

STUDEIS ON SELF-HEALING SUSTAINABLE CONCRETE USING BACTERIAL CARBONATE PRECIPITATE

Submitted in Partial fulfilment of the requirements for the award of bachelor of
engineering degree in Civil Engineering

By

LOKESHWER CJ

(37200036)

MOHAMMED ASIM L

(37200043)



DEPARTMENT OF CIVIL ENGINEERING

SCHOOL OF BUILDING AND ENVIRONMENT

SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY

(DEEMED TO BE UNIVERSITY)

Accredited with Grade “A” by NAAC

JEPPIAAR NAGAR, RAJIV GANDHI SALAI, CHENNAI - 600 119

APRIL - 2020



SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY

(DEEMED TO BE UNIVERSITY)

Accredited with "A" grade by NAAC

Jeppiaar Nagar, Rajiv Gandhi Salai, Chennai – 600 119

www.sathyabama.ac.in

DEPARTMENT OF CIVIL ENGINEERING

BONAFIDE CERTIFICATE

This is to certify that this Project Report is the Bonafide work of **LOKESHWER C J (REG. NO. 37200036)** and **MOHAMMED ASIM L (REG. NO. 37200043)** who carried out the project entitled “**STUDIES ON SELF HEALING SUSTAINABLE CONCRETE USING BACTERIAL CARBONATE PRECIPITATE**” under our supervision from December 2020 to April 2021.

Internal Guide,

Mr. Vanjinathan

Head of the Department

Submitted for Viva voce Examination held on_____

Internal Examiner

External Examiner

DECLARATION

WE, LOKESHWER C J (REG. NO. 37200036) and MOHAMMED ASIM L (REG. NO. 37200043) hereby declare that the Project Report entitled “STUDIES ON SELF HEALING SUSTAINABLE CONCRETE USING BACTERIAL CARBONATE PRECIPITATE” done by us under the guidance of Mr.Vanjinathan is submitted in partial fulfilment of the requirements for the award of Bachelor of Engineering degree in Civil Engineering.

1.

2.

SIGNATURE OF THE CANDIDATES

DATE:

PLACE:

ACKNOWLEDGEMENT

I am pleased to acknowledge my sincere thanks to board of management of **SATHYABAMA** for their kind encouragement in doing this project and for completing it successfully. We are grateful to them.

We convey our thanks to **Dr.Devayani Ganopadhyey B.arch., M.T.P., ph.D.**, Dean School of Building and environment and **Dr.S.Packialakshmi, M.E., ph.D.**, and **Dr.Padmapriya,M.E.,ph.D.**, Head of the department, Dept. of Civil engineering for providing us necessary support and details at the right time during the progressive reviews.

We would like to express our sincere and deep sense of gratitude to our project guide **Mr.Vanjinathan, M.Tech.**, for his valuable guidance, suggestions, and constant paved way for the successful completion of our project work.

We wish to express our thanks to all teaching and non-teaching staff members of the department of Civil Engineering who were helpful in many ways for the completion of the project.

ABSTRACT

Concrete is weak in tension. As a matter of fact, the concrete tends to propagate and crack. These propagation and cracks progress in time and make concrete vulnerable to environmental effects. Thus, self-healing of cracks can be helpful in mitigation of development of cracks. In this project works, the self-healing of cracks by bacterial carbonate precipitation and the efficiency of bacterial deposition on compressive strength between *Bacillus subtilis* and *Bacillus Megaterium* and with conventional concrete were compared. Bacteria were introduced into concrete using light weight aggregate i.e. LECA (light weight expansive clay aggregate). Calcium Lactate is used as nutrient (i.e. organic precursor) for the bacteria. Specimens were made for each type of bacteria and compared for changes in crack healing and compressive strength. Results showed that the concrete incorporated with *Bacillus subtilis* were more effective in crack healing than conventional and *Bacillus Megaterium* based concrete.

TABLE OF CONTENTS

CHAPTER No.	TITLE	PAGE NO.
	ABSTRACT	
	LIST OF TABLES	
	LIST OF FIGURES	
	LIST OF SYMBOLS AND ABBREVIATIONS	
1	INTRODUCTION	12
1.1	GENERAL	12
1.2	CHEMICAL PROCESS	13
1.3	PRINCIPLES GOVERNING BACTERIAL CARBONATE PRECIPITATION FOR BIOCEMENTATION	14
2	OBJECTIVE, SCOPE & LITRATURE REVIEW	15
2.1	OBJECTIVE	15
2.2	SCOPE	15
2.3	LITERATURE REVIEW	15
3	COMPOSITION OF CONCRETE	20
3.1	COMPOSITION OF CONCRETE	20
	3.1.1 Cement	
	3.1.2 Aggregate	

3.1.3	Bacteria	
3.2	MIX DESIGN	25
3.2.1	MIX PROPORTION	
3.2.2	MATERIALS	
3.2.3	TARGET STRENGTH	
3.2.4	WATER CEMENT RATIO	
3.2.5	WATER CONTENT	
3.2.6	CALCULATION OF CEMENT CONTENT	
3.2.7	VOLUME OF COARSE AGGREGATE AND FINE AGGREGATE	
3.2.8	MIX CALCULATIONS	
3.2.9	MIX PROPOTIONS	
3.3	METHODOLOGY	29
3.4	SPECIMEN DETAILS	30
3.4.1	PREPARING THE MOULDS	
3.4.2	CASTING OF SPECIMEN	
3.4.3	DEMOULDING THE TEST CUBES	
3.4.4	SETTING OF MOULDS	
3.4.5	CURING OF CUBES	
3.5	TEST CONDUCTED	33
3.5.1	COMPRESSIVE TESTING MACHINE	
3.5.2	SLUMP CONE TEST	
3.5.3	ENERGY DISPERSIVE X-RAY SPECTROSCOPY (EDAX)	
3.5.4	SCANNING ELECTRON MICROSCOPE (SEM)	
4	RESULTS AND DISCUSSIONS	38
4.1	COMPRESSIVE STRENGTH	38
4.2	MICROSCOPIC EVALUATION OF HEALING CRACK	39
4.3	SCANNING ELECTRON MICROSCOPE	41
4.4	ENERGY DISPERSIVE X-RAY SPECTROSCOPY	42

5	SUMMARY & CONCLUSION	43
6	REFERENCES	44

LIST OF TABLES

TABLE No.	TITLE	PAGE No.
3.1	Properties of fine aggregate	21
3.2	Properties of coarse aggregate	21
4.1	Compressive strength	38

LIST OF FIGURES

FIGURE No.	TITLE	PAGE No.
3.1	The dried powder bacteria	21
3.2	Bacillus Subtilis	23
3.3	Bacillus megaterium	24
3.4	Flowchart of methodology	29
3.5	Mould	30
3.6	Casting of Specimen	30
3.7	Demoulding the cubes	31
3.8	Curing of cubes	32
3.9	Compressive testing machines	34
3.10	Slump cone test	35
3.11	Energy dispersive x-ray spectrem	36
3.12	Scanning electron microscope	37
4.1	Graphical Representaion of Compressive Strength	38
4.2	Microscopic evalution of healing cracks	39
4.3	The evolution of crack healing process in the specimen (initial : 184µm; final : 0µm)	40
4.4	The evolution of crack healing process in the specimen (initial : 507µm; final : 0µm)	40
4.5	SEM Images of Bacillus Subtilis	41

4.6	SEM Image of Bacillus Megaterium	41
4.7	Energy dispersive x-ray spectrum image for Bacillus subtilis	42
4.8	Energy dispersive x-ray spectrum image for Bacillus Megaterium	42

LIST OF SYMBOLS AND ABBREVIATIONS

CO_2	=	Carbon dioxide
H_2O	=	Water
O_2	=	Oxygen
HCO_3	=	Bicarbonate
CO_3	=	Carbonate
OH	=	Hydroxide
Ca_2	=	Calcium ion
CaCO_3	=	Calcium carbonate
SO_4	=	Sulphate
CH_2	=	Methane
MTCC	=	Microbial Type Culture Collection
SEM	=	Scanning Electron Microscope
EDAX	=	Energy Dispersive x-ray Spectrum
OPC	=	Ordinary Portland cement
F_{ck}	=	Characteristic Compressive Strength
F'_{ck}	=	Target average compressive strength
s	=	Standard deviation
w/c	=	Water cement ratio
NDT	=	Non Destructive Testing
MICCP	=	Microbial Induced Calcium Carbonate Precipitate

CHAPTER 1

INTRODUCTION

1.1 GENERAL

Concrete is an important building material that is an essential component of public infrastructure and most buildings. It is most effective when reinforced by steel rebar, mainly because its tensile strength without reinforcement is significantly lower relative to its compressive strength. It is a very brittle material with low tolerance to stress, so cracks are usually expected to occur over time. These cracks, while not compromising structural integrity, expose steel reinforcement to the elements, causing corrosion that increases maintenance costs and compromises structural integrity over the long term. That being said, concrete is a high maintenance material. It worsens severely over decades of its expected service period. It is not flexible and cannot handle significant amounts of stress. Standard concrete will withstand about 0.1% stress before laying out.

