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A Critical Review on the Performance of Microbial Concrete Developed Using E. Coli Bacteria

ABSTRACT

Concrete is a dominant building medium which is sensitive to deterioration, corrosion and cracks. Although it has versatility in construction, it is weak in tension and has lower ductility. Concrete cracks are inevitable and are one of the material's fundamental flaws. Water as well as other salts find its way into these cracks, causing deterioration and reducing the concrete's lifespan. A novel technique in remediating concrete's fissures and cracks by utilizing microbiologically induced calcite ($CaCO_3$) deposition is discussed Calcite precipitation can be induced by Escherichia coli, an environmentally friendly bacterium the microbial system's distinguishing feature is that it initiates concrete self-healing. The utility of microbially induced calcite precipitation (MICP) to improve strength and longevity of cementitious building materials is discussed. This paper inspects the formation of microbial concrete exposed in plain water. 100 mm cubical size concrete samples were distributed and cured for 7, 28, 90 and 365 days with and without bacterial culture. 1 pre-fixed culture density (OD_{600} 0.5±0.1) was used here. 2 different ratios of water to culture (75:25; 50:50) were used in this form of study and were compared to the conventional system (100:0). Specimens were excluded from curing from time to time and tested for compressive and tensile strength. From these tests, it was found that microbial concrete (50:50) showed good resistance under all curing periods.

Concrete specimens were also subjected to ultrasonic pulse velocity (UPV) test and water absorption test. This upv test revealed that specimens with an OD_{600} 0.5± 0.1 had a higher velocity. As a result, the higher pulse velocity will be used to evaluate the material's quality and uniformity. Plain concrete and microbial concrete with varying strengths were used in the water absorption test. The use of microorganisms in concrete reduces absorption of the materials. That is, microorganisms aid in the durability of concrete. Scanning Electron Microscope (SEM) analysis of distinct concrete groups at 28 days revealed that the rate of water substitution by microbial culture increased, resulting in fewer voids.

CONTENTS

	PAGE NO
ABSTRACT	**1**
CONTENTS	**2**
LIST OF TABLES	**5**
LIST OF FIGURES	**6**
CHAPTER 1: INTRODUCTION	**7-11**
1.1 GENERAL	7
1.2 STATEMENT OF THE PROBLEM	8-9
1.3 RESEARCH OBJECTIVES	10
1.4 SCOPE OF THE PROJECT	10
1.5 PROJECT SCHEDULE	11
CHAPTER 2: LITERATURE REVIEW	**12-19**
2.1 GENERAL	12
2.2DIFFERENT SPECIES OF BACTERIA USED IN CONCRETE	12-14
2.3 CHEMICAL PROCESS FOR THE REMEDIATION OF CRACKS BY BACTERIA	14-16
2.4 BENEFITS AND DRAWBACKS OF MICROBIAL CONCRETE	24-26
2.4.1 BENEFITS	17-18
2.4.2 DRAWBACKS	18-19
CHAPTER 3: EXPERIMENTAL INVESTIGATIONS	**20-33**
3.1 GENERAL	20
3.2 BACTERIA CULTURE	20-24

3.2.1 PREPARATION OF LURIA-BERTANI MEDIA 20-21

3.2.2 MAKING MEDIA GERM FREE 21-22

3.2.3 BACTERIA INOCULATION AND GERMINATION 23

3.2.4 BACTERIA CULTURE PROPERTIES 23-24

3.3 MATERIALS USED AND THEIR PROPERTIES 24-29

3.3.1 CEMENT 24

3.3.2 AGGREGATE 25-28

3.3.2.1 FINE AGGREGATE 25-27

3.3.2.2 COARSE AGGREGATE 27-28

3.3.3 BACTERIA 28

3.3.4 WATER 29

3.4 CONCRETE MIXING 30-33

3.4.1 MIX DESIGN OF CONCRETE 30-31

3.4.2 VARIABLES 32

3.4.3 PREPARATION OF SPECIMEN 32

3.5 FLOW CHART OF EXPERIMENTAL INVESTIGATIONS 33

CHAPTER 4: RESULT AND DISCUSSION 34-47

4.1 GENERAL 34

4.2 COMPRESSIVE STRENGTH TEST 34-38

4.3 TENSILE STRENGTH TEST 38-42

4.4 ULTRASONIC PULSE VELOCITY TEST 42-44

4.5 WATER ABSORPTION TEST 44-45

4.6 SCANNING ELECTRON MICROSCOPE (SEM) ANALYSIS 45-47

CHAPTER 5: CONCLUSION AND RECOMMENDATION **48-50**

5.1 GENERAL 48

5.2 CONCLUSION 48-49

5.3 RECOMMENDATION FOR FURTHER STUDY 49-50

REFERRENCES **51-53**

LIST OF TABLES

Table No	Title	Page No
2.1	Luria-Bertani and metabolism process of various Bacterial Groups	19
3.1	Ingredients for Luria-Bertani Media	20
3.2	Physical properties and chemical composition of OPC	24
3.3	Grading of fine aggregate	26
3.4	Physical properties of fine aggregate	27
3.5	Physical properties of coarse aggregate	28
3.6	E. coli bacteria temperature sustainability test	28
3.7	Materials for Conventional Concrete & Microbial Concrete (Design strength: 25 MPa	30
3.8	Materials for Conventional Concrete & Microbial Concrete (Design strength: 35 MPa)	31
3.9	Mix ratios for specimen preparation	32
3.10	Guidelines of concrete quality	35
4.1	Compressive strength results for 25 MPa Concrete	36
4.2	Compressive strength results for 35 MPa Concrete	38
4.3	Strength Behavior Observations	39
4.4	Tensile strength results for 25 MPa Concrete	40
4.5	Tensile strength results for 35 MPa Concrete	43
4.6	UPV data	43
4.7	Water absorption test data	45

LIST OF FIGURES

Figure No	Title	Page No
1.1	Examples of severely damaged concrete support beams of bridge	9
2.1	Classification of Bacteria	13
2.2	Different types of Bacteria used in concrete	13
2.3	Key roles of pH and calcium metabolism in microbial carbonate precipitation (Hammes & Verstraete 2002)	15
2.4	Process of fixing cracks in concrete	16
2.5	Repairing of cracks by Bacteria	17
3.1	Luria-Bertani Media preparation	21
3.2	Autoclave	22
3.3	Media after being sterilized in Autoclave	23
3.4	Cultured bacteria sample	23
3.5	GSD curve of fine aggregate	26
3.6	Mix design by software analysis	29
3.7	Microbial concrete	33
3.8	Flow chart of experimental investigations	33
4.1	Compression testing machine	34
4.2	Graphical representation of compressive strength results	37
4.3	Tensile strength test	39
4.4	Graphical representation of tensile strength result	42
4.5	Ultrasonic Pulse Velocity Test	43
4.6	Concrete specimens kept in oven for water absorption test	44
4.7	SEM imaging of conventional concrete	46
4.8	SEM imaging of E. coli induced concrete	47

CHAPTER 1

INTRODUCTION

1.1 GENERAL

Concrete remains the most widely used construction material in the world due to its widespread availability, low cost, and long service life. It's made up of fine and coarse aggregates, as well as a cement paste that hardens with time. It is a composite substance made up of an aggregate matrix. A binder, such as Portland cement or asphalt, is often used to tie the matrix together. Concrete has poor tensile strength, hardness, and specific strength, making it a quasi-brittle substance. In compression, concrete is solid, but in tension, it is weak. Among the several limitations of concrete one of them is the cracking. To protect the reinforcing steel bar the tension face of concrete has a cover. Its cracks and leads to the corrosion of the reinforcement once the tensile stress at the extreme fiber exceeds the tensile capacity of the concrete.

Cracking means usually the partial or full division of concrete into two or more sections caused by splitting or fracturing. Concrete fails under sustained loading which reduces the life of the structure. The cracks can be of distinctive types like hairline cracks, shrinkage cracks, settlement cracks or structural cracks. The central causes can be due to temperature differences, heavy loads applied, water leakage from concrete surface, corrosion of reinforcement steel, high water cement ratio. Concrete's pore characteristics influence its longevity, and permeability is dependent on the porosity or pore shape.

To protect the concrete from damage many physical and chemical treatments have been applied but none of these were fully favorable due to its non-reversible action and minimal long-term effectiveness. Many experts are looking for new products that can be merged into concrete to help in the reduction of cement used while still improving its properties. Fly ash, silica fume, glass powder, rice husk, coconut shell ash, timber ash, ceramic waste, slag cement generally known as ground granulated blast furnace, clinoptilolite, and other auxiliary cementitious materials are used. Also, e-waste (electronic waste), plastic, high density polythene, glass aggregate, marble etc. to substitute coarse aggregate and ceramic industrial waste to replace fine aggregate have been used in recent years. These admixtures have shown that they can improve the properties of concrete, but they are extremely costly and in short supply.

Recent times, a noble concrete technique has been approved by combining biological and concrete approaches. This technology is known as microbial concrete. Microbial concrete is actually a self-healing concrete using microorganism that is made by combining a cement paste with microbial cells. In this type of concrete, the cracks formed are healed with the help of microbial reaction in the concrete after it has strengthened. It's worth noting that as minor cracks occur in concrete, they repair naturally, while in larger cases, self-healing in concrete is accomplished by introducing mineral-producing bacteria into the mix. The bacteria included in this innovation are acid-producing bacteria that serve as catalysts. Calcium carbonates are precipitated by the microorganism. Calcite precipitation is preferred here because it is pollution-free and sustainable. The calcite fills the voids in the cementitious matrix and thus it enhances the structure's durability. Another advantage of bacteria is that, the oxygen produced is also consumed by the bacteria and in this way, it helps in prevention of corrosion steel also.

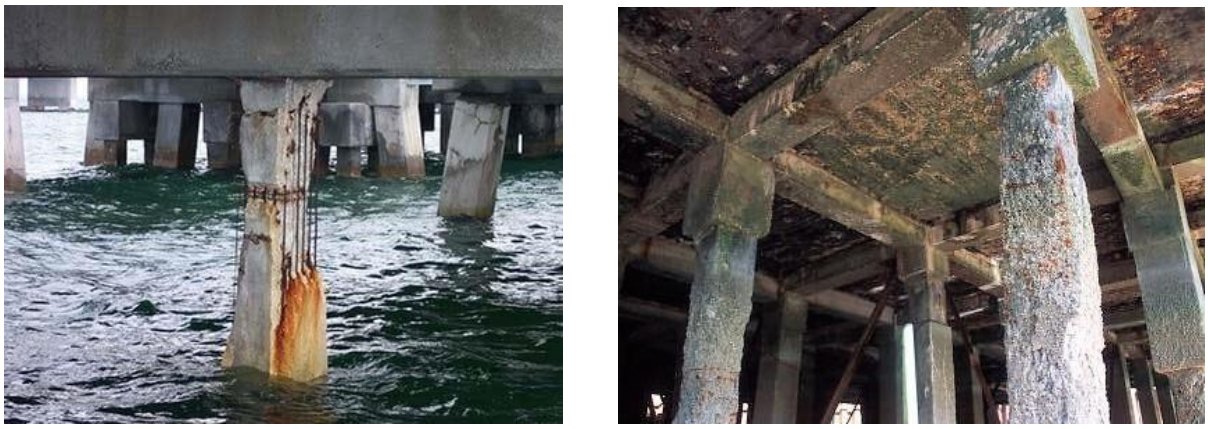
The use of biological approach can be called as a green technology because the production of it does not involve greenhouse gas emission. This method does not deplete natural resources, and the bacteria we use can be grown in a laboratory. Therefore, this process is constructive and recognized as an influential technology for the improvement of strength of building materials.

