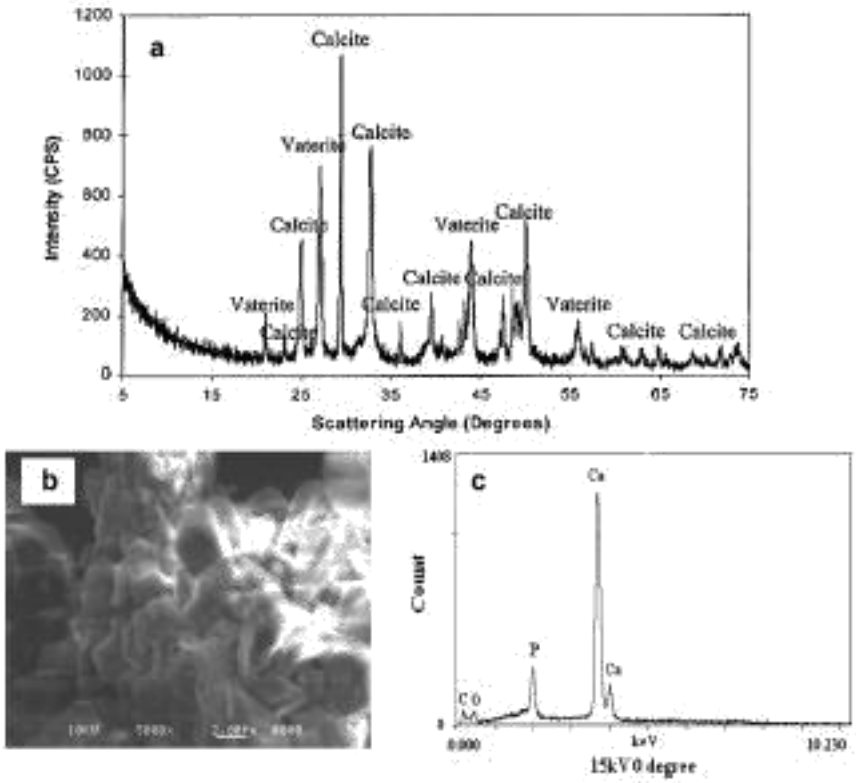



***Fig 3.8 XRD at a continuous scanning rate of 20 min-1 (a) SEM (b) at 5000× magnification and energy dispersive x-ray and (c) analysis of the precipitated calcium carbonate powder*, *Okwadha et al. (2010).***

**Ghosh et al. (2005)** studied on Microbial activity on the microstructure of bacteria modified mortar.OPC 43 and standard sand were used for the study. Standard mortar cubes (70.6mm×70.6mm×70.6mm) by mixing with bacteria were cast. Bacteria cell concentrations were used as 0-$10^7$ cells/ml water. For mortar preparation, the cement to sand ratio was taken as 1:3 and water to cement ratio was fixed 0.4.SEM examinations were made on the broken samples collected from the mortar cubes tested at 28 days.SEM specimens were dried ,then gold coated and stored in desiccators.SEM examination shows that in a mortar made with a $10^5$/ml cell concentration, the pores are almost completely filled with narrow strands of filler *( Fig3.9)* and more modification in pore size distribution is noticed *(Fig 3.10).*SEM examination reveals the growth of fibrous filler material within the pores due to the presence of such organism. This growth is beneficial by the modification of the porosity and pore size distribution of cement mortar which it generates. This compares with the control samples where no filler material was observed *(Fig3. 11).*

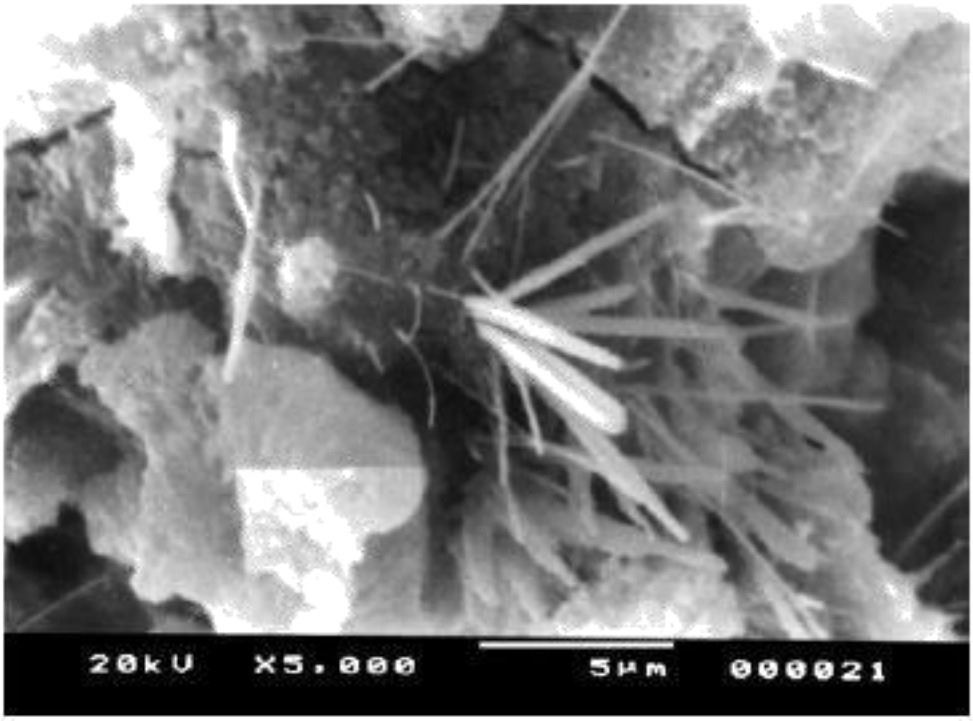



*Fig 3.9 SEM micrograph of mortar with anaerobic microorganism of $10^5$ cells/ml cell concentration, Ghosh et al. ((2005)).*

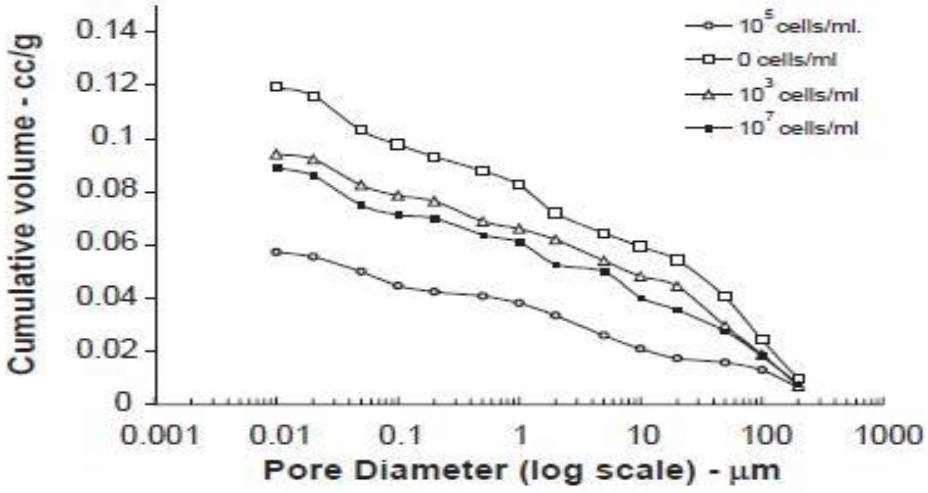



*Fig 3.10 Cumulative volume of pores larger than indicated pore diameter in cement-sand mortar at 28days, (Ghosh et al. (2005)).*

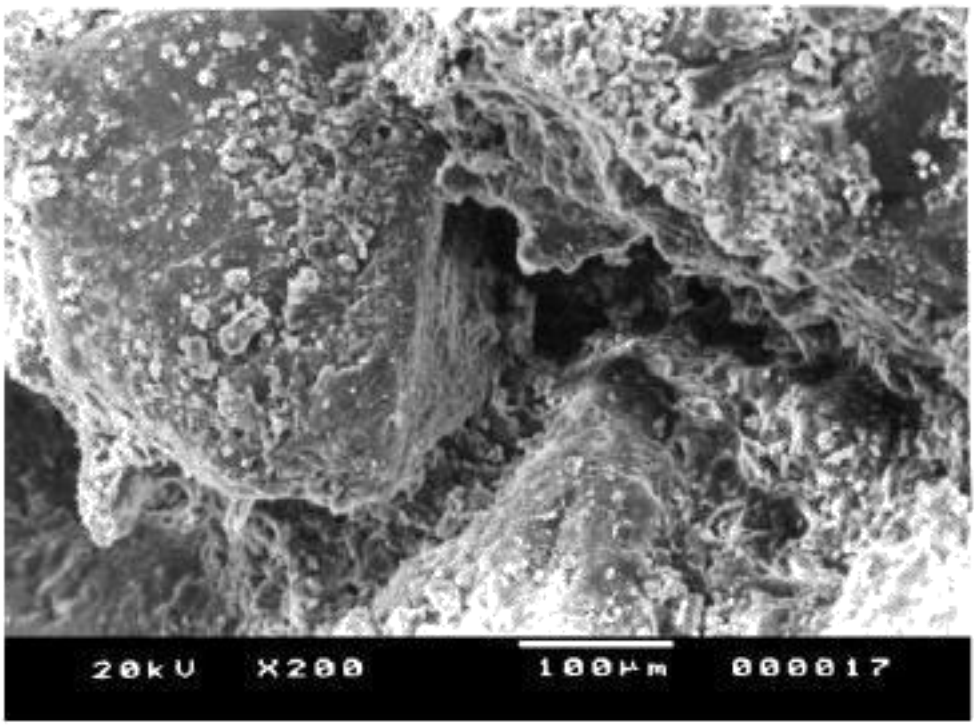



***Fig 3.11 SEM micrograph of mortar without any microorganism,* (Ghosh et al. (2005), (*Ghosh et al. (2005)).***

**Maheswaran et al. (2014)** confirm microbial calcite formation in the cement sand matrix, the powder samples from tested mortar specimens were studied using SEM.Mortar samples CS-I and MTCC 1761 and control mortar, the starin B-pasteurii, Microbial type culture collection,MTCC 1761 and new strain B.cereus (CS-I) grown in the NB-Urea medium were used. Samples were chosen with cell concentration $10^6$ and $10^5$ cells/ml respectively, as they showed the maximum compressive strength. *Fig 3.12a* shows the SEM image of MTCC 1761 incorporated with cell concentration of $10^5$cells/ml. For the cell concentration of $10^5$ cells/ml presence of lamellar rhombohedral crystals of calcite and needle-shaped aragonite crystals of $CaCO_3$ are clearly visible that act as precursors for the formation of calcite crystals of $CaCO_3$, which shows that the system supports the continuous formation of calcite. Moreover, the bacterial impressions on the mortar surface and the precipitation of calcite can be clearly noticed around the edges of the impression as shown in Fig *3.12b.Fib3.12c* shows rhombohedral calcite precipitation for the samples with $10^6$ cells/ml concentration.

