Crack Repair of Concrete Structure using Bacteria

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Declaration by the Scholar

I hereby declare that the work presented in this thesis entitled " Crack Repair of Concrete Structure using Bacteria" in fulfillment of the requirements for the award of Degree of Doctor of Philosophy, submitted in the Maharishi School of Engineering and Technology, Maharishi University of Information Technology, Lucknow is an authentic record of my own research work carried out under the supervision of Dr. Gaurav Shukla. I also declare that the work embodied in the present thesis-

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Date:

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ABSTRACT

The creation of a comprehensive infrastructure, which includes residential regions, industrial sectors, and business sectors, requires the use of Concrete as an essential component. Concrete, on the other hand, is susceptible to its own vulnerabilities, the most notable of which is the formation of fractures. Due to the presence of surface cracks in concrete, a favorable conduit is created for the entry of water, oxygen, and a variety of chemicals. The employment of crystal formation by bacterial species is widely utilized in a variety of industries, including the oil industry and civil engineering, amongst others. Some examples that can be provided are the utilization of rock plugging systems for the purpose of enhancing oil recovery and the implementation of protective measures for stones that are extremely attractive. In these applications, bacteria that are known to be carcinogenic are utilized. These microbes are capable of producing calcium carbonate precipitation. Concrete cracks allow water and chloride to seep into the material, which has a detrimental effect on the material's ability to withstand wear and tear. Therefore, it is of the utmost importance to commence the healing process of concrete as soon as possible in order to minimize water infiltration and damages that could potentially occur. The self-healing concrete approach is the most recent and generally discussed method for tackling various concrete-related concerns. It provides an effective solution to many of these challenges, making it the most recent and discussed methodology. One of the characteristics of self-healing concrete is its capacity to automatically fix cracks that appear in the concrete as soon as they are formed.

This research was conducted with the intention of determining the effects of incorporating a bacillus Subtilis bacterial solution and replacing a portion of the cement with GGBS at the best utilization rates for GGBS. As a substitute for cement, four separate amounts of GGBS were utilized in the construction of M25 grade concrete. These proportions were as follows: 5%, 10%, 15%, and 20%. Each of the three different quantities of bacteria 10^3, 10^5, and 10^7 cells/ml were utilized in the production of the bacterial solution. This bacterial solution was used in place of ten percent of the water used for mixing. This study compared the mechanical properties of concrete that was generated with the addition of a bacterial solution to concrete that was made using only ground granulated blast furnace slag (GGBS). The study was conducted to evaluate the mechanical properties of concrete. Additionally, the rates at which the samples absorbed water were measured in both of the scenarios. In a solution containing 10% GGBS, the results of the tests indicate that the highest mechanical strength is achieved with a bacterial concentration of 10^5 cells per milliliter inside the solution. The

strength of the concrete began to decrease as soon as the percentage of ground granulated blast furnace slag (GGBS) in the concrete reached a 10% level. Attributable to the presence of microorganisms, the concrete's capacity to absorb water was reduced. In this work, regression analysis was utilized to investigate the ways in which ultrasonic pulse velocity and water absorption value are related to the relationship between strength in compression and the two variables discussed. Indicating that there is a substantial link between compressive strength and water absorption, the R^2 values for the GGBS sample and the bacterial solution were 0.9044 and 0.8338, when compared to one another. The R^2 values for the correlation between Ultrasonic Pulse Velocity (UPV) and Strength in compression were 0.8443 and 0.7376 for the GGBS (Ground Granulated Blast Furnace Slag) and Bacterial Samples, respectively. These values suggest that there is a strong relationship between the two variables.

The Bacillus bacteria family has been shown to have extraordinary healing properties for concrete. The current study used a bacterial solution of Bacillus megaterium from the Bacillus bacteria family at concentrations of 10^3, 10^5, and 10^7 CFU. A total 135 number of specimens were made and also tested for durability and strength at one, four, and eight weeks. The findings indicated that flexural, compressive, and split tensile strength improved by 8.83%, 10.10%, and 12.45% MPa, respectively, following 8 weeks of testing at a bacterial concentration of 10^5 cells/ml. The value of absorption of water employing bacteria in concrete mix was found to be lower than that of ordinary concrete mixture. The cracks in the concrete are filled as a result of calcite precipitation generated by the bacterial solution of Bacillus megaterium. The R^2 value of the regression study between compressive and water absorption was 0.6222, while the R^2 value between compressive strength and UPV was 0.4967. The study also included a cluster analysis of water absorption and compressive strength.

Keywords: GGBS (Ground Granulated Blast Furnace Slag), Bacillus Subtilis, Bacillus Megaterium, UPV, Strength, Regression analysis, Compressive Test, Water Absorption, Crack, Repair, Bacteria, Concrete, Structure, Cube, Structures, Durability, bacteria, R^2 value, workability.

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LIST OF ABBREVIATIONS

Abbreviation	Description
CO_2	Carbon dioxide
CA	Coarse Aggregate
FA	Fine Aggregate
GGBS	Ground Granulated Blast Furnace Slag
OPC	Ordinary Portland Cement
SEM	Scanning Electron Microscope
MICP	Microbially Induced Calcium Carbonate Precipitation
CaCo3	Calcium Carbonate
Ph	Potential of Hydrogen
ECC	Engineered Cementitious Composite
SEM	Scanning Electron Microscopy
TGA	Thermo-Gravimetric Analysis
PU	Poly Urethane
SCM	Supplementary Cementitious Material
UPV	Ultrasonic Pulse Velocity
WA	Water Absorption
CS	Compressive Strength
B. Subtilis	Bacillus Subtilis

(Back Cover)

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CHAPTER 1
INTRODUCTION

1.1 OVERVIEW

In reality, concrete is the most consumed substance in the world, second only to water, and the most extensively utilized building material overall. Its relative cost-effectiveness, longevity, and adaptability explain its widespread use. Because of its great compressive strength and malleability, concrete is indispensable in the construction of several constructions, including infrastructure, buildings, roads, bridges, and more. However, there are substantial environmental implications associated with its production, mainly in relation to carbon dioxide emissions from cement manufacturing (a key ingredient in concrete). Significant research has been conducted worldwide to create durable and eco-friendly concrete structures. However, concrete is porous and susceptible to cracking, which can compromise its durability. Cracks in concrete can result from various factors, including shrinkage, mechanical tensile forces, freeze-thaw cycles, and compressive stresses, ultimately leading to structural failure. Microcracks and pores are particularly problematic as they allow water and other harmful substances to infiltrate the structure, causing reinforcement deterioration and reducing the structure's lifespan. Achieving durable concrete is a significant societal concern due to the high costs involved. Effective strategies to repair cracks include proper curing, adding control joints, using the correct water mix, compacting the base, and introducing reinforcements. Nevertheless, the cost of repairing and maintaining concrete structures remains high worldwide. Traditional crack repair methods are often labor-intensive, chemical-based, expensive, and pose environmental and health risks.

1.2 CONCRETE

Over time, a cement paste binds together a variety of fine and coarse components to form concrete. Cement binders made of lime, such as lime putty, were extensively utilized in the past. Concrete made with these ingredients and either Portland cement or calcium aluminate cement is called Portland cement concrete. There are a variety of uses for polymer concretes, but asphalt concrete—which uses a bitumen binder—is most often seen on roads. Contrast this with mortar, which is utilized to join masonry elements like bricks, tiles, and the like; concrete, on other hand, is not. To make concrete, slurry of aggregate, dry Portland cement, and water is mixed until it becomes a fluid, pourable consistency.

Cement is a versatile material with many uses; when combined with water and other ingredients, it forms a strong matrix that bonds the parts into a stone-like solid. Sometimes, mixes include additives like pozzolans or superplasticizers to enhance their physical properties, either in the wet mix or as a finished product. Since concrete lacks tensile strength, steel reinforcement is necessary to create reinforced concrete.

1.2.1 **Composition of Concrete**

Concrete comprises an aggregate matrix and binder, typically stabilized by Portland cement or asphalt. Various types of concrete are produced based on the binder and aggregate used, influencing the final product's strength, density, and chemical and thermal tolerances. Sand, crushed rock (such as limestone or granite), and coarse gravel are the aggregates used to make concrete. The most typical binder is Portland cement, which, when coupled with aggregates and dry powder, forms a semi-liquid slurry when water is added. This slurry undergoes hydration, solidifying and hardening the concrete as water interacts with the cement to form a binding solid. Alternative cementitious materials, such as fly ash and slag cement, can be used in aggregate binders, enhancing concrete's freshness and hardness.

The cure rate and properties of concrete are adjusted with admixture materials. Common admixtures include ground granulated blast furnace slag, silica fume and fly ash, Steel reinforcement is often used in Portland cement-based concrete structures to provide exceptional low tensile strength and compressive strength. When asphalt is used as binder, the resulting material is called asphalt concrete, which is commonly used for joints and pavements where safety and durability are paramount. Figure 1.1 illustrates pavements made from the two types of concrete structures discussed.

(a) Portland Cement Concrete (b) Asphalt Concrete

Figure 1.1: Portland and Asphalt Concrete

1.2.2 Aggregate and Its Types

The process of making concrete involves mixing granular inert materials like sand, gravel, or crushed stone with water and Portland cement. Pure, solid, and long-lasting aggregates devoid of absorbed chemicals, clay coatings, and other microscopic contaminants are essential for concrete to retain its structural integrity. Aggregates, which can be either fine or coarse in texture, can account for as much as 75% of concrete's overall volume. Particles less than 4.75 millimetres in diameter are known as fine aggregates. This category includes crushed stone, natural river sand, and other similar materials. Particles larger than 4.75 millimetres are called coarse aggregates. Crushed stone and gravel make up the bulk of coarse aggregates.

1.2.2.1 River Sand

Superior quality and reduced processing time make river sand a widely preferred choice in the construction industry. However, its use comes with significant environmental costs and impacts on the ecosystem. Overexploitation of riverbed sand deposits can alter river flow, destroy riverbanks, and even trigger floods. It also damages groundwater and harms the habitats of aquatic creatures and microorganisms. Excessive river sand mining can ultimately cause rivers to dry up.

1.2.2.2 Ground Granulated Blast Furnace Slag

The GGBS product is environmentally friendly and made of metal. Furthermore, this material not only has a lower CO_2 footprint, but also exhibits a superior level of quality. The glassy and granular GGBS is made by scalding or water-or steam-extracted molten metal slag from a furnace. As a waste product, molten metal slag contains both metal and mineral components. The maximum particle size utilised in the GGBS was 95.21 micrometres, and its moisture content was 0.14%. A specific gravity of 2.88 is the mark for Ground Granulated Blast Furnace Slag, or GGBS for short. Furthermore, the bulk density of this substance is 1,290 kg/m^3.

1.3 History of Microbiology

Bacteria are multicellular prokaryotic organisms that can take many forms. Soil, acidic hot springs, water, radioactive waste, and the deepest portions of the Earth's crust all contain them, along with biological components and animal and plant carcasses. There are around five billion (5×10^30) bacterial cells per gramme of soil and one million per millilitre of fresh water, respectively, and these bacteria contribute significantly to the planet's biomass. In 1676, Antoine van Leeuwenhoek used a single-lens microscope he had constructed to examine bacteria for the first time. He wrote to the Royal Society about his findings, calling the bacteria "animalcules." According to Christian Gottfried Ehrenberg, the word "bacterium" was first used in 1838.

Louis Pasteur proved in 1859 that fermentation is instigated by microbes. Germ theory of illness was co-founded by Robert Koch and Pasteur. A long-established method for bacterial classification, the Gramme stain test distinguishes between two main groups of bacteria according to the composition of their cell walls: Gram-positive and Gram-negative.

It is common practice to use either solid or liquid media for bacterial growth in labs. Pure bacterial cultures can be isolated using solid media like agar plates, while growth measurements or cell production in large volumes are best accomplished with liquid media. Isolating specific bacteria from liquid media is challenging, but cultivating them in stirred liquid medium results in a uniform cell suspension, which facilitates culture division and transfer. One way to detect certain organisms is by using selective media, which include antibiotics or nutrients that are specific to that organism.

There are three stages of bacterial development. Bacteria go through a lag phase when they initially reach a growth-friendly, nutrient-rich environment. During this period, their cells adjust and generate proteins needed for rapid growth, therefore their growth is delayed. In the second stage, known as the logarithmic (log) or exponential phase, development is extremely fast. Here, the pace of cell multiplication is the growth rate, and generation time is amount of time it receipts for population to double. Log phase lasts until the limiting nutrient is exhausted, during which time nutritional metabolism reaches its peak. In the last stage, known as the stationary phase, cells consume non-essential proteins and metabolic activity drops when nutrients run out.

1.4 Classification of Bacteria

1.4.1 Classification on the Basis of Shapes

The morphologies of bacteria are usually used for classification purposes. Bacilli, which are rod-shaped, cocci, which are spherical, and spiral-shaped, are the three main groups into which bacteria fall.

1.4.2 Classification on the Basis of Gram Strain

The Gramme staining method determines this categorization by binding an agent to the bacterial cell wall. Bacteria are categorised as Gram-positive or Gram-negative depending on their response to stain.

1.4.3 **Classification on Basis of Oxygen Requirement**

The oxygen demand of the bacterium is the basis for this categorization. Two main types of bacteria exist: aerobic bacteria, which absorb electrons from molecules of oxygen, and anaerobic bacteria, which do not.

1.5 Various bacteria used in the concrete

Bacillus Subtilis
Bacillus Megaterium
Bacillus sphaericus
Bacillus pasteurii
Escherichia coli

1.6 Bacillus Subtilis

Several bacteria, including as E. coli, Bacillus sphaericus, and Bacillus pasteurii, have been investigated by researchers for potential application in bacterial concrete. Strain of Bacillus Subtilis is the subject of this investigation. Microbiologically induced calcite precipitation (MICP) is main benefit of adding bacteria to concrete since it allows the bacteria to constantly precipitate calcite. Bacterial calcium carbonate precipitation has important technical and scientific ramifications. Bacillus Subtilis in a controlled environment, is examined in this study to determine its effect on the strength and longevity of concrete.

1.7 Bacillus Megaterium

Megaterium bacteria facilitate the formation of calcite in the pores of concrete specimens during and after the process of healing, hence enhancing the structural density and durability.

Figure.1.2: Bacillus Megaterium in powder form

1.8 Bacterial Concrete

Several new, cost-effective methods have been developed to repair cracks in concrete, with recent innovations focusing on crack-filling materials. One notable advancement is self-healing concrete, which utilizes biomineralization to enhance concrete efficiency. This technique entails infusing the concrete with microorganisms that produce spores and precipitate calcite. As a result of interacting with water through fissures, these bacteria make calcium carbonate, which aids in the mending of the cracks.

Bacterial concrete incorporates bacteria as a component of the concrete mix. The technique of microbiologically induced calcite precipitation (MICP) offers an efficient alternative for repairing micro-cracks and pores in concrete. This eco-friendly, organic, and economical approach not only repairs cracks but also improves durability. The hydrolysis of urea into carbon dioxide and NH_3 is catalyzed by urease enzyme, which is produced by urease-positive bacteria. The pH is raised and calcite (calcium carbonate) can be precipitated in bacterial environment as result of this process. This method was initially used to patch cracks in channels to prevent leaching.

Research has shown that bacillus pasteurii and bacillus sphaericus can improve the compressive strength of concrete and fix cracks in the material through calcite precipitation. Under alkaline, freeze-thaw, and sulphate conditions, concrete treated with Bacillus pasteurii outperformed untreated control specimens, according to research.

To make concrete far less porous, you can add self-healing agents and additional cementitious ingredients like metakaolin and silica fume. The ability to take calcium ions from the environment and create urease enzyme allows bacteria to mend holes and micro-cracks by precipitating calcite. An increase in pH and the subsequent generation of calcite by the reaction of calcium ions with carbon dioxide are the results of this enzyme's hydrolysis of urea into ammonia and carbon dioxide.

Endospore-forming bacteria are particularly useful in concrete as their endospores can survive harsh conditions and remain dormant for up to 200 years. When cracks form and water and air penetrate the concrete, these endospores can germinate and start precipitating calcite to repair the cracks.

Incorporating additional cementitious materials improves concrete's strength and durability while supporting energy and environmental conservation. Silica fume, a byproduct from ferrosilicon and silicon alloy production, enhances concrete's microstructure, resulting in greater durability and strength. Metakaolin, refined kaolin clay calcined under controlled conditions, also contributes to improved engineering properties of concrete. While bacterial concrete may have a higher initial cost due to substrate requirements, using less expensive substrates could reduce costs significantly. Over time, this reduction can lead to savings from decreased repair and maintenance expenses.

1.8.1 **Historical Perspective of Bacteria**

There is wide range in size and shape of bacteria, which are prokaryotic, tiny, single-celled creatures. acidic hot springs, Soil, radioactive waste, the Earth's crust, water, organic materials, and living organisms' bodies are just a few of the many places you could find them. Soil typically contains around 40 million cell units per gramme, whereas fresh water has about 1 million cell units per millilitre.

Christian Gottfried Ehrenberg coined the term "bacteria" in 1838, while Antoine van Leeuwenhoek used a single-lens microscope to detect bacteria in 1676. Louis Pasteur, who demonstrated that germs induce fermentation in 1859, was an early proponent of the germ hypothesis of illness, which he and Robert Koch helped to establish.

There are two main categories of bacteria, Gram-negative and Gram-positive, which are distinguished by the Gramme stain. Bacterial staining in this manner is useful for taxonomy and species identification. The most common ways that bacteria are cultured in labs are in either solid or liquid forms. When working with pure cultures, solid media like agar plates are utilised, whilst liquid media are more suited for growth measurement or cell production in bulk. Even while it can be difficult to isolate a single bacterium from liquid media, moving the medium makes the cells suspension more uniform, which makes dividing and transferring cultures much easier. It is possible to isolate certain bacterial organisms with the help of selective medium that include antibiotics or nutrients.

There are three separate stages to the bacterial life cycle. When bacteria are first exposed to an environment rich in nutrients, they go through a period of adaptation and preparation for fast growth. Crucial proteins are produced in large quantities during this stage. The logarithmic phase, the second stage, is characterised by exponential increase. Time it takes for a population to double is called generation time, and rate of cell division is a measure of growth rate. A limiting nutrient is consumed at a high rate until it is exhausted. Nutrient scarcity triggers the last stage, the stagnant phase. The microbes use up non-essential cellular proteins and slow down their metabolic rate.

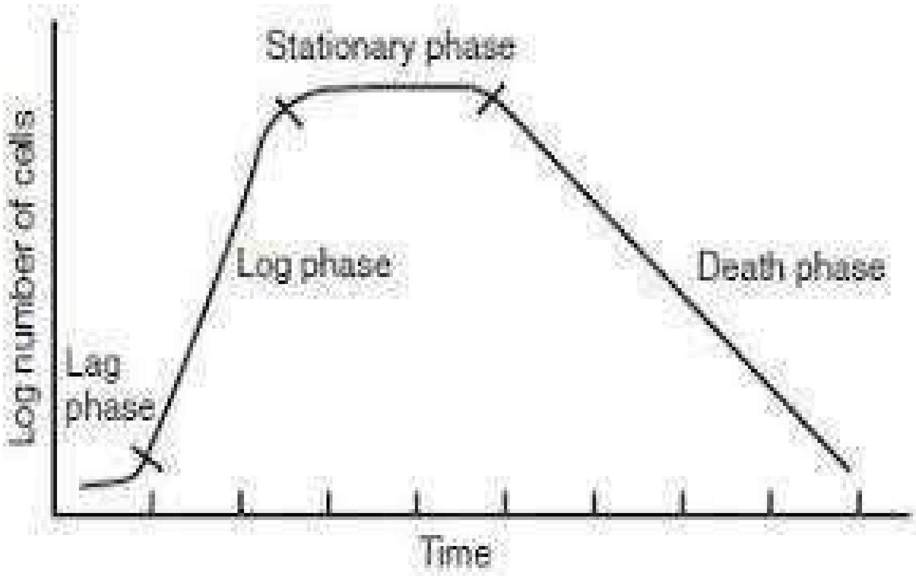


Figure 1.3: Stages of Bacterial Growth
(https://bioisnotdifficult.blogspot.com/2018/08/growth-curve.html?m=1)

1.8.2 Types of Bacteria

Various types of bacteria available and are classified based on gram strain, shapes, and requirement of oxygen.

1.8.2.1 Basis of Shape

Bacteria are divided into three groups based on their shapes: rod- shaped (Bacilli), spherical (Cocci), and spiral (Spirochetes) (Spirilla).

Figure 1.4 depicts several of the bacterial structures.

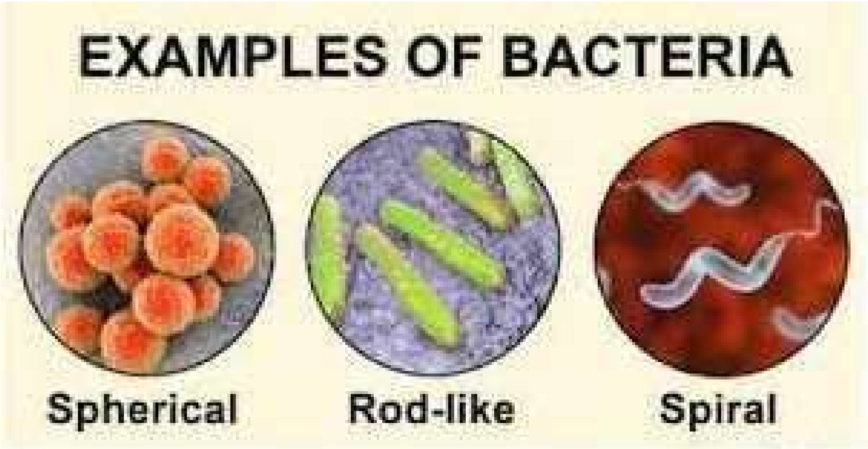


Figure 1.4: Bacterial Classification Based on Shapes

([Examples of Bacteria: Types and Infections | YourDictionary])

1.8.2.2 Basis of Gram Strain

The Gram Staining Method uses a binding agent to bacterial cell wall to determine whether bacteria belong to Gram-positive and Gram- negative categories. Figure 1.5 depicts these microorganisms.

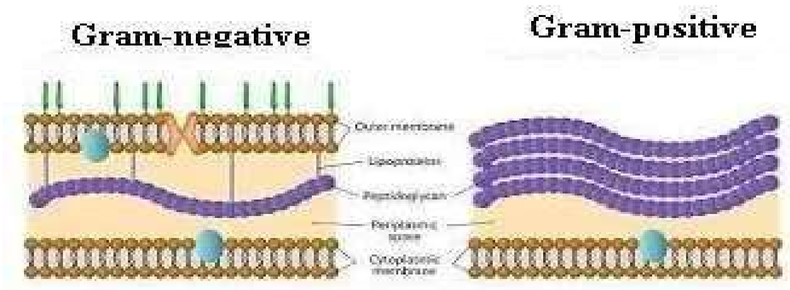


Figure 1.5: Gram Strain Bacteria Classification

([Gram (−)ve bacteria possess peptidoglycan and an extra layer of(A) Protein(B) Lipoprotein(C) Lipopolysaccharide(D) Lipid (vedantu.com)])

1.8.2.3 Basis of Oxygen Requirement

The classification of bacteria based on their oxygen requirements divides them into two main categories:

- Aerobic bacteria, which use molecular oxygen as a terminal electron acceptor for their metabolic processes.

- Anaerobic bacteria, which do not use molecular oxygen for their metabolism.

This classification system is illustrated in Figure 1.6.

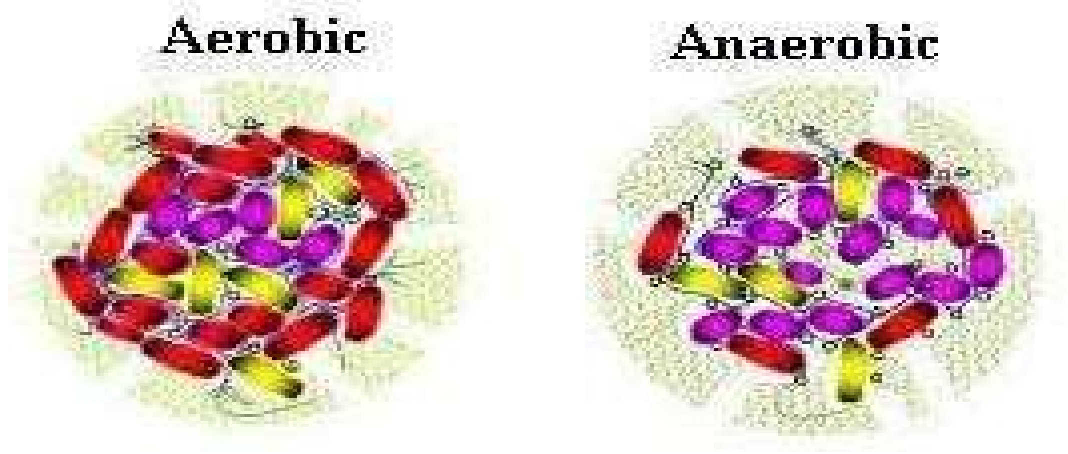


Figure 1.6: Aerobic and Anaerobic Bacteria

(A proposed schematic of the floc structures of anaerobic and aerobic... | Download Scientific Diagram (researchgate.net))

1.9 Self-Healing Concrete

Self-Healing Concrete, also known as Bacterial Concrete, is an innovative method used to repair cracks that form in concrete. This technique involves incorporating bacteria into the concrete mix, which activate to heal the cracks after the concrete has set. Studies show that cracks as narrow as 0.05 to 0.1 mm in diameter can be completely sealed even after multiple cycles of drying and wetting. These small cracks, resembling capillaries, allow water to penetrate, which then hydrates the unreacted or partially reacted cement, causing it to swell and fill the gaps.