Self-healing concrete is commonly sought to rectify these defects to extend the service life of any concrete structure. Self-healing in development is a material in real of concrete, now, which can solve many problems associated with standard concrete. This material is a bacterial self-healing concrete.

When they become active the concrete and calcium lactate mix in the self-healing concrete with the bacteria involved in the food to support those bacteria. Bacteria, feeding on the provided food source, heal the damage done and can also reduce the amount of damage sustained by the concrete structure in place.

Bacteria is a single celled organism. It is a prokaryotic cell that lacks a nucleus and other membrane-attached structure. Typically, bacteria come in three basic shapes, eg, spheroid, rodlike and spiral. Some bacteria do not fit into any of the preceding categories, but rather spindle shape or irregular lobed shape.

Bacteria can be found in every environment such as air, food, soil and water. Many bacteria benefit humans and some can cause disease to humans. Bacteria play an important role in most cycles of the environment such as biochemical cycle, water cycle, carbon cycle, nitrogen cycle and sulfur cycle.

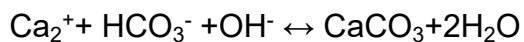
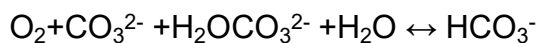
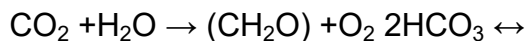
However, there are numbers of bacteria that are not fully characterized and only a few bacteria have been grown in a laboratory for specific application. Cell division in bacteria occurs, usually by binary fission or budding, as opposed to cell division in a higher organism.

In a binary fission, a cell duplicates its components and divides into two cells. Cell division produces 2 identical daughter cells. Bacteria grow in 3 stages, the first phase is the lag phase or rapid growth when the bacteria get a lot of carbon sources and nutrients, the second phase is the logarithm growth or exponential phase, the last stage of growth is the steady growth And the nutrient level is reduced. We are testing the sample to see if the crack is going through the fill material, through the parent material, or through the interface.

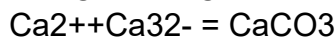
The project will delve deeper, describing the processes behind the self-healing of bacteria in concrete, and the many components involved in this process and how they work independently and collectively.

The project will also transition to practical applications of this self-healing method, including real-world integration into currently steep structures. The sustainability and economics of these materials will also be discussed, as this new improvement on staple construction materials, yet often given, reduces both the environmental and financial impact of concrete production for our modern civilization and presents a wider array of Presents an opportunity to do. Applications

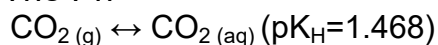
1.2 CHEMICAL PROCESS



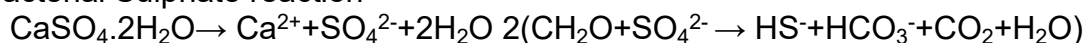
(i) The Calcium Concentration



(ii) The Ph



(iii) Bacterial Sulphate reaction



1.3 PRINCIPLES GOVERNING CALCIUM CARBONATE PRECIPITATION FOR, BIO-CEMENTATION

- Calcium and carbonate concentration have a significant effect on precipitation rate and inhibitory effect on calcium carbonate production by bacteria. Urea hydrolysis produces carbonate ions at a 1: 1 molar ratio. Therefore with increased urea, carbonate concentrations can be increased to facilitate calcium carbonate saturation.
- Microbial induced calcium carbonate precipitation (MICCP) is a naturally occurring biological process in which germs produce inorganic materials as part of their basic metabolic activities.
- Application of carbonate bio-minerals produced by bacteria to improve the durability of buildings, refinement of the environment (water and soil), wear of atmospheric CO₂ filler material and plastics etc.
- The pH of the environment has a significant effect on calcium carbonate precipitation in the medium, the rate of carbonation by bacteria.
- The presence of nucleation sites is important because it controls the symmetry of carbonation as well as the strength of the carbonate produced. In bacterial calcium carbonate precipitation, the first two factors are important for calcium carbonate precipitation, while the third factor is not an important factor because bacteria themselves behave as nucleation sites.

CHAPTER 2

OBJECTIVE, SCOPE & LITERATURE REVIEW

2.1 OBJECTIVE

- The primary objective of this project is to repair the cracks in concrete which shall be done with the help of bacteria.
- The main aim of self-healing concrete is to increase the durability of the structure.
- To view the bacterial mineralization of calcium carbonates in concrete which heals the cracks in concrete.

2.2 SCOPE

In order to achieve the above objective the following are the scope of work;

- The compressive strength of bacterial concrete is to determine the durability of structure.
- Evaluation of Crack healing is viewed through Microscope Using NDT analysis is done by the Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Spectroscopy (EDXS).

2.3 LITERATURE REVIEW

Jianyun Wang (2014) Study was made to understand that the Self-healing strategies are regarded as a promising solution to reduce the high maintenance and repair cost of concrete infrastructures. In the present work, a bacterial-based self-healing by use of hydrogel encapsulated bacterial spores (bio-hydrogels) was investigated. The crack closure behavior of the specimens with/without bio-hydrogels was studied quantitatively by light microscopy. To have a view of the self-healing inside the specimens, a high resolution X-ray computed microtomography (X-ray ICT) was used. The total amount and the distribution of the healing products in the whole matrix were investigated. This study indicates that the specimens incorporated with bio-hydrogels had distinct improved healing efficiency compared to the reference ones with pure hydrogel only. The healing ratios in the specimens with bio-hydrogels were in the range from 70% to 100% for the cracks smaller than 0.3 mm, which is more than 50% higher than for the ones with pure hydrogel; and the maximum crack bridging was about 0.5 mm (in 7 d), while pure hydrogels only allowed healing of cracks of about 0.18 mm. The total volume

ratio of the healing product in the specimens with bio-hydrogels amounted to 2.2%, which was about 60% higher than for the ones with pure hydrogel (1.37%).

Ramesh Kumar .V (2011) study was made to understand that Biomimetics is a field of science that studies biological processes for effectively using them in the development of innovative engineering materials and systems. It is a new field of emergence in materials science and engineering in which lessons learned from biology form the basis for evolution of novel technological materials. Biomimetics is finding application in diverse areas ranging from micro/nano electronics to civil/structural engineering. This article presents a critical review of the literature on biomimetics in civil engineering and its application for the development of sustainable materials in the construction industry. It includes various methodologies such as bio deposition, which has influenced material evolution, and biomineralization, which is a complex phenomenon by which organisms form minerals seen in various geothermal systems. The article deals with the initial usage of *Bacillus pasteurii* for filling up the pores for crack remediation and research work on it by changing the nutrient source and strain improvement of the same towards increasing the strength of concrete. The article also discusses a new type of thermophilic anaerobic microorganism belonging to *Shewanella* species, which when added to concrete, has shown to increase the strength of the concrete. This is due to growth of filler material within the pores of the cement–sand matrix. Thus, there is ample scope for development of biomimetics-based sustainable

Rajesh K. Verma (2015) in this observed that Mortar and concrete are the most widely used building materials all over the world as they are cheap, easily available and convenient to cast. But crack in these materials is a common phenomenon during its service life due to many reasons, if unattended timely, will result in long-term structural deterioration with high level of risk and maintenance cost. Currently, epoxy, resins, epoxy mortar and other synthetic mixtures are used for remediation of cracks. Bacteria from various natural habitats have frequently been reported to precipitate calcium carbonate both in natural and laboratory condition.

J.M.rwan, N.Othman(2013) in this observed that In Malaysia and worldwide, concrete is one of the most popular construction material because of it's strong, durable and inexpensive material. It has specialty of being cast in any desirable shape but plain concrete however is porous, possesses very low tensile strength, limited ductility and little resistance to cracking. These problem become more complicated in various environmental condition like Malaysia to which concrete is exposed. Conventionally, a variety of sealing agent namely, latex emulsions suffer from serious limitations of incompatible interfaces, susceptible to ultraviolet radiations, unstable molecular structure and high cost. Therefore, a novel and more environmental friendly technique is proposed for treating concrete material in structure by employing bacteria induced calcium carbonate precipitation in the form of calcite.