1.2 STATEMENT OF THE PROBLEM

Concrete has extreme load bearing capacity under compression which is very uncertain in case of tension. Concrete in top layers is compressed or shortened under the load and the concrete in the bottom layer are tensioned or stretched. So, after some times when the load increases the beam starts to deflect and crack occurs in the bottom layer. And thus, eventually it fails as concrete is weak in tension. That is why to carry the tensile load steel bars are embedded in the concrete. This bar takes the load when it cracks in tension. At low stress level the elasticity of concrete is comparatively stable but at high stress level it starts decreasing and micro crack develops. If an object expands when it is objected to forces then tensile stress occurs.

All RCC members are porous in nature but in a microscopic scalar. Smaller cracks in concrete may not damage the structure. Typically, a crack which is less than 0.2 mm are not problematic or questionable but such smaller cracks can lead to larger cracks. Larger cracks in concrete hampers a structural integrity. Also, the micro cracks in concrete contributes to the material porosity and permeability. Water seeps in through the tiny cracks to degrade. Entrance of intrusive chemicals such as chlorides, sulfur, and acid may cause the embedded steel framework

to corrode, compromising the structure's long-term longevity. Not only this, the system constructed in a high-water setting, and that also includes underground basements, aquatic structures and also structure like motorway bridges are also vulnerable because of penetration of different chemicals in the concrete structure.



(a) (b)

Figure 1.1: Examples of severely damaged concrete support beams of bridge

Several techniques have also been adopted including epoxy injection, grouting, stitching, routing and sealing, drilling and plugging, gravity filling of cracks in concrete. For large imperfections in concrete mortar mix can be used which is made by one-part Portland cement, three parts masonry sand, and just enough water to prepare a paste. But it has been found that these methods are very costly. And sealing agents used for enhancing the durability of the concrete suffer from serious limitations of incompatibility interfaces, susceptibility of ultra violet radiation and unstable molecular structure. Access of de-icing salts by micro cracks created in the concrete has caused reinforcement corrosion on these pillars, as seen in **Figure 1.1**.

A substitute and more natural self-healing method based on the use of mineral-producing bacteria is being proposed and studied by researchers. Microbial technique is the latest one and it is found to be effective among all the other techniques which is also ecofriendly.

1.3 RESEARCH OBJECTIVE

The study's key goal is to figure out the compressive and tensile strength of the concrete using different compositions and observation with these with plain water. The study's aim is to improve crack tolerance and crack remediation. The main objective of the study are:

1. To observe how the strength of concrete differs with variable percentage of bacteria.
2. To equate bacterial concrete's compressive strength and tensile split strength to those of traditional concrete.
3. To check the quality of concrete and determining the structural concrete's integrity.
4. To investigate concrete's long-term durability.
5. To examine the microstructure of concrete mix using a scanning electron microscope (SEM) that allows to image the microstructure of hydrated cement paste.

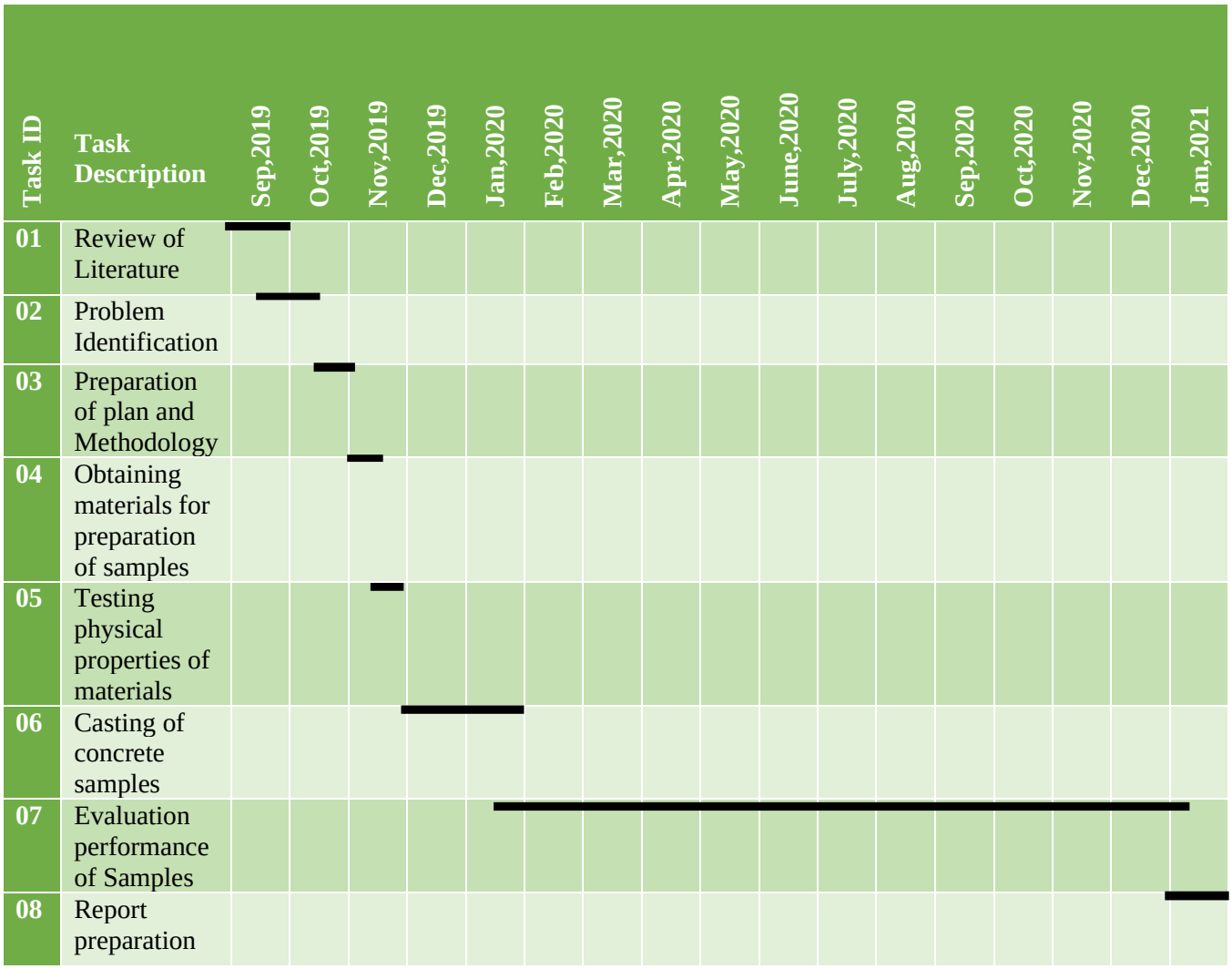
1.4 SCOPE OF THE PROJECT

In this project Microbial Culture was used at ratios of 0:100, 25:75 and 50:50 with potable water. No admixture was used. Mix design was based on material properties and was derived from software analysis (Con-Mix). The compressive strength has been designed between 25 MPa and 35 MPa. Experimental investigation included Compressive Strength, Tensile Strength, ultrasonic pulse velocity test, water absorption test and scanning electron microscope (SEM) analysis. 100 mm cubical specimens were casted. Selected curing periods for the test were taken 7 days, 28 days, 90 days and 365 days. One microbial group having OD_{600} 0.5±0.1 had been studied.

1.5 PROJECT SCHEDULE

A Gantt-Chart showing the project duration is provided below:

Project Name	Project Duration	Project Start Date	Project End Date
A Critical Review on the Performance of Microbial Concrete Developed Using E. COLI Bacteria	17 months	Sep,01, 2019	Jan,28,2021



CHAPTER 2

LITERATURE REVIEW

2.1 GENERAL

Concrete is a very essential element of most construction materials for use in infrastructure as well as buildings. Despite its construction simplicity, this has few shortcomings. It has low tension and the little cracking resistance and limited ductility. As per the researches performed around the globe, to overcome the deficiencies of cement concrete, several adjustments were made through time - to – time.

Microbial Concrete or Bio-Concrete is a vital technique for repairing concrete cracks and fissures by using microbiologically induced calcite precipitation (CaCO3).

Microbiologically mediated calcite precipitation is indeed a method that falls under the bio mineralization branch of science. Living organisms form inorganic solids and improve mechanical strength by this process.

2.2 DIFFERENT SPECIES OF BACTERIA USED IN CONCRETE

Bacteria is basically single celled, living micro-organisms which can be found everywhere. Sometimes they are beneficial but sometimes they could be harmful also for example, while they lead to infection. So, it is necessary to take proper care while dealing with microbes. This is a gel-like simulation made up of water enzymes, nutrient - rich, waste products, and gases. Also, it includes cell structures such as ribosomes, chromosomes, and plasmids. Based on their shape, gram strain and oxygen demand they can be classified into 3 categories as shown in **Figure 2.1**

Selected bacterial strains have been used in concrete to improve the autogenous crack repairing ability of the concrete. Whenever concrete is harmed, water seeps through cracks that show up on the concrete's surface. Different bacterial strains have been used in concrete so that crack formation does not happen in the structure and the structure remains stable. According to the literature review the are the bacteria that are used in the concrete are shown in **Figure 2.2.**

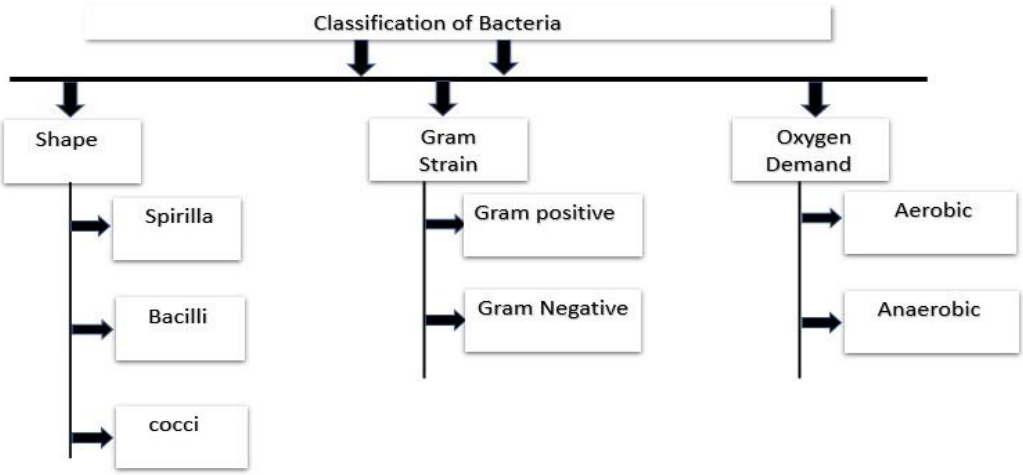


Figure 2.1: Classification of Bacteria

The bacterial strains have been used in concrete matrix are not only helps in crack healing but also, they have other functions. They also have functions like increasing the strength of the concrete, strength enhancement of sand, surface treatment etc. ***Escherichia coli*** has been used in our study. It is obtained from the microbiology department of Chittagong University. It is gram positive and can be found in the nature. Mostly they are harmless and on appropriate media supplemented with such a calcium source, they could indeed generate calcite precipitates.