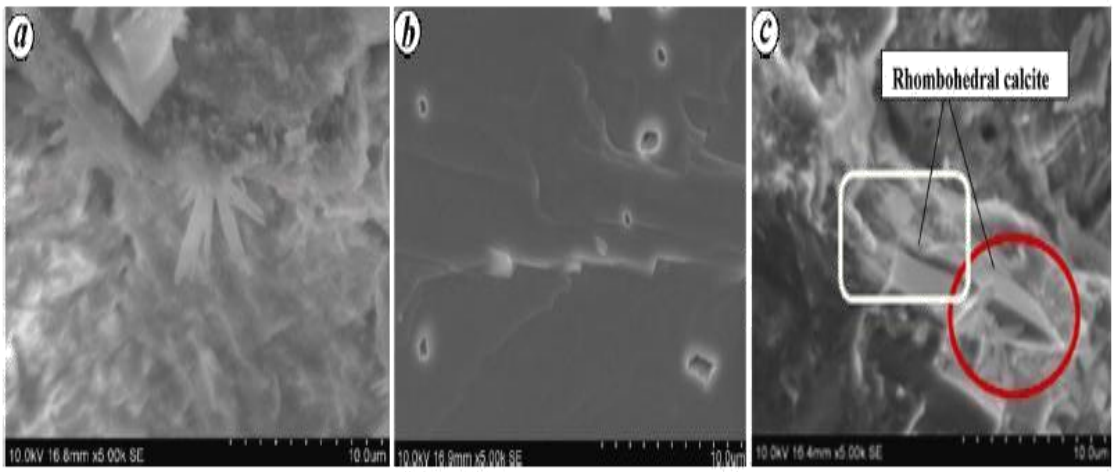



***Fig3. 12 SEM images of bacteria incorporated mortar samples a, MTCC 1761 incorporated mortar with cell concentration of $10^5$ cells/ml. b, Bacterial impression on the mortar surface of MTCC type. c, rhombohedral calcite precipitation for the samples of CS-1 incorporated mortar with cell concentration of $10^6$ cells/ml, Maheswaran et al. (2014).***

**Ramchandran et al. (2001)** examine to check whether increase in compressive strength of the specimens with bacteria and sand in their cracks could be attributed to the microbial calcite precipitation. Bacillus pasteurii was studied in two types of Portland cement mortar specimens: one prepared from mixing with micro-organism, and the other with simulated cracks filled with microbial mixture. Portland cement mortar beams of dimensions 25.4×25.4×125mm containing no cells were cast in which the same amount of water used instead of phosphate buffer. The crack samples within the highest strength values were examined under SEM.*Fig 3.13 (a)* is a SEM of the matrix of bacteria-free Portland cement mortar. *Fig 3.13(b)* through *(d)* includes selected micro-graphs of the specimen taken from the area area close to the surface and interior of the crack. The sample taken from area close to the surface showed calcite crystals grown all over the sand particles and precipitated between them *Fig 3.13(b).*On closer observation of the area containing dense calcite precipitation, it was found that the calcium carbonate crystals were well developed near the surface of the crack *Fig 3.13(c).* They had distinct and sharp edges, indicating a full growth of the crystals. Calcite crystals are seen with rod-shaped holes, which are presumably the space occupied by the bacteria. Much less

consolidation, however, was observed in the sample prepared from an interior area of the crack *Fig 3.13(d).*

*B-pasteurii* is facultative anaerobic. They are able to grow either aerobically or anaerobically .They prefers the presence of oxygen, however, and are metabolically active via aerobic respiration. It is therefore expected o have more calcite precipitation in areas close to the surface in crack remediation. This behaviour, evidenced by SEM examination, suggests that microbial remediation is in general more effective in shallow cracks.

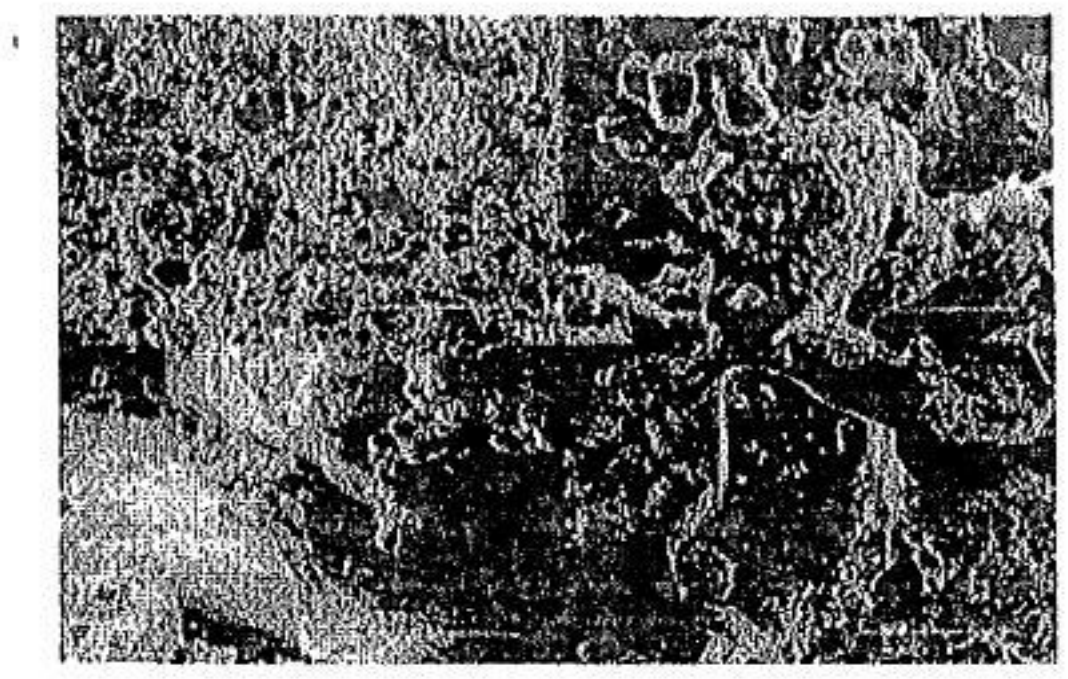


***Fig3. 13 SEM showing microbiological calcite precipitation in concrete cracks: (a) matrix of Portland cement mortar prepared without bacteria: (b) samples taken near surface area of remediated crack, showing dense calcite precipitation between and on surface of sand particle: (c) enlarged portion of calcite crystals found in b, showing calcite crystals with empty holes once housed by B.pasteurii and (d) samples taken from interior of concrete crack showing little calcite precipitation,***
***(Ramchandran et al. (2001)).***

**Muynck et al. (2010)** stated that the morphology and crystal habit of calcium carbonate depend on precipitation conditions such as the initial super saturation ,the temperature and the presence of impurities and additives. Therefore, difference

observed in the size and morphology of carbonates precipitated on limestone specimens treated with different concentrations of calcium and urea could be attributed to the differences in the saturation state of the system and different amounts of additives. Higher super saturation results in the formation of smaller crystals. This could account for the differences in crystal sizes are observed from *Fig 3.14C-E*, decreasing crystal sizes and less developed crystal with increasing concentrations of urea and calcium in the medium. Large rhombohedral crystals, characteristic for calcite were observed on bio deposition treated specimens *Fig 3.14C.*

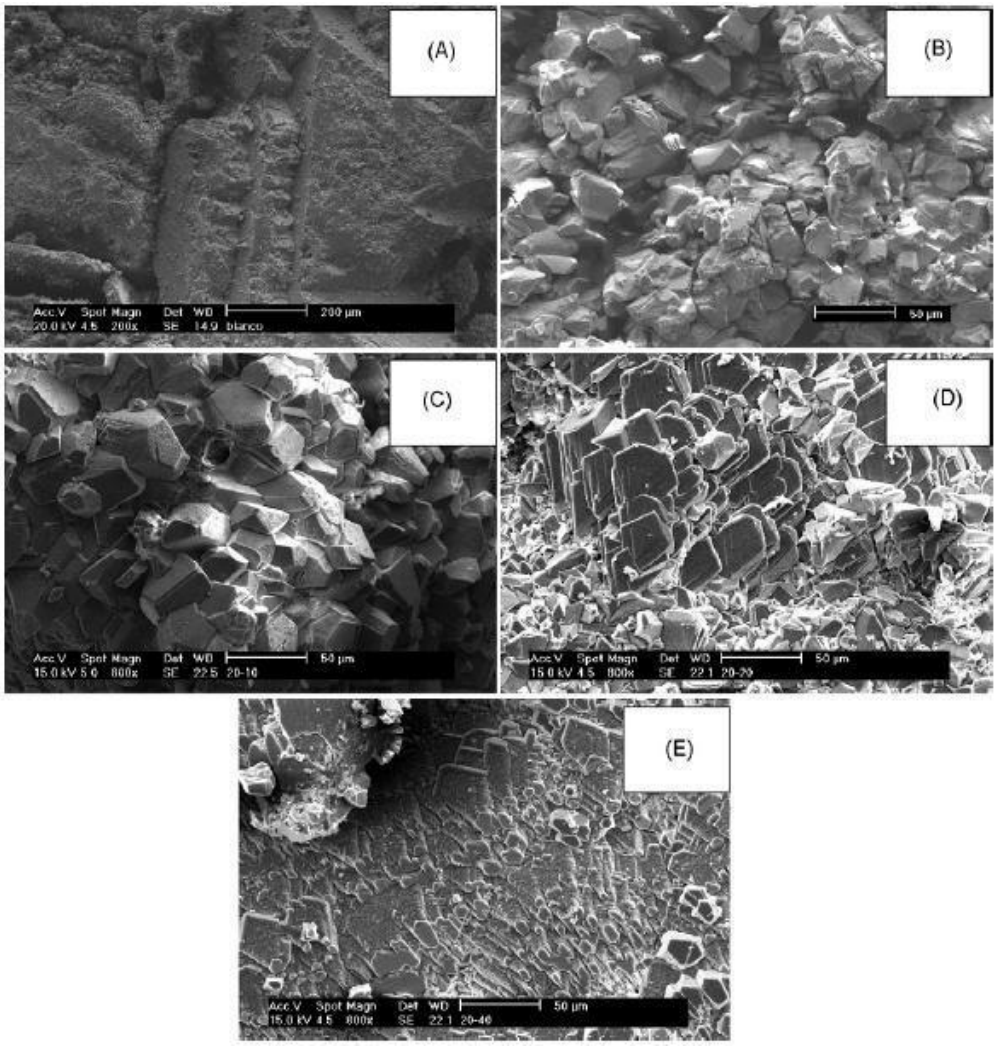



***Fig3. 14-SEM showing the surfaces of an untreated limestone specimen (A) and specimens treated with B.sphaericus and varying concentrations of urea and calcium chloride in the bio deposition media, Muynck et al. (2010).***

**Ghos et al. (2009)** studied on Microbial activity on the microstructure of bacteria modified mortar.OPC 43 and standard sand were used for the study. Standard mortar

cubes (70.6mm×70.6mm×70.6mm) by mixing with bacteria were cast. Bacteria cell concentrations were used as 0-$10^7$ cells/ml water. For mortar preparation, the cement to sand ratio was taken as 1:3 and water to cement ratio was fixed 0.4.All the specimens were cured under water after 24h of casting. Mortar samples of each mix (after 28 days) were taken and crushed into fine powder. The powder samples passing a sieve size of 5 μm were analyzed in a power XRD. The XRD spectrum were taken from $2\theta = 10^0$ to $2\theta = 70^0$.Microstructures examined by SEM (Fig3. 15a-d) of control and bacteria treated mortar samples show contrasting texture of their matrices, The matrix of the untreated ones appears to be amorphous, showing no signature of conspicuous growth. On the other hand, mortar samples that were treated with the microorganism shows crystalline matrix, where individual crystal could be recognized. The degree of crystalinity in the matrix of treated sample is somewhat heterogeneous. There occur concentrations of relatively large crystals at the interface of sand particles and the matrix. This type of textural setting suggests that the coherence between sand particles and the matrix in micro-scale is probably enhanced due to preferential crystallization at the sand-matrix interfaces.

The powder crystal X-ray analysis is of the mortar sample with or without bacteria shows that there were some extra peaks in the XRD spectra of the bacteria treated samples.XRD shows that these new peaks match with the minor peaks of pure calcium aluminium silicate phase. This new phase helps in the modification in pore size distribution and thus increases in the compressive strength.