Srinivasa Reddy V (2013) in this observed that An experimental study on effect of calcifying bacteria on permeation properties (such as absorptivity and sorptivity) of bacterial concrete is outlined in this paper. The rate of water absorption, water absorption capacity, sorptivity and porosity tests were then performed on the samples of concrete treated with bacteria of ordinary (M20), standard (M40) and high strength (M60 and M80) grades, to assess the enhancement of permeation properties due to biomineralization in bacterial concrete. The results show that, absorptivity, sorptivity and porosity of bacterial concrete are much lower than the corresponding characteristics of controlled concrete. These characteristics are noticeably enhanced by the addition of bacillus subtilis JC3 to the concrete. It also indicated from the chemical analysis that the cement-phase composition of bacterial concrete has significant amount of calcium precipitate compound. The study of porosity is fundamental for understanding phenomena of water transport within pore structure and interactions between concrete and water. Water can penetrate a solid because there are interconnected channels (pores) inside the solid that facilitate its transportation. The long-term durability of concrete is affected to a large extent by its permeability. Therefore, the permeation properties, rather than mechanical properties, are the important factors to study in relation to concrete durability. As a consequence, a considerable effort has to be directed toward investigation of such properties in the present study.

F. Pacheco-Torgal (2012) study was made to understand that The deterioration of reinforced concrete structures is a very common problem due to the fact that this material has a high permeability which allows water and other aggressive media to enter, thus leading to corrosion problems. The use of sealers is a common way of contributing to concrete durability. However, the most common ones are based on organic polymers which have some degree of toxicity. The Regulation (EU) 305/2011 related to the Construction Products Regulation emphasizes the need to reduce hazardous substances. Therefore, new low toxicity forms to increase concrete durability are needed. Recent investigations in the field of biotechnology show the potential of bioinspired materials in the development of low toxic solutions. This paper reviews current knowledge on the use of bacteria for concrete with enhanced durability. It covers the use of bacteria in concrete mix and also biomineralization in concrete surface treatments. Investigation gaps are described. Results from practical applications in which there is exposure to environmental conditions are still needed in order to confirm the importance of this new approach.

Vijeth N Kashyap (2013) study was made to understand that Crack is commonly observed failure in the case of concrete. Crack may develop due to addition of excess of water to during mixing of concrete, or may be due to shrinkage and creep. In the present study, crack healing and improvement of physical properties of cement paste, mortar and concrete are studied. It is done by the addition of bacterial strains namely *Bacillus Sphaericus* and *Sporosarcina Pastuerii*. It is found that these bacteria when added at 10^6 concentration of cells/ml of water to cement composites increased by about 39.8% and 33.07% in paste. There is an increment of 50% and 28.2% in mortar for two bacterial strains. The strength increment is found to be 18.3% and 12.2% for *Bacillus Sphaericus* and *Sporosarcina Pastuerii* respectively for concrete. Ultrasonic pulse velocity of the bacterial concrete was in line with conventional concrete. SEM and XRD images revealed presence of CaCO_3 produced microbially. There is overall improvement in the bacterial composites compared to conventional composites.

Silvia Hanah S (2015) in this observed that Quantities of sludge from septic tank and quarry rock dust from industries have been on the rise in recent years due to the rapid improvement in the standard of living and the increase in industrialization. Unfortunately, the majority of sludge from septic tank is not being recycled and hence it causes serious problems on natural resources and environment. For these reasons, this study has been conducted through basic experimental research in order to analyze the possibilities of recycling the sludge from septic tank as a supplementary material in cement concrete. In this research various proportions of septic tank sludge and quarry rock dust are used as supplement to sand in cement concrete and also various proportions of bacterial solutions are used as a supplement to water. The works undertaken here dealt with the use of quarry rock dust, septic tank sludge and bacterial water as a supplementary material in cement concrete. In this investigation, % of water absorption and compressive strength criteria were analyzed by introducing septic tank sludge, quarry rock dust and bacterial solution into the cement concrete.

Dhami, Navdeep Kaur (2013) in this observed that Microbial induced calcium carbonate precipitation (MICCP) is a naturally occurring biological process that has various applications in remediation and restoration of a range of building materials. In the present investigation, five ureolytic bacterial isolates capable of inducing calcium carbonate precipitation were isolated from calcareous soils on the basis of production of urease, carbonic anhydrase, extrapolymeric substances, and biofilm. Bacterial isolates were identified as *Bacillus megaterium*, *B. cereus*, *B. thuringiensis*, *B. subtilis*, and *Lysinibacillus fusiformis* based on 16S rRNA analysis. The calcium carbonate polymorphs produced by various bacterial isolates were analyzed by scanning electron microscopy, confocal laser scanning microscopy, X ray diffraction, and Fourier transmission infrared spectroscopy. A strain specific precipitation of calcium carbonate forms was observed from different bacterial isolates. Based on the type of polymorph precipitated, the technology of MICCP can be applied for remediation of various building materials.

CHAPTER 3

COMPOSITION OF CONCRETE

3.1 COMPOSITION OF CONCRETE

Concrete is made up of three main materials: water, portland cement, and aggregates. The ratio of ingredients changes the properties of the product. Admix is added to accommodate the concrete mix for specific performance criteria. There are several types of concrete available, making different proportions of the material below. The finished product can be tailored to suit its application with varying strength, density or chemical and thermal resistance properties. Mix design depends on the type of structure how it is made, how the concrete is mixed and distributed and how it is laid to form the structure. The ingredients are explained below.

3.1.1 CEMENT

The cement hardens when mixed with water, which binds all the ingredients together. Portland cement is the most common cement used and is made of alumina, silica, lime, iron and gypsum. Small amounts of other ingredients are also included. Ordinary Portland cement is a commonly used cement. It is the basic component of concrete. It contains a mixture of calcium silicates, aluminates, and ferrite compounds that combine calcium, silicon, aluminum, and iron in forms that will react with water.

3.1.2 AGGREGATE

The majority of the concrete mix is made up of both coarse and fine aggregates, which helps to increase the strength of concrete that the cement itself can provide. Sand, gravel and crushed stone are used as aggregates. Fine and coarse aggregates form the bulk of the concrete mixture .. Fine aggregates must completely pass through the sieve 4.75 mm and the coarse use sieve passes through 12.5 mm.

TABLE 3.1 PROPERTIES OF FINE AGGREGATE

SI no:	Property	Test Results
1	Specific Gravity	2.52
2	Fineness Modulus	2.61
3	Water absorption	1%
4	Zone	II

TABLE 3.2 PROPERTIES OF COARSE AGGREGATE

SI no:	Property	Test Results
1	Specific Gravity	2.65
2	Fineness Modulus	8.92
3	Water absorption	0.5%
4	Zone	II

3.1.3 BACTERIA

The bacteria which is used was bought from IMTECH Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology, Sector 39 A, Chandigarh, India.



Fig 3.1 The dry powdered bacteria is in the test tube

(i) REVIVAL OF CULTURE

➤ **Active cultures**

The organism was subcultured immediately onto recommended medium and incubated at recommended temperature and under conditions.

➤ **Freeze - Dried Cultures**

- i. Care must be taken in opening the ampoule as the materials are in vacuum.
- ii. Mark on the ampoule in the middle of cotton wool with a sharp file.
- iii. Disinfect the surface around the scar with alcohol.
- iv. Wrap thick cotton wool around the ampoule and break on the scar area.
- v. Gently remove the pointed top of the ampoule snap, openly pulling the cotton plug to one end. The fine particles of the hastily dried organisms will be released into the laboratory air.
- vi. Carefully, remove the cotton plug and add approximately 0.3 -0.4 ml of specified medium to make the suspension of the culture. For fungi cultures avoid frosting or clogging aerosols, the suspension should be made in approximately 0.4 mL of sterile water and let stand for another twenty minutes before transferring it to solid medium.
- vii. Streak a few drops of the suspension over the recommended medium in a petri.
- viii. Incubate at approximate temperatures and under recommended conditions for culture.
- ix. If proper medium and conditions are followed, the development of culture is visible within a few days. Some fungi also require prolonged incubation periods of up to fifteen days.
- x. All remains of the original ampoule must be considered autoclaved before being infected and released.