For larger cracks, additional maintenance may be required. A promising development in this field involves using microorganisms that directly produce minerals within the concrete mix. These microbes can generate acids that facilitate the repair process. Remarkably, some of these bacteria can remain dormant for up to 200 years in dry conditions, serving as a long-term solution for crack repair. Essentially, these bacteria act as a catalyst for self-repairing fractures in concrete.

1.10 Approach Flow Diagram

Figure 1.7 shows approach flow diagram of the research work.

The methodology for the thesis work is outlined as follows:

1. Preliminary Studies: Initially, research is conducted to identify the presence of bacteria in M-sand. Among the identified bacteria, the most effective calcite-precipitating strain is selected.

2. Mix Proportions: Concrete mixes of M25 grade are prepared according to IS Code 10262:2019.

3. Mechanical and Durability Studies:

- Mechanical Properties: Tests are conducted to evaluate modulus of elasticity, flexural strength, split tensile strength, and compressive strength.
- Durability Properties: Studies include water absorption, sorptivity, acid resistance, rapid chloride permeability, and water permeability tests.
- Statics Analysis: Regression analysis were used to analyze the correlation between the properties of concrete.

4. Bacterial Concentration Testing: Various concentrations of bacteria (ranging from 10^3 to 10^7 cells/ml) are tested, and results are analyzed to determine the optimal bacterial concentration.

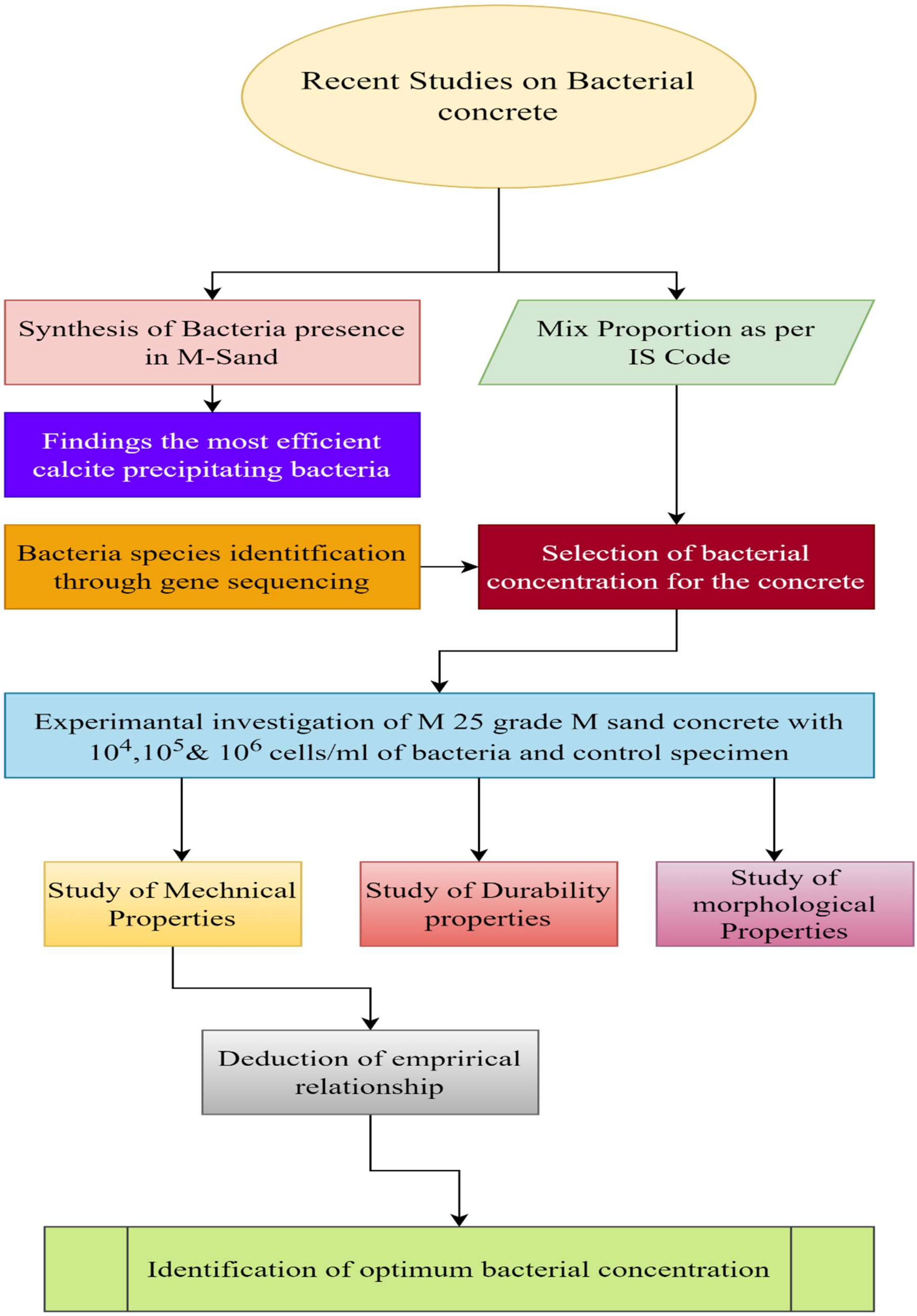


Figure 1.7: Approach Flow Diagram

The process aims to find the most effective concentration of bacteria for enhancing the properties of M-sand concrete.

1.11 Objectives of the Research Work

1. To find out the optimum replacement of cement with GGBS.

   .

2. To find out of maximum dosage of bacterial solution.

3. To find out the mechanical properties of GGBS and bacterial concrete.

4. To find out the durability properties of GGBS and bacterial concrete.

5. To find the correlation of between the properties of concrete.

1.12 Organization of the Thesis

The organization of the remaining chapters of Thesis is as follows:

Chapter 1 presents introduction to bacterial concrete and detailed flow diagram depicting methodology of research.

Chapter 2 Literature review for this research work provides a comprehensive overview of previous studies related to bacterial concrete and M-sand, as well as their applications. It explores various researchers' insights into the development and implementation of bacterial concrete, emphasizing its ability to self-repair through microbial mineralization. The review highlights key findings on different bacterial strains, particularly their efficacy in precipitating calcium carbonate (calcite) and enhancing concrete durability. It also covers the production, properties, and benefits of M-sand as an alternative to natural river sand, noting its impact on concrete's mechanical performance and longevity. Examining the effects of bacterial additions on mechanical parameters such as modulus of elasticity, compressive strength, split tensile strength, flexural strength, and bacterial concrete, this review dives into the subject. Also covered are the long-term effects of several environmental factors on bacterial concrete's water absorption, chloride permeability, acid resistance, and overall durability. The review integrates morphological studies and empirical relationships to provide a holistic understanding of how bacterial concrete and M-sand contribute to improving concrete's properties and durability.

Chapter 3 Details the methodology of developing bacterial concrete with the identified bacteria. Tests conducted to validate the presence of bacteria on the concrete specimens have been illustrated.

Chapter 4 Presents the experimental procedure for preparation of the samples aimed in this research studies. includes the mechanical properties, durability analysis and the morphological of the research work. shows the mechanical properties' experiential relationship based on regression analysis and their validation

Chapter 5. Conclusions of the work presented in the Thesis. The main contribution of the Thesis is highlighted, and suggestions are made for future work.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

A significant number of infrastructure projects all around the world rely heavily on concrete since it is a material that is both extensively used and necessary in the building industry. Given its extensive application and rising demand, the incorporation of advanced materials like pozzolans, silica fume, and bacteria offers not only technical benefits but also economic, ecological, and environmental advantages. Concrete's chemical binding capabilities make it a viable medium for containing toxic elements from industrial wastes, thus contributing to waste management and environmental protection.

Despite the notable technical, environmental, and economic benefits of integrating pozzolans, silica fume, and bacteria into concrete, there remains considerable misinformation and skepticism about their use. Engineers often express concerns that these materials might introduce more issues than advantages to concrete construction. The relationship between material characteristics and structural performance is crucial but not yet fully understood.

Recent attention has focused on the microstructure of concrete, as properties such as durability and strength are inherently linked to its fine microstructure. The incorporation of bacteria can enhance this microstructure through Microbiologically Induced Calcite Precipitation (MICP), which helps to fill cracks in conventional cement concrete.

This literature review aims to provide comprehensive insights into the process of biomineralization, defined as biologically induced precipitation that optimizes extracellular chemical mineral precipitation. MICP involves complex biochemical reactions facilitated by urease, a process that has shown promising results in enhancing strength and durability of materials. This review includes an overview of various studies on bacterial concrete, exploring how different microorganisms and calcium sources contribute to improving concrete properties.

2.2 Self-Healing Concrete

Cracks in buildings are a common occurrence, typically resulting from stress exceeding the material's strength. These cracks can be broadly typed into structural and nonstructural types. Structural cracks arise from poor design, construction flaws, or execution errors, whereas nonstructural cracks are caused by physical phenomena like temperature changes or crazing. Cracks can also be categorized by their width: thin (less than one millimeter), medium (one to two millimeters), and wide (more than two millimeters). These cracks pose significant concerns for concrete structures, impacting aesthetics, structural integrity, and safety. They can result from concrete degradation, reinforcing bar corrosion, construction defects, improper material selection, and shrinkage.

Das Neves et al. (2006) explain that they play a role in waste processing. **Rani et al. (2007)** Microorganisms have diverse effects on human health and the environment. Beneficial microbes are used in the production of yogurt, cheese, wine, and antibiotics.

Van Tittelboom et al. (2013) reported that emerging concept of self-healing concrete addresses these issues by mimicking the body's natural healing process. This innovative concrete includes materials such as fibers or capsules containing adhesive solutions. When cracks occur, these fibers or capsules break open, releasing the healing agent and repairing the damage.

Ivanov et al. (2016) explain that however, some microbes can cause illness, contaminate food, and damage materials like iron pipes and glass lenses. The potential for genetically modified microorganisms to create pharmaceuticals and enhance agricultural productivity is an exciting area of research. Microbial biotechnology, including applications in civil engineering, utilizes microbial processes to produce building materials and improve construction methods.

Wang et al. (2016) investigate to Understanding the properties of individual microbes is crucial, although studying them can be challenging due to their microscopic size. Research often focuses on pure or axenic cultures, where a single microbial species is studied in isolation. The cultural and genetic characteristics of microorganisms are key factors in their suitability for applications in civil engineering and construction.

Amran et al. (2022) conclude Self-healing concrete, also known as self-repairing concrete, automatically repairs small fissures, enhancing the material's durability and extending its lifespan. This approach not only improves the longevity of concrete structures but also their overall sustainability.

2.3 Bio-Concrete Technology

Wang et al. (2014) concludes that the application successfully reduced water penetration and preserved the floor's coating. Urease-producing bacteria are commonly used in bio-healing due to their ability to break down urea into carbonate and ammonia, leading to increased pH and subsequent calcite precipitation. Calcium ions in the environment interact with the bacteria to further promote calcite formation.

Lv et al. (2014) and Suryanto et al. (2017) states that Micro-cracks have been observed to heal through further hydration of cement particles and $CaCO_3$ precipitation during wet-dry curing cycles.

Recent research has turned to self-healing concrete as a more effective alternative for crack repair. **Van Tittelboom (2015), De Belie (2018), Wang (2019)**, **Qureshi et al. (2020)** states that Self-healing concrete can naturally mend cracks in moist conditions through the reactivity of unhydrated cementitious materials and the precipitation of $CaCO_3$.

Choi and Lee et al. (2016) Traditional crack repair methods are mostly manual, involving materials like epoxy systems, polymers, and various chemicals.

However, these methods are often costly, incompatible with concrete, and have durability issues, not to mention their health concerns and environmental.

Zhang et al. (2018) and Li (2001) investigates that ECC creates multiple microcracks, usually less than 100 µm in size, which can self-heal, restoring properties similar to those of new concrete. **Hou et al. (2022)** investigates Engineered Cementitious Composites (ECC), type of fiber-reinforced concrete, have been explored to control crack width more precisely.

Fuhaid et al. (2022) study the presence of cracks in concrete can accelerate carbonation and corrosion of steel reinforcements, thereby compromising structural stability of buildings and increasing maintenance costs.

Rajadesingu et al. (2024) investigates Self-healing processes, which are facilitated by bacterial spores imbedded in concrete, present promising methods to lessen the deterioration of concrete. Because of these mechanisms, which are activated when the material is exposed to moisture, the process of ureolysis is started, which ultimately results in the creation of calcium carbonate and the sealing of cracks.

These studies highlight that incorporating bacteria into concrete can facilitate self-healing, enhancing durability and longevity. Understanding bacterial properties is crucial for selecting the right microorganisms for developing bacterial concrete. This underscores the importance of reviewing microbial characteristics in the context of this research.

2.3.1 Bacterial Concrete

Rodriguez-Navarro et al. (2003) investigate that in bacterial conservation treatments, metastable vaterite is often produced. **Chekroun (2004) and Rodriguez-Navarro et al. (2007)** Research has shown that aragonite and monohydrocalcite can also form. The two most common forms of bacterial calcium carbonate, vaterite and calcite, have been studied. **Meldrum et al. (2008)** investigate Aragonite, most stable polymorph, is followed by vaterite, which is less durable. The selection of CaCO3 polymorphs has significant implications for technological applications. **Rodriguez-Navarro et al. (2012)** Several crystalline forms of calcium carbonate (CaCO3), such as monohydrocalcite (CaCO3·H2O), ikaite (CaCO3·6H2O), and a variety of amorphous phases, can be produced by bacterially-induced precipitation of CaCO3.

Ramakrishnan (2007), Sarda et al. (2009) Research on MICCP technology has focused on a range of applications, such as repairing limestone monuments, restoring cement mortar, and consolidating sand.

Kumar V.R et al. (2011) Bacterial concrete is characterized by incorporation of live bacteria or spores into concrete mixture during production. These bacteria facilitate the precipitation of calcite, which then seals microcracks and voids within the concrete. Biomimetics, which draws inspiration from biological processes, has diverse applications across fields such as micro/nano electronics, civil engineering, and medicine. In concrete, biomimetic principles like bio-deposition and biomineralization are applied, utilizing the natural processes of specific microorganisms, including bacteria.

Dhami (2012), De Belie et al. (2016) stated Concrete technology also makes use of microorganisms to heal cracks, increase durability, and decrease the permeability of water and chloride ion.

Dhami N.K et al. (2013) states Microbially-induced mineralization, a process driven by metabolic activity, results in the formation of extracellular bio-minerals. The most frequently researched of these minerals are carbonates, although they also contain metal sulphides, phosphates, phosphorite, carbonates, iron and iron-alkali silicates, and phosphates. Many fields, including biotechnology, geotechnology, paleobiology, and civil engineering, find MICCP (Microbially Induced Calcium Carbonate Precipitation) to be an important area of study.

2.3.2 Bacteria used in Concrete

Okwadha et al. (2010) conducted factorial experiments to determine factors affecting microbial carbonate precipitation (MICP), such as initial urea concentration, type and concentration of bacteria, calcium ion concentration, ionic strength, pH, and reaction temperature. The optimal conditions for MICP were found to be 666 mM urea and 250 mM Ca2+ with a bacterial cell concentration of 2.3×10^8 cells/mL. In these circumstances, the amount of CO2 sequestration and CaCO3 precipitation increased by 30% at bacterial cell concentration of 10^8 cells/mL, and by 100% at Ca2+ concentration of 250 mM. The presence of vaterite crystals in the precipitate was verified by energy dispersive X-ray, X-ray diffraction, and scanning electron microscopy. The primary component of precipitation was calcite.

Mortensen et al. (2011) investigated environmental conditions affecting bacterium Sporosarcina pasteurii, its metabolism, and calcium carbonate precipitation. The study tested these factors in soil columns and batch systems, finding that microbial growth and mineral precipitation occurred in both freshwater and saltwater environments. Factors such as ammonium concentration, oxygen availability, and the ureolytic activity of live versus lysed cells were examined. Results showed that the biological treatment process performed effectively across different soil types and salinity levels, and was not significantly impacted by lack of oxygen or lysis of cells carrying urease.

Ghashghaei et al. (2013) investigate to improved concrete durability and repair capabilities, it is advisable to utilise bacteria that are resistant to alkaline environments and can withstand the mechanical stresses encountered during the mixing process. Gram-positive bacteria are particularly suitable for this purpose due to their ability to form endospores, which enhances their survival. These bacteria also need to exhibit urease activity to facilitate the precipitation of calcium carbonate ($CaCO_3$), which helps seal microcracks in the concrete. In this research, Bacillus megaterium is chosen as the bacterial strain for experimentation. Bacillus megaterium is gram-positive, endospore-forming, motile, rod-shaped, aerobic bacterium known for its large size.

Wu et al. (2019) studied the durability of the concrete is consequently diminished as a result thereof. Because of this, there is a pressing need to investigate and create a naturally self-healing agent that is derived from natural biological sources and has the ability to repair fractures and fissures in concrete. Methods for the rehabilitation and upkeep of infrastructure have also been developed gradually throughout time. Cracks can be repaired using a variety of techniques; however, these techniques need a significant amount of time and money to complete. It may be difficult to carry out continuous inspections and protection, particularly in large-scale infrastructures, due to the enormous amount of money that is required to make it a reality. In addition, there are some other considerations, such as the location of the structural damage, which can make the process of refurbishment more complicated. On the other hand, bacterial concrete has the ability to successfully repair cracks in concrete. As a result, the idea of autonomous self-healing of such potentially hazardous fissures with minimal resources in structures that are not up to standard has become a very appealing field for academics. As a result, there is an immediate requirement to search for a way of crack mending that is not only sustainable but also cost-effective and does not require any assistance from a human. A strategy that is natural, autonomous, and self-healing can be realized by adding a specific type of bacteria to concrete as it is mixed. This will allow for the resolution of these difficulties. Bacillus subtilis has the ability to cause certain enzymes found in ureolytic bacteria, such as Bacillus Pasteurii, which is often referred to as urease, to be combined with the calcium nutrient source. This results in the formation of CCPs, which are responsible for sealing the newly produced microcracks in concrete. It is feasible to do this through the process of long-lasting hydration. The use of biomineralization has been shown to produce encouraging outcomes in the process of sealing microcracks in concrete buildings. Using this biotechnological method of CCP, it is possible to increase the strength and durability features of structural concrete. The concentrations of bacteria have been optimized in order to achieve better results for the remediation of concrete pores.

2.4 Review Of Literature

2.4.1 Bacterial Species in Crack Remedy

Calcite, aragonite, vaterite, $CaCO_3$ monohydrate, amorphous $CaCO_3$ and CaCO3 hexahydrate, were the six mineral polymorphs of calcium carbonate ($CaCO_3$) that **Manoli and Dalas (2001)** study.

Braissant et al. (2003) investigate Crystal shape may be affected by the amount of urease activity, as pointed in reference to various bacterial cultures. The effects of calcium carbonate precipitation on the form of concrete were investigated. **Böttcher et al. (2005)** research crystal shape is relatively unaffected by the calcium supply. **Gu Z et al. (2022)** study the shape of precipitated calcium carbonate crystals changes from spherical, regular rhombohedral to irregular rhombohedral.

Whiffin et al. (2004) identified Sporosarcina pasteurii and Proteus vulgaris as promising sources of urease for biocementation, considering both biocementation effectiveness and environmental constraints. **Nemati et al. (2005)** demonstrated that bacteria with urease activity can induce $CaCO_3$ production in media containing urea and calcium chloride. **Dick et al. (2006)** confirmed that ureolytic bacteria are responsible for carbonate precipitation in concrete. **Ercole et al. (2007)** study the importance of amino acids in shaping morphology and mineralogy of carbonate precipitation generated by bacteria was brought to light.

De Muynck (2007) study when comparing treatments that used mixed ureolytic cultures with those that used pure Bacillus sphaericus cultures, morphological changes. **Wiktor et al. (2011)** observed that changing the calcium salt used in bio-deposition affects the morphology of precipitated CaCO3, indicating that the choice of reactants influences crystal formation.

2.4.2 Influence and Effect of Bacterial Species in pH

Hammes et al. (2002) identified three key parameters influencing $CaCO_3$ precipitation in bacterial systems: calcium concentration, carbonate concentration, and pH.

Achal et al. (2009, 2011) found that urease-catalyzed ureolysis is temperature-dependent, with an optimal temperature range for bacterial growth between 20°C and 37°C. They also reported that Sporosarcina pasteurii exhibits enhanced urease activity and can tolerate high pH values effectively.

Arunachalam (2010) observed that Bacillus sphaericus bacteria induce $CaCO_3$ precipitation with a predominance of spherical deposits, including vaterite and fluorite, at a pH of 8. **Van Tittelboom et al. (2010)** study the excessively alkaline pH of concrete inhibits the development of microorganisms. **Wang et al. (2011)** use of diatomaceous earth was proposed as a means of preserving Bacillus sphaericus from the high pH of the concrete matrix. This would result in the bacteria being protected and their efficiency being increased.

Fouladi et al. (2023) observed that high pH levels inhibit bacterial growth, with Bacillus pasteurii thriving optimally at a pH around 9.

2.4.3 Bacteria Embedded Concrete

The successful application of biomineralization for calcite precipitation in cement mortar has been widely documented. This technique, which employs microorganisms to induce precipitation of calcium carbonate ($CaCO_3$) as calcite, is used to repair damaged structural formations by selective microbial plugging. Soils, geological formations, freshwater biofilms, seas, and salt lakes are just a few of the natural habitats where different kinds of microbes help bring mineral carbonates to the surface. The possibility of Microbially Induced Calcium Carbonate Precipitation (MICP) to increase resilience and longevity of cement concrete structures has prompted its investigation.

Castanier et al. (1999) investigate Some bacteria and fungi can cause $CaCO_3$ to precipitate out of solution by means of photosynthesis and sulphate reduction.

V. Knorre et al. (2000) investigate Urea is hydrolyzed by the urease enzyme, which results in the production of carbonate ions and ammonium. This is the fundamental process of MICP. The term biomineralization refers to this microbiological process, which represents an exciting new frontier in the field of concrete science and technology. What we know about MICP now is based on a number of studies:
In different habitats, found that bacterial species and abiotic variables both impact CaCO3 precipitation.

Perito (2000) was discovered that six genes involved in crystal formation in Bacillus subtilis.

Örnek et al. (2001) was reported that genetically engineered biofilms from Bacillus subtilis can enhance corrosion inhibition on aluminum. **Nemati et al. (2003)** was documented the precipitation of $CaCO_3$ crystal particles by microbes. **Van Lith et al. (2003)** was emphasized the role of Extracellular Polymeric Substances (EPSs) in forming and aggregating carbonate crystals. **Abo-El-Enein et al. (2012)** was observed that $CaCO_3$ can be used as a sealant in sand, utilizing a biological byproduct. **Torres-Aravena et al. (2018)** was proposed using MICP for removing metal ions from wastewater.

Barabesi et al. (2007) was stated that bacterial $CaCO_3$ production is mineral-based and does not involve specialized structures. **De Muynck et al. (2010**) reported that bounded metal ions like calcium react with carbonate anions to form CaCO3, and highlighted the need for cost-effective raw materials for MICP treatments. **Perito et al. (2011)** found that most bacterial species can precipitate carbonates under alkaline conditions rich in Ca2+ ions. **Al-Salloum et al. (2017)** was noted the active role of microorganisms and microbially mediated mineralization in diverse environments, stressing the importance of nutrient selection in cultivation media. **Chuo et al. (2020)** observed that Bacillus sphaericus induced $CaCO_3$ precipitation at the interface between the medium and concrete, forming biofilms and different crystal shapes depending on ion presence. **Chen et al. (2022)** had identified Sporosarcina pasteurii as an ecologically sound biological construction material. **Kanwal et al. (2022)** demonstrated effectiveness of MICP in reducing reinforcement corrosion. Concrete specimens treated with Bacillus sphaericus CT-5 exhibited enhanced pull-out strength and reduced mass loss in reinforcing bars compared to untreated samples. SEM analysis showed that the bacterial treatment resulted in better shape retention and strength of the reinforcing bars.

Nodehi et al. (2022) was highlighted that biomineralization could occur either through active carbonate precipitation by microorganisms or passive changes in system chemistry induced by bacteria. **Dupraz et al. (2009)** was studied mineral precipitation across different bacterial groups and environmental factors. **Wang et al. (2023)** Groundwater contaminated with heavy metals and radionuclides can be treated with MICP.

These findings collectively emphasize the significant potential of MICP to improve concrete durability, repair structural damage, and offer environmental benefits.

2.4.4 Role of MICP in Crack Remediation

Ramachandran et al. (2001) Introduced a crack-filling method using sand mixed with Bacillus pasteurii which demonstrated increased compressive strength in cement mortar cubes.

Ramakrishnan et al. (2005) refined the immobilization technique, enhancing crack remediation by encapsulating microbial cells to enclose $CaCO_3$ precipitation, thereby improving strength in targeted areas. Ramakrishnan et al. (2005) studied the application of Bacillus pasteurii for MICP in crack remediation.

Van Tittelboom et al. (2010) reported that bacteria like Bacillus sphaericus could repair cracks by inducing $CaCO_3$ precipitation, which decreased water permeability in concrete. They noted that using autoclaved bacteria also achieved similar results in reducing water flow.

Seifan et al. (2018) Utilized both Bacillus lentus and Bacillus sphaericus for calcium carbonate precipitation in crack repair. The concept of autogenous crack healing in concrete, mainly through CaCO3 precipitation during carbonation.

Krajewska (2018) and Shen et al. (2021) Bacillus pasteurii for concrete crack remediation. immobilization of urease enzyme on polyurethane to induce $CaCO_3$ precipitation, while how different filler materials affected cell protection from high pH environments.