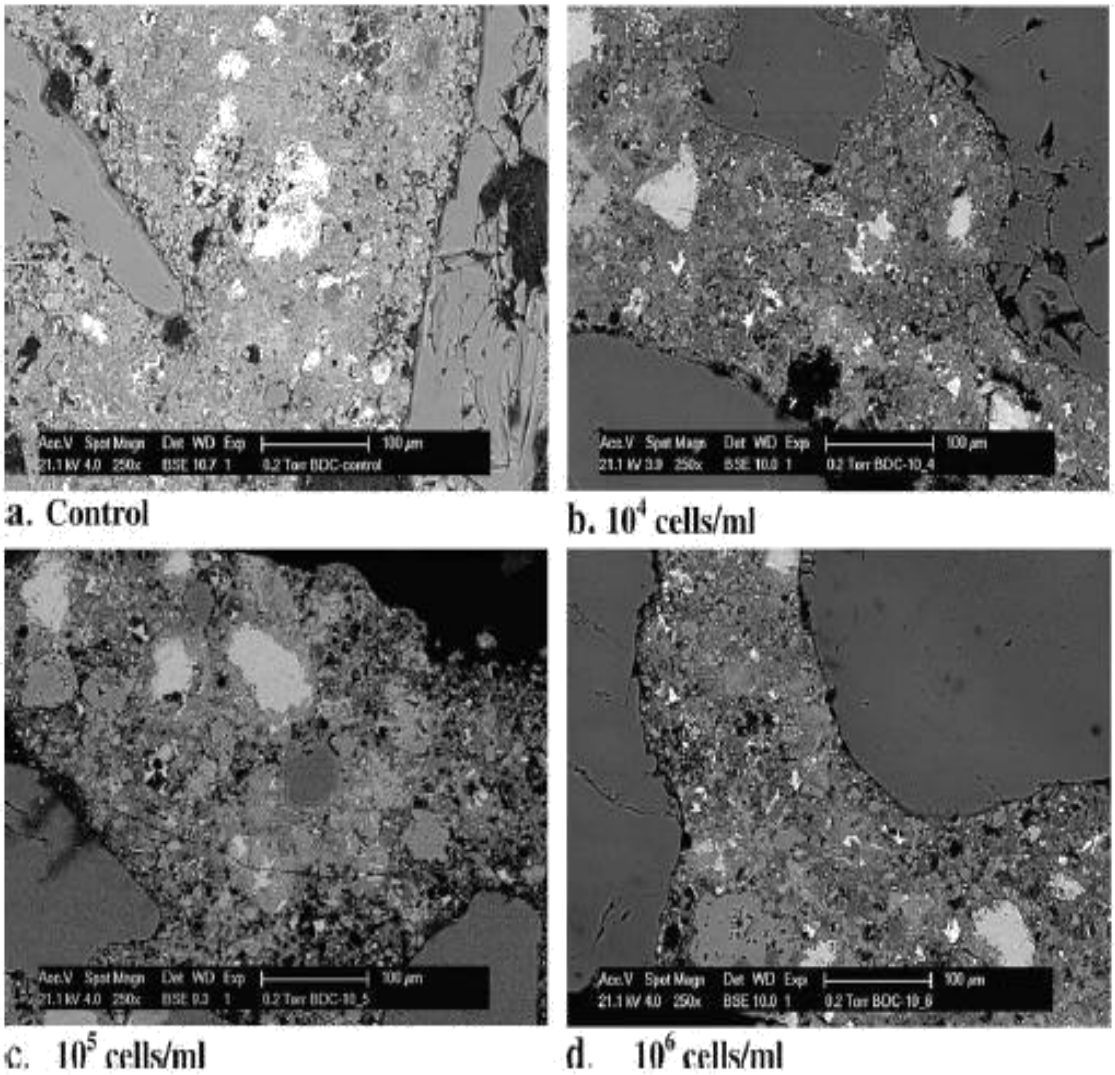



***Fig- 3.15-SEM of mortar samples (a) control sample (b) bacteria treated sample ($10^4$ cells/ml) (c) bacteria treated sample ( $10^5$ cells/ml) (d) bacteria treated sample ($10^6$ cells/ml), Ghos et al. (2009).***

### 3.3.2 EDTA-

**Gavimath et al. (2012)** estimated the amount of calcium carbonate by EDTA method. Using the standard graph of bacterial sample value was generated by carrying titration with EDTA .This was alkalized by using ammonia buffer .End point was obtained by using EBT indicator, which turns steel blue color from reddish pink color. Confirmation of calcium carbonate precipitation in the culture was done using lased Raman spectroscopy.

## 3.4 LITERATURE REVIEW ON DURABILITY-

### 3.4.1-MERCURY INTRUSION POROSIMETRY-

**Muynck et al. (2010)** studied the influence of the deposition treatment on the porosity and pore size distribution of the stone by mercury intrusion porosimetry.Carbonate stone used in the study was Euville limestone which is often used for building and sculpturing. The stone has a rough granulated structure and was created by mixture of fossilized crinoids, cemented together by crystalline calcium carbonate .A characteristic feature of the stone is its higher water absorption. For bio deposition experiments, stone were cut in to size 1cm×1cm×1cm.Biodeposition experiment was performed at 280C under static and non-sterile codition.Limestone specimen were immersed in growth media 0r 1 day culture of *B.sphaericus*.The 1 $cm^3$ cubes were completely immersed in glass tubes containing 10mL,of the growth or bio deposition medium. Analyses were performed in triplicate. Prior to analysis, samples were immersed for 5 min in liquid nitrogen and subsequently freeze-dried. No differences in porosity and pore size distribution could be observed between untreated and 20/50 bio deposition treated specimens shown in *(Table 3.11).*

***Table 3.11-Pore size characteristics as determined from mercury intrusion porosimetry, Muynck et al. (2010).***

| Characteristic | Untreated | Biodeposition Treatment |
|---|---|---|
| Volume-based median pore diameter(μm) | 0.42±0.17 | 0.45±0.17 |
| Are-based median pore diameter(μm) | 0.01±0.00 | 0.01±0.00 |
| (4V/A) Average pore diameter(μm) | 0.02±0.00 | 0.02±0.00 |
| Porosity (%) | 19.78±2.91 | 18.17±1.51 |

## 3.5 pH-

**Okhwadha et al. (2010)** explained for each test, 20ML of the culture medium was mixed with 10mL of the stock culture in a beaker, and the mixture was stirred slowly using magnetic stirrer. A pH meter was then dipped into the solution in succession to measure pH .Measurements were done after 15min,30min,1h,3h,6h,12h,24h and every 24h for 7d.The pH changes generated during urea hydrolysis are presented in

*Fig3.16.*The time taken for the experiments to reach equilibrium pH decreased with increase in bacterial cell concentration (Fig 16 a,c and e ).There is a significant difference between control and inoculated experiment indicating the greater influence urease enzyme has on urea hydrolysis.

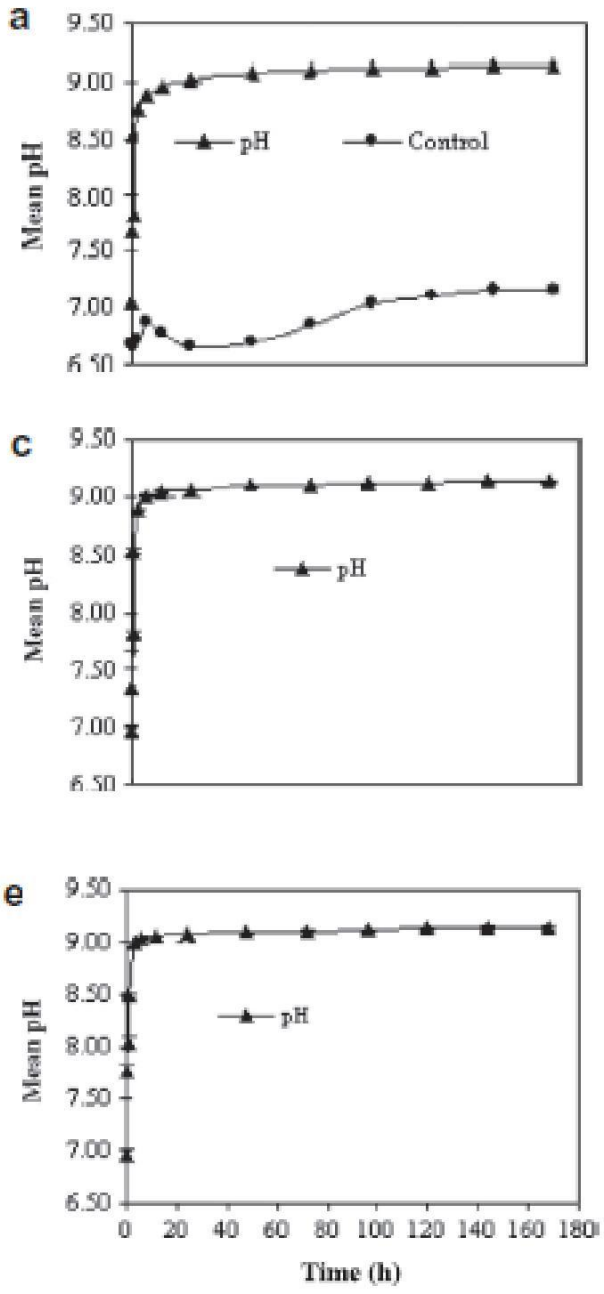



***FIG 3.16-Graphical representation of changes in mean pH generated during urea hydrolysis, Okhwadha et al. (2010).***

**Jagdeesha et al. (2013)** Three bacterial strains Bacillus Flexus, isolated from concrete environment, Bacillus pasturii and Bacillus sphaericus were used.Growth and survival of bacteria is influenced by pH of the environment .Each microbial species possesses definite pH growth range and a distinct pH growth optimum. The nutrient broth of different pH ranging from 4to 12 was prepared in a test tube. Bacterial culture was introduced into it and growth pattern was observed. The test was carried out by measuring turbidity of the sample using photo calorimeter. It was observed that isolate-1 had optimum growth in pH range 7.5-9.0.However the growth was also observed in the extreme pH up to 12.Whereas Bacillus pasteurii had optimum growth in pH range 7-9 for Bacillus sphaericus was 8-9.

### 3.6 CONCLUDING REMARK-

On going through all the research and the results obtained, it may concluded that by addition of any bacteria (Bacillus pasteurii, Bacillus sphaericus etc.) inoculated from different sources like cement, sludge and soil has positive effect on concrete, bricks and ornaments . Compressive strength has found to be increasing with the effective percentage in comparison to control specimen.pH remains constant for all the cases so that bonding properties of cement remains constant. From MIP result it is concluded that microbial treatment reduces water permeability.

# CHAPTER 4

# EXPERIMENTAL PROGRAMME

Following chapter represents experimental set up to evaluate the effectiveness of using bacteria for microbial protection on concrete structure. The effectiveness is monitored in terms of strength and durability by doing different tests.

## 4.1 EXPERIMENTAL PROCEDURE-

The objective of the present work is to explore the effect of bacteria used in concrete to improve its strength and durability by a process known as Biocalcification.Biocalcification is also known as microbiologically induced calcite precipitation (MCIP).

The detailed experimental procedure involves the following steps:-

- Collecting all the basic materials like cement, sand and aggregate as per as Indian standard specification.
- Finding all the basic properties of cement, sand and aggregate by performing basic tests on it.
- Getting cement: sand: aggregate ratio by defining w/c ratio by IS mix design procedure.
- Casting of non microbial concrete cubes without addition of bacteria of size 150mm×150mm×150mm for performing different tests on 7,14 and 28 days.
- Casting of microbial concrete cube of size 150mm×150mm×150mm for performing different tests on 7,14 and 28 days.
- Top and bottom piece of broken sample is preserved for further tests.
- Preserved samples are broken and passes through 300mm sieve and powdered sample obtained is collected.
- pH of powdered samples as top and between on 3,7 and 28days are measured by mixing powdered concrete and water in ratio 1:9.
- Calcium carbonate precipitation amount is calculated from powdered sample on 28days by different methods EDTA, SEM and XRD.
- Pieces form broken samples is cut in 1cm×1cm size for determining porosity by MIP.

## 4.2 MATERIAL TESTING-

The basic materials used in the preparation of specimens are cement, coarse aggregates, fine aggregates, water, and bacterial culture. The properties of these basic materials are as under.

### 4.2.1 Cement-

Portland Pozzolana cement (PPC) is used for the present investigation. The cement is of uniform colour i.e. grey with a light greenish shade and is free from any hard lumps. Summary of the various tests conducted on cement are given in *Table 4.1*All the tests are carried out in accordance with procedure laid down in IS: 8112-1989.*Table 4.2* shows the compressive strength of cubes at 7 and 28 days after curing.

***Table 4.1- Physical properties of cement.***

| S.NO | Characteristics | Obtained result | Standard values |
|---|---|---|---|
| 1- | Normal consistency | 28% | - |
| 2- | Initial setting time | 70min | Should not be less than 30min |
| 3- | Final setting time | 300min | Should not be more then 300min |
| 4- | Fineness | 6% | <10 |
| 5- | Specific gravity | 3.148 | - |

***Table 4.2-Compressive strength of cement (cement: sand=1:1.54).***

| S.No | Days of curing | Compressive strength(MPa) |
|---|---|---|
| 1 | 3 | 18.6 |
| 2 | 7 | 31 |
| 3 | 28 | 38 |

### 4.2.2 Fine Aggregates

The fine aggregates used for the experimental work is locally procured and conformed to grading zone III. Sieve Analysis of the fine aggregate is carried out in the laboratory as per IS 383-1870. The sand is first sieved through 4.75mm sieve to remove any particle greater than 4.75 mm sieve and then washed to remove the dust. The physical properties and sieve analysis of fine aggregates are shown in *Table 4.3*and *Table 4.4* respectively.