3.1.3.1 BAICILLUS SUBTILIS

Bacillus subtilis, also known as grass bacillus or grass bacillus, is a gram-positive, catalytic-positive bacterium found in soil and the gastrointestinal tract of ruminants and humans. Genus *Bacillus*, b. A member of *subtilis* is rod-shaped, and can form a strict, protective spore, allowing it to tolerate extreme environmental conditions. *B. Subtilis* has historically been classified as an oblate aerob, although evidence exists that it is a facultative aerob. *B. Subtilis* is considered to be the best studied gram-positive bacterium and a model organism to study chromosomal replication and cell differentiation of bacteria. It is one of the bacteria champions in secreted enzyme production and is used on an industrial scale by biotechnology companies. *Bacillus subtilis* is a gram-positive bacterium, rod-shaped and catalytic-positive. It was originally named *Vibrio subtilis* by Christian Gottfried Ehrenberg, and *bacillus subtilis* by Ferdinand Cohan in 1872 (for 'fine' in *subtilis* Latin)

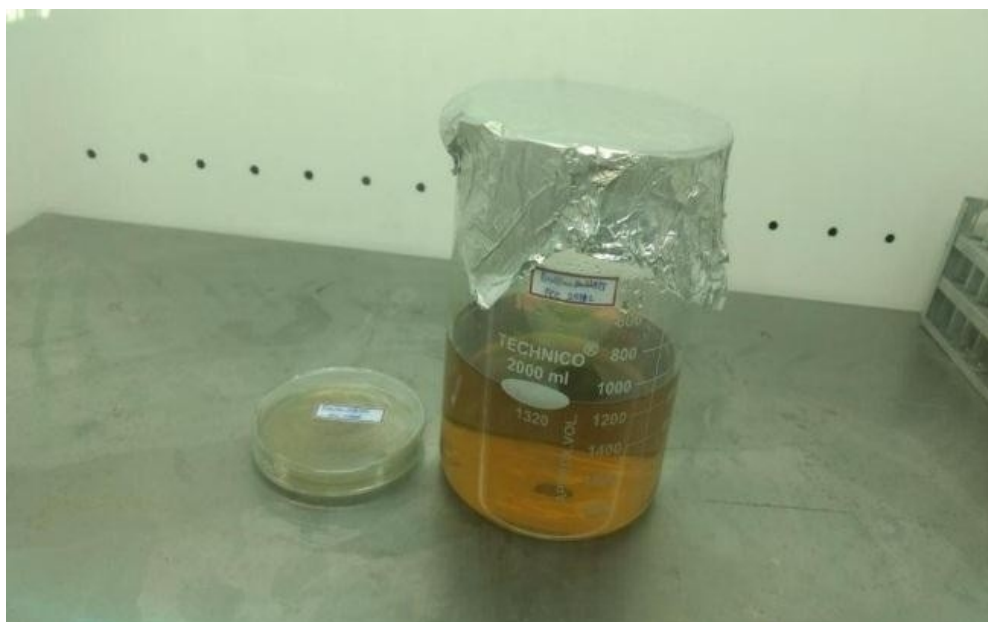


Fig 3.2 *Bacillus subtilis*

B. subtilis cells are typically rod-shaped, and are about 4-10 micrometers (μm) long and 0.25–1.0 μm in diameter, with a cell volume of about 4.6 fL at stationary phase. As with other members of the genus *Bacillus*, it can form an endospore, to survive extreme environmental conditions of temperature and desiccation.

3.1.3.2 BACILLUS MEGATERIUM

Bacillus megaterium is a rod-like, gram-positive, predominantly aerobic spore found in widely diverse habitats. With a cell length of up to 4 μm and a diameter of 1.5 μm , *B. megaterium* is one of the largest known bacteria. Cells often occur in pairs and chains, where cells are joined together by polysaccharides on the walls. In the 1960s, before the development of *Bacillus subtilis*, *B. megaterium* was the main model organism among gram-positive bacteria for in-depth studies on biochemistry, sporulation, and bacteriophages. Recently, its popularity in the field of biotechnology has begun to grow for its recombinant protein production capacity. *B. Megaterium* grows at temperatures ranging from 3 ° C to 45 ° C, with temperatures around 30 ° C. Some isolates from Antarctic geothermal lakes are found at temperatures up to 63 ° C.

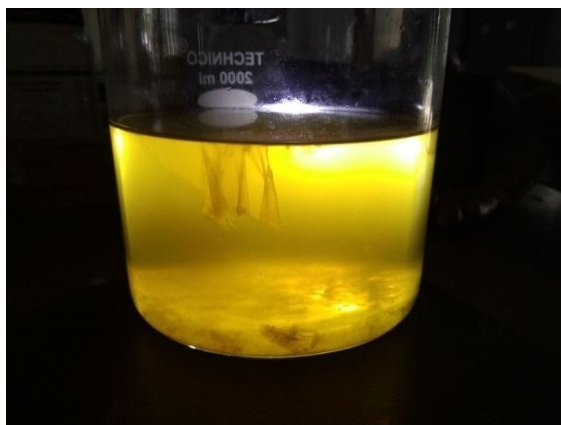


Fig. 3.3 Bacillus Megaterium

B. Megaterium has been recognized as an endophyte and is a potential agent for biocontrol of plant diseases. Nitrogen fixation has been demonstrated in some strains of *B. megaterium*. *B. The megaterium* has been an important industrial organism for decades. It produces the penicillin amidase used to make synthetic penicillin, the various amylase used in the baking industry, and the glucose dehydrogenase used in glucose blood tests. In addition, it is used for the production of pyruvate, vitamin B12, fungicides and drugs with antiviral properties, etc. It produces enzymes to modulate corticosteroids, as well as several amino acids dehydrogenase. *B. Megaterium* is known to produce poly- γ -glutamic acid. The accumulation of the polymer is greatly increased in saline (2–10% NaCl) environments, in which the polymer consists largely of L-glutamate (L-isomer content up to 95%). *B. At least one strain of megaterium* can be considered a halophile, as an increase of up to 15% NaCl has been observed. Phylogenetically, based on 16S rRNA, *b. The megaterium* is strongly associated with *B. flexus*, which a century ago. Differs from *megaterium*, but has recently been confirmed as a separate species. *B. Pathogens in the megaterium b. Anthracis* and *B. There are some phenotypic and phylogenetic similarities with Cereus*, although themselves are relatively harmless.

3.2 MIX DESIGN FOR M40 GRADE

3.2.1 MIX PROPORTION:

Grade Designation	: M40
Type Of Cement	: OPC 53 grade (IS 8112)
Maximum nominal size of aggregates	: 20mm
Minimum Cement Content	: 320 kg/m ³
Maximum water cement ratio	: 0.43
Workability	: 100mm (slump)
Exposure Condition	: Severe (for reinforced concrete)
Method of Concrete Placing	: Not Pumping
Degree of Supervision	: Good
Types of aggregate	: Crushed angular aggregate
Maximum Cement Content	: 450 kg/m ³
Chemical admixture type	: Polycarboxylate ether

3.2.2 MATERIALS

Cement Used : OPC 53 grade confirming to IS8112
Specific gravity of cement : 3.15
Chemical admixture : Superplasticiser confirming to IS9103.

Specific Gravity :

Coarse aggregate : 2.65

Fine aggregate : 2.52

Water Absorption:

Coarse aggregate : 0.5%

Fine aggregate : 1.0%

3.2.3 TARGET STRENGTH

$$f'_{ck} = f_{ck} + 1.65s$$

f'_{ck} = Target average compressive strength at 28 days

f_{ck} = Characteristic compressive strength at 28 days

s = Standard deviation ($s = 5N/\sqrt{m^2}$)

$$\text{Target Strength} = 48.25 \text{ N/mm}^2$$

3.2.4 WATER CEMENT RATIO

Maximum water cement ratio = 0.43

Based on experience, adopt w/c ratio as 0.43 ($0.40 < 0.45$)

Hence it is ok.

3.2.5 WATER CONTENT

Maximum water content for 20mm aggregate = 186 liters (for 25-50 slump)

Estimated Water Content for 100 slump $= 186 + 6/100 \times 186 = 197$ Litres

As super plasticiser is used, the water content can be reduced up to 29 %

Hence the water content arrived $= 197.16 \times 0.71 = 140$ litres.