Frandi (2010), Parashar et al. (2021) commercialized Bacillus Subtilis-induced CaCO3 formation for repairing ornamental stones. The significance of bacterial CaCO3 precipitation in addressing cracks in building materials.

Jonkers et al. (2010) introduced alkali-resistant and spore-forming bacteria, such as Bacillus pseudofirmus and Bacillus cohnii, for self-healing concrete. They demonstrated that these bacteria could autonomously repair micro-cracks and enhance concrete durability.

Qian et al. (2015) discovered that early age cracks in cement-based materials can be healed by a type of bacteria called Bacillus mucilaginosus L3, which produces carbonic anhydrase. Their experimental findings demonstrated that the fractures that emerged during the initial stages were fully repaired (up to 0.4 mm) as a result of bacterial therapy. However, the effectiveness of the healing process diminished as the age of the cracks increased.

Indhumathi et al. (2022) study Crack healing in concrete significantly extends the service life of structures. It described biomineralization as a low-toxic method for crack repair. Various carrier compounds, including lightweight aggregates and graphite nano platelets, for bacterial impregnation in concrete.

Jiang et al. (2024) approach by developing immobilization technique where microbial cells were encapsulated in polymers to facilitate $CaCO_3$ precipitation and improve crack repair in concrete. found that polyurethane was particularly effective in enhancing this process.

2.4.5 Effect of MICP in the Strength of Concrete

HM Jonkers and E Schlangen (2007) Minimizing cracking occurrence in reinforced concrete is essential for both longevity and economic considerations, as repairing cracks can be expensive. Implementing autogenous repair, also known as self-healing, in concrete structures would result in significant cost savings by reducing the need for manual inspection and crack repair. Therefore, the implementation of a dependable self-repairing system for concrete will not only lead to the construction of more long-lasting buildings, but also have positive implications for the worldwide economy. This study utilized the capacity to utilize calcite-precipitating bacteria as a crack-healing agent in concrete. The study examined the capacity of several species to induce the formation of calcite, generate endospores, endure concrete construction, and repair cracks by sealing them with calcite. In addition, the mechanical characteristics of 'bacterial concrete' were examined. Electron Scanning Electron Microscopy (ESEM) examinations revealed that spore-forming bacteria, which are resistant to alkali, are present within the concrete structure. These bacteria have the ability to cause the formation of significant quantities of calcite. The utilization of bacteria appears to be a very promising method for facilitating self-repair in concrete buildings.

Sunil et al. (2010) demonstrated a substantial enhancement in the compressive strength resulting from the inclusion of bacteria at a concentration of 10^5 cells per ml of mixing water. Based on the study using Scanning Electron Micrography, it was observed that the pores were partially filled by the growth of material due to the presence of bacteria. The decrease in pore size resulting from material development will undoubtedly enhance the material's strength. Concrete cubes were made, some with the inclusion of bacteria. It was noted that the cubes with bacteria showed an enhancement in compressive strength. Concrete cylinders were made, some with the addition of bacteria and some without. It was noted that the cylinders with the addition of bacteria showed an improvement in split tensile strength. The durability studies indicate that the weight and strength losses of bacterial concrete are lower than those of conventional concrete when exposed to 5% H_2SO_4. Furthermore, the studies demonstrate that bacterial concrete exhibits higher durability in terms of "Acid Durability Factor" and "Acid Attack Factor" compared to conventional concrete.

N Chahal et al. (2012) reports the findings of an experimental study conducted to assess the impact of Sporoscarcina pasteurii bacteria on the compressive strength and fast chloride permeability of concrete, both with and without fly ash. Cement was substituted with fly ash at three weight percentages: 10%, 20%, and 30%. Three distinct bacterial cell concentrations (0, 10^3, 10^5, 10^7 cells/ml) were utilized in the preparation of the concrete mixtures. Tests were conducted to assess compressive strength, water absorption, and fast chloride permeability at 28 days of age. Test results demonstrated that the incorporation of S. pasteurii in fly ash concrete improved compressive strength while decreasing porosity and permeability. A maximum gain of 22% in compressive strength and a fourfold reduction in water absorption were reported with a bacterial concentration of 105 cells/ml. The enhancement in compressive strength resulted from depositing on the bacterial cell surfaces within the pores. Calcite deposition in concrete resulted in an approximately eightfold decrease in chloride permeability of fly ash concrete. This study emphasizes the impact of bacteria on the characteristics of concrete including supplementary cementitious materials, such as fly ash. The use of bacteria such as S. pasteurii enhances the strength and durability of fly ash concrete via a self-healing mechanism.

Wang et al. (2014) Bacterial-based self-healing represents a promising approach for the sustainable preservation of concrete. This work involved the encapsulation of bacterial spores in hydrogels, which were subsequently integrated into specimens to assess their healing efficacy. Thermogravimetric analysis (TGA) demonstrated the precipitation of $CaCO_3$ by hydrogel-encapsulated spores. The mortar specimens containing hydrogel-encapsulated spores exhibited notable self-healing efficacy: the highest mended crack width was approximately 0.5 mm, and the average reduction in water permeability was 68%. Other specimens in the non-bacterial series exhibited a maximum healed crack width of 0–0.3 mm, and the average water permeability was reduced by just 15–55%.

Xu and Yao (2014) examined the use of Bacillus cohnii spores to generate non-ureolytic bacterially mediated $CaCO_3$ precipitation as a self-healing method for concrete cracking. The researchers proposed that the addition of bacteria and calcium source nutrients as a two-component healing agent in the concrete matrix triggers the creation of CaCO3 when cracks occur. The crack width, ranging from 0.1 to 0.4 mm, was fully sealed, and a coating of precipitates on the specimen's surface was seen during the externally applied healing process. Additionally, they observed that the self-healing efficacy of specimens containing bacterial spores and nutritional supplements was greater than that of the control specimens. Calcium glutamate precursor shown greater efficacy in promoting calcium carbonate precipitation for crack healing in external treatment and self-healing applications compared to calcium lactate.

Silva et al. (2015) conducted a cost analysis of biological self-healing in concrete and underlined the need to produce a bio additive that is more affordable in order to make commercial application of biological self-healing feasible.

Wang et al. (2015) Using a modified alginate-based hydrogel as an encapsulating material for the application of B. sphaericus spores in concrete, Wang et al. conducted their research experiments. The findings of their study shown that the hydrogel was capable of providing effective protection against spores in concrete and had a significant potential for usage in crack self-healing applications in concrete.

eDe Belie et al. (2015) observed that Self-healing concrete offers significant advantages in minimizing expenses associated with concrete maintenance and repair, as it autonomously addresses cracks without human intervention. This study investigated the use of the carbonate-precipitating bacterium Bacillus sphaericus. Concerning the adverse conditions in concrete, B. sphaericus spores were initially encased in a modified-alginate hydrogel (AM-H), which demonstrated favorable compatibility with both the bacteria and concrete in terms of bacterial survival and concrete strength. Experimental findings indicate that the spores remained alive post-encapsulation. Encapsulated spores can induce the precipitation of a significant quantity of CaCO3 within the hydrogel matrix, approximately 70% by weight. Encapsulated B. sphaericus spores were incorporated into mortar specimens, and bacterial in-situ activity was evidenced by oxygen consumption on the simulated fracture surface. Specimens containing free spores exhibited no oxygen consumption. This signifies the effective safeguarding of spores within the hydrogel in concrete. In conclusion, the AM-H encapsulated carbonate-precipitating bacteria provide significant promise for self-healing cracks in concrete applications.

Wang et al. (2016) The optimal concentration of the B. pasteruii bacterium for usage in concrete, as noted, was found to be between 10^6 and 10^8 cells/ml of water. Thermophilic anaerobic microorganisms added to cement mortar increased its compressive strength by 25%, according to research. Additionally, they discovered that adding Escherichia coli bacteria to mortar did not increase its strength. Incorporating live bacteria into concrete by MICP increased its mechanical qualities.

Tziviloglou et al. (2016) introduced a healing agent derived from bacteria into lightweight aggregates. They then mixed this with fresh mortar and assessed the restoration of liquid tightness after cracking and exposure to two distinct healing methods (water immersion and wet-dry cycles) using water permeability tests. Their findings indicated that there was no significant difference in the restoration of water tightness between specimens with or without a healing agent when continuously submerged in water. Nevertheless, the specimens that have the healing agent exhibit a substantial improvement in water tightness recovery compared to the specimens that do not contain it, when exposed to wet-dry cycles.

S Joshi et al. (2017) discovered that early age cracks in cement-based materials can be healed by a type of bacteria called Bacillus mucilaginosus L3, which produces carbonic anhydrase. Their experimental findings demonstrated that the fractures that emerged during the initial stages were fully repaired as a result of bacterial healing. However, the effectiveness of the healing process diminished as the age of the cracks increased.

Rao et al. (2017) investigate that Concrete is susceptible to degradation, corrosion, and fissures, which can result in costly restoration and repair due to the subsequent damage and loss of structural integrity. The requirement for a specialized type of concrete that can automatically repair cracks led to the research and creation of microbial concrete. The microbial concrete operates based on the principle of calcite mineral precipitation through a particular group of alkali-resistant spore-forming bacteria known as Bacillus subtilis JC3, which belongs to the genus Bacillus. This process is referred to as biomineralization or Microbiologically Induced Calcite Crystal Precipitation. Bacillus subtilis JC3, a prevalent bacteria found in soil, possesses an innate capacity to consistently build calcite crystals. This process significantly improves the strength and durability of concrete. This microbial concrete might be referred to as "Self-healing Bacterial Concrete" because to its ability to repair cracks autonomously, without the need for human intervention. This characteristic enhances the durability and sustainability of the concrete. This work examines the integration of the microorganism Bacillus subtilis JC3, which was produced at JNTU, India, into concrete. It shows the findings of experimental research conducted to analyze the enhanced durability and sustainability properties of microbial concrete.

Om Prakash Singh et al. (2023) observed that conventional concrete (CC) is the most widely utilized construction material globally, particularly in harsh settings. This is attributable to the element's dimensions and configuration. The global usage of concrete amounts to approximately 5.5 billion metric tons annually. The progress in concrete technology, alongside the evolution of materials and systems, has led to heightened strength requirements. The maintenance, repair, and rehabilitation of existing concrete structures present numerous challenges, leading to substantial costs. Concrete is susceptible to the formation of microcracks and possesses highly undesirable pores. Therefore, there is an immediate necessity to focus on enhancing the strength properties of concrete. Bacterial concrete (BC) serves as an excellent solution for crack closure, utilizing a process known as Microbiologically Induced Calcite Precipitation (MICP) that occurs continuously within the concrete. The urease enzyme facilitates the deposition of calcium carbonate ($CaCO3$) through bacterial activity. The bacterial remediation method surpasses supplementary approaches. The examinations involved several quantitative assessments of the appropriate concentration of bacterial cells to be gathered in concrete. According to the latest data available in the literature, two distinct bacterial species, Bacillus Megaterium (BM) and Bacillus Subtilis (BS), are quantified in concentrations of 10^4, 10^5, and 10^6 cells/ml in the water mixtures. The ideal bacterial cell concentrations are established at 10^4 cells/ml for BM and 10^5 cells/ml for BS, as determined by compressive strength data. This study examines strength-related parameters, including compressive strength, splitting tensile strength, and flexural strength. BM and BS bacteria are conclusively established as effective agents for enhancing the performance and strength properties of concrete.

This compilation illustrates the diverse approaches and results in microbial cement mortar enhancement, emphasizing the potential of bacterial interventions for improving compressive strength and durability.

2.4.6 Role of MICP on Durability Properties of Concrete

Bang et al. (2001) Bacillus pasteurii cells were immobilized using polyurethane (PU) foam. The immobilized cells demonstrated comparable rates of calcite precipitation and ammonia generation to the free cells. The cells embedded in calcite crystals across PU matrices were discovered using scanning electron micrographs. Calcite in polyurethane (PU) had minimal impact on the elastic modulus and tensile strength of the polymer. However, it enhanced the compressive strength of concrete cubes, which had cracks that were repaired using PU-immobilized cells. Based on these data, we hypothesized that the calcite could persist as a type of precipitation rather than being a bonding substance within the matrices.

De Muynck et al. (2010) Microbially induced carbonate precipitation (MICP) is a naturally occurring phenomenon that has influenced the formation of the Earth from prehistoric times. During this procedure, calcium carbonate minerals are produced through the combination of calcium and carbonate ions. Due to its compatibility with concrete and stone, as well as its environmentally friendly nature, calcium carbonate minerals have been investigated as a potential solution for repairing cracks in stone, known as MICP.

Pei et al. (2013) Bacterial cell walls facilitate microbially induced carbonate precipitation, a chemical reaction that converts Ca^{2+} ions and dissolved CO_2 into $CaCO_3$. In line with this understanding, the addition of bacterial cell walls enhanced the carbonation of $Ca(OH)_2$ and the production of $CaCO_3$ in concrete. In addition, the presence of bacterial cell walls led to a notable 15% increase in the compressive strength of concrete, as well as a reduction in porosity after 28 days of curing. The in vitro assay demonstrated that bacterial cell walls, but not deceased cells, expedited the carbonation of Ca^{2+} ions in a $Ca(OH)_2$ solution. Due to the ability of $CaCO_3$ to fill voids, reduce porosity, and increase compressive strength in concrete, the inclusion of bacterial cell walls as concrete additive shows promise in strengthening mechanical performance and improving other aspects connected to carbonation.

Luo et al. (2015) examined the impact of crack width, curing condition, and age of cracking on concrete when alkali resistant spore generating bacteria were introduced directly into the concrete together with the substrate. Crack widths ranging from 0.1 to 0.5 mm were implemented, and three distinct post-cracking incubation conditions were tested: wet curing, water curing (immersion in water), and wet-dry cycles, all at a temperature of 25°C. The healing process was evaluated by measuring the rate of area restoration at various cracking ages, including 7, 14, 28, 60, and 90 days. When submerged in water, cracks with a width of up to 0.3 mm were seen to be fully repaired, and the healing ratio for cracks between 0.1 and 0.3 mm was approximately 85%. Water curing and wet-dry cycles demonstrated superior efficacy in treating conditions, despite wet-dry cycles having a slower repair rate. Cracks that occurred at an early stage were effectively fixed, whereas cracks that occurred at a later stage were not as effectively healed.

Gupta et al. (2017) Cracking in concrete structures is unavoidable when the material deteriorates over time owing to a combination of load and non-load forces. Hence, it is imperative to carry out repair and maintenance procedures in order to inhibit the propagation of fractures and prolong the lifespan of the buildings. Nevertheless, gaining access to the cracked zone might be challenging. Additionally, these procedures necessitate both financial resources and personnel, while also contributing to pollution as a result of human activities and the use of additional repair materials. Implementing self-healing mechanisms could potentially minimize the need for physical intervention. Many researchers worldwide are studying the environmentally benign technique of autonomous crack closure using bacteria-induced carbonate precipitation. This review specifically examines the assessment of bacterial crack healing in concrete, whether the bacteria is introduced directly into the concrete or enclosed in a protective shell before being added. The effectiveness of bacterial self-healing is determined by four key aspects: the material and encapsulation of bio-agents in capsules, the survival of capsules during concrete mixing, the impact of adding bio-agents or capsules on concrete properties, and the ability of the capsules to seal and restore mechanical and durability properties. Ultimately, this study identifies and discusses areas where further research is needed and the potential scope of future research.

Parashar et al. (2021) Despite employing the highest quality materials and workmanship, it is nearly impossible to prevent the formation of cracks on the surface of concrete. These fissures can lead to the deterioration of concrete in terms of its strength and longevity. Hence, it is crucial to effectively seal these gaps in order to minimize or eliminate the destructive impact of deteriorating agents that may infiltrate the concrete through these openings. This research provides a comprehensive analysis of the impact of different concentrations of bacteria from the bacillus family on the mechanical strength and long-term durability characteristics of concrete. This review focuses on bacteria with a concentration ranging from 100 colony-forming units (CFU) to 108 CFU. This research also discusses the regenerative capacity of many bacteria belonging to the Bacillus family. Self-healing in concrete involves the introduction of bacteria from the bacillus family into the concrete mixture to repair cracks. This process aims to assess the effects of these bacteria on the concrete's strength and durability.

Arun et al. (2021) conducted research on the application of bacteria to enhance concrete performance by minimizing voids. The Bacillus family of bacteria has been discovered to possess remarkable healing properties for concrete. The current investigation employed Bacillus megaterium bacterium from the bacillus family, with a concentration of 108 CFU. 48 specimens were produced and subjected to mechanical strength and water absorption testing after 7 and 28 days of curing. After 28 days of curing, the test results showed that the compressive strength, split tensile strength, and flexural strength rise by

12.91%, 10.28%, and 9.02% correspondingly compared to the conventional M30 grade concrete mix. The water absorption capacity of bacterial concrete was observed to be lower in comparison to the regular concrete mixture. The fissures in the concrete are filled due to the process of calcite precipitation caused by the Bacillus megaterium bacteria. Thus, the Bacillus megaterium bacteria from the bacillus family can be efficiently employed to enhance mechanical strength by minimizing the presence of empty spaces.

Nagar et al. (2021) Utilizing supplemental cementitious materials (SCMs) as a partial substitute for Ordinary Portland Cement (OPC) is the most effective approach to decrease CO2 emissions in the worldwide cement production sector. Calcined clay can be regarded as an effective approach to mitigate CO2 emissions. This paper utilizes calcined clay as supplementary cementitious materials (SCMs) to partially substitute cement at proportions of 10%, 15%, and 20%. In addition, to examine the self-repairing properties of concrete, Bacillus Sphaericus bacteria with a bacterial population of 10^8 cells/ml are introduced, coupled with calcined clay. Specimens underwent testing for water absorption and ew1compressive strength at 7, 14, and 28 days, respectively. The findings indicate that the incorporation of bacteria in concrete, along with the partial substitution of calcined clay, leads to a significant increase in compressive strength. Specifically, the compressive strength is enhanced by 21%, 24%, and 25% respectively after 28 days. As a result, the bacteria-infused concrete with calcined clay has reduced water absorption and improved compressive strength of the concrete sample. This offers a sustainable and cost-effective option for concrete construction.

Parashar et al. (2022) Concrete is a prevalent and extensively used construction material; yet, it is not without its drawbacks, one of which is the occurrence of fractures in the concrete. As the size of the fracture increases, the likelihood of water and carbon dioxide penetrating the construction material also increases. Upon reaching their destination, these chemicals will interact with the other substances present, ultimately leading to a substantial reduction in the strength and durability of the material. If prompt measures are not taken to rectify the cracks, there is a risk of the damage spreading, leading to the formation of larger cracks, increased water loss, reduced structural integrity, and higher expenses for repairs. If this happens, it is crucial to take immediate measures to remedy the fissures. The aim of this study is to give a comprehensive analysis of how different levels of Bacillus bacteria affect the strength and durability properties of concrete. The investigation examined bacterial concentrations ranging from 10° CFU to 108. This research investigates the therapeutic potential of diverse bacteria belonging to the Bacillus family to cure various conditions. The process of remedying concrete cracking by incorporating bacteria from the Bacillus family into the concrete mixture and assessing the impact of these bacteria on the concrete's strength and durability is known as "self-healing." The term "self-healing" is used interchangeably with the phrase "self-repair." The process that yields self-healing concrete is likewise referred to by that term.

Oh et al. (2024) Bacterial concrete is regarded as a very efficient self-repairing substance since it has the ability to independently mend various types of damage, such as cracks, through the process of microbially induced calcium carbonate precipitation (MICP). The objective of this study was to assess the direct integration of bacteria into mortar as part of the concrete damage restoration technology. The bacterium Sporosarcina pasteurii, which can thrive in alkaline environments and stimulate the formation of calcium carbonate through biomineralization, was chosen for this task. Both bacteria-infused and bacteria-free cement mortars were made and subjected to different curing solutions to investigate bacterial reactivity. The solutions used were a media solution that promotes bacterial growth and calcium carbonate precipitation, a urea solution that induces calcium carbonate precipitation, and regular water. The investigation involved the examination of mechanical qualities and microstructural parameters utilizing a range of different methods. The results showed that the bacteria in cement mortar produced calcium carbonate with several phases, including calcite and vaterite, depending on the unique curing solutions used. Specifically, the discovery of well-formed calcium carbonate deposits was determined to greatly improve the strength and resilience of the cement mortar.

2.4.7 Mechanical and Durability Properties

Kaveh (2012), Ling (2017), Jongvivatsakul et al. (2019) studies indicate that utilization of bacteria effectively addresses issues related to the durability and creation of cracks in concrete. A significant downside of mix of concrete is the occurrence of substantial cracks. While in turn, the cracks result in corrosion in the reinforcement, which compromises the internal quality of concrete mix and incurs significant costs for repair. Cracks in concrete result in the infiltration of water and chloride, which negatively impacts the durability of the material.

Chahal (2013), Meharie (2017), Althoey (2023), Zaid et al. (2024) investigate the mechanisms involved include organic carbon oxidation, enzymatic urea hydrolysis and denitrification under anoxic conditions. Among all the above technologies, bacterial concrete with property of self-healing is the most commonly employed. Bacterial species injected into bacteria generate calcium carbonate dense deposition, which subsequently seals the voids formed in concrete. Blocking the cracks reduces the permeability of substances that cause corrosion, preventing them from entering the concrete injection of bacterial species into concrete leads to a reduction in water absorption by up to 85%, as these bacteria generate crystals containing carbonate. Precipitation of calcite has diverse impacts on the characteristics of concrete with varying levels of porosity. The introduction of microorganisms into concrete enhances its durability and prolongs its self-life.

Wang et al. (2016) Concrete cracking is a common occurrence in reinforced concrete (RC) structures and has a substantial impact on the diffusion of chloride and the subsequent deterioration of the structures. Nevertheless, real concrete fissures include intricate properties, and the ways in which they impact chloride penetration have not been thoroughly elucidated. This study provides a meticulous analysis of the geometric characteristics of real concrete cracks, such as density, direction, tortuosity, and width. It also investigates the relationship between these characteristics and the ability of chloride to diffuse through concrete. Concrete sample underwent uni-axial compression testing to induce different levels of cracking severity. Subsequently, a migration approach that does not involve a steady state was used to assess the diffusivity of sound and cracked concretes. After the migration experiments, an image analysis technique was used to quantify the geometry characteristics of concrete fractures. The test results indicate a direct correlation between the fracture tortuosity and the extent of crack orientation. Furthermore, the infiltration of chloride into concrete is heavily influenced by the density and tortuosity of cracks, as well as the width of the cracks. The tortuosity of cracks has a crucial role in determining the extent of chloride penetration, especially when the fracture width falls within the range of 150 to 370 micrometers. The effective crack width and effective crack density are claimed to be connected with the chloride diffusivity of cracked concrete, taking into account the fracture tortuosity and orientation.

Van Tittelboom et al. (2016) Following extensive study conducted at the Magnel Laboratory for Concrete study in Belgium, two of the most promising processes for achieving self-healing qualities in concrete were tested on a bigger scale. One technique involves the process of encapsulating polyurethane and embedding it in the matrix. Self-repair occurs when the formation of cracks leads to the rupture of the capsule, resulting in the release and subsequent solidification of the polyurethane inside the fracture. The second method involves incorporating superabsorbent polymers (SAPs) into the concrete. These superabsorbent polymers (SAPs) absorb water that enters through the gap, causing them to expand and effectively seal the crack. Furthermore, when they subsequently release their water content, they promote ongoing hydration and the formation of calcium carbonate. Concrete beams of actual dimensions (150 mm x 250 mm x 3000 mm), with and without the ability to repair themselves, were manufactured. The effectiveness of self-healing was assessed after inducing cracks in the beams using a four-point bending test. The addition of superabsorbent polymers to the mixture resulted in enhanced autogenous crack healing, as evidenced by the observed reduction in crack width over time. The acoustic emission study provided evidence of glass capsule rupture following the creation of a crack. Cracks were found to be partially filled by hydration products, calcium carbonate crystals, and/or polyurethane that originated from the broken embedded capsules, as confirmed by X-ray tomography, fluorescent light microscopy, and thin section analysis. Although it was anticipated that these findings would lead to a reduction in water penetration into the repaired cracks, this outcome could not be substantiated in this study.

Keerthana et al. (2016) Concrete is the primary building material used in construction industry. Improper design can lead to temperature-related impacts such as freezing, thawing, and shrinkage, which in turn can cause deformation and the formation of cracks in buildings. Cracks are significant vulnerabilities in concrete that can result in structural failure. Bacterial concrete is an innovative approach used to mend cracks in constructions by utilizing microorganisms. During the process of concrete mixing, bacteria generate calcium carbonate precipitate that will naturally heal the fractures. An investigation has been conducted to examine the performance of Bacillus Sphaericus and Escherichia coli in concrete. Additionally, this study focuses on comparing the durability characteristics of bacterial concrete. The calcite precipitate produced by the bacterial strains was analyzed using X-ray diffraction (XRD) and seen using a Scanning Electron Microscope (SEM). The concrete mixture was created by including bacterial strains at concentrations of 10^3, 10^6, and 10^9 cells/ml of water. An assessment was conducted on the durability characteristics of concrete, including its resistance to acid attack and its capability to absorb water. The concrete containing Bacillus Sphaericus at a concentration of 10^9 exhibits greater strength compared to the concrete containing Escherichia coli.