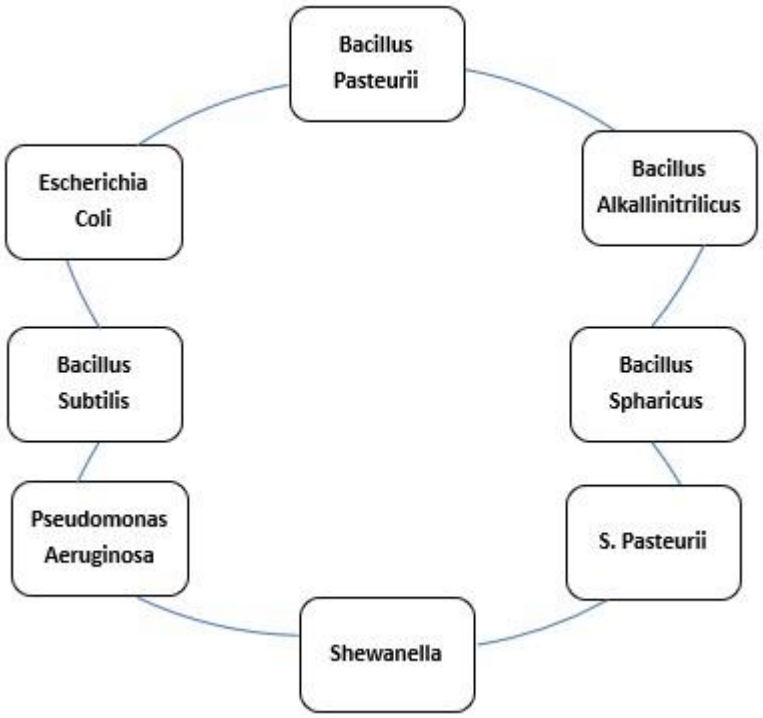


Figure 2.2: Different types of Bacteria used in concrete

Many researchers have used many ways of bacteria in concrete. Because concrete is extremely alkaline, the bacteria in the concrete should meet some criterion. The bacteria must meet two central norms. They are-

1. **Capability to withstand a highly alkaline environment:** Because concrete is a dry material with a PH value of up to 13, it must be capable of withstanding a highly alkaline environment (PH12.8). Most organisms cannot survive in the environment when the PH value reaches higher than 10.
2. **Spore germination capability:** The spore germination of the bacteria must have to continue in the concrete's harsh environmental condition.

2.3 CHEMICAL PROCESS FOR THE REMEDIATION OF CRACKS BY BACTERIA

There are different methods of bacterial participation in calcification were suggested and have been the subject of debate over the last century. This microbial behavior is thought to be influenced by physical-chemical parameters in the atmosphere and to be linked to metabolic processes and cell membrane structures. In addition, a few researchers assume that the metabolic processes of heterotrophic bacteria are perhaps the most important mechanisms of $CaCO_3$ precipitation. Calcium carbonate precipitation is favored by metabolic processes that can increase the pH of the atmosphere against alkalinity with in presence of calcium ions.

In the mechanism of bio-mineralization correlated to microbes, two metabolic pathways are engaged.

(i) Autotrophic mechanism (ii) Heterotrophic mechanism.

Autotrophic mediated mechanism

In autotrophic mediated mechanisms, Microbe's cause $CaCO_3$ precipitation by converting carbon dioxide in the presence of Ca^{2+} throughout the surrounding environment. Non-methylotrophic methanogenesis, anoxygenic photosynthesis, and oxygenic photosynthesis are all examples of autotrophic carbonate precipitation. Carbon dioxide is used as the source of carbon in all three autotrophic mechanisms.

Heterotrophic mediated mechanism

In heterotrophic mediated mechanism, precipitation of carbonate may be caused by either the sulphur or nitrogen cycles.

The first mechanism involves a sulphur cycle, specifically sulphate elimination that is carried out with sulphate reducing agent in anoxic environments by Advanced Topics in

Biomineralization sulphate reducing bacteria. **The second mechanism** involves a nitrogen cycle and particularly-

(i) Oxidative deamination of amino acids through aerobiosis;

(ii) Nitrate reduction in anaerobiosis or microaerophily;

(iii) Urea or uric acid degradation in aerobiosis (by ureolytic bacteria)

Microbially induced calcium carbonate precipitation (MICCP) or microbiologically induced calcite precipitation (MICP) via urea hydrolysis is indeed a simple mechanism wherein ureolytic microbes produce huge quantities of carbonates in such a short time. Because of its simplicity, urea hydrolysis via the enzyme urease inside a calcium-rich atmosphere seems to be the most widely studied precipitation process.

In this technique, the microbial urease enzyme catalyzes the degradation of urea into carbonate as well as ammonium. As shown by the Eqs 1-2, one mole of urea being hydrolyzed intracellularly to yield one mole of ammonia and one mole of carbamate, which then hydrolyzes spontaneously to yield one mole of ammonia and carbonic acid.

$$CO(NH_2)_2 + H_2O \xrightarrow{\text{Bacteria}} NH_2COOH + NH_3 \qquad (1)$$

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

In water, these molecules equalize to produce bicarbonate, one mole of ammonium, and hydroxide ions, leading to an increase in pH levels

$$H_2CO_3 \rightarrow 2\,H^+ + 2\,CO_3^{2-} \qquad (3)$$

Precipitation of calcite by a bacterial cell –

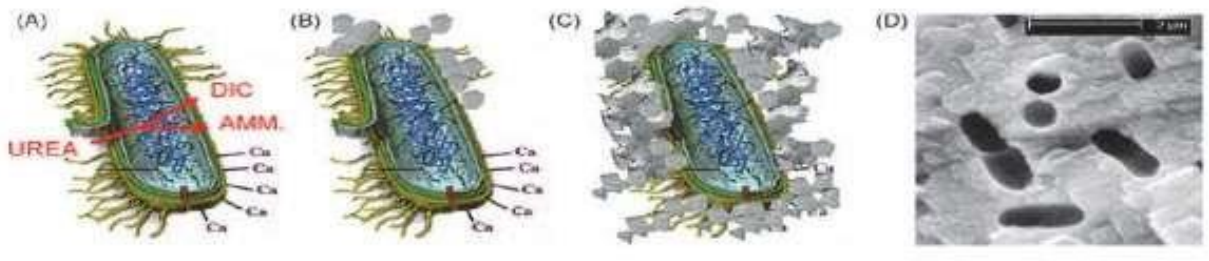


Figure 2.3: Key roles of pH and calcium metabolism in microbial carbonate precipitation (Hammes & Verstraete 2002)

Hammes and Verstraete (2002) looked into the sequence of events that occur while ureolytic calcification, stressing the role of pH and calcium metabolism. The capability of bacteria to produce an alkaline atmosphere through different physiological processes has been attributed to their main role. **Figure 2.3** depicts a condensed timeline of events throughout microbially induced carbonate precipitation (MICP).

That heterogeneous electronegatively loaded bacterial cell membrane serves as a nucleating area for positively charged cations (for example Ca^{2+}, Mg^{2+}) adsorption upon this cell surface. Different negatively charged groups are present at neutral pH, anionic charge dominates the bacterial cell surface results into secretion of divalent positively charged ions on interaction. As shown by the Eqs 4-5, the bacterial cell membrane plays a vital role in the $CaCO_3$ precipitation like a nucleation site.

$$Ca^{2+} + Cell \rightarrow Cell - Ca^{2+} \quad (4)$$

$$Cell - Ca^{2+} + CO_3^{2-} \rightarrow Cell - CaCO_3 \quad (5)$$

The microbes serve as a nucleation site, assisting in the formation of calcite that can gradually seal cracks and pores throughout concrete, improving its durability. This microbiologically induced calcite precipitation (MICP) is the product of a complicated sequence of biological processes. $CaCO_3$ crystals form as a result of this, which expand and develop as the bacteria produce calcium lactate nutrition. The crystals continue to grow until the entire void is filled. Concrete's efficiency is enhanced by this natural and biochemical method.

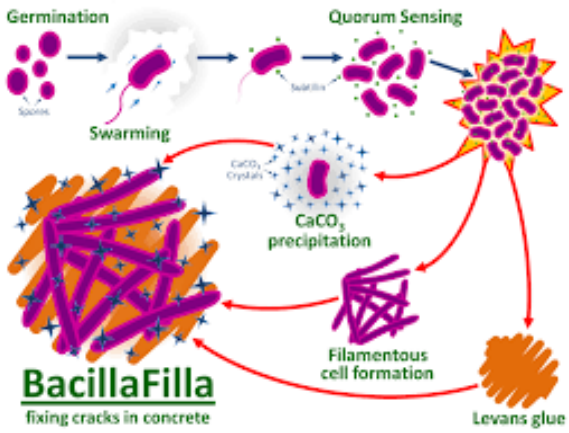


Figure 2.4: Process of fixing cracks in concrete

However, the importance of bacterial precipitation is still debatable. Some writers **(Knorre & Krumbein 2000)** claim that precipitation is indeed an unintended by-product of metabolism, when others **(Ehrlich 1996, Mc Connaughey & Whelan, 1997)** believe this is a distinct phenomenon of environmental benefits for organism's precipitation.

2.4 BENEFITS AND DRAWBACKS OF MICROBIAL CONCRETE

2.4.1 BENEFITS

1. Self-repairing of concrete: this invention will fix concrete cracks where we don't need any construction works. The concrete is mixed with bacteria and other nutrients. So, when crack occurs the water gets into the concrete and activates the bacteria inside. The Bactria produces limestone and fill in the gaps naturally as shown in **Fig 2.5**. The bacteria can fill it within 3 weeks and the bacteria can lie dormant up to 200 years after construction.

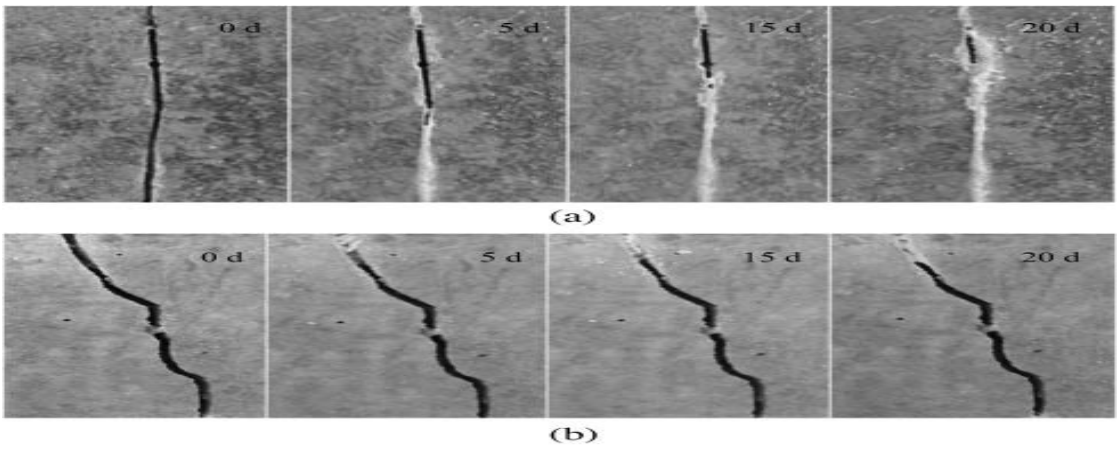


Figure 2.5: Repairing of cracks by Bacteria

2. Reduction in permeability of concrete: many researchers have investigated the importance of bacteria to reduce the permeation. Carbonate producing bacteria helps in this aspect a lot and carbonation test can be used to investigate permeability. Carbonation has been associated to pore interconnection, with larger pores resulting in greater carbonation heights. Microbial calcite precipitation is primarily caused by bacteria's urea lytic interaction and carbonate biofilm formation.