3.2.6 CALCULATION OF CEMENT CONTENT

Water cement ratio = 0.43

Cement content $= 140 / 0.43 = 325 \text{ Kg/m}^3$

Minimum cement content for severe exposure condition $= 320 \text{ Kg/m}^3$

$325 \text{ Kg/m}^3 > 320 \text{ Kg/m}^3$

Hence OK

3.2.7 VOLUME OF COARSE AGGREGATE AND FINE AGGREGATE

Volume of coarse aggregate for w/c ratio of 0.43 $= 0.616$

Volume of coarse aggregate $= 0.616 \times 0.9 = 0.554$

Volume of fine aggregate $= 1 - 0.554 = 0.446$

3.2.8 MIX CALCULATIONS:

The mix calculations per unit volume of concrete

- a) Volume of concrete $= 1 \text{ m}^3$
- b) Volume Of Cement $= [325/3.15] \times [1/1000]$
 $= 0.103 \text{ m}^3$
- c) Volume of Water $= [140/1] \times [1/1000]$
 $= 0.140 \text{ m}^3$
- d) Volume of chemical admixture $= [7/1.145] \times [1/1000]$
 $= 0.006 \text{ m}^3$
- e) Volume of all in aggregates $= a - (b + c + d)$
 $= 1 - (0.103 + 0.140 + 0.006)$
 $= 0.751 \text{ m}^3$
- f) Mass of coarse aggregate $= e \times \text{volume of CA} \times \text{specific gravity of CA}$
 $= 0.751 \times 0.554 \times 2.65 \times 1000$
 $= 1102 \text{ kg/m}^3$
- g) Mass of fine aggregate $= e \times \text{Volume of FA} \times \text{specific gravity of FA}$
 $= 0.751 \times 0.446 \times 2.52 \times 1000$
 $= 844 \text{ kg/m}^3$

3.2.9 MIX PROPORTIONS

- Cement = 325 kg/m³
- Water = 140 litres
- Fine aggregate = 844 kg/m³
- Coarse aggregate = 1102 kg/m³
- Chemical admixture = 7 kg/m³
- Water cement ratio = 0.43

3.3 METHODOLOGY

The Methodology for the casting and testing of specimens are described below:

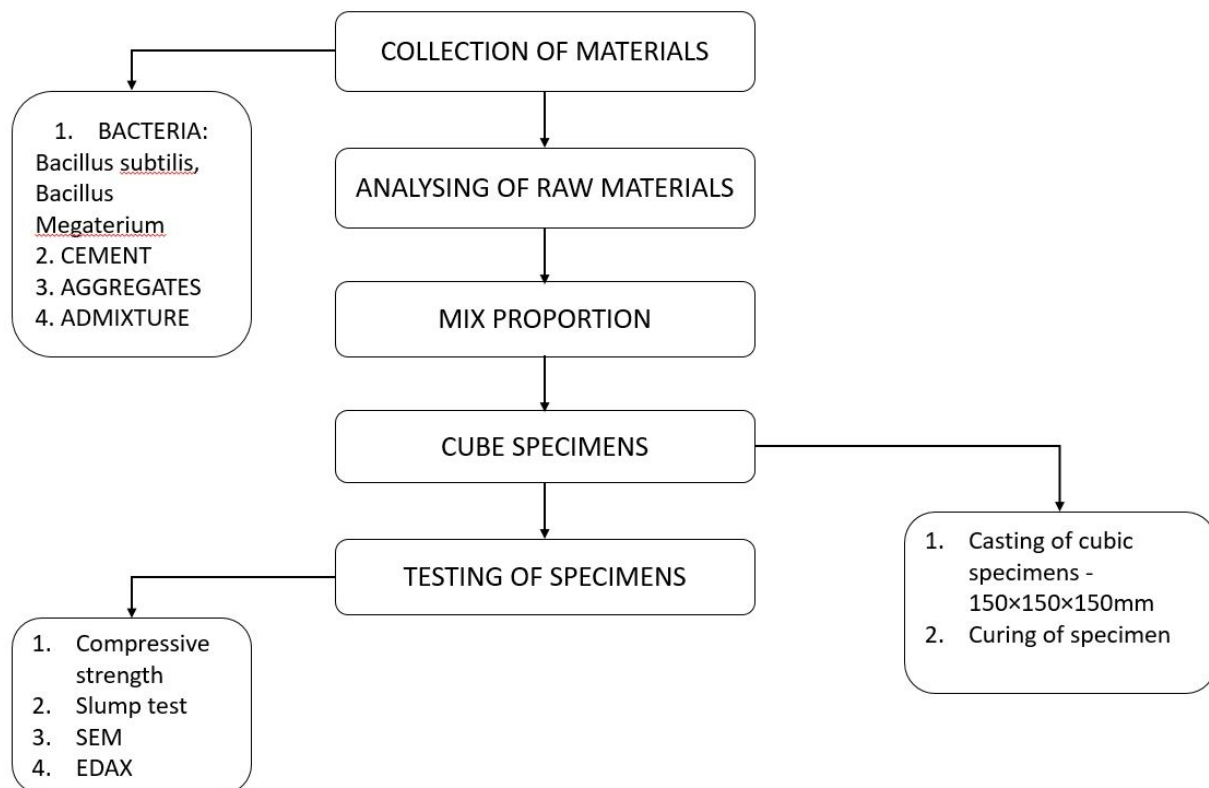


Fig 3.4 Methodology flowchart

3.4 SPECIMEN DETAILS

3.4.1 PREPARING THE MOULDS

Before assembling the moulds, make sure that there is no hardened mortar or dirt on the flange that provided the sections from fitting together closely. These faces must be thinly coated with mould oil to prevent leakage during filling, and a similar oil film should be provided between the content surfaces of the bottom of the mould and the base. The inside of the mould must also be oiled to prevent the concrete from sticking to it. The two sections must be boiled firmly together, and the moulds held down firmly on the base plates.



Fig.3.5 Mould

3.4.2 CASTING OF SPECIMEN

All the ingredients mentioned above [3.2.9] are weighed, mixed and casted in cubic moulds of size 150X150X150mm. While filling the cubic moulds the concrete is compacted with my hand. The casting is done in a wet environment in order to avoid loss of moisture through evaporation. The chemical reaction of water and cement binds the aggregate particle in concrete. This reaction is known as cycle of Hydration, takes place slowly during which it is necessary to avoid the loss of moisture.



Fig. 3.6 Casting of specimen

3.4.3 DEMOULDING THE TEST CUBES

The test cubes are dismantled 24 hours after they are made. If after this period of time the concrete has not acquired sufficient strength to enable it to collapse without damaging the cubes, then the democooling should be delayed for further hours. Separate the mould completely, removing the concrete cubes from the mould. Take care not to damage the cubes, as the compressive strength may decrease if there is a cracking.



Fig. 3.7 Demoulding the cubes

3.4.4 SETTING OF MOULDS

The casted moulds are kept at a place where the temperature is maintained at 27C and then demoulded after 24 hours. After demoulding each cube was marked with a legible identification on the top using a waterproof ink.

3.4.5 CURING OF CUBES

Cubes are cured before they are tested. The cubes are placed immediately after demoulding in the curing tank. The curing temperature of the water in the curing tank should be maintained at 27 to 30°C.



Fig. 3.8 Curing of cubes

3.5 TEST CONDUCTED

3.5.1 COMPRESSIVE TESTING MACHINE

The test is to determine the strength of concrete. Concrete samples are taken and used for casting cubes 15 cm x 15 cm x 15 cm. Concrete is filled in molds in layers about 5 cm deep. It is evenly distributed and compacted by either vibration or hand-tapping. After the top layer is compacted, the concrete surface is finished with the top of the mold using a trowel. Sample stored on site for 24+ d hours under moist mat or sack. After that, samples stored in clean water at $27 \pm 20^\circ\text{C}$; By the time of the test. The sample will be tested immediately upon removal from the water and while they are still in wet condition. The bearing surface of the test specimen is cleaned and any loose material is removed from the surface. In the case of cubes, the machine is placed in the machine in such a way that the load cube is cast, i.e. not up and down. Align the axis of the specimen with the steel plates, do not use any packing. The load will be applied slowly without any shocks and will be continuously increased at a rate of about 140 kg / sqm / min until the resistance of the increased load sample is broken and a greater load can be sustained. The maximum load applied to the specimen is then recorded and any unusual features noted at the time of failure encountered in the report.





Fig 3.9 Compressive test

3.5.2 SLUMP CONE TEST

This test is carried out to measure the stability or practicality of fresh concrete, where the aggregate maximum size does not exceed 38 mm, using the aggregate test. Sampling method and analysis of concrete. Deceleration cone (height = 30 cm, base diameter = 20 cm, top diameter = 10 cm) Tamping rod (length = 60 cm, diameter = 16 mm). The internal surface of the mold is thoroughly cleaned and the superfluous is free of moisture before starting the test. And if the cone is in a completely dry state, wet it using a damp cloth. The mold is then placed on a rigid, horizontal level on a rigid and non-absorbent surface such as a rigid plate. It is kept firmly by standing on two-foot pieces provided in slope cones during filling by the operator. The mold is filled with concrete in four layers, approximately one-quarter of the height of each mold, and each layer is uniformly poured downward with 25 strokes of the tamping rod with pointed ends. After tying the top layer, the concrete is hit level off with a trowel and any mortar leaking between the mold and the base plate is cleaned. The mold is removed from the concrete immediately by slowly and carefully lifting it in a vertical direction. The slope is immediately measured by determining the difference between the height of the mold and the highest point of the specimen.