Babu, Thakur, Singla (2016), Zaid (2024) indicates there are two systems available: a mortar bio-based system and a bio-based repair system containing liquid. The first product is utilized for remedying concrete surface cracks, while the other one addresses more severe issues associated with concrete. The utilization of bacterial solutions for healing of cracks is highly economical and environmentally beneficial. Hence, the imperative of the moment is to develop microbiological self-sustaining concrete. Self-sustaining bacteria possess a unique metabolic mechanism that enables them to repair cracks by producing calcium carbonate precipitate. This precipitate effectively seals the pores and closes the cracks. Three essential bacterial metabolic pathways have been discovered to be highly beneficial for the precipitation of calcium.

Kaveh (2017), Sai (2018), Karimi et al. (2020) promptly initiate the healing process of concrete to prevent water infiltration and subsequent damage. The most recent and widely discussed technique for addressing concrete-related issues is Self-healing concrete, which offers an effective solution to many of these difficulties. Concrete by using the self-healing properties contains the capacity to spontaneously reduce cracks that occur in the concrete. This is accomplished through the incorporation of bacterial substances, which can be of either chemical or biological in nature. Chemical additives used in properties of concrete are beneficial, however, it is crucial to prioritize environmental friendliness, especially since concrete with self-healing properties is expected to be the future of concrete production. There are 2 types of repair procedures employed for reduction of cracks in the concrete.

Mehta (2017), Parashar (2023) Zaid (2024) study the presence of surface cracks in concrete provides a convenient pathway for the infiltration of water, oxygen, and various chemicals. Consequently, this results in the deterioration of concrete. Corrosion results in reduction in the durability and stability of concrete. Failure to promptly address such conditions may lead to expensive repairs. **Bakr et al. (2023**) novel strategies are being contemplated to address these circumstances, and one extraordinary and unconventional concept is the creation of self-repairing concrete by the incorporation of bacterial species.

Nodehi et al. (2022) Cracking is a significant factor that contributes to the deterioration of concrete. It enables the penetration of chemicals and can result in the degradation of the physical, mechanical, and durability characteristics of concrete buildings. The standard procedure for protecting, repairing, and rehabilitating concrete structures involves using various surface coating agents, sealants, binding agents, and adhesives. While these strategies have generally proved useful, their intrinsic differences in mechanism have led to significant obstacles, including delamination and a lack of cost effectiveness. As a result, researchers have been exploring other methods for sealing cracks or achieving self-healing. An innovative method for self-repair involves the utilization of bacterial induced calcite precipitation in concrete mixtures to mend fissures in the concrete. This technique involves the process of bacterial mineralization, also known as biomineralization, where urea and calcium are decomposed to generate calcium carbonate ($CaCO_3$), which can be used to fill fissures. This paper seeks to provide a detailed investigation of the mechanisms governing the precipitation of biomineralization and CaCO3 in bacterial concrete. It will also examine the physico-mechanical, durability, and microstructural aspects of the concrete. In order to accomplish this, more than 70 research articles have been examined. These articles provide valuable data on various aspects such as the types and dosage of bacteria, combination proportions, as well as the outcomes of mechanical and durability tests. This data has been collected, presented, and thoroughly studied. According to this review, it has been determined that biomineralization is mostly influenced by aspects such as the manner of application and the consistent retention of the living bacteria. Furthermore, the environmental consequences of bacterial concrete are strongly correlated with the amount of urea present in the concrete mixture.

Smitha et al. (2022) report the findings of their study on the microbiological stimulation of bacterial biomass (known as biocementation) in concrete mixtures, with the aim of improving the mechanical and durability characteristics. The researchers introduced bacillus megaterium bacteria, which were obtained from produced sand, into concrete mixtures. They then examined the resulting impact on the mechanical and durability aspects of the concrete. The impact of the bacteria concentration was evaluated based on compressive strength, split tensile strength, flexural strength, acid resistance, chloride ion penetration, and water permeability. Furthermore, microstructural analyses were conducted as well. The results of this investigation revealed that the compressive strength, split tensile strength, and flexural strength of concrete produced with a concentration of 10^5 cells/ml of bacteria was 11.3%, 97.5%, and 10.7%, respectively. Concrete containing 10^5 cells/ml of bacteria demonstrated reduced permeability, namely lower penetration of chloride ions and water, as well as increased resistance to acid attack when compared to plain concrete without any added bacteria. The addition of bacillus megaterium to the concrete resulted in enhanced characteristics, which can be attributed to the formation of calcite and the presence of bacterial biomass within the concrete's pores. Microstructural analyses revealed that concretes containing bacteria at a concentration of 10^5 cells/ml had a higher degree of calcite production, as observed in the scanning electron microscopy pictures and confirmed by the elemental composition of the concrete. Therefore, it was determined that utilizing the bacillus megaterium at a concentration of 10^5 cells/ml is the most effective approach to improve the performance of the concrete.

Kumar et al. (2023) Due to increasing environmental concerns, there is a remarkable rise in the demand for construction materials that are sustainable. These materials should require a minimal amount of energy and produce the lowest amount of carbon emissions possible. Consequently, the building sector has observed the rise of modified concretes that utilize industrial waste as a replacement for conventional concrete. This article explores novel techniques that encourage the natural self-repair process in concrete buildings. This procedure is accomplished by skillfully incorporating particular bacterial strains. In addition, the study investigates the impact of integrating several types of industrial waste, such as fly ash, slag, and crystalline additives, on the overall performance of the concrete. Nevertheless, it has been noted that the improved sealing of fractures resulting from the accelerated natural self-repair processes does not always correspond to higher mechanical properties or extended longevity of the cracked specimens. Hence, it is crucial to incorporate performance-based assessments when evaluating cement-based materials. The research also examines the possible contribution of biological methods in developing sustainable construction materials. The text examines the effects of bio-based treatments and bacterial solutions on many tangible factors, providing insight into the factors that influence the choice of bacteria. The emerging field of green biotechnology shows great potential. It provides an environmentally friendly alternative to traditional techniques of fixing problems in various areas, thereby offering a practical option to address a wide range of environmental difficulties.

2.4.8 Utilization of GGBS

Smith Songpiriyakij et al. (2010) utilized rice husk and bark ash (RHBA) as a high SiO_2 content material to substitute a portion of fly ash in the production of geopolymer. As a result, the SiO_2/Al_2O_3 ratio was expanded to a broad range of 4.03–1035. The study examined the impact of the SiO_2/Al_2O_3 ratio on the compressive strength, degree of reaction, and microstructure of the geopolymers. The results indicated that the SiO_2/Al_2O_3 ratio of 15.9 yielded the maximum compressive strength. The reactivity of fly ash surpassed that of RHBA. Additionally, it was demonstrated that both the reactivity of the source materials and the quality of the matrix played a role in improving the compressive strength of the geopolymer paste.

Ali Nazari (2011) and colleagues investigate the effects of fly ash and rice husk bark ash on geopolymer concrete in order to ascertain the material's mechanical and durability characteristics. The compressive strength of the specimens is influenced by a number of factors, including the pattern of ash particle size distribution, the length of time spent curing in the oven, and the amount of time spent curing at room temperature.

ashes with smaller particle sizes are responsible for producing the specimen that is denser and, thus, more robust. GPM has the potential to be utilized as a novel alternative repair material, according to tests on its tensile strength.

J. Guru Jawahar (2013) and colleagues found that overall curing time rise from fifty percent to one hundred percent, which resulted in an increase in the splitting tensile strength. The creation of sodium aluminosilicate hydrate (NASH) and calcium silicate hydrate (CASH) gel can be attributed to an increase in the Al2O3 and SiO2 content of geopolymer concrete. This has resulted in an increase in geopolymerization and an improvement in the strength qualities of geopolymer concrete.

Kupwade-Patil et al. (2013) study Industrial by-products known as slag from blast furnaces (BFS) and slag from steel furnaces (SFS) have been used for a significant period, with a history of around 100 years in the United States and 150 years in Europe.

Kumar, J.B. et al. (2013), Wang (2016), Shumuye (2018) investigate Ground granulated blast furnace slag, commonly referred to as GGBS, has long been employed in the manufacturing of composite cements and as a cementitious ingredient in concrete. In around 1859, the initial utilization of granulated blast furnace slag (GBS) in industrial and commercial settings involved the production of bricks utilizing unprocessed slag obtained from blast furnaces.

P. Saravanakumar et al. (2015) study the use of recycled aggregate (RA) and ground granulated blast furnace slag (GGBFS) in the production of structural concrete was investigated under the condition that the strength of the concrete was reduced to a somewhat lesser degree. Because of this, an effort has been made to investigate the physical properties of recycled aggregate concrete that is based on GGBFS, including its strength and durability. By substituting recycled coarse aggregates for natural coarse aggregate (NA) at a percentage of 0 percent, 25 percent, 50 percent, and 100 percent, four distinct sets of mixes were developed. In each of the groups, the effect of RA was investigated by substituting GGBFS for cement in varying ratios, ranging from forty percent to fifty percent, with a ten percent increment. The impacts of RA and GGBFS on the properties of fresh and hardened concrete were investigated, and the findings were compared with natural aggregate concrete (NAC). Additionally, the optimal replacement of RA and GGBFS was discovered. For all NA replacements with RA for GGBFS concrete, the experimental results suggest that there is an increase in concrete strength of up to forty percent at the age of ninety days, and the addition of more GGBFS shows a tendency in the opposite direction.

J. Guru Jawahar et al. (2016) and colleagues investigate the compressive strength and durability of geopolymer concrete in order to evaluate its effects. The ability of geopolymers to increase their strength is facilitated by an increase in the GGBS replacement level.

F.N. Okoye et al. (2016) stated the tensile strength of the material is what determines the loads at which fractures occur in concrete and collaborators who concentrated on the topic. The tensile strength of fly ash-based geopolymer concrete was shown to rise when the fraction of fly ash substitution with silica fume rise, initially at a gradual rate and later at a rapid rate. This was discovered through an investigation into the impact of silica fume on the quality of the concrete.

Khoa tan Nguyen et al. (2016) and colleagues demonstrated that the split tensile strength of geopolymer concrete is affected by higher curing conditions and longer curing durations throughout the curing process. The results of the sample that was allowed to cure at room temperature indicate that an increase in the ambient curing time is associated with an increase in the split tensile strength. With regard to the early split tensile strength (OPC), each of the samples had a higher value as compared to the control samples. A split tensile strength of 3.10 MPa was demonstrated by the Geopolymer mortar after 24 hours, which is ten times higher than the strength of the OPC mortar.

Prasanna et al. (2017) investigate the cementitious properties of GBS were discovered in the latter half of the 19th century, and by the end of the century, the first GBS-containing cements had been produced. Since the late 1950s, the use of GGBS as a finely ground material added to the concrete mixer together with Portland cement has been increasingly popular. In certain places, the phrase "slag cement" is used to designate to pure GGBS.

Peem Nuaklong et al. (2018) study about metakaolin improve the mechanical property, abrasion and acid resistance of concrete.

Yong Hu et al. (2019). It was detrimental to the compressive strength of the concrete to utilize recycled aggregate instead of natural aggregate because recycled aggregate is less strong than natural aggregate. This was also the case as the percentage of recycled aggregate in the concrete increased. When recycled aggregate was used in place of natural aggregate, it was demonstrated that the geopolymer materials' physical and mechanical properties were negatively affected. Nevertheless, geopolymer composites with recycled aggregate exhibited adequate compressive strength, reaching up to 43.1 MPa and 38.5 MPa, respectively, when the replacement percentages ranged from 50% to 100% with the recycled aggregate.

Over the course of their research, **Ghasan F. Huseien et al. (2019)** demonstrated that the tensile strength of all samples that were cured at room temperature increased when the curing time was increased. With regard to the early split tensile strength (OPC), all of the samples had a higher value when compared to the control samples. It was found that the tensile strength of the geopolymer mortar was 3 MPa after 24 hours, which is higher than the tensile strength of the OPC mortar. Increasing the slag ratio increases the splitting tensile strength of the material, which in turn improves the matrix of geopolymer concrete. This is because the matrix is enhanced. After 28 days, 56 days, and 112 days of ambient curing, the tensile strength values of the GGBS-based geopolymer concrete sample were 3.55 MPa, 3.84 MPa, and 4.13 MPa when measured correspondingly.

Huseien et al. (2020) was found that the optimal temperature for curing geopolymeric specimens was identified as being 80 degrees Celsius. In their research on the compressive strength and durability of geopolymer concrete, Ghasan F. Huseien and colleagues investigate the impacts of curing and alkali activators, including the lengthening of the time required for the curing process and the completion of the polymerization process.

Abdelrahman Albidah et al. (2022) focused primarily on the analysis of water uptake percentage as a function of total molar ratio. The combination of a lower aggregate content and a greater liquid to solids ratio generates a higher rate of water absorption in the material. The water absorption of geopolymer mixtures was comparable to or even greater than that of cement concrete when compared to the water absorption of MK-based GPC and cement concrete mixes alike.

2.5 Summary of Literature Review

Based on the state-of-the-art literature study, it can be understood that the addition of bacteria increases strength and durability properties of concrete, and percentage increase depends on type of bacteria and concentration in which it is incorporated. Therefore, it can be concluded that Bacillus subtilis, Bacillus Subtilis, and Pseudomonas aeruginosa can be effectively used in concrete to enhance its properties. These bacteria can precipitate $CaCO_3$ in high alkaline environment of concrete by converting urea into ammonium and carbonate, thereby improving the material's overall performance.

2.6 Research Findings of the Present Work

2.6.1 General Bacterial Concrete: Self-Healing Mechanism and Durability Enhancement

To make bacterial concrete (BC), bacteria are mixed with regular concrete to create a material that can continuously release calcite. Concrete reinforced with bacteria can detect when a crack is appearing and mend itself automatically. An additional impenetrable layer of calcite can be constantly precipitated onto surface of existing layer of concrete by bacteria. A variety of bacteria are mixed together in the making of bacterial concrete. These compounds have the ability to stay latent in concrete for approximately two hundred years.

Benefits of Bacterial Concrete:

1. Autonomous Healing: Bacterial concrete can self-heal cracks without human intervention, improving the longevity and durability of structures.
2. Increased Strength: The addition of specific bacteria, such as Bacillus Subtilis has been shown to enhance the compressive strength of concrete.
3. Enhanced Durability: By precipitating calcite, these bacteria can reduce the permeability of concrete, thereby protecting against water infiltration and subsequent degradation.
4. Corrosion Prevention: The bio-mineralization process can mitigate the corrosion of steel reinforcements within the concrete, extending the lifespan of the structure.
5. Sustainability: The use of bacterial concrete aligns with sustainable construction practices by reducing the need for repairs and maintenance over the structure's life.

Research Methodology:

1. Bacterial Selection and Cultivation:
   - Bacillus Subtilis (BS)
   - Bacillus Megaterium

These bacteria are chosen for their ability to survive in the high pH environment of concrete and their efficiency in precipitating calcium carbonate ($CaCO_3$).

2. Preparation of Bacterial Concrete

- Bacteria are blended into the concrete mix at various concentrations

Table 2.1: Concrete Mix at various Concentration

Sample ID	Cement (%)	Bacterial Concentration (cells/ml)
S1	100	0
S2	100	10^3
S3	100	10^5
S4	100	10^7

3. Testing and Evaluation:

Table 2.2: Testing and Evaluation

S.No.	Testing	Days
1.	Compressive Strength	7, 28, 56 Days
2.	Split Tensile strength	7, 28, 56 Days
3.	Flexural Strength	7, 28, 56 Days
4.	Water Absorption	28, 56 Days
5.	Ultrasonic Pulse Velocity	28, 56, 90 Days

2.6.2 Scope of the Present Work

The research work is mainly concerned with the mechanical and durability properties of the concrete containing bacteria and also intends to investigate the effect of corrosion on the concrete containing bacteria.

Scope of the research in bacterial concrete is limited to the following:

- To study the mechanical properties and flexural behavior of bacterial concrete.
- To obtain the optimal dosage of bacteria from the compressive strength test results.
- To study the bond strength between concrete and steel for bacterial concrete.

CHAPTER 3
EXPERIMENT PROGRAM

The primary objective of this experimental investigation is to obtain precise empirical data that enhances the understanding of bacterial concrete, focusing on its strength and durability characteristics. The research encompasses the examination of both fresh and hardened properties of regular grade concrete and Ground Granulated Blast Furnace Slag (GGBS) concrete, with and without bacterial addition.

Comprehensive laboratory experiments were conducted on hardened concrete to evaluate various properties, including compressive strength, split tensile strength, flexural behavior, and durability. The study employed Bacillus Subtilis and bacillus megaterium at different concentrations to assess their impact on the mechanical and durability properties of conventional concrete. Furthermore, regression and cluster analysis were performed to investigate the interrelationships between different concrete properties.

3.1 Experimental work in three phases

3.1.1 Phase I: Growth of bacteria

3.1.2 Phase II: Mechanical properties of concrete

3.1.2.1 To study compressive strength
3.1.2.2 To study the split tensile strength
3.1.2.3 To study the flexural strength

3.1.3 Phase III: Durability properties of concrete

3.1.3.1 To study the water absorption capacity of concrete
3.1.3.2 To study the ultra-sonic pulse velocity of concrete

3.1.4 Phase IV: To study the regression analysis of properties of concrete

3.1.5 Phase V: To study the cluster analysis of properties of concrete

3.2 Material

3.2.1 Cement

The investigation employs locally available Ordinary Portland cement of 43 grade. The cement employed for all testing derives from the same batch. The cement employed has been tested for various properties in accordance with the IS: 4031-1988 standard and has been found to comply with the specifications set out in the IS: 12269-1987 standard. The physical and chemical properties of OPC, as established in the laboratory, are presented in Tables 3.1 and 3.2. Figure 3.1 displays the OPC cement utilized in this study.

Table 3.1: Physical properties of OPC

Properties of OPC	Experimental Value	Codal Requirement (IS 8112-2013)
Normal Consistency	30%	25-35%
Initial Setting Time	55 Minutes	>30 Minutes
Final Setting Time	470 Minutes	<600 Minutes
Specific Gravity	3.15	3.15
Fineness	6 %	<10%
Soundness	2 mm	<10 mm
3 Day Compressive Strength	23.00 MPa	>23 N/mm^2
7 Day Compressive Strength	35.33 MPa	>33 N/mm^2
28 Day Compressive Strength	47.00MPa	>43 N/mm^2

Table 3.2. Chemical Composition of Cement

Chemical Composition	Cement (%)
CaO	62.22
SiO_2	21.23
MgO	3.18
Al_2O_3	4.02
Fe_2O_3	1.75
SO_3	2.83

Figure 3.1: OPC Cement

3.2.2 GGBS

The GGBS product is environmentally friendly and composed of metal. Furthermore, this material not only has a lower CO_2 footprint, but also exhibits a superior level of quality. Rapid cooling of molten metal slag with water or steam forms a glassy and granular substance known as Ground Granulated Blast Furnace Slag (GGBS). This by-product is rich in metal and minerals. There was a maximum particle size of 95.21 micrometres and a moisture content of 0.14% in the particular batch of GGBS utilised in this research. The bulk density of GGBS is 1290 kg/m², and its specific gravity is 2.88. The chemical composition of GGBS is detailed in Table 3.3. The appearance of GGBS in its powdered state is shown in Figure 3.2.

Table 3.3. Chemical Composition of GGBS

Chemical Composition	GGBS (%)
CaO	35.24%
SiO_2	29.58%
MgO	6.25%
Al_2O_3	17.02%
Fe_2O_3	0.61%
SO_3	1.78%

Figure 3.2: GGBS (Ground Granulated Blast Furnace Slag)

3.2.3 Aggregates

The aggregate qualities of concrete have a significant impact on the behaviour of concrete since they account for about 80 percent of the entire volume of concrete.

3.2.3.1 Fine aggregate

Sand is a granular substance composed of distinct rock and mineral particles. The sand is stratified, although its granule size is the defining characteristic. Sand particles are smaller than gravel and larger than silt. Sand may also denote a classification of soil, specifically dirt comprising particles that exceed 85 percent of their original size. Table displays results of sieve analysis of fine aggregate. Figure 3.3 was shown the fine aggregate. The specific gravity and moisture content of sand were 2.68 and 1.4%.

Figure 3.3: Fine aggregate

Table 3.4: Sieve analysis of fine aggregate

Sieve Size	Weight Retained (g)	% Weight Retained	Cumulative % Weight Retained	% Passing	Standard % Weight Passing for Zone 3
10.00mm	0	0	0	100	100
4.75mm	60	6	6	94	90-100
2.36mm	72	7.2	13.2	86.8	85-100
1.18mm	68	6.8	20	80	75-100
600µm	107	10.7	30.7	69.3	60-79
300µm	487	48.7	79.4	20.6	12-40
150µm	166	16.6	96	4	0-10
Pan	40	4	-	-	
Total	1000	100	245.3		

Fineness modulus = 245.3/100 = 2.453

Fine aggregate lies in Zone-3 (As per IS 383-2016).

3.2.3.2 Coarse aggregate

When constructing the concrete's fundamental matrix, it is important to take into account the coarse aggregate. In this experiment, natural coarse aggregate with diameters of 20 mm and 10 mm was used.

3.2.3.2.1 Natural coarse aggregate of size 20 mm

The image shows coarse aggregate measuring 20 millimeters in size. According to Table 5, which details the sieve analysis results, the coarse aggregate has a specific gravity of 2.72. Additionally, the aggregate exhibits a water absorption rate of 0.85%. This specific gravity indicates the density of the aggregate compared to water, while the water absorption reflects its porosity and capacity to absorb moisture. These properties are crucial for determining the aggregate’s suitability and performance in concrete mixtures, affecting the overall strength, durability, and workability of the concrete.

Figure 3.4: Coarse aggregate of 20 mm size

Table 3.5: Sieve analysis coarse aggregate of size 20 mm

IS Sieve Size	Weight Retained	% Weight Retained	Cumulative % Weight Retained	% Passing
40mm	0	0	0	100
20mm	315	6.3	6.3	93.7
10mm	4635	92.7	99	1
4.75mm	5	0.9	99.9	0.1
2.36mm	-	0.1	100	0
1.18mm	-	-	100	
600um	-	-	100	
300um	-	-	100	
150um	-	-	100	

Fineness modulus of 20 mm fraction coarse aggregate = 705.2/100 =7.052

3.2.3.2.2 Natural coarse aggregate of 10 mm size

Table 6 and Figure 3.5 present the sieve analysis results for coarse aggregate with a size of 10 millimeters. The coarse aggregate has a specific gravity of 2.70, indicating its density relative to water. Additionally, it has a water absorption rate of 1.0% per unit volume, which signifies its porosity and moisture absorption capacity. These properties are essential for evaluating the aggregate's suitability and performance in concrete mixtures, influencing the concrete's overall strength, durability, and workability.

Figure 3.5: Coarse aggregate of 10mm size

Table 3.6: Sieve analysis of 10 mm size coarse aggregate

IS Sieve Size	Weight Retained	%Weight Retained	Cumulative %Weight Retained	%Passing Weight
20.0mm	0	0	0	100
10.0mm	658	32.9	32.9	67.1
4.75mm	1248	62.4	95.3	4.7
2.36mm	48	2.4	97.7	2.3
1.18mm	40	2	99.7	0.3
600µm	6	0.3	100	-
300 µm	-	-	100	-
150 µm	-	-	100	-

Fineness modulus of 10 mm fraction coarse aggregate = 625.6/100 =6.256

3.2.4 Bacteria:

Bacillus Subtilis and megaterium were utilized in this investigation. These bacteria were purchased from IMTECH (MTCC) Chandigarh. When purchased, the bacteria were in form of dried frozen powder. Through process of inoculating bacteria onto an agar plate, this powdered bacterial culture was transformed into a liquid bacterial culture, which was then applied to concrete. The bacteria known as B. Subtilis are responsible for formation of calcite precipitation in voids of concrete specimens both throughout and after curing process. This precipitation contributes to structure experiencing increased compactness and density. Because of the denseness of the sample, the porosity of the concrete specimens decreases, which helps to prevent the introduction of agents that are not acceptable into the concrete specimens. Precipitation of calcite into concrete pores has been facilitated by the employment of bacteria, which has resulted in the concrete being more long-lasting. Furthermore, the precipitation of calcite on surface of concrete cubes is primary factor responsible for the reduction in water absorption. In Figure 3.6, the Bacillus Subtilis bacteria are depicted in their powdered state.

Figure: 3.6: Bacillus Subtilis bacteria in Powder dried form

Also, Bacillus megaterium bacterial concentration (10^3, 10^5, 10^7 cells/ml) used in this paper and check the impact of bacterial concentration on the mechanical (Flexural, compressive and Split tensile strength) and durability properties (water absorption and Ultrasonic pulse velocity) of cement concrete and cluster analysis of water absorption and compressive strength. Regression analysis between compressive strength and water absorption and compressive strength and ultrasonic pulse velocity were also be done in this study.

Bacterial solution of bacillus megaterium (10^3, 10^5, and 10^7 cells/ml), and poly carboxylic ether have all been used to create an M30 grade concrete mixture. Coarse aggregate, fine aggregate and cement are used to make superplasticizers. A small amount of water was added, and the coarse and fine aggregates were mixed for about two minutes in order to minimize the dust produced by the aggregate mixing process in the mixer pan. After the needed cement amount has been provided, the mixing procedure is repeated twice more. The leftover water was then added to the pan mixer along with the bacterial solution, and for three minutes, the mixture was mixed. After that, the desired moulds are filled with the mixture. The mix id was given below in the table 3.7.

Table 3.7- Mix id of Cement and megaterium bacterial concentration

Sample ID	Cement (%)	Bacterial Concentration (cells/ml)
S1	100	0
S2	100	10^3
S3	100	10^5
S4	100	10^7

3.2.5 Water:

Throughout the entirety of the experiment, the pure water from the tap was utilized for the creation of multiple concrete samples. When the digital TDS meter was used to measure the total dissolved solids of accessible tap water, observed temperature of water was found to be 22 ± 3 degrees Celsius. Additionally, the total dissolved solids were found to be around 300 parts per million. A bacterial solution was created independently, and it was made to have the optimal density that was wanted. When the concrete preparation was being mixed, the available solution was combined with relative replacement of water with bacterial solution. This was done during the stirring process.