***Table 4.3-Physical properties of Fine Aggregate.***

| S.No | Characteristic | Value |
|---|---|---|
| 1 | Specific gravity | 2.567 |
| 2 | Fineness modulus | 2.465 |
| 3 | Water absorption | 1.914% |
| 4 | Grading zone | Zone 3 |
| 5 | Bulk density | Loose=1.48g/cc<br>Compacted=1.6g/cc |

***Table 4.4-Sieve analysis of coarse aggregate.***

| S.No | Sieve size | Mass retained(gm) | Percentage passing (%) |
|---|---|---|---|
| 1 | 10 | 0 | 100 |
| 2 | 4.75 | 12.5 | 98.75 |
| 3 | 2.36 | 49 | 95.1 |
| 4 | 1.18 | 163.5 | 83.65 |
| 5 | 600 | 107.5 | 89.25 |
| 6 | 300 | 289.5 | 71.05 |
| 7 | 150µ | 306 | 69.4 |
| 8 | PAN | 59 | 94.1 |
| Total Weight taken = 1000 gm<br>Fineness Modulus of Fine Aggregate = 2.465 | | | |

### 4.2.3 Coarse Aggregate-

Crushed stone aggregate of size 20 mm and 10mm are used as coarse aggregate throughout the experimental study. The aggregates are washed to remove dust and dirt and are dried to surface dry condition. The aggregates are tested as per IS: 383-1970. The results of various tests conducted on coarse Aggregates are enlisted in *Table 4.5, table 4.6* and *table 4.7.*

***Table 4.5--Physical properties of coarse aggregate of 20mm and 10mm diameter.***

| S.No | Characteristic | Values of 20mm | Values of 10mm |
|---|---|---|---|
| 1 | Type | Crushed | Crushed |
| 2 | Specific gravity | 2.66 | 2.63 |
| 3 | Total water absorption | 0.64% | 0.56% |
| 4 | Fineness modulus | 8.625 | 6.833 |

*Table 4.6-Sieve analysis of coarse aggregate (20mm).*

| S.No | Sieve Size | Mass retained (kg) | Percentage retained | Cumulative percentage retained | Percentage passing |
|---|---|---|---|---|---|
| 1 | 220mm | 0.13 | 3.68 | 3.68 | 96.32 |
| 2 | 10mm | 3.28 | 92.91 | 96.59 | 3.41 |
| 3 | 4.75mm | 0.1 | 2.83 | 99.42 | 0.58 |
| 4 | PAN | 0.02 | 0.56 | ∑=199.69 | |
| Total Mass taken = 3.53 Kg<br>Fineness Modulus of coarse aggregate (20 mm)=<br>199.69+500)/100 = 6.99 | | | | | |

*Table 4.7-Sieve Analysis of Coarse Aggregate (10 mm).*

| S.No | Sieve Size | Mass retained (kg) | Percentage retained | Cumulative retained | Cumulative percentage retained |
|---|---|---|---|---|---|
| 1 | 20mm | 0 | 0 | 0 | 100 |
| 2 | 10mm | 0.7 | 49.64 | 49.64 | 50.36 |
| 3 | 4.75mm | 0.61 | 43.26 | 43.26 | 7.1 |
| 4 | PAN | 0.1 | 7.09 | ∑=142.54 | |

### 4.2.4 Water-

Fresh and clean tap water is used for casting the specimens in the present study. The water is relatively free from organic matter, silt, oil, sugar, chloride and acidic material as per Indian standard.

### 4.2.5 Bacterial culture-

Bacillus is used for this work which is extracted from $CT_5$ strain. The inoculation of the bacteria Bacillus from $CT_5$ strain is done by sequential order fulfilling all conditions of their growth.

### 4.2.6 Inoculation process of bacterial culture-

First of all Nutrient Broth (NB) were added in 50Ml of water. The quantity of NB added was calculated as-

13 gm = 1000 Ml

50mL = ——— = 0.65 gms

0.65 gms of NB were added in water then obtained solution were autoclaved for 30 minutes. After that calcium hydroxide which was act as cementious supplement were added in 50 Ml of water in quantity as calculated below.

1000 ml = 74.09 gms

50 ml = ——— = 3.7045 gms.

The ph of complete 100 ml solution must maintain between 9-12 which is the required pH for growth of bacteria. The solution obtained is kept in shaker for 24hrs at $27^0$C.After 24 hrs calcium acetate, urea and $CT_5$ is added in the solution. The amount of these chemicals and bacterial strain added is calculated below.

- ***Calcium acetate-***

We required 25Mm so $N_1V_1$

$= N_2V_2$

1000mM × X = 25mM × 100 ml

X = ——— = 2.5 Ml

- ***Urea-Consider 40%***

40 × X = 2 × 100

X = —— = 5ml.

- ***Bacterial strain $CT_5$.***

It is always added 0.5% - 0.7% of total solution. Let us adding 0.5% of total solution; 0.5 % of 100 ml = .5 ml

After addition of all these three components in 100 ml solution the final which comes out is known as *Bacterial culture.*

After that Nutrient Agar (NA) which is a solidifying agent through which we can check the growth of bacteria by pouring. Pouring is a process by which NA is poured on Petri plate and leave for 120 seconds so that it gets solidifies after that bacterial

culture is spreaded by spreader over it. For that amount of NA prepared was calculated as;

$$1000 = 28\text{gms}$$

$$200 = \frac{\quad}{\quad} = 5.6 \text{ gms.}$$

5.6 gms of NA were added in 200 ml of water and then prepared samples were kept in cold room where temperature was $-20^0$ C.Culture serial dilution were prepared to check level of growth of bacteria. Culture serial dilution were made in ratio 1:10 that is for each dilution 1 ml of culture were added in 9 ml of water then from each dilution 1ml, 1 ml of culture were taken to add in next 9 ml of water to form continuous serial dilution like $10,10^{-1},10^{-2}$ and $10^{-3}$ cells/ml respectively. After that poring of each dilution were done on Petri plate and kept in BOD chamber for 24 hrs and more than 24hrs to check bacterial growth After observation it was found that $10^{-10}$ cells/ml showed maximum bacteria growth. So, 10-$^{10}$ cells/ml was used for whole set of experiments in this report.

## 4.3 DESIGN OF CONCRETE MIXES-

Concrete mix was designing as IS guidelines. The w/c ratio of the mix was kept as 0.47 and then the ratio of cement: sand: coarse aggregate was obtained. The mix is designed as per Indian Standard Guidelines. The ratio of cement: sand: coarse aggregate was comes out to be 1:1.54:2.86.The water –cement ratio is 0.47 and compressive strength of concrete after 28days is 36 MPa.

### 4.4 SPECIMEN PREPERATION-

The mixes were divided in to two broad categories; control mix and microbial concrete mix. Further microbial concrete was obtained by different procedures of addition of bacteria in to concrete. On this basis following four types of microbial concrete were cast.

Case1-Bacterial mixed and water cured.

Case2-Water mixed and bacterial cured.

Case3-Bacterial mixed and bacterial cured.

Case4-Bacterial mixed and bacterial sprayed.

The specimen preparation procedure for each case is given in detail in the following sections.

### 4.4.1 Casting of control cubes-

Cubes of size 150mm×150mm×150mm were casted by making mixtures taking cement: Sand: aggregate in 1:1.54:2.86 and water-cement ratio of 0.47.Cement, sand and aggregate of size 20mm and 10mm were collected. Mould of size 150mm×150mm×150mm were cleaned and mould properly. After completing the preparation of mould. All sides of mould were greased properly. Dry mix was prepared by mixing cement, sand and water. Dry mix prepared was left for 2 minutes. Then dry mix was filled in mould in three layers by compacting each layer by tampering rod. For complete compaction mould filled with dry mix is kept on vibrator and top surface of mould were prepared evenly. After that mould is kept for 24 hrs.After 24 hrs of casting demoulding was done by removing cube from mould. Then cubes demoulded from mould were dipped in water for specified number of testing days.

### 4.4.2 Preparation of microbial concrete cubes-

Casting of microbial cubes was done by considering different cases. The procedure adopted for each case is explained in the following section.

#### 4.4.2.1 Case 1-Mixed with Bacterial culture and Cured in water-

In this case, bacterial culture was used instead of water for casting the bacterial culture/cement ratio was kept at 0.47.Casting of cubes was same process as adopted for control specimen. After demoulding the cubes were dipped or cured in water for specified testing days.

#### 4.4.2.2 Case 2-Mixed with water and cured in bacterial culture-

In this case, cubes were cast like control specimens only and then after 24hr, demoulded cubes were cured in bacterial culture for specified number of testing days.

#### 4.4.2.3 Case 3-Mixed with bacterial culture and cured in bacterial culture-

In this case, bacterial culture was used instead of water for casting the bacterial culture/cement ratio was kept at 0.47.Casting of cubes was same process as adopted

for control specimen .After 24 hrs of casting demoulded cubes were cured in bacterial culture.

#### 4.4.2.4 Case 4-Mixed with bacterial culture and sprayed by bacterial culture-

In this case, bacterial culture was used instead of water for casting the bacterial culture/cement ratio was kept at 0.47.Casting of cubes was same process as adopted for control specimen .After 24 hrs of casting the demoulded cubes were dipped in water for 24hrs and then cubes were taken out from water and for specified days of testing bacterial culture WAS sprayed on each cube individually.

### 4.5 CURING OF CUBES-

Curing of cubes after demoulding from mould can be done in different ways. In some cases cubes were dipped in water for specific days of testing i.e. 3, 7 and 28 days. In some cases instead of water cubes were cured in bacterial culture for specified days i.e. 3, 7 and 28days.In some case after 24 hrs of curing of cubes in water it was taken out and bacterial spraying were done for specific days of testing i.e. 3, 7 and 28days.
4.7

### 4.6 DESIGNATION OF MIXES-

The five cases which were considered for testing were represented as shown in *Table 4.8.*

***Table 4.8-Designation of mixes.***

| S.No. | Description of Mix | Designation |
|---|---|---|
| 1- | Water mixed and water cured | $M_0$ |
| 2- | Bacterial mixed and water cured | $M_1$ |
| 3- | Water mixed and bacterial cured | $M_2$ |
| 4- | Bacterial mixed and bacterial cured | $M_3$ |
| 5- | Bacterial mixed and bacterial sprayed | $M_4$ |

### 4.7 PROCESS OF TESTING-

Different testing processes which involved in this report are, test for compressive strength, pH value determination, estimation of calcium carbonate precipitation by EDTA, SEM and XRD.

### 4.7.1 Compressive strength test-

Out of many test applied to the concrete, this is the utmost important which gives an idea about all the characteristics of concrete. By this single test one can judge that whether Concreting has been done properly or not. For this ,concrete cubes of size 150mm×150mm×150mm were prepared. The cube which has to be tested for compressive strength was taken out from curing media and then surface water shall be wiped off. Before placing the specimen in the testing machine the bearing surface of the testing machine shall be wiped clean and any loose sand or other material removed from the surface of the specimen. In case of cubes, the specimen shall be placed in the machine in such a manner that the load shall be applied to opposite sides of the cubes as cast, that is, not to the top and bottom. The axis of the specimen shall be carefully aligned with the centre of thrust of the spherically seated platen. The load shall be applied without shock and increased continuously at a rate of approximately 140 $kg/cm^2$/min.The maximum load applied to the specimen shall then be recorded and appearance of the concrete and any unusual features in the type of failure shall be noted. Readings were taken on each 3, 7 and 28days.

### 4.7.2- pH value test-

pH value of concrete specimen determined any detrimental effect that bacteria can cause on the formation of passive layer that prevents rebar from its corrosion. For determining pH of the given samples, crushed concrete obtained after conducting compressive strength was taken were crushed in to powdered form which can pass through 300µ sieve. Powdered sample was mixed with water in ratio of 1:9 in test tube and left for overnight, next day this sample was tested by pH meter to get pH of the mix. The pH was noted corresponding to two layers on concrete cubes. One sample was taken for the top surface to represent surface concrete and other was taken from near the centre of concrete to represent bulk concrete for 3,7 and 28 days.