Fig 3.10 SLUMP CONE TEST

3.5.3 ENERGY DISPERSIVE X-RAY SPECTROSCOPY (EDAX)

Energy dispersive X-ray spectroscopy is an X-ray spectroscopic method for determining the fundamental composition. It can be used with imaging in SEM, TEM (). When SEM is performed in an instrument signal, it can be achieved from a spot, area, line profile, or 2D map. It depends on some source of X-ray excitation and the interaction of a sample. Its characteristic potential is due in large part to the fundamental principle that each element has a unique automated structure that allows unique installation of peaks on its X-ray emission spectrum. In order to excite the X-ray characteristic of the emission from a sample, a high energy beam of charged particles such as electrons and protons or a beam of X-rays is concentrated in the sample being studied. The number and energy of xrays emitted from the sample can be measured by an energy dispersive spectrometer. As the energy of the X-ray is characterized by the energy difference between the two shells and the atomic structure of the emitting element. EDS allows to measure the fundamental structure of the sample.



Fig. 3.11 EDAX

3.5.4 SCANNING ELECTRON MICROSCOPE (SEM)

A scanning electron microscope is a type of electron microscope that produces an image of a sample by scanning it with an electron's focus beam. Electrons interact with the atoms in the sample forming various signals that contain information about the topography and structure of the surface of the samples. The electron beam is typically scanned in a linear scan pattern, and the position of the beam is combined with a known signal to produce an image. SEM can achieve better resolution than a nano meter. Samples can be viewed in a wide range of high vacuum, low vacuum, wet conditions, environmental cell and cryogenic or elevated temperatures. The most common SEM mode is the detection of secondary electrons emitted by atoms excited by the electron beam. Image showing the topography of the surface formed by scanning the samples and collecting secondary electrons to be emitted using a special detector. The basic principle is that a beam of electrons is generated by a suitable source, a specific tungsten filament, or a field emission gun. The electron beam is accelerated through a high voltage (20 kV) beam of electrons. When the beam scans the surface of the sample. Electrons are emitted from the sample by the action of a scanning beam and collected by a suitably positioned detector. Uses electromagnetic lenses, electrons and fluorescent screens to create images.



Fig. 3.12 Scanning Electron Microscope

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 COMPRESSIVE STRENGTH

The following table represents the compressive strength of different types of bacterial concrete cubes and conventional concrete cube.

TABLE 4.1 COMPRESSIVE STRENGTH

DESCRIPTION	COMPRESSIVE STRENGTH		
	7 DAYS	14 DAYS	27 DAYS
Bacillus subtilus	34.14	45.52	58.37
Bacillus Megaterium	17.85	24.35	36.63
Conventional	28.52	47.77	52.37