3.3 Mix Design

Concrete Mix design has been developed to calculate ratio of water to cement, fine aggregates, coarse aggregates, and cement in accordance with IS 10262-2019. This was done in order to adhere to the specifications of the concrete mix design. In this research investigation, M25 grade concrete was utilized to produce bacterial concrete with calcined clay cubes, non-bacterial calcined clay concrete cubes, and controlled concrete cubes. All of these concrete cubes were constructed simultaneously. As a result, the Mix design of concrete has been developed in order to predict the quantity of material that is required to cast cubes. This material includes cement, fine aggregate, coarse aggregate, and calcined clay. The following is the design for the concrete mix for the M25 grade:

3.3.1 Mix design of M25 Grade of concrete

Grade of concrete = M25

Slump of concrete= 25-50 mm

Maximum w/c ratio= 0.50

Minimum cement content= 300 kg/m^3

3.3.2 Adopting w/c ratio= 0.35 < 0.50 OK

Water content= 186 litre

Adding superplasticizer, water content reducing up to 15%

Therefore, Final water content= 186 x 0.15 = 158.1 litre

Cement content = 158.1/0.35 = 451.71 kg/m^3

3.3.3 Volume calculation

Volume of coarse aggregate corresponding to w/c ratio of 0.35 = 0.67

Volume of Fine aggregate = 0.33

Volume of concrete = 1 m^3

Volume of cement = 416.05/3.15 x 1/1000 =0.132 m^3

Volume of water = 158.41/1000 = 0.1581 m^3

Volume of superplasticizer [@ 1.2% by mass] = 4.9926/4.145 x1/1000 = 0.00436

$$\text{Volume of all aggregates} = 1 - (0.132 + 0.1581 + 0.00436) = 0.70554\ \text{m}^3$$

3.3.4 Mass calculation

Mass of Coarse aggregate = 0.70554 x 0.67 x2.74 x1000 = 1295.23 kg

Mass of Fine aggregate = 0.70554 x 0.33 x 2.66 x 1000 = 619.323 kg

Mass of Cement = 451.71 kg

Mass of water = 158.1 kg

Superplasticizer = 4.9926 kg/ m^3

3.4 Mixing of Concrete

The blending procedure is conducted using an electrically driven mixer. The components are layered systematically, starting with coarse aggregate, followed by fine aggregate, and finally the cementitious material. To achieve a uniform mixture, dry blending is performed until the color is homogeneous. Subsequently, an optimal quantity of Bacillus Subtilis bacteria is mixed into the water. This bacterial solution is then added to the dry mix. Immediately after mixing the concrete, workability tests are carried out. The compaction factor testing apparatus is used for these tests, following the guidelines set by IS: 10510-1983. This standard ensures that the compaction factor, which measures the workability of the concrete mix, is accurately determined. The inclusion of Bacillus Subtilis bacteria aims to enhance the concrete's properties, and this testing procedure verifies the effectiveness of the mix before further use.

3.5 Casting of Sample

For a thorough assessment of concrete strength, samples were meticulously prepared in various dimensions to evaluate compressive, split tensile, and flexural strength. The cube samples were created with dimensions of 150 mm x 150 mm x 150 mm, the cylinder samples had a diameter of 150 mm and a height of 300 mm, and the beam samples were made with dimensions of 100 mm x 100 mm x 500 mm. These various sample forms played a crucial role in accurately assessing the mechanical strengths of the concrete and conducting comparative analyses between samples with and without bacterial concentration.

For optimal integrity during the concrete setting process, the moulds were firmly secured to prevent any slurry leakage. This fastening was crucial for preserving the sample's form and guaranteeing consistency in the tests. A vibrating table was used to ensure proper compaction of the concrete in the moulds. Prior to use, the moulds underwent a thorough cleaning and were lubricated to promote effortless demolding and maintain a uniform surface finish for the concrete samples.

A grand total of 120 cubes and 90 cylinders were manufactured, each with specific dimensions. At first, the samples were made using only Ground Granulated Blast Furnace Slag (GGBS) to establish the baseline mechanical properties. Extensive testing was conducted over a period of 28 days to determine the ideal dosage of GGBS in concrete. A suitable period was selected to allow for sufficient reaction and contribution of GGBS to the properties of the concrete.

After finding the best GGBS dosage, a fresh batch of concrete was made using the same process, but with the addition of a bacterial solution. For the project, Bacillus Subtilis was selected as it has the potential to improve the durability and mechanical properties of the concrete. In order to achieve a uniform distribution throughout the material, the bacterial solution was thoroughly mixed with water before being incorporated into the concrete mix.

The compressive strength of the concrete samples was assessed at three different curing periods: 7, 28, and 56 days after production. Conducting this staged testing was crucial in gaining insights into the strength's evolution and the impact of bacteria on its development. For every strength test, we made sure to use the exact same samples to guarantee that the results were consistent and reliable.

Aside from conducting strength tests, water absorption tests were also carried out at 28-day and 56-day intervals. Understanding water absorption is crucial when evaluating the durability of concrete, as it directly impacts the material's ability to withstand weathering and chemical attack. By utilising identical samples for both strength and water absorption tests, the study ensured a direct link between mechanical strength and durability could be established.

The samples prepared and their dispersion for this study are outlined in Table 3.8, offering a comprehensive view of the experimental design and the different conditions examined. By incorporating bacterial solutions alongside GGBS, it was believed that the mechanical properties of the concrete could be enhanced, resulting in a more robust and long-lasting material.

The test results offered valuable insights into the effectiveness of incorporating Bacillus Subtilis into concrete mixtures. It was expected that the bacterial solution would enhance the concrete's self-healing abilities, potentially resulting in fewer microcracks and a longer lifespan for the material. This study sought to enhance our knowledge of the impact of bacterial presence in concrete on its mechanical characteristics. Through a thorough examination of compressive, split tensile, and flexural strengths, along with water absorption tests, a comprehensive dataset was obtained to support future research and potential practical uses of bacterial concrete in the construction field. This approach focused on improving the mechanical properties of concrete and ensuring the long-term sustainability and durability of concrete structures in different environmental conditions.

Table 3.8: Sample ID and Proportion of Materials

S. No	Sample Name	GGBS (%)	Cement (%)	Bacillus Bacterial Solution (cells/ml)
1.	S0	0	100	0
2.	S5	5	95	0
3.	S10	10	90	0
4.	S15	15	85	0
5.	S20	20	80	0
6.	B3S10	10	90	10^3
7.	B5S10	10	90	10^5
8.	B7S10	10	90	10^7

3.6 Growth of Bacteria

In the current study, bacteria belonging to the genus Bacillus Subtilis and bacillus Megaterium are utilized to reduce the porosity of concrete. This is accomplished by causing calcite to precipitate within the areas that are vacant within the concrete. After being freeze-dried, the bacteria known as Bacillus Subtilis and Bacillus Megaterium was acquired from IMTECH MTCC Chandigarh. For the purpose of incorporating the freeze-dried bacteria into concrete, it will be utilized to transform the bacteria into a liquid form with a concentrated concentration. The method entails the creation of nutritional broth media, which is comprised of 0.3 grams of beef extract, 0.5 grams of sodium chloride, and 0.5 grams of peptone for every 100 milliliters of media. After that, the medium is subjected to an autoclaving process that lasts for forty-five minutes. Following the introduction of the Bacillus Subtilis bacteria into a conical flask that contained nutritious broth, steps were taken to avoid the flask from being contaminated by flames that were located in the surrounding area. After that, the flasks were kept at a temperature of four degrees Celsius. Following the passage of twenty-four hours, bacterial adherence will be carried out on the agar plate and the petri dish in order to encourage the proliferation of bacteria. This will be done with the intention of aiding the following expansion of the bacterial culture. A shaker incubator was used to incubate the bacterial culture that was contained within a conical flask for period of 24 hours at temperature of 37

°C. Afterwards, a sample of the bacterial medium containing five milliliters was obtained and placed in culture tubes. The McFarland method was then utilized to determine number of bacterial cells that were present in each milliliter. Following that, the Optimum Density (OD) was determined by employing a spectrophotometer with a wavelength of 600 nm. This was accomplished by directing light through the bacterial media as well as a neutral medium consisting of nutrient broth culture milieu. A pellet was formed by centrifuging the residual bacterial culture in a centrifuge machine for ten minutes at a speed of four thousand revolutions per minute. Additionally, the broth media should be introduced into the culture tube in order to prepare the bacterial concentration for dilution in water, which will be utilized throughout the process of casting concrete.

3.6.1 Preparation of Nutrient Broth

- Take empty conical flask of 250 ml and clean this conical flask with water.
- Then take 0.5 g of peptone, 0.5 g of NaCl and 0.3 g of beef extract in powder form.
- Add 100 ml of Triple Distilled Water in conical flask with these constituents and shake conical flask very well.
- Check pH level 7.2 ± 0.2 of this solution by using pH paper to verify the colour coding of pH level.
- After managing pH level of this solution, make cotton plug for closing the mouth of conical flask and preventing contamination of this solution.

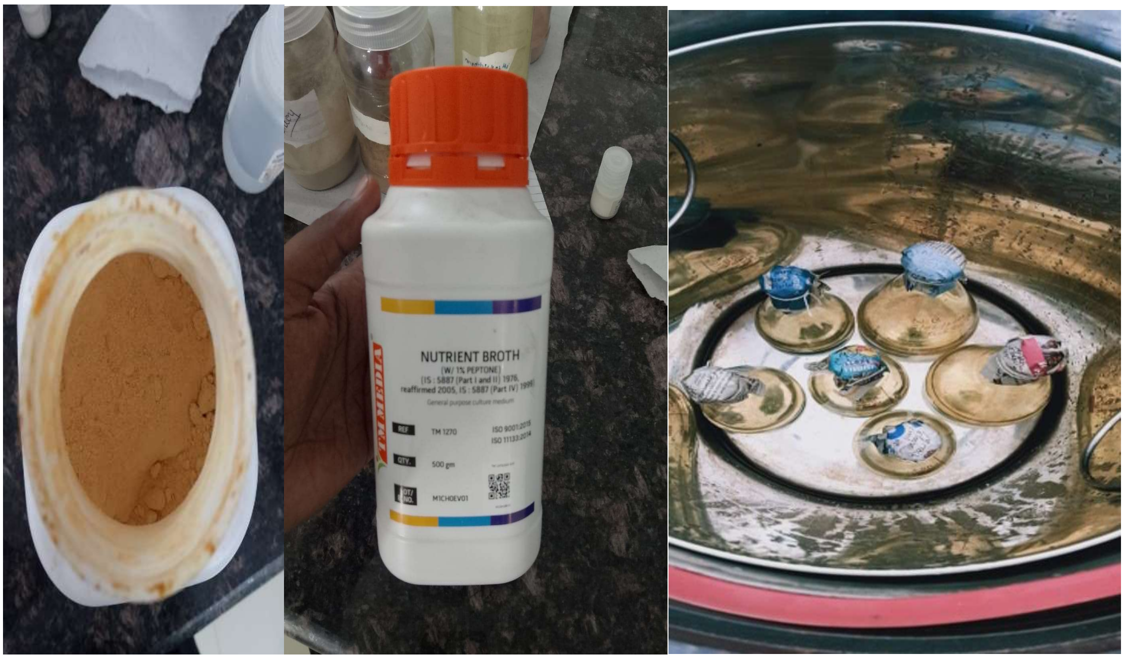


Figure 3.7: Nutrient Broth

3.6.2 Preparation of Nutrient Agar

- Take empty conical flask of 250 ml and clean this conical flask with water.
- Then take 0.5 g of peptone, 0.5 g of NaCl, 0.3 g of beef extract and nutrient agar in powder form.
- Add 100 ml of Triple Distilled Water in conical flask with these constituents and shake conical flask very well.
- Check pH level 7.2 ± 0.2 of this solution by using pH paper to verify the colour coding of pH level.
- After managing pH level of this solution, make cotton plug for closing the mouth of conical flask and preventing contamination of this solution.
- Then autoclave this solution for 45 minutes at 110º C temperature to remove complete contamination of this solution.

- The prepared solution is Nutrient Agar and it is used for bacterial growth on petri dishes. Agar and the final solution of nutrient agar is shown in Fig 3.8.

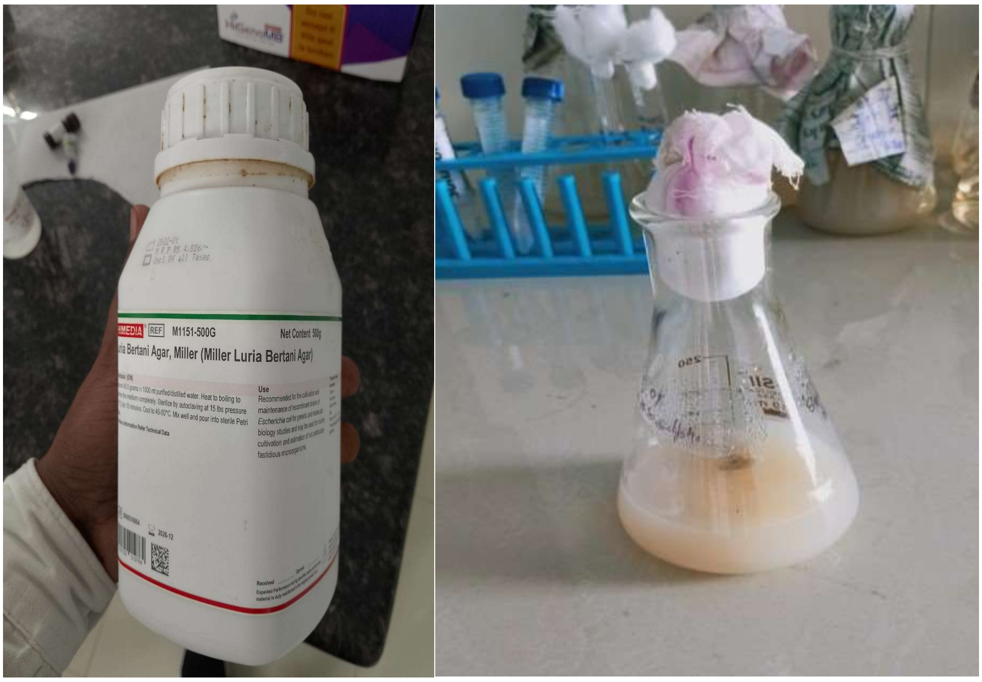


Figure 3.8: Nutrient Agar solution

3.6.3 Preparation of Bacillus Megaterium Bacterial Solution

- The microorganisms were obtained from IMTECH MTCC Chandigarh in both powdered and frozen forms through their acquisition. The bacterial powder has been converted into a liquid solution in order to make the process of incorporating it into concrete easier. To begin, a conical flask was utilized for the cultivation of nourishing broth medium. This flask contained 0.3 grams of beef extract, 0.5 grams of peptone, and 0.5 grams of sodium chloride.
- While, pH of the media is required within the given range of 7 to 7.4 during the entire process. During the cultivation process, the bacteria belonging to the bacillus megaterium species eat the broth solutions.
- Through the process of inoculation, the powdered bacteria were spread out in stripes over agar plates, which led to the creation of a bacterial solution, as shown in Figure 3.9.
- After a period of twenty-four hours, the plates were put into an incubator that was set to 37 degrees Celsius.

- For the purpose of preventing the contamination and degradation of bacillus megaterium bacteria, the nutrient broth media was sterilized by means of an autoclave in a conical flask.
- Additionally, all of the equipment was meticulously cleaned and disinfected.
- Following the inoculation of the liquid solution, it was transferred to the flask having conical shape and then placed at the appropriate height on orbital shaker.
- In order to cultivate the bacteria, it was rotated at a pace of 150 revolutions per minute for a period of twenty-four hours.
- The bacteria that were produced in this manner were utilized in the process of preparing the mixture for the concrete.

Figure 3.9: Bacillus Megaterium Bacteria

3.7 To study the compressive strength

The investigation is conducted to examine the compressive strength of concrete. Cubes were cast and tested to analyse the compressive strength when subjected to axial compression.

3.7.1 Sample Preparation

Cubes with dimensions of 150 millimetres by 150 millimetres by 150 millimetres are created in accordance with the specifications stated in IS: 516-1999. The design mix for standard grade concrete is prepared according to the specifications. Both bacteria-inclusive and bacteria-exclusive manufacturing techniques are utilized in production of cubes. Immediately after casting process is complete, the specimens are extracted from molds and submerged in clean, fresh water within the curing tank. This process occurs after twenty-four hours. Following the curing period, specimens are collected and stored in shaded location.

3.8 To study the split tensile strength of concrete sample

The investigation is conducted to examine the split tensile strength of concrete. Cylinders were cast and tested to analyze the split tensile strength when subjected to axial compression.

3.8.1 Sample Preparation

A cylinder with dimensions of 150 millimeters by 300 millimeters is constructed in accordance with the specifications given in IS: 516-1999. The design mix for standard grade concrete is prepared. The cylinders are manufactured using both methods that are inclusive of bacteria and ways that are exclusive of bacteria. The specimens are removed from the molds once the casting process is complete and are immediately submerged in clean, fresh water within the curing tank. This occurs after twenty-four hours. After the curing period is finished, the specimens are collected and stored in a shaded location.

3.9 Flexural strength of concrete sample

For the purpose of analyzing the flexural behavior of concrete, an investigation is now being carried out. Both bacteria-infused and bacteria-free mixes are employed in the production of the beams, which are then built using traditional and standard grade concrete.

3.9.1 Sample Preparation

The molds are made up of two wooden planks that are located in parallel to one another, and there is a space between them that is equal to the width of the beam that is going to be manufactured. In order to guarantee that the boards are properly spaced apart, wooden pieces of the required width are inserted in between them. In order to complete the casting process, a flat platform is utilized. It should be noted that the molds do not come with a base plate. At the ends of the moulds, bolts and nuts are used to secure the frame in place when it has been assembled.

3.10 Water Absorption Test

The samples were cubes with dimensions of 150 mm x 150 mm x 150 mm, prepared in a manner similar to those used for compressive strength testing. This experiment sought to measure water absorption in both bacteria-embedded concrete and conventional concrete. At first, the cubes underwent a drying process in an oven set at 105°C for 24 hours. The weight of the cubes after drying was noted as (W1). After the drying process, the cubes were placed in water for 24 hours, and their weight while wet was recorded as (W2). The amount of water absorbed by the concrete was then calculated using the formula:

$$\text{Water Absorption} = \frac{(W_2 - W_1)}{W_1} * 100 \tag{1}$$

3.11 Ultrasonic pulse velocity Test

In order to determine the ultrasonic pulse velocity (UPV) of a substance, a pulse transmitter is positioned on one side of the sample, and a receiver is positioned precisely opposite of the transmitter. The amount of time that the ultrasonic pulse must spend travelling through the material is measured using a device that is used for timing calculations. In order to get the UPV, divide the known distance between the transducers by the transit time that was measured. A appropriate coupling agent, such as petroleum jelly, is utilised in order to guarantee accurate readings and remove any air gaps that may exist between the diaphragms of the transducer and the surface of the concrete. In order to guarantee accurate contact, the transducers are firmly fastened to the concrete surfaces, and the duration of the transit time is recorded for the purpose of UPV evaluation.

3.12 Regression Analysis

Regression analysis in statistical modeling comprises a series of statistical methods aimed at estimating the relationships between a dependent variable (commonly referred to as the outcome or response variable, or a label in machine learning terminology) and one or more independent variables devoid of error (often termed regressors, predictors, covariates, explanatory variables, or features). Linear regression is the predominant type of regression analysis, wherein one determines the line (or a more intricate linear combination) that best approximates the data based on a defined mathematical criterion. The ordinary least squares method calculates the distinct line (or hyperplane) that minimizes the aggregate of squared discrepancies between the actual data and that line (or hyperplane).

3.12 Cluster Analysis

Cluster analysis, or clustering, is the categorization of a collection of objects such that items within the same group, termed a cluster, exhibit greater similarity to one another, according to a specified criterion established by the analyst, than to those in different groups. It is a primary objective of exploratory data analysis and a prevalent method for statistical data analysis, employed across various domains, including pattern recognition, image analysis, information retrieval, bioinformatics, data compression, computer graphics, and machine learning.

CHAPTER 4
RESULT AND DISCUSSION

The investigation was conducted in three distinct phases. During the initial phase, the study varied the proportion of Ground Granulated Blast Furnace Slag (GGBS) substituted for Ordinary Portland Cement (OPC) within a range of 0% to 20%. This phase aimed to determine how different levels of GGBS substitution impacted the properties of the concrete.

In the subsequent phase, different bacterial concentrations were introduced into the concrete mixtures that already included GGBS. The bacterial concentrations tested were (10^3), (10^5), and (10^7) cells/ml. This phase sought to assess the effect of varying bacterial concentrations on the concrete's properties when combined with the optimal GGBS dosage. In the last phase used a bacterial solution of Bacillus megaterium from the Bacillus bacteria family at concentrations of 10^3, 10^5, and 10^7 CFU.

Concrete samples from all phases were evaluated for compressive strength at three distinct time intervals: 7, 28, and 56 days after casting. These intervals allowed for a comprehensive analysis of the concrete's strength development over time. By examining compressive strength at these intervals, the study aimed to understand how the combination of GGBS and different bacterial concentrations influenced the concrete's performance. This detailed approach provided insights into the optimal levels of GGBS and bacterial addition for enhancing the mechanical properties of concrete, contributing to the overall effectiveness of the concrete mixture.

4.1 Compressive strength

Compressive strength testing was conducted using equipment in accordance with IS: 516-1959 standards. Concrete samples with GGBS and varying bacterial concentrations were tested, and the results are illustrated in Figures 4.1 and 4.2. The data in Figure 4.1 indicates that substituting GGBS for cement led to an increase in compressive strength up to a 10% replacement level. Beyond this threshold, however, the increase in compressive strength became less pronounced, with substitution levels reaching up to 20%. At testing intervals of 7, 28, and 56 days, the strength differences at various replacement percentages were minimal and nearly indistinguishable.

Table 4.1: Compressive Strength value of GGBS sample

Sample	7 Days	28 Days	56 Days
G0	25.25MPa	31.74 MPa	36.55 MPa
G5	27.92 MPa	34.28 MPa	38.63 MPa
G10	31.02 MPa	36.33 MPa	41.26 MPa
G15	29.24 MPa	35.4 MPa	40.02 MPa
G20	26.11 MPa	33.95 MPa	37.83 MPa

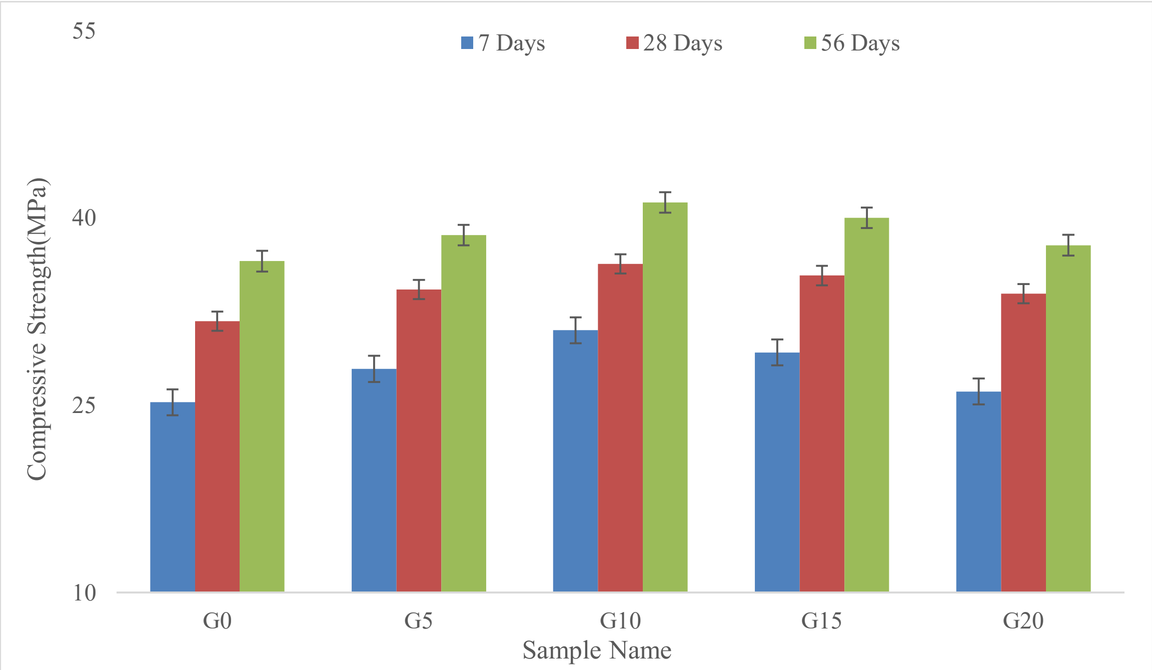


Figure 4.1: Compressive Strength of GGBS sample

As compared to regular concrete, the S10 samples showed a maximum increase in strength of about 22.85%, 14.46%, and 12.88% after 7, 28, and 56 days, respectively. This was the case when compared to the standard concrete. This enhancement can be ascribed to the interaction that takes place between Ground Granulated Blast Furnace Slag (GGBS) and calcium hydroxide. This reaction leads to the creation of calcium silicate hydrate (C-S-H) gel, which increases the strength of concrete.