3. Prevention of corrosion of steel: prevention of corrosion is another importance of microbial concrete. While filling the gaps in concrete, the bacteria consume the oxygen. It aids in the mitigation of steel corrosion. Various reports showed that Microbial calcite increases surface permeation and protects against acid attack.
4. Better resistance towards freeze-thaw attack: the use of bacterial calcite as a result of a bacterial chemical reaction can aid in freeze-thaw resistance. As water cannot go into the pores concrete cannot solidified and turn into ice at temperature below 0 degree in the winter which helps in the reduction of freezing and thawing attack.
5. Reduction of maintenance and repair cost: Fly ash, silica fume, and ground granulated blast furnace slag (GGBS) can all be used to substitute a component of the cement in a concrete mix which has proven the enhancement of durability of concrete up to certain extent. But these materials are very costly and not available. In this sense bacterial concrete is comparatively cheap and also its maintenance is low.
6. Significantly higher flexural and compressive strength than ordinary concrete: reviewing several research's it has been found that the compressive and flexural strength therefore greatly improved for mortar samples that contained bacterial cells. Researchers use to see the result summarizing the 3, 7, 28 and sometimes even 60- or 90-days result of compressive strength of different cement mortar specimen. The compressive strengths were obtained with mortar cubes prepared with bacteria as compared to those of water. It has been found out that the compressive strength had been increased to 35-40% when prepared with bacteria compare to the normal one in most cases.

2.4.2 DRAWBACKS

1. The initial expense of microbial concrete is twice that of conventional concrete. But this cost can be reduced. Conventional concrete needs maintenance and it is costly when repairing the concrete after cracking occurs. But bacterial concrete doesn't need any high maintenance cost.

2. Bacterial growth is unfavorable in any environment or medium – there are particular shorts of media suitable for growing particular types of bacteria since they have an impact on survival, expansion, biofilm as well as crystal development (**Table 2.1**). Although specialized mechanisms are sometimes required for micro-organism and cell culture growth. However, more works should be done in this field on retention of nutrients and metabolic products.

3. Mix design of concrete with microbes- microbial concrete is a new technology and so far, not many works have not been done. As it is not very familiar in construction area till now, no rule given yet for use it. As a result, calculating the dosage of microbes used in concrete to achieve the best performance is difficult.

4. Investigation of calcite precipitation is costly studied- amount of calcite precipitation is not same for all the bacteria. Different types of bacteria precipitates different amount of calcite. To measure the amount of calcite precipitated by a certain bacterium "scanning by electron microscopy" is necessary. But this cost huge and it also requires very good skills to perform the test.

5. The clay pallets which hold the self-healing agent comprises of twenty percent of the concrete volume and it may form a shear zone (or fault zone) throughout the concrete.

Table 2.1: Luria-Bertani and metabolism process of various Bacterial Groups

Micro organisms	Nutrients	Metabolism	References
Bacillus subtilis	Urea hydrolysis	Nutrient broth, Urea, $CaCl_2.2H_2O$, NH_4CL, $NaHCO_3$	Ramachandran (2001)
Bacillus sphaericus	Urea hydrolysis	Yeast/ Beef Extract, Urea, $CaCl_2.2H_2O$	De Muynck (2010)
Bacillus subtilis	Oxidative deamination of amino acids	Peptone: 5 gm/ liter, Yeast/ Beef Extract: 3 gm/ liter, NaCl: 5 gm/ liter	Seshagiri (2012)
Bacillus cereus	Oxidative deamination of amino acids	Growth media (Peptone, extract, yeast, KNO_3.NaCl) + $CaCl_2.2H_2O$, Actical, Natamycine	Seshagiri (2012)

CHAPTER 3

EXPERIMENTAL INVESTIGATIONS

3.1 GENERAL

The major objective of this research is to achieve detailed experimental results that will aid in understanding the behavior of microbial concrete as well as its properties such as strength and density. Further, Studies on the action of fresh and hardened properties of ordinary and normal grade concrete both with and without application of bacteria have also been conducted. This will also include the material properties used, as well as the method of preparing bacteria culture media, concrete mix design, and test procedures, among other things.

3.2 BACTERIA CULTURE

3.2.1 PREPARATION OF LURIA-BERTANI MEDIA:

Escherichia coli strain had been used in this study. For E. coli growth, Luria-Bertani media was used. To make Luria-Bertani media, you'll need peptone, Yeast extract, NaCl, and water. **Table 3.1** shows the amount of ingredients needed for the preparation of the media. First, a 3000 ml conical flask was filled with the necessary volume of peptone, beef extract, and NaCl. Then, at room temperature, the requisite volume of water was applied. Finally, to prepare Luria-Bertani media, the solution was slowly stirred.

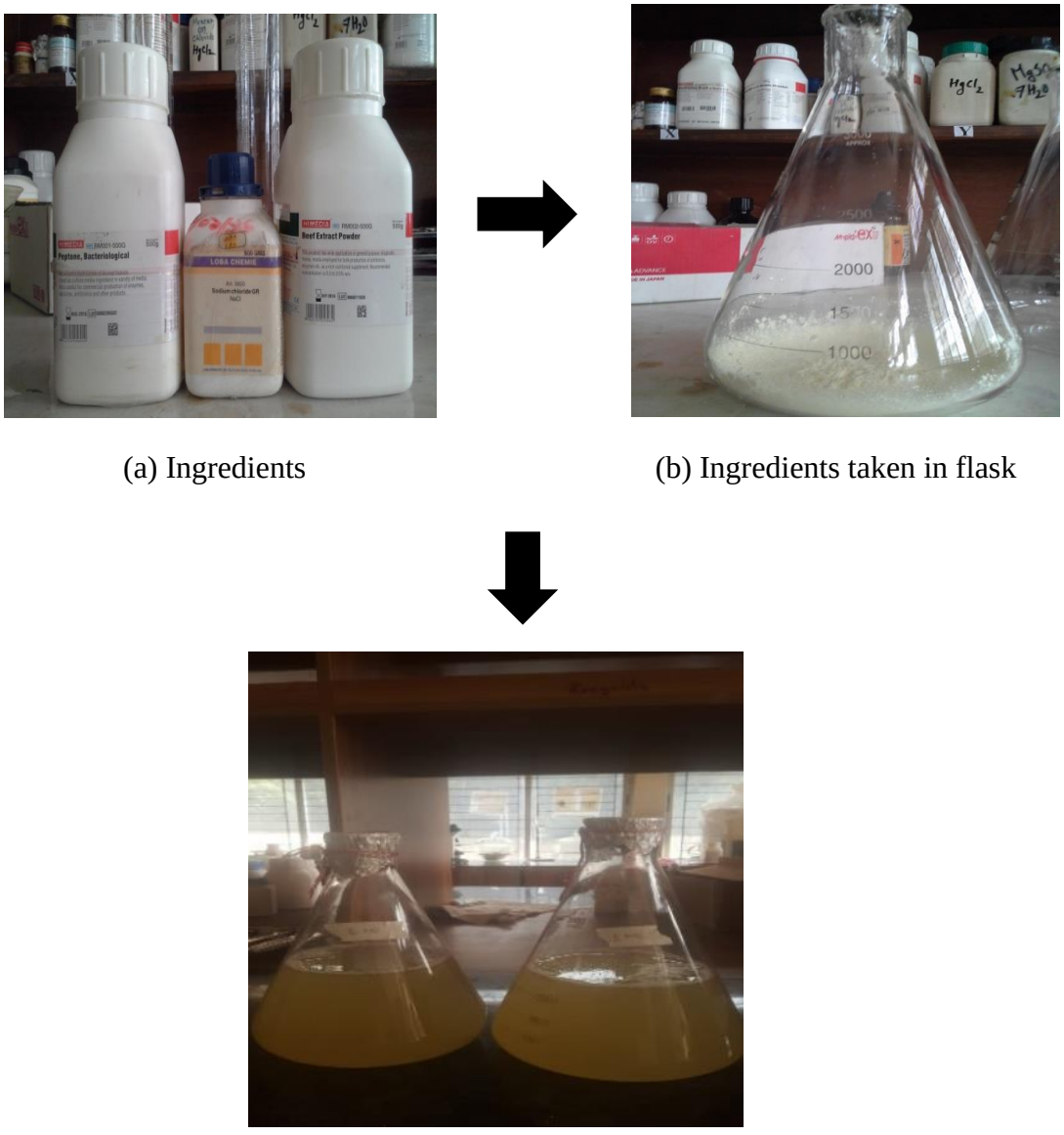
Table 3.1: Ingredients for Luria-Bertani Media

Ingredients for Luria-Bertani Media	
Ingredients	**Quantity**
Tryptone (or Peptone)	10 g/L
Yeast Extract	5 g/L
NaCl	10 g/L
Distilled Water	1 Liter

Bacterial sample had been prepared first in Petri dish and **Figure 3.1** represents the whole procedure related to media preparation.

3.2.2 MAKING MEDIA GERM FREE

It is important to sterilize the media. In microbiology, sterilization autoclaves **(Figure 3.1)** are commonly used. Depending on the media to be sterilized, they differ in size and purpose. Since cylinders are better at withstanding high pressure than boxes, autoclave chambers are usually cylindrical. They self-seal due to the high pressure.



(a) Ingredients

(b) Ingredients taken in flask

(c) Prepared solution

Figure 3.1: Luria-Bertani media preparation

Figure 3.2: Autoclave

If the chamber is enclosed, all of the air is expelled, either by a basic vacuum pump or by forcing the air out of the way with a pumping in stream. The next stream is pumped into the chamber at a greater pressure than natural ambient pressure, resulting in a temperature range of 121 to 140 degree Celsius. A thermostat kicks in and begins a timer until the appropriate temperature is achieved. The precise sterilization period is obtained by a variety of criteria, including the number of contaminants that the products being autoclaved are likely to contain. The same sterility that hots air at $160^{O}C$ can achieve in two hours can be achieved in three minutes with a stream at 134 degree Celsius.

Figure 3.3: Media after being sterilized in Autoclave

3.2.3 BACTERIA INOCULATION AND GERMINATION

The media for Luria-Bertani was sterilized for around 2 hours. The media then became fully germ-free. The insertion of Escherichia coli spores was the next step. As a culture medium, an inoculator is a system that allows microorganisms to be introduced into environments that are conducive to their development. A needle was used to inject spores into prepared media. The spore-containing media was then settled in the refrigerator. The temperature was maintained at a comfortable level. This was the location where bacteria were kept in a binary fission to germinate. Bacterial concentration is determined by the bacteria's growth period and germination time. In this study, four different germination times were used.

Figure 3.4: Cultured bacteria sample

3.2.4 BACTERIA CULTURE PROPERTIES

In the following study, different bacterial groups were investigated. The properties of these prepared samples must be determined. Spectrophotometer method, a fundamental technique in microbiology to monitor bacterial production. A spectrophotometer is needed to determine the optical density of a bacterial colony to evaluate bacterial growth. This method directly measures turbidity. The spectrophotometer can be set to a wavelength of 420–660 nm in general. This wavelength must be consistent, and it may need to be calibrated to the material under study. The maximum absorbance wavelength of different vegetative cells and bacterial spores can differ. To track the growth of E. coli, wave length of 600 nm had been set. It's crucial that the cells are

in an excellently physiological process of growth. The estimated relationship among absorbance and CFU may differ as the cell size differs with growth phase (lag, log, stationery). The concentration of cells differs from optical density and can therefore estimate using the equation below.