### 4.7.3-EDTA

To confirm calcium carbonate precipitation, EDTA test were performed on powdered sample of top surface were tested on 28days.0.5 gms powdered sample were taken in a beaker and 3ml of HCl were mixed in that. Then 43 ml of water were mixed in to the solution obtained and 4 ml of NaOH were added to prepare complete solution. A drop of indicator hydroxyl napthol blue were added which turns whole solution in to light pink colour. The pink solutions obtained were titrated against EDTA which at a

point converts pink solution in to blue colour. This conversion shows the presence of calcium carbonate.0.1ml of EDTA used correspond to 5.006 mg of $CaCO_3$ per gram of samples.

### 4.7.4- XRD

XRD determines the orientation of single crystal or grain. By this size, shape and internal stress of small crystalline regions can also be determined. For performing X-Ray Diffraction (XRD) The powdered samples were further passed through 90 µ sieve. The sample so obtained was mounted on the glass fibre filter using tubular aerosol suspension chamber (TASC). After placing the sample in the chamber, the mass absorption coefficient of the sample was determined by x-ray transmission. The XRD pattern was observed by scanning the sample from 10-80 degrees, 2 theta and having Cu radiation and graphite monochromatic with a current of 30 KV and a voltage of 40Mv by using a vertical x-ray diffract meter. The XRD pattern shows the peakes of different element present in the sample.

### 4.7.5 SEM-

This was performed to validate microbial calcite formation in the concrete. Scanning Electron Microscopy (SEM) is conducted to study the micro-structure properties of the samples. The test was conducted at 28 days of casting and one small piece of broken specimen of concrete was taken to represent the bulk structure of concrete.The specimen was monitored under SEM to see the presence of CaCO3 crystal in bacteria.This test is used to identify the changes which had occurred inside the micro-structure and also the formation and deformation of the phases.

### 4.7.6 MIP-

For determining the porosity of cubes top of the sample piece were taken and 1cm×1cm size of specimen were prepared. The samples were tested on mercury intrusion porosimeter.

# CHAPTER 5
# RESULT AND DISCUSSION

## 5.1 GENERAL-

In this chapter, results obtained from different test investigation are presented. The concrete specimen was cast, as explained in the experimental programme and was tested for various properties at the age of 3, 7 and 28 days. The results obtained are discussed in the following section.

## 5.2 COMPRESSIVE STRENGTH MEARUREMENT-

Control cubes and microbial cubes for four different mixes such as $Mix_1$ - specimens were mixed with bacterial culture and cured in water; $M_2$- specimens were mixed with water and cured in bacterial culture; $M_3$-specimens were mixed with bacterial culture and cured in bacterial culture and $M_4$-specimens were mixed with bacterial culture and after 24 hrs of curing in water sprayed with bacterial culture were cast. After casting, curing of cubes was done in different ways, either in water of in bacterial culture. The cube which has to be tested for compressive strength was taken out from curing media and then surface water shall be wiped off. Before placing the specimen in the testing machine the bearing surface of the testing machine shall be wiped clean and any loose sand or other material removed from the surface of the specimen. In case of cubes, the specimen shall be placed in the machine in such a manner that the load shall be applied to opposite sides of the cubes as cast, that is, not to the top and bottom. The axis of the specimen shall be carefully aligned with the centre of thrust of the spherically seated platen. The load shall be applied without shock and increased continuously at a rate of approximately 140 kg/cm$^2$/min.The maximum load applied to the specimen shall then be recorded and appearance of the concrete and any unusual features in the type of failure shall be noted. Readings were taken on each 3, 7 and 28days.

The results obtained from the testing are tabulated in *Table 5.1*. From *Fig 5.2*, it can be seen that increase in strength is maximum at 3 days. It shows that till 3 days, bacteria grows properly and has completely adopted the atmosphere inside concrete cube. As a bacterium grows, calcium carbonate precipitation starts and increases the

compressive strength of concrete. It is observed by other researcher *(Ghos et al. (2005), Jagdeesha et al. (2013)).*

Layer of calcium carbonates so obtained on concrete cube is known as microbially induced calcite precipitation. Since the bacteria grow only in aerobic condition, the maximum precipitation occurs on the surface of concrete. Slowly After 7 days there were increase in compressive strength were observed due to formation of calcite layer. A significant increase of 21%, 18.33%, 13.88% and 23.88% were observed for different cases i.e. case1, case2, case3 and case 4 in comparison to control case respectively. After 28 days it was observed that, the effective increase in compressive strength was less as compared to 3 days strength. Because once all the pores on surface is blocked, anerobic condition arises in the concrete and the activity of bacteria slows down, with bacteria converting themselves in to spores. This can be explained by several researchers. It is also concluded from the result that there was no as such difference in strength were observed if bacterial source were used in different forms. That means microbial effect influence concrete in same manner whether it is used in any form.

***Table 5.1- Compressive strength.***

| **Mixes** | **Compressive Strength ,MPa** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **M** | **M1** | **% relative Increase with control** | **M2** | **% relative Increase with control** | **M3** | **% relative Increase with control** | **M4** | **% relative Increase with control** |
| 3 Days | 17.2 | 27.6 | 60 | 28.8 | 67.44 | 29 | 68.60 | 28.5 | 65.69 |
| 7 Days | 31.6 | 38.7 | 22.4 | 39.5 | 25 | 37.5 | 18.67 | 38.4 | 21.51 |
| 28 Days | 36 | 43.6 | 21.11 | 42.6 | 18.33 | 41 | 13.88 | 44.6 | 23.88 |

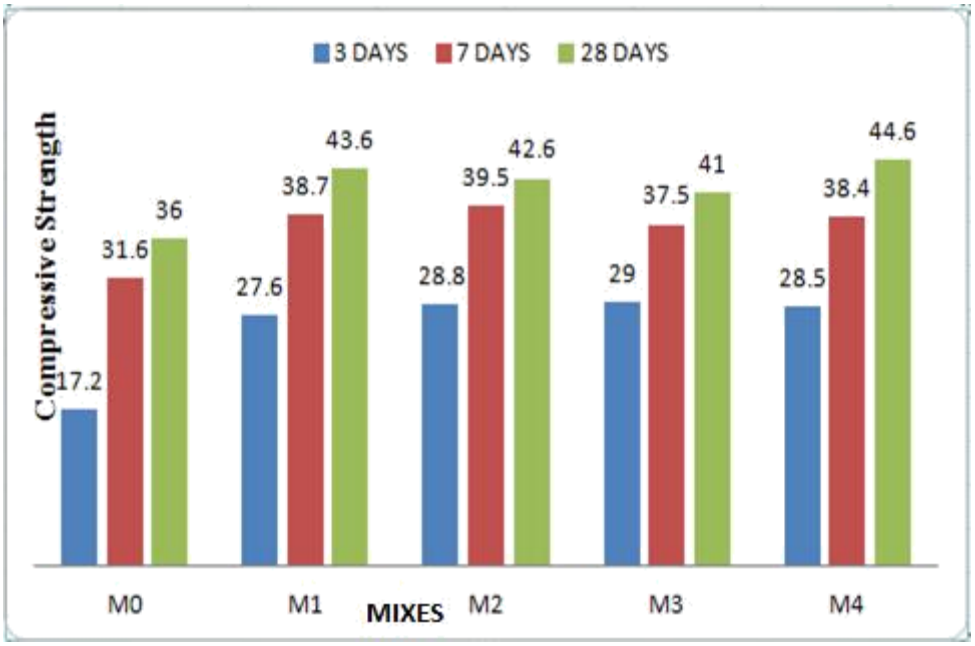



***FIG 5.1- Compressive strength of different mixes.***

**5.3 pH MEASUREMENT-**

For determining pH of the given samples, crushed concrete obtained after conducting compressive strength was taken were crushed in to powdered form which can pass through 300µ sieve. Powdered sample was mixed with water in ratio of 1:9 in test tube and left for overnight, next day this sample was tested by pH meter to get pH of the mix. The pH was noted corresponding to two layers on concrete cubes. One sample was taken for the top surface to represent surface concrete and other was taken from near the centre of concrete to represent bulk concrete for 3,7 and 28 days.

Calcium hydro-oxide in powder form was used as cement supplement for checking growth of bacteria. pH measured in bacterial culture for 1day, 2 days and 3 days were 12.5,12.3 and 12.18 respectively. That means the pH of bacterial culture used for treatment of concrete was maintained at 12.

*Table 5.2* shows pH result of top and bulk surface of concrete cubes for 3, 7 and 28 days. From the *Fig 5.2* and *Fig 5.3*, it is observed for top and between surfaces that for control specimens, the pH observed was approximately 12 on each day. For other cases addition of bacterial culture in concrete in any form do not effect on pH property *(Fig 5.2* and *Fig 5.3).* It is observed that that there is no change in pH value after addition of bacterial culture in any form in concrete. Even if we observed the pH value at different dept of concrete cube that is at top surface and at bulk surface, we did not found any considerable change in pH. That means involvement of bacterial

culture shows detrimental effect that bacteria can cause on the formation of passive layer that prevents rebar from its corrosion.

***Table 5.2- pH of specimens.***

| Days | Ph | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | TOP SURFACE | | | | | BULK SURFACE | | | | |
| | **M0** | **M1** | **M2** | **M3** | **M4** | **M0** | **M1** | **M2** | **M3** | **M4** |
| 3 days | 11.9 | 12.5 | 12.8 | 12.3 | 12.4 | 12.87 | 12.5 | 12.7 | 12.5 | 12.3 |
| 7 days | 12.3 | 12.1 | 12.35 | 12.6 | 12.2 | 12.7 | 12.8 | 12.6 | 12.3 | 12.63 |
| 28days | 12.4 | 12.8 | 12.3 | 12.1 | 12.5 | 12.4 | 12.8 | 12.3 | 12.1 | 12.5 |