The strength of the S10 samples, on the other hand, continued to increase until it reached its highest point at the 56-day mark. There was a noticeable decrease in strength when this milestone was reached. Because of the depletion of calcium hydroxide, which restricts the amount of interaction that can take place between GGBS and calcium hydroxide, this decrease is most likely the result. As the amount of calcium hydroxide decreases, the continuous creation of C-S-H gel is interrupted, which results in a reduction in the gel's strength. During the curing phase of concrete, it is essential to ensure that there is a sufficient supply of calcium hydroxide in order to sustain the positive interactions that occur between GGBS and calcium hydroxide. This behaviour illustrates the necessity of meeting this need.

Table 4.2: Compressive strength of concrete using bacillus bacterial solution

Sample Name	7 Days	28 Days	56 Days
G10	31.02 MPa	36.33 MPa	41.26 MPa
B3G10	34.62 MPa	39.86 MPa	43.82 MPa
B5G10	37.5 MPa	41.72 MPa	46.69 MPa
B7G10	36.25 MPa	40 MPa	44.86 MPa

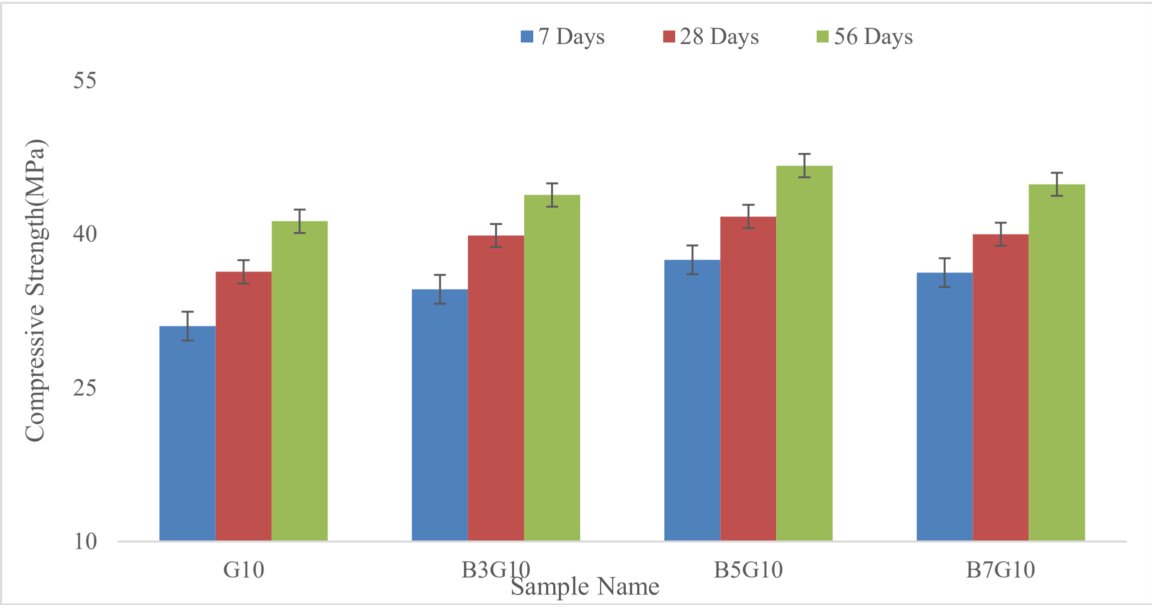


Figure 4.2: **Compressive strength of concrete using bacillus bacterial solution**

Figure 4.2 demonstrates the effect of varying concentrations of Bacillus Subtilis (10^3, 10^5, and 10^7 cells/ml) on the compressive strength of concrete containing 10% Ground Granulated Blast Furnace Slag (GGBS). At a concentration of 10^5 cells/ml, the compressive strength of the concrete exhibited a steady increase over time. However, when the concentration reached 10^7 cells/ml, a decline in compressive strength was observed.

Compared to the G10 samples, the compressive strength of the B5S10 samples showed significant improvements, with increases of approximately 20.92%, 14.83%, and 13.16% at the respective testing intervals. This enhancement in strength is attributed to the growth of Bacillus Subtilis colonies and the subsequent formation of calcite within the concrete matrix. The bacterial activity likely contributed to the filling of pores in the concrete, resulting in increased density and improved durability. This process underscores the role of bacterial-induced calcite precipitation in enhancing the structural properties of concrete, particularly when combined with GGBS.

Table 4.3: Compressive Strength of Megaterium Bacterial Concrete

Sample Name	7 Days	28 Days	56 Days
S1	35.58 MPa	39.26 MPa	41.14 MPa
S2	38.02 MPa	42.85 MPa	43.65 MPa
S3	42.54 MPa	46.05 MPa	47.84 MPa
S4	40.14 MPa	44.92 MPa	43.92 MPa

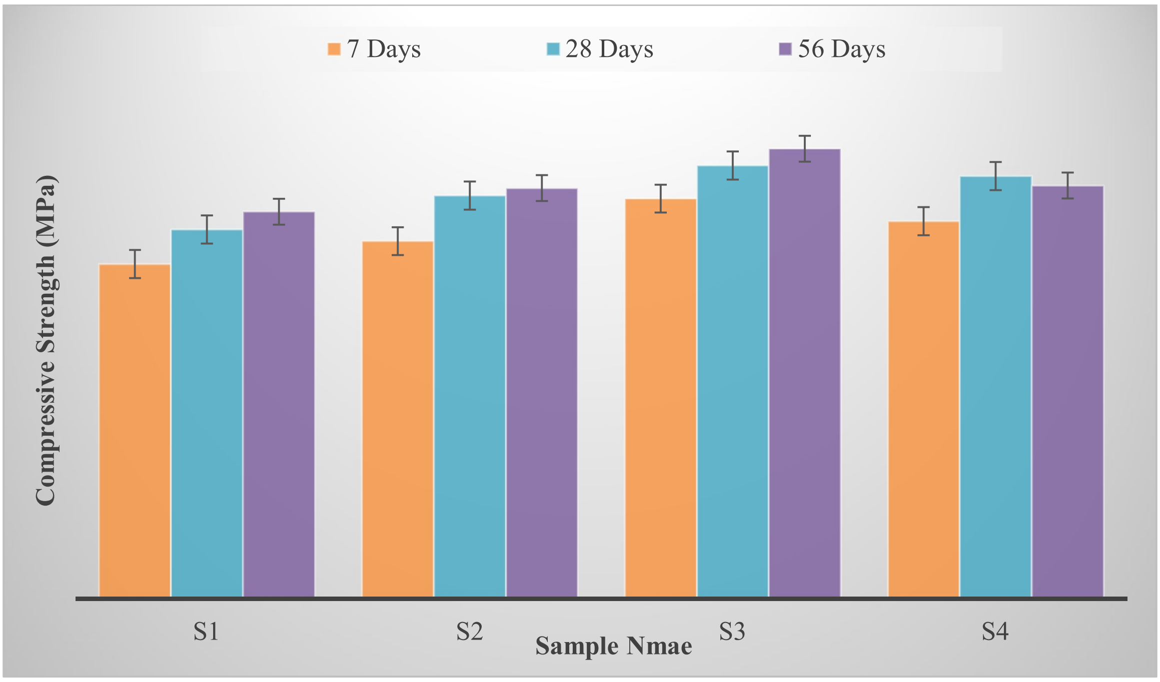


Figure 4.3: Compressive Strength of Megaterium Bacterial Concrete

The effect that different amounts of bacillus megaterium (10^3, 10^5, and 10^7 cells/ml) have on the compressive strength of cement concrete is illustrated in Figure 4.3. At a concentration of 10^7 cells/ml of bacillus megaterium bacteria, the compressive strength began to drop after initially increasing until it reached a 10^5 cells/ml concentration. This was the point at which the compressive strength began to decrease. At 7, 28, and 56 days after testing, the compressive strength of the S3 samples is much higher than that of the cement concrete sample (S1), with an approximate increase of 19.56%, 17.29%, and 15.17% respectively.

4.2 Split Tensile Strength

The strength of concrete cylinders on the 7th, 28th, and 56th days of testing period, following the guidelines outlined in IS: 516-1959 and IS: 5816-1999. The initial measurements aimed to explore the effectiveness of GGBS substitution, Additionally, we evaluated the impact of various bacterial quantities on the concrete, as illustrated in Figure 4.4, to identify the most effective alternative to GGBS.

Table 4.4: Split tensile Strength value of GGBS sample

Sample	7 Days	28 Days	56 Days
G0	1.87 MPa	2.28 MPa	2.55 MPa
G5	2.09 MPa	2.42 MPa	2.7 MPa
G10	2.28 MPa	2.54 MPa	2.82 MPa
G15	2.19 MPa	2.48 MPa	2.7 MPa
G20	2.08 MPa	2.39 MPa	2.59 MPa

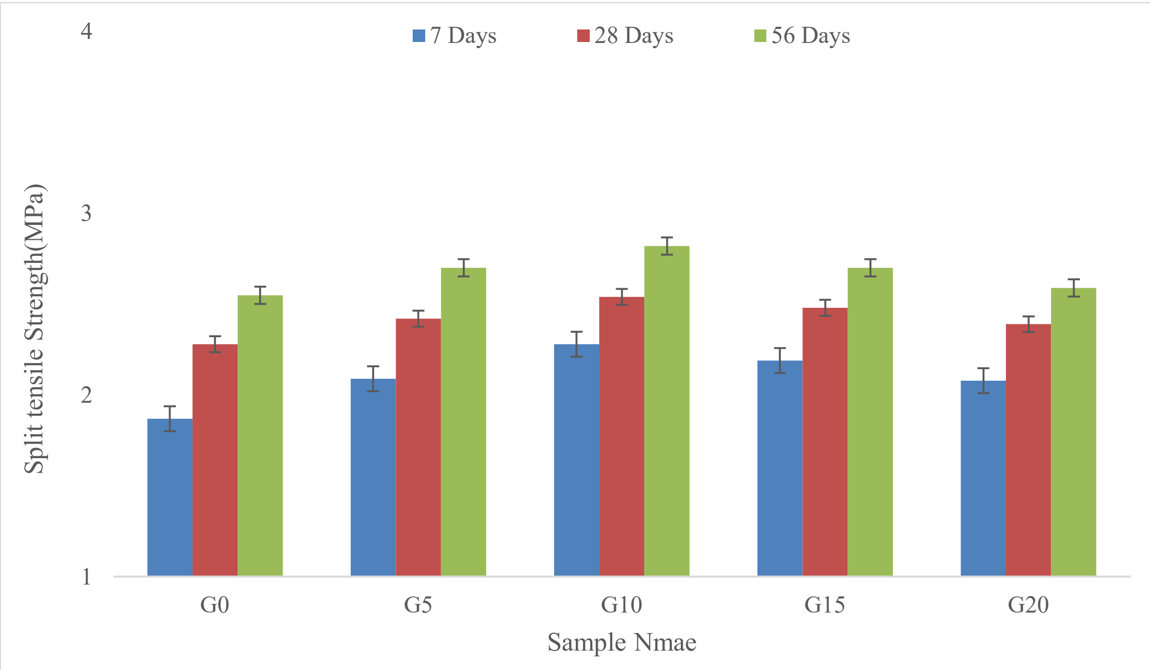


Figure 4.4: Split tensile Strength of GGBS sample

According to results shown in Figure 4.4, maximum split tensile strength was achieved by substituting 10% of cement with GGBS. The G5 and G10 samples initially exhibited increase in strength; however, this was followed by decrease in strength in G15 and G20 samples. This subsequent loss in strength was eventually observed. By replacing 10% of the cement with GGBS, the strength of the material was significantly improved, with improvements of 21.92%, 11.40%, and 10.58% in particular.

Table 4.5: Split tensile strength value of bacillus bacterial solution sample

Sample Name	7 Days	28 Days	56 Days
G10	2.28 MPa	2.54 MPa	2.82 MPa
B3G10	2.48 MPa	2.78 MPa	2.98 MPa
B5G10	2.6 MPa	2.89 MPa	3.08 MPa
B7G10	2.54 MPa	2.68 MPa	2.95 MPa

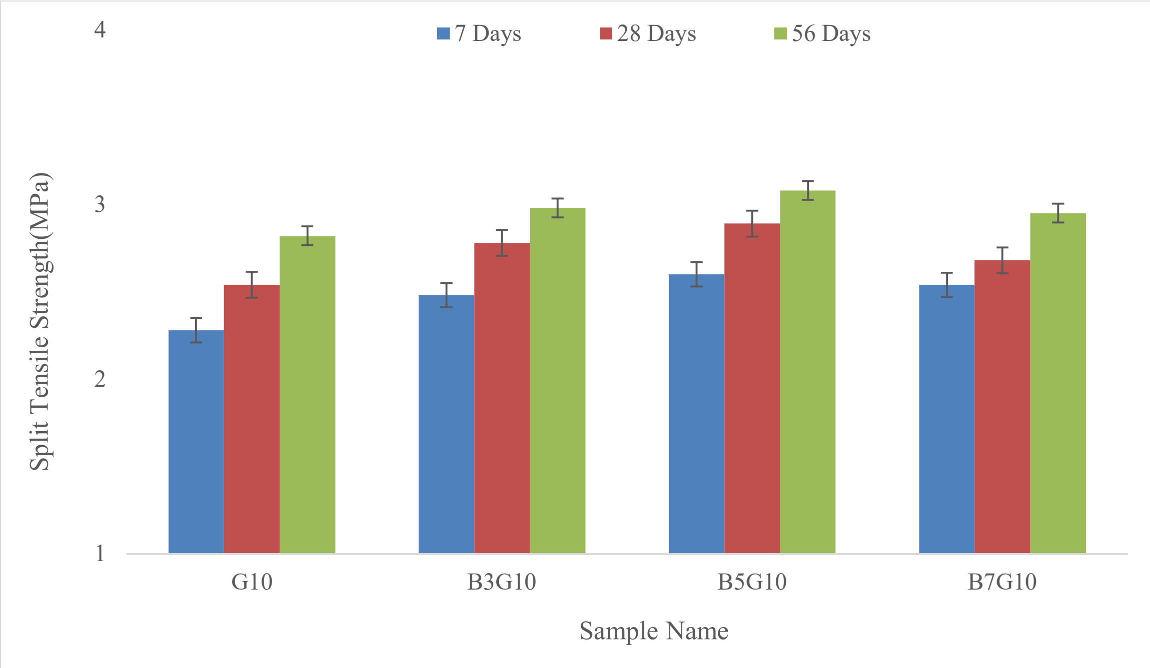


Figure 4.5: Split tensile strength of bacillus bacterial solution sample

The samples with a 10% substitution of GGBS displayed considerable boost in split tensile strength upon introduction of bacterial concentration, as indicated in Figure 4.5. After 56 days of testing, the B3G10, B5G10, and B7G10 mixes showed split tensile strength improvements of 13.44%, 13.77%, and 9.21% respectively, in comparison to the G10 mix. This was the case throughout the whole testing period. 3.12 megapascals was the maximum strength that the B5G10 material could achieve. The observed increase in split tensile strength can likely be attributed to expansion of CSH gel, which occurs with up to 10% substitution of

GGBS.

Table 4.6: Megaterium Bacterial Concrete Split Tensile Strength Value

Sample	7 Days	28 Days	56 Days
S1	2.55 MPa	2.75 MPa	2.87 MPa
S2	2.67 MPa	2.95 MPa	3.02 MPa
S3	2.92 MPa	3.11 MPa	3.16 MPa
S4	2.86 MPa	3.02 MPa	3.09 MPa

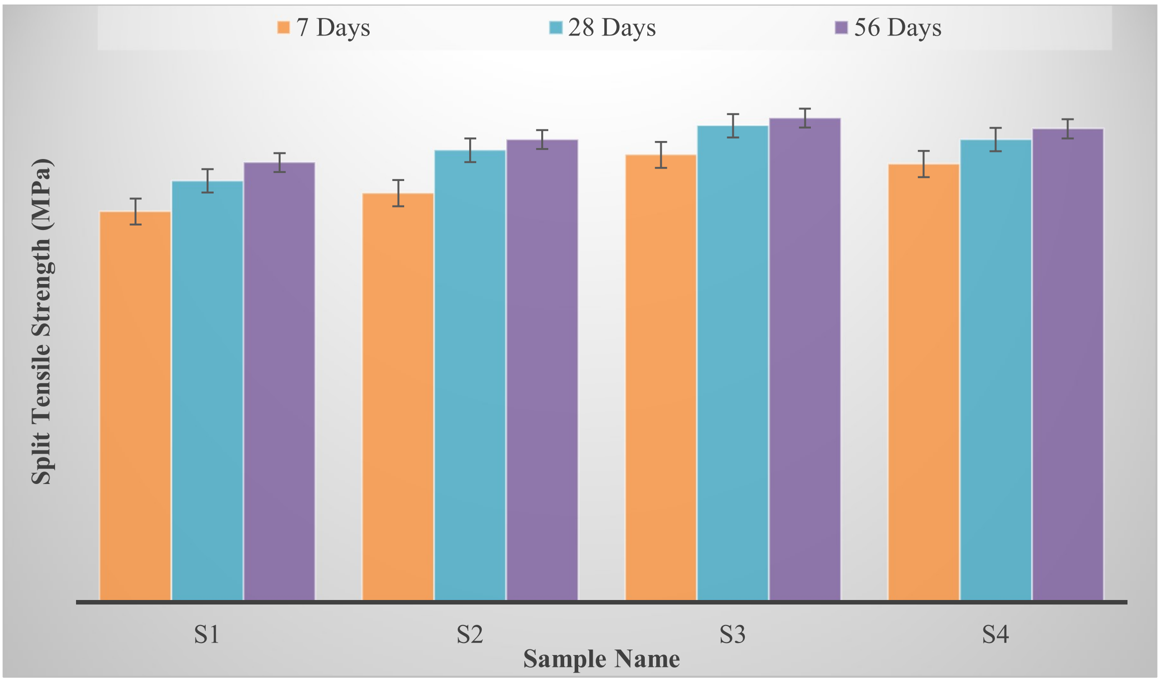


Figure.4.6 Megaterium Bacterial Concrete Split Tensile Strength

Figure 4.6 illustrates the effects that varying concentrations of Bacillus megaterium (10^3, 10^5, and 10^7 cells/ml) have on the split tensile strength of cement concrete materials. Before beginning to fall at 10^7 cells/ml, the split tensile strength first increased up to the concentration of 10^5 bacillus megaterium cells/ml. After reaching this concentration, the strength began to decrease. Over the course of seven, twenty-eight and fifty-six days of testing, the split tensile strength of the S3 samples is significantly higher than that of the cement concrete sample (S1), with estimated increases of 14.50percent, 13.09 percent, and 10.10 percent, respectively. The increase in the concrete split tensile strength that has been bacteria contaminated can be attributed to the development of bacterial colonies as well as the accumulation of calcite within the concrete particle. As an outcome of this process, which entirely filled the pores, the strength and density of concrete have most likely risen.

4.3 Flexural Strength

The flexural strength of concrete beam samples was evaluated at 7, 28, and 56 days after curing in line with the International Standardisation (IS) 516-1959 regulations. This examination was carried out with the use of universal testing equipment and the three-point load technique. Testing was conducted on both the pure form and the form harboring GGBS containing bacteria.

Table 4.7: Flexural Strength value of GGBS sample

Sample Name	7 Days	28 Days	56 Days
G0	4.18 MPa	5.44 MPa	5.79 MPa
G5	5.08 MPa	5.71 MPa	5.98 MPa
G10	5.35 MPa	5.78 MPa	6.18 MPa
G15	5.25 MPa	5.68 MPa	6.08 MPa
G20	4.89 MPa	5.57 MPa	5.91 MPa

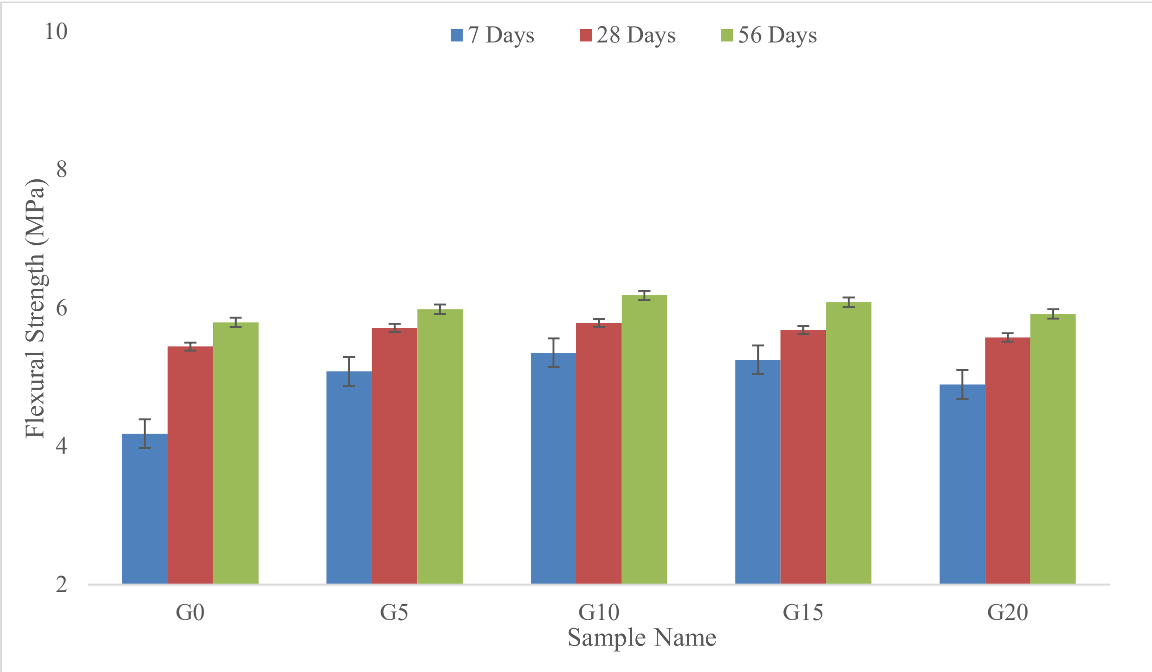


Figure 4.7: Flexural Strength of GGBS sample

As shown in Figure 4.7, the process of replacing 10% of the GGBS with cement resulted in the highest flexural strength being reached. In the beginning, the flexural strength of the G5 and G10 samples was found to be higher than that of the G15 and G20 samples. However, this was followed by a drop in the other two samples. After 28 days, the flexural strength of the material increased significantly by roughly 6.25 percent as a result of the substitution of 10% of the cement with Ground Granulated Blast Furnace Slag (GGBS). In addition to the observed increase in compressive strength, this improvement in flexural strength can be attributed to the production and expansion of calcium silicate hydrate (C-S-H) gel, which occurred as a result of the replacement of 10% GGBS.

The growth of C-S-H gel is one factor that contributes to the improvement of concrete qualities, which includes the enhancement of compressive and flexural strength. The increase in flexural strength that was reported for the S10 samples is supported by the association that exists between the rise in C-S-H gel formation and the improvement in compressive strength. The performance in terms of flexural strength, on the other hand, began to drop once the 10% substitution threshold was reached. This may be attributed to diminishing returns on the benefits of C-S-H gel formation or other variables that impact the concrete matrix.

Table 4.8: Flexural Strength value of bacillus bacterial solution

Sample Name	7 Days	28 Days	56 Days
G10	5.35 MPa	5.78 MPa	6.18 MPa
B3G10	5.7 MPa	6.09 MPa	6.37 MPa
B5G10	5.88 MPa	6.26 MPa	6.6 MPa
B7G10	5.8 MPa	6.07 MPa	6.41 MPa

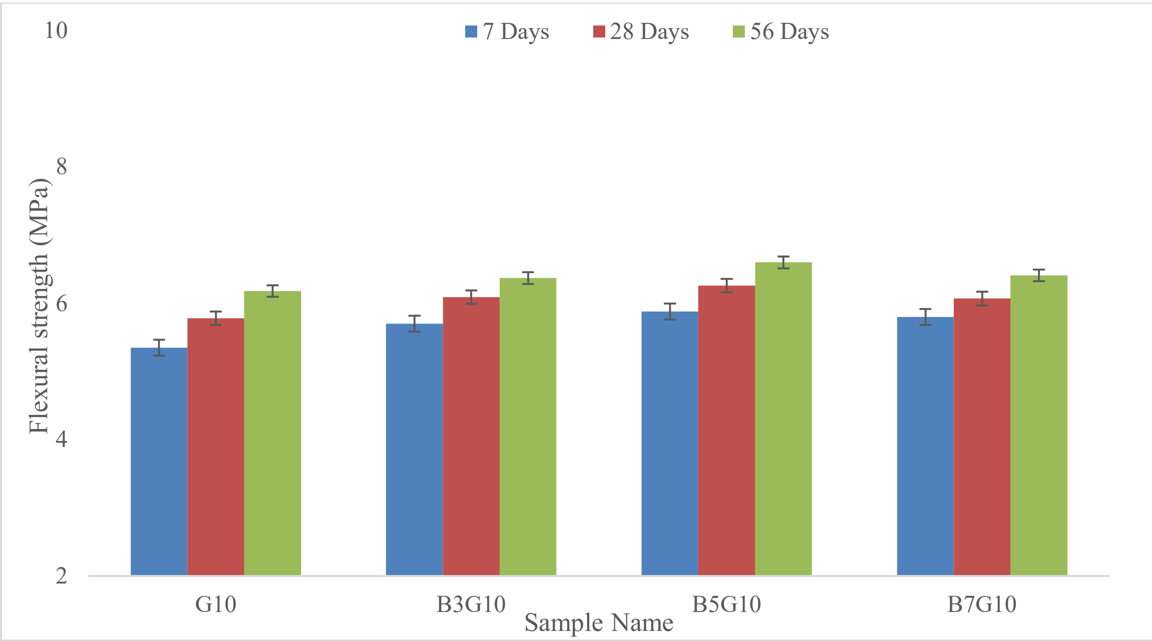


Figure 4.8: Flexural Strength of bacillus bacterial solution

When bacterial content was added to the samples, there was considerable increase in the samples' flexural strength. Figure 4.8, which depicts the improvement that occurred when a 10% substitution of GGBS was employed, shows this improvement. After period of 28 days, flexural strength of B3G10, B5G10, and B7G10 increased by 9.90%, 8.30%, and 6.79% correspondingly, in comparison to the S10 mix. This was the case for all three combinations.