$Y = 8.59 \times 10^7 X^{1.362}$

Here,

X = Reading at optical density 600 nm, Y= Cell concentration per ml

3.3 MATERIALS USED AND THEIR PROPERTIES

3.3.1 CEMENT

As a binding material ordinary Portland cement (OPC) ASTM Type-1, which complies with ASTM C-150 has been chosen. The physical properties and the chemical properties of it are given below in **Table 3.2.**

Table 3.2: Physical properties and chemical composition of OPC

Serial No	Characteristics	Value
1	Blaine's Specific surface (cm^2/gm)	2900
2	Normal Consistency	26%
3	Soundness by Le Chatelier's Test (mm)	4.5mm
4	Specific gravity	3.15
5	Setting Time (a) Initial (min) (b) Final (min)	 70 175
6	Calcium Oxide (CaO)	64%
7	Silicon Dioxide (SiO_2)	21%
8	Aluminum Oxide (Al_2O_3)	6%
9	Ferric Oxide (Fe_2O_3)	3.5%
10	Magnesium Oxide (MgO)	1.2%
11	Sulfur Trioxide (SO_3)	2.5%
12	Loss on ignition	1.2%
13	Insoluble matter	0.6%

3.3.2 AGGREGATE

Natural sand which is locally available as a fine aggregate and crushed stone as a coarse aggregate has been chosen in this experiment.

3.3.2.1 FINE AGGREGATE

The sand passing through 4.75 mm sieve and retaining on 0.075 mm sieve has been used.

Physical properties and grading of fine aggregate are provided in **Table 3.2** and **Table 3.3.**

	Weight (kg)
Sample taken	0.500
Sample (OD)	0.468
Sample (SSD)	0.481
Pycnometer + Water	0.484
Pycnometer + Water + Sample	0.786

Specific Gravity = $\frac{0.468}{0.484 + 0.481 - 0.786}$ = 2.61

Absorption Capacity = $\frac{(0.481 - 0.468)}{0.468} \times 100$ = 2.78%

Water Content = $\frac{(0.500 - 0.468)}{0.468} \times 100$ = 6.84%

Table 3.3: Grading of fine aggregate

Sieve No.	Sieve Size (mm)	Wt. Retained (kg)	% Wt. Retained	Cum. % Wt. Retained	% Finer
#4	4.75	0	0	0	100
#8	2.36	0.012	2.4	2.4	97.6
#16	1.18	0.064	12.8	15.2	84.8
#30	0.6	0.233	46.6	61.8	38.2
#50	0.3	0.087	17.4	79.2	20.8
#100	0.15	0.064	12.8	92	8
#200	0.074	0.012	2.4	94.4	5.6
Pan		0.028	5.6	100	0
Sum		0.500			

Fineness Modulus = $\frac{250.6}{100}$ = 2.51

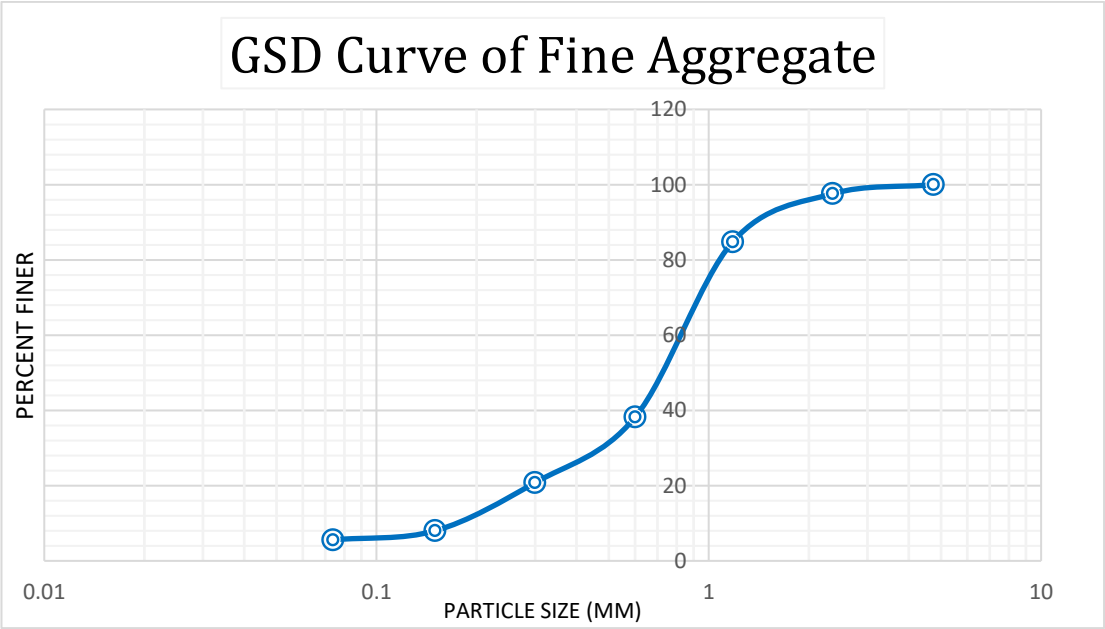


Figure 3.5: GSD curve of fine aggregate

Table 3.4: Physical properties of fine aggregate

Fine Aggregate	
Fineness Modulus	2.51
Specific Gravity	2.61
Absorption Capacity	2.78%
Water Content	6.84%

3.3.2.2 COARSE AGGREGATE:

The nominal size of the crushed stone was 12.5 mm.

Physical properties and grading of Coarse aggregate are provided in **Table 3.5.**

Cylinder Height, h= 7″= 0.175 m

Radius, r= 3″= 0.075 m

Sample, M= 4.887 kg

Unit Weight= $\frac{4.887}{3.1416 \times 0.075 \times 0.075 \times 0.175}$ = 1580.3 kg/m^3

	Weight (kg)
Sample taken	0.500
Sample (OD)	0.472
Sample (SSD)	0.483
Sample in Water	0.311

Specific Gravity = $\frac{0.472}{0.483-0.311}$ = 2.74

Absorption Capacity = $\frac{(0.483-0.472)}{0.472} \times 100$ = 2.33%

Water Content = $\frac{(0.500-0.472)}{0.472} \times 100$ = 5.93%

Table 3.5: Physical properties of Coarse aggregate

Coarse Aggregate	
Density	1580 kg/m^3
Specific Gravity	2.74
Absorption Capacity	2.33%
Water Content	5.93%

3.3.3 BACTERIA

Escherichia coli bacteria has been used in this literature. Rather than sugar and other organic matter, it feeds on carbon dioxide. E. coli is fairly simple to engineer, and its rapid development allows for rapid testing of improvements. E. coli has also been used to synthesize useful chemicals such as insulin. It's also been used to create synthetic forms of human growth hormone. This bacterium can cover concrete cracks and can remain dormant for many years. It will be able to stay in the concrete and at high temperatures. It's a harmless bacterium that divides quickly, according to research. So, one of the benefits of this is that scientists can culture several generations in a limited amount of time. Temperature sustainability test of bacteria is summarized in **Table 3.6.**

Temperature sustainability test:

Table 3.6: E. coli bacteria temperature sustainability test

Temperature	**Bacteria alive condition**
-3^0 C	Alive
10^0 C	Alive
20^0 C	Alive
30^0 C	Alive
40^0 C	Alive
50^0 C	Alive
60^0 C	Alive
70^0 C	Dead

3.3.4 WATER: Water with PH value 7 and turbidity rate zero conforming to requirements of IS456-2000 has been used.

3.4 CONCRETE MIXING

3.4.1 MIX DESIGN OF CONCRETE

Concrete was designed according to material properties. Trial mix designs were made in order to get a rough understanding of cements and total aggregates, and they were collected as a result. And then, software analysis (Conmixer V 1.0) is used to make the mix design. The maximum coarse aggregate size is 12.5 mm, and the slump value is 30-50 mm. It is summarized in **figure 3.6.**

(a) Designed strength **25 MPa** (Target strength **33.5 MPa**)

(b) Designed strength **35 MPa** (Target strength **43.5 MPa**)

Figure 3.6: Mix design by software analysis

Calculation of Amount of Ingredient Materials for 25 MPa designed strength:

Number of concrete groups: There are total 3 groups of concrete for 25 MPa designed strength- Concrete (1 group) without using microbial water and concrete (2 groups) using microbial water.

Number of cubes for each group: 6 (3 cubes for compression test and 3 cubes for tensile test)

Curing Periods: 4 (7 days, 28 days, 90 days, 365 days)

Total number of cubes for 25 MPa concrete: 72 (3 x 6 x 4)

Volume of 72 cubes= 72 x 0.1 x 0.1 x 0.1 = 0.072 m^3. Using factor of safety 1.3 due to change in volume when concrete hardens. Hence total volume = 0.072 x 1.4 = 0.1 m^3.

For a concrete mix design of M25 the ratio of cement, fine aggregate and coarse aggregate was derived to be **1: 2.091: 2.276** for water cement ratio **0.5.**

For traditional concrete, a water cement ratio of **0.5** by mass was chosen. For bacterial concrete, we chose a water cement ratio of **0.375** and a bacterial culture of **0.125**. [Escherichia coli (75:25)] by mass.

For bacterial concrete, we used a water cement ratio of **0.25** and a bacterial culture ratio of **0.25** [Escherichia coli (50:50)] by mass.

Summary of 25 MPa designed strength is given below:

Table 3.7: Materials for Conventional Concrete & Microbial Concrete

(Design strength: 25 MPa)

Groups / **Materials**	**Conventional Concrete** (Concrete volume= 0.034 m^3)	**Microbial Concrete**	
		Escherichia coli (75:25) (Concrete volume= 0.034 m^3)	**Escherichia coli (50:50)** (Concrete volume= 0.034 m^3)
Cement	13.6 kg	13.6 kg	13.6 kg
Fine Aggregate	28.44 kg	28.44 kg	28.44 kg
Coarse Aggregate	30.95 kg	30.95 kg	30.95 kg
Microbial water	0.0 Liter	1.7 Liter	3.4 Liter
Water	6.8 Liter	5.1 Liter	3.4 Liter

Calculation of Amount of Ingredient Materials for 35 MPa designed strength:

Number of concrete groups: There are total 3 groups of concrete for 35 MPa designed strength- Concrete (1 group) without using microbial water and concrete (2 groups) using microbial water.
Number of cubes for each group: 6 (3 cubes for compression test and 3 cubes for tensile test)
Curing Periods: 4 (7 days, 28 days, 90 days, 365 days)
Total number of cubes for 35 MPa concrete: 72 (3 x 6 x 4)

Volume of 72 cubes= 72 x 0.1 x 0.1 x 0.1 = 0.072 m^3. Using factor of safety 1.3 due to change in volume when concrete hardens. Hence total volume = 0.072 x 1.5 = 0.11 m^3.
For a concrete mix design of M35 the ratio of cement, fine aggregate and coarse aggregate was derived to be **1: 1.43: 1.8** for water cement ratio **0.395.**

For traditional concrete, we selected a water cement ratio of **0.395** by mass. We also chose water cement ratio of **0.296** and bacterial culture of **0.099** for bacterial concrete [(Escherichia coli (75:25)].

Water cement ratio of **0.1975** and bacterial culture of **0.1975** for bacterial concrete [Escherichia coli (50:50)] by mass has been selected.

Summary of 35 MPa designed strength is given below:

Table 3.8: Materials for Conventional Concrete & Microbial Concrete
(Design strength: 35 MPa)

Groups / **Materials**	**Conventional Concrete** (Concrete volume= 0.034 m^3)	**Microbial Concrete**	
		Escherichia coli (75:25) (Concrete volume= 0.034 m^3)	**Escherichia coli (50:50)** (Concrete volume= 0.034 m^3)
Cement	17.22 kg	17.22 kg	17.22 kg
Fine Aggregate	24.57 kg	24.57 kg	24.57 kg
Coarse Aggregate	30.95 kg	30.95 kg	30.95 kg
Microbial water	0.0 Liter	1.7 Liter	3.4 Liter
Water	6.8 Liter	5.1 Liter	3.4 Liter

3.4.2 VARIABLES

Concrete quality:

Two separate grades of microbial concrete having OD_{600} (0.5± 0.1) were used. The purpose of casting OPC concrete was to compare its properties with those of microbial concrete.