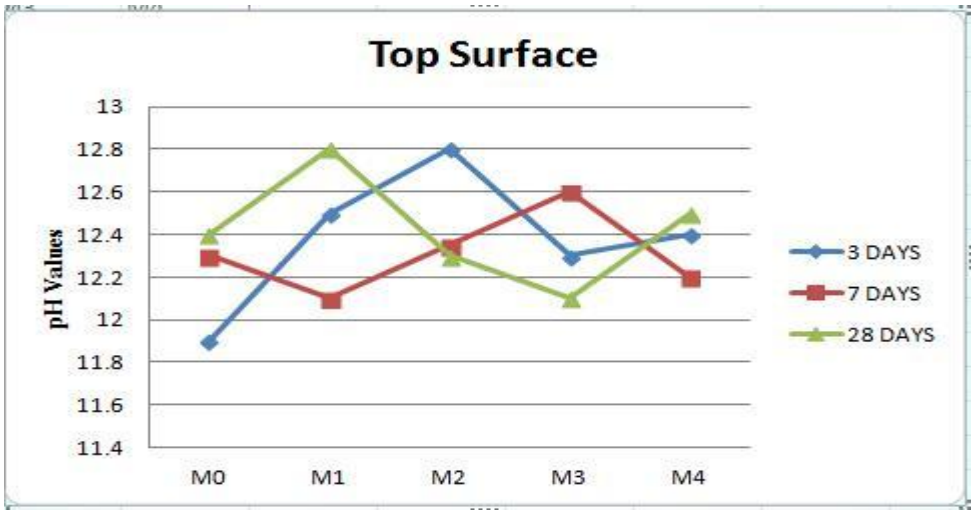



***Fig 5.2-pH of top surfaces.***

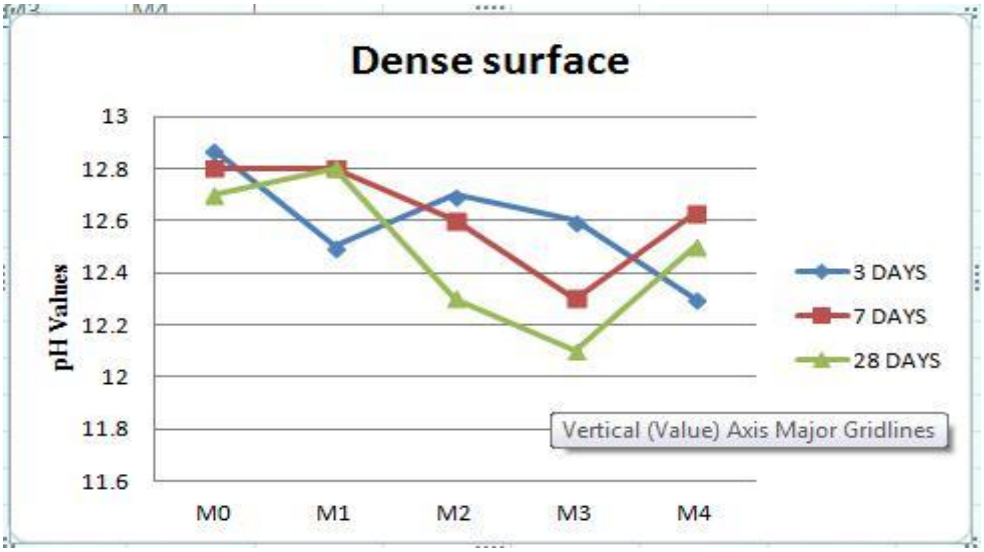



***Fig 5.3-pH of between surfaces.***

## 5.4 EDTA CALCULATION-

By this test we can confirm the presence of calcium carbonate in the powdered sample obtained from broken cubes. Solution was prepared by mixing provided sample of concrete with sodium hydroxide and water. A drop of an indicator, hydroxy napthol blue was added to the obtained solution which gives light pink colour to solution. The pink coloured solution so obtained was further titrated against EDTA.During titration, the pink colour solution changes its colour from pink to violet at a certain point shown in *Fig 5.4*.The variation of pink colour to violet colour confirms the presence of calcium carbonate in the concrete sample. Table 5.3 shows the amount of EDTA added for per gram of sample and the amount of EDTA added can be correlated with the amount of $CaCO_3$ formed *(Maheswaran et al.(2008),Muyunck et al.(2006))*.Since EDTA consumed is more in bacterial concrete, it confirms larger amount of $CaCO_3$ precipitation. From Table 5.3 it is clear that amount of presence of calcium carbonate is more in bacterial mixes in comparison to non-bacterial mix. For all bacterial mixes amount of calcium carbonate consumption is nearly same. This shows that bacterial incorporation leads to more $CaCO_3$ precipitation, irrespective of its process of addition.

1 ml of EDTA used = 5.006 mg of $CaCO_3$/grams.

***Table 5.3 EDTA calculation.***

| **Mixes** | **EDTA changes(mL)** | **EDTA used** | **$CaCO_3$ calculation/grams** | |
|---|---|---|---|---|
| | | | **For 1 gm** | **For 0.5 gm** |
| $M_0$ | 41.4 -42.2 | 1 | 5.006 | 2.503 |
| $M_1$ | 36.3-41.2 | 4.9 | 24.52 | 12.26 |
| $M_2$ | 45.2-52.4 | 7.2 | 36.0432 | 18.216 |
| $M_3$ | 35.5-42 | 6.5 | 32.539 | 17.7695 |
| $M_4$ | 38.7-43.9 | 5.2 | 26.0312 | 13.0156 |


***Fig 5.4-colour changes from pink to violet.***

**5.5 XRD RESULT-**

XRD determine the orientation of a single crystal or grain. Crystal structure of an unknown material can be found out by XRD.By this, size, shape and internal stress of small crystalline regions can also be determined. For performing X-Ray Diffraction (XRD) The powdered samples were further passed through 90 µ sieve. The sample so obtained was mounted on the glass fibre filter using tubular aerosol suspension chamber (TASC). After placing the sample in the chamber, the mass absorption coefficient of the sample was determined by x-ray transmission. The XRD pattern was observed by scanning the sample from 10-80 degrees, 2 theta and having Cu radiation and graphite monochromatic with a current of 30 KV and a voltage of 40Mv by using a vertical x-ray diffract meter. The XRD pattern shows the peaks of different element present in the sample.

*Fig 5.5, 5.7, 5.8, 5.9* shows the XRD images of all specimens including bacterial mixes and non-bacterial mixes. From the figures we can see the peaks of different individual elements like quartz, dicalcium silicate, tricalcium silicate, dicalcium dialuminium silicate and calcite. It can be observed from *Fig 5.5, 5.6, 5.7, 5.8* and *5.9* that control specimen do not have any specific calcite peak. Amount of silicate

present in non bacterial mix was found out to be 65%.In other bacterial mixes, calcite peaks were observed for the angle in between $40^0$-$50^0$.

Calcite peaks confirms the presence of calcium carbonate in the bacterial sample. Amount of silica present in bacterial mixes were more than non bacterial mix. Amount of silica present in bacterial sample was in between 80 to 85%.More amount of silica present in the bacterial mixes form more CSH gel. This large amount of silica can be correlated to more CSH gel and hence increase in compressive strength.

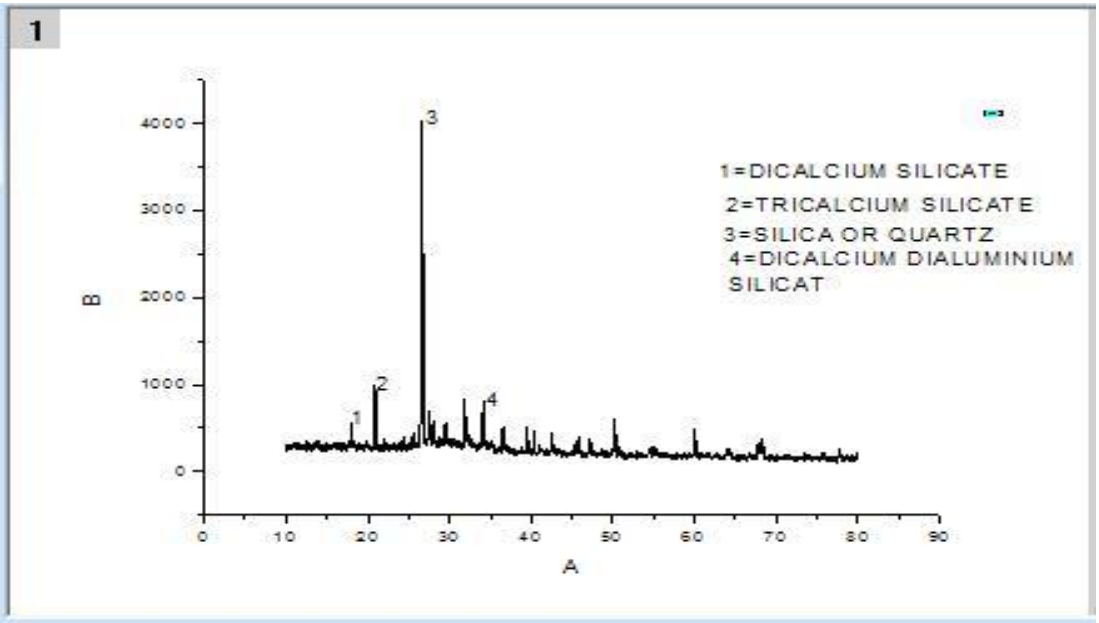



***Fig 5.5-XRD pattern for Control specimen (M0)***

***Table 5.4-Pattern list for Control specimen (M0)***

| Compound name | Displacement (2θ) | Scale factor | Chemical formula | Percentage |
|---|---|---|---|---|
| Quartz | 0.036 | 0.937 | $SiO_2$ | 65 |
| Calcium Hydroxide | 0.131 | 0.112 | $Ca(OH)_2$ | 7 |
| Dicalcium silicate | -0.086 | 0.042 | $Ca_2(SiO_4)$ | 12 |
| Calcium aluminium | 0.003 | 0.045 | $CaAl_2Si2O_8$ | 16 |

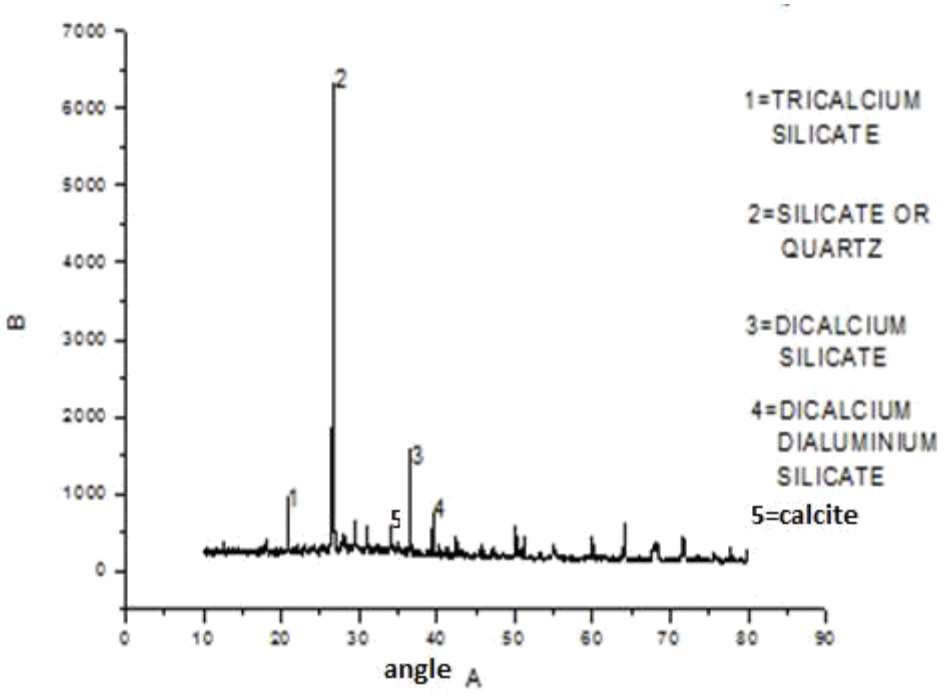



***Fig 5.6-XRD pattern for Case-1(M1)***

***Table 5.5-Pattern list for Case-1 (M1)***

| **Compound name** | **Displacement (2θ)** | **Scale factor** | **Chemical formula** | **Percentage** |
|---|---|---|---|---|
| Quartz | -0.003 | 1.004 | $SiO_2$ | 82 |
| Calcium Hydroxide | 0.039 | 0.045 | $Ca(OH)_2$ | 4 |
| Dicalcium silicate | -0.130 | 0.022 | $Ca_2(SiO_4)$ | 8 |
| Calcium aluminium Silicate | 0.031 | 0.017 | $CaAl_2Si2O_8$ | 6 |

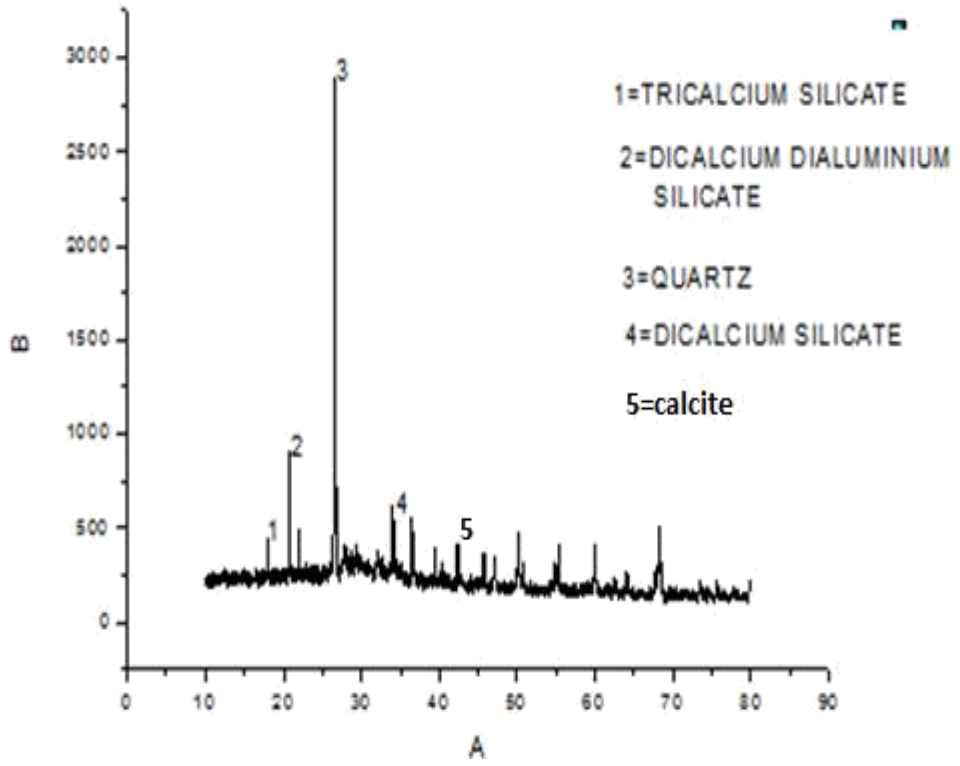



***Fig 5.7-XRD pattern for Case-2 (M2)***

***Table 5.6-Pattern list for Case-2 (M2)***

| **Compound name** | **Displacement (2θ)** | **Scale factor** | **Chemical formula** | **Percentage** |
|---|---|---|---|---|
| Quartz | 0.003 | 0.986 | $SiO_2$ | 80 |
| Calcium Hydroxide | -0.115 | 0.121 | $Ca(OH)_2$ | 9 |
| Dicalcium silicate | -0.037 | 0.035 | $Ca_2(SiO_4)$ | 11 |
| Calcium aluminium Silicate | 0.031 | 0.017 | $CaAl_2Si2O_8$ | 10 |