Graphical Representation

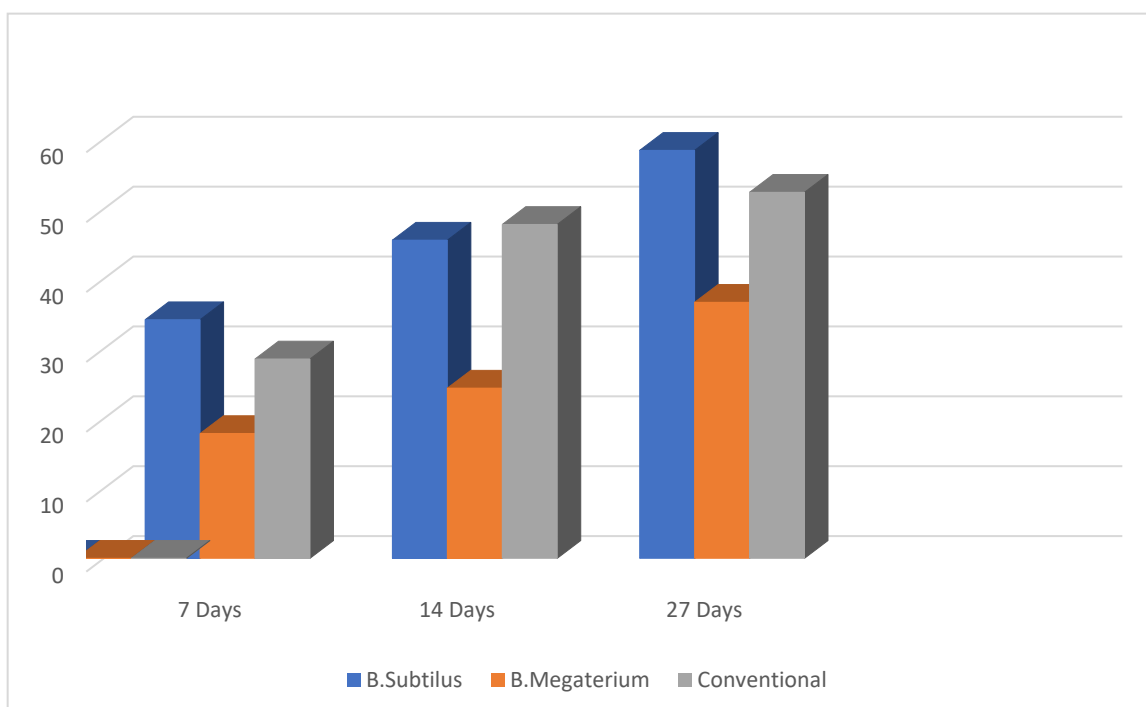


Fig. 4.1 Graphical representation of compressive strength

4.2 MICROSCOPIC EVALUATION OF HEALING CRACK PROCESS

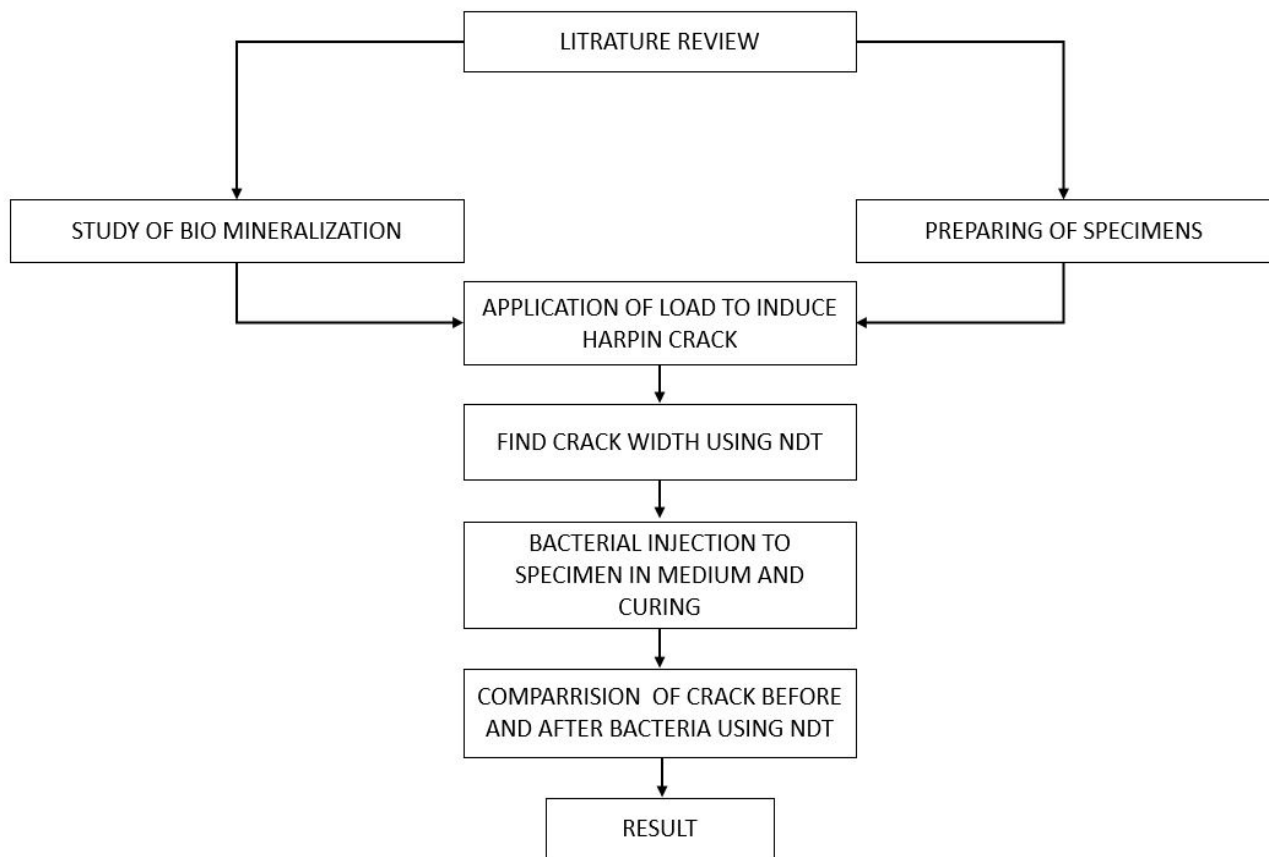
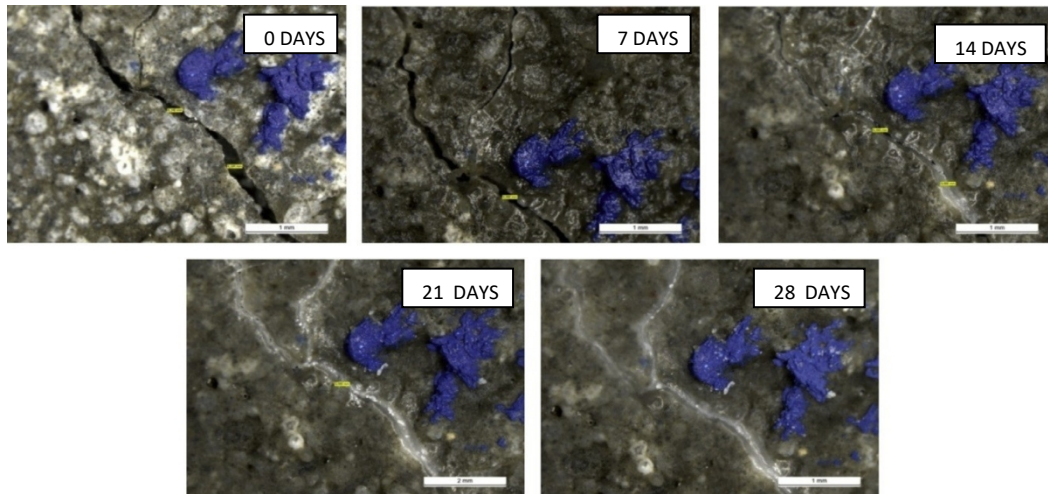
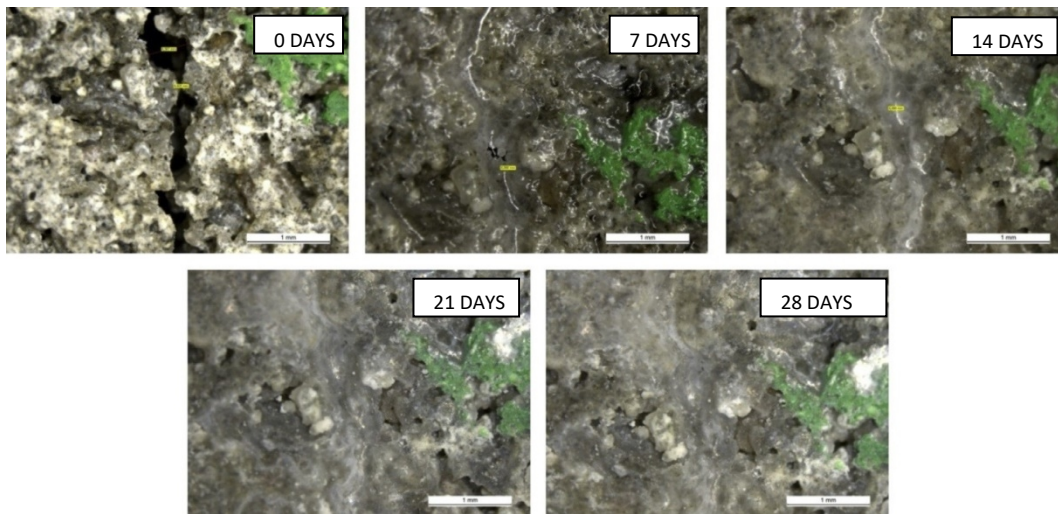


Fig.4.2 Flowchart representing microscopic evaluation process



**Fig. 4.3 The evolution of crack healing process in the specimen (initial: 184 μ m;
final : 0 μ m)**



**Fig. 4.4 The evolution of crack healing process in the specimen (initial : 507 μ m;
final : 0 μ m)**

4.3 SEM (Scanning Electron Microscope)

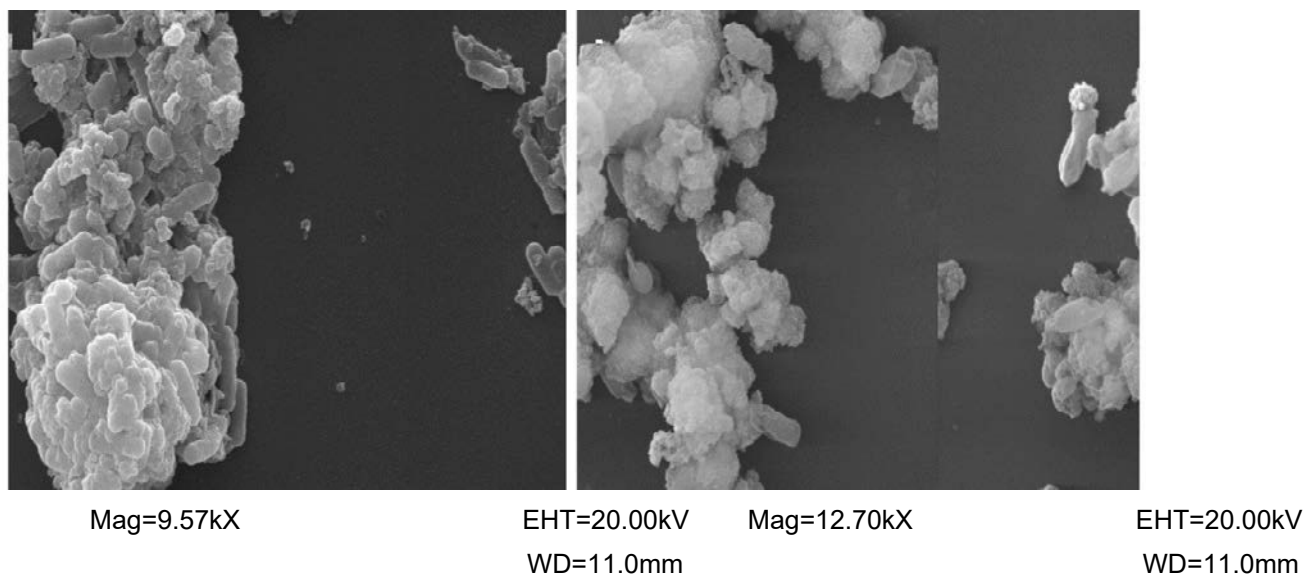


Fig. 4.5 Scanning electron microscopic images of carbonate crystals precipitated by *Bacillus Subtilis*(Bs).

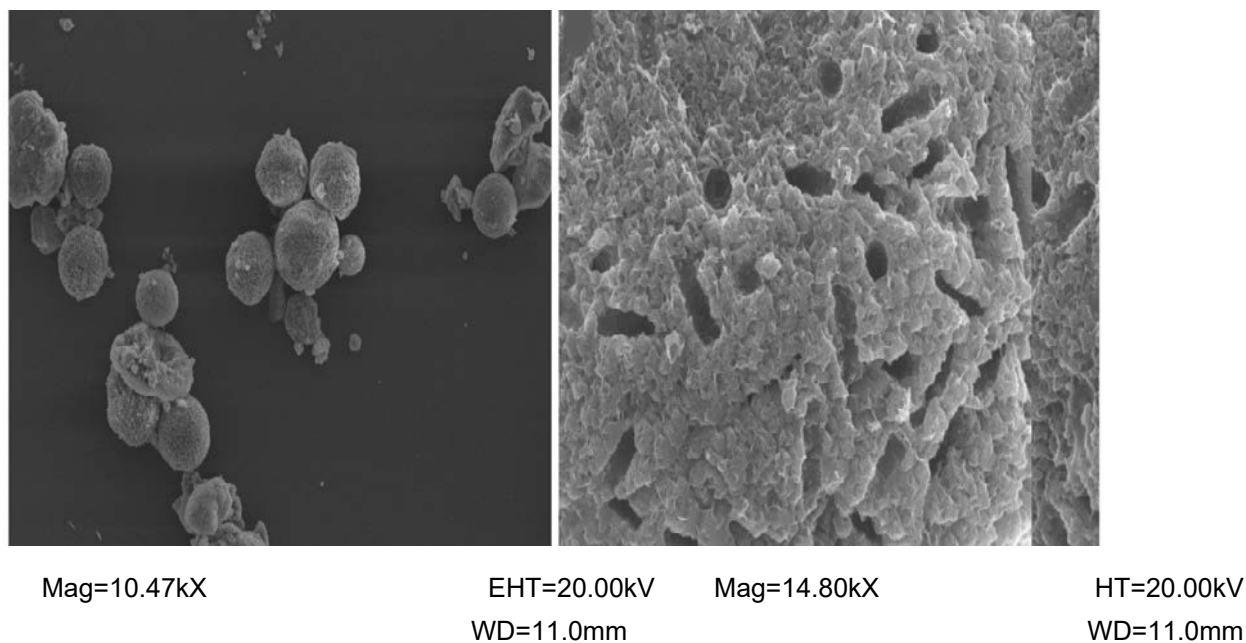


Fig. 4.6 Scanning electron microscopic images of carbonate crystals precipitated by *Bacillus Megaterium*(Bm).