Exposure period:

On a routine basis, specimens were examined after curing in plain water for 7, 28, 90 and 365 days.

Size of specimens:

ASTM standard procedure was used to prepare cube specimens of size 100 mm x 100 mm x 100 mm.

Curing environment:

In the laboratory, 144 concrete specimens were cast. After casting, the samples were held at 27°C and 90% relative humidity for 24 hours. Following the drying process, all specimens were cured in plain water for various amounts of time at room temperature.

3.4.3 PREPARATION OF SPECIMEN

Following ASTM standard procedure cube samples of size 100 mm x 100 mm x 100 mm were prepared. Mixed design was according to material properties. (**Table 3.9**) The needed number of microorganisms are mixed with media and applied to the concrete mix. All specimens were cured in plain water for several periods.

Table 3.9: Mix ratios for specimen preparation

Designed Strength	Cement	Fine Aggregate	Coarse Aggregate	W/C ratio
25 MPa	1	2.091	2.276	0.5
35 MPa	1	1.43	1.8	0.395

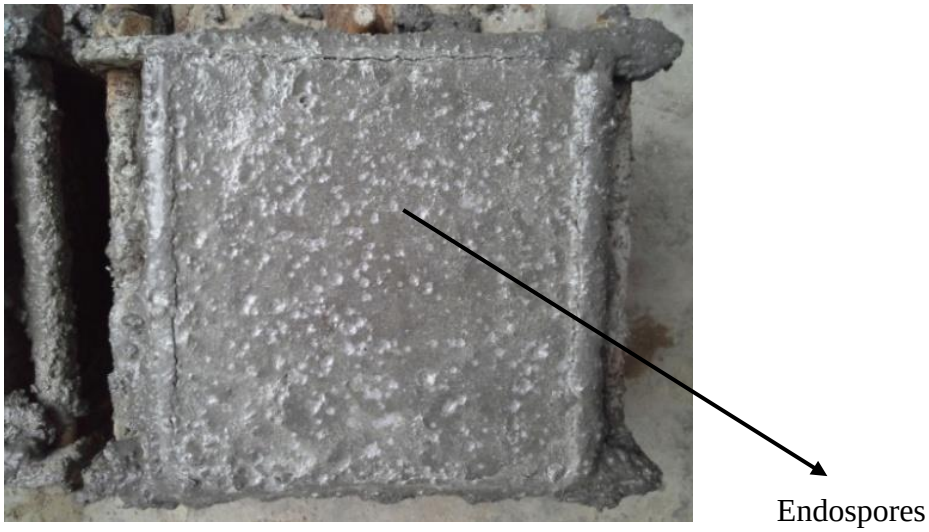


Figure 3.7: Microbial concrete

3.5 FLOW CHART OF EXPERIMENTAL INVESTIGATIONS

The experimental program of the proposed study is given the flow chart shown in **figure 3.8**

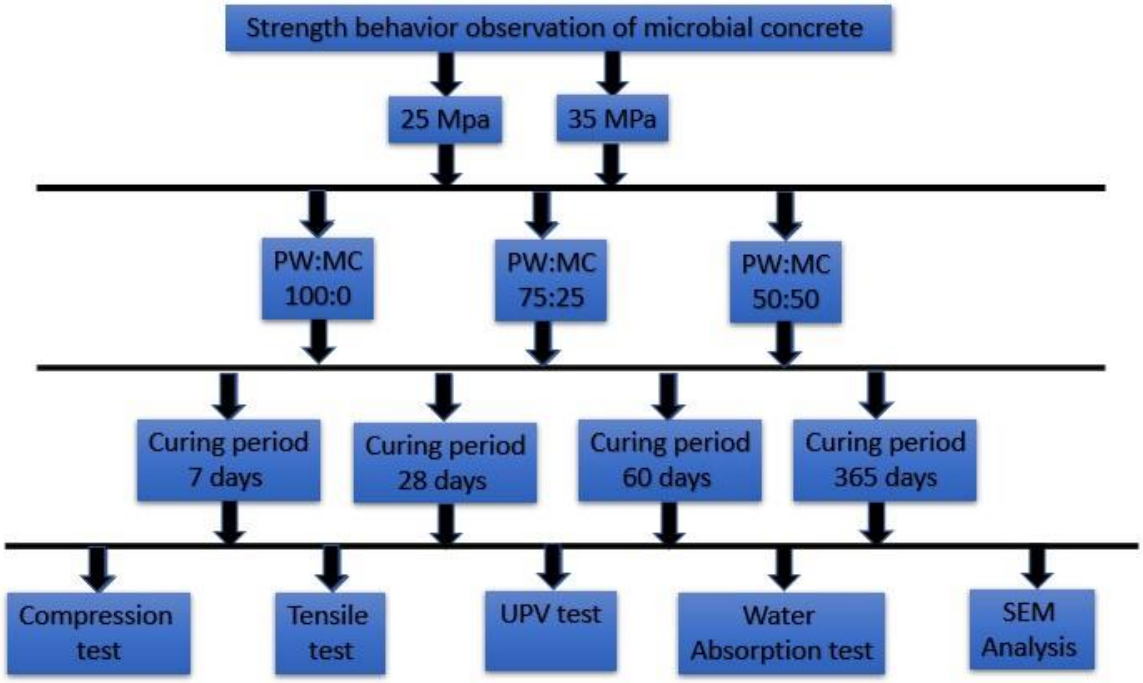


Figure 3.8: Flow chart of experimental investigations

CHAPTER 4

RESULT AND DISCUSSION

4.1 GENERAL

The observed experimental results are discussed in the following section. The study includes compressive strength test, tensile strength test, water absorption, and ultrasonic pulse velocity test (UPV). The results of the tests are displayed in both graphical and numerical format.

4.2 COMPRESSIVE STRENGTH TEST:

This is perhaps the most traditional concrete measure in construction since it provides a general understanding of all the properties of the material. A concrete work may be accepted or rejected based on the outcome of this assessment. Compressive strength is a concrete feature that is influenced by a variety of factors that includes the nature of the components used, the mixing configuration, and quality control during the manufacturing process.

Figure 4.1: Compression testing machine

Compressive test results are shown in **Table 4.1** and **4. 2**

Table 4.1: Compressive strength results for 25 MPa Concrete

Curing Periods	Groups	Compressive Strength (MPa)	Average compressive Strength (MPa)
7 Days	Conventional Concrete	16.7	17.8
		19.37	
		17.3	
	Escherichia coli (75:25)	19.37	19.4
		18.49	
		20.34	
	Escherichia coli (50:50)	18.49	20.9
		22.04	
		22.17	

Curing Periods	Groups	Compressive Strength (MPa)	Average compressive Strength (MPa)
28 Days	Conventional Concrete	27.38	25.6
		25.6	
		23.82	
	Escherichia coli (75:25)	28.56	27.3
		27. 85	
		25.6	
	Escherichia coli (50:50)	27.6	28.4
		27.85	
		29.75	

Curing Periods	Groups	Compressive Strength (MPa)	Average compressive Strength (MPa)
90 Days	Conventional Concrete	29.75	28.5
		27. 85	
		27. 85	
	Escherichia coli (75:25)	30.24	29.6
		32.03	
		26.53	
	Escherichia coli (50:50)	31.53	31.2
		30.04	
		32.03	

Curing Periods	Groups	Compressive Strength (MPa)	Average compressive Strength (MPa)
365 Days	Conventional Concrete	29.14	31.4
		33.82	
		31.24	
	Escherichia coli (75:25)	32.03	32.3
		30.84	
		34.03	
	Escherichia coli (50:50)	34.49	33.7
		31.53	
		35.08	

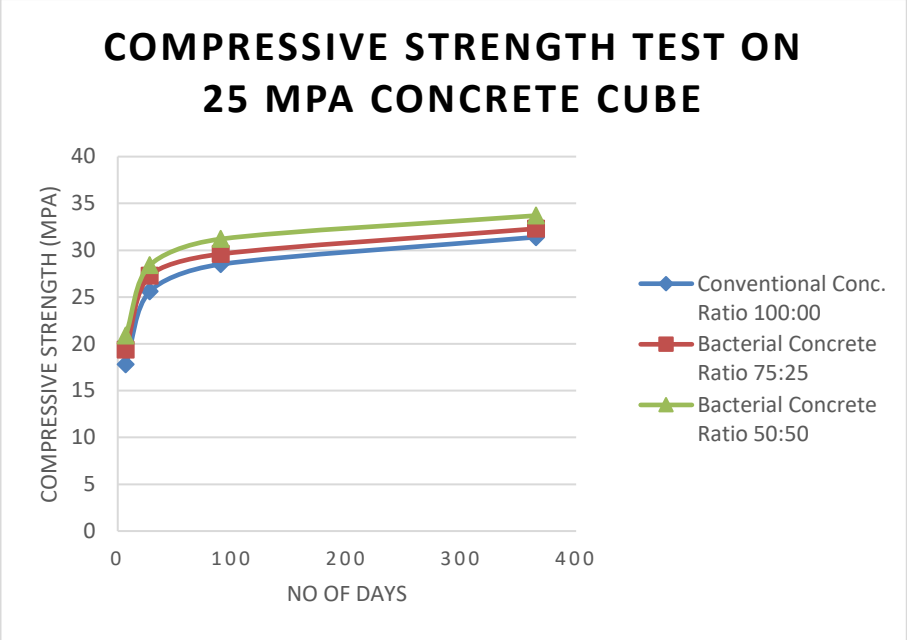
Table 4.2: Compressive strength results for 35 MPa Concrete

Curing Periods	Groups	Compressive Strength (MPa)	Average compressive Strength (MPa)
7 Days	Conventional Concrete	22.64	23.7
		24.04	
		24.42	
	Escherichia coli (75:25)	26.79	26.9
		28.56	
		25.26	
	Escherichia coli (50:50)	29.75	28.8
		29.15	
		27.5	

Curing Periods	Groups	Compressive Strength (MPa)	Average compressive Strength (MPa)
28 Days	Conventional Concrete	36.86	35.9
		33.9	
		36.94	
	Escherichia coli (75:25)	38.66	37.8
		39.06	
		35.69	
	Escherichia coli (50:50)	36.2	39.2
		41.56	
		39.84	

Curing Periods	Groups	Compressive Strength (MPa)	Average compressive Strength (MPa)
90 Days	Conventional Concrete	40.96	39.2
		38.66	
		37.97	
	Escherichia coli (75:25)	41.56	40.8
		42.18	
		38.66	
	Escherichia coli (50:50)	42.75	42.1
		39.6	
		43.94	

Curing Periods	Groups	Compressive Strength (MPa)	Average compressive Strength (MPa)
365 Days	Conventional Concrete	43.94	42.9
		41.56	
		43.2	
	Escherichia coli (75:25)	45.73	44.4
		42.93	
		44.54	
	Escherichia coli (50:50)	48.11	46.3
		46.92	
		43.87	