Table 4.9: - Flexural Strength value of megaterium Bacterial Concrete

Sample	7 Days	28 Days	56 Days
S1	5.72 MPa	5.95 MPa	6.12 MPa
S2	5.92 MPa	6.25 MPa	6.33 MPa
S3	6.22 MPa	6.48 MPa	6.63 MPa
S4	6.07 MPa	6.35 MPa	6.48 MPa

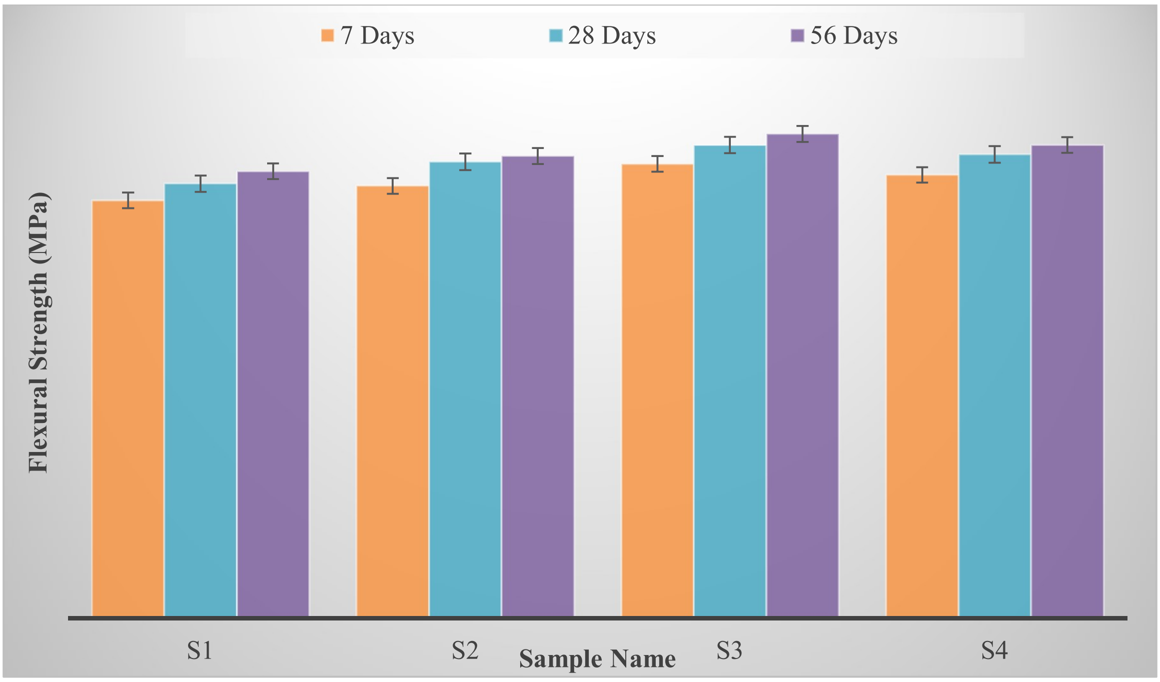


Figure 4.9: Flexural Strength of megaterium Bacterial Concrete

Figure 4.9 shows that at 7, 28, and 56 days of testing, the flexural strength of bacterial solution of bacillus megaterium showed a similar outcomes of strength enhancement as the compressive strength. At 7, 28, and 56 days, respectively, the flexural strength of bacterial concrete demonstrated a maximum increase of 8.74%, 8.90% and 8.33% in comparison to normal concrete, with a bacterial concentration of 10^5cells/ml. The same source that is responsible for the rise in splitting tensile strength is also responsible for the increase in flexural strength of bacterial concrete. The calcite layer that forms on the samples' surface and the bacterially-induced calcite that fills concrete pores are the causes of this phenomenon.

4.4 Water Absorption

Using the ASTM C642 standard, the water absorption (WA) of concrete samples that contained a mixture of Ground Granulated Blast Furnace Slag (GGBS) and bacterial cultures was assessed after 28 and 56 days of water curing. The evaluation was carried out in line with the standard. The water absorption levels for these concrete samples are depicted in Figure 4.10, which highlights the influence that the presence of GGBS and bacteria has on the concrete's capacity to absorb water over time. The investigation demonstrates how the integration of GGBS and bacteria affects the porosity and overall durability of the concrete, with changes being found at various curing periods because of the inclusion of these substances. The evaluation aimed to determine impact of GGBS and bacterial mixture on concrete’s ability to absorb water during the curing period.

Table 4.10: Water Absorption value of GGBS samples

Sample Name	28 Days (%)	56 Days (%)
G0	7.51	6.47
G5	7.01	6.05
G10	6.72	5.72
G15	7.11	6.14
G20	7.47	6.66

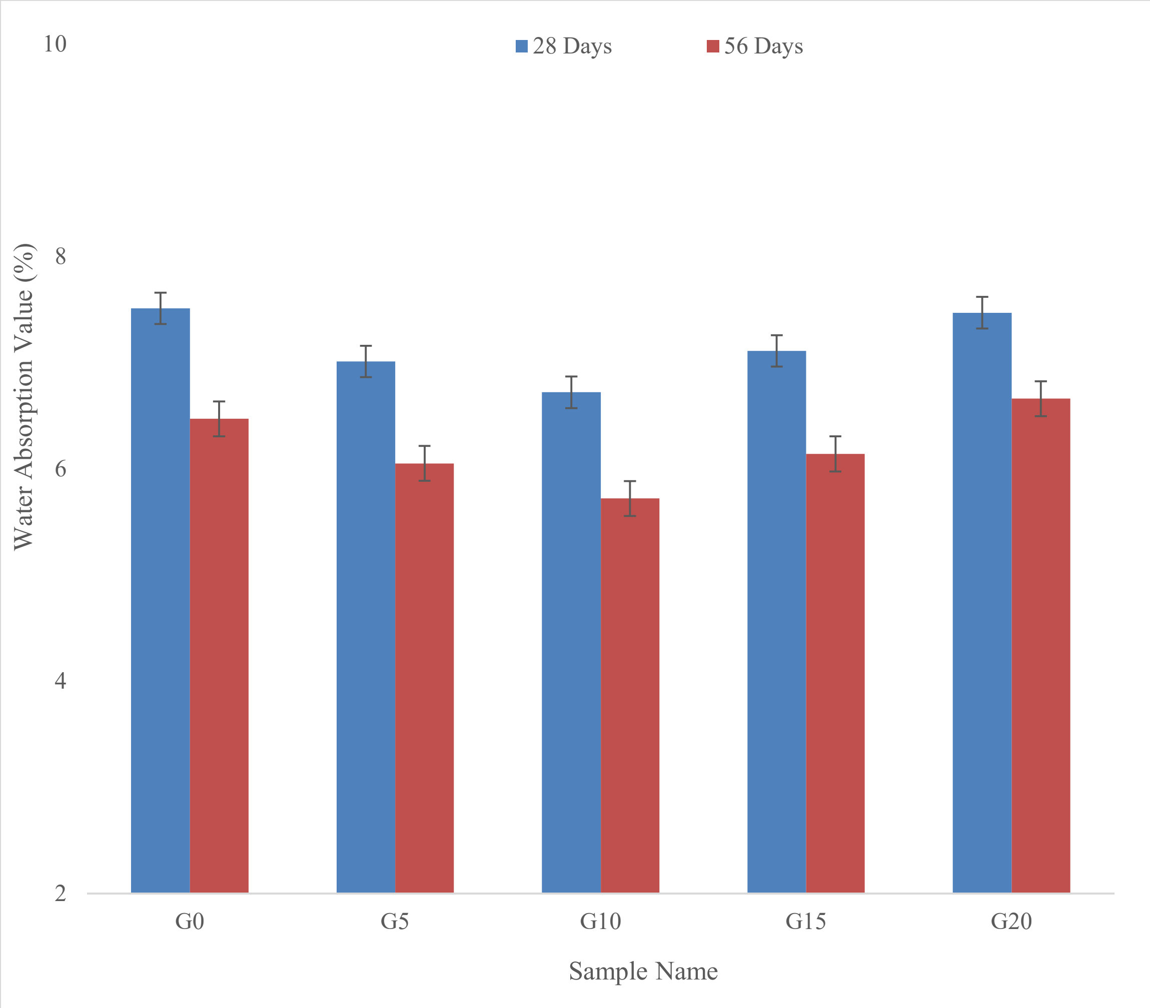


Figure 4.10: Water Absorption value of GGBS samples

Figure 4.10 shows that substituting cement with GGBS led to a reduction in water absorption by up to ten percent. However, as the proportion of GGBS increased, the water absorption value also rise. After 28 days, the water absorption coefficient for a 10% GGBS mixture was 6.72, which improved to 5.72 after 56 days. This reduction is attributed to the gradual constriction of pores over time, which decreases the concrete's water absorption capacity. Without a doubt, the utilization of GGBS results in a substantial reduction in the permeability of pores during the course of an extended period of curing.

Table 4.11: Water Absorption value of bacillus bacterial sample

Sample Name	28 Days (%)	56 Days (%)
G10	6.72	5.72
B3G10	6.42	5.46
B5G10	6.18	4.99
B7G10	5.85	4.78

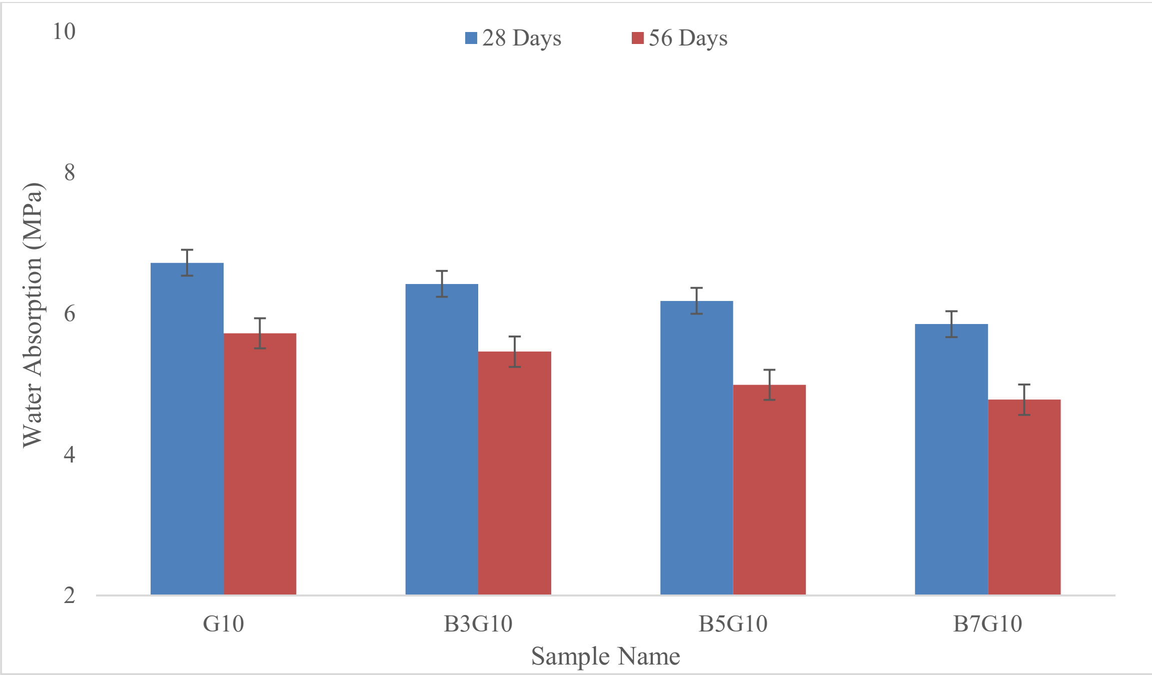


Figure 4.11: Water Absorption value of bacillus bacterial sample

Figure 4.11 shows water absorption measurements for bacterial concrete. Presence of bacteria in GGBS concrete mixture significantly reduced water absorption. This reduction is mainly due to bacterial-induced calcite formation, which fills pores in concrete, thereby decreasing its water absorption (WA).

Table 4.12: Water Absorption Value of Bacillus Megaterium Bacterial Concrete

Sample	28 Days (%)	56 Days (%)	90 Days (%)
S1	6.52	6.14	5.85
S2	6.23	5.85	5.62
S3	6.01	5.64	5.41
S4	5.81	5.48	5.28

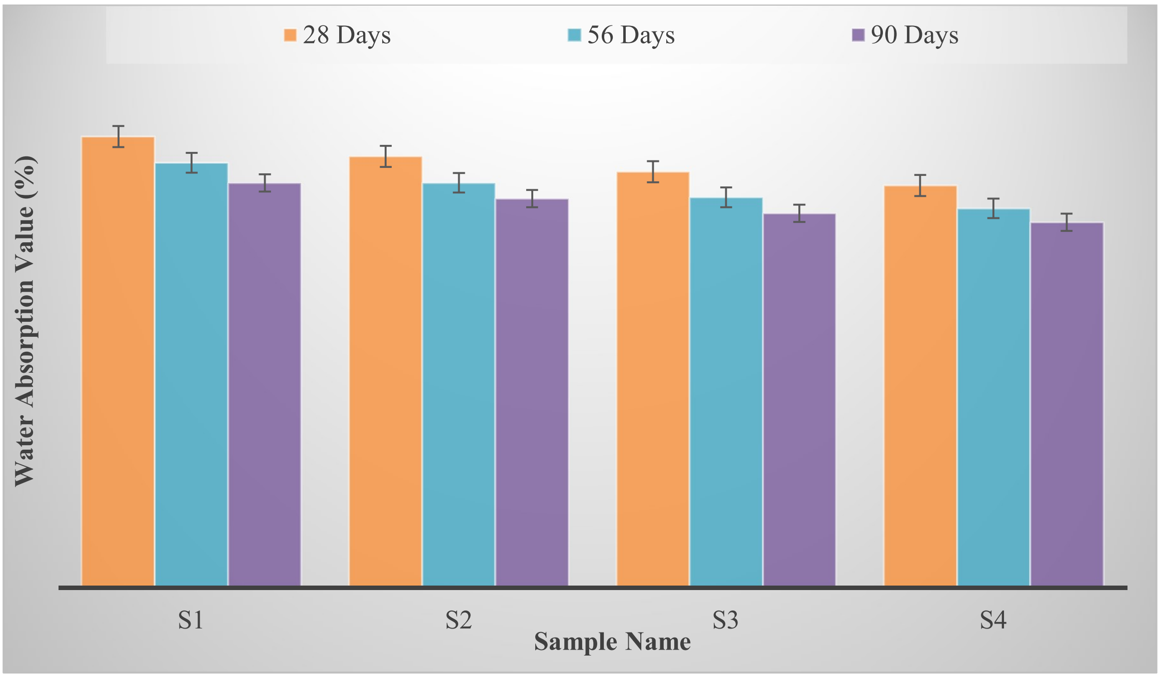


Figure 4.12: Water Absorption Value of Bacillus Megaterium Bacterial Concrete

Figure 4.12 illustrates that the incorporation of bacterial solution into concrete results in a decrease in the water absorption value when compared to the value of conventional concrete. At 28, 56 and 90 days, respectively, when the bacterial concentration was 10^7 cells/ml, the WA of bacterial solution concrete has dropped by 12.22%, 12.04% and 10.79%. This decrease occurred when the compound was exposed to water. The presence of particles of calcite in the presence of voids of bacterial solution concrete ultimately resulted in a decrease in the amount of water that was absorbed by the concrete in contrast to conventional concrete.

4.5 Ultrasonic Pulse Velocity

In order to determine the ultrasonic pulse velocity (UPV) of a substance, a pulse transmitter is placed on one side of the sample, and a receiver is placed on the other side of the sample. The amount of time that the ultrasonic pulse takes to travel through the material is recorded by a device that is used for timing. After that, the UPV is computed by dividing the known distance between the transmitter and the receiver by the amount of time that is necessary for the pulse to travel between those two locations. A coupling agent is utilised in order to reduce air gaps that exist between the transducer diaphragms and the concrete surface. This is done in order to guarantee correct readings. For the purpose of collecting accurate and trustworthy UPV readings, this procedure is very necessary.

Table 4.13: UPV values of GGBS based Cement Concrete

Sample	28 Days (m/s)	56 Days (m/s)	90 Days (m/s)
S0	390	408	416
S5	405	417	425
S10	419	428	439
S15	411	420	431
S20	401	410	422

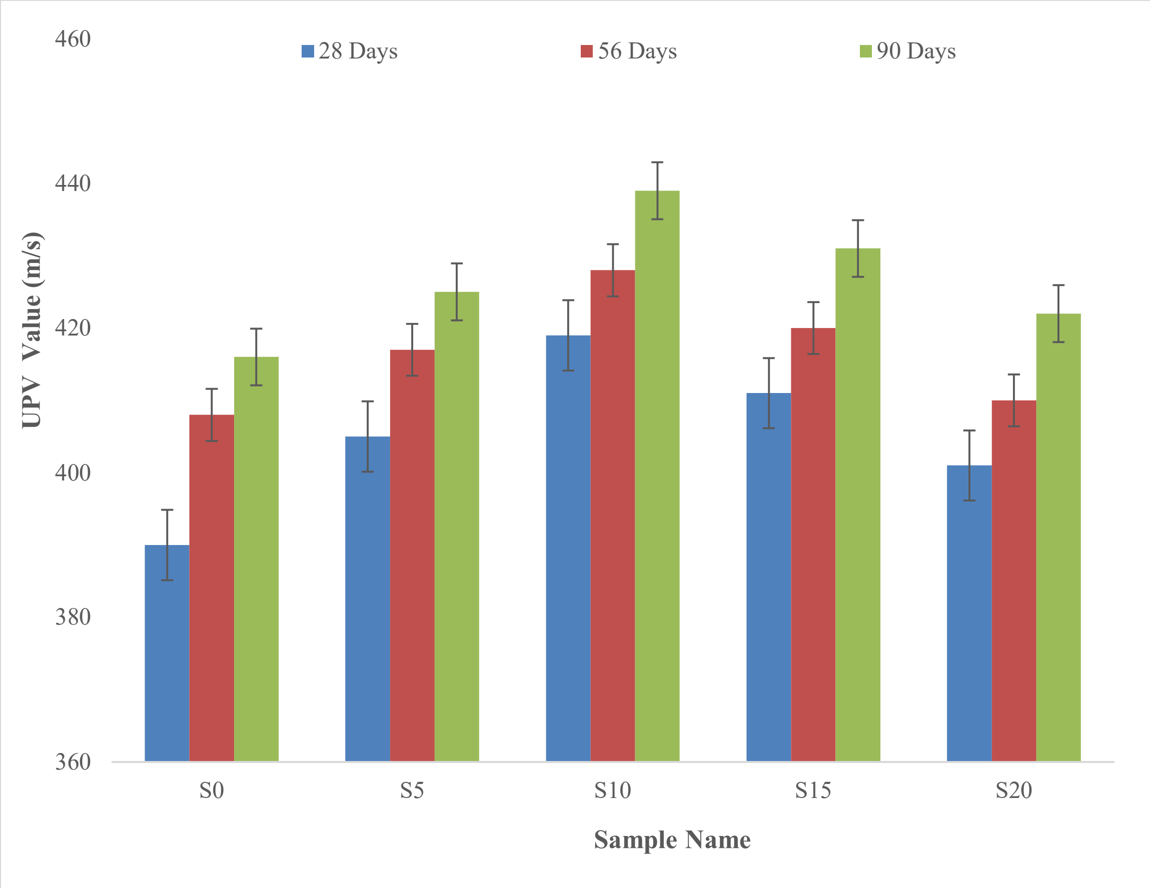


Figure 4.13: UPV values of GGBS based Cement Concrete

Through the use of an ultrasonic pulse, the UPV Test determines whether or not the concrete can be broken through completely. In terms of the density, uniformity, and homogeneity of the material, the UPV Test and the transit time has the potential to provide useful information. The ultrasonic pulse velocity test is a method that is utilized for the purpose of determining whether or not concrete is sound. This method is established in the International Standard 516 (Part 5, Sec-1), 2018. Cubic molds with a diameter of 150 mm^3 were used for the UPV test that was carried out. After replacing cement with ground granulated blast furnace slag (GGBS), Ultrasonic Pulse Velocity (UPV) value showed a maximum increase of 10%. This particular increase was observed. The UPV value, on the other hand, went down after it had reached this point in time. With respect to the standard concrete specimen, the UPV value of sample S10 shown a rise of 7.43%, 4.90%, and 5.52% respectively. Because GGBS particles are smaller in size than cement particles, the UPV value may have increased by as much as ten percent as a result of the filling of empty spaces by GGBS.

Table 4.14: UPV value of bacillus Bacterial Concrete

Sample	28 Days (m/s)	56 Days (m/s)	90 Days (m/s)
S10	419	428	439
B3S10	425	434	445
B5S10	430	442	452
B8S10	434	450	462

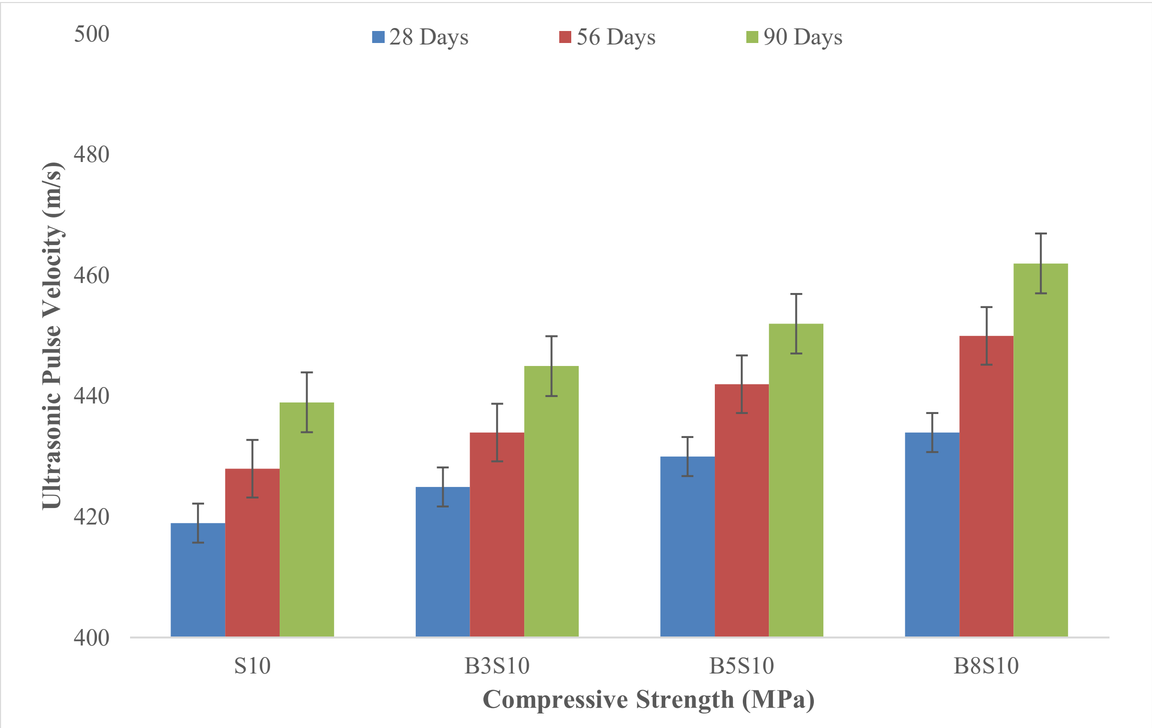


Figure 4.14: UPV value of bacillus Bacterial Concrete

An increase in the UPV values of each individual sample was observed as a result of the introduction of a bacterial solution to the concrete sample, as demonstrated by the data. 439, 445, 452, and 462 meters per second are respective velocities of the samples S10, B3S10, B5S10, and B8S10, respectively. A rise in the UPV value is caused by the incorporation of the bacterial solution, which indicates that it successfully fills the holes or pores in the concrete sample, hence enhancing the sample's uniformity.