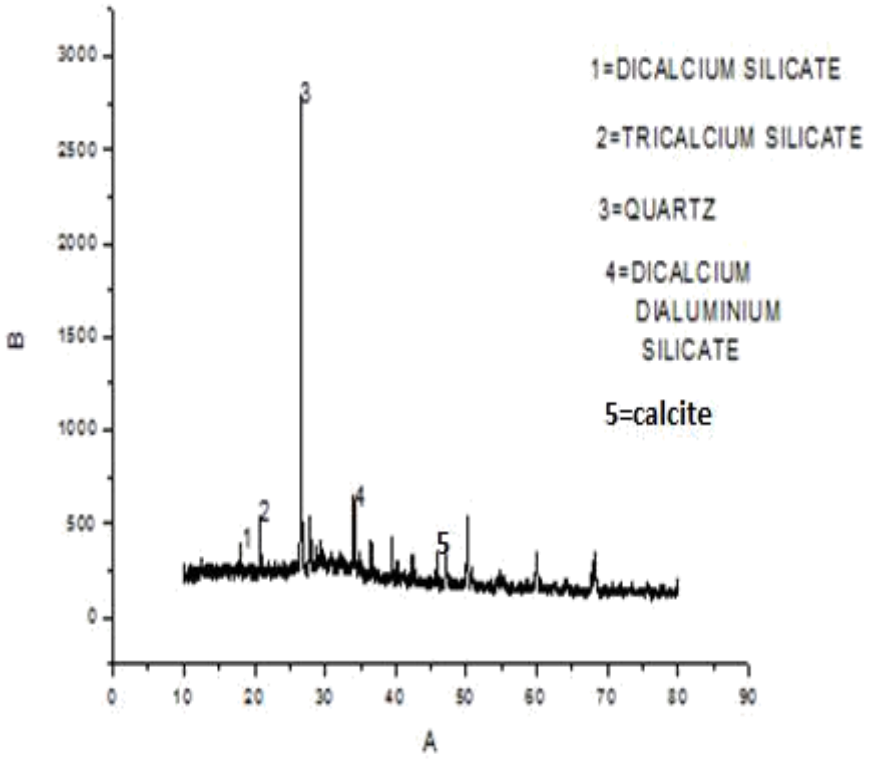



*Fig 5.8-XRD pattern for Case-3 (M3)*

*Table 5.7-Pattern list for Case-3 (M3)*

| Compound name | Displacement (2θ) | Scale factor | Chemical formula | Percentage |
|---|---|---|---|---|
| Quartz | -0.052 | 0.955 | $SiO_2$ | 81 |
| Calcium Hydroxide | 0.184 | 0.106 | $Ca(OH)_2$ | 10 |
| Dicalcium silicate | -0.229 | 0.061 | $Ca_2(SiO_4)$ | 6 |
| Calcium aluminium Silicate | -0.077 | 0.062 | $CaAl_2Si2O_8$ | 20 |

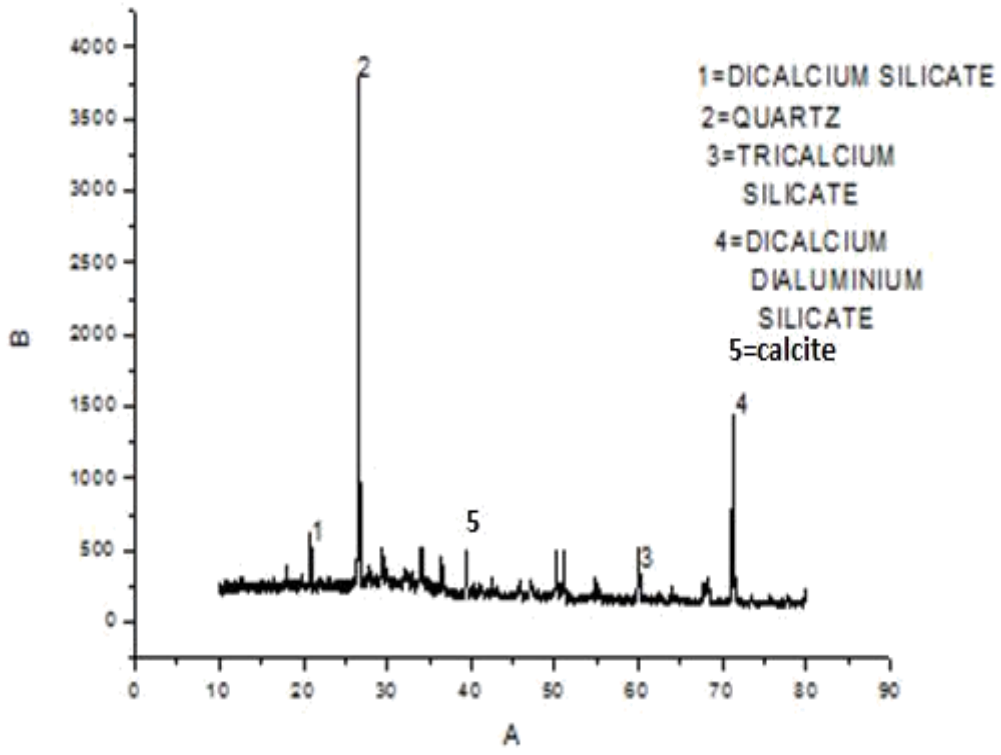



***Fig 5.9-XRD pattern for case-4 (M4)***

***Table 5.8-Pattern list for case-4 (M4)***

| **Compound name** | **Displacement (2θ)** | **Scale factor** | **Chemical formula** | **Percentage** |
|---|---|---|---|---|
| Quartz | -0.018 | 0.994 | $SiO_2$ | 78 |
| Calcium Hydroxide | -0.011 | 0.039 | $Ca(OH)_2$ | 8 |
| Dicalcium silicate | 0.055 | 0.044 | $Ca_2(SiO_4)$ | 5 |
| Calcium aluminium Silicate | 0.032 | 0.026 | $CaAl_2Si2O_8$ | 9 |

## 5.6 SEM RESULT -

To validate calcite formation in the concrete, a piece of broken sample from each mix was further investigated under SEM. The SEM was performed at 28 days. Bacteria incorporated samples in each case on 28 days were tested as they showed maximum compressive strength. *Fig 5.10 a, b, c, d and e* shows the results of all cases. From the figures it is observed that, for control specimens without bacterial culture no crystals of calcite were observed. For all other cases with bacterial culture presence of

lamellar rhombohedral crystals of calcite and needle-shaped aragonite crystals of $CaCO_3$ were observed, which shows that all cases supports continuous formation of calcite. *Fig 5.7 c* further confirms the presence of bacterial cell embedded between concrete. From all the images acquired from SEM, it was very clear that for all cases except control specimen without bacteria, and density and clarity of crystal in rest of the cases were same. This implies that equal amount of calcium carbonate precipitation were observed in all cases which form a layer of calcite on the surface of cube samples.