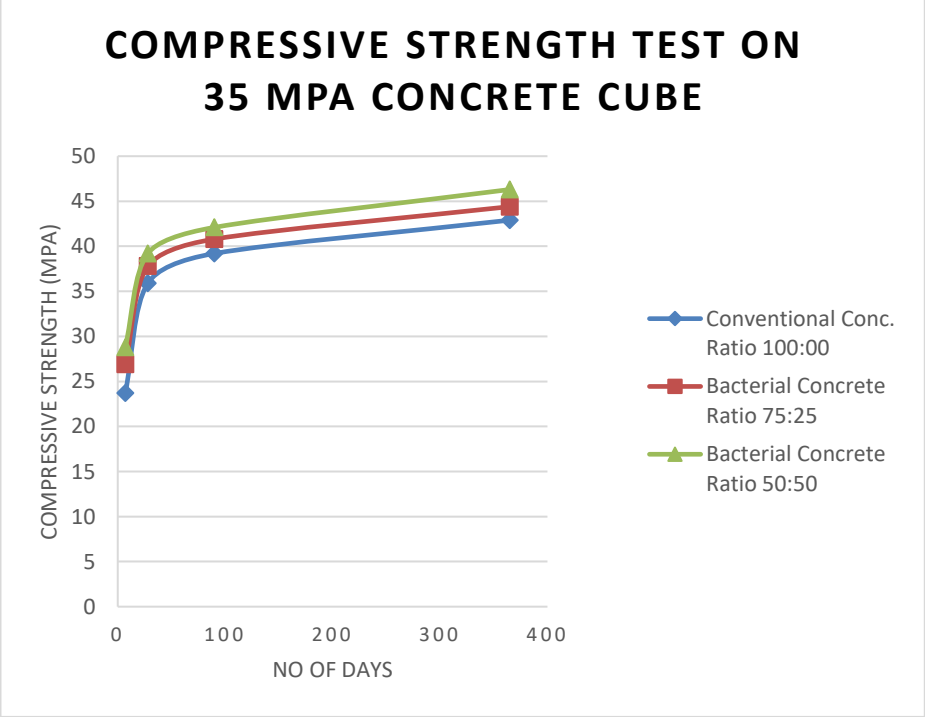


Figure 4.2: Graphical representation of compressive strength results

The study specimen analyses have been thoroughly evaluated and presented via graphical and numerical format. **Figure 4.2** shows the initial findings of microbial concrete compressive strength for multiple strengths and curing periods.

Table 4.3 shows that, for 28 days of curing, the strength of lower-grade concrete increases more than the higher-grade concrete. On the other hand, for 90- or 365-days curing, the strength of higher-grade concrete increases more than the lower-grade concrete.

Again, strength increases more in case of **Escherichia coli (50:50 ratio)** concrete than **Escherichia coli (75:25 ratio)** concrete.

Table 4.3: Strength Behavior Observations

Curing Days	**Increased in Compressive Strength**			
	25 MPa		**35 MPa**	
	Escherichia coli (75:25)	***Escherichia coli (50:50)***	***Escherichia coli (75:25)***	***Escherichia coli (50:50)***
7	8.99%	17.42%	13.50%	21.52%
28	6.64%	10.94%	5.29%	9.19%
90	3.86%	9.47%	4.08%	7.40%
365	2.87%	7.32%	3.50%	7.93%

4.3 TENSILE STRENGTH TEST:

Tensile strength is a valuable property of concrete because structural stresses make it susceptible to tensile cracking. Concrete's tensile strength is significantly weaker than its compressive strength (hence the use of steel to bear stress forces). Concrete's tensile strength is estimated to be about 10% of its compressive strength. Since the direct approach is complex, indirect approaches are used to calculate tensile power. It's worth noting that the results of these methods are better than the results of the uni-axial tensile test.

Figure 4.3: Tensile strength test

Tensile strength results are shown in **Table 4.4** and **4.5**.

Table 4.4: Tensile strength results for 25 MPa Concrete

Curing Periods	Groups	Tensile Strength (MPa)	Average Tensile Strength (MPa)
7 Days	Conventional Concrete	3.57	3.6
		4.26	
		3.1	
	Escherichia coli (75:25)	4.26	3.9
		3.1	
		4.26	
	Escherichia coli (50:50)	3.67	4.2
		4.85	
		4.08	
28 Days	Conventional Concrete	6.15	4.7
		3.1	
		4.85	
	Escherichia coli (75:25)	4.26	4.9
		5.83	
		4.61	
	Escherichia coli (50:50)	5.83	5.1
		4.13	
		5.34	

Curing Periods	Groups	Tensile Strength (MPa)	Average Tensile Strength (MPa)
90 Days	Conventional Concrete	4.85	4.9
		4.13	
		5.83	
	Escherichia coli (75:25)	5.34	5.2
		4.26	
		6	
	Escherichia coli (50:50)	5.83	5.3
		5.34	
		4.73	

Curing Periods	Groups	Tensile Strength (MPa)	Average Tensile Strength (MPa)
365 Days	Conventional Concrete	3.85	5
		5.83	
		5.34	
	Escherichia coli (75:25)	4.43	5.4
		5.34	
		6.43	
	Escherichia coli (50:50)	5.83	5.7
		6.43	
		4.85	

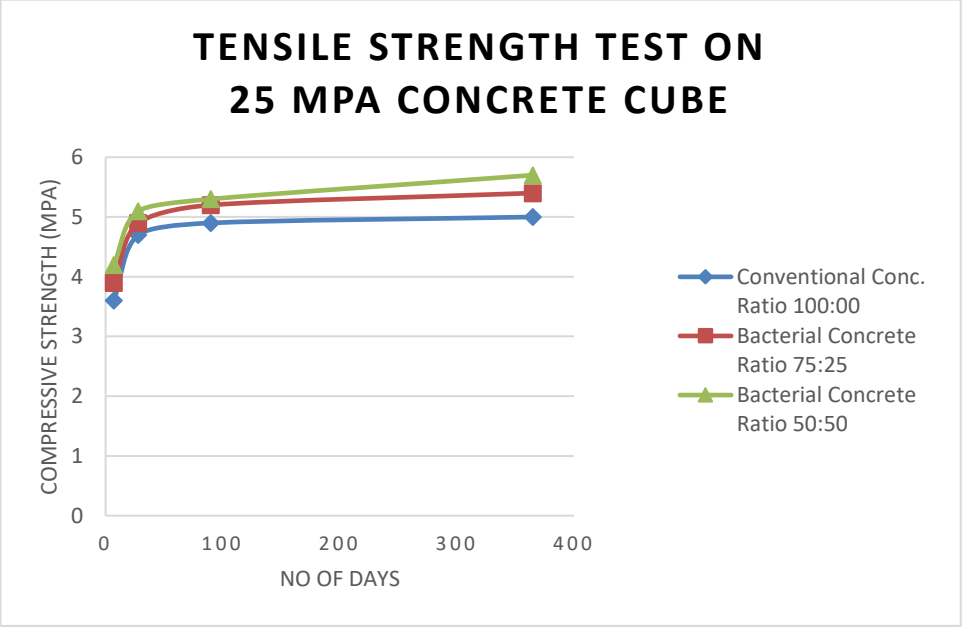
Table 4.5: Tensile strength results for 35 MPa Concrete

Curing Periods	Groups	Tensile Strength (MPa)	Average Tensile Strength (MPa)
7 Days	Conventional Concrete	4.85	4.7
		5.83	
		3.42	
	Escherichia coli (75:25)	5.83	5.4
		4.85	
		5.52	
	Escherichia coli (50:50)	4.85	5.7
		6.43	
		5.83	

Curing Periods	Groups	Tensile Strength (MPa)	Average Tensile Strength (MPa)
28 Days	Conventional Concrete	6.04	5.5
		5.23	
		5.23	
	Escherichia coli (75:25)	6.43	6.2
		5.83	
		6.43	
	Escherichia coli (50:50)	6.73	6.4
		6.43	
		6.04	

Curing Periods	Groups	Tensile Strength (MPa)	Average Tensile Strength (MPa)
90 Days	Conventional Concrete	6.43	6.1
		6.43	
		5.45	
	Escherichia coli (75:25)	5.83	6.9
		7.7	
		7.23	
	Escherichia coli (50:50)	6.43	7.1
		7.23	
		7.7	

Curing Periods	Groups	Tensile Strength (MPa)	Average Tensile Strength (MPa)
365 Days	Conventional Concrete	7.23	6.4
		6.43	
		5.54	
	Escherichia coli (75:25)	7.23	7.2
		7.94	
		6.43	
	Escherichia coli (50:50)	7.23	7.3
		7.23	
		7.7	



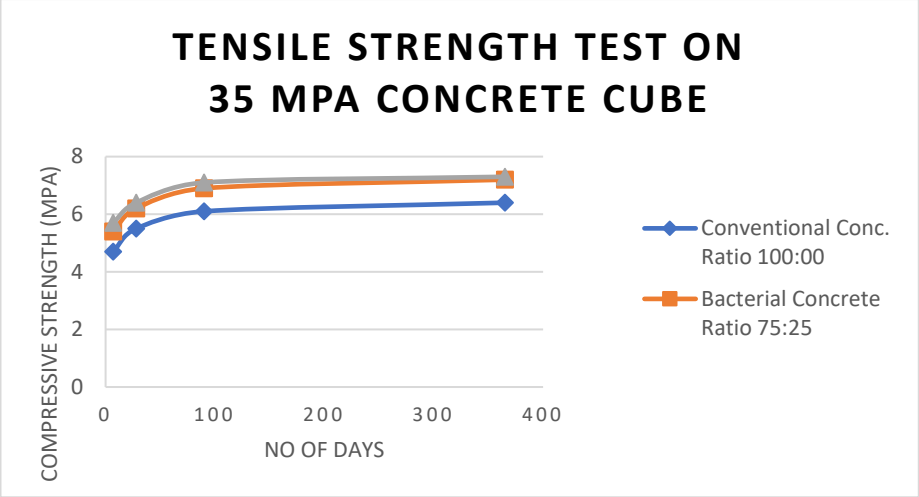


Figure 4.4: Graphical representation of tensile strength result

In split tensile strength tests, plain concrete and microbial concrete of different ratio with different strength were used. Usually, Concrete's tensile strength is just about 10% of its compressive strength. The testing was conducted out after various curing days. The connection of split tensile strength between microbial concrete and handled specimen are shown in graphical and numerical format in **Table 4.4 and 4.4.**

The use of microorganisms in concrete improves the split tensile strength of the material. Also in case of conventional concrete, it rises by 10 to 15%. **Figure 4.4** shows a graphical representation of the relation between split tensile test and microbial concentration.

4.4 ULTRASONIC PULSE VELOCITY (UPV) TEST:

The time it takes for an ultrasonic pulse to propagate through the concrete being studied is measured using this tool. A higher velocity is achieved if the concrete consistency is strong in respect of density, uniformity, homogeneity, and other factors.

UPV test results represent the density of the tested specimens directly. The fewer voids there are, the higher the value. This means that denser concrete specimens would have a higher pulse velocity. Concrete specimens of OD_{600} have a higher velocity, as can be seen. These types of specimens are denser than other groups. The results of the compressive and tensile strength tests also show that specimens with induced microbe have higher strength values than those from other concrete classes. UPV test values are shown in **Table 4.6**.

Ultrasonic pulse velocity was determined for the concrete specimen by using equation given below:

UPV= L/T

Here, L = Distance between transducers

T = Transit time

Figure 4.5: ultrasonic pulse velocity test

Table 3.10: Guidelines of concrete quality

Pulse velocity (km/sec)	Concrete quality (grading)
Above 4.5	Excellent
3.5 to 4.5	Good
3 to 3.5	Medium
Below 3.0	Doubtful

Table 4.6: Ultrasonic pulse velocity test data

Velocity (ms^{-1}) for 25 MPa concrete

Group	**7 Days**	**28 Days**	**90 Days**	**365 Days**
Conventional Concrete	3050	3250	3580	3640
Escherichia coli (75:25)	3140	3290	3630	3710
Escherichia coli (50:50)	3190	3340	3690	3820

Velocity (ms^{-1}) for 35 MPa concrete

Group	7 Days	28 Days	90 Days	365 Days
Conventional Concrete	3120	3270	3340	3420
Escherichia coli (75:25)	3200	3340	3430	3510
Escherichia coli (50:50)	3280	3410	3490	3620

From **Table 4.6,** concrete samples 7 and 28 days were medium quality concrete. On the other hand, 90- and 365-days concrete samples were both medium & good quality concrete for all groups. UPV test indicates that microbial groups are denser than plain concrete.