4.4 EDAX

i. Bacillus Subtilis

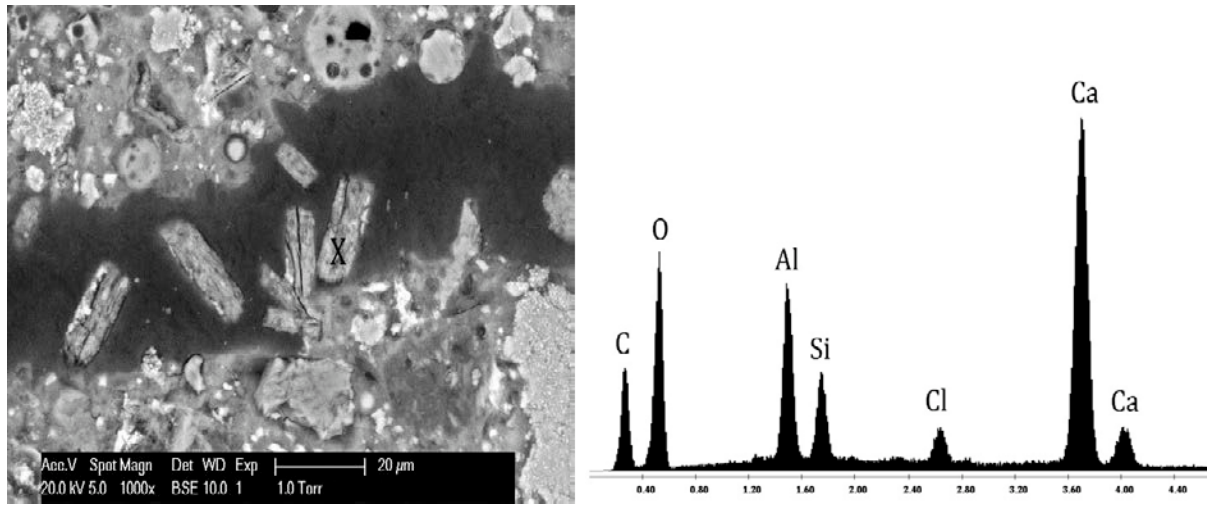


Fig. 4.7 Energy dispersive x-ray spectrum image for Bacillus subtilis

ii. Bacillus megaterium

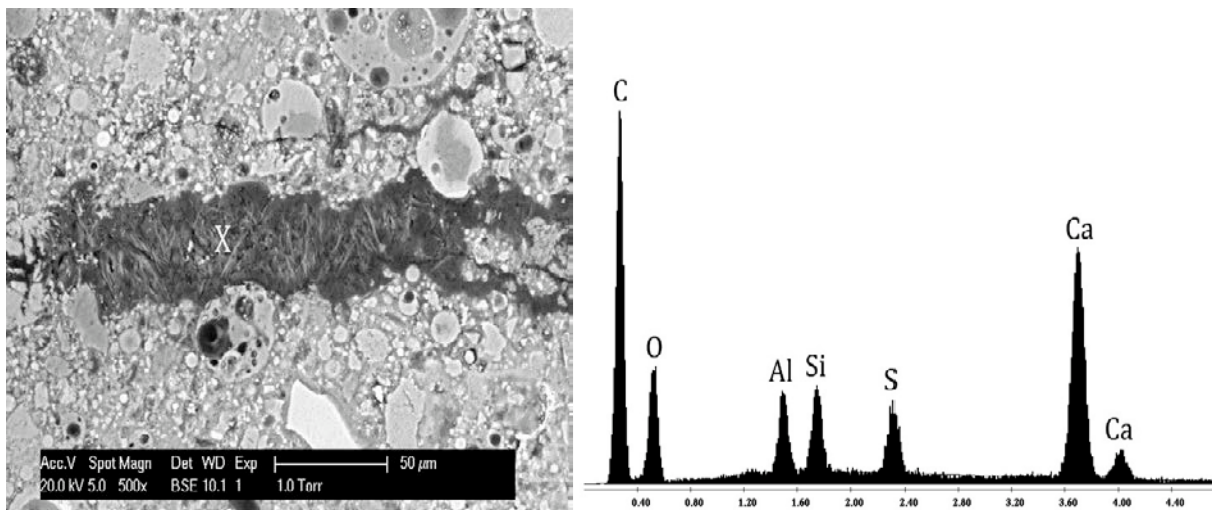


Fig. 4.8 Energy dispersive x-ray spectrum image for Bacillus megaterium.

CHAPTER 5

SUMMARY AND CONCLUSION

5.1 SUMMARY AND CONCLUSION

Thus, the report concludes on the basis of the results:

Bacterial growth for two types of bacteria shows good results in solids.

1. The compressive strength of *Bacillus subtilis* and *Bacillus megaterium* composite concrete is giving more strength than conventional concrete.
2. Calcium carbonate precipitation increases the durability of concrete.
3. The use of bacteria-induced to fill cracks in mortar and concrete is very innovative.
4. Further experiments are to be explored to investigate the durability of the crack repair technique.

REFERENCE

1. Dhami, Navdeep Kaur, M. Sudhakara Reddy and Abijit Mukerjee(2013), "Biom mineralization of Calcium Carbonate Polymorphs by the Bacterial Strains Isolated from Calcareous Sites", Journal of Microbiology and Biotechnology, Vol 23(5), Pg. no. 707-714.
2. Guadalupe Sierra-Beltra. M, H.M. Jonkar, E.Schlangen (2014), "Characterization of Sustainable Bio based mortar for Concrete repair", Construction and Building Materials, Vol 67, pp 344-352.
3. Irwan .J.M., Othman .N, " An Overview of Bioconcrete for structural Repair", Faculty of Civil and Environmental Engineering, Vol 28, 351-366.
4. IS code book 456-2000, IS code book 10262.2009.
5. Jianyun Wang, Jan Dwanckele, Veerle Crudde,(2014), " X-ray Computed Topography proof of bacterial based self-healing in concrete" Cement and Concrete Composites, 53, 289-304.
6. JingXu, Wu Yao, (2014), " Multiscale mechanical quantification of Self-Healing concrete incorporating non-ureolytic bacteria-based Healing agent" , Cement and concrete Research, 64, 1-10.
7. Kim Van Tittelboom, Nele De Belie, Willem De Muynck,Willy Verstrate,(2010), "Use of bacteria to repair cracks in concrete", Cement and Concrete Research,40, 157-166.
8. Mian Luo, Chun-xiang Qian, Rui-yang Li, (2015), " Factors affecting crack repairing capacity of bacteria-based self-healing concrete", Construction and Building materials, 87, 1-7.
9. Navdeep Kaur Dhama, Sudhakara Reddy, "Biofilm and microbial application in biom mineralization concrete".

10. Rajesh K. Varma, Leena Chaurasia, Vishaka Bisht, Manisha Thaku, (2015)
“Biom mineralization and bacterial carbonate precipitation in mortar and concrete”, Vol 1, No.1, pp5-11.
11. Ruoting Pei, Jun Liu, Shuangshunag Wang, Mijia Yang, (2013), “ Use of bacterial cell walls to improve the mechanical performance of concrete”, Cement and Concrete Composites, 39,122-130.
12. Silvia Hannah. S, Sakthieswaran N, (2015), “Mechanical properties of biom mineralized Concrete”, International Journals of Research in Engineering and Technology, Vol 04, Issue 05.
13. Srinivas Reddy V, Sreenivasa Rao D, Seshgiri Rao M V, Sasikala Ch, (2013),
“Permeation Properties of Bacteria Concrete”, Vol 5, Issue 6, pp08-16.
14. Wang .J.Y., Soens .H, Belie De .N,(2014), “Self-/healing Concrete by use of microencapsulated bacterial spores”, Cement and concrete Research 56,139-152.