Table 4.15: Ultrasonic Pulse Velocity value of Megaterium Bacterial Concrete

Sample	28 Days (m/s)	56 Days (m/s)	90 Days (m/s)
S1	397	414	421
S2	412	423	432
S3	424	435	445
S4	435	446	458

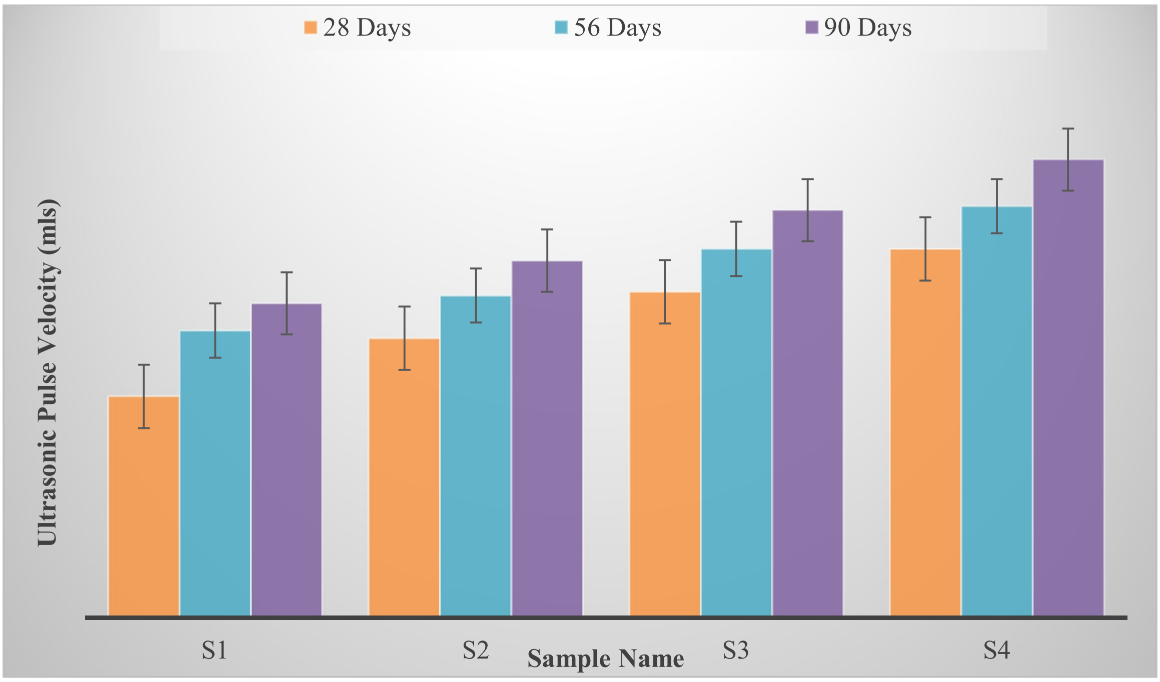


Figure 4.15: Ultrasonic Pulse Velocity of Megaterium Bacterial Concrete

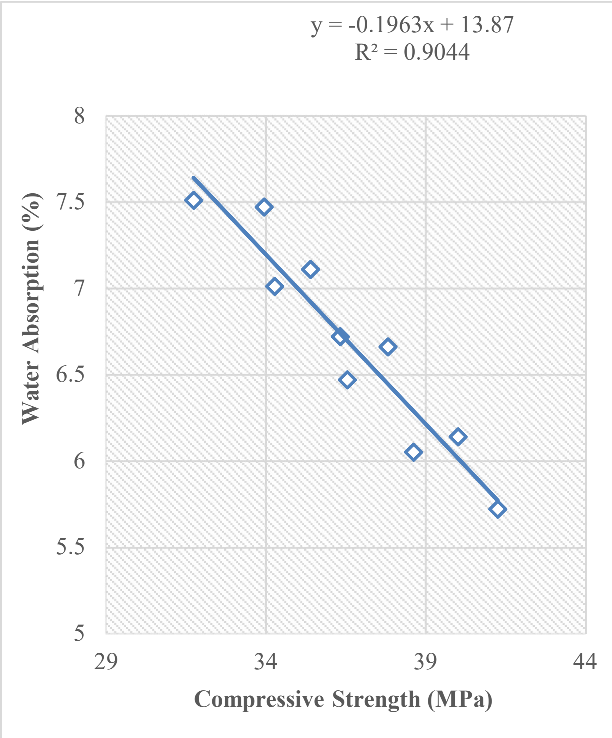
The UPV Test measures the duration of an ultrasonic pulse's penetration into the concrete. The UPV Test and transit time can provide valuable information about the density, uniformity, and homogeneity of the material. The UPV test was conducted on cubical moulds with diameters of 150 mm^3 each. The bacterial concrete underwent a UPV test to confirm its uniformity and structural soundness. The UPV value of Sample S1, S2, S3 and S4 were 421, 432, 445 and 458 m/s at 90 Days of testing. The enhanced UPV results for bacterial samples indicate that the bacterial concrete matrix exhibits a comparatively lower number of pores and cracks. The improvement in UPV readings is attributed to the calcite precipitation that fills the pores in the concrete mixture.

4.6 Regression Analysis of Water Absorption and Compressive Strength

The experiment has revealed a direct relationship between water absorption and compressive strength for both bacterial GGBS concrete and standard GGBS concrete. This relationship is illustrated in Figure 4.16.

Table 4.16: Two variables value for Regression analysis b/w water absorption and compressive strength for GGBS

X-Values	Y-Values
31.74	7.51
34.28	7.01
36.33	6.72
35.4	7.11
33.95	7.47
36.55	6.47
38.63	6.05
41.26	5.72
40.02	6.14
37.83	6.66
36.33	6.72
39.86	6.42
41.72	6.18
40	5.85
41.26	5.72
43.82	5.46
46.69	4.99
44.86	4.78



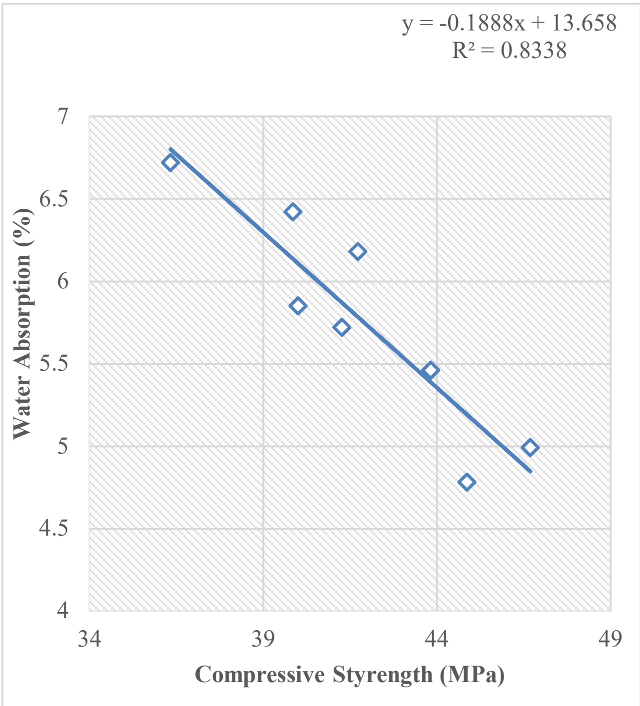


Figure 4.16: Regression analysis b/w water absorption and compressive strength for GGBS sample and bacterial solution

The compressive strength of GGBS concrete is shown to have a favorable correlation with its water absorption performance, as demonstrated in Figure 4.16. Figure 4.16 illustrates the link between the amount of water that is absorbed by the bacterial GGBS concrete and its compressive strength. There is a possibility that the presence of bacteria has decreased the number of empty spaces and porosity, which could be the reason why the mixture is not absorbing water as effectively as it normally would. Compared to the bacterial GGBS mix, which has an R^2 value of 0.8338, the GGBS mix has an R^2 value of 0.9044.

Table 4.17: Two variables value of Compressive Strength and Water Absorption

X-Values	Y-Values
39.26	6.14
42.85	5.85
46.05	5.64
44.92	5.48
41.14	5.85
43.65	5.62
47.84	5.41
43.92	5.28

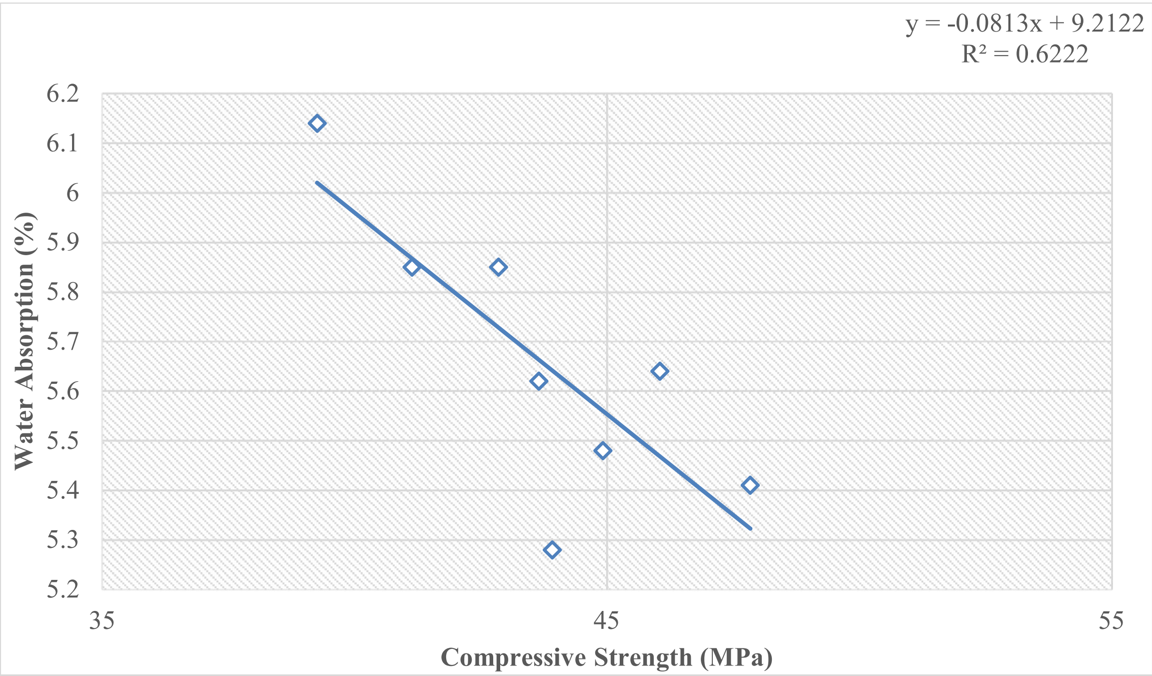


Figure 4.17: Regression Analysis of megaterium between Compressive Strength and Water Absorption

The relationship between the compressive strength of bacterial concrete and its ability to absorb water is illustrated in Figure 4.17. There is a possibility that the presence of bacterial solution, which has resulted in a decrement in pores and voids, is the cause of the diminishing water absorption in the combination. The bacterial concrete mix has R^2 values of 0.6222 for each of its components.

4.7 Regression analysis between compressive and UPV

The link between the compressive strength of GGBS (Ground Granulated Blast Furnace Slag) and bacterial GGBS concrete and the UPV (Ultrasonic Pulse Velocity) for bacterial GGBS concrete is illustrated in Figure 4.18. This relationship was demonstrated in the research study.

Table 4.18: Correlation b/w Split tensile and Compressive Strength of GGBS and Bacterial Samples

X-Values	Y-Values
31.74	390
34.28	405
36.33	419
35.4	411
33.95	401
36.55	408
38.63	417
41.26	428
40.02	420
37.83	410
36.33	419
39.86	425
41.72	430
40	434
41.26	428
43.82	434
46.69	442
44.86	450

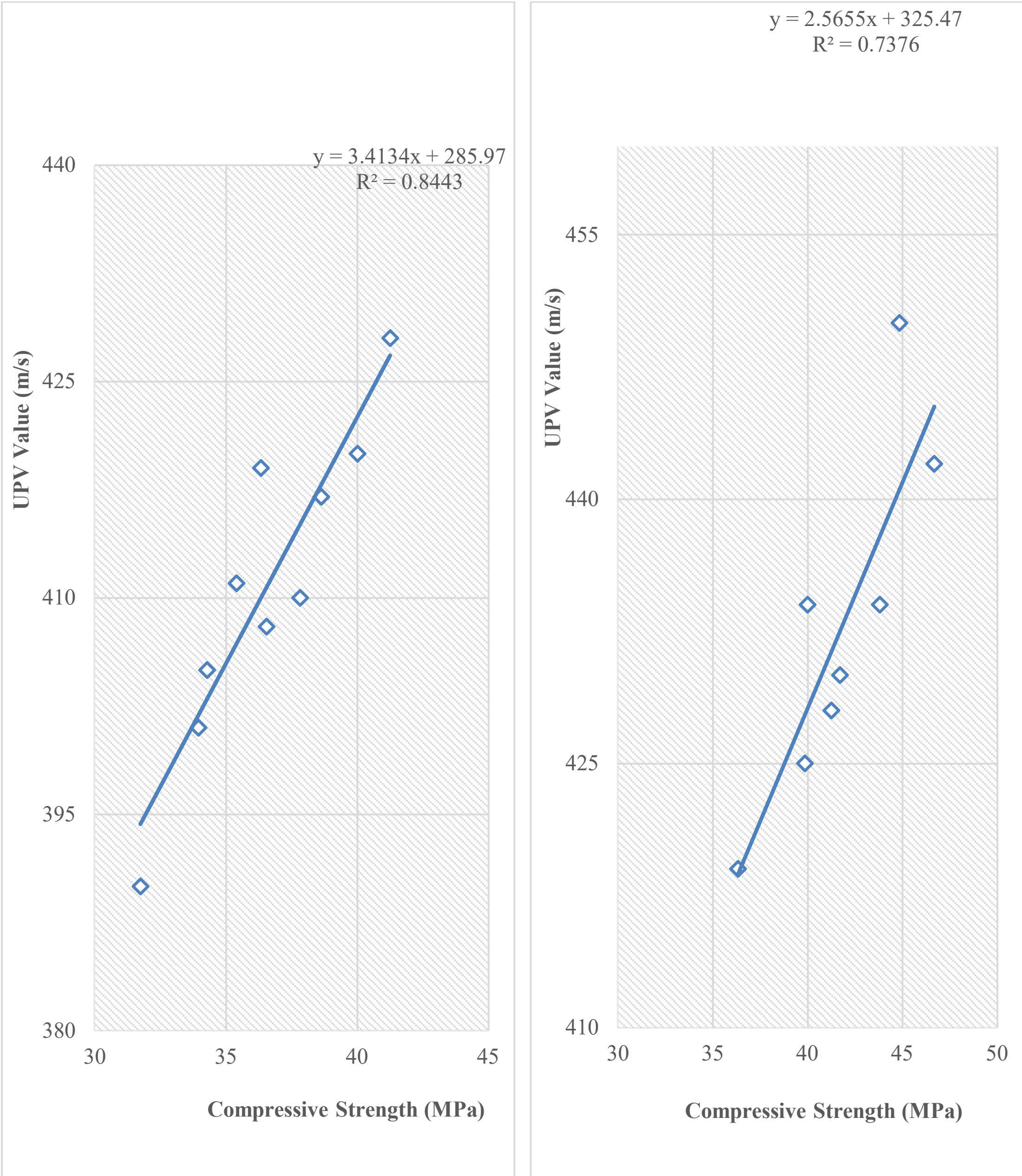


Figure 4.18: Correlation b/w Split tensile and Compressive Strength of GGBS and Bacterial Samples

As can be seen in Figure 4.18, there is a significant relationship between compressive strength and UPV in GGBS concrete. The relationship between the compressive strength of bacterial GGBS (ground granulated blast furnace slag) concrete and the ultrasonic pulse velocity (UPV) is depicted in Figure 4.18. In comparison, the bacterial GGBS mix has an R^2 value of 0.7376, whereas the GGBS mix has an R^2 value of 0.8443 at the moment.

Table 4.19: **Variables for Regression Megaterium Analysis between Compressive Strength and UPV**

X-Values	Y-Values
39.26	405
42.85	412
46.05	424
44.92	435
41.14	414
43.65	423
47.84	435
43.92	446

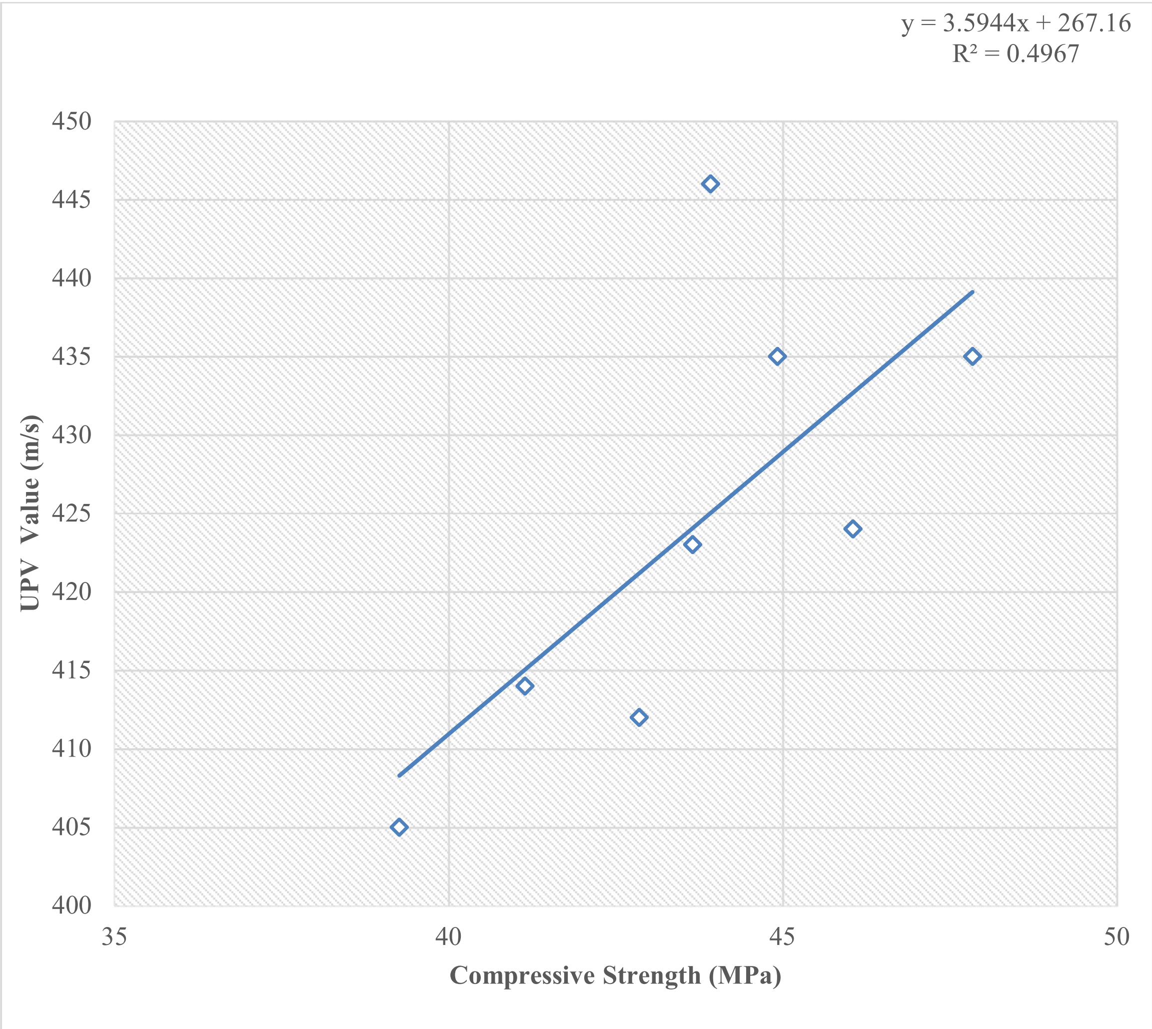


Figure 4.19: Regression Megaterium Analysis between Compressive Strength and UPV

The coefficient of determination ($R^2 = 0.4967$) indicates a weak correlation between the data points and the regression curve of ultrasonic pulse velocity and compressive strength values. Equation (2) shows that the compressive strength increases until a concentration of 10^5cells/ml is reached, and the ultrasonic pulse velocity improves for all samples. The relation between strength in compression and UPV is week because of compressive strength and UPV value was not directly proportional.

4.8 Cluster Analysis of Water Absorption Compressive Strength

Multivariate statistical methods are commonly employed to classify, analyze, and interpret large datasets. These strategies can be used to decrease the complexity of datasets while preserving as much of the actual details as possible. CA (Cluster analysis) is a branch of unsupervised pattern classification. Its main objective is to divide objects into clusters or categories, based on their similar pattern within a category and their differences between categories. Figure 4.20 below illustrates the outcomes of a cluster analysis conducted on the variables of compressive strength and water absorption.

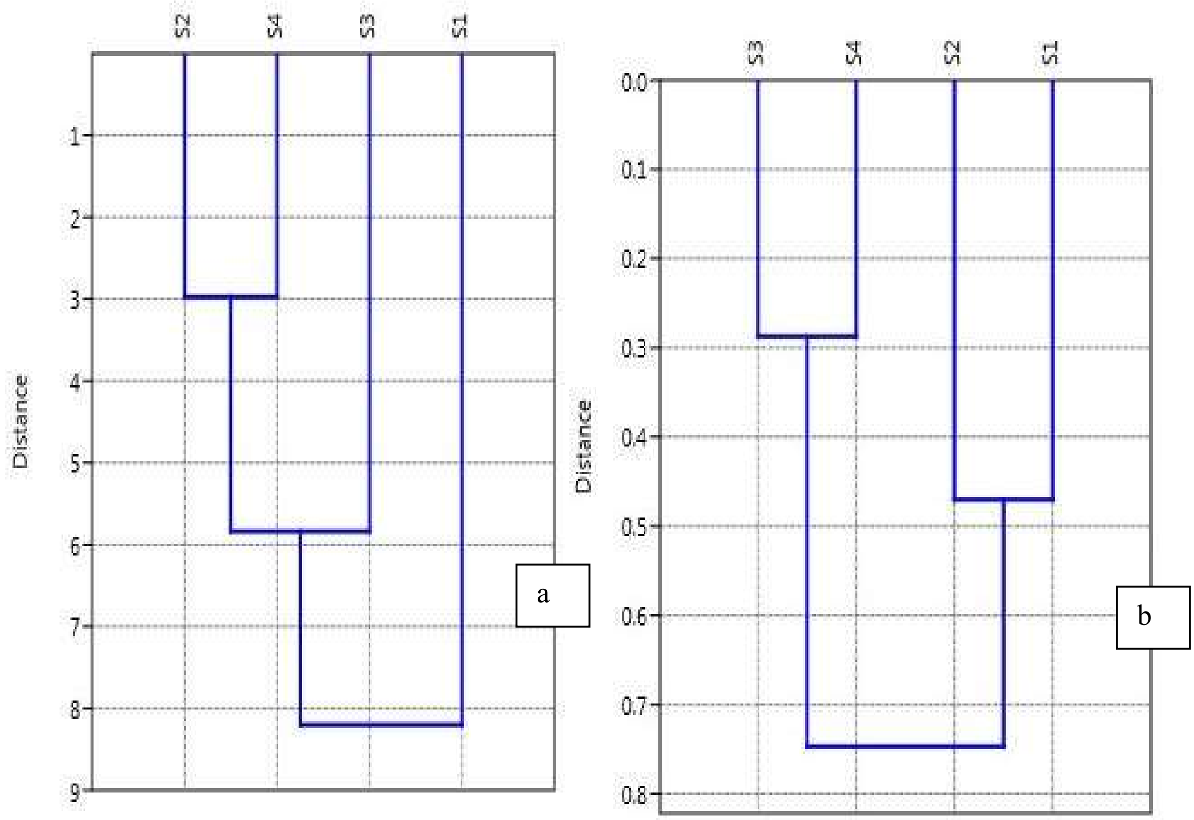


Figure 4.20: Analysis of Cluster (a) Compressive Strength (b) Water Absorption

The results of Cluster processing (CA) are valuable for data processing and uncovering patterns. The Euclidean distance is a commonly employed metric for quantifying the similarity between two circumstances. It is calculated as the square root of the average squared difference between two sets of data. Sample S2 and S4 exhibited comparable trends in compressive strength, while sample S3 and S4 displayed similar trends to sample S2 and S1 for water absorption.

CHAPTER 5
CONCLUSION

Following conclusions can be drawn from the study which are as follows: -

1. The findings indicated that the compressive, split tensile and flexural strength of Bacillus Subtilis bacteria and Megaterium initially increased concentration up to 10^5 cells/ml, after which it decreased when the concentration was further increased to 10^7 cells/ml.

2. Although the use of GGBS in place of cement resulted in an increase in the concrete's strengths up to a 10% replacement level, after that point, the strengths began to decrease.

3. Incorporation of bacteria into GGBS concrete mixture led to reduction in water absorption values. This decrease is primarily due to the calcite formation by the bacteria, which fills the pores in the concrete specimens, thereby lowering water absorption (WA).

4. The water absorption value approximately 10% decreases as the concentration of GGBS increased in place of cement as compared to nominal concrete. While in bacillus megaterium the water absorption (WA) of bacterial solution concrete mix has reduced by 12.22%, 12.04% and 10.79% of 10^7 cells/ml bacterial concentration as compared to nominal concrete.

5. The UPV value are 439, 445, 452, and 462 meters per second are respective velocities of the samples S10, B3S10, B5S10, and B8S10, respectively. A rise in the UPV value is caused by the incorporation of the bacterial solution, which indicates that it successfully fills the holes or pores in the concrete sample, hence enhancing the sample's uniformity. While in megaterium bacteria The UPV value of Sample S1, S2, S3 and S4 were 421, 432, 445 and 458 m/s at 90 Days of testing. The enhanced UPV results for bacterial samples indicate that the bacterial concrete matrix exhibits a

comparatively lower number of pores and cracks.

6. The value of R^2 of GGBS and bacterial samples have relationship between compressive strength and water absorption were 0.771 and 0.818, respectively. While in bacillus megaterium the R^2 value of regression analysis between and compressive and water absorption was 0.6222 and R^2 value between compressive strength and UPV was 0.4967.

CHAPTER 6
FUTURE SCOPE

Future Scope of the Work are-

1. Agricultural and industrials waste can be used with the replacement of cement for minimizing the carbon foot print in the concrete industry.

2. Other bacterial strain of bacillus family can be used in the cement mix to determine the effect on the properties of concrete.

3. Sulphate and acid resistance properties of bacterial concrete can be checked in further study.

4. Microstructural analysis of bacterial concrete sample can be done in further study.

5. Thermal and fire resistance properties of bacterial concrete can be checked in further study.

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