4.5 WATER ABSORPTION TEST:

In laboratory settings, there are a certain effective approach for measuring water absorption. The most widely used Standard approaches for water absorption are ASTM C1585 and ASTM C642.

ASTM C642 method were used in this research work.

The durability of concrete near an unprotected surface is greatly determined by the number of harmful agents which can easily penetrate into the concrete. Water absorption by immersion provides an estimate of the total (reachable) porous volume of the concrete, but it provides no information on the permeability of the concrete, which is more significant in terms of durability. Though absorption test is a standard means of evaluating concrete's water tightness. The lower the absorption, the finer the result. Water absorption test results are shown in **Table 4.7.**

Figure 4.6: Concrete specimen kept in oven for water absorption test

Table 4.7: Water absorption test data

Water absorption for 25 MPa concrete:

Group	7 Days	28 Days	90 Days	365 Days
Conventional Concrete	3.89 %	3.21 %	3.01 %	2.89 %
Escherichia coli (75:25)	3.75 %	2.95 %	2.78 %	2.66 %
Escherichia coli (50:50)	3.64 %	2.72 %	2.65 %	2.51 %

Water absorption for 35 MPa concrete:

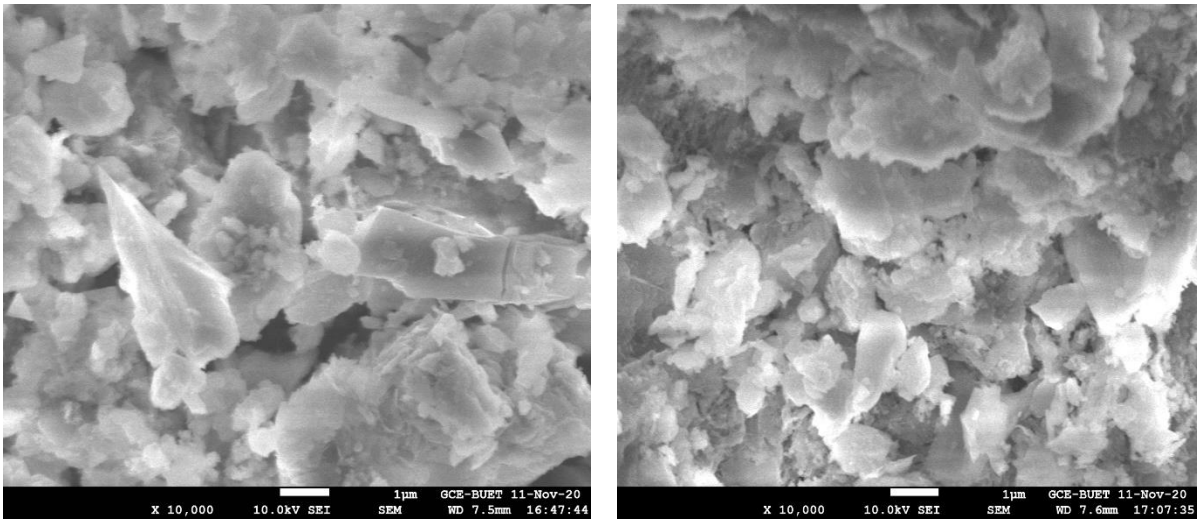
Group	7 Days	28 Days	90 Days	365 Days
Conventional Concrete	3.62 %	3.05 %	2.85 %	2.67 %
Escherichia coli (75:25)	3.41 %	2.74 %	2.51 %	2.32 %
Escherichia coli (50:50)	3.14 %	2.61 %	2.42 %	2.2 %

In Water absorption test, plain concrete and microbial concrete with different strength were used. The testing was conducted out after various curing days. The use of microorganisms in concrete reduces the absorption of the material. That means, microorganisms help concrete making more durable.

4.6 SCANNING ELECTRON MICROSCOPE (SEM) ANALYSIS

SEM analysis was used to study the possible changes in the microstructure of concrete caused by the addition of E. coli bacteria. To explore the microstructure of every concrete group, concrete specimens were taken from all concrete groups at the age of 28 days and studied at various magnifications.

The SEM morphology of distinct concrete groups at 28 days is shown in **Figure 4.7** and **Figure 4.8**. It was found that bacterial inclusion has a significant impact on the microstructure of concrete. Among all concrete samples, conventional concrete **(Figure 4.7)** had the most voids. As the rate of water substitution by microbial culture rose, voids decreased.

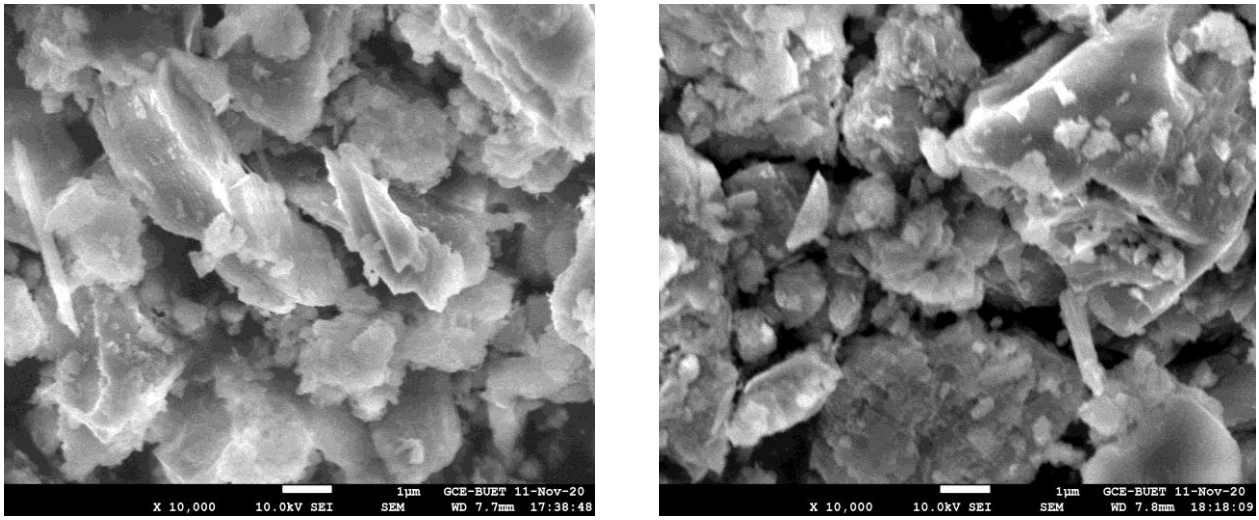


(a) 25 MPa plain concrete (b) 35 MPa plain concrete

Figure 4.7: SEM imaging of conventional concrete

The filling effect of bacterial concrete, which preferred to fill up holes in concrete, attempting to make the microstructure denser, might be ascribed towards the development in concrete microstructure. The microstructure of concrete had a considerable impact on the hardness and durability properties of the material. The minimization in voids in microbial strain-infused concrete mixtures was indeed the primary cause for its better strength and durability attributes when compared to plain concrete group.

The cause for the rise in density of concrete containing microbial strains was also validated by SEM analysis. It demonstrated the presence of calcite precipitation in bacterial concrete, which resulted in fewer cavities and a more compact concrete. Calcite precipitation was detected as the white areas in these images. The density of white patches seen in these photos increased as its concentrations of microbes increased. As a result, it can be stated that the denser microstructure of concrete mixture is primarily responsible for the increase in strength and durability of concrete with inclusion of microbial strain.



(a) 25 MPa plain concrete (b) 35 MPa plain concrete

Figure 4.8: SEM imaging of E. coli induced concrete

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 General

In this study microorganisms were used in concrete to see how they affected the mechanical characteristics of concrete. The bacteria serve as a nucleation site for the formation of calcite, which can pretty quickly fill pores as well as cracks in the concrete and improving its longevity. This biochemical and inherent process aids in the improvement of concrete performance.

5.2 Conclusion

The advantages of bacterial concrete have been observed by many other researchers, including increased compressive strength, reduced permeability, and reinforced corrosion in concrete structures Microbial concrete is becoming incredibly popular in civil engineering.

The experiment might provide some helpful information about the use of microbial concretes in the building of marine reinforced concrete structures both on and off the coast. The following conclusions can be made depending on the available set of test parameters as well as exposure conditions mentioned:

1. Microbial concrete technique has found to be superior to several traditional techniques, for its eco-friendliness, self-healing efficiency, increased durability and simplicity of use.
2. All compressive and tensile strength tests revealed that the bacterial concrete specimens were stronger than standard concrete specimens.
3. The above **Table 4.3** shows that, the microorganism improved the strength of the concrete by gaining high improvement in initial strength.
4. Strength increases more in case of **Escherichia coli (50% bacterial culture)** concrete than **Escherichia coli (25% bacterial culture)** concrete. That means, the strength of higher bacterial ratio concrete increases more than the lower bacterial ratio concrete.

5. As can be seen from **table 4.6**, the microorganism was effective in enhancing the performance of the concrete. UPV test indicates that microbial groups are denser than plain concrete.
6. From **table 4.7** above it can be seen, the use of microorganisms in concrete reduces the absorption of the material. The calcium carbonate formed has occupied a few portions of the voids, trying to make the surface more compact and seepage prone. As a result, the structure's stability is increased by preventing the absorption of liquids and ions that induce reinforcement corrosion.
7. The SEM test showed less voids as the rate of water substitution by microbial culture increased. This inclusion attempt to make the microstructure denser may be attributed to the development of concrete microstructure and had a significant impact on the hardness and durability properties.
8. After observing all the test data, use of Escherichia coli (50% bacterial culture) having OD_{600} 0.5± 0.1 has performed better and use of this ratio will facilitates the production of ecofriendly and cost-effective concrete.

5.3 Recommendations for Future study

Many researchers discovered this superior and intelligent material, but due to its different limitations, further research is needed to get the most value from it.

1. More research into the preservation of nutrients and metabolic products in building materials is required. In order to determine the results of introducing new bacteria into natural microbial populations, as well as the evolution of communities over the short, medium, and long term, Precise microbial ecology experiments are often needed.
2. Bacterial concentration is a feature of bacterial growth process. Better intensity effects can be achieved by monitoring and evaluating the required development process.
3. The gradual deposition of bacterial carbonates could reduce calcite deposition quality. Furthermore, the existence of well-developed rhombohedra calcite crystals has a stronger consolidating effect than the existence of tiny acicular vaterite crystals.

   As a result, further studies into the various nutrients and metabolic products used to develop calcifying microorganisms is required, as these factors affect longevity, development, biofilm formation, and crystal formation.

4. Manufacturing of self-healing bacteria in extensive quantity should be focused on in future studies.
5. Because of the successful use of reinforced concrete for infrastructure building, future work can also rely on the outcome of this process on corrosion.
6. It's worth remembering that the most notable microbial concrete findings in terms of crack repairing and property improvement have only been short-term. The long-term durability of microbial concrete must be tested in order for it to become a viable technology.
7. It is highly recommending that the biotechnology be used in self-healing with sufficient precautions. Appropriate technology that is acceptable in terms of its impact on longevity should be used.

Apart from the scientific aspect, the word "bacteria" has a psychological influence on people due to its widespread perception as “pathogenic”. Making microbial concrete suitable for industrial applications is thus a difficult challenge, and construction community should be educated on microbe pathogenicity.

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