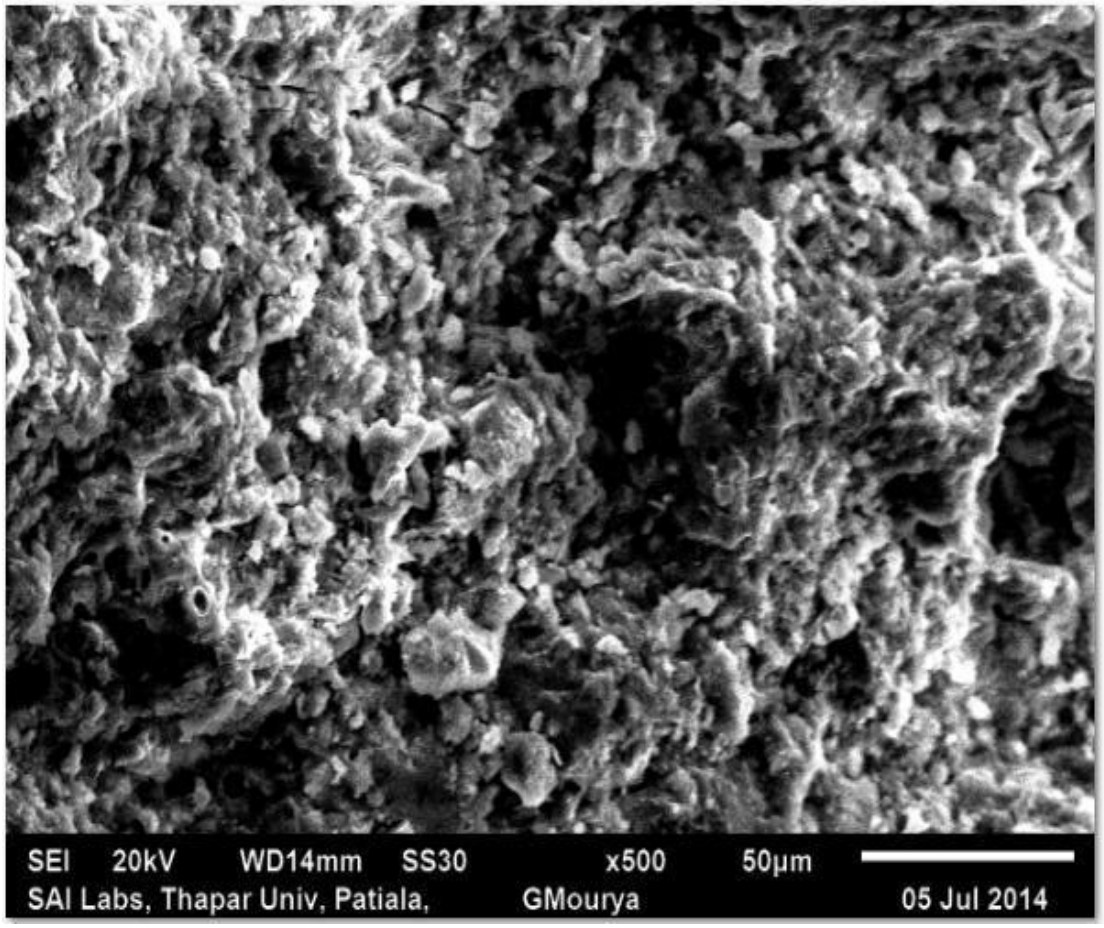



***Fig 5.10 (a)-SEM image of control specimen (M0)***

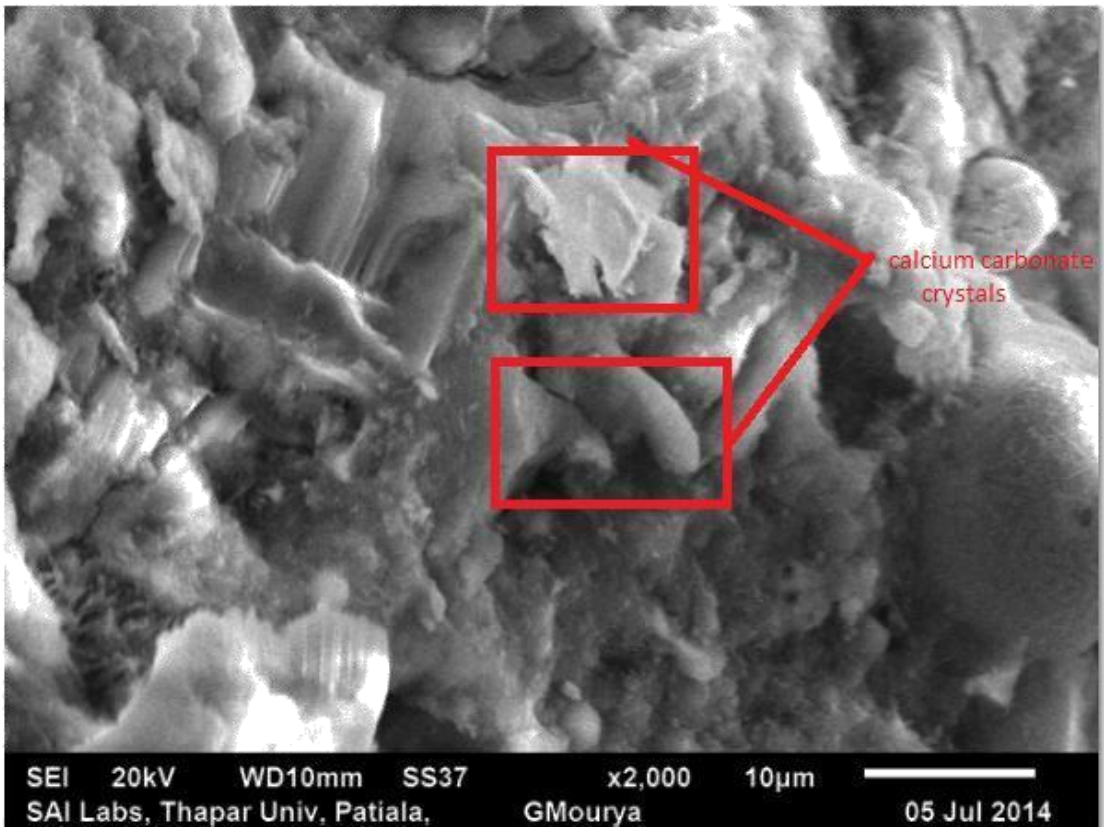



*Fig 5.10 (b)-SEM image of case-1 (M1)*

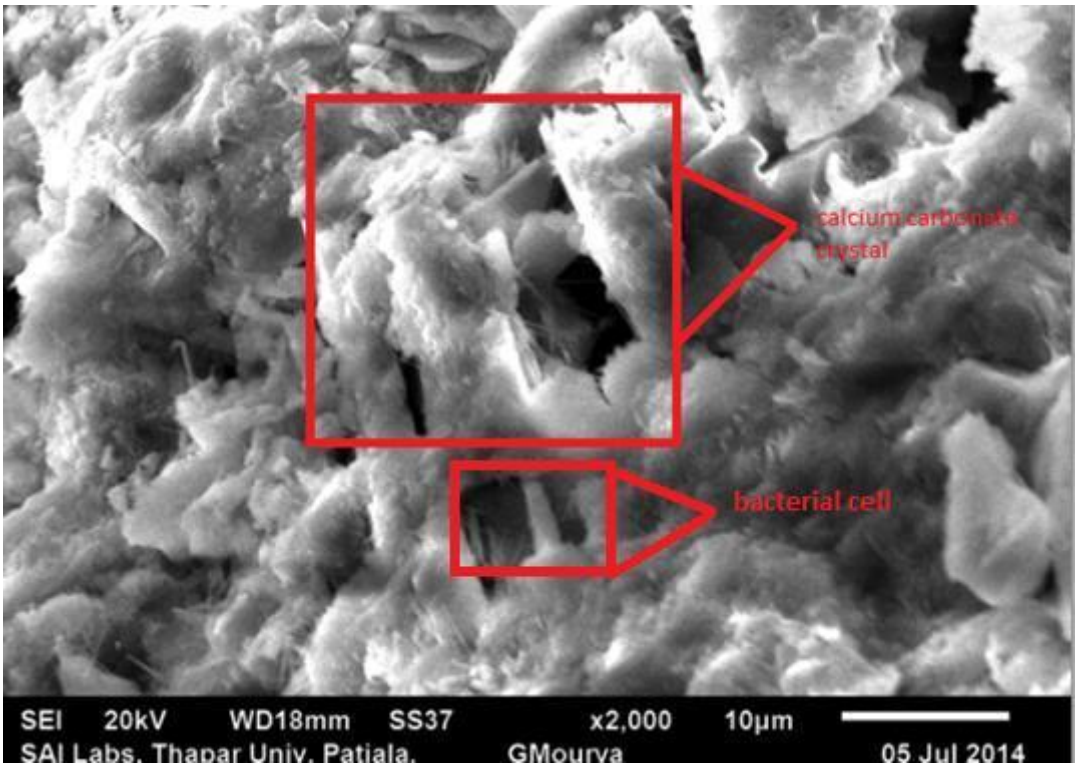



*Fig 5.10 (c)-SEM image of case-2 (M2)*

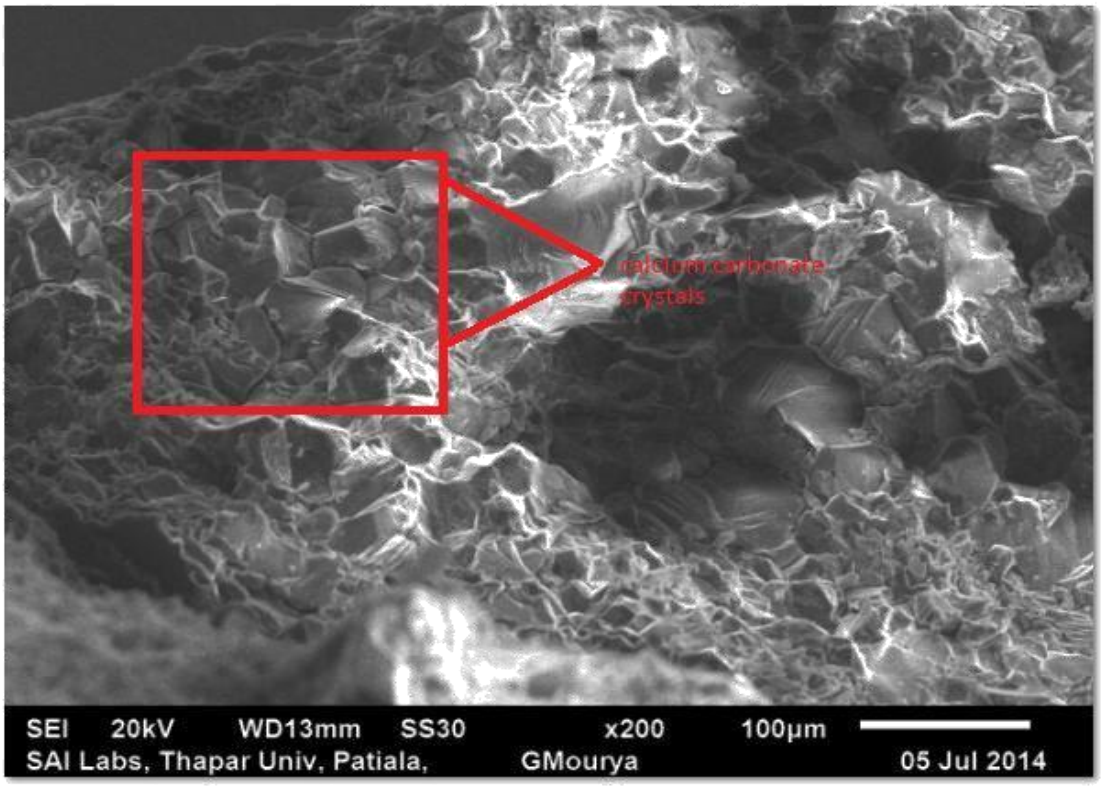



*Fig 5.10 (d)-SEM image of case-3 (M3)*

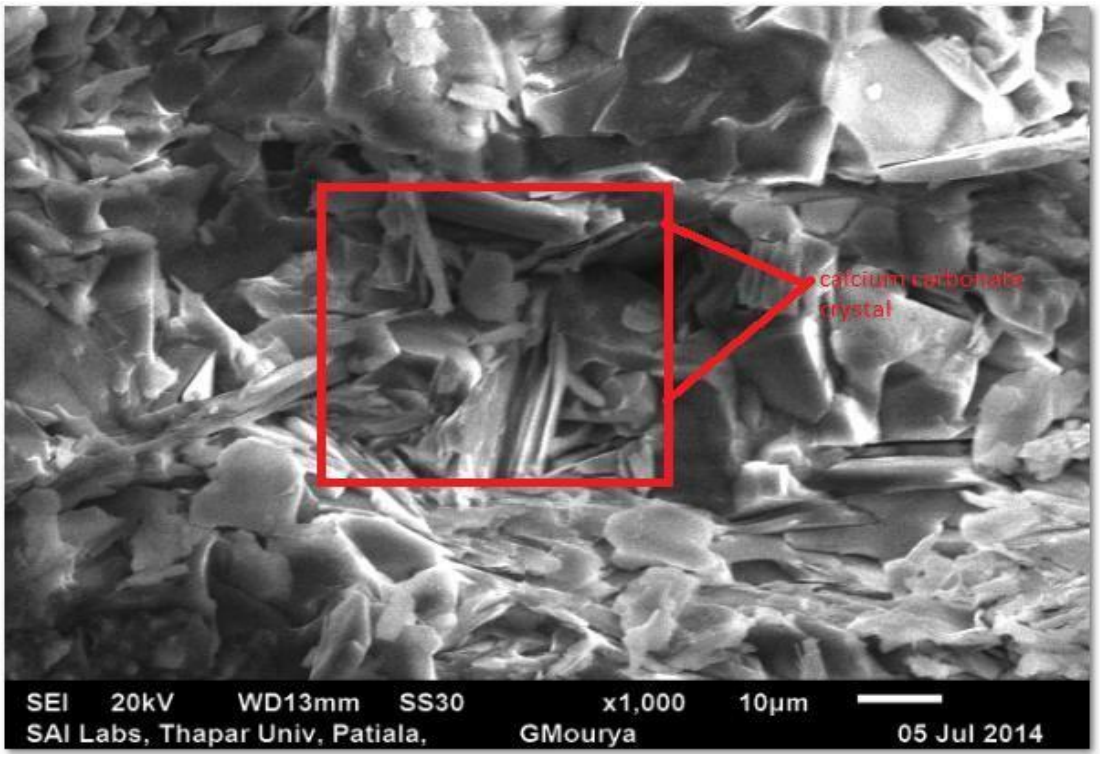



*Fig 5.10 (e)-SEM image of case-4 (M4)*

# CHAPTER 6
# CONCLUSION

In this chapter, results acquired from all the tests performed on cube samples are summarized.

1. From compressive strength result and discussion, it is clear that participation of bacteria in any form in concrete cubes increases the strength in comparison to control cubes. It is observed from outcome result that, initially after 3 days bacteria activities were on its peak. Radical changes of about 60% were observed. After 7 days increase in compressive strength were significant. After 28 days there were considerable increases in strength but not much effective as after 3 days. Because once all the pores on surface is blocked, anerobic condition arises in the concrete and the activity of bacteria slows down, with bacteria converting themselves in to spores. It is also concluded from the result that there was no as such difference in strength were observed if bacterial source were used in different forms. That means microbial effect influence concrete in same manner whether it is used in any form.
2. pH of all samples were calculated and it is found out to be constant for all considered days 3, 7 and 28 days respectively. Involvement of bacterial culture shows detrimental effect that bacteria can cause on the formation of passive layer that prevents rebar from its corrosion.
3. EDTA shows the presence of calcium carbonate precipitation by changing pink colour solution into violet colour.
4. SEM results show rhombohedral crystal structure of calcite in bacterial concrete which confirms the presence of calcium carbonate precipitation. Some bacterial cell embedded in concrete was also observed.
5. XRD shows the peaks of calcite in microbial concrete, which confirm the presence of calcium carbonate in microbial concrete. Amount of silica present in bacterial mixes were more than non bacterial mix. Amount of silica present in bacterial sample was in between 80 to 85%.More amount of silica present in the bacterial mixes form more CSH gel. This large amount of silica can be correlated to more CSH gel and hence increase in compressive strength.

# CHAPTER 7

# FUTURE SCOPE

No research is complete in itself, it has a long distance to cover. Some more area can be covered, like long term durability, long term deterioration, carbonation induced corrosion and sulphate resistance etc if present work will be extended.

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